Extracting organic matter on Mars: a comparison of methods involving subcritical water, surfactant solutions and organic solvents

Duy Luong[a], Richard W. Court[a], Mark R. Sims[b], David C. Cullen[c], Mark A. Sephton[a].

[a]Impacts and Astromaterials Research Centre, Department of Earth Science and Engineering, South Kensington Campus, Imperial College London, SW7 2AZ, UK

[b]Space Research Centre, Michael Atiyah Building, Department of Physics and Astronomy, University of Leicester, Leicester LE1 7RH, UK

[c]Cranfield Health, Vincent Building, Cranfield University, Bedfordshire MK43 0AL, United Kingdom

Correspondence author:

Duy Luong, Department of Earth Science and Engineering, South Kensington Campus

Imperial College London, SW7 2AZ

Email: d.luong12@imperial.ac.uk

Abstract

The first step in many life detection protocols on Mars involves attempts to extract or isolate organic matter from its mineral matrix. A number of extraction options are available and include heat and solvent assisted methods. Recent operations on Mars indicate that heating samples can cause the loss or obfuscation of organic signals from target materials, raising the importance of solvent-based systems for future missions. Several solvent types are available (e.g. organic solvents, surfactant based solvents and subcritical water extraction) but a comparison of their efficiencies in Mars relevant materials is missing. We have spiked the well characterised Mars analogue material JSC Mars-1 with a number of representative organic standards. Extraction of the spiked JSC Mars-1 with the three solvent methods provides insights into the relative efficiency of these methods and indicates how they may be used on future Mars missions.

1. Introduction

1.1. Search for organic matter on Mars

The search for life on Mars employs techniques that rely on the detection and characterisation of organic matter. To date, no confirmed detection of in situ organic matter on Mars has occurred. Yet the efficiency of organic detection and characterisation techniques that can operate on Mars may be assessed by examining their use on samples containing organic matter on Earth. The most convincing organic analyses are those in which the organic target materials are isolated from their mineral matrix, necessitating some form of extraction process. Perhaps the simplest approach is to use heat to liberate organic entities but there is a growing awareness that this approach may initiate mineral decomposition and secondary reactions that can obfuscate the sought after organic data (Abe et al., 1993; Navarro-González et al., 2010; Steininger et al., 2012). A more involved protocol involves the use of a solvent to selectively dissolve the organic targets in a medium that enables transfer and downstream analysis. Several types of solvent system are available and each has advantages and disadvantages for operation on Mars. It should be noted that minerals can retain a fraction of any target analyte precluding complete removal from a mineral matrix (Sephton et al., 2013).

1.2. Organic solvent extraction

Extraction using organic solvents is the standard method in analytical assays that target non-polar organic matter in terrestrial materials. Applications include environmental samples (Tor et al., 2006), active ingredients of foodstuffs (Fang et al., 2005) and characterisation of naturally-occurring polymers for industrial applications (Amnuaypornsri et al., 2010). Organic solvent extraction, in general, offers great efficiency (Bradburn et al., 1995; Guerin, 1999; Qian et al., 2000; Shen and Shao, 2005) and reproducibility (Berset et al., 1999). The efficiency of organic solvent extraction also relies on several parameters such as solvent polarity, agitation, ultra-sonication, extraction duration, and temperature. Moreover, mixed solvent systems can be utilised in some assay extraction methods (Rezić et al., 2005) and the ratio of each solvent in the mixed solvent system can be modified to influence the yield (Kuk et al., 2005).

1.3. Surfactant assisted extraction

Surface active agents or "surfactants" can be added to water to reproduce some of the features of organic solvent extraction. The versatility of surfactant based extraction has been proven in different areas ranging from soil remediation (Chu and Chan, 2003; Villa et al., 2010), waste water analyses (Zou et al., 2012), to petroleum geoscience where constituent organic compounds in petroleum source rock, such as naphthalene and phenanthrene, can be extracted using non-ionic surfactant solutions (Akinlua et al., 2012).

Surfactant solutions were studied as potential solvents for the Life Marker Chip (LMC) which is a Mars life detection strategy (Sims et al., 2012). Surfactant solutions solve the problem of extracting non-polar hydrocarbons from Martian samples in water based solvent while at the same time providing a friendly environment for the antibody based detectors (Court et al., 2010a). Experimental work has demonstrated that the non-ionic surfactant polysorbate 80 is able to extract spiked aliphatic and aromatic hydrocarbons from the Martian soil analogue JSC Mars-1 (Court et al., 2010a). Further work has indicated how other surfactant solutions can obtain similar results to polysorbate 80 (Court et al., 2012).

1.4. Sub-critical water extraction

Subcritical water can be explained as a liquid whose temperature is above 100°C and below 374°C, with enough pressure to maintain the liquid state but not exceeding 22.05 MPa (Sereewatthanawut et al., 2008). During subcritical water extraction, the polarity of water can be altered by fine-tuning the pressure and temperature (Table 1; Amashukeli et al., 2008; Aubrey et al., 2008). The efficacy of subcritical water extraction for isolating organic compounds has been used to extract organic

pollutants from environmental solids (Hawthorne et al., 1994), harmful dioxins from soil (Hashimoto et al., 2004) and antioxidants from fruits and vegetables (Garcia-Marino et al., 2006; Singh and Saldaña, 2011).

Subcritical water extraction has also been tested as a method of extracting organic compounds on Mars. Subcritical water was the intended extraction method for the Urey instrument (Bada et al., 2005) (Aubrey et al., 2008) and a prototype of the extraction system was used to isolate amino acids from a soil sample of the Atacama Desert which acted as a Mars soil analogue (Amashukeli et al., 2008; Amashukeli et al., 2007).

1.5. Purpose of this study

This study aims to provide a comparison of extraction efficiencies between three techniques that could be used to isolate organic matter on Mars, namely organic solvent, surfactant solution and subcritical water-based extraction methods. The data represent the first ever quantitative comparison of the fidelity and extraction efficiency of this suite of key preparative steps for the detection of past or present life on Mars. Results from this study will influence future instrument design for missions intended to operate on the red planet or for possible analysis of materials eventually returned to Earth as part of a Mars Sample Return mission.

2. Experimental

2.1. Mars analogue material

Extraction efficiency studies require a standard material of known organic constitution. In this study the production of a standard material was achieved by spiking a well characterised Mars analogue material with known amounts of representative organic standards. Our work utilised JSC Mars-1 which is an altered volcanic ash derived from a cinder cone on the Island of Hawaii (Allen et al., 1998). Mineralogically, the altered ash from this Hawaiian volcano region is said to bear many similarities to that found on some regions of Mars (Guinness et al., 2007). Physical characterisation of this simulant has been performed before and comparison has been made between JSC Mars-1 and the regolith samples collected by the Viking Landers as well as the Pathfinder rover (Morris et al., 2000; Morris et al., 2001). 75% of the grains of this Mars analogue are larger than 149 μm and only 1% are smaller than 5 μm (Allen et al., 1998). The same batch of Mars JSC Mars-1 was used throughout this spiking study. The technique and the methods described below associated with organic solvent and surfactant based extraction have been used previously (Court et al., 2010a, Court et al., 2012) to measure extraction efficiency and similar methods have been applied to assess

subcritical water extraction in the current study. It should be noted that the spiking of standards, while an effective means of comparing extraction efficiencies, is not completely representative of the natural situation where organic analytes may be occluded or incorporated into the mineral matrix. Moreover the mineralogy of JSC Mars-1, although well characterized (Allen et al., 1998), represents a mixture rather than individual minerals; future work could utilise the individual inorganic constituents with JSC Mars-1 to help constrain their effect on extraction efficiency.

