Search-for-Life Instrument Study

Identifying Scientific Knowledge and Technology Gaps



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Revision A

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ACRONYMS

AA	Amino acid(s)
AFM	Atomic force microscopy
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid (colorimetric protein assay)
C^4D	Capacitively coupled contactless conductivity detection
CA	Carboxylic acid(s)
CE	Capillary electrophoresis
CE-MS	Capillary electrophoresis-mass spectrometry
CRDS	Cavity ring-down spectroscopy
DDA	Data-dependent acquisition
DFAA	Dissolved free amino acids
DIA	Data-independent acquisition
DNA	Deoxyribonucleic acid
DUV	Deep ultraviolet
EC	Electrochemical (analysis)
EI-MS	Electron-ionization mass spectrometry
ELIE	Electronic Life-detection Instrument for Enceladus/Europa
EMILI	Europan Molecular Indicators of Life Investigation
ESA	European Space Agency
ESI-MS	Electrospray-ionization mass spectrometry
FLUMIAS	Fluorescence Microscopy Analyses In Space
GC	Gas chromatography
GC-MS	Gas chromatography–mass spectrometry
HCl	Hydrochloric acid

HEO	Habitable environment origin
HiDAG	Hierarchical directed acyclic graph
HILIC	Hydrophilic interaction liquid chromatography
HMW	High molecular weight
HMWC	High molecular weight compound(s)
HPLC	High-performance liquid chromatography
IC	Ion chromatography
IR	Infrared
ISS	International Space Station
JHU/APL	Johns Hopkins University Applied Physics Laboratory
JPL	Jet Propulsion Laboratory
LC	Liquid chromatography
LC-MS	Liquid chromatography–mass spectrometry
LD-MS	Laser desorption/ablation mass spectrometry
LIF	Laser-induced fluorescence
LLE	Liquid–liquid extraction
LoD	Limit of detection
MECA	Mars Environmental Compatibility Assessment
MeOH	Methanol
MIDAS	Micro-Imaging Dust Analysis System
MMX	Martian Moons eXploration
MS	Mass spectrometry
MSL	Mars Science Laboratory
MS^n	Tandem (multistage) mass spectrometry
m/z	Mass to charge ratio

NASA	National Aeronautics and Space Administration
OWL	Origins, Worlds, and Life (2023 Decadal Survey)
PESTO	Planetary Exploration Science Technology Office (NASA)
PICASSO	Planetary Instrument Concepts for the Advancement of Solar System Observations
PISCES	Planetary In-Situ Capillary Electrophoresis System
PRM	Parallel reaction monitoring
PVEx	Planetary Volatiles Extractor
Q11	Decadal Survey Question 11 (search for life)
RNA	Ribonucleic acid
RP-HPLC	Reversed-phase high-performance liquid chromatography
SA/SPaH	Sample Acquisition, Processing, and Handling (subsystem)
SAM	Sample Analysis at Mars
ScWE	Subcritical water extraction
SDS	Sodium dodecyl sulfate
SDT	Science Definition Team
SEM	Scanning electron microscopy
SHERLOC	Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals
SHIELD	Sterilizing Heat Infrared Emitting LED Device
SOLID	Signs Of Life Detector
SPE	Solid-phase extraction
SRM	Super-resolution microscopy
SSN	Solid-state nanopore
SWaP	Size, weight, and power
THAA	Total hydrolyzable amino acids

TLS	Tunable laser spectroscopy
TRIDENT	The Regolith and Ice Drill for Exploration of New Terrains
UV	Ultraviolet
UV-Vis	Ultraviolet–visible (spectroscopy)
ppb/ppbw	Parts per billion/parts per billion by weight
ppw	Parts per weight (g/g)

FOREWORD

Study Background and Scope

The search for life beyond Earth remains one of the most compelling scientific priorities identified in the 2023 Planetary Science and Astrobiology Decadal Survey, *Origins, Worlds, and Life* (OWL) (National Academies of Sciences, Engineering, and Medicine, 2023). Central to this vision is **Question 11:** ***Is there evidence of past or present life in the solar system beyond Earth, and how do we detect it?***

Answering this question requires more than increasingly sensitive instruments. It demands an end-to-end understanding of the entire detection chain: the biosignatures potentially present in a habitable environment, the physical transport that carries and modifies material from that habitable environment to a sampling location, and the way in which sample acquisition, ingest, processing, and analysis together determine whether a biosignature can be detected and confidently interpreted within complex sample matrices. Table 21-1 of the OWL (National Academies of Sciences, Engineering, and Medicine, 2023) underscores this same need — for technologies that distinguish biological signals from abiotic chemistry, reduce ambiguity, and improve detection limits across complementary measurement types.

The study presented here was commissioned by the NASA Planetary Exploration Science Technology Office (PESTO) to identify the scientific knowledge and technology gaps that could hinder the success of future *in situ* life-detection missions. Its scope, the environments considered, and the specific questions addressed are presented in **Section 1**. **Section 2** describes the framework and methods of the study, **Section 3** provides the results, and **Section 4** discusses the findings. Last, **Section 5** provides a summary.

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EXECUTIVE SUMMARY

Introduction

The search for life beyond Earth is among the most profound goals in planetary science, yet no search-for-life mission has flown since Viking in 1976. In the intervening decades, researchers have greatly advanced the understanding of biosignatures and the false-positive and false-negative challenges inherent to claiming a detection of life. Recent mission concepts — Mars Life Explorer, Europa Lander, Enceladus Life Explorer, and Enceladus Orbilander (Williams et al., 2021; Hand et al., 2022; Reh et al., 2016; MacKenzie et al., 2022) — have identified the biosignatures and contextual measurements that could indicate the presence of life on Mars, Europa, and Enceladus. The instruments capable of making these measurements, however, often have little to no flight heritage and remain at low flight-development suitability. The successful *in situ* identification of life in the solar system will require multiple independent lines of evidence, including data drawn from across the molecular hierarchy that separates living systems from their simpler building blocks — evidence that can be gathered from a single sample as it is progressively interrogated, from intact cells down to their constituent molecules.

Purpose

This study identifies the **scientific knowledge and technology gaps** that could hinder the success of future *in situ* life-detection missions in our solar system, in direct support of Question 11 of the 2023 OWL Decadal Survey — *Is there evidence of past or present life in the solar system beyond Earth, and how do we detect it?* (National Academies of Sciences, Engineering, and Medicine, 2023). Study results are intended to help the astrobiology community target high-value research and to inform the NASA Planetary Exploration Science Technology Office (PESTO) on future technology development needs. Also, rather than recommending which biosignatures or instruments a given mission should select, which is highly mission architecture specific, the study provides a quantitative framework for understanding *where, along the path from cell to analysis signal, detectable material is lost* and correspondingly identifies technology gaps that, if closed, would most benefit detection approaches.

Scope and Approach

This study covers three sampling scenarios corresponding to environments targeted by current mission concepts: **Mars subsurface permafrost** and the **near-surface environments at Europa and Enceladus** (via landed or flythrough sample collections). Technologies considered span those relevant to *in situ* biosignature detection — sample collection and handling, sample preparation and processing, and the measurement instruments themselves — but exclude spacecraft-enabling technologies such as avionics and communications.

To analyze the three sampling scenarios, we developed a **Fermi-style end-to-end model**. Starting from estimated whole-cell inventories, six biosignature classes were evaluated: cells, carboxylic

acids, amino acids, proteins and peptides, high-molecular-weight compounds (HMWC), and nucleic acids. The model begins with an estimated biosignature inventory at each habitable environment origin (HEO). The inventory is bracketed using cell measurements from terrestrial analog sites as true cell densities in the planetary environments are unknown. The model then propagates a biosignature stepwise through physical transport, sample acquisition, and sample processing, applying literature- and laboratory-derived estimates of dilution, concentration, degradation, adsorption, and handling loss. The biosignature density or quantity ultimately delivered to an instrument is then compared against estimated detection limits to determine whether a signal is measurable. Flight suitability for the various techniques is also considered and discussed.

Key Study Points

1. **This study identified representative published "high" and "low" cell densities measured in terrestrial ocean world and Mars analog ecosystems to constrain the modeling effort (see Section 2.3).** These data were used to bound a range of cell densities that could potentially be found in Mars permafrost and ocean world oceans. The "high-density" values selected are moderate (i.e., they are not on the high end reported for the representative analog ecosystems used). Even so, the levels selected for each planetary analog used span 3–4 orders of magnitude. These cell densities were then used to estimate representative levels of each biosignature derived from an "average" microbial cell. Although these average levels vary across different life forms, the orders-of-magnitude range for cell densities exceeds, by far, the biosignature concentrations in different cell types. Past mission concept studies [e.g., Mars Life Explorer, Orbilander, and Europa Lander (Williams et al., 2021; Hand et al., 2022; MacKenzie et al., 2022)] bracketed lower cell densities than terrestrial analog measurements. Therefore, differences exist between those concept studies' bioenergetically derived estimates and this study's estimates, which are grounded in terrestrial analog values. These levels provide a starting point for interpreting the results. The reader can "slide" the vertical lines in the report's results charts (e.g., **Figure 15–Figure 25**) to the left or right to find how various cell/biosignature levels affect the detection ability by different workflows.
2. **The modeling approach applied in this study takes a conservative perspective on searching for signs of life by considering only signatures from cells and their cell-associated biomolecules as the target biosignatures of interest.** Earth ecosystems harbor life and the byproducts of life (which are signs of life themselves). These materials can be abundant and can rival — and, in some cases, even exceed — the levels of molecular biosignatures found in cells. However, this study did not consider the signal increases that extracellular biosignatures could contribute from past or present life, as these data are difficult to track down comprehensively and quantitatively in analog ecosystems, and thus were considered beyond the study's scope. This is an important aspect that could be included in future model scenarios and is equally important for instrument developers and astrobiologists to keep in mind.

Key Study Points — *continued*

3. **This study did not consider potential signals that could be attributed to abiotic sources.** We are aware that abiotic molecules (i.e., dissolved and particulate matter that is not derived from life and geochemical signatures that mimic life but are derived completely abiotically) are important to consider (e.g., Davilla et al., 2026). However, we determined that detailed consideration of these signals for assessing technology gaps was outside the scope of the study, beyond highlighting the importance that missions should have instrumentation to discern between the two.
4. **This study did not consider requirements that would be specific to individual, particular NASA missions.** Specifically, measures required for contamination control and planetary protection were deemed outside the scope of this study. However, we acknowledge the importance of these aspects to sample collection, sample handling, and instrument design with regard to mission requirements.

Key Findings

Cross-cutting

FINDING: The estimated concentration of biosignatures at the point of origin spans several orders of magnitude and is the single dominant source of sensitivity in the model. Improving the fidelity of biosignature density estimates, including incorporating abiotic background signals, is the highest-value knowledge gap to close.

FINDING: Across nearly all biosignature classes, many measurement approaches share the same initial sample processing steps. Developing these shared front-end technologies would enable a large number of downstream measurement techniques, making them a high-leverage technology development opportunity identified by this study.

FINDING: A stepwise analysis strategy, beginning with the simplest, least-destructive measurements to establish environmental context and preserve sample integrity, then proceeding to progressively more targeted and destructive analyses, maximizes scientific return while minimizing irreversible sample loss.

FINDING: The concentration or depletion of biosignature levels during physical transport from a habitable environment to a sampling location, i.e., transport factor, is poorly constrained, particularly for ocean world plume and ice shell pathways. Targeted laboratory, field, and numerical studies are needed both to improve our understanding of the bounds on these transport factor values and to characterize the co-transported compounds and particulates that will accompany biosignatures into sample collection and processing.

Sample Acquisition and Handling

FINDING: There are fundamental technology shortfalls in subsurface and plume sampling architectures. In particular, there is an absence of systems capable of acquiring samples from

depths greater than ~10 cm below the surface, along with the associated hardware for core extraction, preservation, and handling for downstream processing.

FINDING: A critical capability gap, when acquiring and processing liquid or melted samples, is the ability to collect large sample volumes (hundreds of milliliters to a few liters) and concentrate or filter them into a small volume (approximately tens of milliliters or less) suitable for downstream analysis. Supporting needs include miniaturized liquid handling (milli- and micro-fluidics), phase separation, on-particle chemical processing, pH adjustment, purification and desalting, sample splitting, and sample mounting.

FINDING: For the ocean worlds, the inability to extract and promptly process bulk samples from beyond 10-cm depth, compounded by low-gravity sampling challenges and the lack of a reliable bulk liquid-transfer system, is a critical gap that blocks spatially resolved subsurface analysis. For plume flythrough capture, limited sample volume collection remains a fundamental shortcoming.

Biosignature- and Measurement-Specific

FINDING: No single analytical advance is sufficient for any biosignature class; sensitivity and reliability emerge only from tightly coupled, fully automated, end-to-end workflows demonstrated on realistic analog samples under mission-relevant conditions. Study results and findings in **Sections 3 and 4** indicate the sample processing and analysis workflows that provide greater capabilities, with certain specificity, and also indicate developments in flight suitability and workflow sensitivities that are necessary for successful detections. Highlights of the findings include:

1. **Cells:** Microscopes present a highly capable and efficient technique to detect cells, with low cell counts required — warranting dedicated development toward flight readiness.
2. **Carboxylic acids:** Coordinated advances across phase-preserving sample acquisition and handling, extraction chemistry, separation, detection, and system-level integration would enable sensitive, reliable carboxylic acid measurements.
3. **Amino acids:** Developments of ultra-clean, low-background fluidic architectures capable of separation, extraction, and detections from within complex natural samples would substantially enhance capabilities. Chirality provides a high-value biosignature when combined with quantitative and phase-resolved measurements.
4. **Proteins and peptides:** With current sample volume collection capabilities, optical detection methods (ultraviolet fluorescence, colorimetric, spectrophotometric) are not optimal for proteins and peptides. Developments in tandem mass spectrometry (MS/MS) and solid-phase extraction (SPE) would improve detection proficiencies.
5. **HMWC:** Size-based filtration in the 0.1- to 0.22-µm range, paired with downstream lysis, is particularly valuable for this biosignature class (and would contribute to all prior classes

mentioned) and would benefit from near-term flight advancements. Again, MS/MS capabilities and SPE would improve detectability and confident characterization.

6. **Nucleic acids:** With additional development to improve flight robustness and functionality for non-terrestrial base-pair chemistry, nanopore devices offer a powerful capability to detect and characterize nucleic acids, while also providing the ability to rule out terrestrial contamination. Nanogap sensing is a low-flight-suitability technology that could offer advancements with future developments.
7. **Indicators of biotic origin:** Although the study did not consider signals that could be from abiotic sources, certain measurements considered in the study can provide indicators of biotic origin. Tandem mass spectrometry (MS/MS) provides diagnostic fragmentation patterns, enabling more confident molecular identification than precursor mass alone. Additionally, a clumped-isotope measurement of methane is a particularly diagnostic tracer of biological versus abiotic origin. Isotopic ratios are discussed in **Appendix D**.

Development Needs Snapshot

In order of leverage identified by this study, the greatest improvements can be derived from:

1. **Sharing front-end sample processing:** Large-volume collection and concentration/ filtration plus the supporting microfluidic, separation, and purification steps that extract the majority of downstream biosignatures from the cells for downstream measurements at once.
2. **Acquiring subsurface and plume samples:** Deeper (>10 cm) subsurface acquisition; subsurface core extraction, handling, and preservation; low-gravity sampling and handling; and integrity-preserving plume capture and handling.
3. **Using validated, fully automated, end-to-end analytical workflows:** Automated and validated workflows on realistic, low-biomass concentration terrestrial analog samples under mission-relevant conditions.
4. **Closing the biological estimate and transport knowledge gaps:** Targeted laboratory and field analog studies to improve constraints on biosignature concentrations expected at the planetary environment origin, on abiotic backgrounds, and on geophysical transport efficiencies.
5. **Increasing instrument flight suitability and limits of detection:** Maturation of processing and analysis techniques for function in flight environments and with complex samples to improve robustness and detection sensitivities.

1. INTRODUCTION

1.1 Motivation and Context

Since the Viking missions of 1976 first attempted to detect life on Mars, no subsequent mission has been dedicated to that goal. The intervening decades have produced a rich body of work characterizing biosignatures and the false-positive and false-negative pitfalls inherent to claiming a detection (Neveu et al., 2018), and recent concept studies — Mars Life Explorer, Europa Lander, Enceladus Life Explorer, and Enceladus Orbilander (Williams et al., 2021; Hand et al., 2022; Reh et al., 2016; MacKenzie et al., 2022) — have identified the biosignatures and contextual measurements that could indicate life at Mars and the ocean worlds. The instruments capable of making these measurements, however, have little to no flight heritage, and most remain at low flight-development suitability (National Academies of Sciences, Engineering, and Medicine, 2023, Table 21-1).

Because no single measurement can establish the presence of life, a credible detection must rest on multiple independent lines of evidence, drawn from across the molecular hierarchy that distinguishes living systems from their nonliving building blocks (**Figure 1**). At one end of this hierarchy lie simple inorganic and organic compounds, such as carboxylic acids, sugars, and amino acids. These compounds are informative but ambiguous, as they arise readily through abiotic chemistry. At the other end of the hierarchy are the complex, highly organized structures that most strongly indicate life: informational polymers such as DNA, functional proteins, intact cell walls, and whole cells.

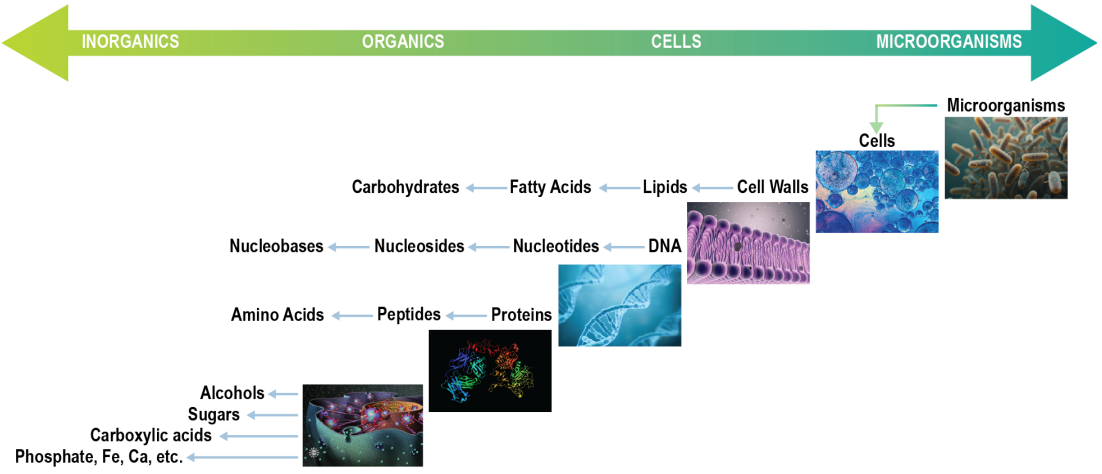


Figure 1. The molecular hierarchy of life, from simple inorganic and organic building blocks (left) to the complex structures and intact cells that most strongly indicate life (right). Reading right to left, each biological structure can be decomposed into progressively simpler constituents — cells into their macromolecules, DNA into nucleotides and nucleobases, and proteins into peptides and amino acids. This decomposition pathway is central to *in situ* life detection: a single sample can be interrogated at multiple levels of this hierarchy as it is progressively processed, with each level offering a distinct line of evidence. *Credit: NASA/JPL; modified from Figure 1 in Lindensmith et al. (2022). Images from Wikimedia Commons, Adobe Stock, Bigstock, and Getty Images.*

These levels are connected by decomposition. A complex structure can be broken into its simpler constituents. Lysis can liberate macromolecules and polymers from cells, DNA can be reduced to nucleotides and nucleobases, and proteins can be digested into peptides and then individual amino acids. During a mission, a single sample can be progressively disassembled and interrogated at each level of the hierarchy, with every stage contributing a distinct line of evidence toward the conclusion that life is, or is not, present.

The complete automation of this end-to-end sample disassembly, processing, and analysis is the single greatest challenge in the field.

1.2 Challenges to Current Approaches

A wide variety of instruments and techniques are used in laboratories worldwide to detect and characterize biological signatures from terrestrial extreme environments. **Figure 2**, from the OWL decadal report, provides examples of biosignature detection techniques and their general range of organic detection limits, as they relate to Earth, Mars, and ocean world estimated organic compositions (National Academies of Sciences, Engineering, and Medicine, 2023).

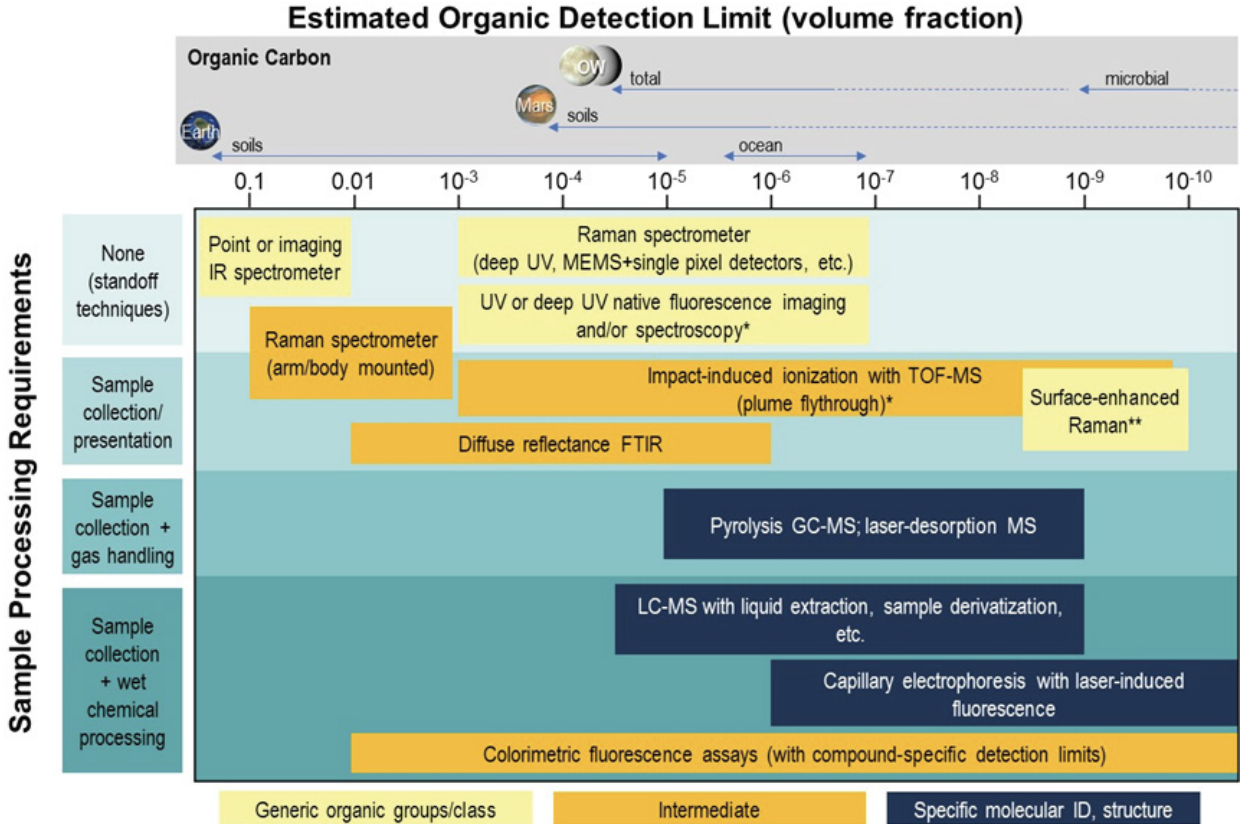


Figure 2. Examples of biosignature detection techniques and their general range of organic detection limits. In general, higher sensitivity measurements and lower limits of detection are enabled by analytical techniques that require sample collection, gas handling, and/or wet-chemical processing. *Used with permission of the National Academies of Sciences, Engineering, and Medicine, from Origins, Worlds, and Life: A Decadal Strategy for Planetary Science and Astrobiology 2023–2032 (National Academies of Sciences, Engineering, and Medicine, 2023); permission conveyed through Copyright Clearance Center, Inc.*

Only a few have flown on space missions, and some are far more amenable to *in situ* implementation and flight development than others. Compounding this suitability gap, the target environments are genuinely extreme: low temperatures and pressures, high radiation, and samples that likely combine very low biomass with high salinity, strongly acidic or basic pH, unknown ice-to-particulate ratios, and complex chemical compositions. Any biological molecules present may further be locked within robust, protective structures such as spores, making the biosignatures within still harder to access.

These realities frame the analysis that follows: the question is not merely whether an instrument can make a measurement, but whether a detectable signal survives the full journey from HEO to detector.

1.3 Concept of the Fermi Model

The Fermi model framework applies Fermi estimation as a first-order, order-of-magnitude extrapolation to bound the sample needs for life-detection measurements, providing realistic high and low estimates rather than single-point predictions. Fermi-style reasoning has been used widely to build intuition and test feasibility across disciplines, from biological scaling and systems thinking to decision science and education, demonstrating its value as a rigorous yet flexible tool for gauging reality (Phillips and Milo, 2009; Gomilsek et al., 2024; Oliveira-Filho et al., 2025). Phillips and Milo describe Fermi-style "back-of-the-envelope" estimation as a complement to direct measurements, arguing that biological and environmental intuition improves when estimates are anchored to a few foundational facts. They point to resources such as the BioNumbers repository and case studies spanning scales from global photosynthetic carbon fixation to the transporter counts needed to sustain rapid *E. coli* growth.

Using this approach, we parameterize Fermi estimates using data from well-characterized Earth-analog sites, translating environmental variability into constrained ranges that reflect plausible mission conditions. The novelty of our work lies in extending these estimates beyond stand-alone calculations by propagating them from point of origin through connected, end-to-end models that include environmental transfer loss, degradation, and concentrating processes. These estimated signals are then further propagated through sample preparation steps and eventual analytical detection, representing multiple possible mission architectures. By explicitly tracking sample preparation steps, cumulative sample loss or concentration, and final detection limits, the model reveals where signal is gained or lost across the workflow. Coupling these results with a qualitative evaluation of a technology's suitability for spaceflight enables clear identification of which processing steps most strongly limit detection performance, providing a quantitative, mission-relevant basis for identifying the key technology gaps limiting detection of a particular biosignature.

1.4 Scope of Study

This study identifies gaps in existing scientific knowledge and technology that could hinder the success of future *in situ* life-detection missions within the solar system. Its scope encompasses the instruments and supporting technologies relevant to biosignature detection, including sample collection and handling systems as well as sample preparation and processing, but excludes spacecraft-enabling technologies such as avionics and communications.

The environments and operational concepts examined correspond to those targeted by current mission concepts: Mars subsurface permafrost and the near-surface regions at Europa and Enceladus, with samples collected by landers or by flythrough plume capture. These map directly to the Mars Life Explorer, Enceladus Orbilander, and Europa Lander concepts.

Within this scope, the study addresses two questions:

1. Given the estimated potential range of biosignature quantity at a sampling location, which measurements can be made with existing technologies, and what is their current suitability for spaceflight and for function within the applicable planetary environment?
2. What are the key technology and science knowledge gaps that limit biosignature detection and what are the most promising strategies to address these challenges?

2. FRAMEWORK AND METHODS

2.1 Integrated End-To-End Modeling Framework

This study used an integrated, end-to-end modeling framework that quantitatively links biosignature density in a potentially inhabited environment to final measurement within flight-relevant instrument systems. As illustrated in **Figure 3, a and b**, the model connects sequential domains: biosignature density at the habitable environment point of origin; geophysical transport to a sampling site; sample acquisition, handling and processing; and, ultimately, detection with analytical instrumentation. For ocean worlds, biosignatures originating in the deep ocean may be transported through convection, fracture networks, sills, cryovolcanic ascent, and plume release prior to surface or flythrough collection, respectively, in surficial or plume sampling mission scenarios. For Mars, biosignatures originating in permafrost or ice-rich layers are accessed through excavation or drilling. Using Fermi-style estimations informed by terrestrial analog sites most relevant to each mission target destination, we define high and low bounds on initial biosignature concentrations (**Section 2.3**). These starting concentrations are then propagated stepwise through transport (**Section 2.4**), collection (**Section 2.5**), and processing using literature-based and experimentally derived estimates of dilution, concentration, degradation, adsorption, and handling losses. The sample processing steps for various possible workflows for each biosignature of interest are described in a hierarchical process flow diagram, represented as a hierarchical directed acyclic graph, or HiDAG, linking sample inputs through processing branches to final detector

analysis (**Section 2.6**). The resulting biosignature concentration delivered to the instrument is compared against estimated limits of detection to determine whether measurable signals can be obtained with current technologies.

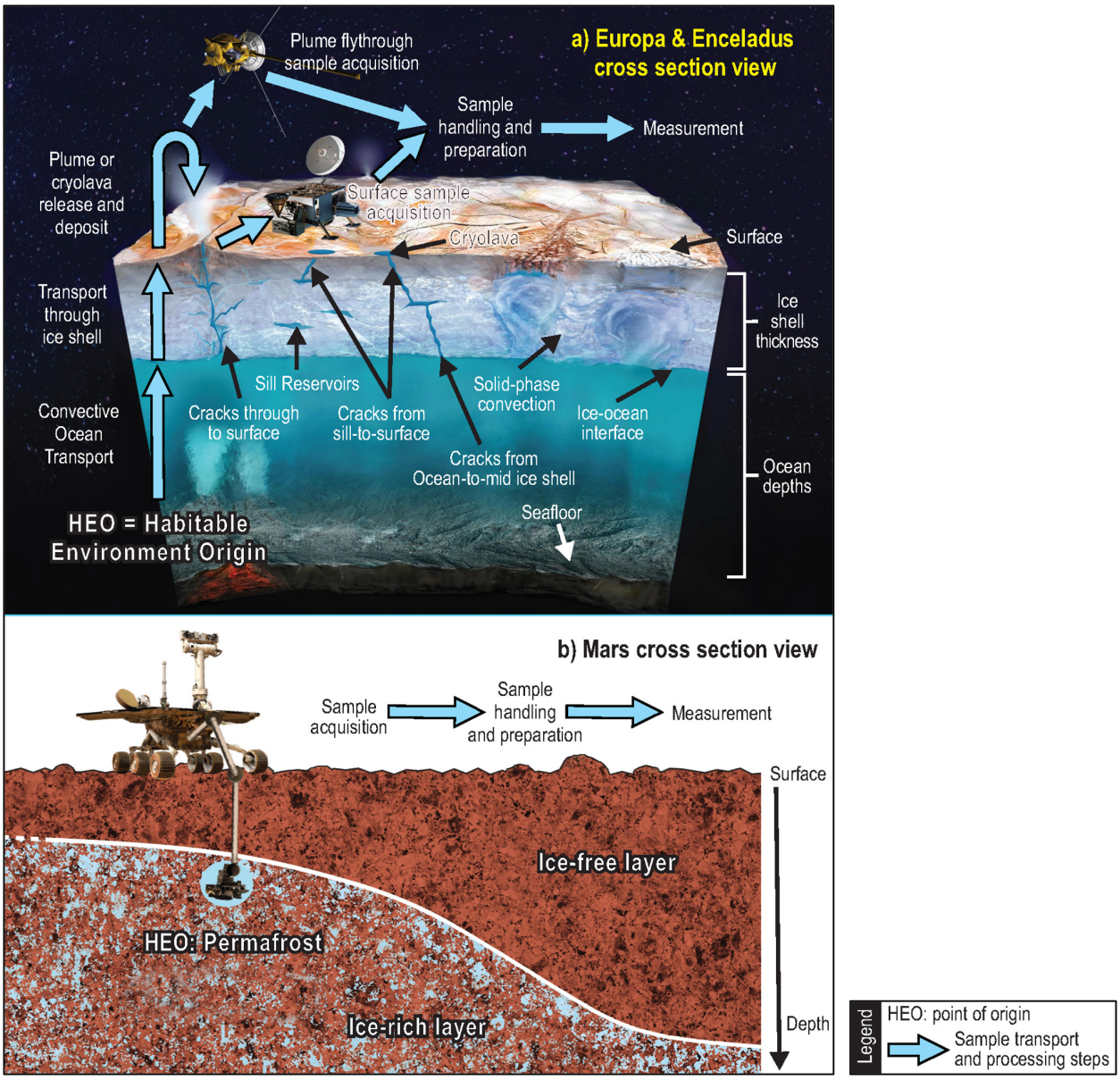


Figure 3. Depiction of sample transport and processing pathways (blue arrows) along which this study estimates the initial amount of a biosignature present in a habitable environment origin (HEO) (**Section 2.3**) for **a.** Europa and Enceladus deep ocean and **b.** Mars permafrost. The biosignature travels from the HEO to the point of sample collection (**Section 2.4**), is collected and transferred into the spacecraft (**Section 2.5**), proceeds through sample handling (**Section 2.6**), and is then analyzed (**Section 2.7**). The quantity of biosignature available at the point of analysis is calculated and compared to the instruments' limits of detection to understand whether analysis would be capable of making a detection and/or further characterization. Shortcomings are considered and brought forward in findings as needed technology developments. *Base images modified from NASA/JPL-Cal Tech, NASA/JPL/Arizona State University, and SwRI TA012956.*

For each biosignature, we construct a hierarchical process tree that traces environmental origin, transport modification, collection efficiency, processing yield, and measurement sensitivity, enabling direct comparison across biomarker types and identification of dominant loss mechanisms. Detection methods evaluated here are not exhaustive but represent a baseline set of capabilities used to identify technology gaps and benchmark alternative or emerging approaches. Performance estimates, including detection limits, required biosignature concentration, processed volumes, throughput, and pre-analytical losses, are derived using Fermi calculations constrained by published and unpublished laboratory data, published literature, engineering considerations, and expert judgment regarding flight suitability. Contamination monitoring and blank processing are assumed as standard operational practices but are not explicitly modeled, allowing the analysis to focus on biosignature survival and recovery efficiency. By integrating environmental biology, planetary transport processes, engineering realities, and analytical sensitivity within a single quantitative framework, this approach reveals where cumulative losses constrain detection and provides actionable guidance for improving collection efficiency, concentration strategies, processing architectures, and instrument integration.

2.2 Biosignature Selection

We considered a number of biosignatures that the astrobiology community has historically considered valuable indicators of life (Neveu et al., 2018). These include cells, carboxylic acids, amino acids, proteins and peptides, HMWC, and nucleic acids. Although this study does not intend to guide decisions on which biosignatures are most ideal for life-detection missions, we anticipate that if these process flow networks are connected, simulations could be run for a specific site targeted. The output of those simulations, once connected, could inform on the most effective techniques and technologies to employ and the most potentially conclusive biosignatures that could be processed in parallel to provide independent measurement to support positive/negative detections.

2.3 Biology Model

Using cell densities and derived estimates of cell-associated biosignatures detected at terrestrial analog sites to create Fermi models is valuable because studies of these terrestrial analog environments provide quantifiable data from environments that are expected to be similar to the extreme physical and chemical conditions expected on distant worlds. For instance, analog sites including hydrothermal vents, polar ice caps, or salt flats offer insights into microbial survival, adaptability, and cell densities under conditions of low temperature, high radiation, and/or chemical scarcity, helping us estimate potential cell densities and biosignature abundances elsewhere. However, Earth analogs, while similar, are not perfect replicas of extraterrestrial environments. Differences in variables such as atmospheric composition, long-term energy flux, water and nutrient availability, and the potential for unknown planetary processes increase the uncertainty in model predictions. Furthermore, Earth life forms are necessarily adapted to Earth-specific biochemistry, so the models may overlook exotic life chemistries or ecological niches

unique to extraterrestrial settings. Even so, by producing Fermi estimates based on the terrestrial extreme environments, this study provides a range of potential biosignature levels for tracing through processing and comparison to instrument detection capabilities.

Estimates for the biosignature levels started at the habitable environment origin (HEO) within each world. For the three environments studied, these were Arctic and Antarctic permafrost environments for Mars and hydrothermal and polar ocean regions for the Europa and Enceladus oceans. We bracketed a high and low cell concentration value for each environment after assessing published field study results for over 100 field sites (see **Appendix A**).

2.3.1 Ocean World Habitable Environment Origin

To estimate the biomass for ocean worlds' oceans, we used hydrothermal vents and polar ocean analog environments (**Table 1**). In particular, the high-cell-density analog location was modeled from a study of seawater from 180 m below the base of the Ross Ice Shelf in Antarctica, which was found to contain 1.2E+5 cells/mL (Martínez-Pérez et al., 2022). For the low-density estimate, the value of 2.6E+4 cells/mL observed in hydrothermal fluids at the Lost City vent was used (Brazelton et al., 2022). Lost City provides a complementary analog for water–rock interactions and hydrothermal chemical energy sources relevant to hypothesized habitable environments within icy ocean worlds. These studies were therefore selected not simply because they report different cell abundances, but because they provide measured biomass concentrations from two environments that bracket distinct, mission-relevant mechanisms for sustaining life in cold, dark ocean systems. Together, these measurements provide empirically grounded high- and low-biomass estimates for first-order Fermi calculations rather than assuming an unconstrained biomass concentration.

2.3.2 Martian Permafrost Habitable Environment Origin

Subterranean Mars is expected to be icy, so we considered permafrost analog environments. Because most permafrost studies have been conducted with climate change considerations in mind, the majority of these studies have been conducted in organic-rich permafrost environments, e.g., peat deposits (Vorobyova et al., 1997; Rivkina et al., 1998), and are significantly more organic rich than we expect the Martian subsurface to be. Fewer studies, several of which are in progress, focus on nutrient-depleted permafrost environments like the Antarctic Dry Valleys and suggest cell densities on the order of 1E+3 to 1E+5 cells/cm^3 (Gilichinsky et al., 2007; Goordial et al., 2016). In this study, we use the range of cells detected by Gilichinsky et al. (2007) as the cell concentration present in the Martian permafrost. This study was selected because Antarctic Dry Valley permafrost combines persistent cold, extreme aridity, low nutrient availability, and very low organic-carbon inputs, providing a more conservative terrestrial analog for potential Martian subsurface biomass than the comparatively organic-rich permafrost environments represented in much of the existing literature. The resulting range is not intended to predict Martian biomass

directly, but instead provides an observationally grounded Earth-analog range from which sample requirements and detection limits can be explored.

Table 1. Habitable environment origin (HEO) starting biosignature values per gram of ice or regolith

Biosignature	High-Density Estimate (fg/g)		Low-Density Estimate (fg/g)	
	Europa/Enceladus Ocean	Mars Permafrost	Europa/Enceladus Ocean	Mars Permafrost
Cells	1.20E+5	5.31E+5	2.60E+4	3.06E+3
Carboxylic Acids	5.70E+6	2.52E+7	6.74E+3	7.94E+2
Amino Acids	2.95E+7	1.31E+8	2.86E+5	3.37E+4
Proteins	2.94E+7	1.30E+8	2.57E+5	3.03E+4
HMWC	3.36E+7	1.49E+8	3.22E+5	3.79 E+4
DNA	5.71E+5	2.53E+6	3.30E+4	3.89E+3
RNA	1.80E+7	7.97E+7	4.94E+4	5.81E+3
DNA+RNA	1.86E+7	8.22E+7	8.24E+4	9.70E+3

Values are based on terrestrial analog studies with high and low cell density measurements (High cell density, ocean world: Martínez-Pérez et al., 2022; Low density ocean world: Brazelton et al., 2022; Martian permafrost high and low density: Gilichinsky et al., 2007), which then are used to calculate the levels of other biosignatures present.

Values (in **Table 1)** for the other biosignatures of interest are calculated based on the cell values as follows:

- Carboxylic Acids:
  - Low: 2.6%/g * dry weight 9.9 fg/cell* cells (low)
  - High: 19%/g * dry weight 250 fg/cell* cells (high) (based on lipid content in cells for Antarctic bacteria and estimated dry mass of bacteria per cell: low: Kendel et al., 2015; high: Akulava et al., 2024)
- Amino Acids:
  - Low: 11 fg/cell (low) * cells (low; Bennett et al., 2009)
  - High: 246 fg/cell * cells (high; Bennett et al., 2009)
- Proteins:
  - Low: 9.9 fg/cell (low; Simon and Azam, 1989) * cells (low)
  - High: 245 fg/cell (high; Bremer and Dennis, 1996) * cells (high)
- HMWC:
  - Low: Total C in ocean bacteria per cell (12.4 fg/cell; Fukuda et al., 1998) * cells (low)
  - High: Total C in ocean bacteria per cell (280 fg/cell; Cayley et al., 1991) * cells (high)
- DNA:
  - Low: 1.27 fg/cell (low; Prondzinsky et al., 2023) * cells (low)
  - High: 4.76 fg/cell (high; Blattner et al., 1997) * cells (high)
- RNA:
  - Low:1.9 fg/cell (low; Simon and Azam, 1989) * cells (low)
  - High: 150 fg/cell (high; Kerkhof and Kemp, 1999) * cells (high)

- DNA+RNA:
  - Low and High: Sum of DNA and RNA values above

Isotopic ratios are also considered in this study, as an example of a proxy biosignature, however they are not a product of cellular abundance and the initial isotopic ratio levels in the planetary environments are challenging to estimate. This study focuses on the efficiencies of collection and processing to identify technology gaps for isotopes, as described in **Appendix D**.

2.4 Transportation Model

The transportation model is designed with the Fermi model to establish realistic bounds for various physical transportation pathways and changes to biosignature levels along those paths, in order to enable the identification of technology needs in sample collection, handling, and analyses. The transportation model focuses on biosignature materials from recent environmental activity and assumes any radiation degradation would primarily be mitigated by mission sample collection strategy (e.g., targeting fresh material or subsurface material). Additional consideration may be required to address other effects and processes specific to a given biosignature-environment combination.

Beginning with the biosignature concentrations estimated at the habitable environments of interest (see **Section 2.3**) in the deep oceans of Europa and Enceladus and the subsurface permafrost on Mars, we estimate the effects on these densities during physical transport.

For the Mars case, we consider that the HEO in the subsurface permafrost is the same as the sampling location and that physical transport loss/gain of the biosignature is not significant. Therefore, the biosignature values are the same as those for the terrestrial analog permafrost environments as described in **Section 2.3**.

For the ocean world targets, Europa and Enceladus, despite their size differences, their interior structure bears a similar layout: underneath the exterior ice shell, a global salty ocean of 10- to 100-km depth is in contact with a rocky core (Nimmo and Pappalardo, 2016). Our study assumes that hypothetical bioactivity occurs near the seafloor, at hydrothermal sites and/or through serpentinization processes and other water–rock processes that release chemicals conducive to habitability (Vance et al., 2023; Vance and Goodman, 2009). The resulting biosignature could then be transported upward through the ocean to be incorporated into or pass through the ice shell,[1] and could then be exposed or deposited at the surface or ejected into space through plume emission. Sample collection for biosignature analyses is expected to occur above the surface for plume ejecta or at the (near-)surface for plume ejecta or cryolava deposits.

[1] Convective transport and the associated ocean dynamics have been the topic of ongoing discussion, especially regarding the possibility of stratification in the ocean of Enceladus (e.g., Zhang et al., 2025). For this report, we assume that convective transport occurs with minor mixing with the ambient ocean during the ascent of biosignature bearing ocean water (e.g., Bouffard et al., 2025).

The associated dilution/enrichment of biological material from the deep ocean, through the considered physical transport processes, to the surface or plume capture sample collection site, is estimated (i.e., transport factor) based on our current understanding of how these processes occur on Earth and how they would occur on Europa or Enceladus, as well as laboratory and numerical modeling studies (see citations in **Table 2**). Most existing studies, however, do not quantify expected biologic transport within the geophysical process, and therefore the values chosen could be significantly different in reality. This study, following the Fermi model estimation process, provides bounds to the loss and/or concentration of biosignatures through geophysical transport path based on study results currently available; however, additional scientific studies would provide more clarification on sample transport processes and loss or concentration. **Figure 4** shows the transport paths considered for physical transport of sample from a deep ocean to the surface at Europa or Enceladus, the geophysical processes occurring, and their estimated transport factor (loss or concentration). Dilution/loss is shown in red, and concentration/enrichment is shown in green.

Table 2. Transport path region within an ocean world, the transport process of interest, and the transport factor estimated for that process

Transport Path Region	Transport Process	Transport Factor Estimated	Reference
Ocean	Convective transport across ocean	0.5x	Choblet et al., 2017; Schoenfeld et al., 2023; Bouffard et al., 2025; Walter et al., 2025
Ocean–Ice Interface	Ice–ocean interface as anchor point	1.2x	Sullivan and Palmisano, 1984
Ocean–Ice Interface	Dilution at ocean-ice interface	0.3x	Schoenfeld et al., 2023
Ice Shell	Crack and fluid straight to surface	1x	Craft et al., 2016
Ice Shell	Aerosol formation in crack (amino acids)	1000x	van Pinxteren et al., 2023
Ice Shell	Aerosol formation in crack (lipids)	10000x	van Pinxteren et al., 2023
Ice Shell	Cell inclusion via droplet dispersion	0.1x	Perera and Cockell, 2023
Ice Shell	Water vapor coating in vent	0.5x	Postberg et al., 2018; Khawaja et al., 2025
Ice Shell	Crack and fluid flow to mid-ice-shell	1x	Craft et al., 2016
Ice Shell	Sill/subsurface water reservoir	1x	Williams et al., 2026
Ice Shell	Freezing sill	1.5x–2x	Lesage et al., 2022; Lesage et al., 2025; Naseem et al., 2023
Ice Shell	Surface crack connects downward to sill and fluid moves to surface	0.95x	Guglielmin et al., 2009; Kornei et al., 2025; Walding et al., 2025
Ice Shell	Diking transports cyromagma to surface	0.95x	Bowling et al., 2025
Ice Shell	Solid-phase convection	0.8x	Howell and Pappalardo, 2018; Showman and Han, 2004
Surface	Plume eruption	0.5x	Ershova et al., 2024
Surface	Plume deposit	1.1x	Southworth et al., 2019
Surface	Radiation damage (assume none as sample is assumed collected from below depth of radiation exposure or is a fresh deposit)	1x	Nordheim et al., 2018

Transport factor (loss or concentration) estimates are based on the studies listed in the far-right "Reference" column. These references provide a base for transport factors chosen; however, many mechanisms lack close analog, laboratory, or modeling studies to provide strong constraints on these values.

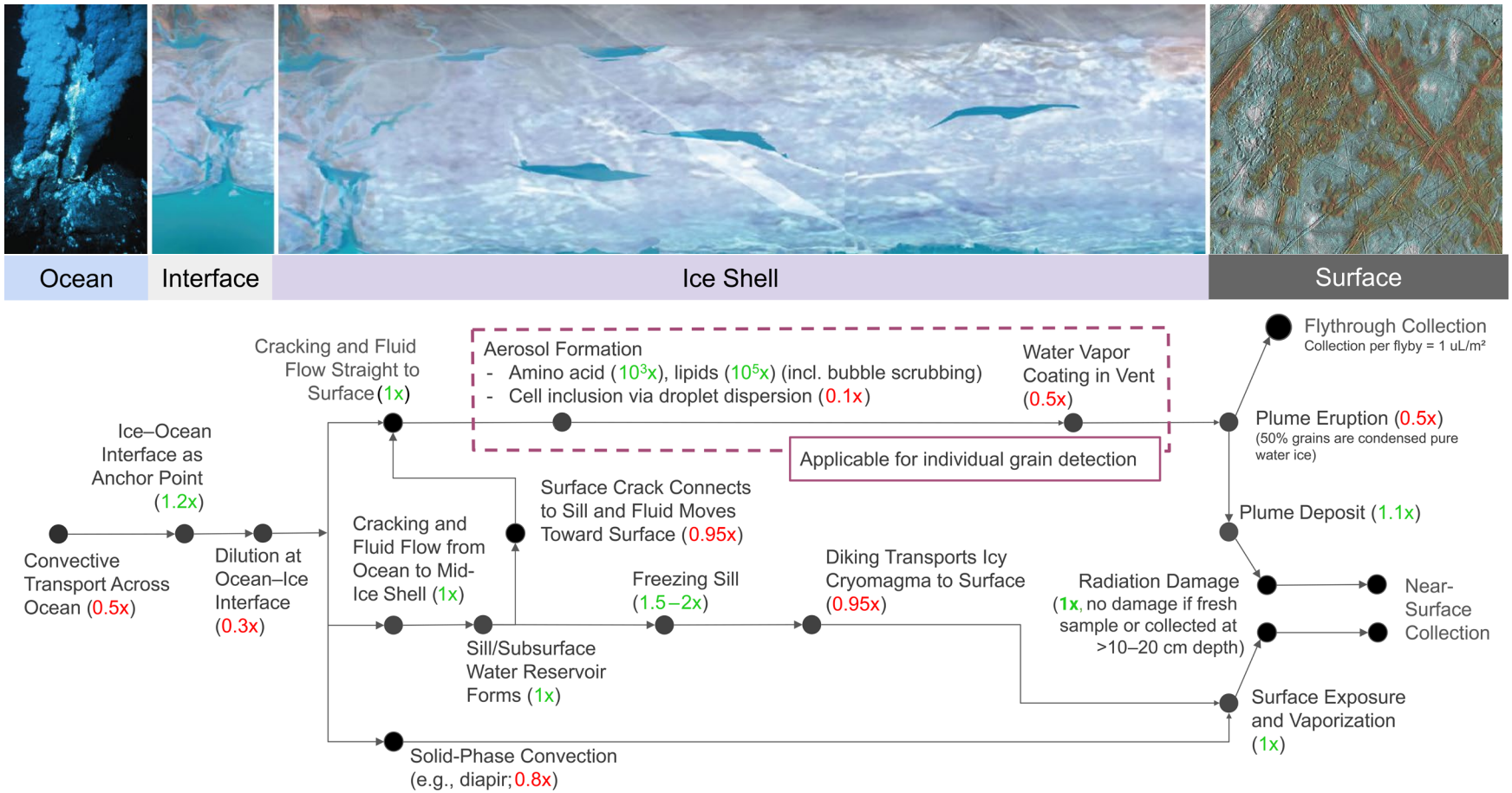


Figure 4. Physical transport model of sample on Europa or Enceladus, moving left to right, from the ocean, through the ice–ocean interface, through the ice shell, to the surface and above (plume ejecta). Loss (red multipliers < 1x) or gain (green multipliers ≥ 1x) for the potential biosignature quantity being transported are shown in parentheses for the various transport processes at each black dot. *Images at the top from NOAA (ocean), NASA/JPL-Caltech (interface and ice shell), and NASA/JPL (surface).*

Within Europa and Enceladus' deep ocean, convective transport may send material up to the ice–ocean interface (Vance and Goodman, 2009). Here we assume that the convective transport occurs with moderate mixing with ocean water during ascent, leading to a 50% reduction of biosignature concentration when arriving at the base of the ice shell. At this ice–ocean interface, the bottom of the ice shell may serve as an anchor point for biology and/or a midway point where the transport of biogenic material stalls. Such accumulation is accounted for with an increase of the biosignature concentration by a factor of 1.2. Another consideration at the ice–ocean interface is the potential for dilution to occur because of the warmer waters ascending from the seafloor that may lead to melting of the ice shell and subsequent mixing with the ice shell meltwater. We expect the biosignature dilution to be less than 50% (e.g., Schoenfeld et al., 2023) for the upwelling water to maintain sufficient buoyancy to continuing ascending through the ice shell.

