Solid Phase Micro Extraction: Potential for Organic Contamination Control for Planetary Protection of Life Detection Missions to the Icy Moons of the Outer Solar System

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Running title: SPME: Planetary Protection of Icy Moons

1. Abstract

Conclusively detecting, or ruling out the possibility of, life on the icy moons of the outer solar system will require spacecraft missions to undergo rigorous planetary protection and contamination control procedures to achieve
extremely low levels of organic terrestrial contamination. Contamination control is necessary to avoid forward contamination of the body of interest and to avoid the detection of false positive signals which could either mask indigenous organic chemistry of interest or cause an astrobiological false alarm. Here we test a new method for rapidly and inexpensively assessing the organic cleanliness of spaceflight hardware surfaces using solid phase micro extraction (SPME) fibres to directly swab surfaces. The results suggest that the method is both time and cost efficient. The SPME-gas chromatography mass spectrometry (GC-MS) method is sensitive to common mid-weight, non-polar contaminant compounds, e.g. aliphatic and aromatic hydrocarbons, which are common contaminants in laboratory settings. While we demonstrate the potential of SPME for surface sampling, the GC-MS instrumentation restricts the SPME-GC-MS technique’s sensitivity to larger polar and non-volatile compounds. Although not used in this study, to increase the potential range of detectable compounds, SPME can also be used in conjunction with high performance liquid chromatography/liquid chromatography-mass spectrometry systems suitable for polar analytes [Kataoka et al., 2000]. Thus, our SPME method presents an opportunity to monitor organic contamination in a relatively rapid and routine way that produces information-rich data sets.

**Key Words:** SPME; Planetary Protection; Organic Contamination; Life Detection; Icy Moons

2. **Introduction**
Numerous past, current and future space missions had and have the detection of extraterrestrial organic matter as a primary goal. Conclusive detection requires the avoidance of forward contamination of the bodies of interest. An extremely low-level of organic (both biological and non-biological) terrestrial contamination is necessary; which translates to a high level of cleanliness for all parts of the spacecraft that may come into contact with samples for analysis [e.g. Blakkolb et al., 2014].

Minimising the contributions of organic contaminants, termed ‘contamination control’, is required for life detection missions. Recently the search for life has turned towards the icy moons of the Jovian and Saturnian systems [Reynolds et al., 1983; Task Group on the Forward Contamination of Europa, 2000; McKay et al., 2008; Parkinson et al., 2008]. False positives (indicating life or pre-biotic chemistry) caused by the detection of organic contaminants could either mask indigenous organic chemistry of interest or cause an astrobiological false alarm leading to unwarranted stringent planetary protection requirements for future missions [Task Group on the Forward Contamination of Europa, 2000; Mahaffy et al., 2003]. It should also be noted that avoiding forward contamination is also important for the reliability of analytical results from organic matter detection missions exploring bodies where we do not expect to find evidence of past or present life, such as those exploring asteroid or cometary bodies [Drake et al., 2011; Nakamura et al., 2012; Tsuda et al., 2013; Westphal et al., 2014; Lauretta et al., 2015].
Contamination knowledge includes the documentation of all known potential organic contaminants to ensure contaminating molecules are not mistaken for compounds of interest during sample analysis. It is useful to define contaminants, in a planetary protection contamination control sense, as those substances that can be detected and that could cause issues for the current life detection mission. As such, contamination control for astrobiology missions may focus on a limited range of key compounds (Table 1).

Organic contamination control in respect to planetary protection is only concerned with affecting the results of the current science mission and is not concerned about contaminating the body itself and the effects on future science missions because, unlike biological contamination control, the compounds of interest are not expected to be self-replicable and would, most probably, stay localised or if dispersed in a liquid medium, such as the subsurface ocean of an icy moon, would simply dilute to undetectable concentrations.

Spacecraft are typically cleaned to a non-volatile residue cleanliness of 1 µg/cm² which, based on the IEST-STD-CC1246D standard, is level A. For astrobiologically-sensitive parts of a mission, such as the Mars Science Laboratory (MSL) sample handling chain [Mahaffy et al., 2003], 100 ng/cm² or level A10 is the standard. The Viking sample handling hardware was cleaned to 1 ng/cm² or Level A1000 – although this is an extreme example, it may well be the level necessary for life detection missions where the types and
concentrations of analytes are uncertain, such as with missions to the icy moons.

To achieve these high levels of cleanliness a variety of techniques are employed:

All work is carried out under clean room conditions. A typical aerospace cleanroom is class 100,000 or ISO 8 (i.e. contains 100,000 or fewer particles of 0.5 \( \mu \text{m} \) in diameter per cubic foot of air, controlled by High Efficiency Particle Air (HEPA) filters and maintaining a positive air pressure between the inside and the outside environments. For the assembly of the most sensitive flight hardware, class 10 or ISO 4 cleanrooms may be employed, containing less than 10 0.5 \( \mu \text{m} \) particles per cubic foot of air and in these environments personnel must be isolated by wearing clean suits at all times.

