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Solvent extraction of organic molecules of exobiological interest for in situ analysis of the Martian soil

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Abstract

A solid–liquid extraction method able to perform in situ extraction of organic compounds on Mars is proposed. The extraction efficiency of various organic solvents was tested and compared to that of water. The selected key compounds are molecules of exobiological interest: glycine, alanine, serine, glutamic acid, oxalic acid, benzoic acid, phthalic acid, isophthalic acid, terephthalic acid and 1,3,5-benzenetricarboxylic acid. Among the organic solvents, propanol gives the highest yield of extraction for all the targeted compounds except for benzoic acid. A mixture of propanol and ethyl acetate increases significantly the extraction yield of benzoic acid. The extraction time was considerably reduced (140 h to 15 min) by using sonication. The method is discussed for an easy automation with coupling to an in situ GC–MS space instrument.
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Keywords: Extraction methods; Solid–liquid extraction; Derivatization, GC; Martian samples; Soil; Amino acids; Carboxylic acids

1. Introduction

This study is part of our continuing effort to develop instrumentation for the sample analysis at Mars (SAM) experiment aiming at performing an in situ chemical analysis of the Martian soil. The main objective of SAM is to detect key organic compounds in rock and soil samples in order to assess whether organic molecules possibly associated with extinct and/or extant life are present on Mars.

The search for organic compounds on Mars began with the Viking lander GC–MS system, which was looking for organic compounds on the surface and subsurface. The GC–MS system of Viking did not detect any organics at the ppm or subppm levels [1]. Despite the negative results, it is possible that organics were present but not detected, because the GC instrument was not designed to detect non-volatile and thermally fragile organic compounds such as organic acids, including benzoic acid and amino acids. Moreover, organic compounds may exist at levels below the detection limit of the Viking instrument as these compounds could be destroyed by a strong oxidant probably present on the surface of Mars. Since meteorites and interplanetary dust must have carried organics to Mars, the currently accepted hypothesis is that these organics are present

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in the Martian subsurface, below the likely oxidative layer. The next exploration of Mars for possible signs of life will focus not only on key organic molecules, such as amino acids which have been detected in several Martian meteorites [2], but also on smaller molecules such as carboxylic acids which could be metastable intermediates of organics under oxidizing conditions [3].

Gas chromatography is, with mass spectrometry, one of the currently rare flight-qualified techniques for the analysis of organic molecules. It has already been used successfully in several space missions to Mars [4] and Venus [5], and it has been selected for the in situ analysis of Titan's atmosphere in the frame of the Cassini–Huygens mission [6,7] and in the forthcoming Rosetta mission to comet P/Wirtanen [8–10]. That is why SAM is a GC–MS based experiment including also a pyrolyzer and a sample treatment subsystem which must extract and transform, prior to analysis, the highly polar and thermally fragile targeted compounds potentially present in the Martian soil [11].

In a previous study, several derivatization methods fully compatible with space constraints, have been studied [12]. In this study, we present the development of an extraction method using an organic solvent able to extract quantitatively both amino and carboxylic acids from the Martian soil. Several methods of extraction are referenced in the literature. Water is the most widely used solvent. It allows the extraction of a large number of organic compounds such as amino acids, some carboxylic acids or humic acid [13]. Using water extraction (liquid–solid), Brinton and Bada [14] have proved the presence of amino acids in the lunar soil by using HPLC to analyse samples from Apollo missions. They have identified aspartic acid, serine, glutamic acid, glycine and alanine. Baziramakenga et al. extracted organic acids from soil with a solution of NaOH within 12 h [15]. Dai et al. [16] extracted humic and fulvic acids with the same method, allowing a better solubilization of the conjugate basic form. Martens et al. used a mixture of trichloroacetic acid and HPO_4^{2-} as a specific extractant of adenosine triphosphate (ATP) [17,18]. They used a multistep extraction process with sonication in order to accelerate extraction. An extraction with a mixture of dimethyl sulfoxide–Na_3PO_4 (pH 1.7) and a quaternary amine detergent was found to be suitable to estimate ATP in agricultural soils.

Despite its high power of extraction, water cannot be used for the in situ analysis of the Martian soil, because the search for water is an important objective of the Mars exploration and water used in the extraction procedure could pollute the Martian environment. In the present work, we compared the use of several organic solvents (ethanol, propanol, acetonitrile, ethyl acetate and dichloromethane) for extracting organic and amino acids present in soil with that of water. Generally, the extraction methods do not use alcohols to extract organic pollutants in soils. Nevertheless, Nam et al. [19] added a small amount of alcohol to alkane mixtures to improve the extraction efficiency of polychlorinated biphenyls and polychlorinated dibenzo-*p*-dioxine, a mixture of alkane–alcohol (5:1) giving the best results.