2.2. Standards

For the experimental work a series of representative standards were used (Figure 1). Hexadecane (99%) was purchased from Sigma-Aldrich (St Louis, MO USA), anthracene (97%) from Sigma-Aldrich (Germany), phytane (analytical grade) purchased from Fluka, Sigma-Aldrich (Norway), stigmasterol (95%) from Sigma-Aldrich (St Louis, MO USA), squalene (98%) from Sigma-Aldrich (Japan), coprostane from Sigma-Aldrich (Norway), pyrene (98%) from Sigma-Aldrich (St Louis, MO USA), atrazine (97.4%) from Sigma-Aldrich (Riedel-de Haën, Germany). The standards represent a number of different molecules and molecular shapes that represent possible biomarkers for abiotic organic matter, extant life constituents or fossil organic markers of past life, informed by our terrestrial experiences of biochemistry and geochemistry.

Standards were weighed using an analytical balance up to 4 decimal places (Table 2). The standards were mixed with 1 L of dichloromethane (DCM) using a volumetric flask and this solution was then ready for sample spiking. Samples of JSC Mars-1 of 0.5053 g were spiked with 1 mL of solution of the 8 different analytes with the masses indicated in Table 2. After spiking, the samples were allowed to dry overnight in a hotbox at 37 ^{o}C under an aluminium foil cover. Unspiked JSC Mars-1 control samples were also prepared to detect any cross contamination of analytes.

2.3. Organic solvent extraction

HPLC grade DCM and methanol (MeOH) were sourced from Fisher Scientific, UK. Three ml of 93:7 DCM:MeOH was added to spiked JSC Mars-1 before sonication using an Sonics & Materials Inc. (USA) VCX-130 Vibra-Cell™ ultrasonic processor with a maximum frequency of 20 kHz for 10 minutes at room temperature, followed by centrifugation for 5 minutes at 2500 rpm to settle suspended particles of JSC Mars-1. No temperatures were measured during extraction but any temperature rise brought about by sonication may have aided extraction efficiency. The solvent supernatants were combined and the total extract was filtered using syringe filters possessing polytetrafluoroethylene (PTFE) membranes with a 0.2 µm pore size. A stream of nitrogen gas was used to evaporate the supernatant to dryness before reconstitution with DCM and transfer to 2 ml gas chromatography

vials for analysis. Each analysis was performed in triplicate to enable calculation of standard deviations.

2.4. Surfactant assisted extraction

Surfactant polysorbate 80 was purchased from Acros Organics (New Jersey, USA). Pure water was prepared on an Elga Ultra-pure Water Maxima water purification system. Surfactant polysorbate 80 concentrations of 1.5 mg/ml in 20:80 methanol: water (vol) were used to extract organic compounds (Court et al., 2010b). The samples were then sonicated, isolated and filtered as outlined above. Three ml of DCM was added to the surfactant solution extracts and the mixture sonicated for 3 minutes followed by centrifugation for 5 minutes at 2500 rpm to settle suspended JSC Mars-1. The mixture was then transferred to separating funnels to which 1 ml of DCM was added. The surfactant solution and DCM mixtures were allowed to stand with the dense DCM settling as a bottom layer that was readily separated using a funnel. The surfactant solution was subjected to this liquid-liquid extraction another two times. The collected DCM layers were combined and evaporated under a stream of nitrogen to a volume of 1 ml and transferred quantitatively to 2 ml gas chromatography vials for analysis. Each analysis was performed in triplicate to enable calculation of standard deviations.

2.5. Sub-critical water extraction

Subcritical water extraction utilised a purpose built system (Figure 2). The system has three main parts: the syringe pump, the sample chamber and cooling coil. Pure deionised water was pumped and pressurised inside the sample cell through the stainless steel tube, the pressure inside the tube and the sample cell was 1500 psi (circa 10 MPa). The sample chamber also includes a pre-heating coil situated within the gas chromatograph oven. The system can be adjusted to run in two different modes namely static mode and dynamic mode. In the static mode, a fixed volume of water is confined within the sample cell at a set temperature for a specific duration. The long residence time in the sample cell can optimise the dissolution of analytes in the subcritical water. Dynamic extraction involves a flow-through extraction process where the analytes are swept from the matrix. Dynamic mode can involve various flow rates and rates of change of temperature. Dynamic extraction can be performed alone or following a period of static extraction. The mode of extraction in this study was static followed by dynamic extraction. We presume that once water was heated to the required temperature on Mars, a static followed by dynamic extraction rather than just dynamic extraction alone would make the most of the utilized resources. Water was first flushed through the entire system and once the system was filled with water, the outlet valve and the eluent valve were shut. The oven with set temperature was then turned on. To stabilise internal pressure during

heating the inlet valve remained open during the temperature ramp and for 5 minutes after the set temperature was attained. The static extraction time was 20 minutes for 100°C, 150°C, 200°C and 250°C temperature steps. Preliminary experiments for 20 minutes at 300°C led to noticeable analyte degradation so the extraction time was reduced to 10 minutes to minimise this effect. The extraction time was further reduced to 5 minutes at 310°C and 320°C for the same purpose. At the end of the set time, the outlet valve and the eluent valve were opened simultaneously. Before it was collected at the eluent valve, the hot water would pass through a cooling coil which was submerged in cold water. The purpose of opening both valves at the same time was to speed up the flow of hot water through the cooling bath. The prolonged exposure to cooling bath can reduce the dielectric constant of heated water and it can decrease the solubility of analytes. The outflow of the eluent through the collection point was also promoted by maintaining the system at high temperature.

The eluent was collected in a large conical flask. The conical flask contained at least 20 ml of DCM prior to eluent collection allowing analytes to readily partition into the organic layer. The aqueous and organic layers were transferred to a separating funnel and shaken. The organic layer was then collected in a beaker. 10 ml of DCM was further added to the aqueous layer and shaken, the organic layer was again collected in the same beaker. Any traces of water in the recovered organic solvent were removed by passing the extract through anhydrous magnesium sulphate (Fluka, Japan puriss grade ≥98% purity). DCM layers were reduced in volume using a rotary evaporator to prepare for further analysis.

2.6. GC-MS analyses

The analytes chosen for the study lend themselves to standard laboratory analyses by gas chromatography-mass spectrometry (GC-MS). Each analysis was performed in triplicate to enable calculation of standard deviations. Analyte separation was achieved using an Agilent Technologies G3172A gas chromatograph fitted with an Agilent HP-5MS column (29.55 m x 250 μm x 0.25 μm). Helium carrier gas flow rate was 1.1 ml/min. Injection volume was 1 μL and injection mode was splitless. The front inlet temperature was 250°C and the oven temperature programme was 50°C held for 1 minute followed by a temperature ramp of 4°C/min to 310°C, where the temperature was held for 20 minutes. Total run time was 86 minutes.