Precision cleaning is the series of processes targeted at removing both particles and molecular films of organic contaminants. Firstly, visible contamination is wiped from the surface. Secondly, a series of rinses with organic and aqueous solvents of varying polarities is performed – which may be coupled with ultrasonic treatment to liberate any contaminants adhering to the surfaces. Thirdly, Freon vapour is used for degreasing. Fourthly, isopropyl alcohol rinses are performed and analysed for remaining particulate levels. The overall process of precision cleaning commonly achieves Level 100 cleanliness, high enough in most cases [Mahaffy et al., 2003]. The use of solvents is not suitable for all materials used in spacecraft assembly (e.g. plastics and polymers can be
dissolved by some solvents) so other techniques are also employed in these cases, using plasma, accelerated CO$_2$ snow, radiation or electron beams to remove organic compounds [e.g. Task Group on the Forward Contamination of Europa, 2000; Committee on preventing the forward contamination of Mars, 2006; ten Kate et al., 2008; Dworkin et al., 2017]. Repeated wiping of surfaces with clean room cloths saturated with isopropyl or ethyl alcohol during assembly prevents the re-build-up of molecular contaminants.

Thermal bakeout is the concluding step to both remove surface contaminants and reduce subsequent outgassing of organic impurities within the materials. Depending on the material, bakeouts may range from 70 °C to 105 °C and last 72 to over 160 hours. The Viking One Lander was baked out after the final assembly as a terminal sterilization step at 112 °C for 30 hours [Martin, 1975].

Monitoring clean room and hardware organic cleanliness is currently a complex process often involving multiple, expensive and time-consuming techniques. [Task Group on the Forward Contamination of Europa, 2000; Mahaffy et al., 2003; Blakkolb et al., 2014; Li et al., 2015]. Effective monitoring is confounded further by the fact that, in contrast to biological contamination, there are currently no strictly defined quantitative limits for organic contamination control. The lack of well-defined guidance is partly due to the fact that the cleanliness level has to be appropriate to the sensitivity of the instruments of the specific mission. If the instrument cannot detect the contaminants they are not an issue. However, initially undetectable...
compounds may be transformed into more problematic species by the harsh environments encountered during the long cruise phase, in-orbit or (if applicable), once landed on the surface. This transformation of organic molecules into other, more problematic, species is particularly a problem for the moons of the Jovian and Saturnian systems due to the increased and highly variable radiation environment the spacecraft will encounter throughout the life of the mission and the highly oxidative surface environment if the mission incorporates a landed element [e.g. Cassidy et al., 2010; Johnson et al., 2012; Kimura and Kitadai, 2015]. With the numerous unknowns associated with exploration of the icy moons, this potential complication highlights the importance of cleanliness.

The OSIRIS-REx sample return mission to the asteroid Bennu had a strict contamination control plan. Procedures to limit the total contamination burden on the returned sample were put in place to limit sensitive surfaces to cleanliness levels (established in IEST-STD-CC1246D) at the 100A/2 level [Borson, 2005]. In addition, due to the unique mission science objectives, specific contaminants of concern were limited to a total accumulation of 180 ng/cm² on the most sensitive surfaces of the sample handling chain [Lauretta et al., 2017]. While not planetary protection-related, optical instruments often have the most stringent organic contamination limits. For instance, for the Hubble Wide Field Planetary Camera, recontamination is limited to a rate of
47 ng/cm²/month and the camera undergoes a monthly decontamination cycle
that reduces the level to 1 ng/cm² [Hedgeland et al., 1994].

Good examples of organic cleanliness monitoring are available from missions
to Mars. Multi-stepped solvent extraction, followed by pre-concentration of
analytes (by evaporation) and analysis by diffuse reflectance infrared Fourier
transform (DRIFT) spectroscopy, Fourier transform infrared (FTIR)
spectroscopy and pyrolysis-gas chromatography-mass spectrometry (Py-GC-
MS) techniques were carried out on swabs from surfaces of the MSL sample
transfer chain hardware at various stage of construction [Blakkolb et al.,
2014]. The use of multiple solvents, however, complicated the analysis of the
data and diluted the contaminants of interest, reducing sensitivity of the
detection. The whole process of extraction, concentration and analysis was
also very time consuming and therefore costly. A similar process to that
employed for MSL is proposed for Mars 2020 [Table 2, Summons et al., 2014]

Various culture dependant assays [Benardini Iii et al., 2014a, 2014b] and
culture independent methods (such as 16s RNA-based diversity; next
generation sequencing; viability-linked metagenomics assays (propidium
monoazide treatment; quantitative polymerase chain reaction) [La Duc et al.,
2004, 2009; Nellen et al., 2006; Probst et al., 2012] have been used to track
the microbial bioburden present on flight instrument surfaces. While the
NASA/ESA standard assay technique [Morris et al., 2010] is a good example
of a standardised planetary protection contamination control method that is
missing from non-biological contamination control, these techniques give no
description of the non-biological organic contamination present.

A diagnostic organic contamination monitoring process is needed. While
useful, witness plates [ten Kate et al., 2008] can only show what is
condensing/falling onto clean metal surfaces, they cannot show transfer from
hands/gloves as they are not handled in the same way as the actual flight
hardware. A standardised technique to directly sample the flight hardware
surfaces in addition to the atmosphere itself, which is rapid, inexpensive and
easy to use would be very useful (alongside the use of witness materials) in
keeping track of clean room cleanliness on a regular basis.