In the present study, we have tested the analytical technique by using standard soil samples prepared by adding given amounts of the compounds of interest to a representative soil (sand of Fontainebleau). This sand is relatively free of organic contamination and was washed with concentrated sulfuric acid before use. The derivatization procedure was performed after extraction to analyse the organic acids by GC. The one-step silylation reaction [21] was selected because of the anhydrous conditions requirements of the derivatization method. Moreover, it can be easily automated and integrated into space instrumentation [12]. In order to reduce the extraction time and make it compatible with space constraints (short analysis time and low energy consumption), sonication-assisted extraction was performed.

2. Experimental

2.1. Reagents

The solid samples used for this study were soil from Fontainebleau (Prolabo, France) of particle size ranging from 230 to 310 μm (volumic mass = 1380 g l^{-1}). All the amino and carboxylic acids used for the preparation of the standard solution: alanine, glycine, serine, glutamic acid, carboxylic acids (oxalic, benzoic, terephthalic, isophthalic, phthalic and 1,3,5 benzenetricarboxylic) were purchased from Aldrich

(99% min). *N,N*-Methyl-*tert.*-butyl(dimethylsilyl)trifluoroacetamide (MTBSTFA) and dimethylformamide (DMF) were obtained from Interchim (France) and from Fluka (France), respectively. Anthracene (99%), used as internal standard, was provided by Prolabo (France). Ethanol (99.5%) and acetonitrile (99.8%) were purchased from Prolabo propanol (99.8%) from Aldrich and dichloromethane (99.9%) from Riedel-de Haen (Germany).

2.2. GC–MS Instruments

The analyses were performed with a Shimadzu QP5050 GC–MS instrument operated with a quadrupole detection mode. The temperature of the split/splitless injector was 300 °C and the detector 270 °C. Helium was used as carrier gas.

A fused CPSIL 5 CB capillary column (15 m× 0.25 mm, 0.25 μm) from Varian-Chrompack (USA) was used for the analyses.

2.3. Standard soil preparation

A sample of Fontainebleau sand was cleaned with sulphuric acid in order to eliminate all organic traces. This soil is free of organic substances as tested with a blank chromatogram obtained after performing a standard extraction and derivatization procedure. The concentration of each organic compound, contained in the sand is determined by weighting. Accurate amounts ($5{\cdot}10^{-5}$ mol) of each organic acid were diluted in 250 ml of water–ethanol (90:10, v/v) and added to 25 g of sand. The suspension was mixed for 24 h using a shaker and the solvent is evaporated to dryness by gentle warming at 40 °C. This procedure was carried out 4 times to eliminate the organic compounds which could be adsorbed on the vessel walls. It enabled us to prepare spiked soil samples of a known final concentration for each organic acid (around $8{\cdot}10^{-7}$ mol g^{-1}).

2.4. Extraction procedure

The extraction was performed in a sealed chamber, in order to avoid the evaporation of solvent, with 1 g of soil and 2.5 ml of solvent. Extraction temperature was kept at 60 °C using an oil bath. The mixture was shaken for several hours. For some experiments, the mixture was sonicated in an ultrasonic bath (frequency 48 kHz, Bransonic 12, Germany) at 60 °C in order to accelerate the extraction step. Then, the supernatant was filtered on a 10 μm MoBiTec filter (MoBiTec, Germany) and the solvent was evaporated to dryness under nitrogen flow at 40 °C. Organic acids were stored in the extraction flask, ready for derivatization and GC–MS analysis.

Six different solvents were tested for extraction: water, ethanol, propanol, ethylene glycol, dichloromethane and acetonitrile. Their main physical characteristics are summarized in Table 1.

Table 1
Characteristics of the extraction solvents [27]

Solvent	Formula	B.p.(°C)	ϵ/ϵ_o
Water	H_2O	100	78.5
Ethanol	C_2H_5OH	78.5	24.3
Propanol	C_2H_7OH	89.5	20.1
Acetonitrile	CH_3N	56.7	63.7
Ethyl acetate	$CH_3COOC_2H_5$	77	6.2
Dichloromethane	CH_2Cl_2	40	9.1(20 °C)

3. Results and discussion

Our experiments focused on the analysis of nine targeted compounds, all of primary exobiological interest: glycine, alanine, serine, glutamic acid, oxalic acid, benzoic acid, phthalic acid, isophthalic acid, terephthalic acid and 1,2,3-benzenetricarboxylic acid. Serine excepted, all the selected amino acids have been found in Martian meteorites [2].