Analyte identification was performed using an Agilent Technologies 5973 Mass Selective Detector set on full scan for a mass range from 50.0 to 550.0 amu. The ionisation source temperature was maintained at 230°C and mass analyser quadrupole temperature was 150°C. A nine minute solvent

delay was employed. Mass spectra were interpreted by reference to the NIST 2008 mass spectral database, and the retention times for this instrumental configuration established by previous runs of standards.

3. Results

Chromatograms for extracts of spiked JSC Mars-1 using the methods are presented in Figure 3. Procedural blanks represented by identical extractions of unspiked JSC Mars-1 are also incuded.

3.1. Organic solvent extraction

The chromatogram in Figure 3a shows the successfully extracted analytes from the spiked sample using the organic solvent mixture. All eight spiked analytes were extracted and detected. The combination of DCM and methanol was able to extract both polar (water soluble) and non-polar compounds. Extraction was performed in triplicate and the peak areas of analytes in 1 ml of standard solution were also measured for comparison as part of an extraction efficiency assessment.

Three different sets of standard organic compounds were used for calibration. One mL of the standard solution represented the masses (in μg) of 19.4, 114.4, 20.5, 6.8, 20.6, 118.9, 19.9, and 2.4 for coprostane, hexadecane, pyrene, atrazine, anthracene, squalene, stigmasterol and phytane respectively. The mass of analytes extracted could then be calculated using the following approach:

$$M_{extracted} = (R_{extracted}/R_{standard}) \times M_{standard}$$

Where $M_{extracted}$ = mass of extracted analyte, $R_{extracted}$ = response (peak area) of extracted analyte, $R_{standard}$ = response of standard, and $M_{standard}$ = mass of standard.

Extraction efficiency can then be calculated thus:

$$\text{Efficiency (\%)} = (M_{extracted}/M_{standard}) \times 100$$

Extraction efficiencies for the organic solvent extraction of all analytes are presented in Table 3. The data indicate that straight chain aliphatic hydrocarbons, branched aliphatic hydrocarbons and polycyclic aliphatic hydrocarbons all have very good extraction yields (almost 70% for hexadecane, more than 82% for phytane and 93% for coprostane). Extraction yields for aromatic hydrocarbons are variable. For the linear aromatic hydrocarbon anthracene, the yield is only around 25% whereas

the yield for the clustered aromatic hydrocarbon pyrene is nearly 90%. Anthracenedione, an oxidation product of anthracene was detected indicating some interaction between analytes and oxidising mineral surfaces. Hence, partial oxidation is one explanation for the lower yield for anthracene. Standard deviations from triplicate analysis for organic solvent extractionof the analytes are listed in table 5 . These figures indicate the relatively good reproducibility of this technique.

3.2. Surfactant assisted extraction

Figure 3b presents a chromatogram of the extracted analytes from the soil sample with polysorbate 80 surfactant solution. All analytes are generally present although stigmasterol failed to extract in one of the extraction experiments.

Surfactant-based extractions were performed in triplicate and to quantify the extracted analytes the same mathematical method as applied to the organic solvent extracts above was used. Similarly, the same calibration standards were used, therefore values of the standard analytes stated previous remained the same. Extraction efficiencies for the surfactant extraction of all analytes are presented in Table 4.

The extraction efficiency data generated in this study can be compared to that in previously published work (Court et al. 2010a, Court et al. 2012) (Table 6). Extraction of hexadecane and squalene in this and previous studies have similar efficiencies. Anthracene and pyrene were extracted less well compared to previous work with polysorbate 80 (Court et al. 2012) (Table 6). As with organic solvent extraction, surfactant assisted extraction produced anthracenedione, an oxidation product of anthracene, was detected most likely indicating some interaction between analytes and oxidising mineral surfaces.

Overall, in the current study, polysorbate 80 can extract aliphatic hydrocarbons such as hexadecane, squalene and coprostane with reasonable efficiencies. Extractions of aromatic hydrocarbons give similar results to aliphatic hydrocarbons. Atrazine is partially water soluble and, therefore, may simply reflect dissolution in water without dependence on the surfactant micelles. Surfactant assisted extraction displays relatively poor reproducibility with standard deviations from triplicate analysis of hexadecane, atrazine, anthracene, phytane, pyrene, coprostane, squalene (Table 5)

3.3. Subcritical water extraction

Temperature investigation:

A range of temperatures were investigated to find the optimum temperature for subcritical water extraction of the standard compounds (Table 7). At 100°C and 150°C, no analytes were detected by GC-MS. Water at 200°C extracted hexadecane, pyrene and coprostane from the spiked JSC Mars-1. A temperature of 250°C allowed the extraction of hexadecane, phytane, pyrene, coprostane from the JSC Mars-1 substrate; small amounts of squalene were also found alongside partial oxidation products of anthracene. It should be noted that as temperature went from 200°C to 250°C, there was an almost 10 fold increase in yield of hexadecane, a straight chain aliphatic hydrocarbon. The temperature of 250°C appears to mark an increase in extraction efficiency for straight chain hydrocarbons. Some standards are either not extracted intact, e.g. stigmasterol, or are extracted at a restricted number of temperatures, e.g. squalene.

The most appropriate temperature for subcritical water extraction of the analytes selected for this study appears to be 300°C. At this temperature the majority of analytes are recovered including anthracene, a polycyclic aromatic hydrocarbon, which could not be extracted at lower temperatures. As with both other extraction techniques anthracenedione, an oxidation product of anthracene was detected indicating some interaction between analytes and the oxidising mineral surface. Moreover, the appearance of oxidation products and reductions in yields suggest that higher temperatures may exacerbate oxidative interaction between the matrix and spiked organic standards leading to negative effects on extraction efficiency. Subcritical water extractions at 300°C, 310°C and 320°C generated a number of oxidation products such as epoxides and aldehydes. Anthracene, itself is absent at higher temperatures, presumably reflecting complete transformation to oxidation products.

Extraction characteristics at 300°C:

Figure 3c displays a chromatogram of subcritical water extracted analytes at 300°C. Most analytes were extracted, with only responses for atrazine and squalene missing. Atrazine is soluble in water at ambient conditions but at such high temperature the physical properties of water, such as the dielectric constant and polarity, are greatly altered. This would mean that atrazine which is water soluble at room temperature will become insoluble at high temperature. Squalene contains many double bonds (Figure 1) and is therefore susceptible to oxidation. It is possible that squalene succumbed to oxidation and fragmentation in the presence of high temperature and pressure water and an oxidised mineral surface. Future work should constrain the effects of mineral phases on hot water mediated organic reactions.

Extraction efficiencies for the subcritical water extraction at 300°C of most analytes are presented in Table 8. Hexadecane, phytane, coprostane could be extracted in reasonable amounts and clustered polycyclic aromatic hydrocarbon pyrene shows some degree of solubility. Extraction efficiencies of 58.1%, 65.7% and 46.9% for hexadecane, phytane, coprostane respectively are reasonably high .At 9.2% and 27.1%, the extraction of anthracene and pyrene are not so efficient. Reproducibility is also reasonable for this technique with standard deviations from triplicate analysis being 13.98% for hexadecane, 0.04% for phytane, 2.69% for pyrene and 0.81% for coprostane (Table 5).