Solid phase micro extraction (SPME) is a sample preparation method
developed for the analysis of organic compounds [Arthur and Pawliszyn,
1990]. In the analysis for organic compounds by SPME, a fused silica optical
fibre coated with liquid organic polymer or solid sorbent is exposed to the
sample matrix wherein a distribution equilibrium of the analytes is established
between the matrix and the coating; this combines sampling and pre-
concentration of analytes into a single step [Harper, 2000]. The analytes
collected are thermally desorbed, in the injector of a gas chromatograph for
analysis. SPME is advantageous for the analysis of organic compounds due to
its high speed (extraction time can be reduced to a few minutes instead of the
hours/days of classical liquid-liquid extraction methods), low cost, elimination
of solvents from both the extraction and analysis steps (although a solvent may
be used instead of thermal desorption for analysis [Arthur and Pawliszyn, 1990]), portability, applicability to gaseous, liquid or solid samples and relative independence of destined analytical instrument design [Arthur and Pawliszyn, 1990; Louch et al., 1992; Otu and Pawliszyn, 1993].

In this study we assessed whether SPME could be employed as a standardised inexpensive, rapid and accurate technique for monitoring the lab atmosphere and flight hardware surfaces for organic contamination control and general cleanliness for future life and organic matter detection space missions. We developed a method to effectively sample hardware surfaces. We assessed sensitivity for detecting organic compounds that have been identified as contaminants of interest for astrobiological missions. This study is timely as there are currently numerous life and/or organic matter detection missions to the icy moons of the outer solar system in various stages of planning and implementation [Powell et al., 2005; Erd, 2012; Pappalardo et al., 2013; Dachwald et al., 2014; Phillips and Pappalardo, 2014; Konstantinidis et al., 2015].

3. Materials and Methods

3.1. SPME fibre selection and sampling procedure development

We chose 30 µm coating thickness polydimethylsiloxane (PDMS) coated SPME fibres (Supelco, USA) as these were suited to detecting the greatest range of non-polar compounds of interest. Other SPME fibre types with different coatings and coating thicknesses could be selected for more specific
contaminants of interest based on the manufacturer’s recommended usage (Table 3).

3.2. SPME fibre preparation

SPME fibres were held in a manual SPME holder. Prior to the use of the SPME fibres they were conditioned by heating in the inlet of the GC for 45 minutes at 300 °C.

3.3. Standard compound selection

Standard organic compounds of interest were selected based on those identified as problematic for astrobiological missions by Mahaffy et al. (2003), Table 1. Only the non-polar compounds were selected to be tested as the more polar compounds would require techniques such as SPME-high performance liquid chromatography (SPME-HPLC) or SPME-liquid chromatography-mass spectrometry (SPME-LC-MS) [Kataoka et al., 2000], which is beyond the scope of this study.

The mid-length C_{18} alkene and alkane compounds 1-octadecene and octadecane, three ring polycyclic aromatic hydrocarbon (PAH) phenanthrene (C_{14}H_{10}), the saturated C_{18} fatty acid octadecanoic acid (CH_{3}(CH_{2})_{16}COOH), the triterpene squalene (C_{30}H_{50}) and the sterol cholesterol (C_{27}H_{46}O) (Figure 1) were selected for their relatively low volatility and non-polarity (to varying degrees).

3.4. Surface spiking
The test surfaces used were the flat portions of a 316 stainless steel cap from Swagelok® (part number SS-20M0-C).

Stainless steel surfaces were prepared for spiking by sonicating the Swagelok® caps in propan-2-ol (isopropyl alcohol; IPA) (HPLC plus grade 99.9%, Sigma-Aldrich) for 10 minutes and then heating them overnight (minimum 15 hours) at 125 °C, to replicate dry heat microbial reduction (DHMR) and ‘bake out’ any initial organic contaminants [Pflug, 1971].

After cleaning, the stainless steel samples were only handled with nitrile gloves that had been wiped with IPA, with no contact being made with the surfaces for analysis. The samples were also kept wrapped in aluminium foil (which had undergone the same overnight heat treatment) between experimental steps to prevent fallout of airborne contaminants onto the surfaces.

10 µl of the solutions of the individual standard organic compounds in dichloromethane (DCM; 99.8% distol-pesticide reagent grade, Fisher Scientific) were syringed onto the stainless steel surface to give contamination levels corresponding to A (1000 ng/cm²), A10 (100 ng/cm²), A100 (10 ng/cm²), A1000 (1 ng/cm²) and the DCM was allowed to fully evaporate.

During the external standard calibration phase of the experimental procedure, if a compound was below the limit of detection (<LOD) at an analysed concentration by liquid injection, the relevant contamination level was not tested (nor contamination levels below that) for SPME-GC-MS sensitivity, as
sensitivity to that compound at astrobiologically relevant levels was shown to be limited by the GC-MS method.

3.5. SPME sampling procedure

Prior to swabbing 5 µl of IPA was syringed onto the test surface. The activated SPME fibre was used to rub over this ‘wetted’ test surface to aid transfer of IPA soluble contaminants from the surface to the fibre. Each experiment was carried out 4 times to test reproducibility of the technique.