The classical method for extracting organic acids from a solid matrix generally uses hot water or a concentrated acid solution to hydrolyze the organic material present in the soil [20]. However, water is one of the main components to be detected in the Martian soil and its presence is currently discussed. Therefore, a possible terrestrial contamination by an onboard experiment should be avoided and water cannot be used for the extraction or derivatization modules. For developing the in situ extraction procedure of Martian soil, we shall test different possible organic solvents and compared their extraction capacity to that of hot water. The derivatization

method selected for this work is silanisation [21] as this technique requires water free conditions.

To estimate the extraction efficiency, given amounts of organic acids were added to a standard soil free of organic material. The acids were dissolved in an organo–aqueous solvent and the solvent was completely evaporated while stirring to obtain an homogeneous standard sample. This method eliminates the possibility of nonreproducible adsorption of each organic acids. Although the levels of the organic acid concentrations introduced in the solid matrix are higher than those expected to be present in the Martian soil, this standard sample enables us to compare, within controlled and reproducible conditions, the extraction efficiency of each tested solvent.

3.1. Derivatization reaction

The advantage of the silanisation procedure used after extraction, filtration and evaporation, is that it is a single-step reaction [22] which does not require separation of the derivatives prior to GC analysis. Previous studies have shown [12,21] that the derivatization of amino acids, using MTBSTFA (30 μl) as reactant in DMF (10 μl), is achieved with a maximum yield close to 100% at a temperature of 75 °C in 30 min. A similar derivatization yield was obtained for the carboxylic acids when using the same operating conditions. The scheme of the one-step derivatization process leading to *N,N*-(*tert.*-butyldimethylsilyl) derivatives of carboxylic acids is presented in Fig. 1.

3.2. GC–MS analysis

Fig. 2 shows the chromatogram obtained with an apolar polydimethylsiloxane capillary column. The silylated derivatives are separated in a single temperature-programmed run from 90 to 250 °C, at a rate of 3 °C/min within 50 min. The analysis of the amino acid derivatives is achieved in less than 30 min. The compounds are separated with a very good resolution and optimization of the temperature program should significantly reduce the analysis time.

The mass fragmentations of the silylated derivatives are given in Table 2. All the targeted analytes were easily identified from their major fragments ions and were quantified using anthracene as internal standard. As this compound does not react either with MTBSTFA or DMF, it is systematically added to the standard solution. A calibration graph was obtained with 5 concentrations of anthracene between $7\cdot10^{-5}$ and $1.4\cdot10^{-3}$ *M* diluted in cyclohexane (1 μl injected). A linear response was obtained: $C=1.1\cdot10^{-10}\pm1.8\cdot10^{-12}A$, ($R^2=0{,}99$) where *C* and *A* are, respectively, the anthracene concentration (*M*) and the area of the chromatographic peak.

3.3. Soil extraction

In order to have constant thermodynamic parameters, soil extraction was performed at 60 °C with different organic solvents. The results are compared with those of water. The relatively low temperature selected for the extraction is due to the objective of reducing energy consumption for a space-compatible experiment. Different extraction times ranging from 3 to 140 h were tested.

The chromatogram of the silylated derivatives after extraction is given in Fig. 3. Quantitative analysis of the extraction method was evaluated from the peak areas. As illustrated in Fig. 3, all the targeted compounds that eluted within 60 min are identified and quantified (Fig. 3).

Fig. 4 summarizes the results obtained with the five organic solvents versus the extraction time. The

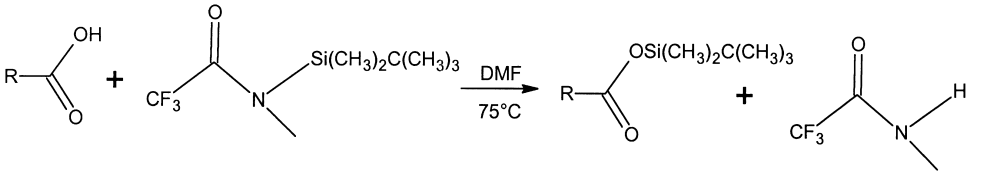

Fig. 1. Scheme of the derivatization reaction.