The ice shells on Europa and Enceladus likely contain cracks and convective processes that act as pathways for subsurface–surface material transport. Studies and observations of Europa and Enceladus have indicated that geophysical mechanisms can provide means for transport of materials between the surface and subsurface (e.g., Vance et al., 2023; Hesse et al., 2022). An example of such a mechanism is an impact. When an impactor hits the ice shell, it not only causes damage directly surrounding the resulting crater, but it can also create cracks at the base of the ice shell, near the ocean (e.g., Sandtorf-McDonald, 2025). The ice directly surrounding the crater is heated, melted, weakened, and/or cracked. Over time, any melted ice on the surface begins to cool and strengthen, which could lead to the formation of subsurface water sills. Sills may also form within ice shells through other means (e.g., Collins and Nimmo, 2009; Michaut and Manga, 2014). As a sill cools, concentration of the composition within the remaining liquid (salts, organics, etc.) can occur and pressure can build up. The increased pressure can cause diking, surface disruption, and potential plume activity (e.g., Quick and Marsh, 2016). Other mechanisms for transporting fluid through the ice on Europa include solid-phase convection (Pappalardo et al., 1998). Solid-state convection at Enceladus is also theorized (Nimmo and Pappalardo, 2006).

Plumes at Europa are not confirmed, and recent evidence suggests they may be regional and sporadic, if occurring at all (Roth et al., 2026). The plume at Enceladus, however, appears to be persistently erupting and provides direct access to fresh subsurface materials in the form of microscopic ice grains and vapor. At Enceladus, spacecraft observations indicate that ocean water is present in the plume grains (the presence of salt, e.g., Postberg et al., 2009), and theories suggest that water enters cracks at the ice–ocean interface and moves directly through to space.

The biosignature concentration in a small fraction of plume ice grains could be enhanced by up to orders of magnitude above the bulk average through processes such as bubble scrubbing, in which biogenic particles may adhere to air bubbles rising through water and when released into the vacuum of space, they break and release highly concentrated droplets (e.g., Perera and Cockell, 2023; van Pinxteren et al., 2023). Such enhancement is only applicable for detection methods capable of detecting and analyzing individual grains, e.g., a dust mass spectrometer (e.g., Kempf

et al., 2025). Biosignature concentrations in bulk sample collected from plume flythrough (collection rate: 0.5–6 mg/m^2 for a 25-km-altitude flyby; Porco et al., 2017; Ershova et al., 2024) would sample the bulk and may not benefit from such enhancement processes. However, based on the grain formation scenario that smaller grains within the plume of Enceladus are likely to form from vapor condensation as pure water ice, and are more likely to escape due to higher ejection speeds, a 10% increase of biosignature concentration is considered likely when collecting falling plume material dominated by larger plume ice grains (maximum mass flux of 0.3g/day/m^2; Southworth et al., 2019) or surface plume deposits. Plume vapor analysis provides essential constraints on the subsurface environment relevant for habitability and geochemistry studies (Waite et al., 2017). No fractionation process is considered.

Radiation damage will occur to biologic materials on the surface of ocean worlds. The rate of damage depends on the type of biologic component and location on the ocean world. For example, Europa, which has an extremely harsh radiation environment near Jupiter, experiences energetic particle fluxes that concentrate in certain regions of the surface as modeled by Nordheim et al. (2018) and reach a certain depth within the ice. For the purposes of this modeling effort, we assume no radiation damage occurs to samples as a landed mission will plan to sample below the depth of radiation damage or from a fresh cryolava or plume deposit. In addition, contamination and impact-driven chemistry caused by micrometeoriod impacts and deposition may affect surfaces, except regions near Enceladus' south pole where plume deposition outweighs exogenous effects.

Table 3 provides the values calculated for the total transport factor (concentration or loss) of a pathway for Europa and Enceladus — from ocean to surface/plume — as shown in **Figure 4**.

Table 3. Cumulative efficiencies on biosignature quantity for each pathway from ocean to surface/plume on Europa or Enceladus

Geophysical Europa/Enceladus Transport Pathway	Transport Factor[+]
Solid-Phase Convection	0.1
Sill/Subsurface Reservoir Transport Through Freezing and Diking	0.3
Plume – Crack to Surface (Grain - Amino Acid)	**50**
Plume – Crack to Surface (Grain - Lipid)	**5000**
Plume – Crack to Surface (Grain - Cell)	**0.005**
Plume – Crack to Surface (Bulk)	0.09
Plume Deposit – Crack to Surface (Bulk)	0.1
Plume – Subsurface Reservoir Through Crack (Grain - Amino Acid)	**40**
Plume – Subsurface Reservoir Through Crack (Grain - Lipid)	**4000**
Plume – Subsurface Reservoir Through Crack (Grain - Cell)	**0.004**
Plume or Plume Fallout – Subsurface Reservoir Through Crack (Bulk)	0.09
Plume Deposit – Subsurface Reservoir Through Crack (Bulk)	0.09

[+]Efficiencies reported at 1 significant figure.
Yellow shaded rows with bolded text are specific to plume grain analyses.

2.5 Sample Collection Model

2.5.1 Permafrost Sampling on Mars (High-Latitude Permafrost and Rocky Terrains)

A portfolio of drilling, scooping, and pneumatic-transfer subsystems — either flight-qualified or in active development — has been assessed for Mars permafrost acquisition, with each concept mapped to its estimated level of suitability and an estimated bulk-sample yield. Five principal collection modalities emerge: (1) a rotary-drill architecture (Curiosity/Perseverance) that drills a few centimeters into the subsurface and collects ~13-cm^3 fine cuttings or a solid core (Anderson et al., 2012); (2) for deeper holes (e.g., 1 m+), a The Regolith and Ice Drill for Exploration of New Terrains (TRIDENT) or ExoMars drill represents the option with the highest flight suitability (medium flight suitability for Mars) (Paulsen et al., 2018); (3) a flight-proven Phoenix-style hinged scoop (high flight suitability) that scrapes surface material, typically providing ~100 cm^3 per scooping event (larger geometry options possible) (Bonitz et al., 2008; Arvidson et al., 2009); (4) core-sampling augers, ranging from a 2- to 6-cm-diameter Planetary Volatiles Extractor (PVEx) auger (medium flight suitability), capable of extracting up to ~1000 cm^3 per meter of core (Zacny et al., 2016) to a compact Mars 2020 Rotary Percussive Coring Drill auger delivering on the order of 8 cm^3 per core (Chu et al., 2014); and, (5) pneumatic extraction mechanisms [PlanetVac/Martian Moons eXploration (MMX)] that entrain loose regolith/ice and convey ~100 cm^3/m to the downstream interface (Zacny et al., 2020). Gravity-assisted transfer and pneumatic handoff are feasible for drill cuttings and scoop collections. The quoted sample-mass figures are subject to refinement through system-level trade studies.

Notably, only the Phoenix-style scoop has been designed to survive dry heat microbial reduction with a biobarrier. Technology under development for *in situ* sterilization is ongoing [vapor-phase hydrogen peroxide and Sterilizing Heat Infrared Emitting LED Device (SHIELD), Seto et al., 2026]. *In situ* sterilization technology will enable systems like TRIDENT and PVEx to penetrate deeper and meet planetary protection requirements.

While high-flight-suitability instruments are already available for surface sampling, the absence of deeper (> 10 cm) subsurface acquisition systems and the associated hardware for core extraction, preservation, and downstream processing constitutes a critical technology deficiency. Several terrestrial deep-drilling techniques exist, including coiled-tubing rotary-percussive drilling, plasma drilling, and millimeter-wave (microwave) drilling. However, little work has been done to assess the feasibility of remote human-in-the-loop operations on another planetary surface. Additional challenges include that cores are subject to fragmentation, resulting in a loss of spatial resolution (and, by extension, the capacity to analyze discrete geological strata representing distinct time periods). The resulting fragmented material can be exceedingly challenging to handle, transport, and process.

2.5.2 Ice Sampling for Ocean Worlds (Europa, Enceladus, and Subsurface Ice)

Four primary technologies for extracting ice samples from icy moons were identified. The leading approaches include: (1) rotary-drill architecture (TRIDENT-type) that fragments ice into fine cuttings and can deliver ≈ 500 cm^3 per meter (medium flight suitability); (2) a flight-proven hinged scoop (medium flight suitability) that scrapes surface ice and collects >100 cm^3 per scoop; and (3) core-sampling augers (medium flight suitability) capable of extracting up to ~1000 mL/m and melt/slush auger, which can displace approximately thousands of milliliters per meter depth. Additionally, gravity-assisted transfer (when there is sufficient gravity) and/or pneumatic handoff are feasible for drill cuttings and scoop collections.

Beyond these near-term surface and shallow-subsurface approaches, several deeper-access technologies are in development. Cryobots are thermal ice-melting probes designed to penetrate kilometers of ice to access subsurface oceans (Howell and Pappalardo, 2020; NASA, 2023; Oleson et al., 2019). Different cryobot architectures exist: laser-powered hot-water jet cryobots (Stone et al., 2014); direct laser ice penetrators using fiber-optic-coupled light (Siegel et al., 2019); and high-voltage electro-resistive closed-cycle hot-water cryobots targeting 500-m or greater subglacial access (Guzman et al., 2022). Additional development efforts include deep wireline drills and hybrid thermomechanical systems that combine mechanical ice-breaking with partial melt for chip removal, designed to overcome the power limitations of pure melt probes while retaining efficient cuttings transport (Palmowski et al., 2019; Stolov et al., 2026). These technologies are generally at lower flight suitability than the surface-sampling approaches listed above.

2.5.3 Plume-Flyby Sampling of Ice and Dust from Europa/Enceladus

Although plume activity at Europa has not been confirmed and likely varies from that at Enceladus, we model sample capture for both moons based on what is known for Enceladus. If plume activity is observed at Europa (and other worlds), these models can be adjusted for the plume dynamics at each specific destination. Here, we reviewed methods for gathering ice particles and dust encountered in a plume flyby. Soft-metal capture surfaces (medium flight suitability) and aerogel samplers (medium flight suitability) are being developed to collect ice at moderate relative speeds ($\lesssim$ 200 m/s) and then deliver the material to a sample preparation interface (Butterworth et al., 2026; New et al., 2020; Neveu et al., 2015). A high-flight-suitability cosmic dust analyzer can directly analyze impact-generated time-of-flight mass spectra for encounter speeds higher than ~1 km/s; however, this technique does not allow for additional sample processing. Sample volumes are dictated by plume dust mass density rather than instrument capacity, and a typical Enceladus plume flythrough at 25-km altitude might intercept ~0.1 µL of ice per pass utilizing a 20-cm-diameter collector.

Several additional funded collector architectures warrant consideration. A large-area (~1 m^2) funnel-style collector is designed to maximize sample yield across multiple flythroughs, with models predicting ~1600 µg of ice per pass at 20- to 60-km altitude (Adams et al., 2018; Davila and Amato,

2020). A hinged, deployable clamshell-style collector, the Gulper-Eel concept, stores flat during cruise and opens on approach, capturing particles via surface adhesion and aerogel embedding before closing a lid for sealed sample transfer to onboard instruments (Backes et al., 2023;Backes et al., 2024). Soft-metal foil capture surfaces (indium and aluminum) have been independently validated at velocities from 0.8 to 3 km/s, demonstrating organic capture efficiencies of 17–90% depending on impact speed and particle size, and have been paired with microfluidic chemical analysis systems capable of detecting organic biosignatures at the 1-nM level (New et al., 2021; Mathies et al., 2021). The overall architecture relies on sequential capture, transfer, and (where possible) *in situ* analysis, with technology maturation needed for reliable bulk collection.

2.6 Sample Processing and Measurement Approach Workflows

Sample processing techniques for sample intake and preparation before analysis are detailed per each biosignature of interest below. **Appendix B** provides tables that list each workflow considered for each biosignature in this study. **Appendix C** lists the processing and measurement steps within each workflow and details on the modeled efficiencies, volume concentrations, minimum quantities, detection limits, flight suitability, and units. The idealized workflow process for permafrost and water ice samples would begin with nondestructive contextual analysis. For example, low-resolution optical imaging followed by non- or semi-destructive chemical analysis [e.g., laser desorption/ablation mass spectrometry (LD-MS), Raman, infrared (IR) spectroscopy] could provide an understanding the scales of heterogeneity and the structure of the bulk-matrix. This would help constrain sample compositions and component distributions, which may inform downstream sample processing and analysis. Also, several analyses overlap in their preparation techniques, which could be considered for sample preparation for multiple signatures, although systems would need flexible designs to optimize the handling and preparation for each sample and biosignature. Lawrence et al. (2023) is one study that considered ocean world sample preparation and provides concepts for coupling of processes.

For the biosignature measurement approach workflows discussed below, we describe techniques and technologies that could potentially be applied in search-for-life missions in order to identify risks and technology development needs. We recognize, however, that laboratory workflows can differ from search-for-life mission workflows when specific instrument and mission requirements are considered. The workflows provided here do not account for specific life-detection requirements. Ultimately, the technologies and analysis pathways used will depend on mission requirements and may differ from those discussed here.

2.6.1 Cells/Microscopy

Our approach to evaluating pathways for a potential search for cell-like features considered, at a top level, samples primarily consisting of permafrost and water ice. The analysis pathways are based on methods that are often employed in terrestrial laboratories to characterize samples that

contain microscopic organisms. However, laboratory workflows can differ from search-for-life mission workflows when specific flight conditions and mission requirements are considered (e.g., longevity of consumables for long-duration flights and sample volume available). The analysis provided here did not consider specific mission requirements, but rather assessed available technologies for general flight suitability and considered techniques that could potentially be applied in search-for-life missions to identify risks and technology gaps.

2.6.1.1 Workflow overview

This workflow would be similar to what was described above for the amino acids analysis — initial analysis to provide organic-level context. While implementation details may differ for permafrost and ice samples, the sample processing steps for both are similar and begin with physical manipulation. For permafrost samples, searching for cell-like structures in the bulk sample is possible but is hampered by particulate and chemical background interferences. To overcome this risk, the measurement workflow for both permafrost and ice begins with separation of the bulk sample. For ice samples, particles from the ice-water melt can be separated, using liquid makeup volume if needed. For permafrost, possible separation methods include pneumatic sorting (e.g., driven in a gas stream), mechanical sieving, or separation from an added liquid phase (e.g., added aqueous buffer). In most cases, sample particles would be size sorted for microscopic imaging and analysis. **Figure 5** shows an example filtration strategy that might be used for biological samples on Earth.

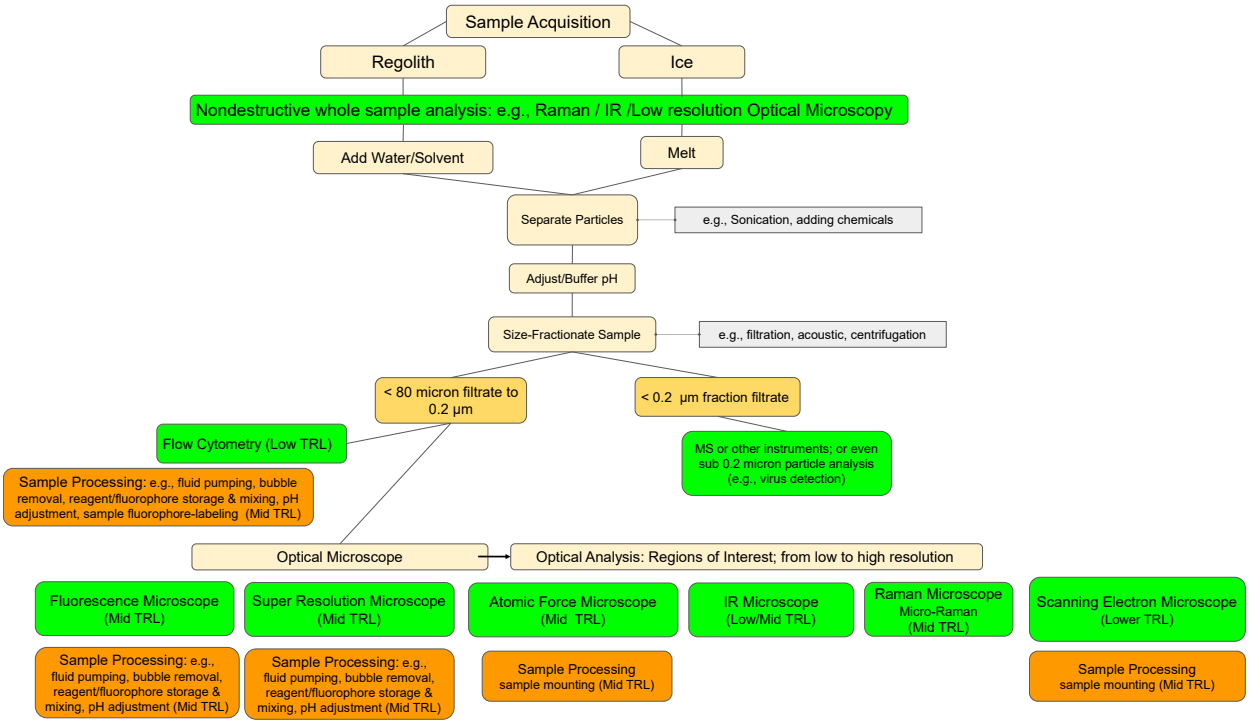


Figure 5. Cell-like feature HiDAG showing the measurement approach workflows.

2.6.1.2 Particle analysis

This study considered a variety of options for cells and cell-feature analysis, many of which are mature for Earth-based sample analyses and will require development for spaceflight if warranted. **Table 4** summarizes these options and provides background literature sources. Optical microscopy is a common method used for microbiology applications and might be considered as a first-line tool in a search for cell-like structures on planetary missions. However, the resolution of visible-light microscopy is diffraction limited, and although submicron resolution is achievable, a life-detection strategy based on morphology alone risks leading to ambiguous or false-positive results. While higher resolution can be achieved by using super-resolution microscopy and scanning electron microscopy (SEM), the risk of ambiguous determination remains high. Therefore, more than one line of evidence in addition to morphology would be needed to establish a robust detection of cellular life.

Potential life-detection approaches shown in **Figure 5** include the co-location of structural and chemical biosignatures (e.g., lipids, proteins, nucleic acids, pigments) potentially achievable using LD-MS or, further downstream, fluorescence microscopy (with or without the use fluorescence stains/labeling), including deep ultraviolet (DUV) protein autofluorescence. Also shown are IR and Raman microscopy, which can allow direct chemical analysis without labeling. Atomic force microscopy (AFM), used previously for regolith grain characterization on Mars (e.g., Goetz et al., 2010) and for dust particle characterization with the Rosetta mission (Bentley et al., 2016a; Bentley et al., 2016b), provides high-resolution, nano-scale sensitivity that can also be applied to cellular structures. Nanomechanical analysis, achievable using AFM, can also provide both high spatial resolution, including 3D topography, and highly sensitive (piconewton) viscoelastic properties (e.g., stiffness, adhesion, viscosity) that may be indicative of cellular life. In addition, AFM-Raman and AFM-IR technologies are used today in Earth-based laboratory microscopic studies, providing simultaneous topographical and biochemical data at high spatial resolution.

Various microscopic technologies (e.g., AFM, optical and visible-near-IR hyperspectral microscopy) have been developed to fly on a variety of missions. Examples include:

- The NASA Mars Phoenix mission to analyze regolith particles [Mars Environmental Compatibility Assessment (MECA) instrument; Hecht et al., 2008, Goetz et al., 2010, Pike et al., 2011]
- Cometary mineralogical analysis using the Micro-Imaging Dust Analysis System (MIDAS) platform on the European Space Agency (ESA) Rosetta mission (Bentley et al., 2016a; Bentley et al., 2016b)
- MicrOmega, a hyperspectral microscope for use on ESA's ExoMars mission (Leroi et al., 2009; Bibring et al., 2017), inspired from the Beagle 2 microscope (Thomas et al., 2004)

Microscopes have also been operated by astronauts on the International Space Station (ISS), for example:

- The Japan Aerospace Exploration Agency's Compact Optical Sensor for Microbial Identification and Counting (COSMIC)
- Structured illumination fluorescence with FLUorescence Microscopy Analyses In Space (FLUMIAS) (Thiel et al., 2019)
- Mochii, a miniature scanning electron microscope with spectroscopy (Own, 2020; Own et al., 2022)
- Miniaturized variable-pressure SEM (Gaskin et al., 2012)

Table 4 summarizes these and certain other key capabilities. Flight suitability is deemed collectively medium for life-detection microscopes because additional developments are still needed to optimize capabilities and autonomous functionality in planetary environments. Some AFM and micro-Raman [e.g., the Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals (SHERLOC) instrument; Bhartia et al., 2021] instruments have flight heritage, although major modifications would likely be needed to adapt them for the detection of microbial cells.

Table 4. Microscope and cytometric technologies and key capabilities

Technology	Capabilities
Optical microscopy	• Essential first step to examine fields of view • Can identify regions of interest for subsequent investigation/instruments • ~> 1 μm resolution
Fluorescence microscopy (e.g., Porter and Feig, 1980; Aguzzi et al., 2020; Case et al., 2024)	• Can supplement optical microscope • Can detect natural DUV, UV, natural fluorescence, and supplemented fluorescence of macromolecular stains (amino acids, nucleic acids, membranes, etc.) • > 0.2 μm (fluorochrome dependent)
Super-resolution microscopy (SRM) (reviews: Schermelleh et al., 2019; Prakash et al., 2022)	• 10–100 nm (fluorochrome dependent) • Bypasses the diffraction limit of light; extends limits of cell and cell-feature detection and can provide 3D imagery
AFM (reviews: Francis et al., 2010; Dazzi et al., 2012; Bonhommeau and Lecomte, 2018; Rodriguez et al., 2025)	• Has spaceflight history (Phoenix and Rosetta missions) • Label-free; nondestructive • > 10 nm resolution • 3D topography of cells and cell-like features with minimal to no preparation • Provides independent physical nanomechanical characterization of sample features; potential for high-resolution (10 nm) chemical characterization using paired Ramen or IR spectrometry
Micro-IR microscopy (review: Soni et al., 2026; planetary science applications: Heinz et al., 2025)	• Label-free; nondestructive; detects spectral signatures of cellular macromolecules • Minimal sample preparation • Traditional IR spectroscopy is governed by limits of diffraction, resulting in 3- to 10-μm resolution; recent improvements (e.g., optical photothermal IR and AFM-IR) have pushed these limits to submicron- and nanometer-level resolution
Raman microspectroscopy (review: Lee et al., 2021; Huang et al., 2010)	• Label-free; nondestructive; detects spectral signatures of cellular macromolecules with different absorption spectra • Minimal sample preparation • Submicron resolution (depending on application)

Technology	Capabilities
SEM (intro Golding et al., 2016)	• Developed for use in ISS science applications • Capable of high-resolution feature detection • Sample preparation typically requires several steps, but newer developments for ISS have mitigated preparation steps • > 10 nm resolution
Digital holographic microscopy (Nadeau et al. 2018; Johnston et al. 2026)	• Evaluated in situ in Earth analog ecosystems • Used on the ISS • Works through recording imagery and reconstructing 3D images of a sample through capturing light waves as a hologram. This results in detecting cell displacement, or motion
Flow cytometry (Wallace et al., 2023; Wallace et al., 2024)	• Minimal to no sample preparation; can detect autofluorescence including DUV; can supplement with macromolecule-targeted fluorochromes to detect polymeric biomolecules • Small format, autonomous microfluidic operations in Earth-based applications • ~ > 100 nm resolution (viruses can be detected)

2.6.2 Carboxylic acids

2.6.2.1 Carboxylic acid analysis of natural samples

Our approach to carboxylic acid analysis is to characterize these compounds across the full range of physical and chemical forms in which they occur within natural samples and to understand how those forms are interconnected. Carboxylic acids are widespread products of biological metabolism, oxidative degradation of organic matter, and abiotic synthesis pathways, and they may exist as free volatile acids, dissolved carboxylates, mineral salts, esterified components of macromolecular material, or species adsorbed onto solid surfaces.

Because carboxylic acids span a wide range of volatilities, acid–base behaviors, and binding modes, their analysis requires a workflow distinct from that used for amino acids. In particular, carboxylic acids are well suited to selective extraction strategies — such as solid-phase extraction (SPE) and liquid–liquid extraction (LLE) — and, for some species, direct gas-phase analysis. These differences are explicitly reflected in the analytical roadmap shown in **Figure 6**.

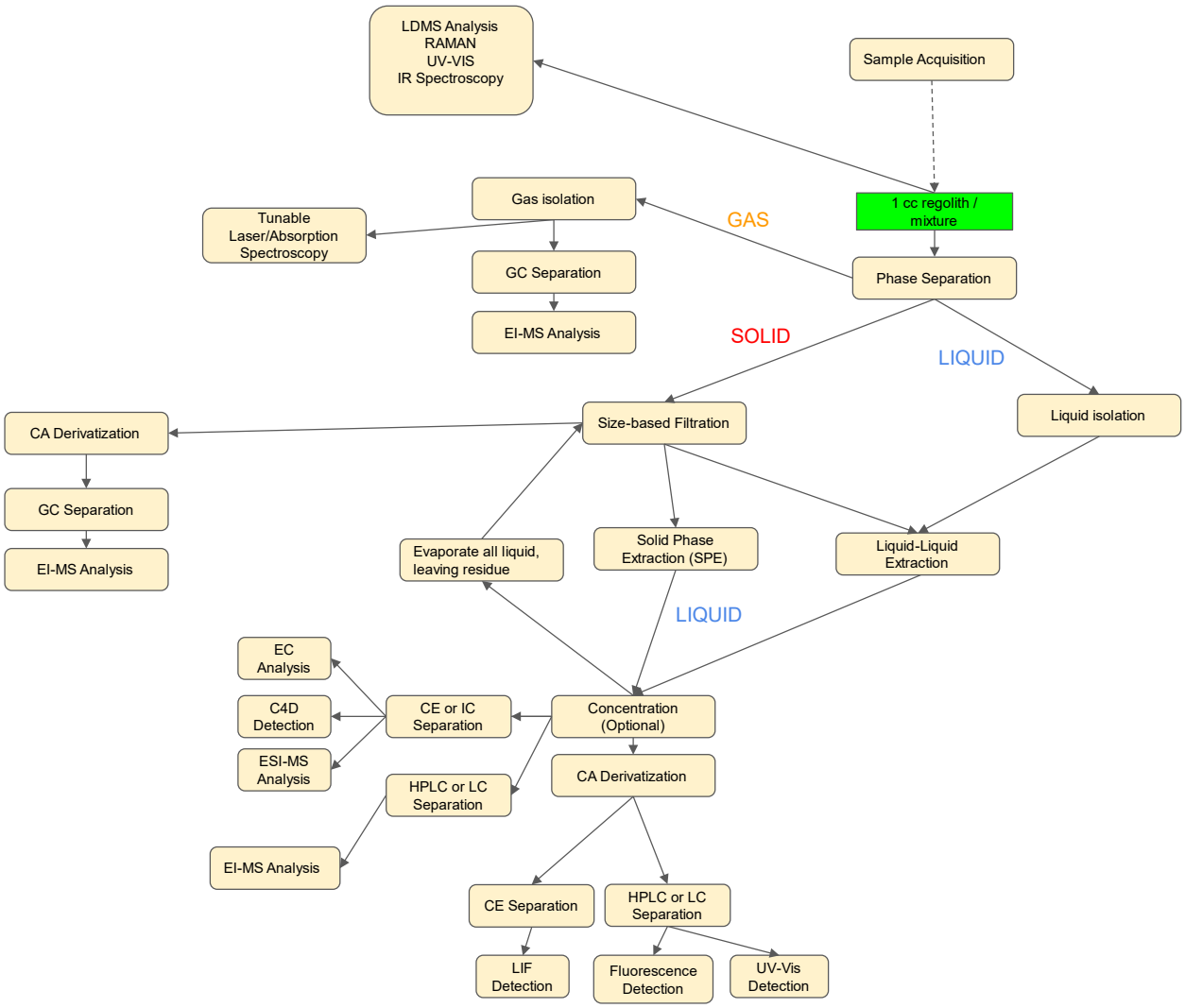


Figure 6. Carboxylic acids HiDAG showing the measurement approach workflows.

2.6.2.2 Analytical workflow overview

Chemical analysis of carboxylic acids follows a multistep workflow consisting of sample preparation, phase separation, extraction or release, derivatization (where required), separation, detection, and quantification. After sample acquisition, non-/semi-destructive reconnaissance measurements, including LD-MS, Raman spectroscopy, ultraviolet–visible (UV-Vis) spectroscopy, and tunable laser-based IR absorption spectroscopy, may be used to assess mineralogy, bulk organic content, and the potential presence of volatile organic species.

The workflow explicitly supports gas-, solid-, and liquid-phase analytical pathways. The dominant methodological distinction from amino acid analysis lies in the availability of chemically selective extraction methods, particularly SPE and LLE, as well as direct gas-phase access for some low-molecular-weight carboxylic acids.

2.6.2.3 Phase separation and initial isolation

A representative starting sample (~1 mL) is assumed to consist of a heterogeneous mixture of solids (e.g., minerals, salts, ice) and liquids. Upon controlled warming above 0 °C, volatile species may be released to a gas phase, liquid phases are isolated, and solid residues are retained for further processing. This phase separation enables parallel analysis of gaseous, liquid, and solid fractions. Notably, the solid fraction could be returned and reexamined by the same spectroscopic techniques applied previously (e.g., LD-MS, Raman, UV-Vis, and IR spectroscopy).

Temperature strongly influences both solubility and volatility of carboxylic acids. In cryogenic, ice-rich samples, carboxylic acids may be concentrated within brines, grain boundaries, or interstitial liquid films, whereas in desiccated or salt-rich materials, they may persist primarily as mineral-bound carboxylates with reduced extractability. These distinctions directly affect recovery efficiency and downstream analytical sensitivity.

2.6.2.4 Gas-phase analysis pathway

Some low-molecular-weight carboxylic acids are accessible through direct gas isolation after warming or thermal processing. Gas-phase species may be examined using tunable laser absorption spectroscopy to detect characteristic vibrational features, providing rapid, noncontact compositional screening.

Isolated gases may then be routed to gas chromatography (GC) followed by electron-ionization mass spectrometry (EI-MS) for compound-specific identification and quantification. This pathway offers high sensitivity and molecular specificity for volatile and semi-volatile carboxylic acids and provides a complementary perspective to condensed-phase measurements.

For plume-derived or otherwise highly dilute samples, gas-phase detection may be limited by extreme dilution, short residence times, and adsorption losses on sampling surfaces, necessitating preconcentration or signal integration across multiple sampling events. In addition, thermal and shock processes associated with natural plume ejection may selectively degrade or decarboxylate more labile species, biasing observed gas-phase inventories toward thermally robust carboxylic acids.

2.6.2.5 Solid-phase workflow

Carboxylic acids in solid materials may occur as mineral salts, surface-bound species, or esterified components of complex organic matter. Solid-phase processing therefore emphasizes chemical release and selective extraction rather than bulk hydrolysis. Solid-phase analytical pathways may include:

- SPE following aqueous or buffered extraction, using ion-exchange or mixed-mode sorbents to selectively retain carboxylates
- Acid or base treatments to liberate carboxylic acids from salt forms or ester linkages

- Optional desalting, solvent exchange, or concentration steps to improve compatibility with downstream separations and detection methods

Extracted carboxylic acids may be analyzed by GC for volatile or derivatized species, or by capillary electrophoresis (CE), ion chromatography (IC), or high-performance liquid chromatography (HPLC) for soluble components. Detection methods include MS and conductivity-based detection, with optical detection employed when derivatization is used.

2.6.2.6 Liquid-phase workflow

Liquid isolation enables direct analysis of dissolved carboxylic acids or further chemical refinement prior to separation. Liquid-phase processing emphasizes pH-controlled extraction, as the carboxylic acid ionization state strongly influences partitioning behavior, electrophoretic mobility, and chromatographic retention. Solvent choice and phase behavior further influence recovery. Longer-chain or amphiphilic carboxylic acids may preferentially partition into organic solvents during LLE, associate with micellar or colloidal material, or be excluded from aqueous phases under high-ionic-strength conditions. These effects must be considered when selecting extraction strategies and interpreting abundance patterns.

Liquid-phase workflows may include:

- LLE following pH adjustment
- SPE for cleanup, fractionation, or preconcentration
- Optional concentration by evaporation to improve detection limits

Final separations could be performed using CE, IC, HPLC, or liquid chromatography (LC), with detection by electrochemical (EC) analysis, UV-Vis absorbance, fluorescence (after derivatization), or electrospray ionization mass spectrometry (ESI-MS). Internal standards are used throughout to track extraction efficiency and analytical recovery.

2.6.2.7 Quantification and chemical interpretation

After separation and detection, carboxylic acids are identified and quantified through peak integration that is corrected using internal standards and process blanks. Final data products include compound-specific concentrations and their distributions across gas, liquid, and solid phases. Interpretation emphasizes molecular distributions, chain-length patterns, functional substitution, volatility, and phase associations rather than chirality. Observed inventories must be interpreted in light of extraction efficiency, phase partitioning, and potential thermal alteration, particularly when comparing cryogenic, plume-derived, and desiccated sample types. When integrated with amino acid measurements and mineralogical context, carboxylic acid data provide complementary constraints on organic synthesis pathways, degradation processes, and environmental history.

2.6.3 Amino Acids

2.6.3.1 Amino acid analysis of natural samples

Because amino acids are central to terrestrial biology as the building blocks of proteins and are widespread throughout the cosmos (Cronin and Pizzarello, 1983; Ehrenfreund et al., 2001), they represent key molecular targets for life-detection investigations (MacKenzie et al., 2022; Willis et al., 2021; Hand et al., 2021). Given the heterogeneity of natural samples, a structured analytical roadmap is required. The guiding philosophy for the search-for-life is to first characterize samples nondestructively with spectroscopy to provide physical and chemical context, and then to apply increasingly selective and sensitive wet-chemical analyses to fully characterize biosignature inventories and distributions. For the analyses presented here, as mentioned previously, we assume amino acid levels as estimated from cellular abundances in the terrestrial analog environments.

2.6.3.2 Analytical workflow overview

Chemical analysis of amino acids follows a multistep hierarchical workflow represented as a HiDAG consisting of sample preparation, extraction or hydrolysis, derivatization, separation, detection, and quantification (Cieslarova et al., 2024). After acquisition, samples may be examined using non- or only semi-destructive spectroscopic techniques, such as LD-MS, Raman spectroscopy, and UV-Vis spectroscopy, to assess mineralogy and bulk organic content and identify spatially resolved correlations between these phases. While Raman and UV-Vis spectroscopic techniques provide contextual information, they lack the sensitivity and molecular selectivity required for amino acid analysis. In contrast, LD-MS techniques, particularly those that leverage high-resolution mass analyzers capable of determining exact masses (and, by extension, informing on molecular formulas; e.g., Arevalo, 2018), can effectively build 2D chemical images of mineralogy and organic inventory, enabling insights into co-located features.

The workflow (**Figure 7**) is therefore designed to support both solid- and liquid-phase analyses using well-established methodologies with demonstrated or emerging suitability for spaceflight implementation.

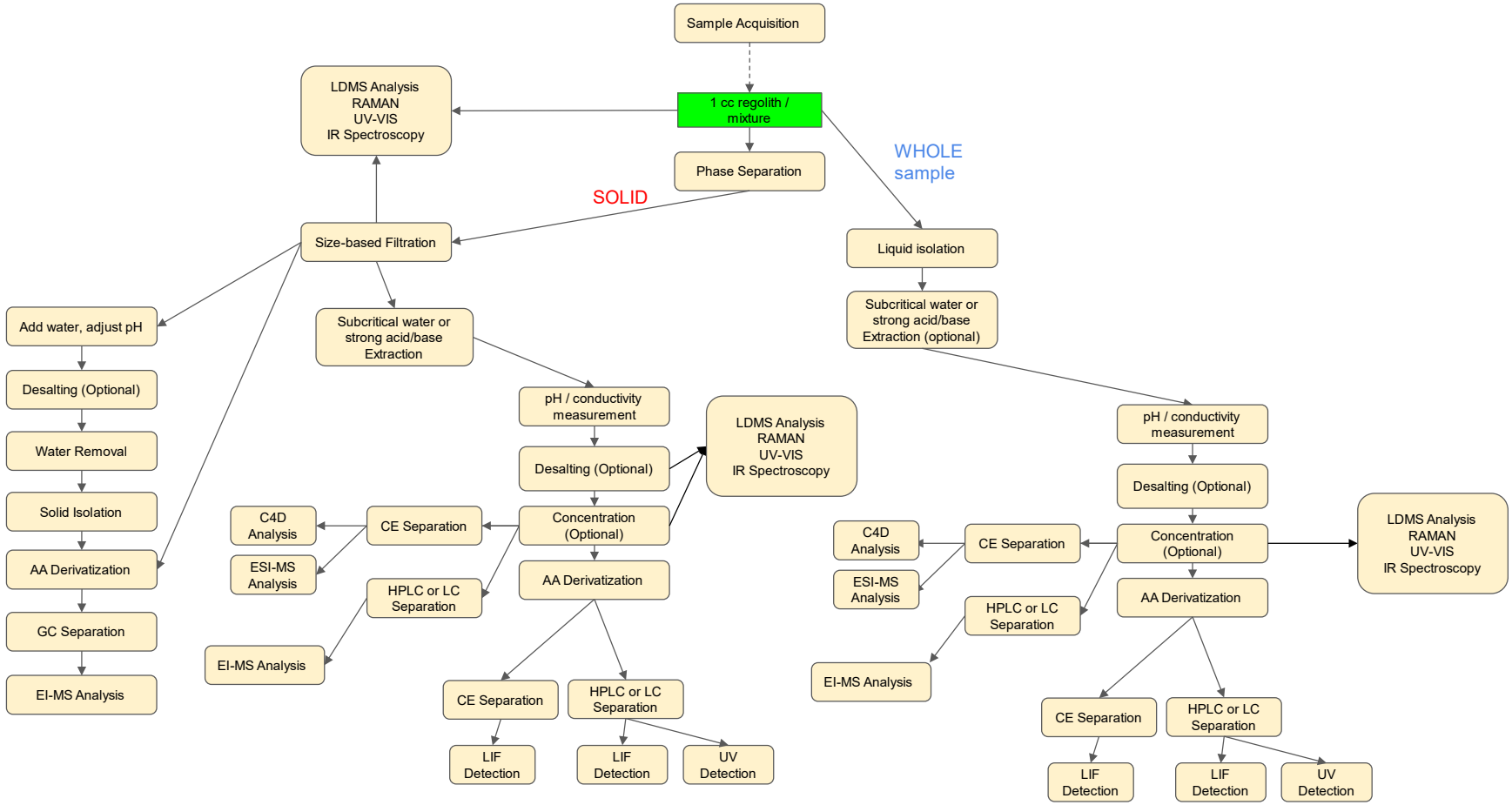


Figure 7. Amino acids HiDAG showing the measurement approach workflows.

2.6.3.3 Phase separation and size fractionation

A representative starting sample (~1 mL) is assumed to contain a heterogeneous mixture of solids (e.g., minerals, salts, ice). Upon warming above 0°C, liquid phases are separated from solid residues by filtration or settling. This initial phase separation enables size-fractionated analysis of coarse solids, fine particulates, ultrafine or colloidal material, and dissolved species, and ultimately concentration of amino acids and other nonvolatile organic molecules that would otherwise be diluted in the water-rich matrix. Size fractionation provides insight into whether amino acids occur as free solutes, are associated with particles or minerals, or are concentrated in ultrafine fractions, informing interpretations of aqueous processing, transport, and preservation. After separation, samples may be reexamined using the same spectroscopic techniques applied previously, namely LD-MS, Raman spectroscopy, and UV-Vis spectroscopy. Subsequent analytical steps in both solid- and liquid-phase workflows involve controlled additions of liquid to optimize sensitivity.

2.6.3.4 Solid-phase workflow

Direct derivatization of intact solids is possible in principle but is inefficient because of poor reagent access and matrix effects, and, therefore, does not meet sensitivity requirements. Instead, solid materials undergo extraction or hydrolysis to mobilize amino acids into solution. These treatments may include water extraction, strong-acid or strong-base hydrolysis, or subcritical water extraction (ScWE) (Kehl et al., 2019), each with known trade-offs in amino acid recovery and preservation. Optional desalting and water removal reduce ionic load prior to separation.

Extracted samples are analyzed using GC of volatile derivatives and CE (National Academies of Sciences, Engineering, and Medicine, 2023) or HPLC for soluble species. Detection methods include laser-induced fluorescence coupled with MS (Brinckerhoff et al., 2022) and capacitively coupled contactless conductivity detection (C^4D) (Shen et al., 2013), enabling quantitative comparison of amino acid distributions across size fractions and solid matrices.

2.6.3.5 Liquid-phase workflow

Liquid extracts may be analyzed directly using CE or HPLC to assess the presence of free amino acids. Where appropriate, additional extraction or hydrolysis steps may be applied to mobilize weakly bound material. Sample characterization includes measurement of pH and conductivity to guide method selection, along with optional desalting or concentration to improve analytical performance.

Amino acids are typically detected after pre- or post-column derivatization to enhance UV or fluorescence sensitivity, with internal standards used to track recovery. Final analyses employ CE or HPLC coupled to UV, fluorescence (Takizawa and Nakamura, 1998; Oborny et al., 2021), C^4D, or ESI-MS (Mora et al., 2022). CE-based microfluidic systems represent a leading spaceflight architecture because of their automation (Mora et al., 2020), low mass and power requirements (Willis et al., 2015), and minimal reagent consumption (National Academies of Sciences, Engineering, and Medicine, 2023; Zamuruyev et al., 2021); these systems are the only ones that

have demonstrated liquid-phase separations in a simulated mission scenario (Mora et al., 2020; Stoker et al., 2023).

2.6.3.6 Chirality, quantification, and life-detection interpretation

After separation and detection, amino acids are identified and quantified through peak integration that is corrected using internal standards and process blanks. Final data products include the identities and concentrations (relative and absolute) of individual amino acids, their distributions across size fractions and physical phases, and, where applicable, their enantiomeric ratios.

Amino acid chirality provides a critical interpretive dimension for detecting signs of extant or past life (Pietrogrande, 2013). Terrestrial biology exhibits a strong preference for L-amino acids, whereas abiotic syntheses typically produce racemic mixtures. Deviations from racemic abundance, or position-specific enantiomer enrichments, therefore represent high-value biosignatures. In addition to chirality, amino acid distributions may also provide complementary biotic/abiotic discrimination, including recently proposed HOMO–LUMO-gap-based metrics that compare molecular energy-gap patterns across amino acid assemblages to help reduce false-positive interpretations (Carr et al., 2023; Ramírez-Colón et al., 2026). Chiral analyses may be performed using chiral-CE with laser-induced fluorescence detection, chiral LC, and/or mass spectrometric confirmation of enantiomer-specific derivatives (Schultz and Moini, 2003).

The greatest interpretive power arises from combining quantitative abundance measurements with chirality and size-fraction information. For example, racemic amino acids concentrated in ultrafine or particulate fractions alongside non-racemic signatures in dissolved or selectively extracted fractions may indicate partial biological processing, degradation, or differential preservation. Conversely, non-racemic amino acids preserved within solid matrices may reflect ancient biological input protected from subsequent alteration.

Together, quantitative amino acid inventories, phase-resolved distributions, and chiral signatures provide a multidimensional framework for distinguishing biological, abiotic, and mixed chemical histories in natural samples, enabling robust interpretation of amino acids as potential biosignatures.

2.6.4 Proteins and peptides

Protein structure and folding follow well-described chemical rules, including constraints from hydrophobicity, hydrogen bonding, and backbone torsion angles. These rules enable interpretation of sequence distributions using thermodynamic and kinetic models developed for terrestrial proteins. Once extracted, peptides and proteins can be interrogated through several complementary analytical modes, making them ideal biosignatures. After acid hydrolysis, amino acid distributions and chirality patterns can potentially constrain origin and diagenetic state. Additionally, intact proteins and peptides can be measured by high-resolution MS to obtain mass to charge (m/z) distributions, then fragmented in a tandem mass spectrometer to produce MS spectra. In collision-based fragmentation, the inherent cleavage of amide bonds along the peptide backbone yields a

series of *b*- and *y*-ions whose masses are predictable from the underlying sequence and complement one another because of the forward (*b*-ions) and reverse (*y*-ions) nature of the sequence; this yields the characteristic fragmentation ladder used to derive peptide sequence identity and validate spectral assignments.

These fragmentation patterns enable reconstruction of the original amino acid sequence and comparison against terrestrial protein databases, emphasizing the importance of understanding protein chemistry of extreme environments on Earth. Because peptide sequences can be compared to functional domains conserved across diverse Earth-based life, their analysis may provide insight into the environmental specificity of the protein function they represent. Therefore, when peptide sequence information is integrated with surrounding chemical and physical parameters obtained from complementary analytical techniques, it provides both structural context and environmental relevance for the detected informational polymer, thereby meeting several criteria required in the Standards of Evidence for Life Detection (Meadows et al., 2022).

2.6.4.1 Solid-phase extraction and lysis

In the protein/peptide HiDAG, we assumed that most peptide and protein biosignatures are initially associated with a solid phase as cell-like structures or organic-rich particles that can be physically separated from surrounding mineral or ice matrices. For sediment-dominated systems such as Mars permafrost, and for icy matrices such as those expected on Europa and Enceladus surface materials or plume fallout, the first major objective is to liberate these cell-like structures into a soluble phase. This requires an extraction step that both releases cell-like structures from grains and solubilizes mineral-associated organics.

Several extraction chemistries that are commonly used in terrestrial and marine geomicrobiology were considered as candidate steps in the HiDAG (Benndorf et al., 2007; Nunn and Keil, 2006; Keiblinger et al., 2016; Géron et al., 2019). Sodium hydroxide is a strong base that can saponify membrane lipids, denature proteins, and disrupt hydrogen bonding, which helps detach cells and organic coatings from mineral surfaces (Nunn and Keil, 2006; Chen et al., 2023). Water rinses (hot, cold, or pressurized) provide a gentle alternative that can remove loosely bound or water-soluble organic matter without introducing additional reagents that require downstream cleanup (Amashukeli et al., 2007; Simkus et al., 2019). Surfactants such as sodium dodecyl sulfate (SDS) or Triton X-100 disrupt lipid bilayers; above the critical micelle concentration, they incorporate membrane lipids and proteins into micelles and drive them into solution (Mattei et al., 2015; Andersen et al., 2009; Bjørnestad and Lund, 2023). Addition of chaotropic agents, such as urea, can disrupt hydrogen bonding networks and help unfold proteins and destabilize membranes, enhancing solubilization of cellular material (Bennion and Daggett, 2003; Chen et al., 2023). Once biological particles and associated organics are in the soluble phase, the HiDAG routes them through one or more optional lysis operations, including mechanical disruption, sonication, pressure cycling, surfactant treatment, or chemical lysis, to ensure release of intracellular proteins and peptides (**Figure 8**) (Islam et al., 2017; Cai et al., 2022).

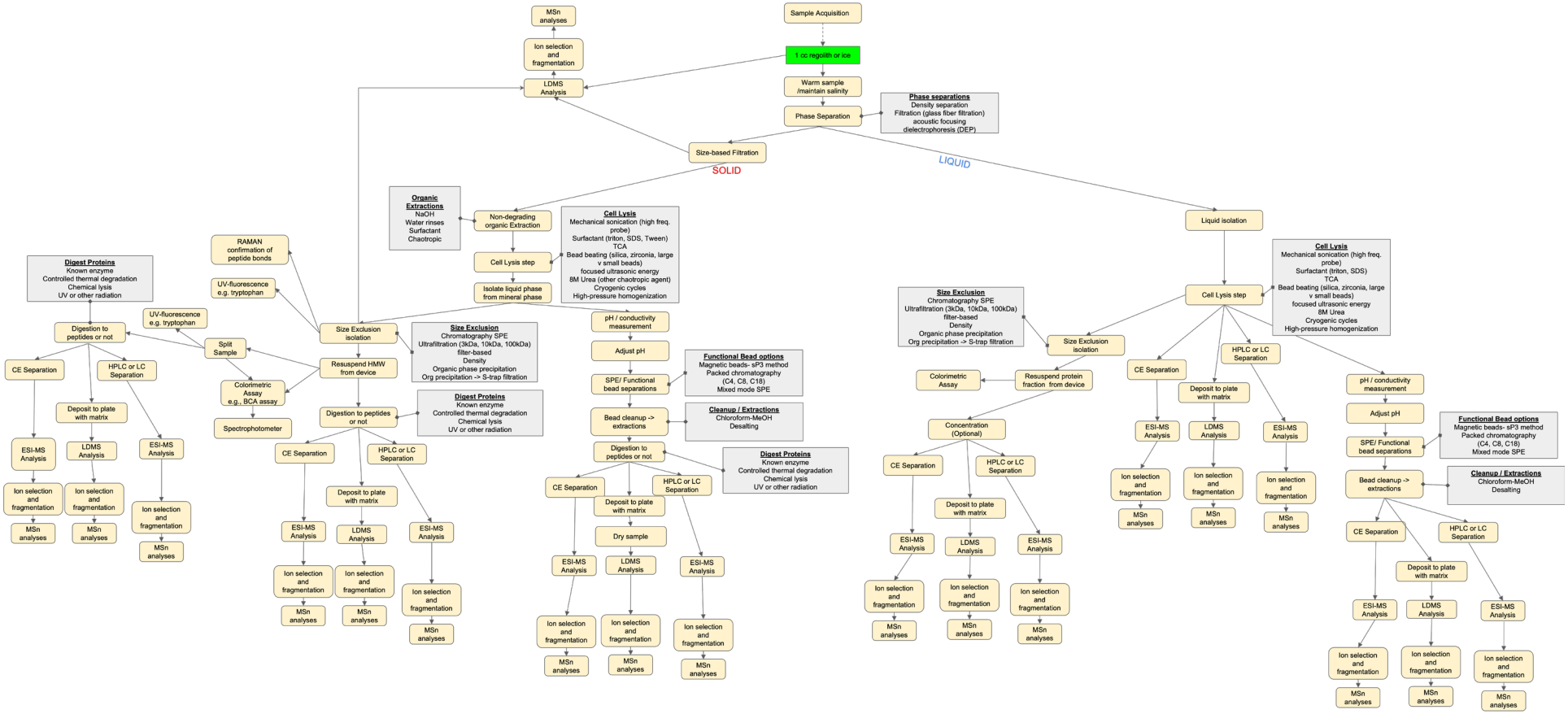


Figure 8. Protein and peptide HiDAG showing the measurement approach workflows.