Experimental blanks (referred to in the results as ‘surface blanks’) were performed by carrying out the sampling procedure on the test surface after the cleaning procedure, without spiking with the standard compounds.

3.6. SPME-Gas Chromatography-Mass Spectrometry (SPME-GC-MS)

Analysis of the volatiles adsorbed onto the SPME fibre was carried out via SPME-GC-MS analysis. The SPME fibre was inserted directly into the injector of a Perkin Elmer Clarus 580 gas chromatograph coupled to a Clarus SQ85 mass spectrometer (GC-MS). Analytes were desorbed from the SPME fibre for 10 minutes into the injector, which was operated in split mode with a 20:1 split ratio and held at 290 °C, with a column flow rate of 1.1 ml min⁻¹. Separation was performed on a J&W DB-5 ((5%-Phenyl)-methylpolysiloxane) column (30 m x 250 µm x 0.25 µm). The GC oven was held for 2 min at 60 °C and then ramped at a rate of 10 °C min⁻¹ to 310 °C where it was held for 5 min.
Mass spectra were acquired simultaneously in full scan (45-550 m/z) and selective ion monitoring (SIM), the ions detected were m/z 55, 57, 69, 73, 178, 217 with a dwell time of 50 ms. Recoveries were calculated from external standards injected in solution under the same conditions.

Analytical blanks were performed by the insertion of the SPME fibre into the injector of the GC-MS directly after activation.

4. Results

Integrated peak area in the extracted ion chromatogram of the characteristic ion fragment for that compound (m/z 55 = 1-octadecene; m/z 57 = octadecane and octadecanoic acid; m/z 69 = squalene; m/z 178 = phenanthrene; m/z 217 = cholesterol) was used as a proxy for relative detectability (data used for calculation shown in Table 4). Total ion chromatograms were used to confirm peak identity.

The SPME recovery was thereby calculated by comparing the peak areas produced via SPME-GC-MS (using the average of 4 SPME swabbings) against that which was expected based on the liquid injection calibration curve and expressing this as a percentage (Table 4).

SPME-GC-MS selected ion current chromatograms produced for all compounds are shown in Figures 2-7.

5. Discussion
5.1. Relative sensitivity of SPME-GC-MS relative to liquid injection GC-MS calibration

While recovery was reduced, in the majority of cases, if a standard organic compound was detected by the liquid injection technique, it was also detected by SPME-GC-MS at the equivalent surface spiking concentration. The only cases where SPME-GC-MS failed to recover a compound over the LOD which had been detected by liquid injection (at the relevant concentration) were at the lowest concentrations detectable (by liquid injection) for octadecane, 1-octadecane, octadecanoic acid and cholesterol. There are in fact low responses at the correct retention times in the SPME-GC-MS selected ion chromatograms for A1000 level octadecane and 1-octadecene and A1 level cholesterol that do not appear in the surface blanks. These low responses are, however, too weak to be quantifiable.

This all suggests that the limiting factor in this technique, at astrobiologically relevant contamination levels, is the sensitivity of the GC-MS technique used.

Variation in the percentage recovery by the SPME technique between compounds is likely due to the partition co-efficient between the compound, the IPA solvent and the fibre coating. The more polar compounds such as octadecanoic acid (with its polar carboxyl group) are less soluble in IPA and so more likely to stick to the stainless steel surface, reducing recovery.

5.2. Reproducibility – True positive rate (sensitivity)
Whilst quantitative reproducibility of the SPME-GC-MS technique was poor and standard deviation between repetitions was high (Table 4), qualitative reproducibility was good. A standard organic compound at a certain contamination level was either detected in all of the SPME swabs or none, this is expressed in terms of sensitivity.

Sensitivity and Specificity are statistical terms relevant in life detection missions in the solar system, and specifically those to the Icy Moons [Sephton et al., 2018].

The sensitivity (or true positive rate) of a technique is its ability to make a correct detection of organic matter, this is investigated by calculating the rate of true positive detections in a sample with known composition.

\[
\text{Sensitivity} = \frac{\# \text{ true positives}}{\# \text{ true positives} + \# \text{ false negatives}}
\]

Specificity (or the true negative rate) is the technique’s ability to correctly identify a negative response, i.e. not detecting a false positive in a blank sample.

\[
\text{Specificity} = \frac{\# \text{ true negatives}}{\# \text{ true negatives} + \# \text{ false positives}}
\]

A true negative represents a null detection in a sample that contains nothing, an example is the surface blanks after the steel test surface had undergone the cleaning procedures. A null detection in this context is a sample analysis producing no detectable compounds other than the IPA used in the method.
As all surface blanks showed no detectable compounds the specificity of the technique for surfaces that have undergone contamination control was 100%.

Measurements of the spiked test surfaces were used to investigate the sensitivity of the technique for different standard compounds and concentrations. In this case, detecting the standard compound, e.g. squalene, on the spiked test surface would represent a true positive whereas not detecting the standard compound would represent a false negative (as it is known to be present). Sensitivity of the SPME sampling technique was found to be highly variable between the compounds tested and the results are shown in table 5.