In the previous spiking studies, Hawthorne et al. (1994) demonstrated that sequential subcritical water extraction at 250°C (plus 150°C and 50°C) was capable of extracting anthracene with an efficiency of 95%, benzo[a]pyrene at an efficiency of 86% and heptadecane at an efficiency of 50% (Hawthorne et al., 1994). However, it should be noted that the Hawthorne et al. (1994) spiking study, the extractions were carried out as soon as the normal sand was spiked, this did not allow the spiked analytes time to bind to the sand surface. The Hawthorne et al. (1994) extractions were also performed in dynamic mode at a different temperature. Normal sand was used in their spiking study, unlike in this spiking study where JSC Mars-1 was employed and it is known to contain iron (III) oxide and manganese oxide (Allen et al., 1998) both minerals are known for their oxidising power. It should be no surprise, therefore, that our study reveals more oxidation effects compared to that of Hawthorne et al. (1994).

In a separate study (Yang et al., 1997) the spiking technique was used to determine the collection efficiencies of aliphatic hydrocarbons and polycyclic aromatic hydrocarbons following subcritical extraction. It was shown that n-alkanes ranging from C_8 to C_{28} were not efficiently extracted in liquid water at 250°C. Instead, n-alkanes were extracted with great efficiency using steam at 250°C (82% to 102%). Spiked polycyclic aromatic hydrocarbons were extracted with efficiencies ranging from 80-94% for polycyclic aromatic hydrocarbons ranging from naphthalene to benzo[ghi]perylene using both steam and liquid water at 250°C. No results were reported for 300°C in the previously published spiking study (Yang et al., 1997). The extraction time in the Yang et al. (1997) study was kept very short from 5 to 7 minutes and such a short exposure to high temperature would be expected to produce fewer oxidative degradation products than our Mars analogue focused study.

Our subcritical water study of Mars biomarkers reveals that the best extraction efficiencies for saturated hydrocarbons occur at a temperature of 300°C and pressure of 20 MPa. The aromatic hydrocarbons extractions, in this study, are not as efficient as in other experiments reported in the literature (Hawthorne et al., 1994; Yang et al., 1997) probably due to the fact that the aromatic hydrocarbon analytes were given time to adsorb on the JSC Mars-1 substrates and juxtaposition with

oxidising minerals appears to have led to degradation of these analytes. Our findings concur with previous work that has identified that subcritical water extraction can cause molecular transformations (Amashukeli et al., 2007).

3.4. Comparison of extraction methods

Organic solvent extraction is the most reproducible extraction method out of the three techniques for the majority of analytes and it is also the technique that produced the highest efficiency (Figure 4). Subcritical water extraction appears to be a reasonably reproducible technique. Surfactant extraction shows comparatively poor reproducibility compared to other two techniques

Each technique manages to extract most of the analytes, but subcritical water extraction is most selective with squalene and atrazine lacking detectable responses at high temperature (>300 °C) with oxidation and insolubility being two possible explanations. Notably, diminished responses for compounds that are susceptible to oxidation such as anthracene occurs during each technique implying that oxidised mineral surfaces such as those presented by the JSC Mars-1 analogue material are common interfering factors.

For those analytes extracted by all three methods, extraction efficiencies reveal that organic solvent extraction is the superior technique followed by subcritical water extraction and finally surfactant solutions. The non-polar aliphatic hydrocarbons are particularly well extracted by organic solvents and high temperature subcritical water.

3.5. Recommendations for future extractions on Mars

High temperature subcritical water and organic solvent are good at extracting aliphatic hydrocarbons. The aliphatic hydrocarbon fraction contains many diagnostic biomerkers and is relatively resistant to degradation. If life existed on Mars in its near or distant past, then aliphatic hydrocarbons would be the most likely biomarkers to survive the current inhospitable conditions owing to their high preservation potential (Sephton et al., 2013). Surfactant solutions display reasonable efficiency but with relatively low reproducibility. Surfactant solutions are, however, irreplaceable when extraction of non-polar organic compounds at low temperatures in aqueous solutions is required.

An additional factor to be considered for any in-situ analysis mission is the complexity of implementation for a real space instrument. All space instruments involve technology development to produce a compact and light instrument with a combination of low volume and low mass to make them compatible with mission resources. Subcritical water implementation is certainly possible as

indicated by published development work (Bada et al., 2005) (Aubrey et al., 2008) (Amashukeli et al., 2008; Amashukeli et al., 2007). Similarly an implementation strategy and flight type configuration is possible for surfactant based systems as illustrated by Life Marker Chip designs (Sims et al., 2012). Organic solvent extraction however presents some problems in terms of material compatibilities for valve seals and similar components. Moreover, with organic solvents there is the need to isolate and evaporate the excess solvent to concentrate the extracted analytes. When extraction methods are being considered for space mission use, the benefits of each approach must be balanced against their disadvantages. Subcritical water presents a suitable compromise method with the greatest degree of flexibility in accommodating all possible target compounds

4. Conclusions

Extraction of organic matter on Mars can be achieved by a number of methods with variable efficiencies and repeatabilities. Each method has strengths and weaknesses depending on target materials to be analysed. Subcritical water extraction outperformed an alternative aqueous based method involving surfactants, but still lagged behind organic solvent extraction in terms of both efficiencies and reproducibility. Oxidation appears to occur under high temperature water conditions in the presence of oxidising minerals leading to diminished responses for certain analytes. Mineral-mediated reactions during subcritical water extractions appear to be an important consideration for future work.

5. References

Abe, I., Rohmer, M., Prestwich, G.D., 1993. ENZYMATIC CYCLIZATION OF SQUALENE AND OXIDOSQUALENE TO STEROLS AND TRITERPENES. Chemical Reviews 93, 2189-2206.

Akinlua, A., Jochmann, M.A., Qian, Y., Sulkowski, M., Schmidt, T.C., 2012. Factors Controlling Leaching of Polycyclic Aromatic Hydrocarbons from Petroleum Source Rock Using Nonionic Surfactant. Chromatographia 75, 213-221.

Allen, C.C., Jager, K.M., Morris, R.V., Lindstrom, D.J., Lindtsrom, M.M., Lockwood, J.P., 1998. Martian soil simulant available for scientific, educational study. Eos, Transactions American Geophysical Union 79, 405-409.

Amashukeli, X., Grunthaner, F.J., Patrick, S.B., Yung, P.T., 2008. Subcritical water extractor for Mars analog soil analysis. Astrobiology 8, 597-604.

Amashukeli, X., Pelletier, C.C., Kirby, J.P., Grunthaner, F.J., 2007. Subcritical water extraction of amino acids from Atacama Desert soils. J. Geophys. Res. 112, G04S16.

Amnuaypornsri, S., Tarachiwin, L., Sakdapipanich, J.T., 2010. Character of Long-Chain Branching in Highly Purified Natural Rubber. Journal of Applied Polymer Science 115, 3645-3650.