2.6.4.2 Concentration and cleanup of peptides and proteins

After solubilization and lysis, the protein/peptide HiDAG includes a set of concentration and cleanup steps that selectively enrich peptides and proteins while removing inorganic salts and low-molecular-weight interferers (e.g., Rappsilber et al., 2003; Wiśniewski et al., 2009; Hughes et al., 2014). In terrestrial laboratories, SPE resins with carbon-based alkyl chains bound to silica beads (i.e., C4, C8, or C18), and other similarly functionalized magnetic beads are widely used to reversibly bind peptides and proteins, allowing washing of contaminants and elution of the target fraction in small volumes of organic solvent. These materials are chemically robust and many remain stable in storage for years. Magnetic-bead-based workflows, which can be automated on robotic platforms, are particularly attractive for planetary missions because they combine high surface area with straightforward fluid handling (e.g., Hughes et al., 2019; Hughes et al., 2014). For example, once the peptides or proteins are bound to the magnetic beads, robotic handling can transfer and elute the purified biosignature in very small volumes with minimal loss and no potential downstream clogging issues. To date, few of these specific chemistries have been evaluated at a high suitability for flight, but analogous sorbents are used in current spaceflight systems (e.g., Mahaffy et al., 2012; Goesmann et al., 2017; Willis et al., 2015).

Within the HiDAG, concentration steps are modeled as the primary means to transform dilute peptide and protein solutions, potentially at sub-femtomolar levels, into enriched fractions suitable for MS analysis or paralleled analysis to complete hydrolysis and amino acid analyses. Microchip CE and related microfluidic systems for planetary exploration already demonstrate parts-per-trillion sensitivity for amino acids and related biosignatures, indicating that comparable detection limits may be achievable for peptides with appropriate enrichment (Mora et al., 2012; Chiesl et al., 2009).

If life on Mars, Europa, or Enceladus uses long-chain amino acid polymers, it may be advantageous for sequence determination to convert large proteins into shorter peptides, typically on the order of 5 to 25 residues, to increase the detectability (e.g., Aebersold and Mann, 2003; Steen and Mann, 2004; Olsen et al., 2004). Peptides in this size range ionize efficiently, fall within the optimal mass ranges for common flight-compatible mass analyzers, and generate fragmentation spectra that are more informative for sequence reconstruction due to the consistency of ionization and fragmentation (i.e., fewer missed ions).

In the HiDAG, we include several possible digestion modes, recognizing that transporting labile enzymes into space is not trivial. Options include thermal degradation, cyanogen bromide cleavage at methionine residues, strong-acid hydrolysis, and use of proteases immobilized on solid supports that can be stored dry and activated as needed (e.g., Kaiser and Metzka, 1999; Freije et al., 2005; Nagy et al., 2022). When proteases such as trypsin are employed, their well-defined cleavage specificity at lysine and arginine residues produces peptides that terminate in basic amino acids. These C-terminal basic residues provide two protonation sites, yielding predominantly +2 or higher charge states upon electrospray ionization. Higher charge states fragment more uniformly

along the backbone, improving sequence ladder completeness and facilitating confident peptide reconstruction. Moreover, mass spectrometers can apply charge-state filtering to selectively transmit ions of +2 and above, enriching for peptides that yield the most diagnostic fragmentation patterns and thereby increasing confidence in downstream identifications (e.g., Tabb et al., 2003; Meyer et al., 2012).

2.6.4.3 Chromatographic and electrophoretic separation before MS

The final concentration, separation, and conditioning step in the protein/peptide HiDAG is analytical separation before ionization. Separation reduces sample complexity, increases dynamic range, and provides additional retention time information that can help discriminate isobaric species. For liquid-phase workflows, we considered both HPLC and CE as front ends to ESI-MS. CE and microchip CE are particularly attractive for planetary missions because they require very small samples and offer high efficiency separations; several CE instruments, including the Planetary In-Situ Capillary Electrophoresis System (PISCES) and related systems, are being developed specifically for *in situ* planetary biosignature analyses (Mora et al., 2012). HPLC remains the workhorse separation method for peptide-based proteomics on Earth and provides a large body of operational experience. Although the last few decades have emphasized the power of nano-flow LC (i.e., 100–500 µl/minute flow rates), recent developments resulting from the increased speed of mass spectrometers demonstrate microflow yields with convincingly sharper peaks and better detection.

In typical reversed-phase nano-flow LC workflows, peptides are retained on C18 columns and eluted with gradients of increasing acetonitrile in water with 0.1% formic acid. Typical gradients for complex peptide mixtures range from about 2% to 40% acetonitrile over tens of minutes, and many tryptic peptides elute between about 10% and 30% acetonitrile under these conditions. Such systems routinely load peptides at nanomolar concentrations or lower, making them directly relevant to the low-abundance conditions expected for planetary samples. Direct infusion without prior separation is also possible, particularly for LD-MS instruments that analyze dried sample spots. Although direct infusion generally yields more complex spectra and places a greater burden on downstream data deconvolution, recent advances in computational approaches can deconvolute complex spectra that result from multiple peptides fragmenting simultaneously.

Laser desorption or LD-MS is another promising approach for *in situ* organic analysis that can interface at several break-off points within the protein/peptide HiDAG (**Figure 8**). LD-MS instruments, including Orbitrap-based prototypes, have demonstrated high mass resolution and sensitivity for complex organic mixtures and are under active development for planetary missions (Arevalo et al., 2018). Such instrumentation has been shown to successfully sequence short peptides (Ni et al., 2023). In our framework, LD-MS can operate either as a stand-alone detection endpoint or in combination with limited front-end cleanup steps where fluids are available (**Figure 8**).

Across these approaches, the primary mass spectrometer in the protein/peptide HiDAG is assumed to operate over a precursor *m/z* range of roughly 350 to 1600, which encompasses most peptide charge states accessed in bottom-up proteomics experiments on commercial Orbitrap instruments. This range is compatible with both data-dependent acquisition (DDA) and data-independent acquisition (DIA) strategies. As mentioned above, charge-state filtering in the mass spectrometer can select ions of +2 and above, enabling the enrichment for peptides with more diagnostic fragmentation patterns and thereby increasing confidence in identifications.

2.6.4.4 Tandem mass spectrometry (MS^n) and sequence-level biosignatures

A critical capability for confirming peptides as informational polymers is tandem MS (i.e., MS^n) (Aebersold and Mann, 2003; Steen and Mann, 2004). In MS^n, precursor ions of interest are isolated and fragmented, and the resulting fragment ions are analyzed to derive structural information. The protein/peptide HiDAG allows for several operational modes. In DDA, the instrument selects the most intense precursor ions in real time for fragmentation. In DIA, the instrument systematically fragments all ions within defined *m/z* windows, generating comprehensive but multiplexed fragment spectra (Gillet et al., 2012; Ludwig et al., 2018). Although this method is promising because all signals are detected, data interpretation can be more complex, and experimental design and sample preparation are more critical. In parallel reaction monitoring (PRM) or other targeted modes, predetermined *m/z* values corresponding to candidate peptides are isolated and fragmented with optimized collision energies (Peterson et al., 2012).

Peptide fragmentation in collision-induced or higher-energy collisional dissociation tends to occur along the backbone amide bonds and yields a series of N-terminal *b*-ions and C-terminal *y*-ions whose masses are predictable from the sequence (Biemann, 1988; Steen and Mann, 2004). Although not every theoretical fragment is observed, the pattern and spacing of *b*- and *y*-ions in high-resolution MSn spectra strongly constrain the underlying sequence. Modern algorithms can match experimental spectra to candidate sequences and assign statistical confidence, and emerging machine learning approaches now predict fragment ion intensities with high accuracy (Käll et al., 2007; Fondrie and Noble, 2021; Gessulat et al., 2019; Wen et al., 2025). Within a mission context, putative peptide sequences inferred by onboard or ground-based analysis could be validated post-mission by synthesizing the candidate peptides and reanalyzing them on identical instruments on Earth, providing a rigorous confirmation pathway. Biological nanopore-based peptide sequencing is a nascent capability (e.g., Lu et al., 2025; Wang et al., 2026) and is not considered further in this section because of its low flight suitability, although it represents a promising option for future missions subject to caveats similar to those discussed above related to biological nanopores.

2.6.4.5 Orthogonal measurements and amino acid context

The protein/peptide HiDAG includes several branch points where material can be diverted for orthogonal measurements that strengthen biosignature confidence. Colorimetric assays such as

bicinchoninic acid (BCA) estimate total peptide bond or amine content and are widely used for protein quantification in laboratory settings (Smith et al., 1985). Raman spectroscopy can probe the amide vibrational modes characteristic of peptide bonds, providing an independent measure of total peptide or protein content (e.g., Pelton and McLean, 2000; Mikhonin et al., 1997). In addition, aliquots of the peptide or protein fraction can be hydrolyzed using hot 6N hydrochloric acid (HCl) to yield total hydrolyzable amino acids (THAA), which are then analyzed by GC-MS (post-derivatization) or HPLC (e.g., Keil and Kirchman, 1991; Hutchings et al., 2018). Comparing THAA to dissolved free amino acids (DFAA) from the same sample, and tracking changes in amino acid composition and chirality, provides insight into the proportion of organic nitrogen present in polymers versus monomers and the degree of biological processing (e.g., Dauwe and Middelburg, 1998; Dauwe et al., 1999; Pietrogrande, 2013). Additionally, comparing the hydrolyzed and unhydrolyzed fractions provides insight into polymeric versus monomeric amino acid pools and can provide confidence in final molecular characterization when completed on the same samples split and analyzed in parallel.

2.6.4.6 Liquid-phase branch of the protein/peptide HiDAG

When the initial extraction of a sample yields a liquid phase (e.g., brine-rich, brecciated permafrost on Mars or melted ice from Europa or Enceladus), the downstream branches of the protein/peptide HiDAG are identical to those described above once the sample is in solution. In all cases, tracking intact peptides and proteins with tandem MS while simultaneously characterizing THAA and DFAA using higher flight suitability instrumentation, such as in LD-MS, GC-MS, or capillary electrophoresis-mass spectrometry (CE-MS) methods, offers multiple, converging lines of evidence for the presence of larger informational polymers. This combination of orthogonal measurements, embedded in a quantitatively parameterized HiDAG, provides a robust framework for assessing peptide and protein biosignatures in future missions to search for life on Mars and the ocean worlds.

2.6.5 High-Molecular-Weight Compounds

Similar to carboxylic acids, peptides, and proteins, high-molecular-weight organic compounds (HMWC) are valuable biosignatures because polymer size, composition, distribution patterns, and abundances can reflect synthesis constraints and processing history (Marshall et al., 2021). HMWC material can include biogenic polymers (e.g., extracellular polymeric substances, polysaccharides, peptidoglycans, proteinaceous matrices, and complex lipid assemblies) as well as abiotic macromolecular organics formed by irradiation, thermal alteration (e.g., humic acids), or aqueous chemistry (e.g., Decho, 2017; Hedges et al., 2000). The mission challenge is therefore not just detecting “big organics,” but distinguishing organized, information-driven signatures from abiotic backgrounds using chemistry plus context.

In the HMWC HiDAG (**Figure 9**), samples from permafrost, ice, plume particles, or brines are routed through (1) physical partitioning and cleanup to concentrate organic-rich particles, (2) extraction to liberate macromolecules from mineral or ice matrices, and (3) measurement paths optimized for macromolecular mixtures. Pyrolysis or thermal extraction plays two roles: it can (1) volatilize bound or trapped organics for direct detection, and (2) deliberately break macromolecules into smaller diagnostic fragments whose distributions are easier to analyze with heritage MS- or GC-MS-class methods (e.g., Mahaffy et al., 2012; Freissinet et al., 2015; Goesmann et al., 2017). Complementary "intact" approaches, including LD-MS and high-resolution MS of enriched fractions, preserve higher-order mass envelopes that may retain signatures of repeating units or polymer families (e.g., Goesmann et al., 2017; Li et al., 2017).

To help separate biogenic from abiotic polymers, the HMWC HiDAG emphasizes generalized identification that does not require full structural elucidation: quantify the size distribution and abundance of HMWC material before and after controlled thermal steps, then interpret those distributions using assembly theory as a guiding framework or training models with machine learning and science-autonomy approaches (Marshall et al., 2021; Theiling et al., 2022; Cleaves et al., 2023; Da Poian et al., 2025). In practice, this means treating polymer size, diversity, and relative abundance patterns as a first-pass indicator of chemical "assembly" complexity, then strengthening or rejecting biogenic interpretations using orthogonal context described in earlier sections (mineralogy, salts, redox indicators, and monomer-level measurements such as amino acid distributions and chirality) (Marshall et al., 2021; Arevalo et al., 2023; Riedo et al., 2026). This combined approach supports robust, quantitative triage of HMWC biosignatures while explicitly accounting for abiotic macromolecular sources.

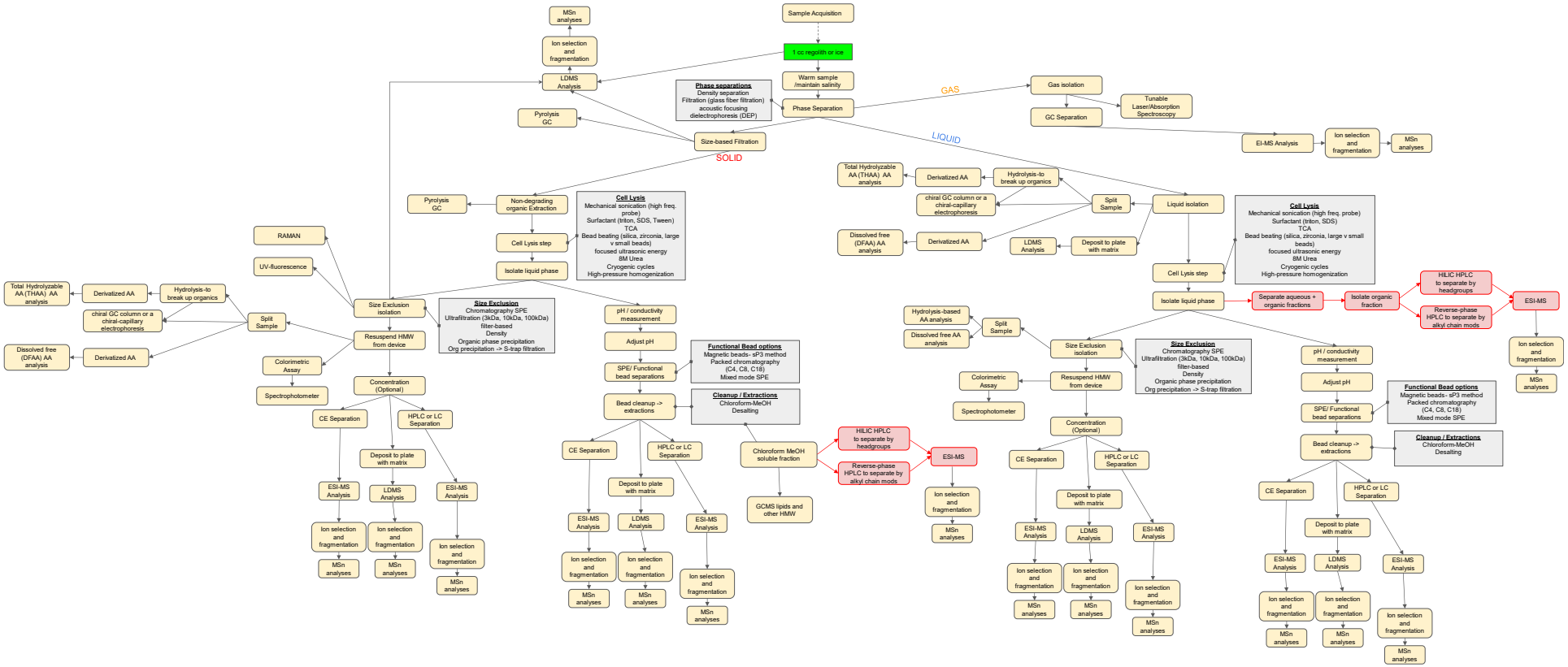


Figure 9. HMWC HiDAG showing the measurement approach workflows.

2.6.6 Nucleic Acids

Several steps are required to process samples to ultimately quantify and characterize DNA- and/or RNA-like nucleic acids. **Figure 10** depicts various possible sample processing pathways for preparing a sample before these analyses. Assumptions in this processing are that the nucleic acids have similar structure and charge as terrestrial DNA/RNA.

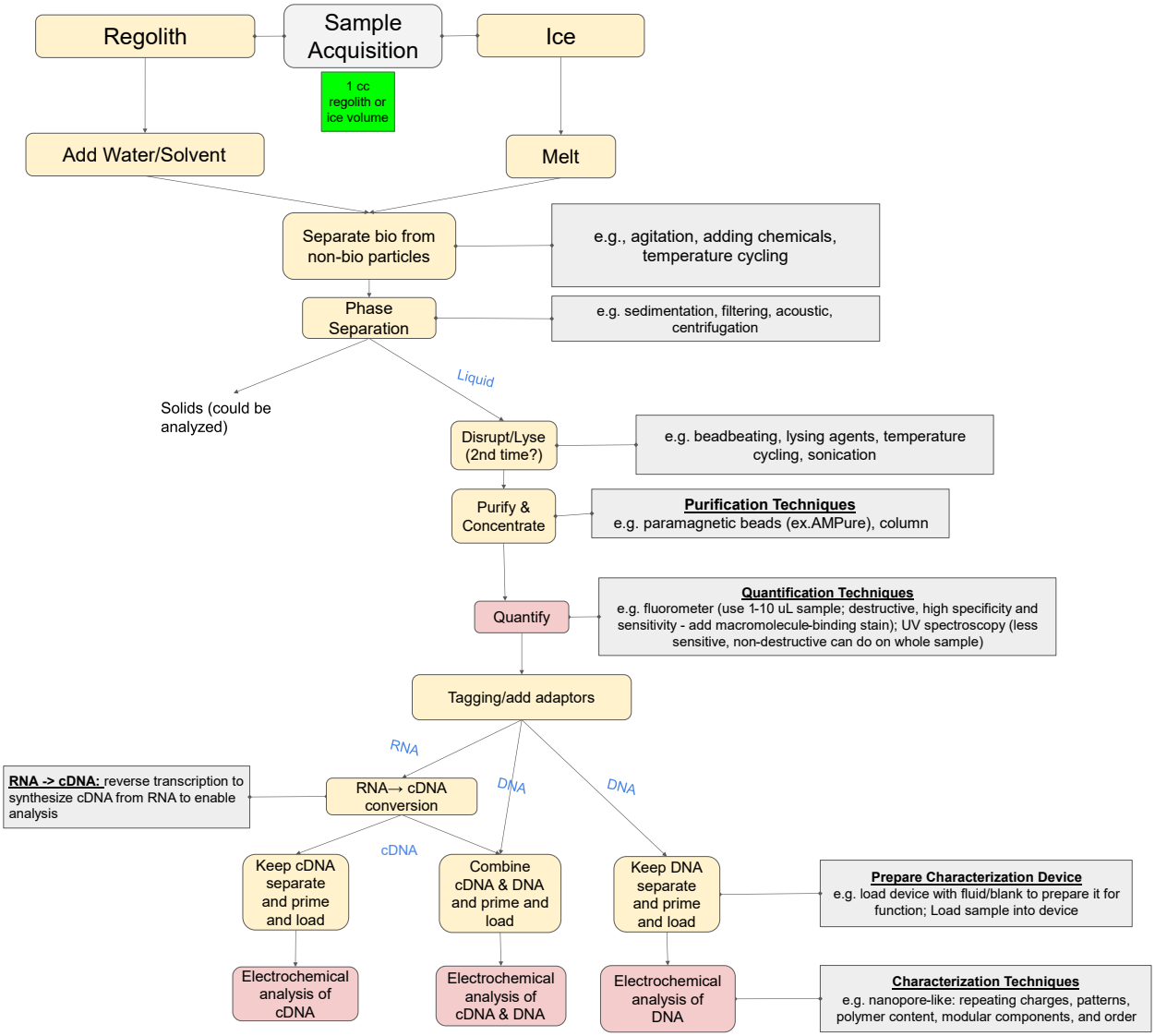


Figure 10. Nucleic acids HiDAG showing the measurement approach workflows.

2.6.6.1 Sample preparation

When a sample of ice or permafrost is received, it would first be warmed to melt any ice present and a lysing agent would be added to begin breaking down cell walls. A separation step can then be performed to decouple cells and other biologic materials that may be attached to particulates in the sample. This separation step can also further lyse and disrupt any cells and spores present to

release the nucleic acids into solution. Next, a phase separation process can be performed to separate the non-biologic particulates from the nucleic acids now in solution. Based on our experience with lab preparations, where some biosignature is not retained through these steps, each step has an estimated efficiency of 0.8.

Continuing with the solution portion of the sample, another lysing step could be performed if the sample is expected to contain spores and other biologic organisms that are especially hard to extract from their protective outer membranes. Based on our laboratory experience, this step is expected to be only about 50% efficient in releasing biomolecules for analysis. Next a purification step can be performed to extract the nucleic acids from any complex chemistry that was contained within the acquired sample that might interfere with downstream analysis. An example of potentially challenging chemistries include salts, acids, and bases that would challenge quantification, detection, and characterizations. According to our laboratory experience, the purification step would likely be about 50% efficient for retaining the biosignature.

2.6.6.2 Quantification

With purified DNA- or RNA-type biomolecules, an analysis can be performed to quantify the amount of the biosignature(s) present. Options for this step include tagging the biomolecules with a fluorescent dye, exciting the sample at a certain wavelength, and measuring the emissions to quantify the presence of the biosignature(s). Lab bench, automated technologies, including the Qubit fluorometer device (*Thermo Fisher Scientific Inc.*), are used to perform this analysis. Another option for quantification is to use UV spectroscopy. Overall, although fluorescence is more specific and sensitive, it is a destructive method. UV spectroscopy is nondestructive, yet less sensitive.

2.6.6.3 Electrochemical analysis

Novel, small-SWaP (size, weight, and power) commercial nanopore devices can detect — and, in some cases, characterize — nucleic acids and proteins as they pass through the pore. The MinION nanopore sequencer (*Oxford Nanopore Technologies Ltd.*) is the current state-of-the art, flash-drive-sized device. Essentially, the nanopore sequencer drives strands of DNA, with an electric charge, through a pore opening of a specific diameter. As the bases pass through the opening, they block the normal open-pore current and cause changes to the current amplitude, which are read out by the device and can be matched, through bioinformatic processes, to specific bases or patterns of bases to enable characterization of the organisms present in a sample (**Figure 11**; Wang et al., 2021; Jain et al., 2016; Xue et al., 2020).

The MinION has detected and sequenced DNA and RNA from low-biomass samples with certain sample preparation steps (Mojarro et al., 2017; Mojarro et al., 2019) and has been demonstrated on the ISS (Castro-Wallace et al., 2017). The MinION's functionality in micro-g and low SWaP make it appealing for spaceflight. However, it uses biological nanopores on polymeric membranes that have limited shelf life (years), making it untenable for some long-duration missions. While

biological reagents would generally tolerate space radiation for typical mission lifetimes (Carr et al., 2013), the nanopores would face challenges in strong radiation environments (e.g., at Europa) (Sutton et al., 2019). Additionally, for signal to be interpretable, the current MinION configurations require binding a motor protein to the nucleic acid strands in order to regulate their translocation through the pore. Because this protein is specific to terrestrial DNA, it is possibly incompatible with extraterrestrial nucleic acids with different molecular characteristics. Solid-state nanopore (SSN) systems (e.g., Xue et al., 2020), another nanopore technology in development, are also small-SWaP nucleic acid detectors. These devices are made with a range of pore opening diameters that enable targeting of particular molecules and use materials that are more robust to spaceflight (e.g., Waugh et al., 2020; Liu et al., 2020; Pearson et al., 2019). However, detection and high-resolution characterization will require solving the challenges in regulating the translocation of the nucleic acids through the pore opening. Ongoing commercial developments aim to improve the characterization capabilities for various biomolecules (Chen and Muthukumar, 2023; Kwok et al., 2014; Tang et al., 2015).

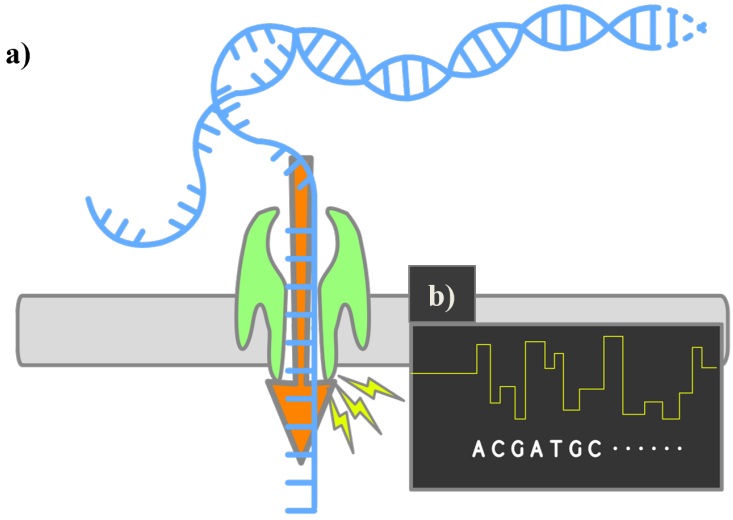


Figure 11. Depiction of nanopore sequencer function. **a**) Cross-sectional view showing nucleic acid strand passing through a nanopore (green) set into a device membrane (gray bar). **b)** As bases pass through, the electrical current levels change (yellow line) and those changes are used to characterize the strand sequence *Image credit: DataBase Center for Life Science (DBCLS), https://doi.org/10.7875/togopic.2020.01, CC BY 4.0, via Wikimedia Commons.*

A typical nanopore sequencing sample analysis would include the following steps:

- **Repair:** DNA is first converted to blunt-ended double-stranded molecules, repairing any nicks or lesions and removing overhangs.
- **Tagging/add adapters:** Next, adapters are added. They attach to the nucleic acids and then to the motor protein to regulate the nucleic acid strands' translocation speed and allow characterization. No loss of material or concentration is modeled during this step, although, in practice, retaining 30–50% of DNA is typical during library preparation (from purified DNA to prepared library; Oxford Nanopore Technologies, 2025).

- **RNA to cDNA conversion:** RNA can be converted to cDNA to enable detection. This involves performing a reverse transcription that synthesizes cDNA from the RNA. A small loss is expected during this process, so an efficiency of 0.8 is conservatively estimated. cDNA and DNA can be loaded separately into the nanopore device or combined and run simultaneously.
- **Sample loading and analyses:** These steps entail a modest loss, estimated here about 0.8, and combining cDNA and DNA may run the risk of overloading the pores available for analysis and cause additional loss (captured here by another 0.8 efficiency for the combined cDNA and DNA analysis path). This may or may not be a factor for synthetic pores but has been noted for the MinION protein-based nanopore sequencer (Craft et al., 2025; Bradburne et al., 2026).

2.6.6.4 Nanogap sensing

An additional analysis technique that could be applied is a nascent method employing quantum-electron tunneling to detect DNA or RNA bases between a sub-nanometer gap (Ohshiro et al., 2012; Ohshiro et al., 2021). This technique can also detect individual amino acids and peptides and has previously been funded by the Planetary Instrument Concepts for the Advancement of Solar System Observations (PICASSO) program as a low-flight-suitability concept (Carr et al., 2023). Subsequences of DNA, RNA, and peptides can be obtained by using electrophoresis to enhance molecular control (direction); however, robust sequencing has not yet been demonstrated.

2.6.7 Considerations Across Biosignature Workflows

The measurement approach workflows demonstrate the interconnected nature of models, including reliance on shared methodologies, and key common processes across biosignature measurement workflows (**Appendices B** and **C**). In laboratory workflows, we perform analyses in a specific order, typically addressing pH, conductivity, and salinity, before conducting all analyses that require that information and before moving on to analyze biological molecules. The measurement approach workflow models highlight how these shared workflows that we may take for granted in the laboratory show up in mission analytical workflows and underline the importance of efficient integration in multi-biosignature workflows. By leveraging these synergies, the flowcharts serve as a guide to design modular, adaptable frameworks for biochemical detection in future astrobiology missions.

The measurement approach workflow models for various biosignature workflows highlights the ways in which analyses considered in the search for signs of life demonstrate the shared sample preparation and processing steps establishing cross-dependencies across multiple biosignature analyses. These shared processing steps include:

1. Desalting and cleanup
2. Lysing and/or Digestion

3. Purification and filtration
4. Sample preparation
5. Shared instrumentation

Peptides, amino acids, and other macromolecules such as nucleic acids, commonly depend on desalting steps to remove interfering ions through purification and concentration methods. This commonality extends across workflows that aim to study derived fragments or the intact molecules themselves. Techniques such as particle filtration (<80 μm or <0.2 μm) and buffering/pH adjustment, highlighted in sample processing paths, are critical for isolating and preparing both peptides and amino acids. Such preprocessing ensures compatibility with downstream techniques, such as electrophoresis or HPLC. With the exception of nondestructive analyses (e.g., Raman) and long-chained polymers, the considered organic chemistry and isotopic analyses require phase separation upon collection of the sample. For both the solid and liquid phases, the next shared step is cell lysis, which liberates cellular contents for follow-on analyses. Processes such as fluorophore labeling and derivatization techniques are applicable to peptides, amino acids, and other macromolecules (e.g., nucleic acids) and improve detection sensitivity for multiple analytical workflows. Common separation techniques such as chromatography (CE, HPLC, or LC) are used to target specific biosignatures for highly sensitive downstream analyses. Devices such as HPLC or LC/MS systems and processes such as bead-based cleanup are vital to multiple biosignature workflows, and processes could be performed on one system to minimize redundancies in equipment requirements and enable co-analysis of peptides, amino acids, and nucleic acids. The reliance on shared resources would streamline experimentation but would also necessitate careful coordination to ensure that these dependencies did not introduce contamination or inefficiencies.

2.7 Model Integration

2.7.1 Model Construction and Example

The model begins by estimating the biosignature density available based on cellular density estimates for the HEO, specifically the subsurface ocean for Europa or Enceladus and subsurface permafrost for Mars. As a reminder, this study does not cover the abiotic background and leaves that to important future work. The sample then undergoes physical transport through the ocean and ice shell on Europa or Enceladus, but has no transport for the Mars scenario. Here we provide an example of estimating the amino acid sample concentration from HEO to sample collection site for a low- and high-density sample on Europa/Enceladus, transported through a crack and landing on the surface as a plume deposit.

2.7.1.1 Starting biosignature density (amino acids in this example)

Calculate the quantity of amino acids available based on cellular density estimates:

- Cellular densities at ocean (**low-density estimate**):
  - Hydrothermal vent at Lost City = 2.6E+4 cells/ml/(density of water, 1 g/mL) = 2.6E+4 cells/g (Brazelton et al., 2022)
  - Amino acids available = (low estimate of 11 fg/cell) * 2.6E+4 cells/g = 2.9E+5 fg/g *10^{-15} g/fg = 2.9E–10 g/g
- Biology available at ocean (**high-density estimate**):
  - Ross Sea under ice shelf = 1.2E+5 cells/ml/(density of water, 1 g/mL) = 1.2E+5 cells/g (Martínez-Pérez et al., 2022)
  - Amino acids available = (high estimate of 246 fg/cell) * 1.2E+5 cells/g = 3.0E+7 fg/g * 10^{-15} g/fg = 3.0E-8 g/g

2.7.1.2 Transport

Transport path and transport factor (loss or concentration): Assume the amino acids are transported through the ocean, through a crack to the surface, and are expelled in a plume that then deposits onto the surface — referred to as Plume or Plume Fallout – Crack to Surface (Bulk) in **Section 2.4**, **Figure 4**, and **Table 3**. The cumulative transport factor for this path = 0.09.

- Low-density biosignature * transport factor = 2.9E-10 g/g * 0.09 = 2.6E–11 g/g biosignature concentration at sampling site
- High-density biosignature * transport factor = 3.0E-8 g/g * 0.09 = 2.7E-9 g/g biosignature concentration at sampling site

2.7.1.3 Sample collection and concentration

The sample can then be collected and provided to an instrument for sample preparation and analysis. In this example, we assume a scoop and pneumatic transfer, which collects 400 mL of sample. If we assume the sampled material is mostly ice and density is about = 0.917 g/mL, then:

- **Low bio density** estimate: 400 mL * 0.917 g/mL * 2.6E-11 g/g = 9.4E-9 g of amino acids
- **High bio density** estimate: 400 mL * 0.917 g/mL * 2.7E-9 g/g = 9.7E-7 g of amino acids

The sample volume concentration to 1 mL is assumed such that the 9.44E-9 g or 9.7E-7 g of amino acids collected are eluted from the concentration process into 1 mL of water:

- **Low bio concentration** estimate of collected sample = 9.44E-9 g/mL
- **High bio concentration** estimate of collected sample = 9.74E-7 g/mL

2.7.1.4 Analysis

An ideal measurement approach would be highly likely to detect its targets and would be straightforward to implement for flight. To determine whether a measurement workflow from

environment source (i.e., HEO) to instrument detection could be successful, we run the concentrated 1 mL volume of sample through the processing steps (with biosignature and volume concentration efficiencies) of each workflow pathway, or HiDAG. (Tables showing all detailed paths are in **Appendix B**, and parameter values for each step — e.g., processing biosignature efficiency, volume concentration, and detection limits — are in **Appendix C**.) If the concentration or quantity of biosignature at the analysis step is comparable to or less than the analysis technique's capability (limit of detection, or LoD), the measurement approach is considered feasible.

A measurement approach is modeled as a series of processing steps terminated by an analysis step. For example, the ScWE LD-MS measurement approach consists of the following steps:

1. Phase separation
2. Size-based filtration
3. ScWE
4. pH/conductivity measurement
5. Desalting (optional)
6. LD-MS analysis

Each step in the measurement approach has a biosignature mass efficiency that captures whether any biosignature is lost during a process, as well as a concentration factor to track whether the volumes change at all during processing steps. For the ScWE LD-MS measurement approach, biosignature efficiencies accumulate to 0.16, and the overall volume concentration factor = 30x.

For a 400 mL sample that is concentrated 30x during analysis, the final volume available for analysis = 400 mL/30 = 13 mL. Biosignature concentration available after processing in that 13 mL is:

- **Low bio sample** (9.44E-9 g/mL) * 0.16 efficiency * (400 mL/13 mL) / molar mass of amino acids (110 g/mol) = 4.2E-10 mol/mL
- **High bio sample** (9.74E-7 g/mL) * 0.16 efficiency * (400 mL/13 mL) / molar mass of amino acids (110 g/mol) = 4.3E-8 mol/mL

The analysis step (in this case, liquid chromatography–mass spectrometry, or LC-MS, analysis) is characterized by a LoD and a minimum sample quantity for performing the analysis. The LoD is the concentration of biosignature required for a high probability of detection, in units of parts per weight (ppw) or mol/mL. The minimum quantity is the smallest practical quantity of solution or material the analysis system can process, expressed in grams or milliliters. If, for example, we specify LoD for LC-MS analysis of a specific biosignature to be 1E–9 mol/mL and minimum quantity is 1 mL, using the levels of biosignature calculated for the low and high starting bio densities, low density samples would not have a high enough concentration of amino acids to be detected, while high density samples would:

- **Low bio sample:** 4.2E-10 mol/mL < LoD for LC-MS (1E-9 mol/mL)
- **High bio sample:** 4.3E-8 mol/mL > LoD for LC-MS (1E-9 mol/mL)

This then indicates that other amino acid measurement processes that have higher inherent processing concentration factors and/or higher biosignature mass efficiencies would also be capable of detecting biosignatures. Additionally, larger collected and concentrated sample volumes also contribute significantly to the ability to detect a biosignature. See **Sections 3 and 4** for results and findings.

To estimate flight suitability, the suitability of every step in a measurement approach is evaluated on a three-point scale of low, medium, or high suitability. Flight suitability is a qualitative evaluation of the practicality of integrating a technology into a spaceflight payload, encompassing flight suitability, SWaP, data volume, and any technology-specific constraints. High suitability indicates either that the technology has spaceflight heritage or that routine engineering development efforts would be sufficient to prepare it for flight. Medium suitability indicates that substantial additional development is required. Low suitability indicates that one or more notable challenges would need to be overcome before the technology is practical for spaceflight. For each individual step in a measurement approach evaluated for flight suitability, we count the number of high-, medium-, and low-suitability steps for a total, qualitative evaluation of the measurement approach's flight suitability.

2.7.2 Validation

Our biological models rely on existing terrestrial analog data. By inputting the estimated cell densities in Earth environments into the various measurement approach workflows, we can validate our models' efficiencies and sensitivities, to within an order of magnitude, when compared to Earth-based studies performed in analog environments.

2.7.2.1 Mars

The primary uncertainty within the Mars biological models was the limited datasets available for Mars analog permafrost sites. Most of the permafrost literature focuses on relatively organic rich (e.g., peat deposits) permafrost, as microbial activity in these sites will play a major role in climate feedback as warming occurs (Ernakovich et al., 2022; Schuur et al., 2015; Jansson and Taş, 2014). These sites are significantly more organic rich than we expect the Martian subsurface to be, resulting in cell counts on the order of 1E+6 to 1E+10 cells/g dry weight sediment (Vorobyova et al., 1997; Rivkina et al., 1998). Previous work in the McMurdo Dry Valleys suggest cell counts on the order of 1E+3 to 1E+5 cells/g (Gilichinsky et al., 2007; Goordial et al., 2016); however, more research in low-nutrient permafrost on Earth is needed to improve our understanding of the bounds for the limits of life within Mars analog permafrost.

Ongoing research is attempting to begin filling these gaps in our understanding of microbial population sizes, community structures, and activity within these systems (Quevedo-Caraballo et al., 2026; Phillips-Lander et al., 2024). These preliminary studies detected evidence of a sulfate reducer with the Signs Of Life Detector (SOLID) in only one sample collected in permafrost within the Haughton impact structure (Quevedo-Caraballo et al., 2026). Using values estimated for adenosine triphosphate (ATP) (White et al., 1979), and for DNA (Prondzinsky et al., 2023; Blattner et al., 1997) with the preliminary data collected from these Haughton impact structure samples, active cell concentrations are estimated at levels as low as ~1E+3 active cells/g (the lowest measurable quantity observed by these investigators) and total cell counts (active + dead/dormant) as high as 1E+6 to 1E+7 cells/g. These analyses assume all of the DNA extracted from the samples is intracellular DNA (Phillips-Lander et al., 2024; unpublished data).

Because the sample handling pathway for Mars is similar to that for Earth (direct sample acquisition and no physical transport), the biological model for Mars is essentially the same as it would be if the measurement approach workflows were applied to Earth analogs. The high-end model estimate of approximately 246 parts per billion by weight (ppbw) amino acid concentration, is approximately 3.5x the upper value reported from Atacama Desert sediments (e.g., glycine, alanine, valine, aspartic acid, serine, and glutamic acid yield, 1–70 ppbw; Amashukeli et al., 2007). Agreement to within a factor of ~4 at the high end suggests that the cell count to biomolecule conversion produces reasonable outputs where biomass is comparatively high. The low end of the model remains unvalidated as amino acid contents are not available for the McMurdo Dry Valleys or other low-nutrient permafrost sites. The only relevant measurements to date are non-quantitative detections of amino acid derivatives by solid-phase microextraction in samples from the Great Kobuk Sand Dunes in the active layer in March 2025 (Phillips-Lander et al., 2026). This underscores that quantifying biosignatures in Mars analog permafrost similar to what will be drilled during a future Mars Life Explorer-type mission (Williams et al., 2021) is needed to validate flight-like analysis workflows.

2.7.2.2 Europa and Enceladus

Model outputs for Europa and Enceladus are influenced by sample physical transport pathways from their oceans through the ice shells. Fractionation processes during the geophysical transport likely significantly influence the signal. By comparing input cell densities from Earth environments to cell densities from transported and emplaced ocean world models, we can constrain how these transport processes on and within ocean worlds influence biosignature concentrations and, in turn, the instrument requirements to detect these biosignatures.

Using amino acids as a benchmark for validation requires care in selecting terrestrial comparison values. Our Fermi model calculates strictly the intracellular amino acids that would be liberated during cell lysis. Reported literature values for amino acids in Earth's oceans frequently report DFAA concentrations, which represent a fraction (1–5%) of the total amino acid pool, with the

rest of the amino acids partitioned between dissolved combined and particulate (largely cells associated) fraction. Because the particulate fraction values are sparse, we compare model values to a subset of studies that report total amino acids (dissolved + particulate), recognizing this comparison biases the terrestrial values high relative to what the model computes.

Our model predicts amino acid concentrations in the ocean of ~268 nM for the high estimate, based on cell count data from the Ross Ice Shelf, Antarctica (Martínez-Pérez et al., 2022). This falls within the range of productive surface ocean waters, the Southern Ocean winter total dissolved amino acids (1–5.4E+3 nM), measured subglacial Lake Whillans free amino acids, and data from the Lost City Hydrothermal Vent Field (Keil and Kirchman, 1991; Dittmar et al., 2001; Shen et al., 2018; Turetta et al., 2023; Shen et al., 2017). The model low estimate for amino acid concentrations comes from cell counts in hydrothermal fluids measured at the Lost City Vent Field, 2.6E+4 (Brazelton et al., 2022) and falls below all reported values in the literature, which is expected given the model resolves only the intracellular pool.

Fermi model predictions for amino acids in Europa and Enceladus ice shell emplacement mechanisms are most directly comparable to terrestrial glacial environments. The high estimate that is calculated in this model = ~48 nM (following that the high estimate of amino acid concentration in the ocean ~268 nM (Martínez-Pérez et al., 2022) is multiplied by this model's transport factors for incorporation into ice of 0.5*1.2*0.3 (see Figure 4)] is consistent with detections within Lake Vostok Type II accreted ice (0.8–160 nM; Christner et al., 2006), which forms by the freezing of subglacial lake water at the ice–water interface. Cell densities from the Ross Ice Shelf (~1E+5 cells/mL; Martínez-Pérez et al., 2022), which underpin the model's high-end estimate, are higher than the cell densities measured in Vostok ice, 83–260 cells/mL depending on ice type (Christner et al., 2006). The model's low-end estimate of amino acids (0.11 nM) falls below the 0.8–160 nM values for Vostok Type II accreted ice by nearly a magnitude. This again reflects the intracellular-only scope of the model and highlights the difficulty of detecting biological signals in ultra-low-biomass environments.

No terrestrial environment replicates the plume transport scenario. Despite this, we validate the Fermi estimate of plume grain transport by comparison to marine aerosol literature. The model's 62.5x fractionation factor is within an order of magnitude of the amino acid concentration factor derived for natural sea spray (Triesch et al., 2021). Submicron sea spray has higher concentration factors by 1E+3 to 1E+4, suggesting that amino acids may be higher in some smaller grains within the plume. One caveat is that marine aerosols and their formation differ from Enceladus's plume in terms of salinity, temperature, and pressure differential.

3. RESULTS

3.1 Physical Transport

Much remains unknown about the physical processes and effects on biosignature quantity and concentration as ocean water travels through an ice shell on Europa or Enceladus. The overall transport factors calculated for the physical transport paths, shown in **Table 3**, **Section 2.4**, range from 0.004 for a grain containing a cell in a plume to a concentration of 4500x through aerosol grain formation and detection of a lipid in a plume. This large range emphasizes the importance of understanding these physical transport mechanisms at ocean worlds. Effects of the overall transport factor on the ability to make detections of biosignatures at Europa and Enceladus, for the various transport paths considered in this study, are described further in **Section 3.2.3**.

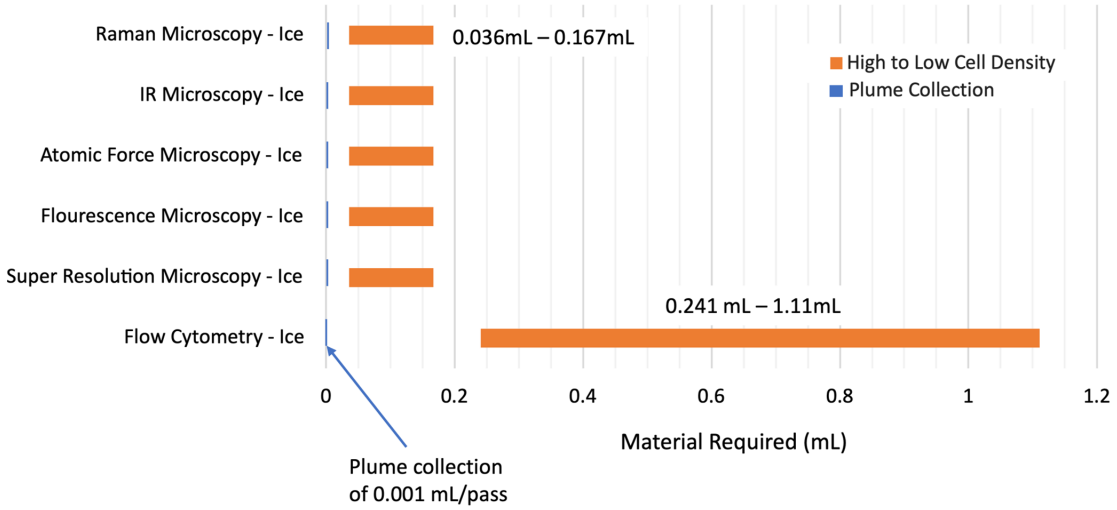
3.2 Cells, Carboxylic Acids, Amino Acids, Peptides and Proteins, High-Molecular-Weight Compounds, and Nucleic Acids

3.2.1 Cells

The model considered three sample types (plume, ice, and permafrost) followed by sample processing through six different instrument types. Although most of the measurement paths have similar sample processing requirements — and thus similar efficiencies (**Figure 5** and **Appendix C**; Cells model) — one notable difference was identified between examination of cells on a filter/membrane versus suspensions in liquid that are examined with a flow cytometer. For the models, we assumed that flow cytometry would have a detection limit of ~100 cells (Santibañez et al., 2016) and the other microscope-based technologies would have a detection limit of 15 cells (**Appendix C**; Cells model).

Overall, for the models estimated here, the detection of cells after microscopic or cytometric methods requires small collection volumes for ice (0.036–1.11 mL) or permafrost (0.01–0.075 cm^3) per sample, depending on the environment and microscopy technology used (**Figure 12** and **Figure 13)**. After routine Earth-based sample handling and steps, a modest number of processing steps (as laid out here, 5–9 steps) are required to characterize the cells and cell-associated properties. Flight suitability for each processing step in an analysis workflow is estimated and shown in **Figure 14.**

a) Cells - Material Required for Plume Flythrough Collection and Successful Analyses Transport: Subsurface Reservoir Through Crack (Grain)



b) Cells - Material Required for Landed Europa/Enceladus Collection and Successful Analyses Transport: Subsurface Reservoir Through Freezing and Diking

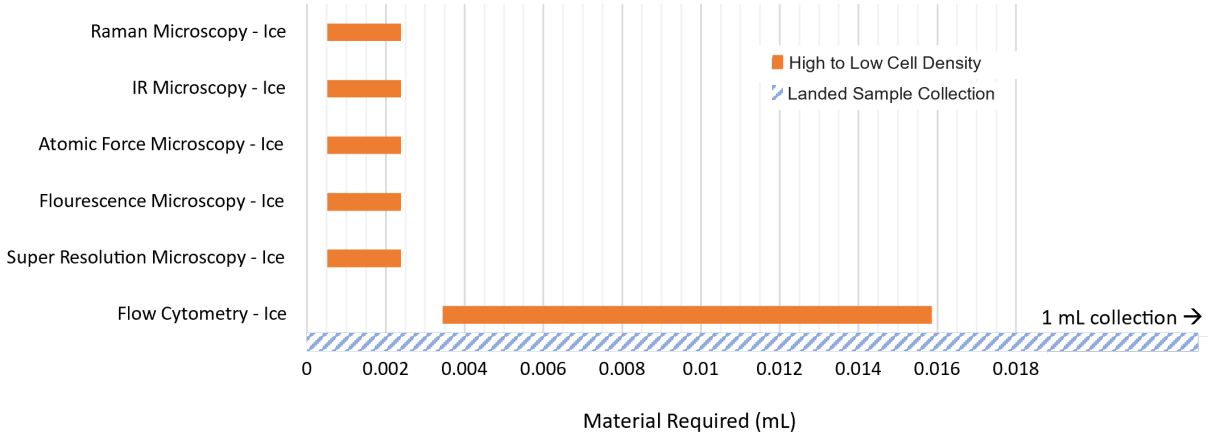


Figure 12. Cells analyses results for Europa/Enceladus. Solid orange bars indicate the amount of sample required to be collected (range required considers high to low cell density estimates) for successful cell analyses at Europa/Enceladus for two collection scenarios: **a)** plume flythrough or **b)** landed, after the sample was transported from the ocean, to a subsurface reservoir, through a crack, and to the plume or on to the surface, respectively. The labeled blue line in **a**) represents the expected amount of sample (~0.001 mL) collected per each plume flythrough, and in **b**) the blue diagonal-lined bar represents a 1 mL sample volume collected at the surface (the length of which goes to the right off the graph). For the landed scenario in **b**), the collected volume is much higher than the required volume for successful analysis, while the plume scenario in **a**) would require multiple collections.