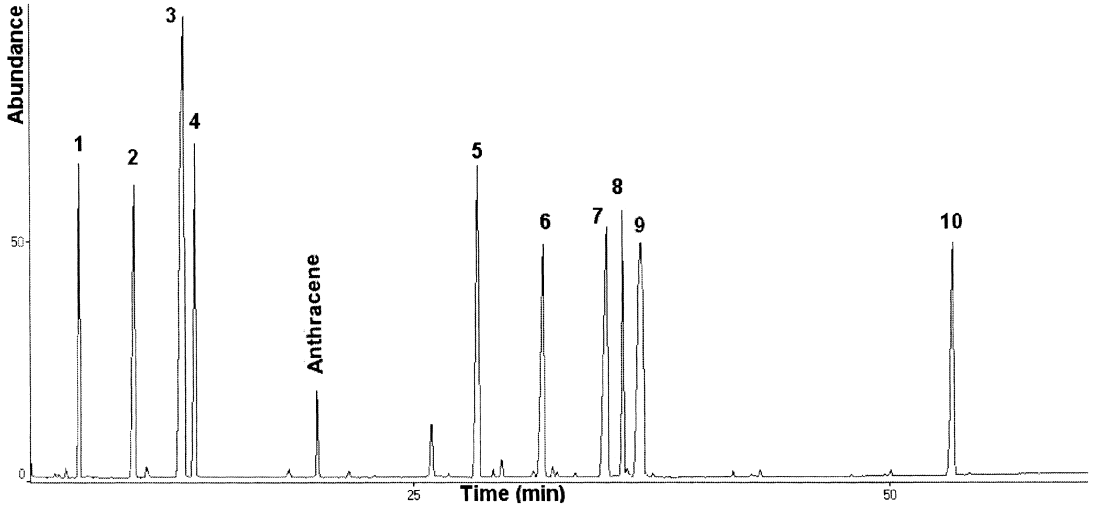

Fig. 2. GC–MS analysis of the carboxylic acids and amino acids standard mixture (10^{-3} *M* each) derivatized by MTBSTFA treatment. A 15 m×0.25 mm CPSIL 5 CB fused-silica WCOT column, operated in the split mode (1:100) was programmed at 3 °C min^{-1} from 90 °C to 250 with an inlet internal helium pressure of 19.5 kPa; 1 = Oxalic acid, 2 = benzoic acid, 3 = alanine, 4 = glycine, 5 = serine, 6 = phthalic acid, 7 = terephthalic acid, 8 = glutamic acid, 9 = isophthalic acid, 10 = 1,3,5-benzenetricarboxylic acid.

best results are obtained with propanol, except for the extraction of benzoic acid. Dichloromethane does not extract amino acids. The only extracted acids are the three benzenedicarboxylic acids with a maximum yield of only 20%. This low extraction yield can be explained since CH_2Cl_2 is a nonpolar solvent.

All carboxylic acids, except benzoic acid, can be extracted with acetonitrile. For the amino acids, the extraction recovery is poor, close to 5% for serine and a quantitative analysis cannot be achieved. Ethyl acetate is the best solvent for the extraction of carboxylic acids: unlike acetonitrile, it is able to extract benzoic acid with an extraction yield close to 30%, but does not extract any of the targeted amino acids.

With ethanol, the extraction yield of amino acids does not exceed 20%. Compared to the extraction power of propanol, such a poor result could be explained by the shorter alkyl chain. Therefore, the solubility of organic compounds with long alkyl chains will be higher in propanol. Ethanol cannot be used as an extraction solvent for amino acids, as the analysis is not quantitative.

Propanol is the best compromise for the extraction of amino and carboxylic acids. It enables a quantitative extraction of target compounds, except for

Table 2
Major fragment ions of the silylated derivatives of amino and carboxylic acids in GC–MS

Peak no.	Silylated acid derivative	M_r	Fragments (intensities)
1	Oxalic acid	303	276(2) 234(10) 233(32) 189(5) 147(100) 133(7) 117(8) 73(43) 57(10)
2	Benzoic acid	317	179(100) 149(1) 135(40) 105(72) 77(90) 57(40)
3	Alanine	450	302(1) 260(47) 232 (72) 189 (2) 158(89) 147(74) 73(96)
4	Glycine	489	288(1) 260(1) 247(10) 246(50) 218(67) 189(26) 147(90) 73(100)
5	Serine	318	432(1) 391(12) 390(32) 363(12) 362(40) 302(26) 288(36) 147(25) 73(100)
6	Glutamic acid	236	474(1) 433(28) 432(50) 330(40) 272(40) 147(30) 73(100)
7,8,9	Phthalic, isophthalic, terephthalic acid	394	379(1) 339(12) 338(27) 337(100) 279(14) 178(29) 104(33) 73(67) 57(15)
10	1,2,5 Benzenetri-carboxylic acid	552	537(2) 498(2) 497(9) 495(20) 495(70) 177(15) 73(100) 57(20)