However, as sensitivity was either 100% or 0% (compounds were either detected in all 4 replicates or not at all) the reproducibility of the SPME-GC-MS technique was excellent.

5.3. Standard organic compound relative selectivity and contamination control relevance

The SPME-GC-MS technique tested was found to have variable sensitivity to the different standard organic compounds tested. Based on the characteristics of the molecules (Table 6) it is possible to say something about the potential of this technique in detecting different classes of compounds relevant to organic contamination control for planetary protection.

The SPME-GC-MS method employed here proved very sensitive to phenanthrene from levels A to A1000 where there has been a 100%
successful positive detection. Phenanthrene is a typical PAH, there is great interest in PAHs from an astrobiological point of view as evidence of their presence has been detected in meteorites [Sephton, 2002], atmospheric hazes [Trainer et al., 2004] and even interstellar space [Tielens, 2008] so are important in understanding prebiotic chemistry. However, as PAHs are common products of combustion processes, especially diesel (and to a lesser extent) petrol exhaust emissions [Haefliger et al., 2000; Botta et al., 2008] they are often recorded as contaminants in otherwise organically-lean environments/samples [Botta et al., 2008; Calaway et al., 2014]. PAHs are often a component of particulate contamination, falling out from the atmosphere onto surfaces [Giger and Schaffner, 1978; Hodge et al., 2003], keeping track of particulate contamination on actual spaceflight surfaces throughout the build, as well as just on witness plates, is important to track change over time (effectiveness of cleaning procedures, etc.) and in case differences in molecular affinity to the surfaces or airflow patterns cause differential distribution of contaminants.

The SPME-GC-MS method also proved very sensitive to squalene from levels A to A1000 where there was a 100% successful positive detection rate. This is likely to be as a result of squalene being reasonably volatile and non-polar. The effectiveness of the SPME-GC-MS technique in detecting squalene at a range of concentrations will be particularly useful in planetary protection contamination control. Squalene is one of the major components of human
sebum [Kim and Karadeniz, 2012] and a terrestrial contaminant attributed to human contamination. Human sebum will inevitably cause a false positive in life detection missions therefore effective monitoring to check the cleanliness of spacecraft hardware is fundamentally necessary. At a minimum, gloves that are wiped off with IPA should be worn at all times to avoid human contamination and this SPME-GC-MS technique proves a quick and effective way to check for accidental human recontamination of surfaces. Octadecane and 1-octadecane are typical mid-chain length aliphatic hydrocarbons which are common contaminants of biological source, from the breakdown of biopolymers, for example from (terrestrial) microbial life [Biller et al., 2015], organic oils such as those in lubricants [Grosjean and Logan, 2007], for example in vacuum pumps that are present in laboratory settings [Illing et al., 2014], plastic polymers [Grosjean and Logan, 2007; Brocks et al., 2008], diesel fumes [Hauser and Pattison, 2019]. The detection of pairs of alkenes and alkanes, especially at these longer chain lengths, could thus be taken as an indicator of biological activity if detected on mission leading to a false positive life detection. Hints of their presence in the A1000 level SPME-GC-MS selected ion chromatograms suggest that with a more sensitive GC-MS method (lower split ratio, etc.) these would be above detection limits even at the levels of contamination necessary in the most astrobiologically-sensitive areas of a spacecraft (A1000 level).
Octadecanoic acid, and other long chain length fatty acids are often used as biomarkers as they are indicative of a biological source [O’leary, 1962; Volkman et al., 1989; Alfaro et al., 2006; Tan et al., 2018], however the polar carboxyl group makes detection via the GC-MS technique used difficult without derivatization of the molecule. Amino acids, a potential target biomarker of life-detection astrobiological missions and interesting in the context of icy moon prebiotic chemistry [Elsila et al., 2009; Martins and Sephton, 2010; Neish et al., 2010; Johnson et al., 2012; Dworkin et al., 2017] are more polar and so are not detectable by the column chromatography used, hence why they were not tested in the current study, however they too may be detectable by SPME-LC-MS or SPME-HPLC.

The lack of detection even at level A indicates that cholesterol does not desorb from the fibre, possibly due to its low volatility indicated by its large size and relatively high enthalpy of vaporization (Table 6). Large, low volatility compounds are less mobile and less likely to be transferred to surfaces in a clean lab environment and so may be not so important.

Thus the SPME-GC-MS technique employed is not suited to larger molecular weight compounds, like cholesterol, or those which are more polar, like fatty acids. However, if these larger-weight molecules are contaminants of concern for a particular mission, then SPME-LC-MS or SPME-HPLC need to be investigated to overcome the current limitations to small and volatile compounds due to the GC-MS instrumentation, potentially enabling the
detection of a much wider range of compounds at astrobiologically relevant concentrations.