An important factor in comparing sample types is differences in efficiencies between processing water ice versus permafrost samples, in which an efficiency of 0.5 was imposed on the permafrost size fractionation step versus 0.9 for the water ice melted samples. This translates to a need for

larger permafrost sample collection volumes compared with the ice sample; yet the required volumes for either type are small, at less than 1 cm^3 per sample.

A plume flythrough sample presents the greatest challenge for cell detection because of the low sample volumes captured per flythrough (~1 µL; Adams et al., 2018; Davila and Amato, 2020). For the least efficient transport pathway modeled, Plume – Subsurface Reservoir Through Crack (Grain – Cell), with a transport factor of 0.004 (see **Table 3**), results suggest that 0.036–0.167 mL of sample is needed (considering the high to low biologic density estimates, respectively) for a successful detection by the different cells HiDAG workflows. Flow cytometry would require an estimated 0.241–1.11 mL of sample (**Figure 12a**). These values indicate that successful detection would not be possible for a 1-µL plume flythrough sample, although multiple collections could potentially build up adequate volume. As shown for the transport scenario of biologic material transported from the ocean, through a crack, to a sill/subsurface reservoir, through freezing and diking, to the surface (**Figure 12b)**, the modeled cumulative transport factor of 0.3 (**Table 3**) and the cells HiDAG workflow efficiencies indicate that only very small volumes of sample are required for successful biosignature detection.

In **Figure 12,** plume collection is estimated at 1 µL/pass for an altitude range of 25–50 km, although suggested ranges by Porco et al. (2017) did have an upper range of ~6 µL at 50 km altitude. Following the Porco et al. (2017) estimate, about nine flythrough collections could potentially collect enough sample for a successful detection, rather than the 36 passes to reach the higher density range detection level.

In **Figure 13**, collection at Mars permafrost, all required volumes for successful analysis are low, at less than 0.075 mL. Therefore, a 1 mL sample collection (shown with a blue hash-marked bar) would be more than adequate to enable a detection via the analysis workflows specified on the y-axis.

Cells – Regolith Sample Volume Required for Successful Analyses of Mars Permafrost

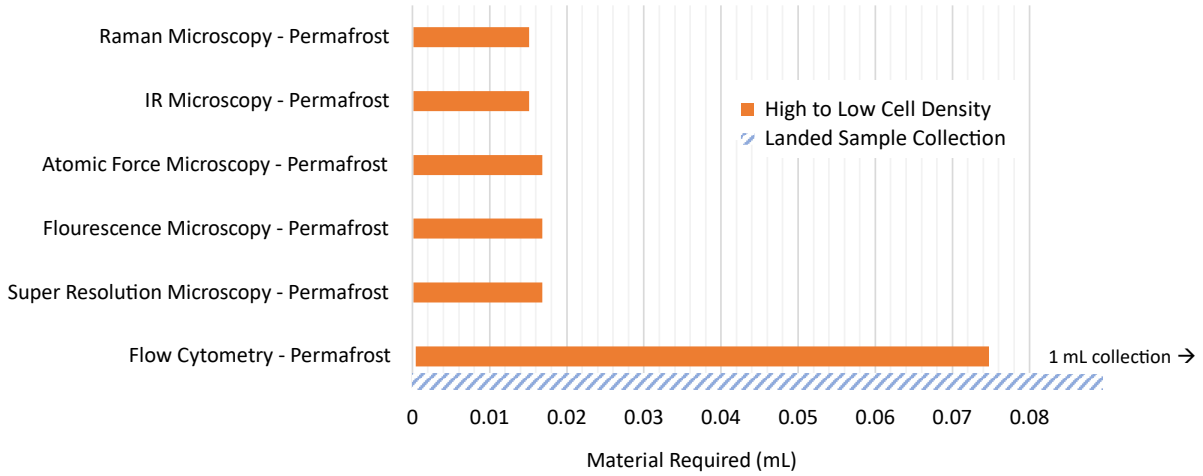


Figure 13. Cells analyses results for Mars permafrost. Required permafrost material volumes (solid orange bar showing range for high to low cell density estimates) for successful analysis of cells from Martian permafrost. These required values are much lower than landed sample collection capabilities of ~1 mL (represented by blue diagonal-lined bar) up to ~1000 mL.

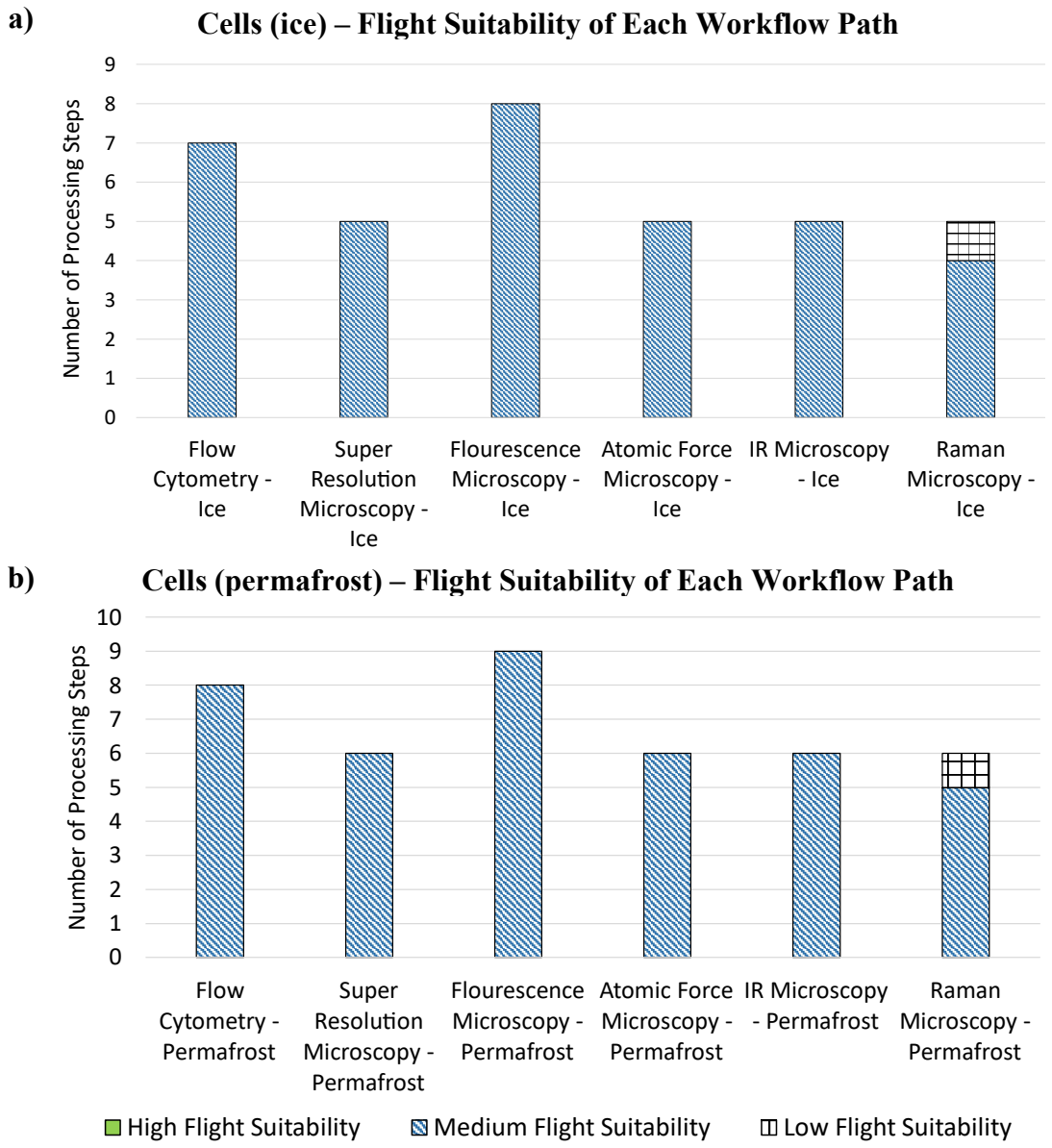


Figure 14. Cells processing steps approximate flight suitability (low in the open check pattern and medium in the solid blue diagonal-line pattern) for each processing step performed in the modeled workflow paths for **a)** Europa or Enceladus ice and **b)** Mars permafrost.

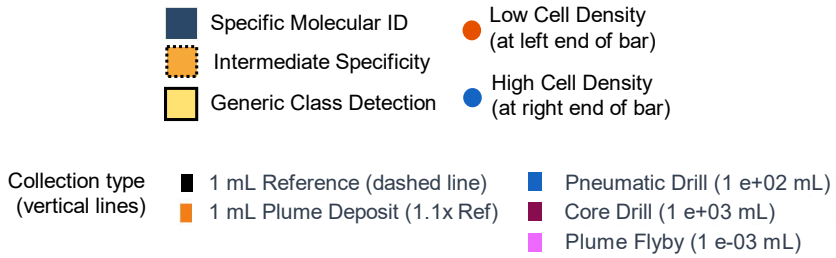
3.2.2 Europa/Enceladus Plume and Plume Deposit (Crack to Surface, Bulk)

We also evaluated if the other biosignatures, in addition to cells, could be detected in the scenario of a Europa or Enceladus surface sample collection after the sample was transported from the ocean through a crack in the ice shell, to a plume, and then redeposited onto the surface. **Figure 15–Figure 19** denote the biosignature signal required over measurement LoD in log10 scale for the various biosignatures for each measurement approach flow path. Flight suitability charts are also provided.

Europa/Enceladus Figure Definitions

For each chart, the measurement approach workflow path is described on the y-axis and correlates to the colored horizontal bars. These colored bars denote the sample signal range, in log10 scale on the x-axis, that is at the sensor divided by the LoD required to make a successful analysis for a high-density sample (blue dot at right end of bar) to a low-density sample (orange dot at left end of bar) and across that density range. Horizontal bar colors indicate the specificity of the analysis (yellow/heavy outline, orange/dashed outline, or blue/no outline, with analysis specificity increasing from yellow/heavy outline to blue/no outline). The vertical black dashed line denotes an example 1 mL collected sample volume and for that 1 mL, if sample signal/LoD falls to the left in the red shaded area, then the analysis is not successful. If sample signal/LoD falls to the right in the green shaded area, then analysis is successful. For the plume scenario, a sample may concentrate biosignatures as a plume deposit builds up over time (modeled as ~1.1x concentration; orange vertical line at x = –log10(1.1 mL/1 mL) =–0.04). The orange vertical line and two other colored lines are placed and labeled to provide examples of how the detection boundary would change location for different collection types. Examples include a pneumatic drill collection of 100 mL of concentrated plume deposit (green line at x = –2.04), a core drill collection of 1000 mL of concentrated plume deposit (red vertical line at x = –3.04), and an unconcentrated plume sample collection of 1 µL (pink vertical line at x = –log10(.001 mL/1 mL) = 3). Additional collection volumes could be considered to determine whether analyses could be successful for scenarios of high/low cell density, workflow, and collection volume.

Legend for all Europa/Enceladus charts (**Figure 15–Figure 19**):



3.2.2.2 *Carboxylic acids*

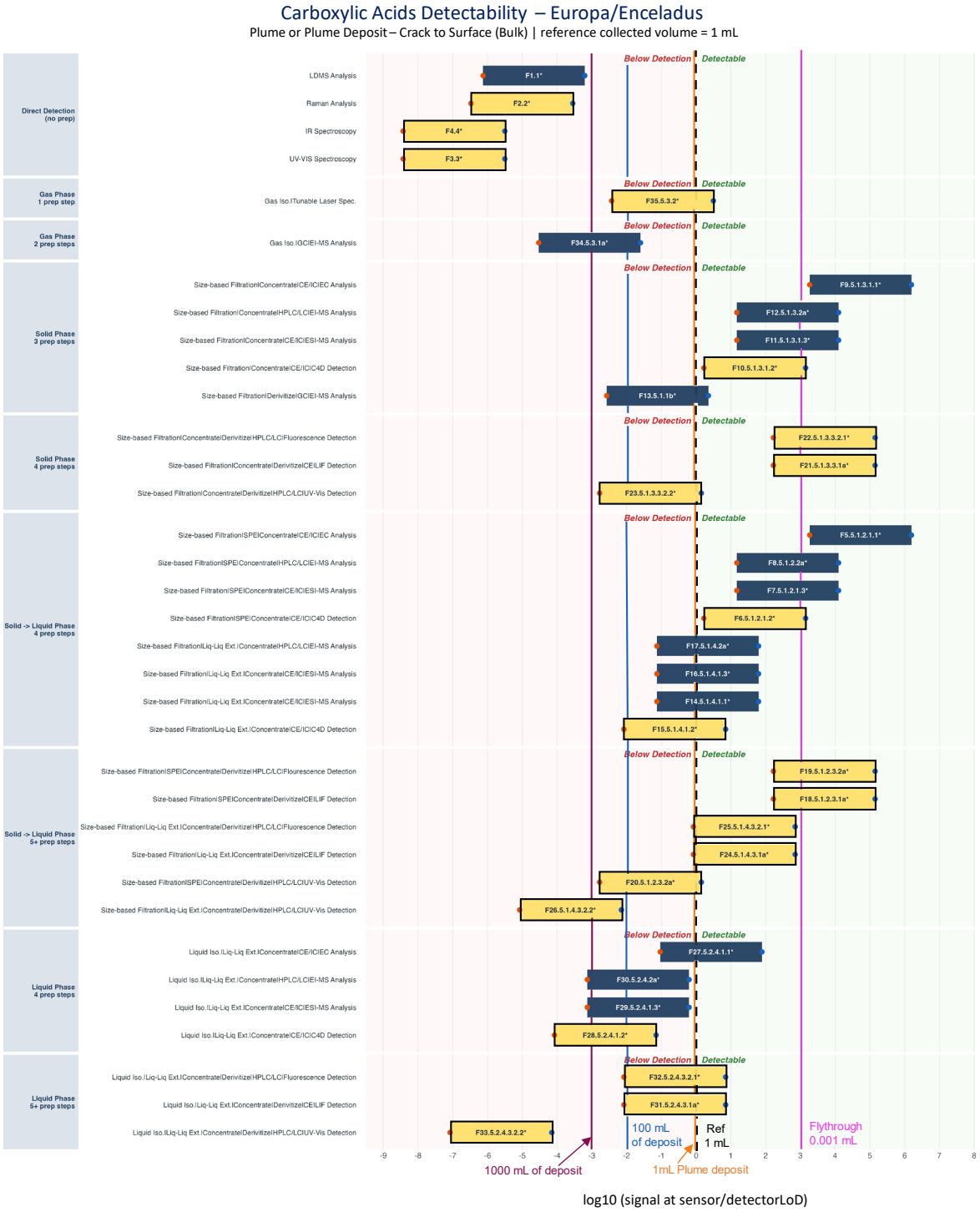


Figure 15. Plume or Plume Deposit carboxylic acid detection and characterization results for each sample measurement approach workflow shown on the y-axis (Workflow ID given within yellow/blue box). The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a surface sample collected after the sample has transported from the ocean through a crack in the ice shell, to a plume, and deposited on the surface. See "**Europa/Enceladus Figure Definitions**" for chart legend and further details.

3.2.2.3 *Amino acids*

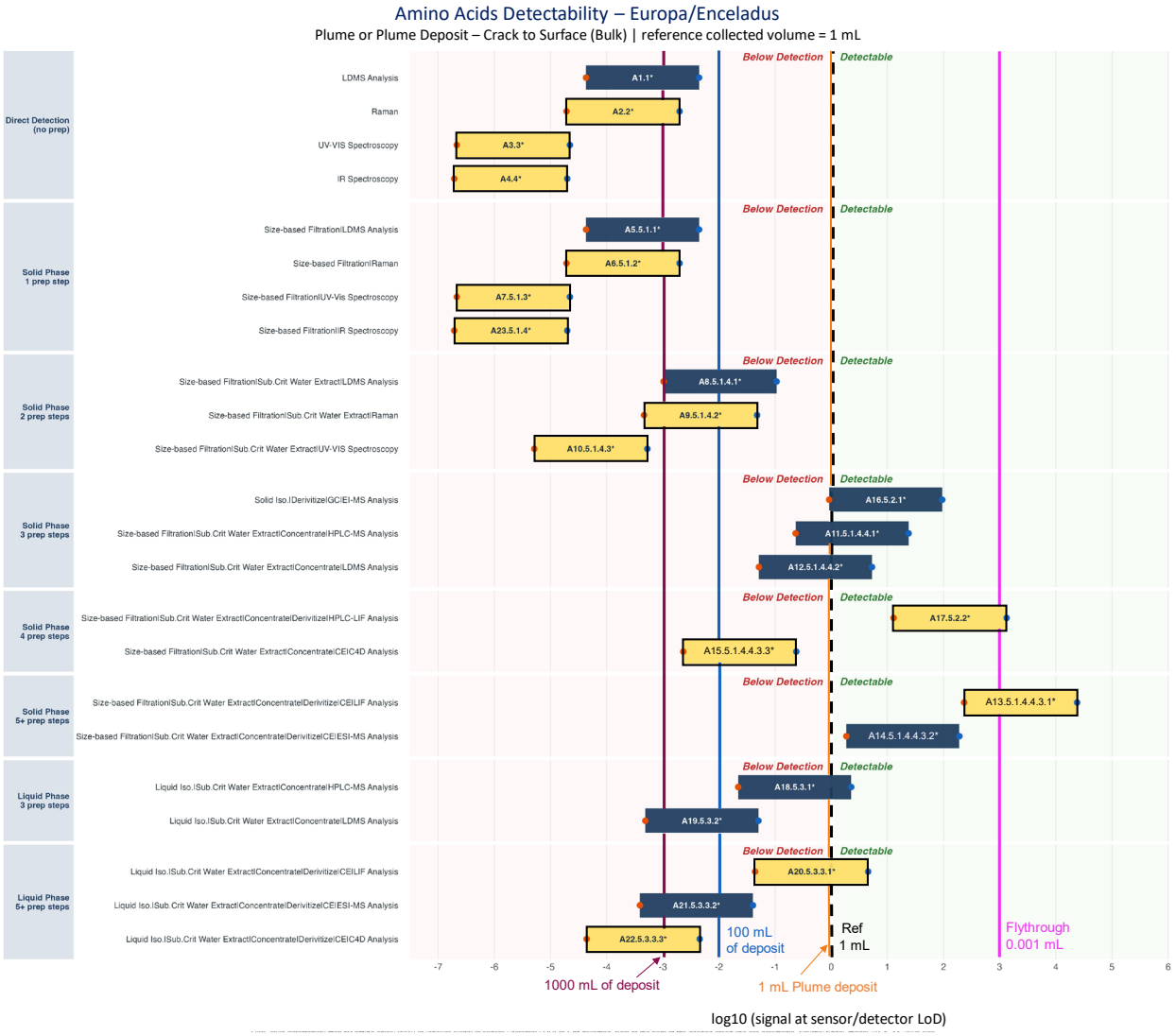


Figure 16. Plume or Plume Deposit amino acid detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a surface sample collected after the sample has transported from the ocean through a crack in the ice shell, to a plume, and deposited on the surface. See "**Europa/Enceladus Figure Definitions**" for chart legend and further details.

3.2.2.4 *Proteins and peptides*

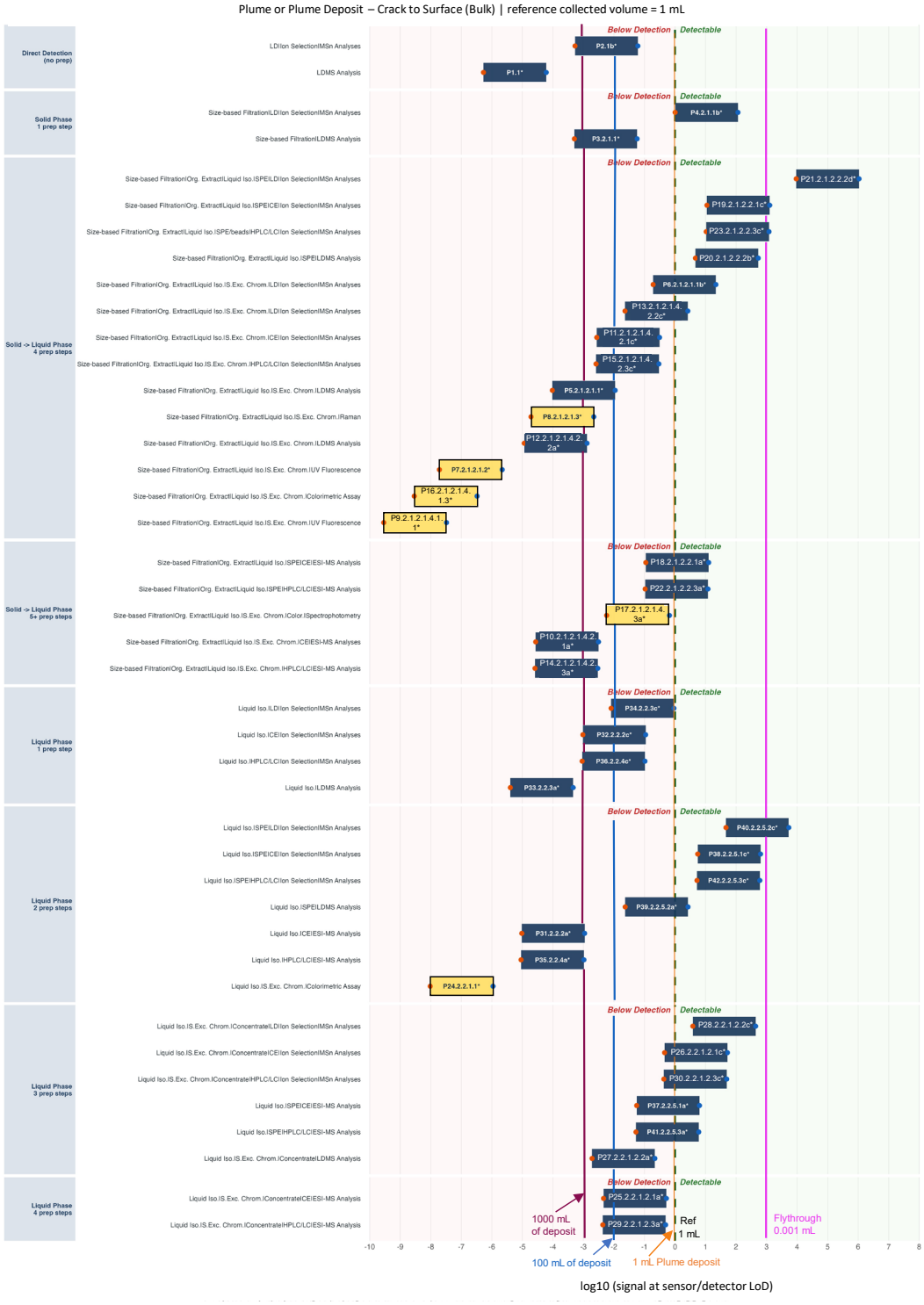


Figure 17. Plume or Plume Deposit protein and peptide detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a surface sample collected after the sample has transported from the ocean through a crack in the ice shell, to a plume, and deposited on the surface. See "**Europa/Enceladus Figure Definitions**" for chart legend and further details.

3.2.2.5 *High-molecular-weight compounds*

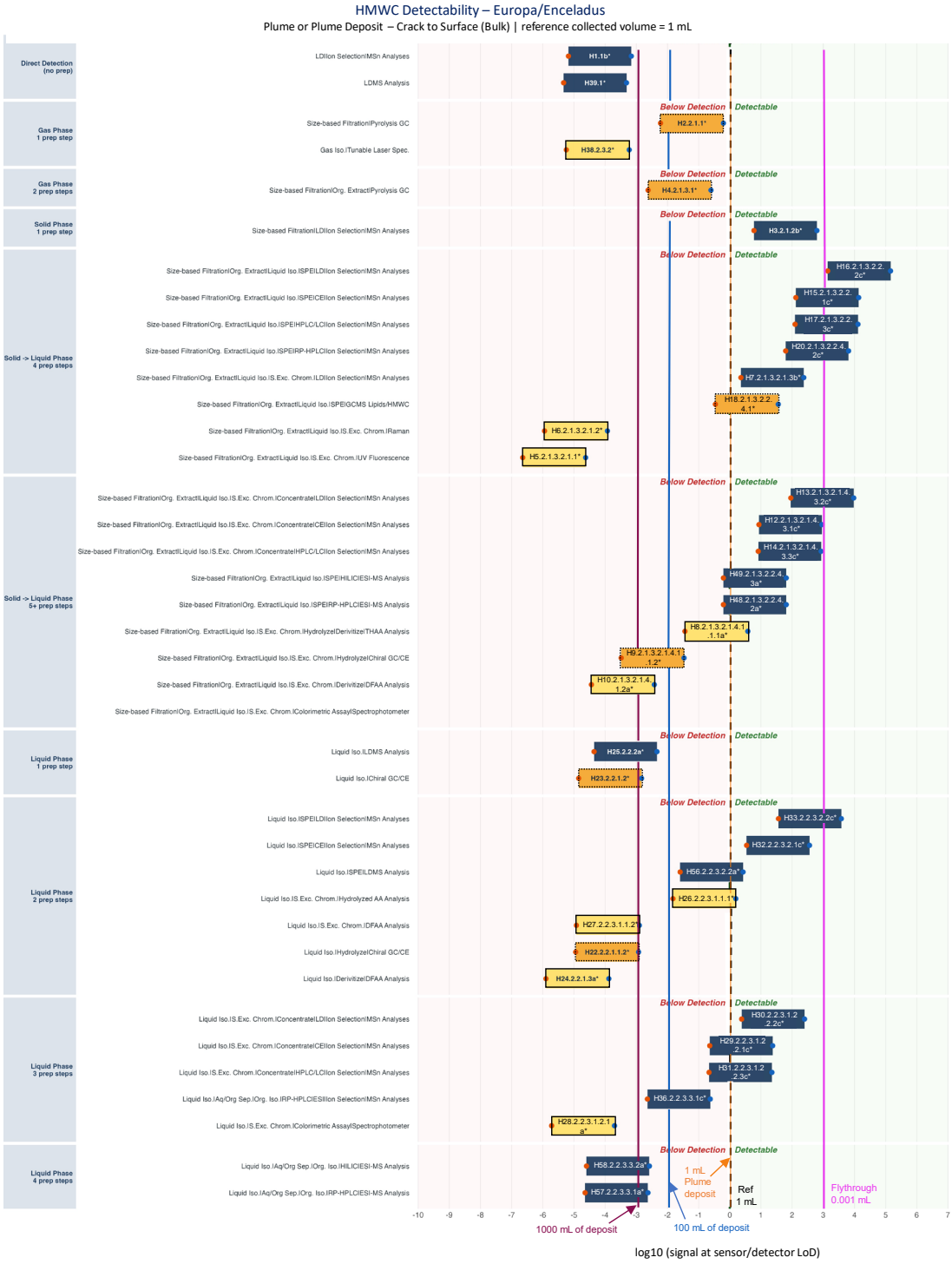


Figure 18. Plume or Plume Deposit high-molecular-weight detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a plume sample collected after the sample has transported from the ocean through a crack in the ice shell to a plume and then collected in a flythrough or deposited on the surface. See "**Europa/Enceladus Figure Definitions**" for chart legend and further details.

3.2.2.6 *Nucleic acids*

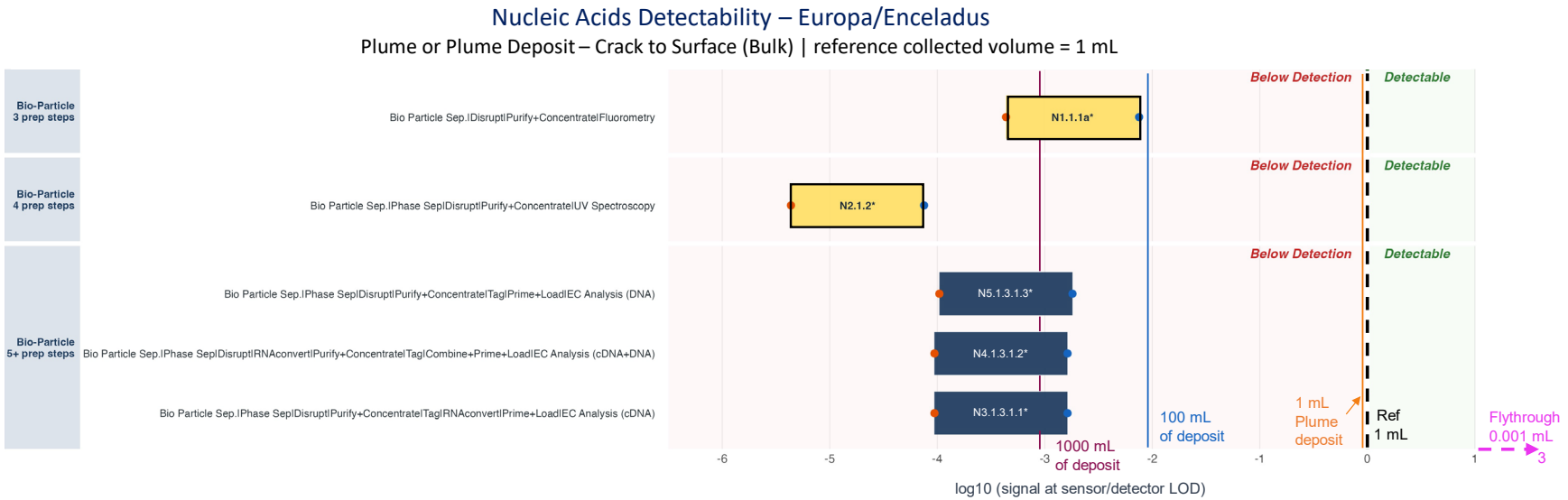


Figure 19. Plume or Plume Deposit nucleic acid detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a plume sample collected after the sample has transported from the ocean through a crack in the ice shell to a plume and then collected in a flythrough or deposited on the surface. See "**Europa/Enceladus Figure Definitions**" for chart legend and further details.

3.2.3 Europa/Enceladus – Other Physical Transport Scenarios

Other physical transport scenarios for Europa/Enceladus ocean to surface transport (see **Section 2.4**) that would change the biosignature signal that reaches the surface can be considered as a shift in available signal level relative to what is shown in **Figure 15–Figure 19**, based on the transport path factors calculated in **Table 3**. Values for the various transport paths relative to the Plume – Crack to Surface (Bulk) value of 0.09 are shown in **Table 5**, as well as a multiplier factor, M, that can be used to shift the effective detection boundary for the reference Plume – Crack to Surface (Bulk) 1 mL collection and consider the change to the amount of biosignature reaching the sampling location. With the reference bars held fixed, the effective detection boundary is $-\log_{10}$ (M) at the reference volume: enrichment shifts it left and depletion shifts it right. For a collected sample volume V, the boundary is $-\log_{10}$ [M × (V/1 mL)], assuming that signal scales with collected volume and analytical recovery and LoD remain unchanged. Note that although M can be used to understand the capabilities of the Plume transport pathways that end in a grain analysis (distinguished by a yellow highlight and bold text in **Table 5**), an instrument provider should determine whether a particular analysis technique could be applied to grains or would analyze a "bulk" sample. Additionally, only a small fraction of plume grains formed are expected to receive the concentration/depletion during aerosol formation, and this would also need to be considered for detection capabilities.

For the bulk type of samples, as shown in **Table 5**, three transport scenarios — Plume Deposit – Crack to Surface (Bulk), Plume – Subsurface Reservoir Through Crack (Bulk), and Plume Deposit – Subsurface Reservoir Through Crack (Bulk) — result in biosignature signal at the sampling location that is comparable (to 1 significant figure) to the Plume – Crack to Surface (Bulk) pathway shown in **Figure 15–Figure 19**. The other transport scenarios — Solid-Phase Convection to Surface and Crack to Sill/Subsurface Reservoir Through Freezing and Diking to Surface — increase signal 2- and 3-fold. **Figure 20** provides an example depiction for the transport Scenario A = Crack to Sill/Subsurface Reservoir Through Freezing and Diking to Surface, with its relative multiplier of ~3 (to 1 significant figure) compared to the original Plume – Crack to Surface (Bulk) value. Scenario A's 3-fold multiplier moves the effective detection boundary for the original 1 mL sample collection (black dashed line) to the left to $-\log_{10}(3) = -0.48$ in **Figure 20**, shown by the vertical solid orange line. This new detection boundary represents the higher biosignature signal at an unchanged instrument LoD and shows what workflow measurements would be possible, for those techniques that analyze bulk samples, as represented by the portions of the horizontal bars that fall to the right of the vertical orange line. As in **Figure 15–Figure 19**, other collection volumes could also be considered that would shift the detection boundary further left for larger (>1 mL) collection volumes or right for smaller (<1 mL) volumes.

Table 5. Transport factors as compared to the Plume – Crack to Surface (Bulk) pathway

Geophysical Europa/Enceladus Transport Pathway	Cumulative Transport Factor	Value Relative to Plume or Plume Deposit – Crack to Surface (Bulk)	Multiplier (M) at 1 Significant Figure
Solid-Phase Convection to Surface	0.144	1.60	2
Crack to Sill/Subsurface Reservoir Through Freezing and Diking to Surface	0.257	2.850	3
Plume – Crack to Surface (Grain - Amino Acid)	**45**	**500**	**500**
Plume – Crack to Surface (Grain - Lipid)	**4500**	**50000**	**50000**
Plume – Crack to Surface (Grain - Cell)	**0.00450**	**0.050**	**0.05**
Plume – Crack to Surface (Bulk)	0.090	1.0	1
Plume Deposit – Crack to Surface (Bulk)	0.10	1.10	1
Plume – Subsurface Reservoir Through Crack (Grain - Amino Acid)	**42.75**	**475**	**500**
Plume – Subsurface Reservoir Through Crack (Grain - Lipid)	**4275**	**47500**	**50000**
Plume – Subsurface Reservoir Through Crack (Grain - Cell)	**0.0043**	**0.048**	**0.05**
Plume – Subsurface Reservoir Through Crack (Bulk)	0.086	0.950	1
Plume Deposit – Subsurface Reservoir Through Crack (Bulk)	0.094	1.045	1

Values shown are transport pathways' cumulative efficiencies, values relative to the Plume or Plume Deposit – Crack to Surface (Bulk) pathway, and an approximate multiplier for shifting values in **Figure 15–Figure 19** to determine successful workflows for alternative transport pathways. Yellow highlight and bold type indicates pathways ending in analyses of grains.

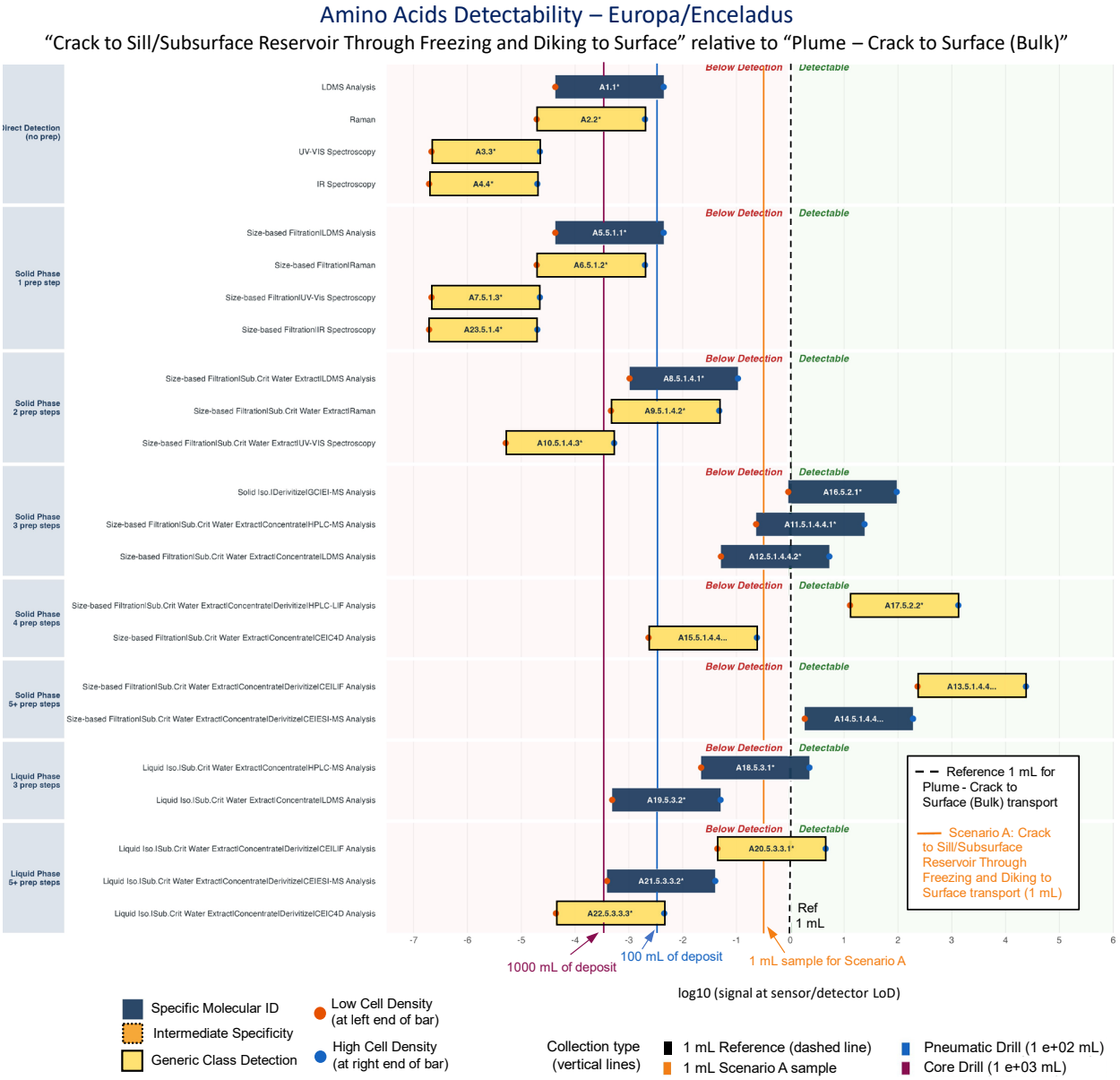


Figure 20. Changes for alternative Europa/Enceladus physical transport scenario. The chart here shows an example of how an alternative physical transport scenario, labeled Scenario A, would change the signal value available and what workflow models can make successful analyses (as shown by portions of horizontal bars that fall to the right of the vertical line). For this example, Scenario A, Crack to Sill/Subsurface Reservoir Through Freezing and Diking to Surface has its effective detection boundary shown as an orange vertical line, with a relative multiplier of about 3 (see **Table 5**) compared to the original Plume – Crack to Surface (Bulk) value (black dashed line). The line shifts left to $-\log_{10}(3) = -0.48$ to where the orange line occurs. Successful workflow measurements are those with horizontal density ranges that fall to the right of the orange line (or portions of the plotted range, dependent on the high/low biosignature density estimates). The horizontal bars, background shading, and detection labels retain the bulk-plume reference values. Blue and red lines (labeled with volumes) show examples of effective detection boundaries for other collection volumes of 100 mL and 1000 mL, respectively, with unchanged instrument LoD.

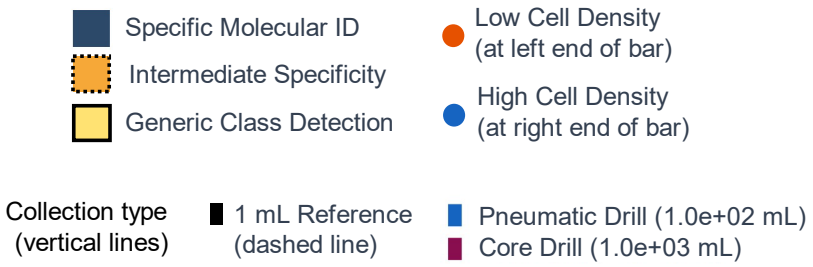
3.2.4 Mars Permafrost

We analyzed the biosignatures considered in addition to cells for detection in the scenario of sample collection from a Mars subsurface permafrost environment. **Figure 21–Figure 25** denote the results for the various biosignatures.

Mars Permafrost Figure Definitions

The measurement approach flow path + analysis is described on the y-axis and correlates to the colored horizontal bars, with colors indicating the specificity of the analysis (yellow/heavy outline, orange/dashed outline, or blue/no outline, with analysis specificity increasing from yellow/heavy outline to blue/no outline). The x-axis denotes the biosignature signal at the sensor divided by the LoD required to make a successful analysis for a high-density sample (blue dot at right end of bar) to a low-density sample (orange dot at left end of bar) and across that density range. The vertical red dashed line denotes an example 1 mL collected sample volume and for that 1 mL, if sample concentration ranges fall to the left in the red shaded area, then the analysis is not successful. If sample concentration ranges fall to the right in the green shaded area, then analysis is successful. Sampling systems in development for flight that can collect more sample (e.g., drills, scoops, corers, etc.) change the analyses capabilities and move the vertical line to the left, ranging from the example 1 mL collection up to 2800 mL for a melt drill collection.

Legend for Mars charts (**Figure 21–Figure 25)**:



3.2.4.1 Carboxylic acids

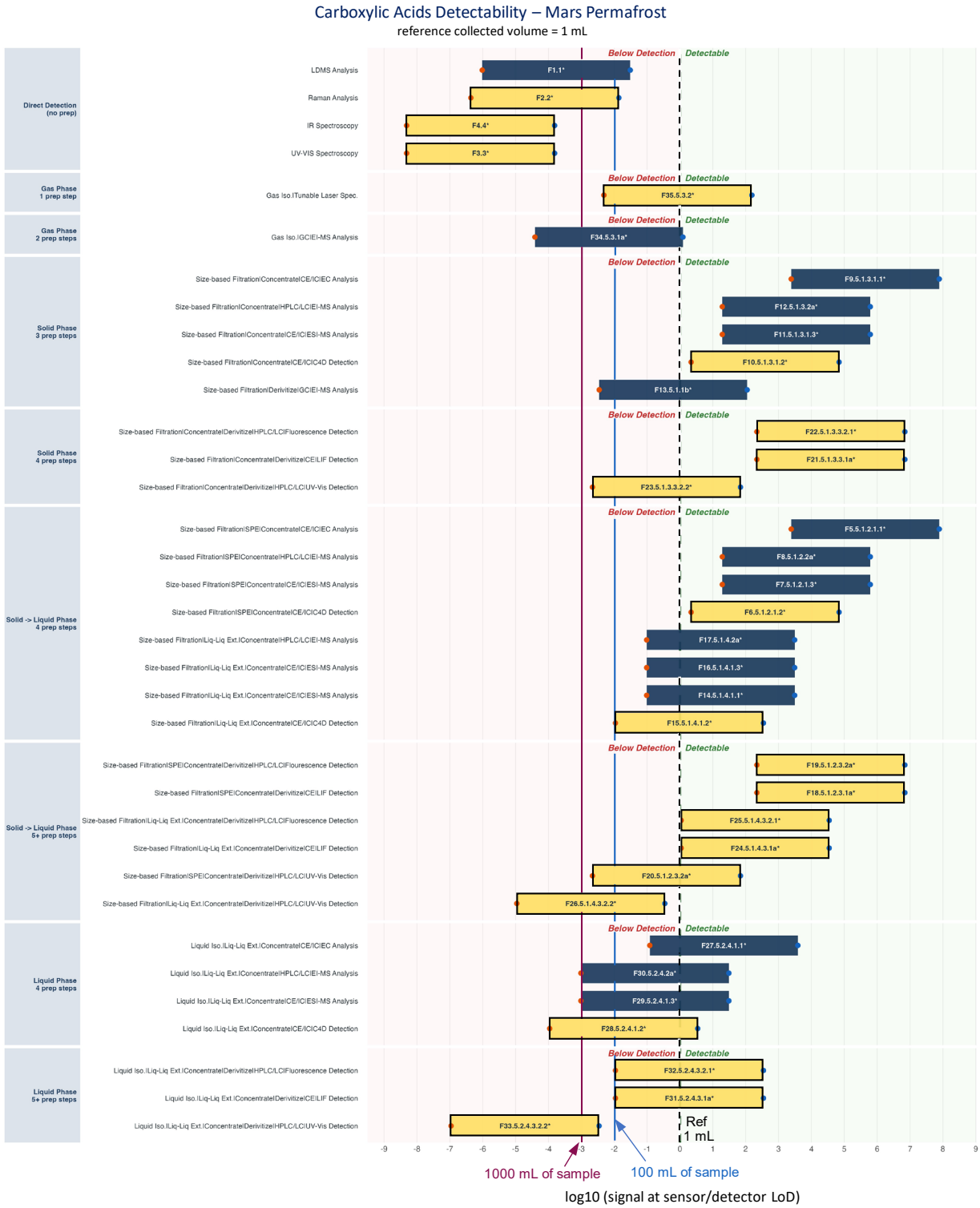


Figure 21. Mars permafrost carboxylic acids detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a surface sample collected after the sample has transported from the ocean through a crack in the ice shell, to a plume, and deposited on the surface. See "**Mars Permafrost Figure Definitions**" for further chart details.

3.2.4.2 Amino acids

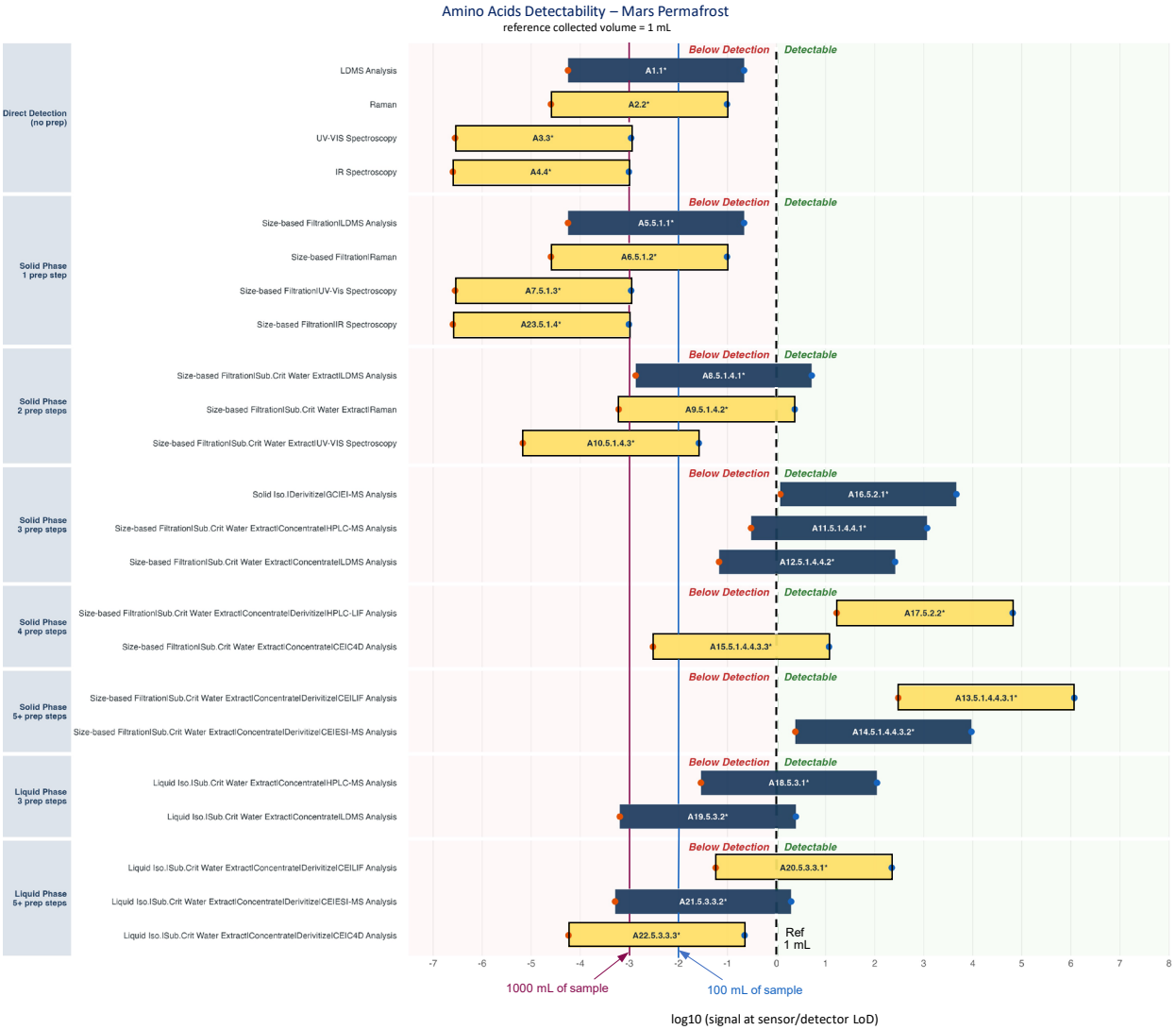


Figure 22. Mars permafrost amino acid detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a surface sample collected after the sample has transported from the ocean through a crack in the ice shell, to a plume, and deposited on the surface. See "**Mars Permafrost Figure Definitions**" for further chart details.