Aubrey, A.D., Chalmers, J.H., Bada, J.L., Grunthaner, F.J., Amashukeli, X., Willis, P., Skelley, A.M., Mathies, R.A., Quinn, R.C., Zent, A.P., Ehrenfreund, P., Amundson, R., Glavin, D.P., Botta, O., Barron, L., Blaney, D.L., Clark, B.C., Coleman, M., Hofmann, B.A., Josset, J.L., Rettberg, P., Ride, S.,

Robert, F., Sephton, M.A., Yen, A., 2008. The Urey instrument: An advanced in situ organic and oxidant detector for Mars exploration. Astrobiology 8, 583-595.
Bada, J.L., Sephton, M.A., Ehrenfreund, P., Mathies, R.A., Skelley, A.M., Grunthaner, F.J., Zent, A.P., Quinn, R.C., Josset, J.-L., Robert, F., Botta, O., Glavin, D.P., 2005. New strategies to detect life on Mars. Astronomy & Geophysics 46, 6.26-26.27.
Berset, J.D., Ejem, M., Holzer, R., Lischer, P., 1999. Comparison of different drying, extraction and detection techniques for the determination of priority polycyclic aromatic hydrocarbons in background contaminated soil samples. Analytica Chimica Acta 383, 263-275.
Bradburn, N., Coker, R.D., Blunden, G., 1995. A comparative study of solvent extraction efficiency and the performance of immunoaffinity and solid phase columns on the determination of aflatoxin B1. Food Chemistry 52, 179-185.
Chu, W., Chan, K.H., 2003. The mechanism of the surfactant-aided soil washing system for hydrophobic and partial hydrophobic organics. Sci. Total Environ. 307, 83-92.
Court, R.W., Baki, A.O., M.R., S., David Cullen, D., Sephton, M.A., 2010a. Novel solvent systems for in situ extraterrestrial sample analysis. Planet Space Sci. 58, 1470-1474.
Court, R.W., Baki, A.O., Sims, M.R., Cullen, D., Sephton, M.A., 2010b. Novel solvent systems for in situ extraterrestrial sample analysis. Planetary and Space Science 58, 1470-1474.
Court, R.W., Rix, C.S., Sims, M.R., Cullen, D.C., Sephton, M.A., 2012. Extraction of polar and nonpolar biomarkers from the martian soil using aqueous surfactant solutions. Planetary and Space Science 67, 109-118.
Fang, F., Sang, S.M., Chen, K.Y., Gosslau, A., Ho, C.T., Rosen, R.T., 2005. Isolation and identification of cytotoxic compounds from Bay leaf (Laurus nobilis). Food Chemistry 93, 497-501.
Garcia-Marino, M., Rivas-Gonzalo, J.C., Ibanez, E., Garcia-Moreno, C., 2006. Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. Analytica Chimica Acta 563, 44-50.
Guerin, T.F., 1999. The extraction of aged polycyclic aromatic hydrocarbon (PAH) residues from a clay soil using sonication and a Soxhlet procedure: a comparative study. Journal of Environmental Monitoring 1, 63-67.
Guinness, E.A., Arvidson, R.E., Jolliff, B.L., Seelos, K.D., Seelos, F.P., Ming, D.W., Morris, R.V., Graff, T.G., 2007. Hyperspectral reflectance mapping of cinder cones at the summit of Mauna Kea and implications for equivalent observations on Mars. Journal of Geophysical Research: Planets 112, E08S11.
Hashimoto, S., Watanabe, K., Nose, K., Morita, M., 2004. Remediation of soil contaminated with dioxins by subcritical water extraction. Chemosphere 54, 89-96.
Hawthorne, S.B., Yang, Y., Miller, D.J., 1994. Extraction of Organic Pollutants from Environmental Solids with Sub- and Supercritical Water. Analytical Chemistry 66, 2912-2920.
Kuk, M.S., Tetlow, R., Dowd, M.K., 2005. Cottonseed extraction with mixtures of acetone and hexane. Journal of the American Oil Chemists Society 82, 609-612.
Morris, R.V., Golden, D.C., Bell, J.F., Shelfer, T.D., Scheinost, A.C., Hinman, N.W., Furniss, G., Mertzman, S.A., Bishop, J.L., Ming, D.W., Allen, C.C., Britt, D.T., 2000. Mineralogy, composition, and alteration of Mars Pathfinder rocks and soils: Evidence from multispectral, elemental, and magnetic data on terrestrial analogue, SNC meteorite, and Pathfinder samples. Journal of Geophysical Research: Planets 105, 1757-1817.
Morris, R.V., Golden, D.C., Ming, D.W., Shelfer, T.D., Jørgensen, L.C., Bell, J.F., Graff, T.G., Mertzman, S.A., 2001. Phyllosilicate-poor palagonitic dust from Mauna Kea Volcano (Hawaii): A mineralogical analogue for magnetic Martian dust? Journal of Geophysical Research: Planets 106, 5057-5083.
Navarro-González, R., Vargas, E., de la Rosa, J., Raga, A.C., McKay, C.P., 2010. Reanalysis of the Viking results suggests perchlorate and organics at midlatitudes on Mars. Journal of Geophysical Research: Planets 115, E12010.

Qian, J., Skyllberg, U., Tu, Q., Bleam, W.F., Frech, W., 2000. Efficiency of solvent extraction methods for the determination of methyl mercury in forest soils. Fresenius Journal of Analytical Chemistry 367, 467-473.

Rezić, I., Horvat, A.J.M., Babić, S., Kaštelan-Macan, M., 2005. Determination of pesticides in honey by ultrasonic solvent extraction and thin-layer chromatography. Ultrasonics Sonochemistry 12, 477-481.

Sephton, M.A., Sims, M.R., Court, R.W., Luong, D., Cullen, D.C., 2013. Searching for biomolecules on Mars: Considerations for operation of a life marker chip instrument. Planet Space Sci.

Sereewatthanawut, I., Prapintip, S., Watchiraruji, K., Goto, M., Sasaki, M., Shotipruk, A., 2008. Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. Bioresource Technology 99, 555-561.

Shen, J.C., Shao, X.G., 2005. A comparison of accelerated solvent extraction, Soxhlet extraction, and ultrasonic-assisted extraction for analysis of terpenoids and sterols in tobacco. Analytical and Bioanalytical Chemistry 383, 1003-1008.

Sims, M.R., Cullen, D.C., Rix, C.S., Buckley, A., Derveni, M., Evans, D., Miguel García-Con, L., Rhodes, A., Rato, C.C., Stefinovic, M., Sephton, M.A., Court, R.W., Bulloch, C., Kitchingman, I., Ali, Z., Pullan, D., Holt, J., Blake, O., Sykes, J., Samara-Ratna, P., Canali, M., Borst, G., Leeuwis, H., Prak, A., Norfini, A., Geraci, E., Tavanti, M., Brucato, J., Holm, N., 2012. Development Status of the Life Marker Chip Instrument for ExoMars. Planet Space Sci. 72, 129–137.

Singh, P.P., Saldaña, M.D.A., 2011. Subcritical water extraction of phenolic compounds from potato peel. Food Research International 44, 2452-2458.

Steininger, H., Goesmann, F., Goetz, W., 2012. Influence of magnesium perchlorate on the pyrolysis of organic compounds in Mars analogue soils. Planet Space Sci. 71, 9-17.

Tor, A., Aydin, M.E., Ozcan, S., 2006. Ultrasonic solvent extraction of organochlorine pesticides from soil. Analytica Chimica Acta 559, 173-180.

Villa, R.D., Trovo, A.G., Nogueira, R.F., 2010. Soil remediation using a coupled process: soil washing with surfactant followed by photo-Fenton oxidation. J Hazard Mater 174, 770-775.