Conclusion

- A new method that utilises SPME fibres to swab spacecraft hardware surfaces is demonstrated for the monitoring of cleanliness in planetary protection contaminant control procedures.
- The SPME-GC-MS method is convenient, both time and cost efficient. It can be employed into many stages of space missions.
- The SPME-GC-MS method is particularly sensitive to squalene and therefore human contamination at all levels tested.
- The SPME-GC-MS method is sensitive to common mid-weight, non-polar contaminant compounds e.g. aliphatic and aromatic hydrocarbons.
- The SPME-GC-MS method is not particularly sensitive to larger polar and non-volatile compounds as it is limited by the GC-MS instrumentation.
- While the potential of SPME for surface sampling is demonstrated here, future work needs to demonstrate the effectiveness of other desorption/detection techniques (SPME-LC-MS/HPLC) on specific compound classes, especially the higher molecular weight and polar species that are undetectable by the GC-MS technique employed here.

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7. References


Borson, N. (2005), IEST-STD-CC1246D: product cleanliness levels and contamination control program, *Clean Rooms*.


Committee on preventing the forward contamination of Mars (2006), *Preventing the Forward Contamination of Mars*, National Academies Press, Washington, D.C.


Dworkin, J. P. et al. (2017), OSIRIS-REx Contamination Control Strategy and Implementation,


Erd, C. (2012), Planetary Protection for the JUpiter ICy moons Explorer


ten Kate, I. L., J. S. Canham, P. G. Conrad, T. Errigo, I. Katz, and P. R. Mahaffy (2008), Mitigation of the Impact of Terrestrial Contamination on


Li, X., R. M. Danell, W. B. Brinckerhoff, V. T. Pinnick, F. van Amerom, R. D. Arevalo, S. A. Getty, P. R. Mahaffy, H. Steininger, and F. Goesmann (2015), Detection of Trace Organics in Mars Analog Samples Containing
Perchlorate by Laser Desorption/Ionization Mass Spectrometry,


Louch, D., S. Motlagh, and J. Pawliszyn (1992), Dynamics of organic
compound extraction from water using liquid-coated fused silica fibers,


Steering Group*.

Martin, J. S. (1975), *Viking '75 program lander capsule sterilization plan*,
Hampton, Virginia.

Martins, Z., and M. A. Sephton (2010), Extraterrestrial Amino Acids, in
*Amino Acids, Peptides and Proteins in Organic Chemistry*, pp. 1–42,
Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

The Possible Origin and Persistence of Life on Enceladus and Detection
of Biomarkers in the Plume, *Astrobiology, 8*(5), 909–919,

Morris, H. C., L. A. Monaco, A. Steele, and N. Wainwright (2010), Setting a
Standard: The *Limulus* Amebocyte Lysate Assay and the Assessment of
Microbial Contamination on Spacecraft Surfaces, *Astrobiology, 10*(8),

Nakamura, E. et al. (2012), Space environment of an asteroid preserved on


Figure 1 Standard organic compounds selected for SPME organic contamination control method development
Figure 2 SPME-GC-MS mass 57 chromatograms of octadecane spiked onto test surface at varying concentrations. Octadecane peak is at ~16 min 40 s, below LOD at A1000 contamination level, although a small peak is visible.
Figure 3 SPME-GC-MS mass 55 chromatograms of 1-octadecene spiked onto test surface at varying concentrations. 1-octadecene peak is at ~16 min 30 s, null detection at A1000 contamination level.
Figure 4 SPME-GC-MS mass 178 chromatograms of phenanthrene spiked onto test surface at varying concentrations. Phenanthrene peak is at ~16 min 40 s.
Figure 5 SPME-GC-MS mass 57 chromatograms of octadecanoic acid spiked onto test surface at varying concentrations, experiments at concentrations equal to A1000 and A100 were not run as the liquid injection experiments showed the GC-MS method to be insufficiently sensitive at these concentrations. Octadecanoic acid peak is at ~20 min 10 s, below LOD at A1000 contamination level, background peak at 20 min 40 sec appears in blank and may obscure small octadecenoic acid peak.
Figure 6 SPME-GC-MS mass 69 chromatograms of squalene spiked onto test surface at varying concentrations. Squalene peak is at ~25 min 45 s.
Figure 7 SPME-GC-MS mass 217 chromatograms of cholesterol spiked onto test surface at varying concentrations, experiments at concentrations equal to A1000, A100, A10 were not run as the liquid injection experiments showed the GCMS method to be insufficiently sensitive at these concentrations. Cholesterol peak is at ~29 min 20 s, this is below LOD even at A1 contamination level, although a small broad hump is observed corresponding with this retention time. The positive detection of cholesterol in the 1:1 (mg to ml) liquid injected sample is shown for comparison.
### Table 1 Contaminants of concern for astrobiological missions [after Mahaffy et al., 2003]