3.2.4.3 *Proteins and peptides*

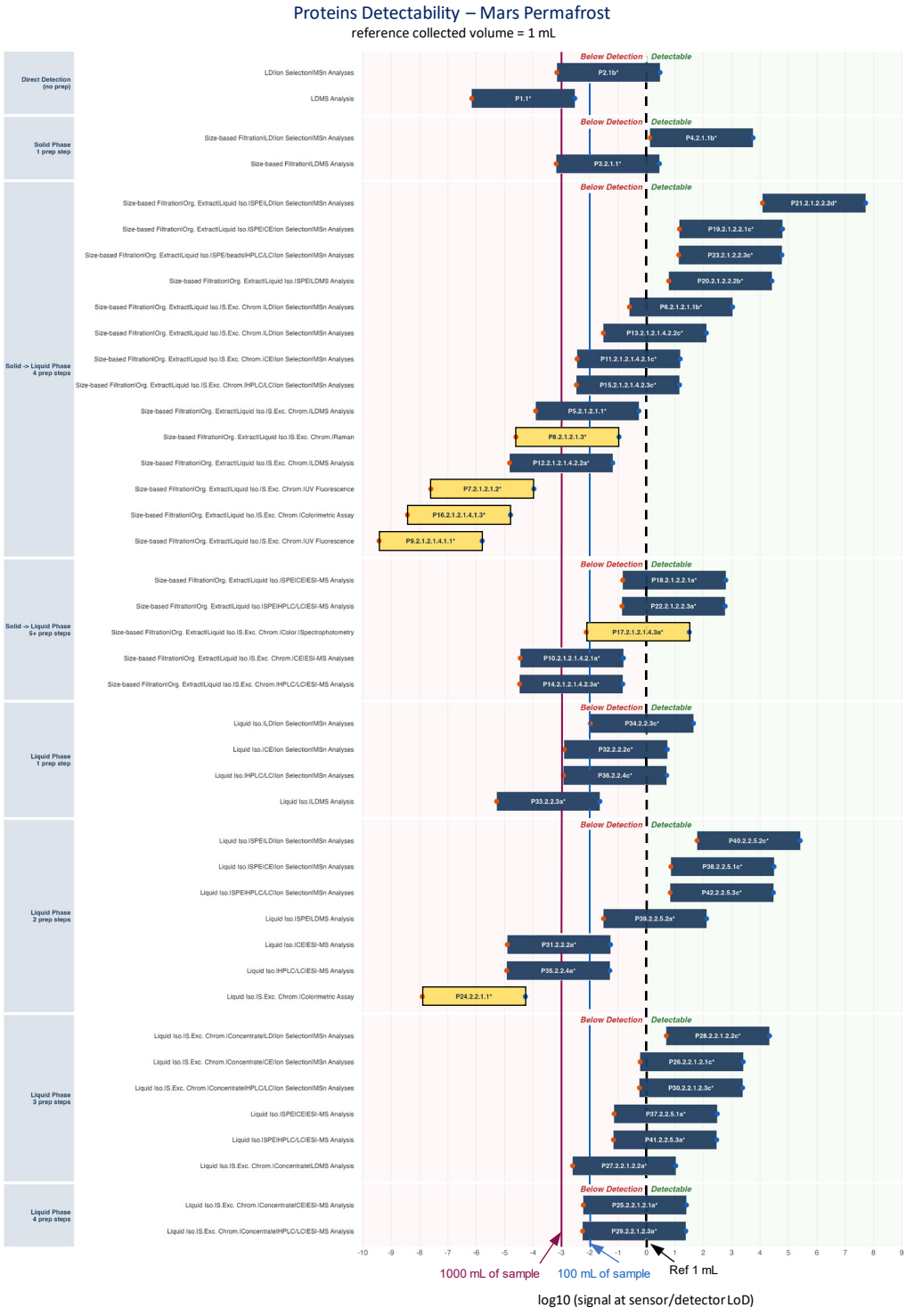


Figure 23. Mars permafrost proteins and peptides detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a surface sample collected after the sample has transported from the ocean through a crack in the ice shell, to a plume, and deposited on the surface. See "**Mars Permafrost Figure Definitions**" for further chart details.

3.2.4.4 *High-molecular-weight compounds*

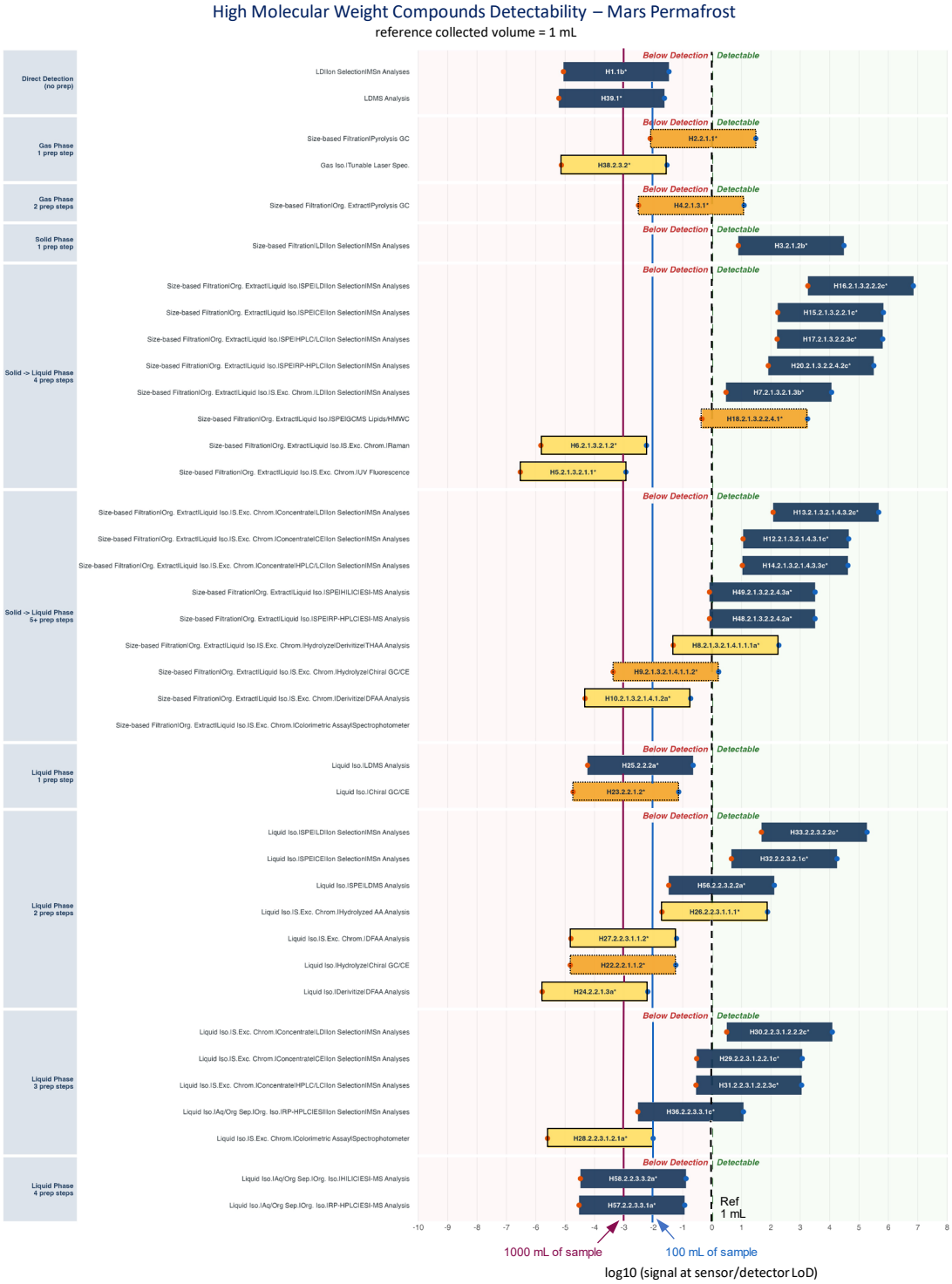


Figure 24. Mars permafrost HMWC detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a surface sample collected after the sample has transported from the ocean through a crack in the ice shell, to a plume, and deposited on the surface. See "**Mars Permafrost Figure Definitions**" for further chart details.

3.2.4.5 *Nucleic acids*

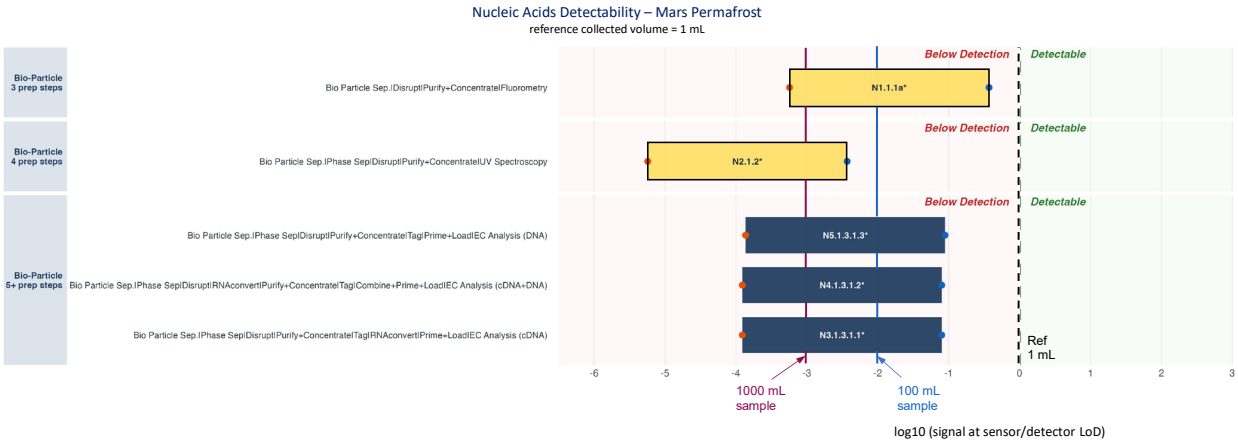


Figure 25. Mars permafrost nucleic acid detection and characterization results for each sample measurement approach workflow shown on the y-axis. The x-axis denotes the log10 value of sample signal/detector LoD for high to low estimated cell densities at Europa or Enceladus for a surface sample collected after the sample has transported from the ocean through a crack in the ice shell, to a plume, and deposited on the surface. See "**Mars Permafrost Figure Definitions**" for further chart details.

3.2.5 Flight Suitability

Flight suitability of each workflow process step for each biosignature are shown in **Figure 26**–**Figure 30**. Green horizontal-striped columns denote high flight suitability, blue cross-patterned columns denote medium flight suitability, and red diagonal-striped columns denote low flight suitability. Workflow ID along x-axis pairs with workflow in results charts. Suitability estimate is the same for both Mars and Europa/Enceladus environments.

3.2.5.1 Carboxylic acids

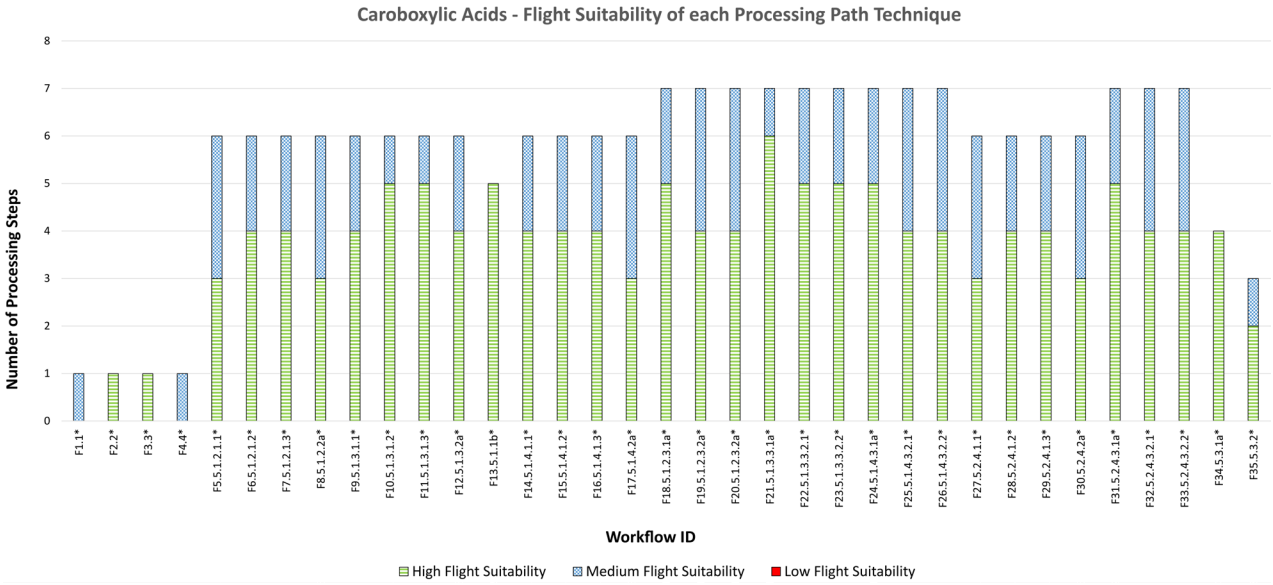


Figure 26. Flight suitability for techniques in carboxylic acids processing.

3.2.5.2 *Amino acids*

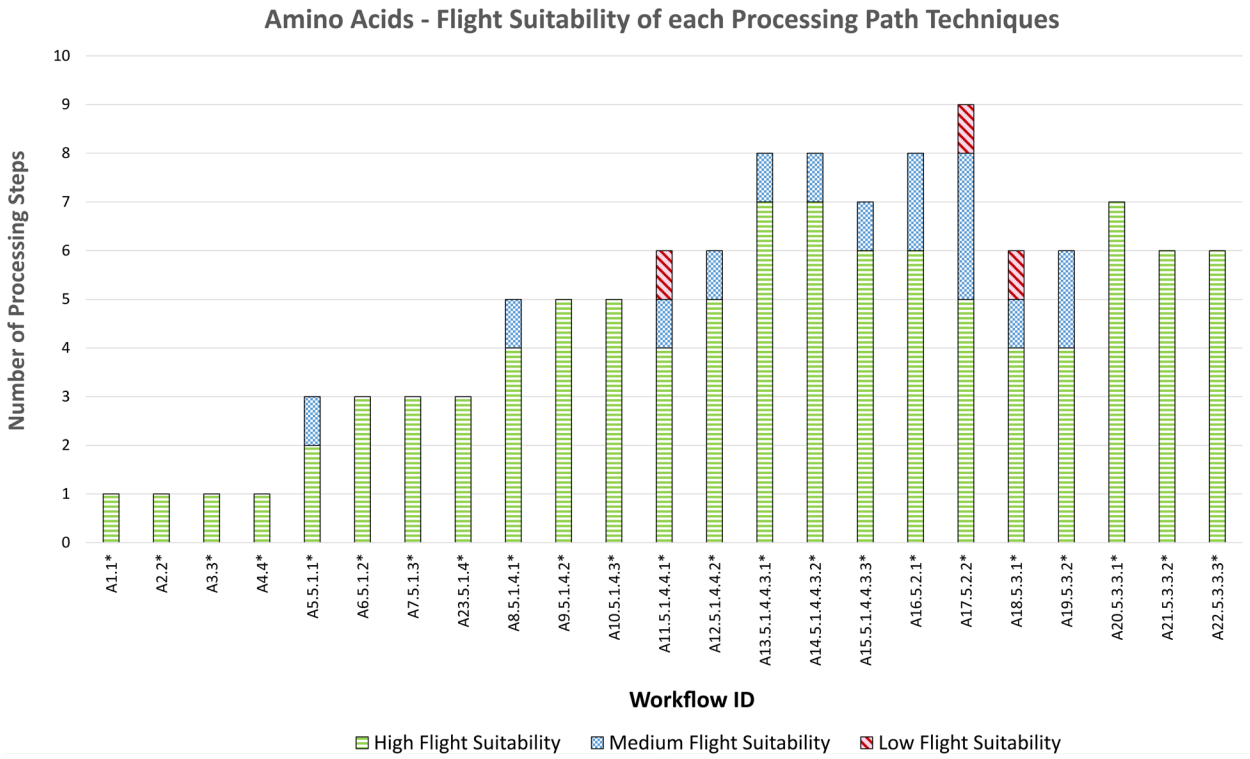


Figure 27. Flight suitability for techniques in amino acids processing.

3.2.5.3 *Proteins and peptides*

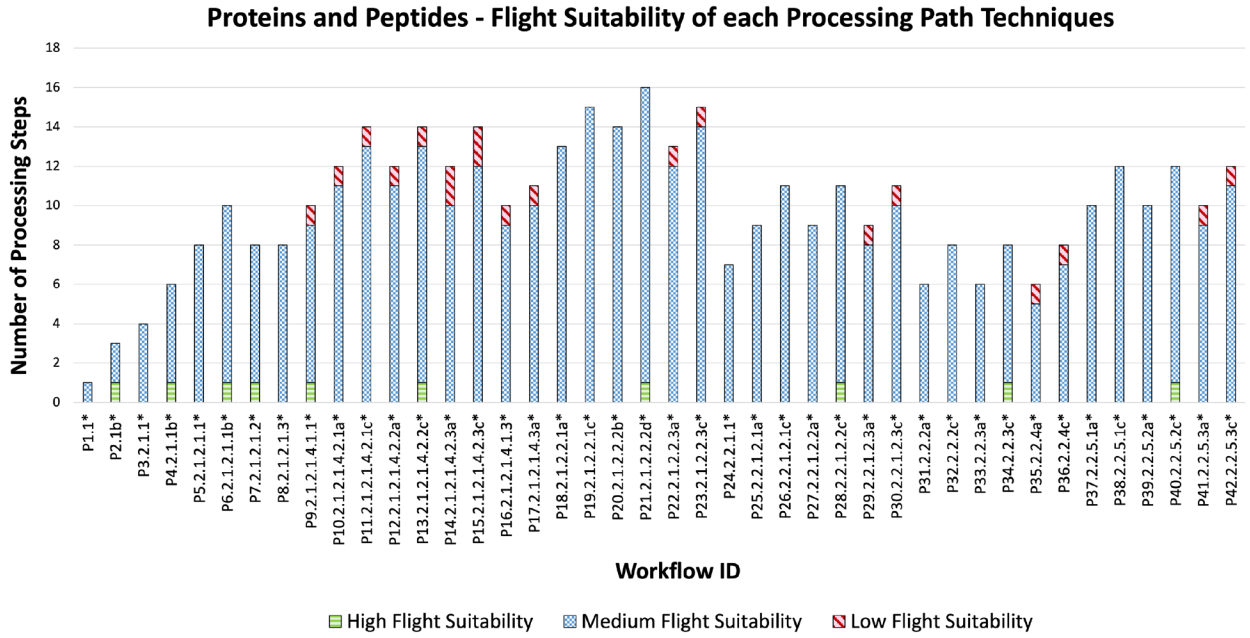


Figure 28. Flight suitability for techniques in proteins and peptides processing.

3.2.5.4 *High-molecular-weight compounds*

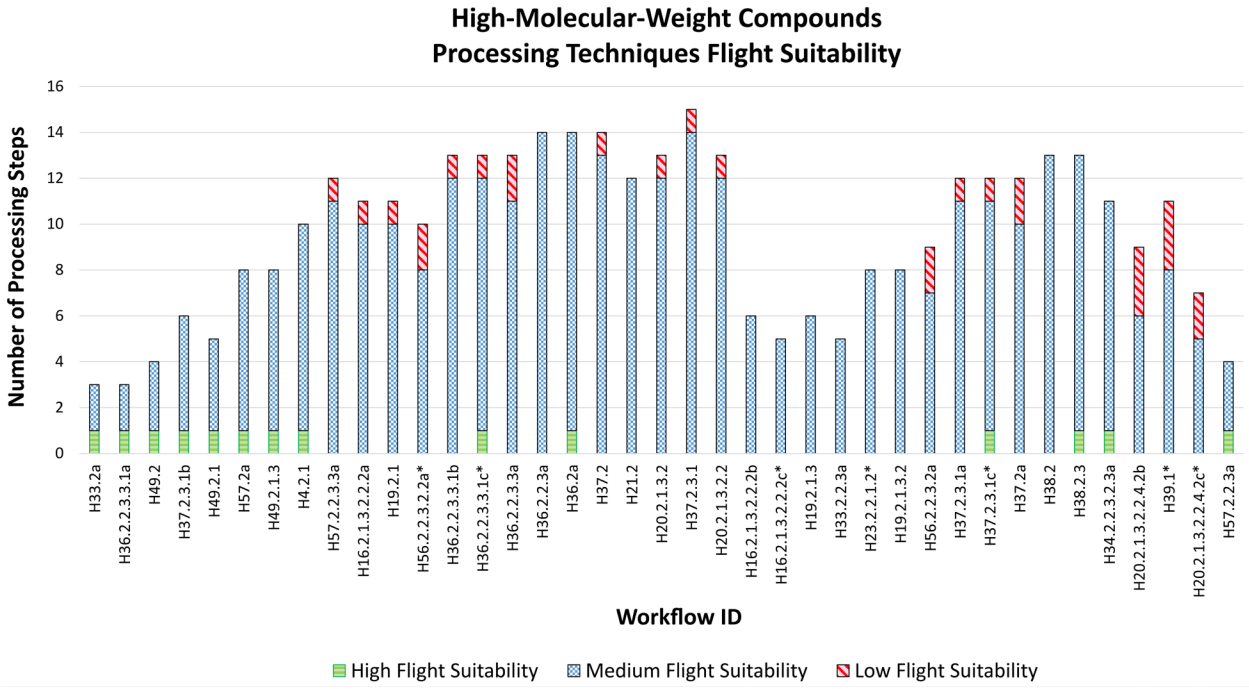


Figure 29. Flight suitability for techniques in HMWC processing.

3.2.5.5 *Nucleic acids*

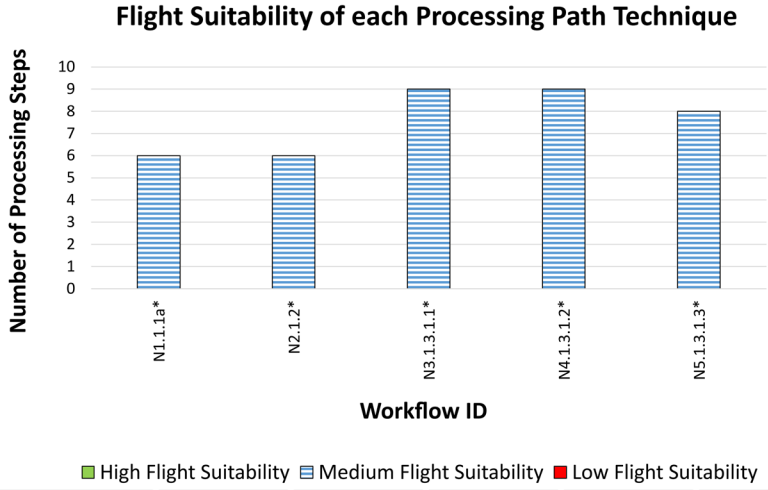


Figure 30. Flight suitability for techniques in nucleic acids processing.

4. FINDINGS

4.1 Analysis Methodology

To search for key technology gaps, for each measurement approach we investigated two questions:

1. How likely is this measurement approach to detect a desired biosignature at one of our prototypical target environments given transport and sampling methodologies?
2. What is the flight suitability of the measurement techniques and supporting sample processing technologies for spaceflight?

4.2 General Findings

Several general findings are derived independent of the individual biosignature models.

Biological model uncertainty dominates: The estimates of biosignature concentration vary by orders of magnitude and are the dominant source of uncertainty in the model. A driving challenge for any payload architecture will be determining what sensitivity is required to detect the presence of biosignatures and whether any pre-analysis collection or preparation steps can raise the "floor" of expected biosignature levels and/or reduce contextual "noise" to improve overall payload design.

Several search-for-life mission concept studies (e.g., Williams et al., 2021; MacKenzie et al., 2022; and Hand et al., 2022) have made predictions of lower biomass concentrations in the planetary environments, estimating concentrations at 1–2 orders of magnitude lower than this study's "low" terrestrial analog starting concentrations. To understand the impacts of a lower cell concentration, one can easily shift the result values calculated for each measurement flow path on the y-axis to the left **(Figure 15–Figure 25)** and evaluate how biosignature detection limits are affected for various analytical techniques. However, our results from evaluating analog environments demonstrate that even under terrestrial extreme environmental conditions, many collection, preparation, and measurement techniques require improvement to effectively detect biosignatures.

We acknowledge that biomass, as shown also in terrestrial analog environments, is distributed heterogeneously, and, thus, the floor for detection is sample dependent. Sample to sample variation is outside the scope of this effort; however, adjusting the vertical bars on **Figure 15–Figure 25** enables the reader to evaluate how this variation could show up during analysis.

Diminishing returns of efficiency improvements: There is minimal value in improving the efficiency of processes that are already quite efficient. Improving a 90% efficient process to 99% is typically a substantial technical challenge; biosignature loss is reduced by an order of magnitude. But the figure of merit is biosignature available for detection, which only improves by 10%, a marginal improvement compared to the orders-of-magnitude uncertainty ranges.

Measurement-agnostic sample processing improvement: For almost all biosignatures, many measurement approaches share the same initial processing steps. Supporting development of these enabling technologies would enhance the feasibility of many measurement technologies at once, allowing pursuit of a broad portfolio of sample processing enhancements.

Several sample handling and preparation steps would benefit all biosignature analyses, but they need technical development. Of critical importance, highlighted by the findings of this study, is the ability to **collect larger (hundreds of milliliters to a few liters) sample volumes and concentrate/filter that large volume into a smaller volume (approximately tens of milliliters or less**) such that a sample handling and processing system can perform further steps and analysis. Additionally, several sample processing needs common to many biosignature analysis include:

- Milli-/microfluidics to perform liquid handling
- Filtration, size-based filtration
- Phase separation techniques and devices (to separate particles from fluids) — e.g., size exclusion, density filtration, centrifugation
- Capabilities to perform chemistry on cells/particles themselves (staining, advanced chemistry, organic extraction)
- Measurements of, and adjustments to, pH
- Purification, extraction (desalting, separation of cells/molecular biosignatures from sample matrix)
- Particle concentration (e.g., amicons, hollow fiber filter)
- Sample splitting
- Sample mounting (e.g., microscopes)

In many environments, a substantial fraction of biosignature mass may be retained within cellular compartments or associated with particulate material. Additionally, organic compounds can adsorb onto particles through electrostatic interactions, hydrophobic interactions, ionic bonding, mineral surface complexation, or biofilm-associated adhesion. Under these conditions, size-based filtration is an example of one of the most efficient means of increasing biosignature concentration. Additionally, because filtration operations can process progressively larger sample volumes, size-based filtration workflows can scale effectively with increasing collection capacity.

These methods are intentionally simplistic — both to keep the scope of the study tractable and to allow comparison across a broad range of technologies. This is mitigated by the coarse precision goals and large uncertainty inherent in the biological density estimates. Particularly noteworthy opportunities for improvement include improving the fidelity of the biological density estimates and incorporating estimated abiotic background signals.

Finally, **system-level integration, data synthesis, and autonomy** are critical for mission readiness. Fully automated operation, adaptive analytical decision-making based on initial measurements, and fault-tolerant designs capable of executing complex workflows with minimal

ground intervention would enable robust *in situ* analyses for reduced resources. End-to-end testing with realistic analog samples will be essential to validate sensitivity claims and ensure reliable performance in planetary environments.

4.3 Physical Transport Studies Findings

The Mars permafrost environment was modeled as being sampled directly, and therefore no geophysical transport of the sample was considered. Yet at the ocean worlds Europa and Enceladus, geophysical transport plays an important role in the levels of biomolecules that could be present at a near surface sampling site. This study was able to provide estimates on biosignature concentration or losses expected during various possible geophysical transport pathways of sample from the deep ocean, through the ice shell, and out to the surface or plume sample collection location. **Table 3** and **Table 5** provides transport factors for the various transport pathways. Five transport scenarios have comparable transport factors of about 0.09 to 0.10 (reported at 1 significant figure): Solid-Phase Convection to Surface, Plume – Crack to Surface (Bulk), Plume Deposit – Crack to Surface (Bulk), Plume – Subsurface Reservoir Through Crack (Bulk), and Plume Deposit – Subsurface Reservoir Through Crack (Bulk). Another transport pathway, Crack to Sill/Subsurface Reservoir Through Freezing and Diking to Surface, has a higher transport efficiency at about 0.3 (reported at 1 significant figure). Other transport pathways are particular for "grain" analysis of plume material collected during a plume flythrough. Aerosol formation of the grains can increase or decrease signal for different biosignatures and the transport pathways with this process results in concentration factors of 4000–5000 for lipids and 40–50 for amino acids. Such enhancement is consistent with the Cassini detection of concentrated amounts of complex organics in individual ice grains (Postberg et al., 2018; Klenner et al., 2024; Khawaja et al., 2025) despite the bulk composition of ice grains in the plume of Enceladus and Saturn's E ring being dominated by water ice. In contrast, cell concentration in grains is shown to depend on droplet sizes (Perera and Cockell, 2023), leading to a reduction and overall transport factor of about 0.004-0.005, making sampling cells in plume material more challenging (cf. Porco et al., 2017; Klenner et al., 2024).

Although these estimates of ocean world sample transport from ocean to surface/plume provide reasonable bounds for these processes, the range of biosignature level that reaches the sampling site is large and needs better constrained. Laboratory and numerical studies should be performed to improve our understanding of and reduce the transport factor range for these processes. Additionally, such studies can help to identify other compounds that are likely to be present within samples, which would enable improvements to sample preparation, biosignature analyses, and detection biases. Future investigations of the targeted sampling environment should be considered in order to better inform sampling strategy and collection efficiency.

4.4 General Sample Collection Findings

There are fundamental technology shortfalls in subsurface and plume sampling architectures. The technology gap of core acquisition and processing directly impedes the ability to perform quantitative, high-resolution investigations of stratigraphic and compositional variability below the Martian regolith surface. Coring technology on Mars is essential because potential biosignatures and organic molecules are likely best preserved beneath the irradiated and oxidizing surface and may be located and potentially concentrated within stratified sediments and ancient groundwater-altered rocks accessible by drilling (Summons et al., 2011; Michalski et al., 2013). By obtaining intact cores, missions can analyze the sequence of layers and their mineralogy, capturing a time-resolved record of past environments and habitability that loose surface samples cannot provide (Summons et al., 2011). This is why missions such as the ExoMars rover were specifically designed with a drill capable of reaching ~2 m depth to search for preserved biosignatures in subsurface cores (Vago et al., 2017).

For ocean worlds such as Europa and Enceladus, the inability to extract bulk cores from depths beyond 10 cm — and to process them promptly — combined with the lack of a reliable liquid-transfer system for bulk samples, is a critical technology gap that prevents spatially resolved subsurface ice analysis. Compounding the problem, low-gravity environments make sample acquisition difficult, and the current surface-sampling technologies for such conditions are underdeveloped. In the context of plume flyby sampling at Europa and Enceladus, even though slower flyby speeds can improve conditions, it remains difficult to acquire a sample without compromising biosignature integrity, highlighting a fundamental technology challenge that needs solutions to enable a capability for capturing pristine material suitable for detailed chemical and biosignature analyses.

4.5 Cells — Findings for Future Technology Advancements

This study indicates that microscopy can detect cells in very small volumes of ice or permafrost at Europa, Enceladus, and Mars, yet certain technology and instrument developments are still needed (see **Table 6**). Devices and techniques to capture plume material during a flythrough and preserve cellular structure would be enabling for detection of cells within plume material. Laboratory analyses that investigate further strategies for discerning cellular signal from abiotic background are also of importance.

Table 6. Summary of recognized technology gaps identified for microscope/cytometric technologies for biosignature detection

Microscope Technology	Technology Gaps
Optical microscopy	• Automated sample processing • Requirement of flexibility with downstream microscopy approaches
Fluorescence microscopy	• Microfluidic capacity for handling supplemental stains • Sample preparation to deliver cell-associated particles to filters
SRM	• Sample processing to deliver cell-associated particles to SRM • Evaluate potential for spaceflight adaptations
AFM	• Sample processing to deliver cell-associated particles to AFM • Upgrade previous instrument design to nanomechanical sensing • Automated image analysis — and extracting features of interest
Micro- IR microscopy	• Adaptation of technology for autonomous, space-based applications
Raman microspectroscopy	• Adaptation of technology for autonomous, space-based applications
SEM	• Mature ISS instrument for autonomous operations • Improve sample preparations
Digital holographic microscopy	• Adaptation of technology for autonomous, space-based applications • Autonomous image analysis mitigating large data transfer
Flow cytometry	• Mature ISS instrument for autonomous operations • Evaluate adaptability for heterogeneous sample handling

4.6 Carboxylic Acids — Findings for Future Technology Advancements

Coordinated advances across sample acquisition, extraction chemistry, separation, detection, and system-level integration would enable sensitive, reliable carboxylic acid measurements on future planetary missions. As with other biosignature processing, no single technological advancement is sufficient; rather, robust performance emerges from tightly integrated, end-to-end workflows demonstrated on realistic analog samples under mission-relevant conditions.

Phase-preserving sample acquisition and handling represent a foundational area for advancement. Future missions would benefit from collection architectures capable of preserving volatile, semi-volatile, and condensed-phase carboxylic acids without fractionation or loss during transfer. This includes low-adsorption materials, temperature-controlled sampling pathways, and interfaces that allow parallel access to gas, liquid, and solid phases, particularly for plume-derived or cryogenic environments.

Selective extraction technologies offer the greatest leverage for improving sensitivity. Continued development of miniaturized SPE systems with tunable ion-exchange and mixed-mode chemistries can enable efficient, selective isolation of carboxylates from complex matrices. Similarly, spaceflight-compatible LLE architectures that allow precise pH control, solvent exchange, and phase separation in microgravity would substantially improve recovery and preconcentration for trace-level detection.

Thermal release and gas-phase handling technologies warrant focused development. Controlled thermal desorption systems that minimize decomposition while enabling reproducible release of volatile carboxylic acids would strengthen gas-phase measurements. Advances in low-loss gas handling, preconcentration, and adsorption-resistant surfaces would be particularly enabling for plume sampling and other dilute environments where biosignature availability is limited.

Separation technologies remain a key capability in need of advancement. Further maturation of CE, IC, and LC systems optimized for small organic acids — including improved control of pH, ionic strength, and temperature — will directly enhance selectivity and sensitivity. Integration of on-chip preconcentration and fractionation methods offers a high-payoff path toward detecting low-abundance species.

Detection systems should continue to advance in parallel. Compact, low-power mass spectrometers compatible with both gas- and liquid-phase separations provide molecular specificity and resilience against false positives. Complementary advances in conductivity-based detection and TLS can enable rapid screening and cross-validation of carboxylic acid inventories across multiple phases.

Reagent chemistry and calibration strategies are also critical. Long-lived, low-background derivatization reagents, solvent systems, and internal standards must be developed with spaceflight stability in mind. The inclusion of compound-class-specific internal standards should be treated as an integral design requirement rather than an optional enhancement.

Finally, system-level integration and autonomy are essential for mission readiness. Future instruments should emphasize fully automated workflows, adaptive decision-making based on initial phase or concentration measurements, and fault-tolerant designs capable of executing complex extraction and analytical sequences with minimal ground intervention. End-to-end testing using cryogenic, plume-derived, and desiccated analog samples will be essential to validate performance and interpretability under realistic mission conditions.

Collectively, these technology advancements would enable the transition from laboratory-scale demonstrations to flight-ready systems capable of robust, sensitive carboxylic acid analysis, providing critical chemical context to complement amino acid measurements in the search for life beyond Earth.

4.7 Amino Acids — Findings for Future Technology Advancements

To achieve extremely sensitive and unambiguous amino acid detection on future planetary missions, continued advancement across multiple layers of instrument development is needed, spanning sample handling, chemical processing, separations, detection, and system-level integration. No single analytical advance is sufficient; rather, sensitivity and reliability emerge from tightly coupled improvements throughout fully automated end-to-end workflows, which must be demonstrated on real analog samples. Additionally, when paired with specific molecular

identification of polymeric amino acid classes, such as peptides and proteins, these measurements can provide complementary information related to biological function, structural organization, and potential taxonomic relationships to analog organisms on Earth. Because robust life-detection claims will likely require multiple independent and confirmatory lines of evidence, continued technology development focused on parallel characterization of amino acids, peptides, and proteins represents a valuable consideration for future missions.

Sample handling and contamination control remain foundational. Prioritizing ultra-clean, low-background fluidic architectures capable of processing complex natural samples without introducing terrestrial amino acids or interfering residues would substantially enhance biosignature detection while supporting required contamination control measures. This includes improved materials compatibility, reagent storage and delivery systems, and *in situ* blanks and calibration strategies to enable rigorous contamination tracking and quantification at parts-per-trillion levels and below.

Extraction and hydrolysis technologies represent a major leverage point for sensitivity. Continued development of tunable, low-volume extraction methods, such as controlled subcritical water processing and miniaturized acid/base hydrolysis, can improve recovery from diverse matrices while minimizing amino acid degradation and racemization. Emphasis should be placed on extraction efficiency, reproducibility, and compatibility with downstream separations.

Microfluidic separations are a critical enabling technology for spaceflight amino acid analysis. Further maturation of CE-based systems, including improved injection control, longer effective separation lengths, and robust chiral separation chemistries, will directly translate to improved sensitivity and selectivity. Integrated preconcentration methods, such as stacking or on-chip SPE, offer particularly high payoff for trace-level detection.

Detection technologies should continue to advance in parallel. Laser-induced fluorescence remains one of the most sensitive detection approaches for amino acids. Complementary development of mass spectrometric interfaces compatible with microfluidic separations can provide molecular confirmation and reduce ambiguity in complex samples.

Reagent chemistry and derivatization strategies also warrant sustained attention. Future efforts should focus on high-yield, low-background derivatization chemistries with long-term stability under spaceflight conditions. The availability of robust internal standards, including isotopically labeled amino acids, is essential for quantitative analysis and should be considered an integral part of instrument design rather than an ancillary capability.

4.8 Proteins and Peptides — Findings for Future Technology Advancements

The modeling results for protein and peptide detection are encouraging and point to a clear set of advancement opportunities. Across the 42 protein workflows evaluated, 25 are detectable in both

high and low cell density estimates at the 1000 mL collected sample volume, rising to 29 at 2800 mL. The strongest workflows achieve detection margins 3–6 orders of magnitude above the detector limit at the reference volume of 1 mL, and several remain detectable at sample volumes as small as 0.001 mL for the high-cell-density estimate. This reflects two structural advantages of the protein analytical landscape: the higher per-molecule signal accessible through tandem MS and the powerful concentration leverage of SPE. Peptide and protein measurements also provide complementary and largely independent lines of evidence to the amino acid characterization, revealing sequence information, polymer length distributions, and structural features that bear on biological origin and functional organization. Considering that robust life detection will require multiple converging biosignature measurements, advances in protein and peptide processing and analysis should be considered a high-priority complement to the amino acid technologies.

SPE paired with size-based filtration is the single highest-leverage technology area. Every protein workflow that achieves detection at sub-nanoliter processed volumes depends on SPE concentration factors of 10^5 to 10^8, and these workflows define the detectable frontier at small sample volumes. Size-based filtration is an equally critical front-end step because Earth-based cellular life is upward of 90% protein by dry mass, meaning that whole-cell and cellular debris captured by filtration provides a direct and highly efficient route to concentrate the biosignature of interest before any chemical processing. Priorities for technology advancements include flight-compatible SPE chemistries spanning affinity, hydrophobic-interaction, mixed-mode, and bead-based architectures; robust filtration membranes (or other) across the size ranges relevant to cells, cellular debris, and extracellular protein; miniaturized low dead-volume cartridges; and integrated on-chip configurations compatible with downstream separation.

Mass efficiency is the most quantifiable improvement. The highest-performing workflows in this analysis currently operate at end-to-end mass efficiencies of only 0.9–2.1%, even as their concentration factors approach the practical ceiling. Raising mass efficiency toward 10–20% through improved transfer chemistries, reduced surface losses, and optimized elution and digestion protocols would translate directly into a 10-fold reduction in sample volume required for detections and would push several additional workflows into the robustly detectable category. Because inherent processing concentration factors are already high, further gains there yield diminishing returns; additional improvements in mass efficiencies would be most enabling.

Tandem MS with upstream ion selection is the analytical centerpiece for identification. Tandem mass spectrometry (MS/MS), including higher-order MS^n approaches that enable additional rounds of ion selection and fragmentation, appears in essentially every workflow that achieves detection at low cell densities, and the modeling shows that no single upstream separation modality dominates when SPE is included: CE, HPLC/LC, and direct laser desorption coupled to MS^n produce detection margins within about one order of magnitude of each other. This argues for parallel maturation of multiple flight-compatible MS^n architectures (ion trap, Orbitrap-class, time-of-flight) and multiple upstream separation modalities engineered for low-flow, low-volume

operation, rather than premature down-selection. The standard flexibility in tandem MS architecture allows for *in situ* experimental design as information is retrieved at the site. Fragmentation of a molecule is one of the most, if not the most, valuable methods for deciphering the underlying chemistry and molecular class and structure (for all biosignatures discussed in this study). High mass accuracy Orbitrap-class instruments are particularly valuable for resolving ambiguous or near-isobaric masses arising from modified or chemically altered residues, because peptides fragment in a reproducible manner along the peptide bond when subjected to collisional activation in a tandem MS system, producing predictable *b*- and *y*-ion series that enable confident sequence assignment even in the presence of post-translational modifications or environmental degradation products.

Extraction and digestion chemistry provides an opportunity to significantly enhance measurement sensitivity. The protein workflows modeled here assume conservative recovery values from organic extraction. Targeted advances in extraction methods optimized for cold, salty, oxidizing, or otherwise challenging planetary matrices, together with miniaturized, reagent-stable digestion modules that generate tractable peptide fragments for MS^n, could yield one to two orders of magnitude in overall sensitivity. Preserving labile post-translational modifications and minimizing in-process degradation also carries biological interpretation value beyond simple detection.

Optical detection methods should be deprioritized for peptides and proteins. Workflows based on UV fluorescence, colorimetric assays, and spectrophotometry fail by four to 10 orders of magnitude at the 1 mL reference volume and remain undetectable in the low cell density estimate, even at the largest collectible volumes considered. This is a meaningful divergence from the amino acid roadmap, where laser-induced fluorescence remains a top-tier detection mode, and reflects the lower per-molecule chromophore density and greater structural diversity of polymeric biosignatures. This is due to the inherent size of proteins assumed in this model (compared to average size of an amino acid). Optical methods retain value as complementary triage or fraction-monitoring capabilities but may not be reliable as primary protein detection pathways. Similarly, direct laser desorption approaches without upstream extraction or concentration fail by three to five orders of magnitude. However, they retain utility when integrated into a larger workflow that includes solid-phase preconcentration.

Sample handling and contamination control are essential for this biosignature class. Terrestrial protein contamination is ubiquitous and persistent, and contamination thresholds are correspondingly stringent. Ultra-clean, low-background fluidic architectures with materials selection and surface passivation strategies that minimize protein adsorption and carryover, combined with *in situ* blanks and isotope-labeled internal standards, are essential to enable contamination tracking at parts-per-trillion levels. Reagent stability and quantification capabilities, including peptide standards and stable derivatization chemistries with multi-year cruise

compatibility, should be designed into the instrument architecture from the outset rather than treated as ancillary capabilities.

System-level integration and analog validation are critical for mission readiness. Protein workflows can involve five or more sequential processing steps, placing a particularly high premium on fault tolerance, reagent management, and adaptive analytical decision-making based on initial measurements. That said, once optimized, the resulting biosignature class isolated is far more homogeneous, making it easier to interpret the data. End-to-end validation on realistic analog samples, including matrices that capture the salinity, oxidation chemistry, and radiolytic or photochemical alteration products expected at the target body, is essential to confirm the sensitivity gains modeled here.

Collectively, these advances would push the number of robustly detectable workflows in the low cell density estimate from the current 25 at 1000 mL toward the full complement of MS-based methods, and would bring protein and peptide detection into reach at sample volumes obtainable from a scoop or pneumatic drill rather than requiring a melt drill or deep core. In combination with parallel amino acid capability, this would provide one of the strongest available foundations for a credible, multi-line-of-evidence search for life beyond Earth.

4.9 High-Molecular-Weight Compounds — Findings for Future Technology Advancements

The modeling results for HMWC detection are favorable and broadly parallel the picture for proteins and peptides, although with somewhat tighter sensitivity margins. Of the 39 HMW workflows evaluated, 23 are detectable in both high and low cell density estimates at the 1000 mL collected volume, and 12 reach detection at the 1 mL unit sample volume for the high-cell-density estimate. The strongest workflows achieve detection margins three to five orders of magnitude above the detector limit and follow the same architectural pattern that dominates the protein analysis: size-based filtration, organic extraction, liquid isolation, SPE, and tandem MS. Size-based filtration in the 0.1- to 0.22-μm range, paired with downstream lysis to release intracellular HMW content, is particularly valuable for this biosignature class and would benefit from near-term flight advancements. Chromatographic separation [HPLC/LC, CE, or reversed-phase HPLC (RP-HPLC)] provides an additional one to two orders of magnitude of sensitivity and dramatically improves downstream mass spectrometric interpretability by reducing matrix interference at the ion source. Most importantly, tandem MS with upstream ion selection is again the analytical centerpiece: it dominates every workflow that achieves detection at low cell densities and provides the fragmentation data needed to reconstruct parent masses and infer structural features of complex polymeric biosignatures. As with proteins and peptides (**Section 4.8**), optical methods (UV fluorescence, Raman, colorimetric assays) fail by four or more orders of magnitude at the 1 mL reference volume for HMWC and, thus, provide complementary triage capabilities, while direct laser desorption without upstream concentration is not viable as a stand-alone strategy. The

advancements outlined for proteins and peptides, particularly improvements in mass efficiency, flight-compatible SPE, and MS^n maturation, apply directly to HMWC analysis and would yield comparable sensitivity gains, bringing robust detection of this biosignature class into reach at sample volumes obtainable from a scoop or pneumatic drill.

4.10 Nucleic Acids — Findings for Future Technology Advancements

Current nanopore sequencing technologies use biological nanopores on polymeric membranes with limited shelf life that make them untenable for long-duration missions with strong radiation environments. Additionally, for signal to be interpretable, the current configurations require the nucleic acid strands to bind to a motor protein to stabilize their translocation through the pore. This protein is specific to terrestrial DNA and is therefore possibly incompatible with extraterrestrial nucleic acids with different molecular characteristics. Materials that are robust to radiation and that can be used to make precise pore openings would enable these technologies to be used in flight. Ways to ratchet or slow the translocation of the nucleic acids through a pore opening would also potentially make identification of bases and genetic sequences possible. Additionally, new technologies, including nanogap, may also enable detections and characterizations of DNA- or RNA-like nucleic acids and their components, as well as other molecular biosignatures (including amino acids and peptides).

5. SUMMARY

This study describes an end-to-end investigation into the levels of biosignatures that would be available for analysis from Martian permafrost and Europa/Enceladus ocean world environments. We performed Fermi estimations to model the changes in biosignature levels (based on terrestrial analog environments; **Section 2.3**) in a sample as it is physically transported from its habitable environment origin (**Section 2.4**; transport considered for Europa and Enceladus only) to sample acquisition location, through sample collection and handling (**Section 2.5**), sample preparation/processing, and finally through to analysis with current laboratory and flight instrumentation (**Section 2.6**). We then assessed the capability to make a detection, or not, for multiple transport and workflows scenarios by comparing biosignature availability at a detector to the detector's LoD (**Section 3**). We also estimated flight suitability for sample acquisition and each of the processing steps to assist with understanding technology development needs. Based on these assessments, we then identified scientific and technology gaps that would most benefit from development to ensure robust *in situ* search-for-life capabilities (see Findings in **Executive Summary** and **Section 4**).

Collectively, addressing these science and technology development gaps would enable flight-ready instruments capable of detecting biosignatures at extremely low abundances, preserving chemical context, and providing robust, interpretable measurements in the search-for-life beyond Earth.