Yang, Y., Hawthorne, S.B., Miller, D.J., 1997. Class-Selective Extraction of Polar, Moderately Polar, and Nonpolar Organics from Hydrocarbon Wastes Using Subcritical Water. Environmental Science & Technology 31, 430-437.

Zou, Y., Li, Y.H., Jin, H., Zou, D.Q., Liu, M.S., Yang, Y.L., 2012. Ultrasound-Assisted Surfactant-Enhanced Emulsification Microextraction Combined with HPLC for the Determination of Estrogens in Water. Journal of the Brazilian Chemical Society 23, 694-701.

Table 1. A comparison of subcritical water dielectric constants at various temperatures and 20 MPa with common organic solvents at room temperature and pressure (after (Amashukeli et al., 2008).

Subcritical water at 20 MPa		Organic solvents at 25°C and 0.1MPa	
Temperature	Dielectric constant	Solvent type	Dielectric constant
25 °C	78.5	Water	79.5
85 °C	59.7	Formic acid	58.0
145 °C	45.4	Dimethtylsulfoxide	47.0
185 °C	37.8	Acetonitrile	37.0
225 °C	31.5	Methanol	32.6
275 °C	24.2	Ethanol	24.6
325 °C	17.5	Acetone	20.7

Table 2. Masses of standards and JSC Mars-1 used to prepare the spiked samples and the resulting concentrations expressed as ppm.

Analyte	Mass of standards (μg) in 1 ml DCM	Concentration of analyte on JSC Mars-1 (ppm)
Hexadecane	114.4	226.4
Atrazine	6.8	13.5
Anthracene	20.6	40.8
Phytane	2.4	4.7
Pyrene	20.5	40.6
Coprostane	19.4	38.4
Squalene	118.9	235.3
Stigmasterol	19.9	39.4

Table 3. Extraction yields (means of three separate extractions) for organic solvent extraction of spiked JSC Mars-1.

Analyte	Mass of standards (μg)	Mass of extracted analyte of spiked JSC Mars-1 01 (μg)	Mass of extracted analyte of spiked JSC Mars-1 02 (μg)	Mass of extracted analyte of spiked JSC Mars-1 03 (μg)	Average mass of extracted analyte of triplicate (μg)	Extraction efficiency (%)
Hexadecane	114.4	76.8	83.7	69.2	76.6	66.9
Atrazine	6.8	4.8	5.4	5.0	5.1	74.9
Anthracene	20.6	6.6	4.3	4.4	5.1	24.7
Phytane	2.4	2.0	2.1	1.9	2.0	82.4
Pyrene	20.5	17.9	19.4	17.8	18.4	89.7
Coprostane	19.4	17.9	18.8	17.4	18.0	93.0
Squalene	118.9	65.1	63.8	60.9	63.3	53.2
Stigmasterol	19.9	12.6	13.7	13.0	13.1	65.8

Table 4. Extraction yields (means of three separate extractions) for surfactant assisted extraction of spiked JSC Mars-1.

Analyte	Mass of standards (μg)	Mass of extracted analyte of spiked JSC Mars-1 01 (μg)	Mass of extracted analyte of spiked JSC Mars-1 02 (μg)	Mass of extracted analyte of spiked JSC Mars-1 03 (μg)	Average mass of extracted analyte of triplicate (μg)	Extraction efficiency (%)
Hexadecane	114.4	20.8	26.7	31.4	26.3	23.0
Atrazine	6.8	4.0	5.2	6.5	5.2	76.9
Anthracene	20.6	2.7	3.5	5.4	3.9	18.8
Phytane	2.4	0.1	0.1	0.1	0.1	4.2
Pyrene	20.5	4.7	6.6	8.1	6.5	31.5
Coprostane	19.4	3.4	5.0	6.8	5.1	26.2
Squalene	118.9	13.1	20.7	28.5	20.8	17.5
Stigmasterol	19.9	0.0	0.2	0.2	0.2	1.1

Table 5. Reproducibilties of triplicate analyses for organic solvent extraction, surfactant extraction and sub-critical water extraction of spiked JSC Mars-1

Analyte	Reproducibility of organic solvent extraction (%)	Reproducibility of surfactant extraction (%)	Reproducibility of sub-critical water extraction (%)
Hexadecane	7.28	5.31	13.98
Atrazine	0.29	1.23	N/A
Anthracene	1.27	1.39	N/A
Phytane	0.08	0.02	0.04
Pyrene	0.9	1.73	2.69
Coprostane	0.71	1.72	0.81
Squalene	2.16	7.68	N/A
Stigmasterol	0.56	N/A	N/A

Table 6. Comparison of extraction efficiencies between surfactant extraction of this study and a previous study (Court et al., 2012). Numerical figures in first parentheses represent extraction efficiencies, standard deviations of the triplicate are quoted in the second parentheses.

Extraction efficiencies comparison (%) between two studies								
	Hexadecane	Phytane	Atrazine	Pyrene	Anthracene	Squalene	Stigmasterol	Coprostane
This study	23.0 (5.31)	4.2(0.02)	76.9(1.23)	31.5(1.73)	18.8(1.39)	17.5(7.68)	1.1(0)	26.2(1.72)
Previous study	28.3	24.3	92.2	74.6	49.3	16.6	7.7	Not reported

Table 7. Extraction efficiency as a function of temperature for sub-critical water extraction of the various analytes. Highlighted is the extraction at 300°C as this temperature extracted most analytes with highest efficiencies. Single analyses were carried out for this particular part of the work.

	Extraction efficiency (%) as a function of temperature (%)						
	100°C	150°C	200°C	250°C	300°C	310°C	320°C
Hexadecane	0.00	0.00	3.22	42.72	58.13	64.55	56.68
Atrazine	0.00	0.00	7.58	0.00	15.12	0.00	0.00
Anthracene	0.00	0.00	0.00	0.00	9.19	0.00	0.00
Phytane	0.00	0.00	0.00	23.89	65.74	64.18	65.84
Pyrene	0.00	0.00	16.19	29.14	27.13	19.51	17.75
Coprostane	0.00	0.00	0.00	8.21	46.86	47.18	41.48
Squalene	0.00	0.00	0.00	0.69	0.00	0.00	0.00
Stigmasterol	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 8. Extraction yields (means of three separate extractions) for subcritical water extraction of spiked JSC Mars-1 at 300 ^{o}C.