<table>
<thead>
<tr>
<th>Molecular class</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Aromatic hydrocarbons</td>
<td>Benzene, toluene, higher molecular weight aromatics, polyaromatic hydrocarbons</td>
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<tr>
<td>S, N, O heterocyclic aromatics</td>
<td>Furan, pyridine, pyramadine, benzothiophene</td>
</tr>
<tr>
<td>Carboxylic acids &amp; their salts</td>
<td>Alkyl &amp; aromatic acids, fatty acids</td>
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<tr>
<td>Aliphatic hydrocarbons</td>
<td>Alkenes, alkanes</td>
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<td>Nitrogen containing compounds</td>
<td>Amino acids, amines, amides, purines, pyrimidines, porphyrins</td>
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<td>Alcohols</td>
<td>Methanol, higher molecular weight linear and branched chain alcohols</td>
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<tr>
<td>Carbonyl</td>
<td>Esters, ketones, aldehydes</td>
</tr>
<tr>
<td>Sulfonylic, phosphonic acids</td>
<td>Methanesulfonic acid</td>
</tr>
<tr>
<td>Lipids and derivatives</td>
<td>Hydrocarbon chains, fatty acids, fats, phospholipids, hopanes, steranes</td>
</tr>
<tr>
<td>Sugars and derivatives</td>
<td>Glucose</td>
</tr>
<tr>
<td>Proteins</td>
<td>Polar and non-polar</td>
</tr>
<tr>
<td>Nucleotides</td>
<td>DNA fragment</td>
</tr>
</tbody>
</table>
**Table 2** List of methods and their sensitivities suggested for testing contamination levels on the Mars 2020 rover, adapted from Summons et al. [2014]

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Sampling/Form</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vibrational Spectroscopy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRIFT spectroscopy</td>
<td>&lt;1 ng/cm² (from 100 cm²)</td>
<td>Witness plate or solvent extract*</td>
<td>Provides broad range of chemical functional groups and/or identification. Applied to numerous spacecraft mission, detects common airborne contaminants (AC) and spacecraft molecular contamination. Large spacecraft database.</td>
</tr>
<tr>
<td>FTIR-Grazing angle attenuated total reflection IR (GATR)</td>
<td>Sub-monolayer 0.5 ng/cm²</td>
<td>Witness plate or solvent extract</td>
<td>Provides chemical functional groups and identification, detects common AC. Rapid</td>
</tr>
<tr>
<td>FTIR-Microscopy</td>
<td>Sub-nanogram particles</td>
<td>Specialized witness plate</td>
<td>Requires specialized witness plates or particle sampling. Rapid.</td>
</tr>
<tr>
<td>Raman-Microprobe</td>
<td>Sub-nanogram particles</td>
<td>Specialized witness plate</td>
<td>Requires specialized witness plates or particle sampling. Rapid.</td>
</tr>
<tr>
<td><strong>Mass Spectrometry (MS)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC-MS</td>
<td>&lt;0.1 ng/cm² (from 100 cm²)</td>
<td>Witness plate or solvent extract</td>
<td>Identification of components in a complex mixture. Non-volatile components not detected, detects common AC.</td>
</tr>
<tr>
<td>Pyrolysis GC-MS</td>
<td>&lt;0.1 ng/cm² (from 100 cm²)</td>
<td>Witness plate or solvent extract</td>
<td>Detects non-volatile components, can run in series with GC-MS.</td>
</tr>
<tr>
<td>Direct Analysis in Real Time (DART)-MS</td>
<td>&lt;0.001 ng/cm² (from 100 cm²)</td>
<td>Witness plate or solvent extract</td>
<td>Identification of components in a complex mixture, molecular weight &gt;1000 amu requires pyrolysis, detects common AC, very sensitive, rapid</td>
</tr>
<tr>
<td>Liquid Chromatography (LC)-MS</td>
<td>&lt;0.1 ng/cm² (from 100 cm²)</td>
<td>Witness plate or solvent extract</td>
<td>Identification of components in a complex mixture, somewhat complex procedures and method development, particularly well-suited for some biological analyses.</td>
</tr>
<tr>
<td>Laser-assisted Desorption (LD)-MS</td>
<td>&lt;1 ng/cm² (from 100 cm²)</td>
<td>Witness plate or solvent extract</td>
<td>Identification of components in a complex mixture, suited for high molecular weight bio-analytes, complex procedures and method development, expensive instrumentation.</td>
</tr>
<tr>
<td>Secondary-Ion MS (SIMS)</td>
<td>Sub-monolayer</td>
<td>Witness plate</td>
<td>Quantitation difficult, limited molecular identification for organics, very sensitive, detects common AC, complex, expensive instrumentation</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray Photoelectron Spectroscopy (XPS)/Auger</td>
<td>Sub-monolayer</td>
<td>Witness plate</td>
<td>Sensitive, elemental information, limited molecular identification, detects common AC, complex, expensive instrumentation</td>
</tr>
<tr>
<td>Total Organic Carbon (TOC) Instruments (pyrolysis and electrochemical)</td>
<td>~3 ng/cm²</td>
<td>Witness plate</td>
<td>No chemical information, no identification, does not quantify incombustible components</td>
</tr>
</tbody>
</table>

*It should be noted that all methods require specialized hardware sampling and/or witness plates

**Solvent extracts may use a surface rinse or specialized solvent swabs of hardware surfaces
Table 3: Potential organic contaminants and the Supelco recommended fibres for their analysis (by headspace extraction) (adapted from https://www.sigmaaldrich.com/technical-documents/articles/analytical/selecting-spme-fibers.html#fiber)