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Appendix A. Examples of the Terrestrial Studies Considered for Determining the HEO Cell Concentrations

Location	Sample matrix	Reference
Mt. Baker, WA (lake)	Clean snow	Lutz et al. 2014
Franklin Bay, Northwest Territories, Canada	Arctic sea ice, arctic brine	Collins et al., 2008
Midtre Lovénbreen, Svalbard	Supraglacial water, surface glacier ice cores	Irvine-Fynn et al., 2012
Antarctic Dry Valleys	Active layer, permafrost	Gilichinsky et al., 2007
Greenland (GISP2) glacial ice	Glacial ice, silty	Price et al., 2007
Thule, Greenland	Surface ice	Stibal et al., 2015
Dronning Maud Land, Antarctica	Continental ice (11 m depth)	Antony et al., 2012
Russell Glacier, Greenland	Basal Ice	Yde et al., 2010
Yungay Region, Atacama Desert, Chile, A2	Desiccated soil	Connon et al., 2007
Yungay, Atacama Desert, Chile	Surface soil, various depths	Lester et al., 2007
Atacama Desert	Surface soil (1 cm deep)	Glavin et al., 2004
Washington coast sediments	Marine sediment (various depths)	Nunn and Keil, 2005
Washington coast, Northeastern Pacific	Ocean water (various depths)	Bridoux et al., 2015
Subtropical Gyre, Tropical North Pacific Ocean	Ocean water (various depths)	Bridoux et al., 2015
Sargasso Sea, North Atlantic	Ocean water (various depths)	Bridoux et al., 2015
Bering Sea	Ocean water (various depths), sinking particle trap material (various depths), post-bloom shelf sediment	Moore et al., 2012
Chuckchi Sea, Alaska	Coastal fast ice core	Junge et al., 2004
Barrow Permafrost Tunnel, Utqiaġvik, Alaska	Intra-ice brine, sackhole brine from sea ice	Cooper et al., 2019
Lost City Hydrothermal Field	Hydrothermal fluid	Lang et al., 2013; Lang et al., 2021; Brazelton et al., 2022
Lost City Hydrothermal Field	Carbonate chimney, hydrothermal fluid	Brazelton et al., 2006; Schrenk et al., 2004
Tablelands Ophiolite Ultrabasic Serpentinite Springs, Gros Morne National Park, Newfoundland, Canada	Serpentinite springs fluid	Brazelton et al., 2013
Amundsen Gulf, Canadian Arctic Shelf	Surface seawater; thin, medium, and thick ice; sackhole brine; surface seawater; sea ice	Collins & Deming, 2011
Mariana Arc, near Guam	Hydrothermal fluid	Huber et al., 2010
Baby Bare Seamount, Juan de Fuca Ridge	Ridge flank crustal fluid	Huber et al., 2005

Location	Sample matrix	Reference
Axial Seamount, Juan de Fuca Ridge	Basalt hosted diffuse fluids	Huber et al., 2006
South Shetland Islands, Antarctic Peninsula	Winter water, circumpolar deep water, transitional weddell water, hot spots	Shen et al., 2017
Kolyma, Lena, Ob, Yenisei, and Mackenzie Arctic Rivers	River water	Kaiser et al., 2017
Chukchi Sea, Beaufort Sea, Amundsen Gulf, Archipelago, Baffin Bay, Davis Strait, Nordic Seas, Barents Sea, Svalbard, Kara Sea, Laptev Sea, E. Siberian Margin, E. Siberian Sea	Ocean water	Shen et al., 2018
Continental Rise East of the Gulf of Maine	Interstitial water (various depths), sediment hydrolysate (0–2 cm)	Henrichs & Farrington, 1979
Lake Vostok, Antarctica	Accretion ice (Type I, Type II), glacial Ice 2610 below surface	Christner et al., 2006
Under the Ross Ice Shelf, Ross Sea, Antarctica	Seawater (various depths)	Michel et al., 1979
Southern Ocean	Seawater (various depths)	Shen et al., 2017
Greenland Surface Ice	Surface glacial ice (various sites)	Stibal et al., 2015

Appendix B. Tables of Biosignature Measurement Approach Workflows and Analysis Workflow IDs

Cells 1 of 5

Flow Cytometry - Regolith	Super-Resolution Microscopy - Regolith
Add Water/Solvent or Melt	Add Water/Solvent or Melt
Separate Particles	Separate Particles
Adjust Salinity/Buffer pH (Optional)	Adjust Salinity/Buffer pH (Optional)
Size-Fractionate Sample	Size-Fractionate Sample
Select <80 micron Filtrate	Observe Each Particle Size Bin
Bubble Removal (Optional)	**Super-Resolution Microscopy (C5.4.2.1*)**
Sample Fluorophore Labeling (Optional)	
Laser Scatter Measurement (C4.4.1e*)	

Cells 2 of 5

Fluorescence Microscopy - Regolith	Atomic Force Microscopy (AFM) - Regolith	IR Microscopy - Regolith
Add Water/Solvent or Melt	Add Water/Solvent or Melt	Add Water/Solvent or Melt
Separate Particles	Separate Particles	Separate Particles
Adjust Salinity/Buffer pH (Optional)	Adjust Salinity/Buffer pH (Optional)	Adjust Salinity/Buffer pH (Optional)
Size-Fractionate Sample	Size-Fractionate Sample	Size-Fractionate Sample
Select 0.2 micron to 80 micron Filtrate	Select 0.2 micron to 80 micron Filtrate	Select 0.2 micron to 80 micron Filtrate
Bubble Removal (Optional)	**Atomic Force Microscopy (C7.4.2.3*)**	**IR Microscopy (C8.4.2.4*)**
Autofluorescence Analysis		
Macromolecule Analysis		
Fluorescence Microscopy (C6.4.2.2e*)		

Cells 3 of 5

Raman Microscopy - Regolith	Flow Cytometry - Ice	Super-Resolution Microscopy - Ice
Add Water/Solvent or Melt	Add Water/Solvent or Melt	Add Water/Solvent or Melt
Separate Particles	Adjust Salinity/Buffer pH (Optional)	Adjust Salinity/Buffer pH (Optional)
Adjust Salinity/Buffer pH (Optional)	Size-Fractionate Sample	Size-Fractionate Sample
Size-Fractionate Sample	Select <80 micron Filtrate	Observe Each Particle Size Bin
Select 0.2 micron to 80 micron Filtrate	Bubble Removal (Optional)	**Super-Resolution Microscopy (C11.4.2.1*)**
Raman Microscopy (C9.4.2.4*)	Sample Fluorophore Labeling (Optional)	
	Laser Scatter Measurement (C10.4.1e*)	

Cells 4 of 5

Fluorescence Microscopy - Ice	AFM - Ice	IR Microscopy - Ice
Add Water/Solvent or Melt	Add Water/Solvent or Melt	Add Water/Solvent or Melt
Adjust Salinity/Buffer pH (Optional)	Adjust Salinity/Buffer pH (Optional)	Adjust Salinity/Buffer pH (Optional)
Size-Fractionate Sample	Size-Fractionate Sample	Size-Fractionate Sample
Select 0.2 micron to 80 micron Filtrate	Select 0.2 micron to 80 micron Filtrate	Select 0.2 micron to 80 micron Filtrate
Bubble Removal (Optional)	**AFM (C13.4.2.3*)**	**IR Microscopy (C14.4.2.4*)**
Autofluorescence Analysis		
Macromolecule Analysis		
Fluorescence Microscopy (C12.4.2.2e*)		

Cells 5 of 5

Raman Microscopy - Ice		
Add Water/Solvent or Melt		
Adjust Salinity/Buffer pH (Optional)		
Size-Fractionate Sample		
Select 0.2 micron to 80 micron Filtrate		
Raman Microscopy (C15.4.2.4*)		

Carboxylic Acids 1 of 12

Unseparated LD-MS Analysis	Unseparated Raman	Unseparated UV-Vis Spectroscopy
LD-MS Analysis (F1.1*)	**Raman Analysis (F2.2*)**	**UV-Vis Spectroscopy (F3.3*)**

Carboxylic Acids 2 of 12

Unseparated IR Spectroscopy	Solid-Phase SPE EC Analysis	Solid-Phase SPE C4D Detection
IR Spectroscopy (F4.4*)	Phase Separation	Phase Separation
	Size-based Filtration	Size-based Filtration
	Solid-Phase Extraction	Solid-Phase Extraction (SPE)
	Concentration (Optional)	Concentration (Optional)
	CE or IC Separation	CE or IC Separation
	EC Analysis (F5.5.1.2.1.1*)	**C4D Detection (F6.5.1.2.1.2*)**

Carboxylic Acids 3 of 12

Solid-Phase SPE ESI-MS Analysis	Solid-Phase SPE EI-MS Analysis	Solid-Phase Evaporated EC Analysis
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Solid-Phase Extraction	Solid-Phase Extraction	Evaporate All Liquid, Leaving Residue
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CE or IC Separation	HPLC or LC Separation	CE or IC Separation
ESI-MS Analysis (F7.5.1.2.1.3*)	**EI-MS Analysis (F8.5.1.2.2a*)**	**EC Analysis (F9.5.1.3.1.1*)**

Carboxylic Acids 4 of 12

Solid-Phase Evaporated C4D Detection	Solid-Phase Evaporated ESI-MS Analysis	Solid-Phase Evaporated EI-MS Analysis
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Evaporate All Liquid, Leaving Residue	Evaporate All Liquid, Leaving Residue	Evaporate All Liquid, Leaving Residue
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CE or IC Separation	CE or IC Separation	HPLC or LC Separation
C4D Detection (F10.5.1.3.1.2*)	**ESI-MS Analysis (F11.5.1.3.1.3*)**	**EI-MS Analysis (F12.5.1.3.2a*)**

Carboxylic Acids 5 of 12

Solid-Phase Unextracted EI-MS Analysis	Solid-Phase Liquid–Liquid Extracted EC-MS Analysis	Solid-Phase Liquid–Liquid Extracted C4D Detection
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
CA Derivatization	Liquid–Liquid Extraction	Liquid–Liquid Extraction
GC Separation	Concentration (Optional)	Concentration (Optional)
EI-MS Analysis (F13.5.1.1b*)	CE or IC Separation	CE or IC Separation
	ESI-MS Analysis (F14.5.1.4.1.1*)	**C4D Detection (F15.5.1.4.1.2*)**

Carboxylic Acids 6 of 12

Solid-Phase Liquid–Liquid Extracted ESI-MS Analysis	Solid-Phase Liquid–Liquid Extracted EI-MS Analysis	Solid-Phase SPE LIF Detection
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Liquid–Liquid Extraction	Liquid–Liquid Extraction	SPE
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CE or IC Separation	HPLC or LC Separation	CA Derivatization
ESI-MS Analysis (F16.5.1.4.1.3*)	**EI-MS Analysis (F17.5.1.4.2a*)**	CE Separation
		LIF Detection (F18.5.1.2.3.1a*)

Carboxylic Acids 7 of 12

Solid-Phase SPE Fluorescence Detection	Solid-Phase SPE UV-Vis Detection	Solid-Phase Evaporated LIF Detection
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Solid-Phase Extraction	Solid-Phase Extraction	Evaporate All Liquid, Leaving Residue
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CA Derivatization	CA Derivatization	CA Derivatization
HPLC or LC Separation	HPLC or LC Separation	CE Separation
Fluorescence Detection (F19.5.1.2.3.2a*)	**UV-Vis Detection (F20.5.1.2.3.2a*)**	**LIF Detection (F21.5.1.3.3.1a*)**

Carboxylic Acids 8 of 12

Solid-Phase Evaporated Fluorescence Detection	Solid-Phase Evaporated UV-Vis Detection	Solid-Phase Liquid–Liquid Extraction LIF Detection
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Evaporate All Liquid, Leaving Residue	Evaporate All Liquid, Leaving Residue	Liquid–Liquid Extraction
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CA Derivatization	CA Derivatization	CA Derivatization
HPLC or LC Separation	HPLC or LC Separation	CE Separation
Fluorescence Detection (F22.5.1.3.3.2.1*)	**UV-Vis Detection (F23.5.1.3.3.2.2*)**	**LIF Detection (F24.5.1.4.3.1a*)**

Carboxylic Acids 9 of 12

Solid-Phase Liquid–Liquid Extraction Fluorescence Detection	Solid-Phase Liquid–Liquid Extraction UV-Vis Detection	Liquid-Phase EC Analysis
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Isolate Liquid Phase
Liquid–Liquid Extraction	Liquid–Liquid Extraction	Liquid–Liquid Extraction
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CA Derivatization	CA Derivatization	CE or IC Separation
HPLC or LC Separation	HPLC or LC Separation	**EC Analysis (F27.5.2.4.1.1*)**
Fluorescence Detection (F25.5.1.4.3.2.1*)	**UV-Vis Detection (F26.5.1.4.3.2.2*)**	

Carboxylic Acids 10 of 12

Liquid-Phase C4D Detection	Liquid-Phase ESI-MS Analysis	Liquid-Phase EI-MS Analysis
Phase Separation	Phase Separation	Phase Separation
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Liquid–Liquid Extraction	Liquid–Liquid Extraction	Liquid–Liquid Extraction
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CE or IC Separation	CE or IC Separation	HPLC or LC Separation
C4D Detection (F28.5.2.4.1.2*)	**ESI-MS Analysis (F29.5.2.4.1.3*)**	**EI-MS Analysis (F30.5.2.4.2a*)**

Carboxylic Acids 11 of 12

Liquid-Phase LIF Detection	Liquid-Phase Fluorescence Detection	Liquid-Phase UV-Vis Detection
Phase Separation	Phase Separation	Phase Separation
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Liquid–Liquid Extraction	Liquid–Liquid Extraction	Liquid–Liquid Extraction
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CA Derivatization	CA Derivatization	CA Derivatization
CE Separation	HPLC or LC Separation	HPLC or LC Separation
LIF Detection (F31.5.2.4.3.1a*)	**Fluorescence Detection (F32.5.2.4.3.2.1*)**	**UV-Vis Detection (F33.5.2.4.3.2.2*)**

Carboxylic Acids 12 of 12

Gas-Phase EI-MS Analysis	Gas-Phase Tunable Laser/Absorption Spectroscopy	
Phase Separation	Phase Separation	
Isolate Gas Phase	Isolate Gas Phase	
GC Separation	**Tunable Laser/Absorption Spectroscopy (F35.5.3.2*)**	
EI-MS Analysis (F34.5.3.1a*)		

Amino Acids 1 of 8

LD-MS Analysis	Raman	UV-Vis Spectroscopy
LD-MS Analysis (A1.1*)	**Raman (A2.2*)**	**UV-Vis Spectroscopy (A3.3*)**

Amino Acids 2 of 8

IR Spectroscopy	Solid Isolated LD-MS Analysis	Solid Isolated Raman
IR Spectroscopy (A4.4*)	Phase Separation	Phase Separation
	Size-Based Filtration	Size-Based Filtration
	LD-MS Analysis (A5.5.1.1*)	**Raman (A6.5.1.2*)**

Amino Acids 3 of 8

Solid Isolated UV-Vis Spectroscopy	Subcritical Water Extraction (ScWE) LD-MS Analysis	ScWE Raman
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
UV-Vis Spectroscopy (A7.5.1.3*)	ScWE	ScWE
	pH/Conductivity Measurement	pH/Conductivity Measurement
	Desalting (Optional)	Desalting (Optional)
	LD-MS Analysis (A8.5.1.4.1*)	**Raman (A9.5.1.4.2*)**

Amino Acids 4 of 8

Subcritical ScWE UV-Vis Spectroscopy	Solid-Phase HPLC-MS Analysis	Solid-Phase LD-MS Analysis
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
ScWE	ScWE	ScWE
pH/Conductivity Measurement	pH/Conductivity Measurement	pH/Conductivity Measurement
Desalting (Optional)	Desalting (Optional)	Desalting (Optional)
UV-Vis Spectroscopy (A10.5.1.4.3*)	Concentration (Optional)	Concentration (Optional)
	HPLC-MS Analysis (A11.5.1.4.4.1*)	**LD-MS Analysis (A12.5.1.4.4.2*)**

Amino Acids 5 of 8

Solid-Phase LIF Analysis	Solid-Phase ESI-MS Analysis	CE-C4D Analysis
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
ScWE	ScWE	ScWE
pH/Conductivity Measurement	pH/Conductivity Measurement	pH/Conductivity Measurement
Desalting (Optional)	Desalting (Optional)	Desalting (Optional)
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
AA Derivatization	AA Derivatization	AA Derivatization
CE Separation	CE Separation	CE Separation
LIF Analysis (A13.5.1.4.4.3.1*)	**ESI-MS Analysis (A14.5.1.4.4.3.2*)**	**C4D Analysis (A15.5.1.4.4.3.3*)**

Amino Acids 6 of 8

GC-MS Analysis	HPLC-LIF Analysis	Liquid-Phase HPLC-MS Analysis
Phase Separation	Phase Separation	Phase Separation
Add Water, Adjust pH	Size-Based Filtration	Liquid Isolation
Desalting (Optional)	ScWE	ScWE
Solid Isolation	pH/Conductivity Measurement	pH/Conductivity Measurement
Water Removal	Desalting (Optional)	Desalting (Optional)
AA Derivatization	Concentration (Optional)	Concentration (Optional)
GC Separation	AA Derivatization	**HPLC-MS Analysis (A18.5.3.1*)**
EI-MS Analysis (A16.5.2.1*)	HPLC Separation	
	LIF Analysis (A17.5.2.2*)	

Amino Acids 7 of 8

Liquid-Phase LD-MS Analysis	Liquid-Phase LIF Analysis	Liquid-Phase CE-MS Analysis
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Liquid Isolation	Liquid Isolation
ScWE	ScWE	ScWE
pH/Conductivity Measurement	pH/Conductivity Measurement	pH/Conductivity Measurement
Desalting (Optional)	Desalting (Optional)	Desalting (Optional)
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
LD-MS Analysis (A19.5.3.2*)	AA Derivatization	AA Derivatization
	CE Separation	CE Separation
	LIF Analysis (A20.5.3.3.1*)	**ESI-MS Analysis (A21.5.3.3.2*)**

Amino Acids 8 of 8

Liquid-Phase C4D Analysis	Solid Isolated IR	
Phase Separation	Phase Separation	
Liquid Isolation	Size-based Filtration	
ScWE	**UV-Vis Spectroscopy (A23.5.1.4*)**	
pH/Conductivity Measurement		
Desalting (Optional)		
Concentration (Optional)		
AA Derivatization		
CE Separation		
C4D Analysis (A22.5.3.3.3*)		

Proteins 1 of 14

LD-MS Analysis	MS^n Analyses	Solid-Phase LD-MS Analysis
LD-MS Analysis (P1.1*)	Laser Desorption	Warm Sample/Maintain Salinity
	Ion Selection and Fragmentation	Phase Separation
	MS^n Analyses (P2.1b*)	Size-Based Filtration
		LD-MS Analysis (P3.2.1.1*)

Proteins 2 of 14

Solid-Phase MS^n Analyses	Solid-Phase Size Exclusion Isolated LD-MS Analysis	Solid-Phase Size Exclusion Isolate MSn Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
Laser Desorption	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Ion Selection and Fragmentation	Cell Lysis Step	Cell Lysis Step
MS^n Analyses (P4.2.1.1b*)	Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase
	Size Exclusion Isolation	Size Exclusion Isolation
	LD-MS Analysis (P5.2.1.2.1.1*)	Laser Desorption
		Ion Selection and Fragmentation
		MS^n Analyses (P6.2.1.2.1.1b*)

Proteins 3 of 14

Solid-Phase Size Exclusion Isolated UV Fluorescence	Solid-Phase Size Exclusion Isolated Raman	Solid-Phase Size Exclusion Isolated Resuspended UV Fluorescence
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase
Size Exclusion Isolation	Size Exclusion Isolation	Size Exclusion Isolation
UV Fluorescence (P7.2.1.2.1.2*)	**Raman Confirmation of Peptide Bonds (P8.2.1.2.1.3*)**	Resuspend HMW from Device
		Split Sample
		UV Fluorescence (P9.2.1.2.1.4.1.1*)

Proteins 4 of 14

Solid-Phase Size Exclusion Isolated Resuspended CE Separated ESI-MS Analyses	Solid-Phase Size Exclusion Isolated Resuspended CE Separated MS^n Analyses	Solid-Phase Size Exclusion Isolated Resuspended Deposited LD-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase
Size Exclusion Isolation	Size Exclusion Isolation	Size Exclusion Isolation
Resuspend HMW from Device	Resuspend HMW from Device	Resuspend HMW from Device
Split Sample	Split Sample	Split Sample
Digestion to Peptides or Not	Digestion to Peptides or Not	Digestion to Peptides or Not
CE Separation	CE Separation	Deposit to Plate with Matrix
ESI-MS Analyses (P10.2.1.2.1.4.2.1a*)	Electrospray Ionization	**LD-MS Analysis (P12.2.1.2.1.4.2.2a*)**
	Ion Selection and Fragmentation	
	MS^n Analyses (P11.2.1.2.1.4.2.1c*)	

Proteins 5 of 14

Solid-Phase Size Exclusion Isolated Resuspended Deposited MS^n Analyses	Solid-Phase Size Exclusion Isolated Resuspended HPLC or LC Separated ESI-MS Analysis	Solid-Phase Size Exclusion Isolated Resuspended HPLC or LC Separated MSn Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase
Size Exclusion Isolation	Size Exclusion Isolation	Size Exclusion Isolation
Resuspend HMW from Device	Resuspend HMW from Device	Resuspend HMW from Device
Split Sample	Split Sample	Split Sample
Digestion to Peptides or Not	Digestion to Peptides or Not	Digestion to Peptides or Not
Deposit to Plate with Matrix	HPLC or LC Separation	HPLC or LC Separation
Laser Desorption	**ESI-MS Analysis (P14.2.1.2.1.4.2.3a*)**	Electrospray Ionization
Ion Selection and Fragmentation		Ion Selection and Fragmentation
MS^n Analyses (13.2.1.2.1.4.2.2c*)		**MS^n Analyses (P15.2.1.2.1.4.2.3c*)**

Proteins 6 of 14

Solid-Phase Size Exclusion Isolated Resuspended Colorimetric Assay	Solid-Phase Size Exclusion Isolated Resuspended Spectrophotometry	Solid-Phase pH Adjusted CE Separated ESI-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase
Size Exclusion Isolation	Size Exclusion Isolation	pH/Conductivity Measurement
Resuspend HMW from Device	Resuspend HMW from Device	Adjust pH
Split Sample	Split Sample	SPE/Functional Bead Separations
Colorimetric Assay (P16.2.1.2.1.4.1.3*)	Colorimetry	Bead Cleanup/Extractions
	Spectrophotometry (P17.2.1.2.1.4.3a*)	Digestion to Peptides or Not
		CE Separation
		ESI-MS Analysis (P18.2.1.2.2.1a*)

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Solid-Phase pH Adjusted CE Separated MS^n Analyses	Solid-Phase pH Adjusted Deposited LD-MS Analysis	Solid-Phase pH Adjusted Deposited MSn Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Size-Based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase
pH/Conductivity Measurement	pH/Conductivity Measurement	pH/Conductivity Measurement
Adjust pH	Adjust pH	Adjust pH
SPE/Functional Bead Separations	SPE/Functional Bead Separations	SPE/Functional Bead Separations
Bead Cleanup/Extractions	Bead Cleanup/Extractions	Bead Cleanup/Extractions
Digestion to Peptides or Not	Digestion to Peptides or Not	Digestion to Peptides or Not
CE Separation	Deposit to Plate with Matrix	Deposit to Plate with Matrix
Electrospray Ionization	Dry Sample	Dry Sample
Ion Selection and Fragmentation	**LD-MS Analysis (P20.2.1.2.2.2b*)**	Laser Desorption
MS^n Analyses (P19.2.1.2.2.1c*)		Ion Selection and Fragmentation
		MS^n Analyses (P21.2.1.2.2.2d*)

Proteins 8 of 14

Solid-Phase pH Adjusted HPLC/LC Separated ESI-MS Analysis	Solid-Phase pH Adjusted HPLC/LC Separated MS^n Analyses	Liquid-Phase Colorimetric Assay
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-Based Filtration	Size-Based Filtration	Liquid Isolation
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Cell Lysis Step
Cell Lysis Step	Cell Lysis Step	Size Exclusion Isolation
Isolate Liquid from Solid Phase	Isolate Liquid from Solid Phase	Resuspend Protein Fraction from Device
pH/Conductivity Measurement	pH/Conductivity Measurement	**Colorimetric Assay (P24.2.2.1.1*)**
Adjust pH	Adjust pH	
SPE/Functional Bead Separations	SPE/Functional Bead Separations	
Bead Cleanup/Extractions	Bead Cleanup/Extractions	
Digestion to Peptides or Not	Digestion to Peptides or Not	
HPLC or LC Separation	HPLC or LC Separation	
ESI-MS Analysis (P22.2.1.2.2.3a*)	Electrospray Ionization	
	Ion Selection and Fragmentation	
	MS^n Analyses (P23.2.1.2.2.3c*)	

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Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	Liquid-Phase Size Exclusion Isolated CE Separated MS^n Analyses	Liquid-Phase Size Exclusion Isolated Deposited LD-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Size Exclusion Isolation	Size Exclusion Isolation	Size Exclusion Isolation
Resuspend Protein Fraction from Device	Resuspend Protein Fraction from Device	Resuspend Protein Fraction from Device
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
CE Separation	CE Separation	Deposit to Plate with Matrix
ESI-MS Analysis (P25.2.2.1.2.1a*)	Electrospray Ionization	**LD-MS Analysis (P27.2.2.1.2.2a*)**
	Ion Selection and Fragmentation	
	MS^n Analyses (P26.2.2.1.2.1c*)	

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Liquid-Phase Size Exclusion Isolated Deposited MS^n Analyses	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated MS^n Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Size Exclusion Isolation	Size Exclusion Isolation	Size Exclusion Isolation
Resuspend Protein Fraction from Device	Resuspend Protein Fraction from Device	Resuspend Protein Fraction from Device
Concentration (Optional)	Concentration (Optional)	Concentration (Optional)
Deposit to Plate with Matrix	HPLC or LC Separation	HPLC or LC Separation
Laser Desorption	**ESI-MS Analysis (P29.2.2.1.2.3a*)**	Electrospray Ionization
Ion Selection and Fragmentation		Ion Selection and Fragmentation
MS^n Analyses (P28.2.2.1.2.2c*)		**MS^n Analyses (P30.2.2.1.2.3c*)**

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Liquid-Phase CE Separated ESI-MS Analysis	Liquid-Phase CE Separated MS^n Analyses	Liquid-Phase Deposited LD-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
CE Separation	CE Separation	Deposit to Plate with Matrix
ESI-MS Analysis (P31.2.2.2a*)	Electrospray Ionization	**LD-MS Analysis (P33.2.2.3a*)**
	Ion Selection and Fragmentation	
	MS^n Analyses (P32.2.2.2c*)	

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Liquid-Phase Deposited MS^n Analyses	Liquid-Phase HPLC/LC Separated ESI-MS Analysis	Liquid-Phase HPLC/LC Separated MS^n Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Deposit to Plate with Matrix	HPLC or LC Separation	HPLC or LC Separation
Laser Desorption	**ESI-MS Analysis (P35.2.2.4a*)**	Electrospray Ionization
Ion Selection and Fragmentation		Ion Selection and Fragmentation
MS^n Analyses (P34.2.2.3c*)		**MS^n Analyses (P36.2.2.4c*)**

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Liquid-Phase pH Adjusted CE Separated ESI-MS Analysis	Liquid-Phase pH Adjusted CE Separated MS^n Analyses	Liquid-Phase pH Adjusted Deposited LD-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
pH/Conductivity Measurement	pH/Conductivity Measurement	pH/Conductivity Measurement
Adjust pH	Adjust pH	Adjust pH
SPE/Functional Bead Separations	SPE/Functional Bead Separations	SPE/Functional Bead Separations
Bead Cleanup/Extractions	Bead Cleanup/Extractions	Bead Cleanup/Extractions
CE Separation	CE Separation	Deposit to Plate with Matrix
ESI-MS Analysis (P37.2.2.5.1a*)	Electrospray Ionization	**LD-MS Analysis (P39.2.2.5.2a*)**
	Ion Selection and Fragmentation	
	MS^n Analyses (P38.2.2.5.1c*)	

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Liquid-Phase pH Adjusted Deposited MS^n Analyses	Liquid-Phase pH Adjusted HPLC/LC Separated ESI-MS Analysis	Liquid-Phase pH Adjusted HPLC/LC Separated MS^n Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
pH/Conductivity Measurement	pH/Conductivity Measurement	pH/Conductivity Measurement
Adjust pH	Adjust pH	Adjust pH
SPE/Functional Bead Separations	SPE/Functional Bead Separations	SPE/Functional Bead Separations
Bead Cleanup/Extractions	Bead Cleanup/Extractions	Bead Cleanup/Extractions
Deposit to Plate with Matrix	HPLC or LC Separation	HPLC or LC Separation
Laser Desorption	**ESI-MS Analysis (P41.2.2.5.3a*)**	Electrospray Ionization
Ion Selection and Fragmentation		Ion Selection and Fragmentation
MS^n Analyses (P40.2.2.5.2c*)		**MS^n Analyses (P42.2.2.5.3c*)**

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Unprocessed Sample MS^n Analyses	Pyrolysis GC	Solid-Phase MS^n Analyses
Laser Desorption	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Ion Selection and Fragmentation	Phase Separation	Phase Separation
MS^n Analyses (H1.1b*)	Size-based Filtration	Size-based Filtration
	Pyrolysis GC (H2.2.1.1*)	Laser Desorption
		Ion Selection and Fragmentation
		MS^n Analyses (H3.2.1.2b*)

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Non-Degrading Organic Extraction Pyrolysis GC	UV Fluorescence	Raman
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Pyrolysis GC (H4.2.1.3.1*)	Cell Lysis Step	Cell Lysis Step
	Isolate Liquid Phase	Isolate Liquid Phase
	Size Exclusion Isolation	Size Exclusion Isolation
	UV Fluorescence (H5.2.1.3.2.1.1*)	**Raman (H6.2.1.3.2.1.2*)**

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Solid-Phase Size Exclusion Isolated MS^n Analyses	Solid-Phase Total Hydrolyzable AA (THAA) Analysis	Solid-Phase Chiral GC Column or a Chiral-Capillary Electrophoresis (CE)
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Size Exclusion Isolation	Size Exclusion Isolation	Size Exclusion Isolation
Laser Desorption	Resuspend HMW from Device	Resuspend HMW from Device
Ion Selection and Fragmentation	Split Sample	Split Sample
MS^n Analyses (H7.2.1.3.2.1.3b*)	Hydrolysis to Break Up Organics	Hydrolysis to Break Up Organics
	Derivatized AA	**Chiral GC Column or a Chiral-CE (H9.2.1.3.2.1.4.1.1.2*)**
	Total Hydrolyzable AA (THAA) Analysis (H8.2.1.3.2.1.4.1.1.1a*)	

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Solid-Phase Dissolved Free (DFAA) AA Analysis	Solid-Phase Spectrophotometer	Solid-Phase Size Exclusion Isolated CE Separated MS^n Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Size Exclusion Isolation	Size Exclusion Isolation	Size Exclusion Isolation
Resuspend HMW from Device	Resuspend HMW from Device	Resuspend HMW from Device
Split Sample	Colorimetric Assay	Concentration (Optional)
Derivatized AA	**Spectrophotometer (H11.2.1.3.2.1.4.2a*)**	CE Separation
Dissolved Free (DFAA) AA Analysis (H10.2.1.3.2.1.4.1.2a*)		Electrospray Ionization
		Ion Selection and Fragmentation
		MS^n Analyses (H12.2.1.3.2.1.4.3.1c*)

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Solid-Phase Size Exclusion Isolated CE Separated Concentrated Laser Desorption MS^n Analyses	Solid-Phase Size Exclusion Isolated HPLC/LC Separated MS^n Analyses	Solid-Phase Bead Separated CE Separated MS^n Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Size Exclusion Isolation	Size Exclusion Isolation	pH/Conductivity Measurement
Resuspend HMW from Device	Resuspend HMW from Device	Adjust pH
Concentration (Optional)	Concentration (Optional)	SPE/Functional Bead Separations
Deposit to Plate with Matrix	HPLC or LC Separation	Bead Cleanup/Extractions
Laser Desorption	Electrospray Ionization	CE Separation
Ion Selection and Fragmentation	Ion Selection and Fragmentation	Electrospray Ionization
MS^n Analyses (H13.2.1.3.2.1.4.3.2c*)	**MS^n Analyses (H14.2.1.3.2.1.4.3.3c***	Ion Selection and Fragmentation
		MS^n Analyses (H15.2.1.3.2.2.1c*)

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Solid-Phase Bead Separated Laser Desorption MS^n Analyses	Solid-Phase Bead Separated HPLC or LC Separated MS^n Analyses	GC-MS Lipids and Other HMWC Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
pH/Conductivity Measurement	pH/Conductivity Measurement	pH/Conductivity Measurement
Adjust pH	Adjust pH	Adjust pH
SPE/Functional Bead Separations	SPE/Functional Bead Separations	SPE/Functional Bead Separations
Bead Cleanup/Extractions	Bead Cleanup/Extractions	Bead Cleanup/Extractions
Deposit to Plate with Matrix	HPLC or LC Separation	Chloroform MeOH Soluble Fraction
Laser Desorption	Electrospray Ionization	**GC-MS Lipids and Other HMWC Analysis (H18.2.1.3.2.2.4.1*)**
Ion Selection and Fragmentation	Ion Selection and Fragmentation	
MS^n Analyses (H16.2.1.3.2.2.2c*)	**MS^n Analyses (H17.2.1.3.2.2.3c*)**	

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Solid-Phase HILIC HPLC MS^n Analyses	Solid-Phase Reverse-Phase HPLC MS^n Analyses	
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	
Phase Separation	Phase Separation	
Size-based Filtration	Size-based Filtration	
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	
Cell Lysis Step	Cell Lysis Step	
Isolate Liquid Phase	Isolate Liquid Phase	
pH/Conductivity Measurement	pH/Conductivity Measurement	
Adjust pH	Adjust pH	
SPE/Functional Bead Separations	SPE/Functional Bead Separations	
Bead Cleanup/Extractions	Bead Cleanup/Extractions	
Chloroform MeOH Soluble Fraction	Chloroform MeOH Soluble Fraction	
HILIC HPLC to separate by headgroups	Reverse-Phase HPLC to Separate by Alkyl Chain Mods	
Electrospray Ionization	Electrospray Ionization	
Ion Selection and Fragmentation	Ion Selection and Fragmentation	
MS^n Analyses (H19.2.1.3.2.2.4.1c*)	**MS^n Analyses (H20.2.1.3.2.2.4.2c*)**	

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Liquid-Phase Hydrolyzed Chiral GC Column or Chiral-CE	Liquid-Phase Non-Hydrolyzed Chiral GC Column or Chiral-CE	Liquid-Phase Dissolved Free (DFAA) AA Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Split Sample	Split Sample	Split Sample
Hydrolysis to Break Up Organics	**Chiral GC Column or Chiral-CE (H23.2.2.1.2*)**	Derivatized AA
Chiral GC Column or Chiral-CE (H22.2.2.1.1.2*)		**Dissolved Free (DFAA) AA Analysis (H24.2.2.1.3a*)**

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Liquid-Phase LD-MS Analysis	Liquid-Phase Cell Lysed Hydrolysis-Based AA Analysis	Liquid-Phase Cell Lysed Dissolved Free AA Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Deposit to Plate with Matrix	Cell Lysis	Cell Lysis
LD-MS Analysis (H25.2.2.2a*)	Isolate Liquid Phase	Isolate Liquid Phase
	Size Exclusion Isolation	Size Exclusion Isolation
	Split Sample	Split Sample
	Hydrolysis-Based AA Analysis (H26.2.2.3.1.1.1*)	**Dissolved Free AA Analysis (H27.2.2.3.1.1.2*)**

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Liquid-Phase Spectrophotometer	Liquid-Phase Size Exclusion Isolated CE Separated MS^n Analyses	Liquid-Phase Size Exclusion Isolated Laser Desorption MS^n Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis	Cell Lysis	Cell Lysis
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Size Exclusion Isolation	Size Exclusion Isolation	Size Exclusion Isolation
Resuspend HMW from Device	Resuspend HMW from Device	Resuspend HMW from Device
Colorimetric Assay	Concentration (Optional)	Concentration (Optional)
Spectrophotometer Analysis (H28.2.2.3.1.2.1a*)	CE Separation	Deposit to Plate with Matrix
	Electrospray Ionization	Laser Desorption
	Ion Selection and Fragmentation	Ion Selection and Fragmentation
	MS^n Analyses (H29.2.2.3.1.2.2.1c*)	**MS^n Analyses (H30.2.2.3.1.2.2.2c*)**

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Liquid-Phase Size Exclusion Isolated HPLC/LC Separated MS^n Analyses	Liquid-Phase Bead Extracted CE Separated MS^n Analyses	Liquid-Phase Bead Extracted Laser Desorption MS^n Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis	Cell Lysis	Cell Lysis
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Size Exclusion Isolation	pH/Conductivity Measurement	pH/Conductivity Measurement
Resuspend HMW from Device	Adjust pH	Adjust pH
Concentration (Optional)	SPE/Functional Bead Separations	SPE/Functional Bead Separations
HPLC or LC Separation	Bead Cleanup/Extractions	Bead Cleanup/Extractions
Electrospray Ionization	CE Separation	Laser Desorption
Ion Selection and Fragmentation	Electrospray Ionization	Ion Selection and Fragmentation
MS^n Analyses (H31.2.2.3.1.2.2.3c*)	Ion Selection and Fragmentation	**MS^n Analyses (H33.2.2.3.2.2c*)**
	MS^n Analyses (H32.2.2.3.2.1c*)	

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Liquid-Phase Bead Extracted MS^n Analysis	Liquid-Phase HILIC HPLC MS^n Analyses	Liquid-Phase Reverse-Phase HPLC MS^n Analyses
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis	Cell Lysis	Cell Lysis
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
pH/Conductivity Measurement	Separate Aqueous and Organic Fraction	Separate Aqueous and Organic Fraction
Adjust pH	Isolate Organic Fraction	Isolate Organic Fraction
SPE/Functional Bead Separations	HILIC HPLC to Separate by Headgroups	Reverse-Phase HPLC to Separate by Alkyl Chain Mods
Bead Cleanup/Extractions	Electrospray Analysis	Electrospray Analysis
HPLC or LC Separation	Ion Selection and Fragmentation	Ion Selection and Fragmentation
Electrospray Ionization	**MS^n Analyses (H35.2.2.3.3.1c*)**	**MS^n Analyses (H36.2.2.3.3.1c*)**
Ion Selection and Fragmentation		
MS^n Analyses (H34.2.2.3.2.3c*)		

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Gas-Phase MS^n Analyses	Gas-Phase Tunable Laser/Absorption Spectroscopy	LD-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	**LD-MS Analysis (H39.1*)**
Phase Separation	Phase Separation	
Gas Isolation	Gas Isolation	
GC Separation	**Tunable Laser/Absorption Spectroscopy (H38.2.3.2*)**	
Electron Ionization		
Ion Selection and Fragmentation		
MS^n Analyses (H37.2.3.1c*)		

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Solid-Phase LD-MS Analysis	Solid-Phase Size Exclusion Isolated LD-MS Analysis	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
LD-MS Analysis (H40.2.1.2*)	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
	Cell Lysis Step	Cell Lysis Step
	Isolate Liquid Phase	Isolate Liquid Phase
	Size Exclusion Isolation	Size Exclusion Isolation
	LD-MS Analysis (H41.2.1.3.2.1.3)	Resuspend HMW from Device
		Concentration (Optional)
		CE Separation
		ESI-MS Analysis (H42.2.1.3.2.1.4.3.1a*)

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Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	Solid-Phase Bead Separated CE Separation ESI-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Size Exclusion Isolation	Size Exclusion Isolation	pH/Conductivity Measurement
Resuspend HMW from Device	Resuspend HMW from Device	Adjust pH
Concentration (Optional)	Concentration (Optional)	SPE/Functional Bead Separations
Deposit to Plate with Matrix	HPLC or LC Separation	Bead Cleanup/Extractions
LD-MS Analysis (H43.2.1.3.2.1.4.3.2a*)	**ESI-MS Analysis (H44.2.1.3.2.1.4.3.3a*)**	CE Separation
		ESI-MS Analysis (H45.2.1.3.2.2.1a*)

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Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	Solid-Phase Reverse-Phase HPLC ESI-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Size-based Filtration	Size-based Filtration	Size-based Filtration
Non-Degrading Organic Extraction	Non-Degrading Organic Extraction	Non-Degrading Organic Extraction
Cell Lysis Step	Cell Lysis Step	Cell Lysis Step
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
pH/Conductivity Measurement	pH/Conductivity Measurement	pH/Conductivity Measurement
Adjust pH	Adjust pH	Adjust pH
SPE/Functional Bead Separations	SPE/Functional Bead Separations	SPE/Functional Bead Separations
Bead Cleanup/Extractions	Bead Cleanup/Extractions	Bead Cleanup/Extractions
Deposit to Plate with Matrix	HPLC or LC Separation	Chloroform MeOH Soluble Fraction
LD-MS Analysis (H46.2.1.3.2.2.2a*)	**ESI-MS Analysis (H47.2.1.3.2.2.3a*)**	Reverse-Phase HPLC to Separate by Alkyl Chain Mods
		ESI-MS Analysis (H48.2.1.3.2.2.4.2a*)

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Solid-Phase HILIC HPLC ESI-MS Analysis	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation
Size-based Filtration	Liquid Isolation
Non-Degrading Organic Extraction	Cell Lysis
Cell Lysis Step	Isolate Liquid Phase
Isolate Liquid Phase	Size Exclusion Isolation
pH/Conductivity Measurement	Resuspend HMW from Device
Adjust pH	Concentration (Optional)
SPE/Functional Bead Separations	CE Separation
Bead Cleanup/Extractions	**ESI-MS Analysis (H51.2.2.3.1.2.2.1a*)**
Chloroform MeOH Soluble Fraction	
HILIC HPLC to separate by headgroups	
ESI-MS Analysis (H49.2.1.3.2.2.4.3a*)	

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Liquid-Phase Size Exclusion Isolated LD-MS Analysis	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation	Liquid Isolation
Cell Lysis	Cell Lysis	Cell Lysis
Isolate Liquid Phase	Isolate Liquid Phase	Isolate Liquid Phase
Size Exclusion Isolation	Size Exclusion Isolation	pH/Conductivity Measurement
Resuspend HMW from Device	Resuspend HMW from Device	Adjust pH
Concentration (Optional)	Concentration (Optional)	SPE/Functional Bead Separations
Deposit to Plate with Matrix	HPLC or LC Separation	Bead Cleanup/Extractions
LD-MS Analysis (H52.2.2.3.1.2.2.2a*)	**ESI-MS Analysis (H53.2.2.3.1.2.2.3a*)**	CE Separation
		ESI-MS Analysis (H54.2.2.3.2.1a*)

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Liquid-Phase Bead Extracted LD-MS Analysis	Liquid-Phase Reverse-Phase HPLC ESI-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation
Liquid Isolation	Liquid Isolation
Cell Lysis	Cell Lysis
Isolate Liquid Phase	Isolate Liquid Phase
pH/Conductivity Measurement	Separate Aqueous and Organic Fraction
Adjust pH	Isolate Organic Fraction
SPE/Functional Bead Separations	Reverse-Phase HPLC to Separate by Alkyl Chain Mods
Bead Cleanup/Extractions	**ESI-MS Analysis (H57.2.2.3.3.1a*)**
Deposit to Plate with Matrix	
LD-MS Analysis (H56.2.2.3.2.2a*)	

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Liquid-Phase HILIC HPLC ESI-MS Analysis	Gas-Phase EI-MS Analysis
Warm Sample/Maintain Salinity	Warm Sample/Maintain Salinity
Phase Separation	Phase Separation
Liquid Isolation	Gas Isolation
Cell Lysis	GC Separation
Isolate Liquid Phase	**EI-MS Analysis (H60.2.3.1a*)**
Separate Aqueous and Organic Fraction	
Isolate Organic Fraction	
HILIC HPLC to Separate by Headgroups	
ESI-MS Analysis (H58.2.2.3.3.2a*)	

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Fluorometry	UV Spectroscopy
Add Solvent or Melt	Add Solvent or Melt
Separate Bio from Non-Bio Particles (Icey Regolith Only)	Separate Bio from Non-Bio Particles (Icey Regolith Only)
Phase Separation – Liquid	Phase Separation – Liquid
Disrupt/Lyse	Disrupt/Lyse
Purity & Concentrate	Purity & Concentrate
Fluorometry Analysis (N1.1.1a*)	**UV Spectroscopy Analysis (N2.1.2*)**

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cDNA Electrochemical (EC) Analysis	cDNA and DNA EC Analysis	DNA EC Analysis
Add Solvent or Melt	Add Solvent or Melt	Add Solvent or Melt
Separate Bio from Non-Bio Particles (Icey Regolith Only)	Separate Bio from Non-Bio Particles (Icey Regolith Only)	Separate Bio from Non-Bio Particles (Icey Regolith Only)
Phase Separation – Liquid	Phase Separation – Liquid	Phase Separation – Liquid
Disrupt/Lyse	Disrupt/Lyse	Disrupt/Lyse
Purity & Concentrate	Purity & Concentrate	Purity & Concentrate
Tagging/Add Adapters	Tagging/Add Adapters	Tagging/Add Adapters
RNA to cDNA Conversion (if RNA)	RNA to cDNA Conversion (if RNA)	RNA to cDNA Conversion (if RNA)
Prime and Load	Combine cDNA and DNA then Prime and Load	Prime and Load
EC Analysis cDNA (N3.1.3.1.1*)	**EC Analysis cDNA and DNA (N4.1.3.1.2*)**	**EC Analysis DNA (N5.1.3.1.3*)**

Appendix C. Tables of Measurement Approach Workflow Parameter Values

The tables below provide the information on the measurement approach workflows and their parameters for each biosignature of interest (indicated in top row of each chart). Listed are the function name, type of function performed, the measurement approach workflow name, the workflow ID, and workflow parameter values estimated for the calculations in the models. Details on each label or parameter include:

- Function name: the processing or measurement step within a particular Measurement Approach Workflow for which the parameters to the right pertain to
- Type: the type of step in the workflow = "Processing" or "Measurement"
- Workflow ID: label that ties the function to the step in the Measurement Approach Workflow
- Biosignature processing Efficiency (dimensionless): estimated amount of biosignature retained during workflow steps
- Volume Concentration Factor (dimensionless; only included in certain analyses where applicable): estimate of the volume change (increase or decrease) during a workflow step
- Measurement specific:
  - Detection Limit (mol/mL or ppw = g/g): value at which the biosignature could be detected for that Measurement technique
  - Minimum Quantity (g or mL): indicates the minimum amount of the biosignature or working volume required to perform a Measurement step
- Flight Suitability (low, medium, high): Suitability of the processing step technology for flight
- Units
- Justifications/References added as applicable/possible. Otherwise, values are estimates based on research experience by study team members

Cells

Function Name	Type	Process	Workflow ID	Efficiency (Dimension-less)	Detection Limit (Cells)	Minimum Quantity (cells)	Flight Suitability	Units	Justifications/References
Add Water/Solvent or Melt	Processing	Flow Cytometry - Regolith	C4.4	0.8	N/A	N/A	Medium	N/A	
Select <80 micron Filtrate	Processing	Flow Cytometry - Regolith	C4.4.1	1	N/A	N/A	Medium	N/A	
Bubble Removal (Optional)	Processing	Flow Cytometry - Regolith	C4.4.1b	1	N/A	N/A	Medium	N/A	
Sample Fluorophore Labeling (Optional)	Processing	Flow Cytometry - Regolith	C4.4.1d	1	N/A	N/A	Medium	N/A	
Laser Scatter Measurement	Measurement	Flow Cytometry - Regolith	C4.4.1e*	1	100	0.1	Medium	Cells	Santibañez et al., 2016
Separate Particles	Processing	Flow Cytometry - Regolith	C4.4b	0.8	N/A	N/A	Medium	N/A	
Adjust Salinity/Buffer pH	Processing	Flow Cytometry - Regolith	C4.4c	0.9	N/A	N/A	Medium	N/A	
Size-Fractionate Sample	Processing	Flow Cytometry - Regolith	C4.4d	0.75	N/A	N/A	Medium	N/A	

Cells

Function Name	Type	Process	Workflow ID	Efficiency (Dimension-less)	Detection Limit (Cells)	Minimum Quantity (cells)	Flight Suitability	Units	Justifications/References
Add Water/Solvent or Melt	Processing	Super-Resolution Microscopy - Regolith	C5.4	0.8	N/A	N/A	Medium	N/A	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
Observe Each Particle Size Bin	Processing	Super-Resolution Microscopy - Regolith	C5.4.2	1	N/A	N/A	Medium	N/A	
Super-Resolution Microscopy	Measurement	Super-Resolution Microscopy - Regolith	C5.4.2.1*	1	15	0.1	Medium	Cells	
Separate Particles	Processing	Super-Resolution Microscopy - Regolith	C5.4b	0.8	N/A	N/A	Medium	N/A	
Adjust Salinity/Buffer pH	Processing	Super-Resolution Microscopy - Regolith	C5.4c	0.9	N/A	N/A	Medium	N/A	
Size-Fractionate Sample	Processing	Super-Resolution Microscopy - Regolith	C5.4d	0.5	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	Fluorescence Microscopy - Regolith	C6.4	0.9	N/A	N/A	Medium	N/A	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Select 0.2 micron to 80 micron Filtrate	Processing	Fluorescence Microscopy - Regolith	C6.4.2	1	N/A	N/A	Medium	N/A	
Bubble Removal (Optional)	Processing	Fluorescence Microscopy - Regolith	C6.4.2.2a	1	N/A	N/A	Medium	N/A	
Autofluorescence Analysis	Processing	Fluorescence Microscopy - Regolith	C6.4.2.2c	1	N/A	N/A	Medium	N/A	
Macromolecule Analysis	Processing	Fluorescence Microscopy - Regolith	C6.4.2.2d	1	N/A	N/A	Medium	N/A	
Fluorescence Microscopy	Measurement	Fluorescence Microscopy - Regolith	C6.4.2.2e*	1	15	0.1	Medium	Cells	
Separate Particles	Processing	Fluorescence Microscopy - Regolith	C6.4b	0.8	N/A	N/A	Medium	N/A	
Adjust Salinity/Buffer pH	Processing	Fluorescence Microscopy - Regolith	C6.4c	0.9	N/A	N/A	Medium	N/A	
Size-Fractionate Sample	Processing	Fluorescence Microscopy - Regolith	C6.4d	0.5	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	Atomic Force Microscopy (AFM) - Regolith	C7.4	0.9	N/A	N/A	Medium	N/A	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Select 0.2 micron to 80 micron Filtrate	Processing	AFM - Regolith	C7.4.2	1	N/A	N/A	Medium	N/A	
Atomic Force Microscopy	Measurement	AFM - Regolith	C7.4.2.3*	1	15	0.1	Medium	Cells	
Separate Particles	Processing	AFM - Regolith	C7.4b	0.8	N/A	N/A	Medium	N/A	
Adjust Salinity/Buffer pH	Processing	AFM - Regolith	C7.4c	0.9	N/A	N/A	Medium	N/A	
Size-Fractionate Sample	Processing	AFM - Regolith	C7.4d	0.5	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	IR Microscopy - Regolith	C8.4	0.9	N/A	N/A	Medium	N/A	
Select 0.2 micron to 80 micron Filtrate	Processing	IR Microscopy - Regolith	C8.4.2	1	N/A	N/A	Medium	N/A	
IR Microscopy	Measurement	IR Microscopy - Regolith	C8.4.2.4*	1	15	0.1	Medium	Cells	
Separate Particles	Processing	IR Microscopy - Regolith	C8.4b	0.8	N/A	N/A	Medium	N/A	

Cells

Function Name	Type	Process	Workflow ID	Efficiency (Dimension-less)	Detection Limit (Cells)	Minimum Quantity (cells)	Flight Suitability	Units	Justifications/References
Adjust Salinity/Buffer pH	Processing	IR Microscopy - Regolith	C8.4c	0.9	N/A	N/A	Medium	N/A	
Size-Fractionate Sample	Processing	IR Microscopy - Regolith	C8.4d	0.5	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	Raman Microscopy - Regolith	C9.4	0.9	N/A	N/A	Low	N/A	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Select 0.2 micron to 80 micron Filtrate	Processing	Raman Microscopy - Regolith	C9.4.2	1	N/A	N/A	Medium	N/A	
Raman Microscopy	Measurement	Raman Microscopy - Regolith	C9.4.2.4*	1	15	0.1	Medium	Cells	
Separate Particles	Processing	Raman Microscopy - Regolith	C9.4b	0.8	N/A	N/A	Medium	N/A	
Adjust Salinity/Buffer pH	Processing	Raman Microscopy - Regolith	C9.4c	0.9	N/A	N/A	Medium	N/A	
Size-Fractionate Sample	Processing	Raman Microscopy - Regolith	C9.4d	0.5	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	Flow Cytometry - Ice	C10.4	0.9	N/A	N/A	Medium	N/A	
Select <80 micron Filtrate	Processing	Flow Cytometry - Ice	C10.4.1	1	N/A	N/A	Medium	N/A	
Bubble Removal (Optional)	Processing	Flow Cytometry - Ice	C10.4.1b	1	N/A	N/A	Medium	N/A	
Sample Fluorophore Labeling (Optional)	Processing	Flow Cytometry - Ice	C10.4.1d	1	N/A	N/A	Medium	N/A	
Laser Scatter Measurement	Measurement	Flow Cytometry - Ice	C10.4.1e*	1	100	0.1	Medium	Cells	
Adjust Salinity/Buffer pH	Processing	Flow Cytometry - Ice	C10.4c	1	N/A	N/A	Medium	N/A	
Size-Fractionate Sample	Processing	Flow Cytometry - Ice	C10.4d	0.9	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	Super-Resolution Microscopy - Ice	C11.4	0.9	N/A	N/A	Medium	N/A	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
Observe Each Particle Size Bin	Processing	Super-Resolution Microscopy - Ice	C11.4.2	1	N/A	N/A	Medium	N/A	
Super-Resolution Microscopy	Measurement	Super-Resolution Microscopy - Ice	C11.4.2.1*	1	15	0.1	Medium	Cells	
Adjust Salinity/Buffer pH	Processing	Super-Resolution Microscopy - Ice	C11.4c	1	N/A	N/A	Medium	N/A	
Size-Fractionate Sample (ICE)	Processing	Super-Resolution Microscopy - Ice	C11.4d	0.9	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	Fluorescence Microscopy - Ice	C12.4	0.9	N/A	N/A	Medium	N/A	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Select 0.2 micron to 80 micron Filtrate	Processing	Fluorescence Microscopy - Ice	C12.4.2	1	N/A	N/A	Medium	N/A	
Bubble Removal (Optional)	Processing	Fluorescence Microscopy - Ice	C12.4.2.2a	1	N/A	N/A	Medium	N/A	
Autofluorescence Analysis	Processing	Fluorescence Microscopy - Ice	C12.4.2.2c	1	N/A	N/A	Medium	N/A	
Macromolecule Analysis	Processing	Fluorescence Microscopy - Ice	C12.4.2.2d	1	N/A	N/A	Medium	N/A	