Analyte	Mass of standards (μg)	Mass of extracted analyte of spiked JSC Mars-1 01 (μg)	Mass of extracted analyte of spiked JSC Mars-1 02 (μg)	Mass of extracted analyte of spiked JSC Mars-1 03 (μg)	Average mass of extracted analyte of triplicate (μg)	Extraction efficiency (%)
Hexadecane	114.4	82.4	56.0	61.1	66.5	58.1
Atrazine	6.8	0.0	1.0	0.0	1.0	15.1
Anthracene	20.6	1.6	2.2	0.0	1.9	9.2
Phytane	2.4	1.6	1.6	1.5	1.6	65.7
Pyrene	20.5	5.0	8.5	3.2	5.6	27.1
Coprostane	19.4	8.5	10.0	8.8	9.1	46.9
Squalene	118.9	0.0	0.0	0.0	0.0	0.0
Stigmasterol	19.9	0.0	0.0	0.0	0.0	0.0

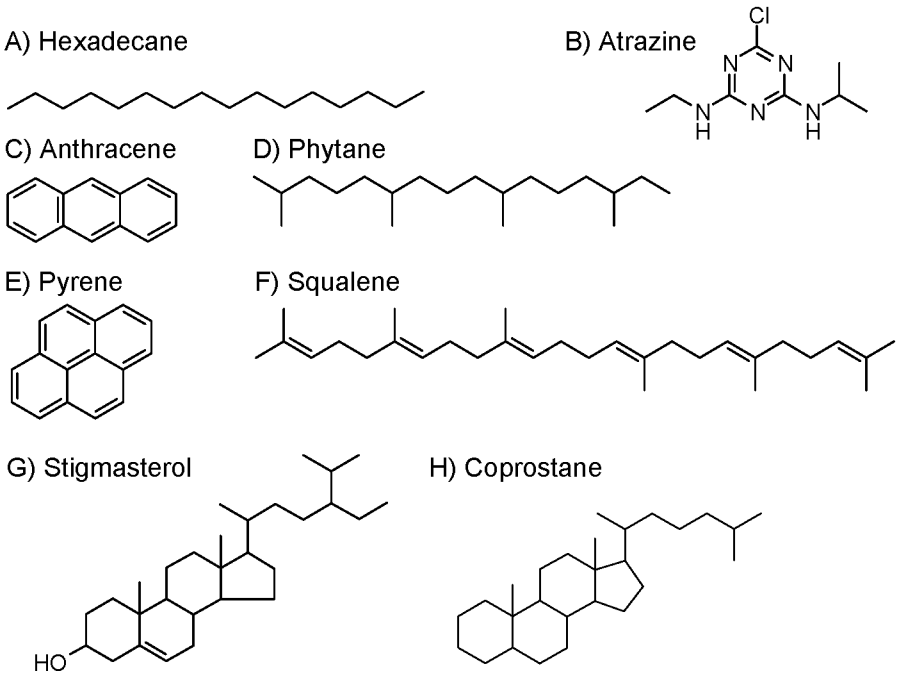


Figure 1. Structures of the organic compounds used to spike the JSC Mars-1 sample in preparation for extraction by the various methods. Hexadecane is an aliphatic hydrocarbon, phytane is an isoprenoid, coprostane is a steroid. These represent the organic compound class that is most likely to present evidence of past life on Mars owing to their relatively high preservation potential. Anthracene and pyrene - polycyclic aromatic hydrocarbons (PAHs) – are also included in this study to explore the extractability of this particular class of compound using subcritical water. Atrazine is partially water soluble at ambient conditions; the solubility of this compound at high temperature is part of the discussion in this study. Squalene and stigmasterol are both unsaturated hydrocarbons, the subcritical water extraction outcomes can shed light on the stability of these compounds in hot aqueous conditions.

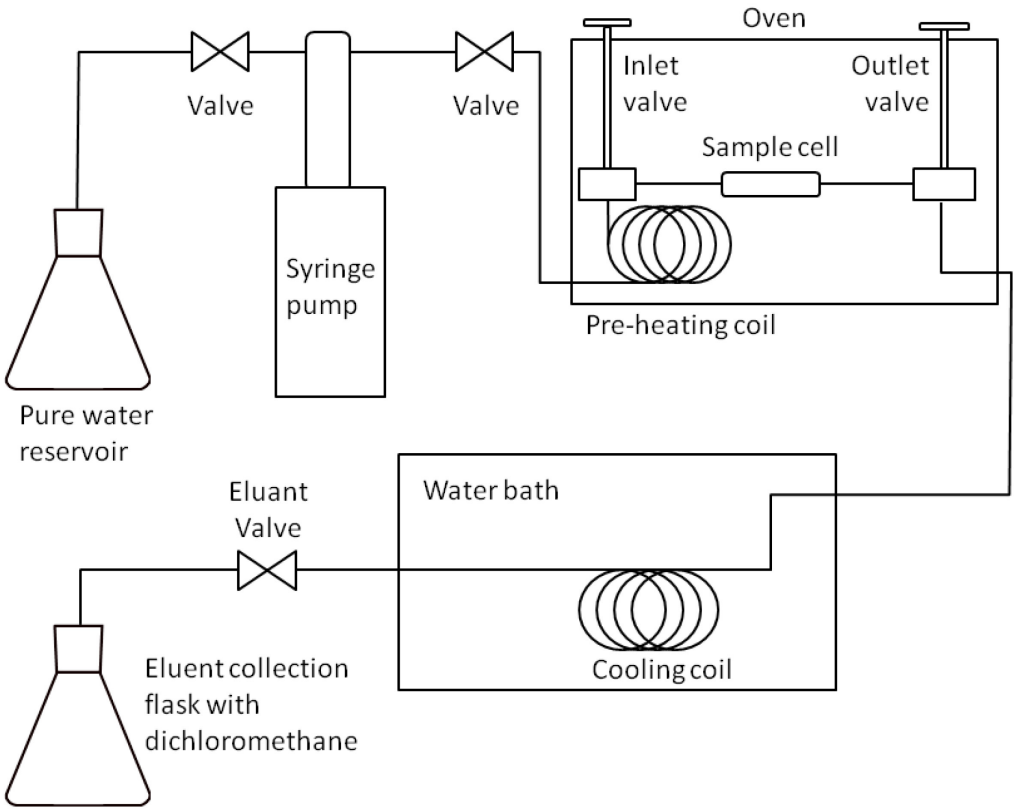
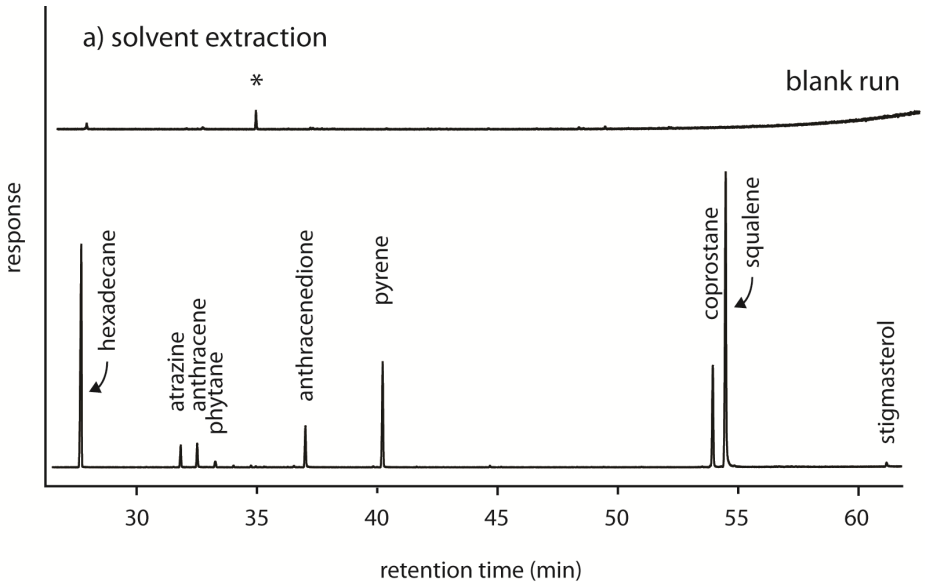
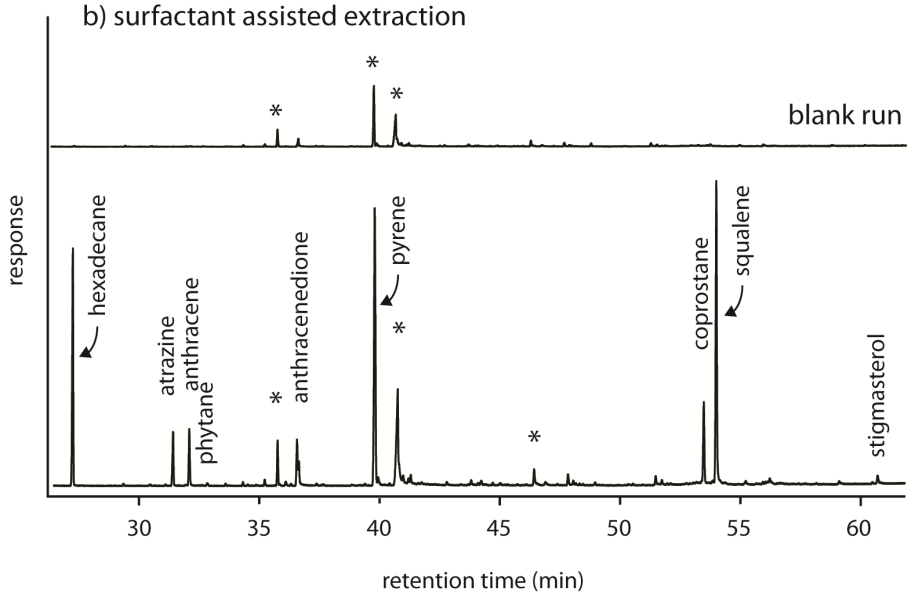