<table>
<thead>
<tr>
<th>Analyte type</th>
<th>Recommended Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low molecular weight</td>
<td>75 µm/85 µm Carboxen/Polydimethylsiloxane</td>
</tr>
<tr>
<td>Volatiles</td>
<td>100 µm polydimethylsiloxane</td>
</tr>
<tr>
<td>Volatile, amines and nitro-aromatics</td>
<td>65 µm polydimethylsiloxane/divinylbenzene</td>
</tr>
<tr>
<td>Polar semi-volatiles</td>
<td>85 µm polyacrylate</td>
</tr>
<tr>
<td>Non-polar high molecular weight compounds</td>
<td>7 µm polydimethylsiloxane</td>
</tr>
<tr>
<td><strong>Non-polar semi-volatiles</strong></td>
<td><strong>30 µm polydimethylsiloxane</strong></td>
</tr>
<tr>
<td>Alcohols and polar compounds</td>
<td>60 µm Carbowax</td>
</tr>
</tbody>
</table>
Table 4 Integrated peak areas from selected ion chromatograms produced by both liquid injection and SPME-GC-MS of standard organic compound solutions. Percentage recovery of standard compounds against liquid injection external standard calibration (N/A = Not applicable, <LOD = below limit of detection)

<table>
<thead>
<tr>
<th>Solution concentrated (ng·ml)</th>
<th>1 µl direct liquid injection (peak area)</th>
<th>Contamination level equivalent SPME average (peak area)</th>
<th>SPME Standard deviation (% of mean)</th>
<th>% recovery at contamination level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octadecane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>&lt;LOD</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>941</td>
<td>A1000</td>
<td>&lt;LOD</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>5207</td>
<td>A1000</td>
<td>1723</td>
<td>24 %</td>
</tr>
<tr>
<td>100</td>
<td>61663</td>
<td>A10</td>
<td>8694</td>
<td>50 %</td>
</tr>
<tr>
<td>1000</td>
<td>1859793</td>
<td>A</td>
<td>368767</td>
<td>63 %</td>
</tr>
<tr>
<td>1-Octadecene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>&lt;LOD</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>1688</td>
<td>A1000</td>
<td>&lt;LOD</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>4677</td>
<td>A1000</td>
<td>9411</td>
<td>18 %</td>
</tr>
<tr>
<td>100</td>
<td>90213</td>
<td>A10</td>
<td>20120</td>
<td>14 %</td>
</tr>
<tr>
<td>1000</td>
<td>1650655</td>
<td>A</td>
<td>913332</td>
<td>48 %</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>577</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>3309</td>
<td>A1000</td>
<td>1379</td>
<td>8 %</td>
</tr>
<tr>
<td>10</td>
<td>23632</td>
<td>A1000</td>
<td>6227</td>
<td>23 %</td>
</tr>
<tr>
<td>100</td>
<td>434883</td>
<td>A10</td>
<td>101058</td>
<td>34 %</td>
</tr>
<tr>
<td>1000</td>
<td>5510655</td>
<td>A</td>
<td>1761618</td>
<td>18 %</td>
</tr>
<tr>
<td>Octadecanoic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>&lt;LOD</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>&lt;LOD</td>
<td>A1000</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>&lt;LOD</td>
<td>A1000</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>100</td>
<td>&lt;LOD</td>
<td>A10</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>1000</td>
<td>549876</td>
<td>A</td>
<td>14439</td>
<td>31 %</td>
</tr>
<tr>
<td>Squalene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>85</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>336</td>
<td>A1000</td>
<td>611</td>
<td>20 %</td>
</tr>
<tr>
<td>10</td>
<td>4388</td>
<td>A1000</td>
<td>4030</td>
<td>9 %</td>
</tr>
<tr>
<td>100</td>
<td>72063</td>
<td>A10</td>
<td>48410</td>
<td>19 %</td>
</tr>
<tr>
<td>1000</td>
<td>653790</td>
<td>A</td>
<td>509926</td>
<td>67 %</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>&lt;LOD</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>&lt;LOD</td>
<td>A1000</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>&lt;LOD</td>
<td>A1000</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>100</td>
<td>&lt;LOD</td>
<td>A10</td>
<td>N/A</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>1000</td>
<td>863</td>
<td>A</td>
<td>&lt;LOD</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Table 5 Calculated sensitivities for each standard compound and concentration (expressed as percentages)

<table>
<thead>
<tr>
<th>Level</th>
<th>Octadecane</th>
<th>1-Octadecene</th>
<th>Phenanthrene</th>
<th>Steric acid</th>
<th>Cholesterol</th>
<th>Squalene</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>A10</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>A100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>A1000</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 6 molecular weights and enthalpy of vaporization of standard compounds tested (from NIST)

<table>
<thead>
<tr>
<th>Standard compound</th>
<th>Molecular weight</th>
<th>Enthalpy of vaporization (KJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Octadecene</td>
<td>252</td>
<td>55</td>
</tr>
<tr>
<td>Octadecane</td>
<td>255</td>
<td>92</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178</td>
<td>78</td>
</tr>
<tr>
<td>Steric acid</td>
<td>284</td>
<td>79</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>387</td>
<td>154</td>
</tr>
<tr>
<td>Squalene</td>
<td>410</td>
<td>83</td>
</tr>
</tbody>
</table>