Cells

Function Name	Type	Process	Workflow ID	Efficiency (Dimension-less)	Detection Limit (Cells)	Minimum Quantity (cells)	Flight Suitability	Units	Justifications/References
Fluorescence Microscopy	Measurement	Fluorescence Microscopy - Ice	C12.4.2.2e*	1	15	0.1	Medium	Cells	
Adjust Salinity/Buffer pH	Processing	Fluorescence Microscopy - Ice	C12.4c	1	N/A	N/A	Medium	N/A	
Size-Fractionate Sample	Processing	Fluorescence Microscopy - Ice	C12.4d	0.9	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	AFM - Ice	C13.4	0.9	N/A	N/A	Medium	N/A	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Select 0.2 micron to 80 micron Filtrate	Processing	AFM - Ice	C13.4.2	1	N/A	N/A	Medium	N/A	
Atomic Force Microscopy	Measurement	AFM - Ice	C13.4.2.3*	1	15	0.1	Medium	Cells	
Adjust Salinity/Buffer pH	Processing	AFM - Ice	C13.4c	1	N/A	N/A	Medium	N/A	
Size-Fractionate Sample (ICE)	Processing	AFM - Ice	C13.4d	0.9	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	IR Microscopy - Ice	C14.4	0.9	N/A	N/A	Medium	N/A	
Select 0.2 micron to 80 micron Filtrate	Processing	IR Microscopy - Ice	C14.4.2	1	N/A	N/A	Medium	N/A	
IR Microscopy	Measurement	IR Microscopy - Ice	C14.4.2.4*	1	15	0.1	Medium	Cells	
Adjust Salinity/Buffer pH	Processing	IR Microscopy - Ice	C14.4c	1	N/A	N/A	Medium	N/A	
Size-Fractionate Sample (ICE)	Processing	IR Microscopy - Ice	C14.4d	0.9	N/A	N/A	Medium	N/A	
Add Water/Solvent or Melt	Processing	Raman Microscopy - Ice	C15.4	0.9	N/A	N/A	Low	N/A	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Select 0.2 micron to 80 micron Filtrate	Processing	Raman Microscopy - Ice	C15.4.2	1	N/A	N/A	Medium	N/A	
Raman Microscopy	Measurement	Raman Microscopy - Ice	C15.4.2.4*	1	15	0.1	Medium	Cells	
Adjust Salinity/Buffer pH	Processing	Raman Microscopy - Ice	C15.4c	1	N/A	N/A	Medium	N/A	
Size-Fractionate Sample (ICE)	Processing	Raman Microscopy - Ice	C15.4d	0.9	N/A	N/A	Medium	N/A	

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suitability	Units
C4D Detection	Measurement	Size-based Filtration | SPE | Concentrate | CE/IC | C4D Detection	F6.5.1.2.1.2*	0.9	1	1.00E-09	0.1	High	ppw
C4D Detection	Measurement	Size-based Filtration | Concentrate | CE/IC | C4D Detection	F10.5.1.3.1.2*	0.9	1	1.00E-09	0.1	High	ppw

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
C4D Detection	Measurement	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F15.5.1.4.1.2*	0.9	1	1.00E-09	0.1	High	ppw
C4D Detection	Measurement	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F28.5.2.4.1.2*	0.9	1	1.00E-09	0.1	High	ppw
CA Derivatization	Processing	Size-based Filtration | Derivatize | GC | EI-MS Analysis	F13.5.1.1	0.9	1	N/A	N/A	High	mol/mL
CA Derivatization	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | CE | LIF Detection	F18.5.1.2.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F19.5.1.2.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F20.5.1.2.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Size-based Filtration | Concentrate | Derivatize | CE | LIF Detection	F21.5.1.3.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F22.5.1.3.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F23.5.1.3.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F24.5.1.4.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F25.5.1.4.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F26.5.1.4.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F31.5.2.4.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F32.5.2.4.3	0.9	1	N/A	N/A	High	N/A
CA Derivatization	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F33.5.2.4.3	0.9	1	N/A	N/A	High	N/A
CE or IC Separation	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | EC Analysis	F5.5.1.2.1	1	1	N/A	N/A	High	N/A
CE or IC Separation	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | C4D Detection	F6.5.1.2.1	1	1	N/A	N/A	High	N/A
CE or IC Separation	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | ESI-MS Analysis	F7.5.1.2.1	1	1	N/A	N/A	High	mol/mL
CE or IC Separation	Processing	Size-based Filtration | Concentrate | CE/IC | EC Analysis	F9.5.1.3.1	1	1	N/A	N/A	High	N/A
CE or IC Separation	Processing	Size-based Filtration | Concentrate | CE/IC | C4D Detection	F10.5.1.3.1	1	1	N/A	N/A	High	N/A

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
CE or IC Separation	Processing	Size-based Filtration | Concentrate | CE/IC | ESI-MS Analysis	F11.5.1.3.1	1	1	N/A	N/A	High	mol/mL
CE or IC Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F14.5.1.4.1	1	1	N/A	N/A	High	N/A
CE or IC Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F15.5.1.4.1	1	1	N/A	N/A	High	N/A
CE or IC Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F16.5.1.4.1	1	1	N/A	N/A	High	mol/mL
CE or IC Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | EC Analysis	F27.5.2.4.1	1	1	N/A	N/A	High	N/A
CE or IC Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F28.5.2.4.1	1	1	N/A	N/A	High	N/A
CE or IC Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F29.5.2.4.1	1	1	N/A	N/A	High	mol/mL
CE Separation	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | CE | LIF Detection	F18.5.1.2.3.1	1	1	N/A	N/A	High	N/A
CE Separation	Processing	Size-based Filtration | Concentrate | Derivatize | CE | LIF Detection	F21.5.1.3.3.1	1	1	N/A	N/A	High	N/A
CE Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F24.5.1.4.3.1	1	1	N/A	N/A	High	N/A
CE Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F31.5.2.4.3.1	1	1	N/A	N/A	High	N/A
Concentration (Optional)	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | EC Analysis	F5.5.1.2a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | C4D Detection	F6.5.1.2a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | ESI-MS Analysis	F7.5.1.2a	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Size-based Filtration | SPE | Concentrate | HPLC/LC | EI-MS Analysis	F8.5.1.2a	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Size-based Filtration | Concentrate | CE/IC | EC Analysis	F9.5.1.3a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Concentrate | CE/IC | C4D Detection	F10.5.1.3a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Concentrate | CE/IC | ESI-MS Analysis	F11.5.1.3a	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Size-based Filtration | Concentrate | HPLC/LC | EI-MS Analysis	F12.5.1.3a	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F14.5.1.4a	0.5	100	N/A	N/A	Medium	N/A

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Concentration (Optional)	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F15.5.1.4a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F16.5.1.4a	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F17.5.1.4a	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | CE | LIF Detection	F18.5.1.2a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F19.5.1.2a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F20.5.1.2a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Concentrate | Derivatize | CE | LIF Detection	F21.5.1.3a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F22.5.1.3a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F23.5.1.3a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F24.5.1.4a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F25.5.1.4a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F26.5.1.4a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | EC Analysis	F27.5.2.4a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F28.5.2.4a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F29.5.2.4a	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F30.5.2.4a	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F31.5.2.4a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F32.5.2.4a	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F33.5.2.4a	0.5	100	N/A	N/A	Medium	N/A
EC Analysis	Measurement	Size-based Filtration | SPE | Concentrate | CE/IC | EC Analysis	F5.5.1.2.1.1*	1	1	1E-12	10	Medium	mol/mL

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
EC Analysis	Measurement	Size-based Filtration | Concentrate | CE/IC | EC Analysis	F9.5.1.3.1.1*	1	1	1E-12	10	Medium	mol/mL
EC Analysis	Measurement	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | EC Analysis	F27.5.2.4.1.1*	1	1	1E-12	10	Medium	mol/mL
EI-MS Analysis	Measurement	Size-based Filtration | SPE | Concentrate | HPLC/LC | EI-MS Analysis	F8.5.1.2.2a*	0.8	1	1.00E-10	1	High	mol/mL
EI-MS Analysis	Measurement	Size-based Filtration | Concentrate | HPLC/LC | EI-MS Analysis	F12.5.1.3.2a*	0.8	1	1.00E-10	1	High	mol/mL
EI-MS Analysis	Measurement	Size-based Filtration | Derivatize | GC | EI-MS Analysis	F13.5.1.1b*	0.8	1	1.00E-10	1	High	mol/mL
EI-MS Analysis	Measurement	Size-based Filtration | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F17.5.1.4.2a*	0.8	1	1.00E-10	1	High	mol/mL
EI-MS Analysis	Measurement	Liquid Iso. | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F30.5.2.4.2a*	0.8	1	1.00E-10	1	High	mol/mL
EI-MS Analysis	Measurement	Gas Iso. | GC | EI-MS Analysis	F34.5.3.1a*	0.8	1	1.00E-10	1	High	mol/mL
ESI-MS Analysis	Measurement	Size-based Filtration | SPE | Concentrate | CE/IC | ESI-MS Analysis	F7.5.1.2.1.3*	0.8	1	1.00E-10	1	High	mol/mL
ESI-MS Analysis	Measurement	Size-based Filtration | Concentrate | CE/IC | ESI-MS Analysis	F11.5.1.3.1.3*	0.8	1	1.00E-10	1	High	mol/mL
ESI-MS Analysis	Measurement	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F14.5.1.4.1.1*	0.8	1	1.00E-10	1	High	mol/mL
ESI-MS Analysis	Measurement	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F16.5.1.4.1.3*	0.8	1	1.00E-10	1	High	mol/mL
ESI-MS Analysis	Measurement	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F29.5.2.4.1.3*	0.8	1	1.00E-10	1	High	mol/mL
Evaporate All Liquid, Leaving Residue	Processing	Size-based Filtration | Concentrate | CE/IC | EC Analysis	F9.5.1.3	1	100	N/A	N/A	High	N/A
Evaporate All Liquid, Leaving Residue	Processing	Size-based Filtration | Concentrate | CE/IC | C4D Detection	F10.5.1.3	1	100	N/A	N/A	High	N/A
Evaporate All Liquid, Leaving Residue	Processing	Size-based Filtration | Concentrate | CE/IC | ESI-MS Analysis	F11.5.1.3	1	100	N/A	N/A	High	mol/mL
Evaporate All Liquid, Leaving Residue	Processing	Size-based Filtration | Concentrate | HPLC/LC | EI-MS Analysis	F12.5.1.3	1	100	N/A	N/A	High	mol/mL
Evaporate All Liquid, Leaving Residue	Processing	Size-based Filtration | Concentrate | Derivatize | CE | LIF Detection	F21.5.1.3	1	100	N/A	N/A	High	N/A
Evaporate All Liquid, Leaving Residue	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F22.5.1.3	1	100	N/A	N/A	High	N/A
Evaporate All Liquid, Leaving Residue	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F23.5.1.3	1	100	N/A	N/A	High	N/A
Fluorescence Detection	Measurement	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F19.5.1.2.3.2a*	1	1	1.00E-11	10	High	mol/mL

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Fluorescence Detection	Measurement	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F22.5.1.3.3.2.1*	1	1	1.00E-11	10	High	mol/mL
Fluorescence Detection	Measurement	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F25.5.1.4.3.2.1*	1	1	1.00E-11	10	High	mol/mL
Fluorescence Detection	Measurement	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F32.5.2.4.3.2.1*	1	1	1.00E-11	10	High	mol/mL
GC Separation	Processing	Size-based Filtration | Derivatize | GC | EI-MS Analysis	F13.5.1.1a	1	1	N/A	N/A	High	mol/mL
GC Separation	Processing	Gas Iso. | GC | EI-MS Analysis	F34.5.3.1	1	1	N/A	N/A	High	mol/mL
HPLC or LC Separation	Processing	Size-based Filtration | SPE | Concentrate | HPLC/LC | EI-MS Analysis	F8.5.1.2.2	1	1	N/A	N/A	Medium	mol/mL
HPLC or LC Separation	Processing	Size-based Filtration | Concentrate | HPLC/LC | EI-MS Analysis	F12.5.1.3.2	1	1	N/A	N/A	Medium	mol/mL
HPLC or LC Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F17.5.1.4.2	1	1	N/A	N/A	Medium	mol/mL
HPLC or LC Separation	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F19.5.1.2.3.2	1	1	N/A	N/A	Medium	N/A
HPLC or LC Separation	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F20.5.1.2.3.2	1	1	N/A	N/A	Medium	N/A
HPLC or LC Separation	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F22.5.1.3.3.2	1	1	N/A	N/A	Medium	N/A
HPLC or LC Separation	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F23.5.1.3.3.2	1	1	N/A	N/A	Medium	N/A
HPLC or LC Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F25.5.1.4.3.2	1	1	N/A	N/A	Medium	N/A
HPLC or LC Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F26.5.1.4.3.2	1	1	N/A	N/A	Medium	N/A
HPLC or LC Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F30.5.2.4.2	1	1	N/A	N/A	Medium	mol/mL
HPLC or LC Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F32.5.2.4.3.2	1	1	N/A	N/A	Medium	N/A
HPLC or LC Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F33.5.2.4.3.2	1	1	N/A	N/A	Medium	N/A
Isolate Gas Phase	Processing	Gas Iso. | GC | EI-MS Analysis	F34.5.3	1	1	N/A	N/A	High	mol/mL
Isolate Gas Phase	Processing	Gas Iso. | Tunable Laser Spec.	F35.5.3	1	1	N/A	N/A	High	N/A
Isolate Liquid Phase	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | EC Analysis	F27.5.2	1	1	N/A	N/A	High	N/A
Isolate Liquid Phase	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F28.5.2	1	1	N/A	N/A	High	N/A
Isolate Liquid Phase	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F29.5.2	1	1	N/A	N/A	High	mol/mL

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Isolate Liquid Phase	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F30.5.2	1	1	N/A	N/A	High	mol/mL
Isolate Liquid Phase	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F31.5.2	1	1	N/A	N/A	High	N/A
Isolate Liquid Phase	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F32.5.2	1	1	N/A	N/A	High	N/A
Isolate Liquid Phase	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F33.5.2	1	1	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | EC Analysis	F5.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | C4D Detection	F6.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | ESI-MS Analysis	F7.5.1	1	100	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | SPE | Concentrate | HPLC/LC | EI-MS Analysis	F8.5.1	1	100	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | Concentrate | CE/IC | EC Analysis	F9.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Concentrate | CE/IC | C4D Detection	F10.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Concentrate | CE/IC | ESI-MS Analysis	F11.5.1	1	100	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | Concentrate | HPLC/LC | EI-MS Analysis	F12.5.1	1	100	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | Derivatize | GC | EI-MS Analysis	F13.5.1	1	100	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F14.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F15.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F16.5.1	1	100	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F17.5.1	1	100	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | CE | LIF Detection	F18.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F19.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F20.5.1	1	100	N/A	N/A	High	N/A

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Size-based Filtration	Processing	Size-based Filtration | Concentrate | Derivatize | CE | LIF Detection	F21.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F22.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F23.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F24.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F25.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F26.5.1	1	100	N/A	N/A	High	N/A
LD-MS Analysis	Measurement	LD-MS Analysis	F1.1*	0.2	1	1.00E-09	1	Medium	mol/mL
LIF Detection	Measurement	Size-based Filtration | SPE | Concentrate | Derivatize | CE | LIF Detection	F18.5.1.2.3.1a*	1	1	1.00E-11	1	High	mol/mL
LIF Detection	Measurement	Size-based Filtration | Concentrate | Derivatize | CE | LIF Detection	F21.5.1.3.3.1a*	1	1	1.00E-11	1	High	mol/mL
LIF Detection	Measurement	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F24.5.1.4.3.1a*	1	1	1.00E-11	1	High	mol/mL
LIF Detection	Measurement	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F31.5.2.4.3.1a*	1	1	1.00E-11	1	High	mol/mL
Liquid–Liquid Extraction	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F14.5.1.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F15.5.1.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F16.5.1.4	1	0.5	N/A	N/A	Medium	mol/mL
Liquid–Liquid Extraction	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F17.5.1.4	1	0.5	N/A	N/A	Medium	mol/mL
Liquid–Liquid Extraction	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F24.5.1.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F25.5.1.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F26.5.1.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | EC Analysis	F27.5.2.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F28.5.2.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F29.5.2.4	1	0.5	N/A	N/A	Medium	mol/mL

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Liquid–Liquid Extraction	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F30.5.2.4	1	0.5	N/A	N/A	Medium	mol/mL
Liquid–Liquid Extraction	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F31.5.2.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F32.5.2.4	1	0.5	N/A	N/A	Medium	N/A
Liquid–Liquid Extraction	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F33.5.2.4	1	0.5	N/A	N/A	Medium	N/A
Phase Separation	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | EC Analysis	F5.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | C4D Detection	F6.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | ESI-MS Analysis	F7.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | SPE | Concentrate | HPLC/LC | EI-MS Analysis	F8.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | Concentrate | CE/IC | EC Analysis	F9.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Concentrate | CE/IC | C4D Detection	F10.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Concentrate | CE/IC | ESI-MS Analysis	F11.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | Concentrate | HPLC/LC | EI-MS Analysis	F12.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | Derivatize | GC | EI-MS Analysis	F13.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F14.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F15.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F16.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F17.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | CE | LIF Detection	F18.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F19.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F20.5	1	1	N/A	N/A	High	N/A

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Phase Separation	Processing	Size-based Filtration | Concentrate | Derivatize | CE | LIF Detection	F21.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F22.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F23.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F24.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F25.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F26.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | EC Analysis	F27.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | C4D Detection	F28.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | CE/IC | ESI-MS Analysis	F29.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | HPLC/LC | EI-MS Analysis	F30.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | CE | LIF Detection	F31.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F32.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F33.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Gas Iso. | GC | EI-MS Analysis	F34.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Gas Iso. | Tunable Laser Spec.	F35.5	1	1	N/A	N/A	High	N/A
Raman Analysis	Measurement	Unseparated Raman Analysis	F2.2*	0.9	1	1.00E-08	10	High	ppw
Solid-Phase Extraction	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | EC Analysis	F5.5.1.2	1	100	N/A	N/A	Medium	N/A
Solid-Phase Extraction	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | C4D Detection	F6.5.1.2	1	100	N/A	N/A	Medium	N/A
Solid-Phase Extraction	Processing	Size-based Filtration | SPE | Concentrate | CE/IC | ESI-MS Analysis	F7.5.1.2	1	100	N/A	N/A	Medium	mol/mL
Solid-Phase Extraction	Processing	Size-based Filtration | SPE | Concentrate | HPLC/LC | EI-MS Analysis	F8.5.1.2	1	100	N/A	N/A	Medium	mol/mL
Solid-Phase Extraction	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | CE | LIF Detection	F18.5.1.2	1	100	N/A	N/A	Medium	N/A
Solid-Phase Extraction	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | Fluorescence Detection	F19.5.1.2	1	100	N/A	N/A	Medium	N/A

Carboxylic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Solid-Phase Extraction	Processing	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F20.5.1.2	1	100	N/A	N/A	Medium	N/A
Tunable Laser/Absorption Spectroscopy	Measurement	Gas Iso. | Tunable Laser Spec.	F35.5.3.2*	1	1	1E-12	10	Medium	mol/mL
Unseparated IR Spectroscopy	Measurement	IR Spectroscopy	F4.4*	1	1	1.00E-06	10	Medium	mol/mL
UV-Vis Detection	Measurement	Size-based Filtration | SPE | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F20.5.1.2.3.2a*	1	1	1.00E-06	1	High	mol/mL
UV-Vis Detection	Measurement	Size-based Filtration | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F23.5.1.3.3.2.2*	1	1	1.00E-06	1	High	mol/mL
UV-Vis Detection	Measurement	Size-based Filtration | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F26.5.1.4.3.2.2*	1	1	1.00E-06	1	High	mol/mL
UV-Vis Detection	Measurement	Liquid Iso. | Liq-Liq Ext. | Concentrate | Derivatize | HPLC/LC | UV-Vis Detection	F33.5.2.4.3.2.2*	1	1	1.00E-06	1	High	mol/mL
UV-Vis Spectroscopy	Measurement	UV-Vis Spectroscopy	F3.3*	1	1	1.00E-06	1	High	mol/mL

Amino Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
AA Derivatization	Processing	Solid Iso. | Derivatize | GC | EI-MS Analysis	A16.5.2d	0.9	1	N/A	N/A	High	mol/mL
Concentration (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5.2d	0.5	100	N/A	N/A	High	N/A
AA Derivatization	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5.3.3	0.9	1	N/A	N/A	High	N/A
AA Derivatization	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5.3.3	0.9	1	N/A	N/A	High	mol/mL
AA Derivatization	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5.3.3	0.9	1	N/A	N/A	High	N/A
AA Derivatization	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5.1.4.4.3	0.9	1	N/A	N/A	High	N/A
AA Derivatization	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5.1.4.4.3	0.9	1	N/A	N/A	High	mol/mL
AA Derivatization	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5.1.4.4.3	0.9	1	N/A	N/A	High	N/A
Add Water, Adjust pH	Processing	Solid Iso. | Derivatize | GC | EI-MS Analysis	A16.5.2	0.5	0.5	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5.2	1	100	N/A	N/A	High	N/A
C4D Analysis	Measurement	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5.3.3.3*	0.9	1	0.000000001	0.1	High	mol/mL
C4D Analysis	Measurement	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5.1.4.4.3.3*	0.9	1	0.000000001	0.1	High	mol/mL
CE Separation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5.3.3a	1	1	N/A	N/A	High	N/A
CE Separation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5.3.3a	1	1	N/A	N/A	High	mol/mL
CE Separation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5.3.3a	1	1	N/A	N/A	High	N/A
CE Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5.1.4.4.3a	1	1	N/A	N/A	High	N/A
CE Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5.1.4.4.3a	1	1	N/A	N/A	High	mol/mL
CE Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5.1.4.4.3a	1	1	N/A	N/A	High	N/A
Concentration (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5.3d	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5.3d	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A18.5.3d	0.5	100	N/A	N/A	Medium	N/A

Amino Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Concentration (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A19.5.3d	0.5	100	N/A	N/A	Medium	ppw
Concentration (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5.3d	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5.1.4.4	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5.1.4.4	0.5	100	N/A	N/A	Medium	mol/mL
Concentration (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A11.5.1.4.4	0.5	100	N/A	N/A	Medium	N/A
Concentration (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A12.5.1.4.4	0.5	100	N/A	N/A	Medium	ppw
Concentration (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5.1.4.4	0.5	100	N/A	N/A	Medium	N/A
Desalting (Optional)	Processing	Solid Iso. | Derivatize | GC | EI-MS Analysis	A16.5.2a	0.5	100	N/A	N/A	Medium	mol/mL
Subcritical Water Extraction (ScWE)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5.2a	0.8	0.3	N/A	N/A	Medium	N/A
Desalting (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5.3c	0.5	100	N/A	N/A	Medium	N/A
Desalting (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5.3c	0.5	100	N/A	N/A	Medium	mol/mL
Desalting (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A18.5.3c	0.5	100	N/A	N/A	Medium	N/A
Desalting (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A19.5.3c	0.5	100	N/A	N/A	Medium	ppw
Desalting (Optional)	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5.3c	0.5	100	N/A	N/A	Medium	N/A
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5.1.4b	0.5	100	N/A	N/A	Medium	N/A
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5.1.4b	0.5	100	N/A	N/A	Medium	mol/mL
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A11.5.1.4b	0.5	100	N/A	N/A	Medium	N/A
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A12.5.1.4b	0.5	100	N/A	N/A	Medium	ppw
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5.1.4b	0.5	100	N/A	N/A	Medium	N/A
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | LD-MS Analysis	A8.5.1.4b	0.5	100	N/A	N/A	Medium	ppw
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Raman	A9.5.1.4b	0.5	100	N/A	N/A	Medium	N/A

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | UV-Vis Spectroscopy	A10.5.1.4b	0.5	100	N/A	N/A	Medium	N/A
EI-MS Analysis	Measurement	Solid Iso. | Derivatize | GC | EI-MS Analysis	A16.5.2.1*	0.8	1	1E-10	1	High	mol/mL
ESI-MS Analysis	Measurement	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5.3.3.2*	0.8	1	1E-10	1	High	mol/mL
ESI-MS Analysis	Measurement	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5.1.4.4.3.2*	0.8	1	11E-10	1	High	mol/mL
GC Separation	Processing	Solid Iso. | Derivatize | GC | EI-MS Analysis	A16.5.2e	1	1	N/A	N/A	High	mol/mL
HPLC Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5.2f	1	1	N/A	N/A	Medium	mol/mL
AA Derivatization	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5.2e	1	1	N/A	N/A	High	N/A
HPLC-LIF Analysis	Measurement	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5.2.2*	1	1	0.000000001	0.1	Low	mol/mL
HPLC-MS Analysis	Measurement	Liquid Iso. | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A18.5.3.1*	0.9	1	1E-10	1	Low	mol/mL
HPLC-MS Analysis	Measurement	Size-based Filtration | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A11.5.1.4.4.1*	0.9	1	0.000000001	0.1	Low	mol/mL
IR Spectroscopy	Measurement	IR Spectroscopy	A4.4*	0.9	1	0.000001	1	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | LD-MS Analysis	A5.5.1	1	1	N/A	N/A	High	ppw
Size-based Filtration	Processing	Size-based Filtration | Raman	A6.5.1	1	1	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | UV-Vis Spectroscopy	A7.5.1	1	1	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | IR Spectroscopy	A23.5.1	1	1	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5.1	1	1	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5.1	1	100	N/A	N/A	High	mol/mL
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A11.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A12.5.1	1	100	N/A	N/A	High	ppw
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | LD-MS Analysis	A8.5.1	1	100	N/A	N/A	High	ppw
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | Raman	A9.5.1	1	100	N/A	N/A	High	N/A
Size-based Filtration	Processing	Size-based Filtration | Sub.Crit Water Extract | UV-Vis Spectroscopy	A10.5.1	1	100	N/A	N/A	High	N/A
LD-MS Analysis	Measurement	LD-MS Analysis	A1.1*	0.2	1	0.000000001	1	High	mol/mL
LD-MS Analysis	Measurement	Liquid Iso. | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A19.5.3.2*	0.2	1	0.000000001	1	Medium	mol/mL

Amino Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
LD-MS Analysis	Measurement	Size-based Filtration | LD-MS Analysis	A5.5.1.1*	0.2	1	0.000000001	1	Medium	mol/mL
LD-MS Analysis	Measurement	Size-based Filtration | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A12.5.1.4.4.2*	0.2	1	0.000000001	1	High	mol/mL
LD-MS Analysis	Measurement	Size-based Filtration | Sub.Crit Water Extract | LD-MS Analysis	A8.5.1.4.1*	0.2	1	0.000000001	1	Medium	mol/mL
LIF Analysis	Measurement	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5.3.3.1*	1	1	1E-12	0.1	High	mol/mL
LIF Analysis	Measurement	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5.1.4.4.3.1*	1	1	1E-12	0.1	High	mol/mL
Liquid Isolation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5.3	0.95	1	N/A	N/A	High	N/A
Liquid Isolation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5.3	0.95	1	N/A	N/A	High	mol/mL
Liquid Isolation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A18.5.3	0.95	1	N/A	N/A	High	N/A
Liquid Isolation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A19.5.3	0.95	1	N/A	N/A	High	ppw
Liquid Isolation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5.3	0.95	1	N/A	N/A	High	N/A
pH/Conductivity Measurement	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5.3b	1	1	N/A	N/A	High	N/A
pH/Conductivity Measurement	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5.3b	1	1	N/A	N/A	High	mol/mL
pH/Conductivity Measurement	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A18.5.3b	1	1	N/A	N/A	High	N/A
pH/Conductivity Measurement	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A19.5.3b	1	1	N/A	N/A	High	ppw
pH/Conductivity Measurement	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5.3b	1	1	N/A	N/A	High	N/A
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5.1.4a	1	1	N/A	N/A	High	N/A
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5.1.4a	1	1	N/A	N/A	High	mol/mL
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A11.5.1.4a	1	1	N/A	N/A	High	N/A
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A12.5.1.4a	1	1	N/A	N/A	High	ppw
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5.1.4a	1	1	N/A	N/A	High	N/A
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | LD-MS Analysis	A8.5.1.4a	1	1	N/A	N/A	High	ppw

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | Raman	A9.5.1.4a	1	1	N/A	N/A	High	N/A
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | UV-Vis Spectroscopy	A10.5.1.4a	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Solid Iso. | Derivatize | GC | EI-MS Analysis	A16.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A18.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A19.5	1	1	N/A	N/A	High	ppw
Phase Separation	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | LD-MS Analysis	A5.5	1	1	N/A	N/A	High	ppw
Phase Separation	Processing	Size-based Filtration | Raman	A6.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | UV-Vis Spectroscopy	A7.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | IR Spectroscopy	A23.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5	1	1	N/A	N/A	High	mol/mL
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A11.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A12.5	1	1	N/A	N/A	High	ppw
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | LD-MS Analysis	A8.5	1	1	N/A	N/A	High	ppw
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | Raman	A9.5	1	1	N/A	N/A	High	N/A
Phase Separation	Processing	Size-based Filtration | Sub.Crit Water Extract | UV-Vis Spectroscopy	A10.5	1	1	N/A	N/A	High	N/A
Raman	Measurement	Raman	A2.2*	0.9	1	0.00000001	1	High	mol/mL
Raman	Measurement	Size-based Filtration | Raman	A6.5.1.2*	0.9	1	0.00000001	1	High	mol/mL
Raman	Measurement	Size-based Filtration | Sub.Crit Water Extract | Raman	A9.5.1.4.2*	0.9	1	0.00000001	10	High	mol/mL
Solid Isolation	Processing	Solid Iso. | Derivatize | GC | EI-MS Analysis	A16.5.2b	0.95	1	N/A	N/A	Medium	mol/mL

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentration Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units
pH/Conductivity Measurement	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5.2b	1	1	N/A	N/A	Medium	N/A
ScWE	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | C4D Analysis	A22.5.3a	0.8	0.3	N/A	N/A	High	N/A
ScWE	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A21.5.3a	0.8	0.3	N/A	N/A	High	mol/mL
ScWE	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A18.5.3a	0.8	0.3	N/A	N/A	High	N/A
ScWE	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A19.5.3a	0.8	0.3	N/A	N/A	High	ppw
ScWE	Processing	Liquid Iso. | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A20.5.3a	0.8	0.3	N/A	N/A	High	N/A
ScWE	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | CE | C4D Analysis	A15.5.1.4	0.8	0.3	N/A	N/A	High	N/A
ScWE	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | ESI-MS Analysis	A14.5.1.4	0.8	0.3	N/A	N/A	High	mol/mL
ScWE	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | HPLC-MS Analysis	A11.5.1.4	0.8	0.3	N/A	N/A	High	N/A
ScWE	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | LD-MS Analysis	A12.5.1.4	0.8	0.3	N/A	N/A	High	ppw
ScWE	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | CE | LIF Analysis	A13.5.1.4	0.8	0.3	N/A	N/A	High	N/A
ScWE	Processing	Size-based Filtration | Sub.Crit Water Extract | LD-MS Analysis	A8.5.1.4	0.8	0.3	N/A	N/A	High	ppw
ScWE	Processing	Size-based Filtration | Sub.Crit Water Extract | Raman	A9.5.1.4	0.8	0.3	N/A	N/A	High	N/A
ScWE	Processing	Size-based Filtration | Sub.Crit Water Extract | UV-Vis Spectroscopy	A10.5.1.4	0.8	0.3	N/A	N/A	High	N/A
IR Spectroscopy	Measurement	Size-based Filtration | IR Spectroscopy	A23.5.1.4*	0.9	1	0.000001	1	High	mol/mL
UV-Vis Spectroscopy	Measurement	Size-based Filtration | UV-Vis Spectroscopy	A7.5.1.3*	1	1	0.000001	1	High	mol/mL
UV-Vis Spectroscopy	Measurement	Size-based Filtration | Sub.Crit Water Extract | UV-Vis Spectroscopy	A10.5.1.4.3*	1	1	0.000001	0.1	High	mol/mL
UV-Vis Spectroscopy	Measurement	UV-Vis Spectroscopy	A3.3*	1	1	0.000001	10	High	mol/mL
Water Removal	Processing	Solid Iso. | Derivatize | GC | EI-MS Analysis	A16.5.2c	0.5	100	N/A	N/A	High	mol/mL
Desalting (Optional)	Processing	Size-based Filtration | Sub.Crit Water Extract | Concentrate | Derivatize | HPLC-LIF Analysis	A17.5.2c	0.5	100	N/A	N/A	High	N/A

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
LD-MS Analysis	Measurement	LD-MS Analysis	P1.1*	0.5	1	1.00E-09	1	Medium	mol/mL	
Laser Desorption	Processing	LD | Ion Selection | MSn Analyses	P2.1	0.5	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	LD | Ion Selection | MSn Analyses	P2.1a	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	LD | Ion Selection | MSn Analyses	P2.1b*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | LD-MS Analysis	P3.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | LD-MS Analysis	P3.2a	1	1	N/A	1	Medium	ppw	
Size-based Filtration	Processing	Size-based Filtration | LD-MS Analysis	P3.2.1	0.95	1000	N/A	1	Medium	ppw	
LD-MS Analysis	Measurement	Size-based Filtration | LD-MS Analysis	P3.2.1.1*	0.5	1	1.00E-09	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	P4.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	P4.2a	1	1	N/A	1	Medium	N/A	
Size-based Filtration	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	P4.2.1	0.95	1000	N/A	1	Medium	ppw	
Laser Desorption	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	P4.2.1.1	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	P4.2.1.1a	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | LD | Ion Selection | MSn Analyses	P4.2.1.1b*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P5.2	1	1	N/A	0.000001	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P5.2a	1	1	N/A	1	Medium	ppw	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P5.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P5.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P5.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P5.2.1.2b	0.05	1	N/A	N/A	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P5.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
LD-MS Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P5.2.1.2.1.1*	0.5	1	1.00E-09	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2a	1	1	N/A	1	Medium	N/A	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2.1.2b	0.05	1	N/A	N/A	Medium	N/A	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Laser Desorption	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2.1.2.1.1	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2.1.2.1.1a	1	1	**N/A**	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P6.2.1.2.1.1b*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extraction | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P7.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extraction | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P7.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extraction | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P7.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extraction | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P7.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extraction | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P7.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extraction | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P7.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extraction | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P7.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
UV Fluorescence	Measurement	Size-based Filtration | Org. Extraction | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P7.2.1.2.1.2*	1	1	1.00E-05	1	High	mol/mL	Zhang et al., 2021; Hobira Scientific, 2021
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	P8.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	P8.2a	1	1	N/A	1	Medium	N/A	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	P8.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	P8.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	P8.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	P8.2.1.2b	0.05	1	N/A	N/A	Medium	N/A	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	P8.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Raman Confirmation of Peptide Bonds	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	P8.2.1.2.1.3*	1	1	1.00E-08	10	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2.1.2	0.8	0.5	N/A	1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2.1.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
UV Fluorescence	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	P9.2.1.2.1.4.1.1*	1	1	1.00E-05	1	High	mol/mL	Zhang et al, 2021; Hobira Scientific, 2021
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2.1.4.2	1	1	N/A	N/A	Medium	mol/mL	
CE Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2.1.4.2.1	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
ESI-MS Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | ESI-MS Analyses	P10.2.1.2.1.4.2.1a*	1	1	1.00E-10	10	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2.1.4.2	1	1	N/A	N/A	Medium	mol/mL	
CE Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2.1.4.2.1	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2.1.4.2.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2.1.4.2.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | CE | Ion Selection | MSn Analyses	P11.2.1.2.1.4.2.1c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2a	1	1	N/A	1	Medium	ppw	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1	0.95	100	N/A	1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2b	0.05	1	N/A	N/A	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2.1.4.2	1	1	N/A	N/A	Medium	ppw	
Deposit to Plate with Matrix	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2.1.4.2.2	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
LD-MS Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD-MS Analysis	P12.2.1.2.1.4.2.2a*	0.5	1	1.00E-09	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2a	1	1	N/A	1	Medium	ppw	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1	0.95	100	N/A	1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2b	0.05	1	N/A	N/A	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2.1.4.2	1	1	N/A	N/A	Medium	ppw	
Deposit to Plate with Matrix	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2.1.4.2.2	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
Laser Desorption	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2.1.4.2.2a	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2.1.4.2.2b	1	1	N/A	N/A	Medium	Gas	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	P13.2.1.2.1.4.2.2c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2.1.4.2	1	1	N/A	N/A	Medium	mol/mL	
HPLC or LC Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2.1.4.2.3	0.9	**1**	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | ESI-MS Analysis	P14.2.1.2.1.4.2.3a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2a	1	1	N/A	1	Medium	N/A	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2b	0.05	1	N/A	N/A	Medium	N/A	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2.1.4.2	1	1	N/A	N/A	Medium	N/A	
HPLC or LC Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2.1.4.2.3	0.9	**1**	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2.1.4.2.3a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2.1.4.2.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | HPLC/LC | Ion Selection | MSn Analyses	P15.2.1.2.1.4.2.3c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2a	1	1	N/A	1	Medium	N/A	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2.1.2	0.8	0.5	N/A	1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2.1.2b	0.05	1	N/A	N/A	Medium	N/A	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2.1.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Colorimetric Assay	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P16.2.1.2.1.4.1.3*	1	1	1.00E-06	10	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1.2.1.4	0.6	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1.2.1.4.1	1	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Colorimetry	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1.2.1.4.1.3	1	1	1.00E-06	N/A	Medium	mol/mL	
Spectrophotometry	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Color. | Spectrophotometry	P17.2.1.2.1.4.3a*	1	1	1.00E-12	10	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2.2	1	1	N/A	**1**	Medium	mol/mL	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2.2a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2.2b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2.2c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2.2d	1	1	N/A	N/A	Medium	mol/mL	
CE Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2.2.1	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
ESI-MS Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | ESI-MS Analysis	P18.2.1.2.2.1a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2	1	1	N/A	**1**	Medium	mol/mL	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2d	1	1	N/A	N/A	Medium	mol/mL	
CE Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2.1	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P19.2.1.2.2.1c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2a	1	1	N/A	1	Medium	ppw	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2	0.8	0.5	N/A	1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2b	0.05	1	N/A	N/A	Medium	ppw	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2.2	1	1	N/A	**1**	Medium	ppw	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2.2a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2.2b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2.2c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2.2d	1	1	N/A	N/A	Medium	ppw	
Deposit to Plate with Matrix	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2.2.2	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
Dry Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2.2.2a	1	100	N/A	N/A	Medium	ppw	
LD-MS Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD-MS Analysis	P20.2.1.2.2.2b*	0.5	1	1.00E-09	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2a	1	1	N/A	1	Medium	ppw	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1	0.95	100	N/A	1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2b	0.05	1	N/A	N/A	Medium	ppw	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2	1	1	N/A	**1**	Medium	ppw	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2d	1	1	N/A	N/A	Medium	ppw	
Deposit to Plate with Matrix	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2.2	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
Dry Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2.2a	1	100	N/A	N/A	Medium	ppw	
Laser Desorption	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2.2b	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2.2c	1	1	N/A	N/A	Medium	Gas	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P21.2.1.2.2.2d*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2a	1	1	N/A	1	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2b	0.05	1	N/A	N/A	Medium	mol/mL	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2.2	1	1	N/A	**1**	Medium	mol/mL	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2.2a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2.2b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2.2c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2.2d	1	1	N/A	N/A	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
HPLC or LC Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2.2.3	0.9	**1**	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P22.2.1.2.2.3a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2a	1	1	N/A	1	Medium	N/A	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1	0.95	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2a	1	1	1.00E-04	0.1	Medium	ppw	
Isolate Liquid Phase from Solid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2b	0.05	1	N/A	N/A	Medium	N/A	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2	1	1	N/A	**1**	Medium	N/A	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Digestion to Peptides or Not	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2d	1	1	N/A	N/A	Medium	N/A	
HPLC or LC Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2.3	0.9	**1**	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2.3a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE/beads | HPLC/LC | Ion Selection | MSn Analyses	P23.2.1.2.2.3c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P24.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P24.2a	1	1	N/A	1	Medium	N/A	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P24.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P24.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P24.2.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend Protein Fraction from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P24.2.2.1a	1	0.1	N/A	N/A	Medium	N/A	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Colorimetric Assay	Measurement	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay	P24.2.2.1.1*	1	1	1.00E-06	10	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2a	1	1	N/A	1	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend Protein Fraction from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2.2.1a	1	0.1	N/A	N/A	Medium	mol/mL	
Concentration (Optional)	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2.2.1.2	0.5	**100**	N/A	0.000001	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
CE Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2.2.1.2.1	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
ESI-MS Analysis	Measurement	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | ESI-MS Analysis	P25.2.2.1.2.1a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2a	1	1	N/A	1	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2	0.95	1	N/A	0.01	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Cell Lysis Step	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend Protein Fraction from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2.1a	1	0.1	N/A	N/A	Medium	mol/mL	
Concentration (Optional)	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2.1.2	0.5	100	N/A	0.000001	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
CE Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2.1.2.1	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
Electrospray Ionization	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2.1.2.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2.1.2.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	P26.2.2.1.2.1c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2a	1	1	N/A	1	Medium	ppw	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend Protein Fraction from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2.2.1a	1	0.1	N/A	N/A	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Concentration (Optional)	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2.2.1.2	0.5	**100**	N/A	0.000001	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
Deposit to Plate with Matrix	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2.2.1.2.2	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
LD-MS Analysis	Measurement	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD-MS Analysis	P27.2.2.1.2.2a*	0.5	1	1.00E-09	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2a	1	1	N/A	1	Medium	ppw	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend Protein Fraction from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2.1a	1	0.1	N/A	N/A	Medium	ppw	
Concentration (Optional)	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2.1.2	0.5	100	N/A	0.000001	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
Deposit to Plate with Matrix	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2.1.2.2	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Laser Desorption	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2.1.2.2a	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2.1.2.2b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	P28.2.2.1.2.2c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2a	1	1	N/A	1	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend Protein Fraction from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2.2.1a	1	0.1	N/A	N/A	Medium	mol/mL	
Concentration (Optional)	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2.2.1.2	0.5	100	N/A	0.000001	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
HPLC or LC Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2.2.1.2.3	0.9	**1**	1.00E-09	0.00001	Low	mol/mL	making assumption that most of the HMWC are reversibly taken off a chromatography column.

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
ESI-MS Analysis	Measurement	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | ESI-MS Analysis	P29.2.2.1.2.3a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2a	1	1	N/A	1	Medium	N/A	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend Protein Fraction from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2.1a	1	0.1	N/A	N/A	Medium	N/A	
Concentration (Optional)	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2.1.2	0.5	100	N/A	0.000001	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
HPLC or LC Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2.1.2.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2.1.2.3a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2.1.2.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	P30.2.2.1.2.3c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | CE | ESI-MS Analysis	P31.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | CE | ESI-MS Analysis	P31.2a	1	1	N/A	1	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | CE | ESI-MS Analysis	P31.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | CE | ESI-MS Analysis	P31.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
CE Separation	Processing	Liquid Iso. | CE | ESI-MS Analysis	P31.2.2.2	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
ESI-MS Analysis	Measurement	Liquid Iso. | CE | ESI-MS Analysis	P31.2.2.2a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | CE | Ion Selection | MSn Analyses	P32.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | CE | Ion Selection | MSn Analyses	P32.2a	1	1	N/A	1	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | CE | Ion Selection | MSn Analyses	P32.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | CE | Ion Selection | MSn Analyses	P32.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
CE Separation	Processing	Liquid Iso. | CE | Ion Selection | MSn Analyses	P32.2.2.2	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
Electrospray Ionization	Processing	Liquid Iso. | CE | Ion Selection | MSn Analyses	P32.2.2.2a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Separation and Fragmentation	Processing	Liquid Iso. | CE | Ion Selection | MSn Analyses	P32.2.2.2b	1	1	N/A	N/A	Medium	mol/mL	
MSn Analyses	Measurement	Liquid Iso. | CE | Ion Selection | MSn Analyses	P32.2.2.2c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | LD-MS Analysis	P33.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | LD-MS Analysis	P33.2a	1	1	N/A	1	Medium	ppw	
Liquid Isolation	Processing	Liquid Iso. | LD-MS Analysis	P33.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | LD-MS Analysis	P33.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Deposit to Plate with Matrix	Processing	Liquid Iso. | LD-MS Analysis	P33.2.2.3	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
LD-MS Analysis	Measurement	Liquid Iso. | LD-MS Analysis	P33.2.2.3a*	0.5	1	1.00E-09	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | LD | Ion Selection | MSn Analyses	P34.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | LD | Ion Selection | MSn Analyses	P34.2a	1	1	N/A	1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Liquid Isolation	Processing	Liquid Iso. | LD | Ion Selection | MSn Analyses	P34.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | LD | Ion Selection | MSn Analyses	P34.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
Deposit to Plate with Matrix	Processing	Liquid Iso. | LD | Ion Selection | MSn Analyses	P34.2.2.3	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
Laser Desorption	Processing	Liquid Iso. | LD | Ion Selection | MSn Analyses	P34.2.2.3a	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Liquid Iso. | LD | Ion Selection | MSn Analyses	P34.2.2.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | LD | Ion Selection | MSn Analyses	P34.2.2.3c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | HPLC/LC | ESI-MS Analysis	P35.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | HPLC/LC | ESI-MS Analysis	P35.2a	1	1	N/A	1	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | HPLC/LC | ESI-MS Analysis	P35.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | HPLC/LC | ESI-MS Analysis	P35.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
HPLC or LC Separation	Processing	Liquid Iso. | HPLC/LC | ESI-MS Analysis	P35.2.2.4	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Liquid Iso. | HPLC/LC | ESI-MS Analysis	P35.2.2.4a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | HPLC/LC | Ion Selection | MSn Analyses	P36.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | HPLC/LC | Ion Selection | MSn Analyses	P36.2a	1	1	N/A	1	Medium	N/A	
Liquid Isolation	Processing	Liquid Iso. | HPLC/LC | Ion Selection | MSn Analyses	P36.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | HPLC/LC | Ion Selection | MSn Analyses	P36.2.2a	1	1	1.00E-04	0.1	Medium	ppw	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
HPLC or LC Separation	Processing	Liquid Iso. | HPLC/LC | Ion Selection | MSn Analyses	P36.2.2.4	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Liquid Iso. | HPLC/LC | Ion Selection | MSn Analyses	P36.2.2.4a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Separation and Fragmentation	Processing	Liquid Iso. | HPLC/LC | Ion Selection | MSn Analyses	P36.2.2.4b	1	1	N/A	N/A	Medium	N/A	
MSn Analyses	Measurement	Liquid Iso. | HPLC/LC | Ion Selection | MSn Analyses	P36.2.2.4c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2a	1	1	N/A	1	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2.2.5	1	1	N/A	1	Medium	mol/mL	
Adjust pH	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2.2.5a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2.2.5b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/ Extractions	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2.2.5c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
CE Separation	Processing	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2.2.5.1	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
ESI-MS Analysis	Measurement	Liquid Iso. | SPE | CE | ESI-MS Analysis	P37.2.2.5.1a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2a	1	1	N/A	1	Medium	mol/mL	

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Liquid Isolation	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2.5	1	1	N/A	1	Medium	mol/mL	
Adjust pH	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2.5a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2.5b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2.5c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
CE Separation	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2.5.1	0.95	1	1.00E-06	0.0001	Medium	mol/mL	
Electrospray Ionization	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2.5.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2.5.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	P38.2.2.5.1c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2a	1	1	N/A	1	Medium	ppw	
Liquid Isolation	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2.2.5	1	1	N/A	1	Medium	mol/mL	
Adjust pH	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2.2.5a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2.2.5b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2.2.5c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Deposit to Plate with Matrix	Processing	Liquid Iso. | SPE | LD-MS Analysis	P39.2.2.5.2	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
LD-MS Analysis	Measurement	Liquid Iso. | SPE | LD-MS Analysis	P39.2.2.5.2a*	0.5	1	1.00E-09	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2a	1	1	N/A	1	Medium	ppw	
Liquid Isolation	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2.5	1	1	N/A	1	Medium	mol/mL	
Adjust pH	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2.5a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2.5b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2.5c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Deposit to Plate with Matrix	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2.5.2	0.8	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
Laser Desorption	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2.5.2a	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)

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Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Ion Selection and Fragmentation	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2.5.2b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	P40.2.2.5.2c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2a	1	1	N/A	1	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2.2a	1	1	1.00E-04	0.1	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2.2.5	1	1	N/A	1	Medium	mol/mL	
Adjust pH	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2.2.5a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2.2.5b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2.2.5c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
HPLC or LC Separation	Processing	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2.2.5.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Liquid Iso. | SPE | HPLC/LC | ESI-MS Analysis	P41.2.2.5.3a*	1	1	1.00E-10	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2	1	1	N/A	0.000001	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2a	1	1	N/A	1	Medium	N/A	
Liquid Isolation	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis Step	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2a	1	1	1.00E-04	0.1	Medium	ppw	

Proteins & Peptides

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Bio-signature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2.5	1	1	N/A	1	Medium	mol/mL	
Adjust pH	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2.5a	0.8	1	N/A	0.001	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2.5b	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2.5c	0.85	100	1.00E-06	0.0001	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
HPLC or LC Separation	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2.5.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2.5.3a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2.5.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	P42.2.2.5.3c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Laser Desorption	Processing	LD | Ion Selection | MSn Analyses	H1.1	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Ion Selection and Fragmentation	Processing	LD | Ion Selection | MSn Analyses	H1.1a	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	LD | Ion Selection | MSn Analyses	H1.1b*	1	1	1.00E-09	1	Medium	N/A	MS has flown in the past. Tandem MS has not been developed.
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1.3.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1.3.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Derivatized AA	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1.3.2.1.4.1.2	1	1	1.00E-06	0.01	Medium	mol/mL	Options to connect this to AA processing paths
Dissolved Free (DFAA) AA Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1.3.2.1.4.1.2a*	0.8	1	1.00E-09	1	Medium	mol/mL	Noell et al., 2018; Nunn & Keil, 2006
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Derivatize | DFAA Analysis	H10.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension -less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2.1.3.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Colorimetric Assay	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2.1.3.2.1.4.2	0.5	1	1.00E-06	1.00E-04	Low	mol/mL	Pigments can be a problem, reagents have a specific shelf life, range of methods -not all HMWC detected with colorimetric assays
Spectrophotometer	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2.1.3.2.1.4.2a*	0.8	1	1.00E + 03	0.1	Medium	mol/mL	Liquid handling needs mission development
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H11.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2.1.4.3	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
CE Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2.1.4.3.1	0.95	1	1.00E-06	1.00E-04	Medium	mol/mL	
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2.1.4.3.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2.1.4.3.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2.1.4.3.1c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H12.2a	1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Concentration (Optional)	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2.1.4.3	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
Deposit to Plate with Matrix	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2.1.4.3.2	1	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
Laser Desorption	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2.1.4.3.2a	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2.1.4.3.2b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2.1.4.3.2c*	1	1	1.00E-12	1	Medium	ppw	MS has flown in the past. Tandem MS has not been developed.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H13.2a	1	1	N/A	1	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2.1.4.3	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
HPLC or LC Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2.1.4.3.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2.1.4.3.3a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2.1.4.3.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2.1.4.3.3c*	1	1	1.00E-12	1	Medium	N/A	MS has flown in the past. Tandem MS has not been developed.