Figure 2.The schematic of SCWE system which illustrates the three main components of the instrument. The syringe pump takes pure water from the reservoir and fills up the extraction system which is confined to a gas chromatograph oven. The water first passes through a pre-heating coil at set temperature before entry to the extraction chamber. Following the exposure to high temperature, hot extract was channelled to the cooling coil where cooling occurs swiftly. The extract is then collected at the end of the eluent valve into a flask of organic solvent.


a) solvent extraction
blank run
response
hexadecane
atrazine
anthracene
phytane
anthracenedione
pyrene
coprostane
squalene
stigmasterol
30
35
40
45
50
55
60
retention time (min)


b) surfactant assisted extraction
blank run
response
hexadecane
atrazine
anthracene
phytane
anthracenedione
pyrene
coprostane
squalene
stigmasterol
30
35
40
45
50
55
60
retention time (min)

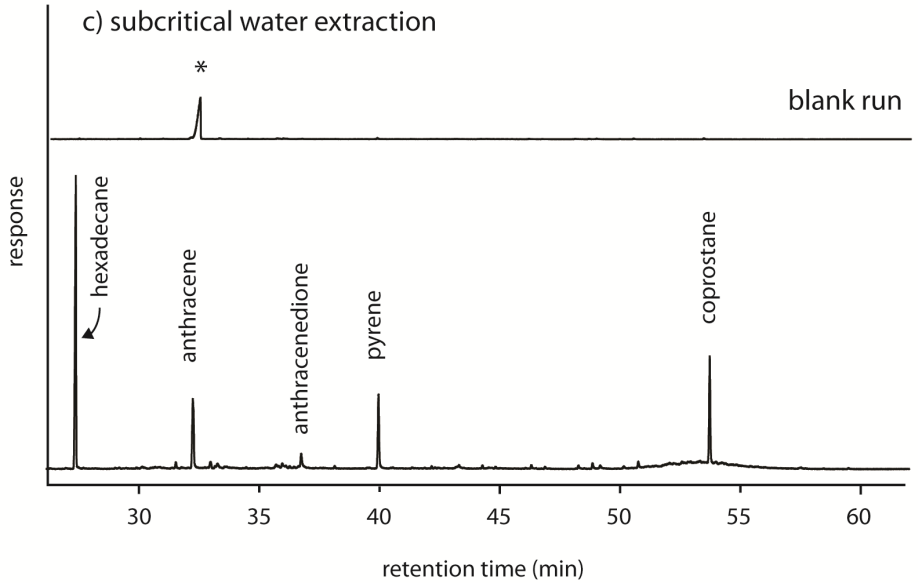

c) subcritical water extraction
blank run
response
hexadecane
anthracene
anthracenedione
pyrene
coprostane
30
35
40
45
50
55
60
retention time (min)

Figure 3. Representative total ion chromatograms a) solvent extraction, b) surfactant extraction c) subcritical water extraction. Procedural blank runs are also included for each method. For chromatogram a, various side reactions products are present in small quantities, possibly the results of oxidation. Also note that anthraquinone is present in all samples, which may suggest that all techniques encourage interaction with the mineral matrix. For chromatogram b, certain surfactant (polysorbate 80) degradation products, indicated by asterisks, coelute with the extracted standards. In the case of coelution, quantification is performed using chromatograms of specific *m*/*z* ratios.

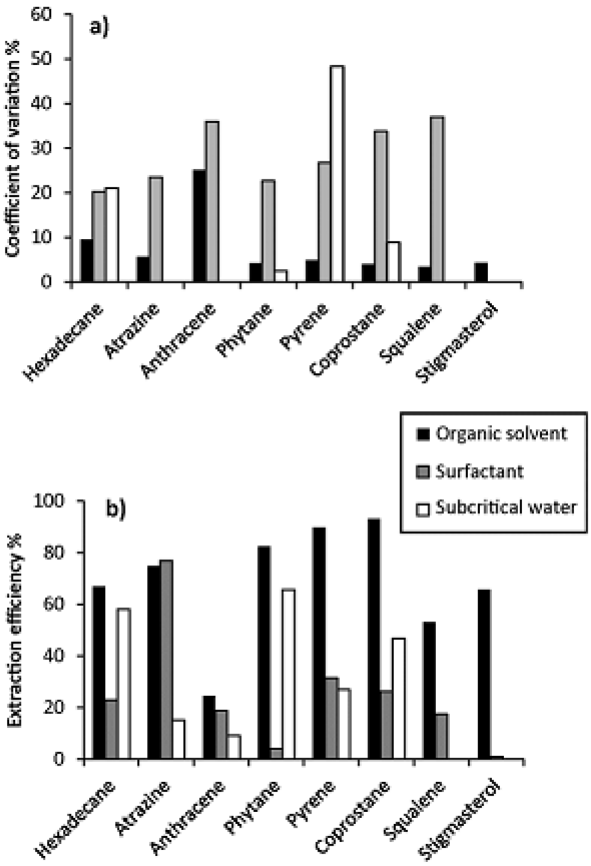


Figure 4. The comparison of a) repeatability and b) extraction efficiency between the three extraction techniques. Repeatability of each technique is measured by the coefficient of variation of that technique. Coefficient of variation is the ratio of standard deviation to mean values. Coefficients of variation are unitless and therefore can be used in comparison studies of repeatability where standard deviations are unsuitable for the same purpose. Repeatability measures the closeness between extraction results of each replicate obtained with the same extraction procedure under same conditions, as defined by the International Union of Pure and Applied Chemistry (IUPAC). Extraction efficiency of the technique measures the amount of analytes extracted with the respective technique relative to the amount of analytes initially added to the substrate (JSC Mars-1).