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Concentrate | HPLC/LC | Ion Selection | MSn Analyses	H14.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
CE Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2.2.1	0.95	1	1.00E-06	1.00E-04	Medium	mol/mL	
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2.2.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2.2.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2.2.1c*	1	1	1.00E-12	0.1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed. Berensmeier, 2006; Moggridge et al., 2018.
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/ Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H15.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
Deposit to Plate with Matrix	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2.2.2	1	10	5.00E-04	0.00001	Medium	mol/mL	
Laser Desorption	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2.2.2a	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities))

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2.2.2b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2.2.2c*	1	1	1.00E-12	0.1	Medium	ppw	MS has flown in the past. Tandem MS has not been developed. Berensmeier, 2006; Moggridge et al., 2018.
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/ Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H16.2a	1	1	N/A	1	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
HPLC or LC Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2.2.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2.2.3a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2.2.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2.2.3c*	1	1	1.00E-12	0.1	Medium	N/A	MS has flown in the past. Tandem MS has not been developed. Berensmeier, 2006; Moggridge et al., 2018.

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/ Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HPLC/LC | Ion Selection | MSn Analyses	H17.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
Chloroform MeOH Soluble Fraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3.2.2.4	0.5	1	1.00E-04	1.00E-04	Medium	mol/mL	
GC-MS Lipids and Other HMWC Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3.2.2.4.1*	0.5	1	1.00E-10	10	Medium	N/A	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | GC-MS Lipids/HMWC	H18.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
pH/Conductivity Measurement	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
Chloroform MeOH Soluble Fraction	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2.4	0.5	1	1.00E-04	1.00E-04	Medium	mol/mL	
HILIC HPLC to Separate by Headgroups	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2.4.1	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2.4.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2.4.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2.4.1c*	1	1	1.00E-12	1	Medium	N/A	MS has flown in the past. Tandem MS has not been developed.
Adjust pH	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Solid-Phase HILIC HPLC MSn Analyses	H19.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Pyrolysis GC	H2.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Pyrolysis GC	H2.2.1	0.9	100	N/A	1	Medium	ppw	
Pyrolysis GC	Measurement	Size-based Filtration | Pyrolysis GC	H2.2.1.1*	1	1	1.00E-10	0.5	High	N/A	Most HMW material will burn up in pyrol. GC- limited to lipids
Phase Separation	Processing	Size-based Filtration | Pyrolysis GC	H2.2a	1	1	N/A	1	Medium	N/A	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
Chloroform MeOH Soluble Fraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2.4	0.5	1	1.00E-04	1.00E-04	Medium	mol/mL	
Reverse-Phase HPLC to Separate by Alkyl Chain Mods	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2.4.2	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2.4.2a	1	1	1.00E-05	0.01	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2.4.2b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2.4.2c*	1	1	1.00E-12	1	Medium	N/A	MS has flown in the past. Tandem MS has not been developed.
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | Ion Selection | MSn Analyses	H20.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Liquid-Phase THAA Analysis	H21.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid-Phase THAA Analysis	H21.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Split Sample	Processing	Liquid-Phase THAA Analysis	H21.2.2.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Hydrolysis to Break Up Organics	Processing	Liquid-Phase THAA Analysis	H21.2.2.1.1	0.8	1	N/A	0.01	Medium	mol/mL	Pressurized hot acid is used to do complete hydrolysis. Great for comparison to non-hydrolyzed sample to understand inherent complexity
Derivatized AA	Processing	Liquid-Phase THAA Analysis	H21.2.2.1.1.1	1	1	1.00E-06	0.01	Medium	mol/mL	Options to connect this to AA chart…
Total Hydrolyzable AA (THAA) AA Analysis	Measurement	Liquid-Phase THAA Analysis	H21.2.2.1.1.1a*	1	1	1.00E-12	10	Medium	N/A	
Phase Separation	Processing	Liquid-Phase THAA Analysis	H21.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | Hydrolyze | Chiral GC/CE	H22.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | Hydrolyze | Chiral GC/CE	H22.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Split Sample	Processing	Liquid Iso. | Hydrolyze | Chiral GC/CE	H22.2.2.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Hydrolysis to Break Up Organics	Processing	Liquid Iso. | Hydrolyze | Chiral GC/CE	H22.2.2.1.1	0.8	1	N/A	0.01	Medium	mol/mL	Pressurized hot acid is used to do complete hydrolysis. Great for comparison to non-hydrolyzed sample to understand inherent complexity
Chiral GC Column or Chiral-Capillary Electrophoresis (CE)	Measurement	Liquid Iso. | Hydrolyze | Chiral GC/CE	H22.2.2.1.1.2*	0.9	1	1.00E-10	1	Medium	mol/mL	Pietrogrande & Basaglia, 2010
Phase Separation	Processing	Liquid Iso. | Hydrolyze | Chiral GC/CE	H22.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | Chiral GC/CE	H23.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | Chiral GC/CE	H23.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Split Sample	Processing	Liquid Iso. | Chiral GC/CE	H23.2.2.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Chiral GC Column or Chiral-CE	Measurement	Liquid Iso. | Chiral GC/CE	H23.2.2.1.2*	0.9	1	1.00E-10	1	Medium	mol/mL	Pietrogrande & Basaglia, 2010
Phase Separation	Processing	Liquid Iso. | Chiral GC/CE	H23.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | Derivatize | DFAA Analysis	H24.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Liquid Isolation	Processing	Liquid Iso. | Derivatize | DFAA Analysis	H24.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Split Sample	Processing	Liquid Iso. | Derivatize | DFAA Analysis	H24.2.2.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Derivatized AA	Processing	Liquid Iso. | Derivatize | DFAA Analysis	H24.2.2.1.3	1	1	1.00E-06	0.01	Medium	mol/mL	Options to connect this to AA chart…
Dissolved Free (DFAA) AA Analysis	Measurement	Liquid Iso. | Derivatize | DFAA Analysis	H24.2.2.1.3a*	0.8	1	1.00E-09	1	Medium	mol/mL	Noell et al., 2018; Nunn & Keil, 2006
Phase Separation	Processing	Liquid Iso. | Derivatize | DFAA Analysis	H24.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | LD-MS Analysis	H25.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | LD-MS Analysis	H25.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Deposit to Plate with Matrix	Processing	Liquid Iso. | LD-MS Analysis	H25.2.2.2	1	10	5.00E-04	0.00001	Medium	mol/mL	
LD-MS Analysis	Measurement	Liquid Iso. | LD-MS Analysis	H25.2.2.2a*	0.7	1	1.00E-09	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | LD-MS Analysis	H25.2a	1	1	N/A	1	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Hydrolyzed AA Analysis	H26.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Hydrolyzed AA Analysis	H26.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | S.Exc. Chrom. | Hydrolyzed AA Analysis	H26.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Hydrolyzed AA Analysis	H26.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% Efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Split Sample	Processing	Liquid Iso. | S.Exc. Chrom. | Hydrolyzed AA Analysis	H26.2.2.3.1.1	0.5	0.5	N/A	0.01	Medium	mol/mL	liquid handling systems to divert half the sample- I think this is developed already

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Hydrolysis-Based AA Analysis	Measurement	Liquid Iso. | S.Exc. Chrom. | Hydrolyzed AA Analysis	H26.2.2.3.1.1.1*	1	1	1.00E-12	10	Medium	mol/mL	
Isolate Liquid Phase	Processing	Liquid Iso. | S.Exc. Chrom. | Hydrolyzed AA Analysis	H26.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Hydrolyzed AA Analysis	H26.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | DFAA Analysis	H27.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | DFAA Analysis	H27.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | S.Exc. Chrom. | DFAA Analysis	H27.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | DFAA Analysis	H27.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Split Sample	Processing	Liquid Iso. | S.Exc. Chrom. | DFAA Analysis	H27.2.2.3.1.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Dissolved Free AA Analysis	Measurement	Liquid Iso. | S.Exc. Chrom. | DFAA Analysis	H27.2.2.3.1.1.2*	0.8	1	1.00E-09	1	Medium	mol/mL	Noell et al., 2018; Nunn & Keil, 2006
Isolate Liquid Phase	Processing	Liquid Iso. | S.Exc. Chrom. | DFAA Analysis	H27.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | DFAA Analysis	H27.2a	1	1	N/A	1	Medium	N/A	

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2.2.3.1.2	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Colorimetric Assay	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2.2.3.1.2.1	0.5	1	1.00E-06	1.00E-04	Low	mol/mL	Pigments can be a challenge, reagents have a specific shelf life, range of methods -not all HMWC detected with colorimetric assays
Spectrophotometer Analysis	Measurement	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2.2.3.1.2.1a*	0.8	1	1.00E-09	1	Medium	mol/mL	Noell et al., 2018; Nunn & Keil, 2006
Isolate Liquid Phase	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Colorimetric Assay | Spectrophotometer	H28.2a	1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3.1.2	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3.1.2.2	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
CE Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3.1.2.2.1	0.95	1	1.00E-06	1.00E-04	Medium	mol/mL	
ESI-MS Analysis	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3.1.2.2.1a	1	1	1.00E-10	1	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3.1.2.2.1b	1	1	N/A	N/A	Medium	Gas	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
MSn Analyses	Measurement	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3.1.2.2.1c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Isolate Liquid Phase	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | CE | Ion Selection | MSn Analyses	H29.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	H3.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	H3.2.1	0.9	1000	N/A	1	Medium	ppw	
Laser Desorption	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	H3.2.1.2	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	H3.2.1.2a	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | LD | Ion Selection | MSn Analyses	H3.2.1.2b*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Phase Separation	Processing	Size-based Filtration | LD | Ion Selection | MSn Analyses	H3.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2	0.95	1	N/A	0.01	Medium	mol/mL	

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Cell Lysis	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3.1.2	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3.1.2.2	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
Deposit to Plate with Matrix	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3.1.2.2.2	1	10	5.00E-04	0.00001	Medium	mol/mL	
Laser Desorption	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3.1.2.2.2a	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3.1.2.2.2b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3.1.2.2.2c*	1	1	1.00E-12	1	Medium	ppw	MS has flown in the past. Tandem MS has not been developed.
Isolate Liquid Phase	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Phase Separation	Processing	Liquid Iso. | S.Exc. Chrom. | Concentrate | LD | Ion Selection | MSn Analyses	H30.2a	1	1	N/A	1	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be challenging for flight
Resuspend HMW from Device	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3.1.2	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3.1.2.2	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
HPLC or LC Separation	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3.1.2.2.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3.1.2.2.3a	1	1	1.00E-05	0.01	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Ion Selection and Fragmentation	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3.1.2.2.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3.1.2.2.3c*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Isolate Liquid Phase	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid-Phase Size Exclusion Isolated ESI-MS Analysis MSn Analyses	H31.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3.2	1	1	N/A	1	Medium	mol/mL	
CE Separation	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3.2.1	0.95	1	1.00E-06	1.00E-04	Medium	mol/mL	
Electrospray Ionization	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3.2.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3.2.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3.2.1c*	1	1	1.00E-12	0.1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed. Berensmeier, 2006; Moggridge et al., 2018.

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Adjust pH	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | CE | Ion Selection | MSn Analyses	H32.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3.2	1	1	N/A	1	Medium	mol/mL	
Deposit to Plate with Matrix	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3.2.2	1	10	5.00E-04	0.00001	Medium	mol/mL	
Laser Desorption	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3.2.2a	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3.2.2b	1	1	N/A	N/A	Medium	Gas	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
MSn Analyses	Measurement	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3.2.2c*	1	1	1.00E-12	0.1	Medium	ppw	MS has flown in the past. Tandem MS has not been developed. Berensmeier, 2006; Moggridge et al., 2018.
Adjust pH	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | LD | Ion Selection | MSn Analyses	H33.2a	1	1	N/A	1	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3.2	1	1	N/A	1	Medium	mol/mL	
HPLC or LC Separation	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3.2.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3.2.3a	1	1	1.00E-05	0.01	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Ion Selection and Fragmentation	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3.2.3b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3.2.3c*	1	1	1.00E-12	0.1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed. Berensmeier, 2006; Moggridge et al., 2018.
Adjust pH	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid-Phase Bead Extracted ESI-MS MSn Analysis	H34.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Separate Aqueous and Organic Fraction	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2.3.3	0.5	10	N/A	1	Low	mol/mL	
HILIC HPLC to Separate by Headgroups	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2.3.3.1	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Analysis	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2.3.3.1a	1	1	1.00E-05	0.01	Medium	mol/mL	
Ion Selection and Fragmentation	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2.3.3.1b	1	1	N/A	N/A	Medium	Gas	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
MSn Analyses	Measurement	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2.3.3.1c*	1	1	1.00E-12	1	Medium	N/A	MS has flown in the past. Tandem MS has not been developed.
Isolate Organic Fraction	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2.3.3a	0.8	1	N/A	1	Low	mol/mL	
Isolate Liquid Phase	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid-Phase HILIC HPLC MSn Analyses	H35.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Separate Aqueous and Organic Fraction	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2.3.3	0.5	10	N/A	1	Low	mol/mL	
Reverse-Phase HPLC to Separate by Alkyl Chain Mods	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2.3.3.1	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
Electrospray Ionization	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2.3.3.1a	1	1	1.00E-05	0.01	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Ion Selection and Fragmentation	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2.3.3.1b	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2.3.3.1c*	1	1	1.00E-12	1	Medium	N/A	MS has flown in the past. Tandem MS has not been developed.
Isolate Organic Fraction	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2.3.3a	0.8	1	N/A	1	Low	mol/mL	
Isolate Liquid Phase	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI | Ion Selection | MSn Analyses	H36.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Gas-Phase MSn Analyses	H37.2	1	1	N/A	0.000001	Medium	mol/mL	
Gas Isolation	Processing	Gas-Phase MSn Analyses	H37.2.3	0.95	1	N/A	1.00E-01	Medium	Gas	To detect HMW via GC you must derivatize
GC Separation	Processing	Gas-Phase MSn Analyses	H37.2.3.1	0.5	1	N/A	1.00E-01	Medium	Gas	To detect HMW via GC you must derivatize
EI-MS Analysis	Processing	Gas-Phase MSn Analyses	H37.2.3.1a	0.8	1	1.00E-10	0.01	Medium	mol/mL	
Ion Fragmentation and Analysis	Processing	Gas-Phase MSn Analyses	H37.2.3.1b	1	1	1.00E-12	N/A	Medium	Gas	
MSn Analyses	Measurement	Gas-Phase MSn Analyses	H37.2.3.1c*	1	1	1.00E-12	1	Medium	N/A	MS has flown in the past. Tandem MS has not been developed.
Phase Separation	Processing	Gas-Phase MSn Analyses	H37.2a	1	1	N/A	1	Medium	N/A	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Gas Iso. | Tunable Laser Spec.	H38.2	1	1	N/A	0.000001	Medium	mol/mL	
Gas Isolation	Processing	Gas Iso. | Tunable Laser Spec.	H38.2.3	0.95	1	N/A	1.00E-01	Medium	Gas	To detect HMW via GC you must derivatize
Tunable Laser/Absorption Spectroscopy	Measurement	Gas Iso. | Tunable Laser Spec.	H38.2.3.2*	0.9	1	1.00E-09	1	High	N/A	https://arc.aiaa.org/doi/pdf/10.2514/6.2011-7316
Phase Separation	Processing	Gas Iso. | Tunable Laser Spec.	H38.2a	1	1	N/A	1	Medium	N/A	
LD-MS Analysis	Measurement	LD-MS Analysis	H39.1*	0.7	1	1.00E-09	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Pyrolysis GC	H4.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Pyrolysis GC	H4.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Pyrolysis GC	H4.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Pyrolysis GC	Measurement	Size-based Filtration | Org. Extract | Pyrolysis GC	H4.2.1.3.1*	1	1	1.00E-10	0.5	High	N/A	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Pyrolysis GC	H4.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Solid-Phase LD-MS Analysis	H40.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Solid-Phase LD-MS Analysis	H40.2.1	0.9	1000	N/A	1	Medium	ppw	
LD-MS Analysis	Measurement	Solid-Phase LD-MS Analysis	H40.2.1.2*	0.7	1	1.00E-09	1	High	mol/mL	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Phase Separation	Processing	Solid-Phase LD-MS Analysis	H40.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Solid-Phase Size Exclusion Isolated LD-MS Analysis	H41.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size-based Filtration	Processing	Solid-Phase Size Exclusion Isolated LD-MS Analysis	H41.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Solid-Phase Size Exclusion Isolated LD-MS Analysis	H41.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Solid-Phase Size Exclusion Isolated LD-MS Analysis	H41.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Solid-Phase Size Exclusion Isolated LD-MS Analysis	H41.2.1.3.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
LD-MS Analysis	Measurement	Solid-Phase Size Exclusion Isolated LD-MS Analysis	H41.2.1.3.2.1.3	0.7	1	1.00E-09	1	High	mol/mL	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Isolate Liquid Phase	Processing	Solid-Phase Size Exclusion Isolated LD-MS Analysis	H41.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Solid-Phase Size Exclusion Isolated LD-MS Analysis	H41.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size Exclusion Isolation	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1.3.2.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1.3.2.1.4.3	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
CE Separation	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1.3.2.1.4.3.1	0.95	1	1.00E-06	1.00E-04	Medium	mol/mL	
ESI-MS Analysis	Measurement	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1.3.2.1.4.3.1a*	1	1	1.00E-10	1	Medium	mol/mL	
Isolate Liquid Phase	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Solid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H42.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size-based Filtration	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1.3.2.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1.3.2.1.4.3	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Deposit to Plate with Matrix	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1.3.2.1.4.3.2	1	10	5.00E-04	0.00001	Medium	mol/mL	Ni et al., 2023
LD-MS Analysis	Measurement	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1.3.2.1.4.3.2a*	0.7	1	1.00E-09	1	High	mol/mL	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Isolate Liquid Phase	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Solid-Phase Size Exclusion Isolated CE Separated Concentrated Deposited LD-MS Analysis	H43.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size Exclusion Isolation	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1.3.2.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1.3.2.1.4.3	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
HPLC or LC Separation	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1.3.2.1.4.3.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1.3.2.1.4.3.3a*	1	1	1.00E-10	1	Medium	mol/mL	
Isolate Liquid Phase	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Solid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H44.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1	0.9	100	N/A	1	Medium	ppw	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Non-Degrading Organic Extraction	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
CE Separation	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3.2.2.1	0.95	1	1.00E-06	1.00E-04	Medium	mol/mL	
ESI-MS Analysis	Measurement	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3.2.2.1a*	1	1	1.00E-10	1	Medium	mol/mL	
Adjust pH	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Solid-Phase Bead Separated CE Separation ESI-MS Analysis	H45.2a	1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
Deposit to Plate with Matrix	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3.2.2.2	1	10	5.00E-04	0.00001	Medium	mol/mL	
LD-MS Analysis	Measurement	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3.2.2.2a*	0.7	1	1.00E-09	1	High	mol/mL	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Adjust pH	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Isolate Liquid Phase	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Solid-Phase Bead Separated Laser Desorption LD-MS Analysis	H46.2a	1	1	N/A	1	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
HPLC or LC Separation	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3.2.2.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3.2.2.3a*	1	1	1.00E-10	1	Medium	mol/mL	
Adjust pH	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
SPE/Functional Bead Separations	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Solid-Phase Bead Separated HPLC or LC Separated ESI-Ms Analysis	H47.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
Chloroform MeOH Soluble Fraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2.2.4	0.5	1	1.00E-04	1.00E-04	Medium	mol/mL	

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Reverse-Phase HPLC to Separate by Alkyl Chain Mods	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2.2.4.2	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2.2.4.2a*	1	1	1.00E-10	1	Medium	mol/mL	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | RP-HPLC | ESI-MS Analysis	H48.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3	0.8	0.5	N/A	1	Medium	ppw	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2.2	1	1	N/A	1	Medium	mol/mL	
Chloroform MeOH Soluble Fraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2.2.4	0.5	1	1.00E-04	1.00E-04	Medium	mol/mL	
HILIC HPLC to separate by headgroups	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2.2.4.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2.2.4.3a*	1	1	1.00E-10	1	Medium	mol/mL	
Adjust pH	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | SPE | HILIC | ESI-MS Analysis	H49.2a	1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	H5.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	H5.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	H5.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	H5.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	H5.2.1.3.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
UV Fluorescence	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	H5.2.1.3.2.1.1*	1	1	1.00E-05	1	High	mol/mL	Zhang et al, 2021; Hobira Scientific, 2021
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	H5.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | UV Fluorescence	H5.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2.2	0.95	1	N/A	0.01	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Cell Lysis	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2.2.3.1.2	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2.2.3.1.2.2	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
CE Separation	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2.2.3.1.2.2.1	0.95	1	1.00E-06	1.00E-04	Medium	mol/mL	
ESI-MS Analysis	Measurement	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2.2.3.1.2.2.1a*	1	1	1.00E-10	1	Medium	mol/mL	
Isolate Liquid Phase	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid-Phase Size Exclusion Isolated CE Separated ESI-MS Analysis	H51.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Liquid Isolation	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2.2.3.1.2	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2.2.3.1.2.2	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
Deposit to Plate with Matrix	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2.2.3.1.2.2.2	1	10	5.00E-04	0.00001	Medium	mol/mL	
LD-MS Analysis	Measurement	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2.2.3.1.2.2.2a*	0.7	1	1.00E-09	1	High	mol/mL	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Isolate Liquid Phase	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid-Phase Size Exclusion Isolated LD-MS Analysis	H52.2a	1	1	N/A	1	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Liquid Isolation	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2.2.3.1	0.95	100	1.00E-06	0.00005	Medium	mol/mL	50% efficient filter aided extraction: Wiśniewski et al., 2009, personal tests and experience also suggest this for HMW ultrafiltration methods that are nonspecific. These are liquid handling systems so may be hard for flight
Resuspend HMW from Device	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2.2.3.1.2	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Concentration (Optional)	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2.2.3.1.2.2	0.5	100	N/A	1.00E-06	Medium	mol/mL	Range of ways to concentrate samples, depends on how much you reduce the volume or isolate the compounds.
HPLC or LC Separation	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2.2.3.1.2.2.3	0.9	1	1.00E-09	0.00001	Low	mol/mL	making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2.2.3.1.2.2.3a*	1	1	1.00E-10	1	Medium	mol/mL	
Isolate Liquid Phase	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid-Phase Size Exclusion Isolated HPLC/LC Separated ESI-MS Analysis	H53.2a	1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Warm Sample/Maintain Salinity	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2.3.2	1	1	N/A	1	Medium	mol/mL	
CE Separation	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2.3.2.1	0.95	1	1.00E-06	1.00E-04	Medium	mol/mL	
ESI-MS Analysis	Measurement	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2.3.2.1a*	1	1	1.00E-10	1	Medium	mol/mL	
Adjust pH	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2.3.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2.3.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2.3.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Phase Separation	Processing	Liquid-Phase Bead Extracted CE Separated ESI-MS Analysis	H54.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
pH/Conductivity Measurement	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2.3.2	1	1	N/A	1	Medium	mol/mL	
Deposit to Plate with Matrix	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2.3.2.2	1	10	5.00E-04	0.00001	Medium	mol/mL	
LD-MS Analysis	Measurement	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2.3.2.2a*	0.7	1	1.00E-09	1	High	mol/mL	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Adjust pH	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2.3.2a	0.8	1	N/A	1.00E-03	Medium	mol/mL	Expect loss of some HMWC due to the fact they are soluble at range of different pHs
SPE/Functional Bead Separations	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2.3.2b	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Bead Cleanup/Extractions	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2.3.2c	0.85	100.00	1.00E-06	1.00E-04	Medium	mol/mL	Hughes et al., 2014; challenging for HMW undefined compounds with all functionalities.
Isolate Liquid Phase	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | SPE | LD-MS Analysis	H56.2a	1	1	N/A	1	Medium	ppw	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Liquid Isolation	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	
Separate Aqueous and Organic Fraction	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2.2.3.3	0.5	10	N/A	1	Low	mol/mL	
Reverse-Phase HPLC to Separate by Alkyl Chain Mods	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2.2.3.3.1	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2.2.3.3.1a*	1	1	1.00E-10	1	Medium	mol/mL	
Isolate Organic Fraction	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2.2.3.3a	0.8	1	N/A	1	Low	mol/mL	
Isolate Liquid Phase	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | RP-HPLC | ESI-MS Analysis	H57.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2	1	1	N/A	0.000001	Medium	mol/mL	
Liquid Isolation	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2.2	0.95	1	N/A	0.01	Medium	mol/mL	
Cell Lysis	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2.2.3	1	1	1.00E-04	1.00E-01	Medium	ppw	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Separate Aqueous and Organic Fraction	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2.2.3.3	0.5	10	N/A	1	Low	mol/mL	
HILIC HPLC to Separate by Headgroups	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2.2.3.3.2	0.9	1	1.00E-09	0.00001	Low	mol/mL	Making assumption that most of the HMWC are reversibly taken off a chromatography column.
ESI-MS Analysis	Measurement	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2.2.3.3.2a*	1	1	1.00E-10	1	Medium	mol/mL	
Isolate Organic Fraction	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2.2.3.3a	0.8	1	N/A	1	Low	mol/mL	
Isolate Liquid Phase	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2.2.3a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Liquid Iso. | Aq/Org Sep. | Org. Iso. | HILIC | ESI-MS Analysis	H58.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	H6.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	H6.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	H6.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	H6.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	H6.2.1.3.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Raman	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	H6.2.1.3.2.1.2*	0.5	1	1.00E-06	10	High	ppw	
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	H6.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Raman	H6.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Gas-Phase EI-MS Analysis	H60.2	1	1	N/A	0.000001	Medium	mol/mL	
Gas Isolation	Processing	Gas-Phase EI-MS Analysis	H60.2.3	0.95	1	N/A	1.00E-01	Medium	Gas	To detect HMW via GC you must derivatize
GC Separation	Processing	Gas-Phase EI-MS Analysis	H60.2.3.1	0.5	1	N/A	1.00E-01	Medium	Gas	To detect HMW via GC you must derivatize
EI-MS Analysis	Measurement	Gas-Phase EI-MS Analysis	H60.2.3.1a*	0.8	1	1.00E-10	0.01	Medium	mol/mL	
Phase Separation	Processing	Gas-Phase EI-MS Analysis	H60.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2.1.3	0.8	0.5	N/A	1	Medium	ppw	

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2.1.3.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Laser Desorption	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2.1.3.2.1.3	1	1	1.00E-09	0.001	High	ppw	Arevalo et al., 2018; Arevalo et al., 2023 Orbitrap references on flight suitable LD-MS (does not have tandem MS capabilities)
Ion Selection and Fragmentation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2.1.3.2.1.3a	1	1	N/A	N/A	Medium	Gas	
MSn Analyses	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2.1.3.2.1.3b*	1	1	1.00E-12	1	Medium	mol/mL	MS has flown in the past. Tandem MS has not been developed.
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | LD | Ion Selection | MSn Analyses	H7.2a	1	1	N/A	1	Medium	mol/mL	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2	1	1	N/A	0.000001	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Hydrolysis to Break Up Organics	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3.2.1.4.1.1	0.8	1	N/A	0.01	Medium	mol/mL	Pressurized hot acid is used to do complete hydrolysis. Great for comparison to non-hydrolyzed sample to understand inherent complexity
Derivatized AA	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3.2.1.4.1.1.1	1	1	1.00E-06	0.01	Medium	mol/mL	Options to connect this to AA chart…

High-Molecular-Weight Compounds										
Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
THAA Analysis	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3.2.1.4.1.1.1a *	1	1	1.00E-12	10	Medium	N/A	
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Derivatize | THAA Analysis	H8.2a	1	1	N/A	1	Medium	N/A	
Warm Sample/Maintain Salinity	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2	1	1	N/A	0.000001	Medium	mol/mL	
Size-based Filtration	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1	0.9	100	N/A	1	Medium	ppw	
Non-Degrading Organic Extraction	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1.3	0.8	0.5	N/A	1	Medium	ppw	
Cell Lysis Step	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1.3.2	1	1	1.00E-04	1.00E-01	Medium	ppw	
Size Exclusion Isolation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1.3.2.1	0.95	100	1.00E-03	N/A	Medium	mol/mL	

High-Molecular-Weight Compounds

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Resuspend HMW from Device	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1.3.2.1.4	0.8	0.1	N/A	0.01	Low	mol/mL	Estimating this might be challenging to do in flight as it can be challenging in lab
Split Sample	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1.3.2.1.4.1	0.5	0.5	N/A	0.01	Medium	mol/mL	Liquid handling systems to divert half the sample
Hydrolysis to Break Up Organics	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1.3.2.1.4.1.1	0.8	1	N/A	0.01	Medium	mol/mL	Pressurized hot acid is used to do complete hydrolysis. Great for comparison to non-hydrolyzed sample to understand inherent complexity
Chiral GC Column or a Chiral-CE	Measurement	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1.3.2.1.4.1.1.2*	0.9	1	1.00E-10	1	Medium	mol/mL	Pietrogrande & Basaglia, 2010
Isolate Liquid Phase	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2.1.3.2a	0.1	1	N/A	1	Medium	mol/mL	
Phase Separation	Processing	Size-based Filtration | Org. Extract | Liquid Iso. | S.Exc. Chrom. | Hydrolyze | Chiral GC/CE	H9.2a	1	1	N/A	1	Medium	mol/mL	

Nucleic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Add Solvent or Melt	Processing	Bio Particle Sep. | Disrupt | Purify + Concentrate | Fluorometry	N1.1	1	0.8	N/A	N/A	Medium	N/A	
Separate Bio from Non-Bio Particles	Processing	Bio Particle Sep. | Disrupt | Purify + Concentrate | Fluorometry	N1.1a	0.8	1	N/A	N/A	Medium	N/A	Higher for liquid, lower for particulate heavy (should make these separate steps (one for liquid, one for particulate heavy)
Phase Separation - Liquid	Processing	Bio Particle Sep. | Disrupt | Purify + Concentrate | Fluorometry	N1.1b	1	1	N/A	N/A	Medium	N/A	
Disrupt/Lyse	Processing	Bio Particle Sep. | Disrupt | Purify + Concentrate | Fluorometry	N1.1c	1	1	N/A	N/A	Medium	N/A	
Purify & Concentrate	Processing	Bio Particle Sep. | Disrupt | Purify + Concentrate | Fluorometry	N1.1d	0.7	36	N/A	N/A	Medium	N/A	AMPure bead purification step is typically 70-90% efficient but as low as 60% in suboptimal situations (Beckman Coulter Life Sciences, n.d.); if assume 1 mL working sample, eluted into 40uL after purification = 25x vol concentration
Fluorometry	Measurement	Bio Particle Sep. | Disrupt | Purify + Concentrate | Fluorometry	N1.1.1a*	1	1	1.00E-07	0.005	Medium	ppw	0.1 ng is min detection, 1 ng as good bottom line; detection limit: 1ng DNA in 10uL water = 1e-9g/.01g =1e-7
Add Solvent or Melt	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | UV Spectroscopy	N2.1	1	0.8	N/A	N/A	Medium	N/A	
Separate Bio from Non-Bio Particles	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | UV Spectroscopy	N2.1a	0.8	1	N/A	N/A	Medium	N/A	
Phase Separation - Liquid	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | UV Spectroscopy	N2.1b	1	1	N/A	N/A	Medium	N/A	

Nucleic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension -less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Disrupt/Lyse	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | UV Spectroscopy	N2.1c	1	1	N/A	N/A	Medium	N/A	
Purify & Concentrate	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | UV Spectroscopy	N2.1d	0.7	36	N/A	N/A	Medium	N/A	Chosen to reach vol of 35 uL at analysis
UV Spectroscopy	Measurement	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | UV Spectroscopy	N2.1.2*	1	1	1.00E-05	0.005	Medium	ppw	
Add Solvent or Melt	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1	1	0.8	N/A	N/A	Medium	N/A	
Separate Bio from Non-Bio Particles	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1a	0.8	1	N/A	N/A	Medium	N/A	
Phase Separation - Liquid	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1b	1	1	N/A	N/A	Medium	N/A	
Disrupt/Lyse	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1c	1	1	N/A	N/A	Medium	N/A	
Purify & Concentrate	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1d	0.7	36	N/A	N/A	Medium	N/A	Chosen to reach vol of 35 uL at analysis
Tagging/Add Adapters	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1.3	1	1	N/A	N/A	Medium	N/A	

Nucleic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension -less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
RNA to cDNA Conversion (if RNA)	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1.3.1	0.9	1	N/A	N/A	Medium	N/A	
Prime and load	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1.3.1a	0.8	1	N/A	N/A	Medium	N/A	
Electrochemical (EC) Analysis cDNA	Measurement	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | RNAconvert | Prime + Load | EC Analysis (cDNA)	N3.1.3.1.1*	1	1	1.00E-08	0.03	Medium	g	10 ng cDNA
Add Solvent or Melt	Processing	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1	1	0.8	N/A	N/A	Medium	N/A	
Separate Bio from Non-Bio Particles	Processing	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1a	0.8	1	N/A	N/A	Medium	N/A	
Phase Separation - Liquid	Processing	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1b	1	1	N/A	N/A	Medium	N/A	
Disrupt/Lyse	Processing	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1c	1	1	N/A	N/A	Medium	N/A	

Nucleic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension-less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Purify & Concentrate	Processing	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1d	0.7	36	N/A	N/A	Medium	N/A	Chosen to reach vol of 35 uL at analysis
Tagging/Add Adapters	Processing	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1.3	1	1	N/A	N/A	Medium	N/A	
RNA to cDNA Conversion (if RNA)	Processing	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1.3.1	0.9	1	N/A	N/A	Medium	N/A	
Combine cDNA & DNA, Prime and Load	Processing	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1.3.1a	0.8	1	N/A	N/A	Medium	N/A	
EC Analysis cDNA and DNA	Measurement	Bio Particle Sep. | Phase Sep | Disrupt | RNAconvert | Purify + Concentrate | Tag | Combine + Prime + Load | EC Analysis (cDNA + DNA)	N4.1.3.1.2*	1	1	0.00000001	0.03	Medium	g	10 ng cDNA and DNA
Add Solvent or Melt	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | Prime + Load | EC Analysis (DNA)	N5.1	1	0.8	N/A	N/A	Medium	N/A	
Separate Bio from Non-Bio Particles	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | Prime + Load | EC Analysis (DNA)	N5.1a	0.8	1	N/A	N/A	Medium	N/A	

Nucleic Acids

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Biosignature Efficiency (Dimension-less)	Volume Concentra-tion Factor (Dimension -less)	Detection Limit (mol/mL or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Units	Justifications/ References
Phase Separation - Liquid	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | Prime + Load | EC Analysis (DNA)	N5.1b	1	1	N/A	N/A	Medium	N/A	
Disrupt/Lyse	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | Prime + Load | EC Analysis (DNA)	N5.1c	1	1	N/A	N/A	Medium	N/A	
Purify & Concentrate	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | Prime + Load | EC Analysis (DNA)	N5.1d	0.7	36	N/A	N/A	Medium	N/A	Chosen to reach vol of 35 uL at analysis
Tagging/Add Adapters	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | Prime + Load | EC Analysis (DNA)	N5.1.3	1	1	N/A	N/A	Medium	N/A	
Prime and load	Processing	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | Prime + Load | EC Analysis (DNA)	N5.1.3.1	0.8	1	N/A	N/A	Medium	N/A	
EC Analysis DNA	Measurement	Bio Particle Sep. | Phase Sep | Disrupt | Purify + Concentrate | Tag | Prime + Load | EC Analysis (DNA)	N5.1.3.1.3*	1	1	0.00000001	0.03	Medium	g	10 ng DNA

Appendix D. Isotopic Ratios

While stable isotopic compositions are not typically used as stand-alone biosignatures, they can provide powerful confirmation of biological processes within a broader contextual framework. A thorough exploration of potential isotope bio/geosignatures is beyond the scope of this study. While many isotope systems have been proposed to determine biogenicity, interpretation of isotopes as biosignatures is highly dependent on context. Therefore, we chose to focus on C isotopes of methane as a biosignature because this measurement has been implemented on another planetary surface, and, thus, system parameters dealing with collection, concentration, and measuring these isotopes are well known (e.g., Kueter et al., 2025). In addition, the dual and clumped isotopes of methane are diagnostic of biogenicity in ways that bulk C isotopes of solids are not (Sivan et al., 2025). Other potential isotope measurements include bulk C, N, S, of solid materials (e.g., Franz et al., 2020). Other promising measurements include position specific isotopes (Fox et al., 2021).

Isotopic measurements of gas, liquid, and solid substances each require different processes, which are further refined depending on whether a bulk (e.g., total carbon) or compound-specific isotopic signature is desired. Existing technology to measure isotopic signatures *in situ* and with the highest precision requires conversion of the sample to the gas phase. This is usually achieved through heating or laser ablation, or in the case of a liquid, equilibration with headspace. Once in the gas phase, the isotopic composition of the sample can be measured using mass spectrometry (MS), absorption spectroscopy, or other applicable methods.

Because isotope techniques are usually highly tailored toward targeted measurements, here we focus on the workflow for a single sample already in the gas phase or as a solid (**Figure 31**). The carbon and hydrogen isotopic composition of methane can fingerprint its formation mechanism and/or source, (e.g., Lollar and McCollom, 2006). In particular, the "clumped" carbon and hydrogen isotope composition of methane is a powerful tracer of the methane source and formation mechanism (e.g., Young et al., 2017). This measurement has been proposed for Enceladus as well as Mars missions (Taubner et al., 2018).

D.1 Measurement approach workflow overview

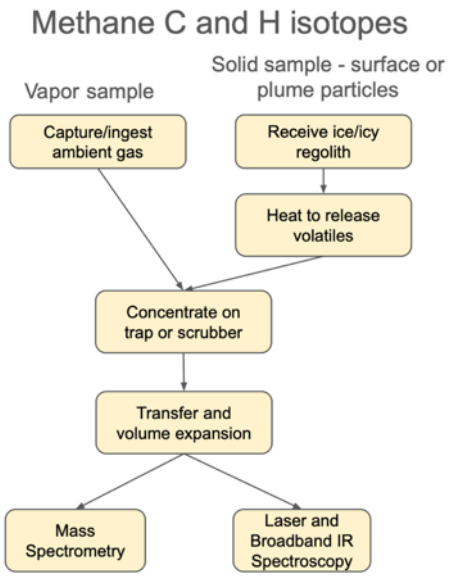


Figure 31. Isotopic ratios HiDAG showing the measurement approach workflows.

Capture/concentration

Detecting trace quantities of targeted compounds, such as methane, in the Martian atmosphere, the plumes of Enceladus/Europa, or in icy solid samples (e.g., Martian permafrost or Europa/Enceladus near-surface samples) presents significant challenges because of their low abundances. Once collected, solid samples can be heated to release volatiles for processing (the efficiency depends on whether methane is trapped in rock or ice and whether it is chemically bound or free, but it probably does not exceed a factor of 0.9), while vapor samples are ingested directly. Incorporating a concentration step as part of gas ingestion or capture by flowing a gas through a trap — for example, chemical scrubbing [e.g., Sample Analysis at Mars (SAM) for methane enrichment], cold trapping, or a combination of the two — enhances detection capability. However, alternative methods, including multiple ingestions of gas into a system based on pressure differential or exposure of the trap to the local environment over a period of time, can also be used to concentrate compound signatures by increasing trapping efficiency (e.g., Kueter et al., 2025). Each method should be characterized for its trapping efficiency or enrichment factor, which depends greatly on parameters such as trap size and temperature. For example, the trap and scrubber system on the SAM instrument on Curiosity can enrich methane for measurement by a factor of approximately 23 because it allows for both trapping of methane and removal of other gas species over multiple ingestions (Webster et al., 2015). While the overall effect of trapping is to concentrate a compound's signature, trapping and release of gases is never 100% efficient, so our model includes an efficiency factor of 0.7 to represent a reasonably efficient system, based on our laboratory experience and with the SAM instrument on the Curiosity rover.

Sample handling

During capture and concentration, gases are restricted to a smaller volume. Gases are then transported either by pressure differential or by a carrier gas to the detector, which has distinct implications for detection and measurement. Volumetric expansion occurs during transportation to the detector as gases are released from a trap through heating. This causes the gas to expand and equilibrate into the body of the detector cavity or cell, resulting in signal dilution, but allows highly repeatable static measurements that enhance precision. The resulting decrease in signal strength depends on the ratio between the trapping volume and expansion volume, which is dependent on system architecture; we use a straw man efficiency factor of 0.9, reflecting only ~10% expansion, as an example of minimal dilution and maximum efficiency. If a carrier gas is used and the trap is heated under flow, a "plug" of gas sample will flow through the detector where it will be detected as a temporary increase in signal. This method of "continuous flow" is often used in commercial instruments but is less common/lower flight suitability.

Detection

Many examples of stable isotope detectors exist in the literature, but few are as routinely used in the laboratory and as well understood as MS and absorption spectroscopy. Both are high suitability for spaceflight (e.g., Webster et al., 2023; Waite et al., 2024) and are being further refined for future mission concepts. High-flight-suitability absorption spectroscopy methods such as cavity ring-down spectroscopy (CRDS) and tunable laser spectroscopy (TLS) are well suited and in development for the specific measurement of clumped methane isotopes (Webster et al., 2023; Zhang et al., 2025; Liu et al., 2026). These methods leverage laser-based techniques to resolve isotopic signatures and are currently under development for spaceflight with applications targeting the astrobiological investigation of extraterrestrial environments. Their ability to measure the isotopic compositions of methane *in situ* may provide new pathways for *in situ* isotopic analyses. Measurement techniques and interpretation frameworks for other isotopes, e.g., position-specific isotopes, are also under investigation and could be considered for detection technologies; however, evaluation of these techniques and frameworks is outside the scope of this study.

Table 7. Isotopic ratio measurement approach workflows and analysis workflow IDs

Isotopic Ratios 1 of 2	
Solid_Orbitrap MS, TOF, QMS	**Gas_Orbitrap MS, TOF, QMS**
Heat to release volatiles	Ingest vapor
Concentrate on trap or scrubber	Concentrate on trap or scrubber
Transfer and Volume Expansion	Transfer and Volume Expansion
MS Analysis (I1.1c*)	**MS Analysis (I2.1c*)**

Isotopic Ratios 2 of 2	
Solid_Spectroscopy	**Gas_Spectroscopy**
Heat to release volatiles	Ingest vapor
Concentrate on trap or scrubber	Concentrate on trap or scrubber
Transfer and Volume Expansion	Transfer and Volume Expansion
Spectroscopy Analysis (I3.1c*)	**Spectroscopy Analysis (I4.1c*)**

Table 8. Isotopic ratio parameter values for each measurement workflow step

Isotopic Ratios									
Function Name	**Type**	**Measurement Approach Workflow Name**	**Workflow ID**	**Efficiency (Dimension-less)**	**Concentration Factor (Dimension-less)**	**Detection Limit (mol/mL, or ppw=g/g)**	**Minimum Quantity (g or mL)**	**Flight Suit-ability**	**Justifications/ References**
Heat to release volatiles	Processing	(Solid) Heat | Concentrate | Transfer & Expand | MS Analysis	I1.1	0.9	1	N/A	N/A	High	Heritage from SAM, TEGA on Phoenix, Viking
Concentrate on trap or scrubber	Processing	(Solid) Heat | Concentrate | Transfer & Expand | MS Analysis	I1.1a	0.7	10	N/A	N/A	Medium	SAM (Webster et al., 2015; Webster and Mahaffy, 2011)
Transfer and Volume Expansion	Processing	(Solid) Heat | Concentrate | Transfer & Expand | MS Analysis	I1.1b	0.9	0.6	N/A	N/A	Medium	King et al., 2025; King et al., 2026
MS Analysis	Measurement	(Solid) Heat | Concentrate | Transfer & Expand | MS Analysis	I1.1c*	1	1	ppm/vol	100 mL	High	SAM (Mahaffy et al., 2012)
Ingest vapor	Processing	(Gas) Ingest | Concentrate | Transfer & Expand | MS Analysis	I2.1	1	1	N/A	N/A	Medium	King et al., 2025; King et al., 2026
Concentrate on trap or scrubber	Processing	(Gas) Ingest | Concentrate | Transfer & Expand | MS Analysis	I2.1a	0.7	10	N/A	N/A	Medium	SAM (Webster et al., 2015; Webster and Mahaffy, 2011)
Transfer and Volume Expansion	Processing	(Gas) Ingest | Concentrate | Transfer & Expand | MS Analysis	I2.1b	0.9	0.6	N/A	N/A	Medium	King et al., 2025; King et al., 2026
MS Analysis	Measurement	(Gas) Ingest | Concentrate | Transfer & Expand | MS Analysis	I2.1c*	1	1	ppm/vol	100 mL	High	SAM (Mahaffy et al., 2012)
Heat to release volatiles	Processing	(Solid) Heat | Concentrate | Transfer & Expand | Spectroscopy	I3.1	0.9	1	N/A	N/A	High	Heritage from SAM, DraMS, TEGA on Phoenix, Viking
Concentrate on trap or scrubber	Processing	(Solid) Heat | Concentrate | Transfer & Expand | Spectroscopy	I3.1a	0.7	10	N/A	N/A	Medium	SAM (Webster et al., 2015; Webster and Mahaffy, 2011)
Transfer and Volume Expansion	Processing	(Solid) Heat | Concentrate | Transfer & Expand | Spectroscopy	I3.1b	0.9	0.6	N/A	N/A	Medium	King et al., 2025; King et al., 2026

Isotopic Ratios

Function Name	Type	Measurement Approach Workflow Name	Workflow ID	Efficiency (Dimension-less)	Concentration Factor (Dimension-less)	Detection Limit (mol/mL, or ppw=g/g)	Minimum Quantity (g or mL)	Flight Suit-ability	Justifications/ References
Spectroscopy	Measurement	(Solid) Heat | Concentrate | Transfer & Expand | Spectroscopy	I3.1c*	1	1	10-100 ppb/vol	500 mL	High	TLS (Webster et al., 2015; Webster and Mahaffy, 2011; Kriesel et al., 2023)
Ingest vapor	Processing	(Gas) Ingest | Concentrate | Transfer & Expand | Spectroscopy	I4.1	1	1	N/A	N/A	Medium	King et al., 2025; King et al., 2026
Concentrate on trap or scrubber	Processing	(Gas) Ingest | Concentrate | Transfer & Expand | Spectroscopy	I4.1a	0.7	10	N/A	N/A	Medium	SAM (Webster et al., 2015; Webster and Mahaffy, 2011)
Transfer and Volume Expansion	Processing	(Gas) Ingest | Concentrate | Transfer & Expand | Spectroscopy	I4.1b	0.9	0.6	N/A	N/A	Medium	King et al., 2025; King et al., 2026
Spectroscopy	Measurement	(Gas) Ingest | Concentrate | Transfer & Expand | Spectroscopy	I4.1c*	1	1	10-100 ppb/vol	500 mL	High	TLS (Webster et al., 2015; Webster and Mahaffy, 2011; Kriesel et al., 2023)

SAM = Sample Analysis at Mars (Mahaffy et al., 2012)
TLS = tunable laser spectroscopy (Webster et al., 2015; Webster and Mahaffy, 2011)

D.2 Findings for Future Technology Advancements

Table 9 shows the results for isotopic ratio measurement approach workflow cumulative volume concentration and biosignature mass processing efficiencies. The ability to make detections of isotopic ratios in the Mars and Europa/Enceladus environments would be determined by the amount of this biosignature within the sample and these processing efficiencies.

Table 9. Cumulative volume concentration factor and cumulative biosignature mass efficiencies for each isotopic ratio measurement approach workflow

Isotopic Ratio Measurement Approach	Cumulative Volume Concentration Factor (Dimensionless)	Cumulative Mass Efficiency (Dimensionless)
Solid_MS	12	0.57
Gas_MS	12	0.63
Solid_Spectroscopy	12	0.57
Gas_Spectroscopy	12	0.63

Of greatest importance to enabling isotopic ratio measurements is improving the sample capture capability and concentration techniques to increase the presence of the biosignatures. The cumulative volume concentration factor could be improved by increasing trapping efficiency or enrichment factor, which depends greatly on parameters such as trap size and temperature. Traps, scrubber systems, removal of other gas species, and ingestion of multiple samples can enrich the biosignature of interest for measurement.