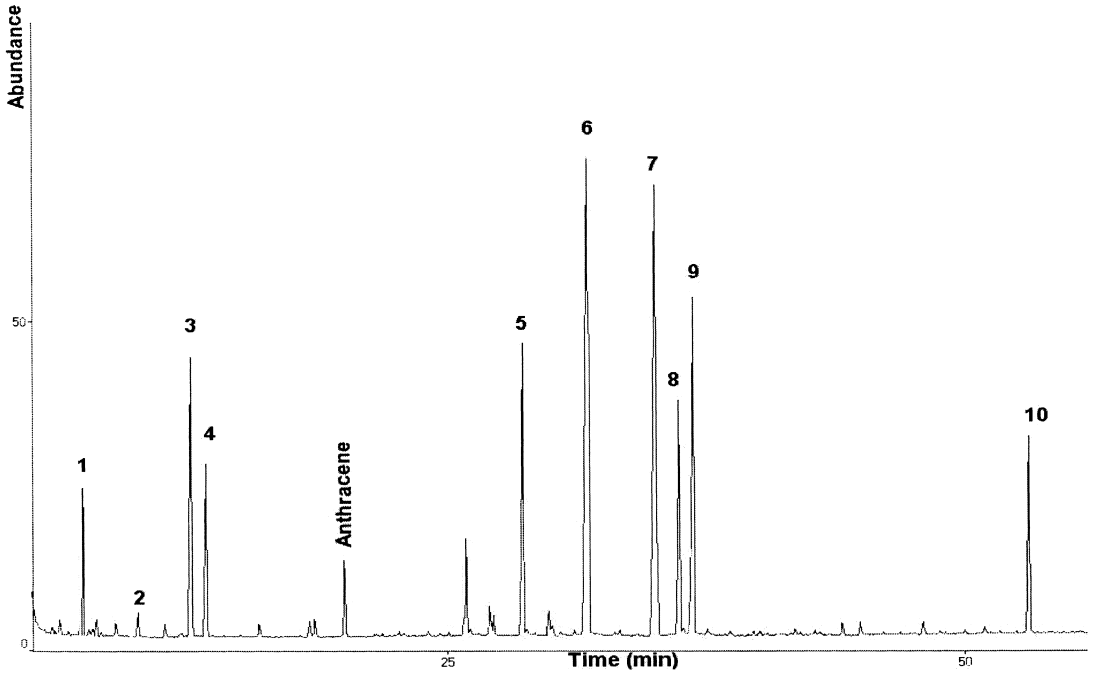

Fig. 3. GC–MS analysis of the silylated carboxylic and amino acids derivatives extracted with propanol. Same experimental conditions as in Fig. 2.

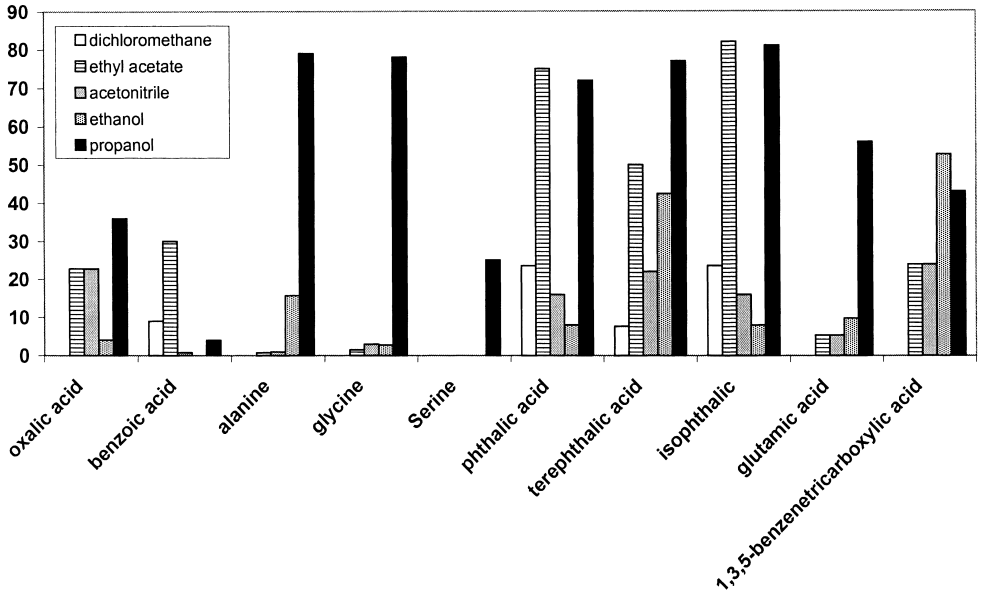

Fig. 4. Comparison of the recoveries of organic acids with different organic solvents.

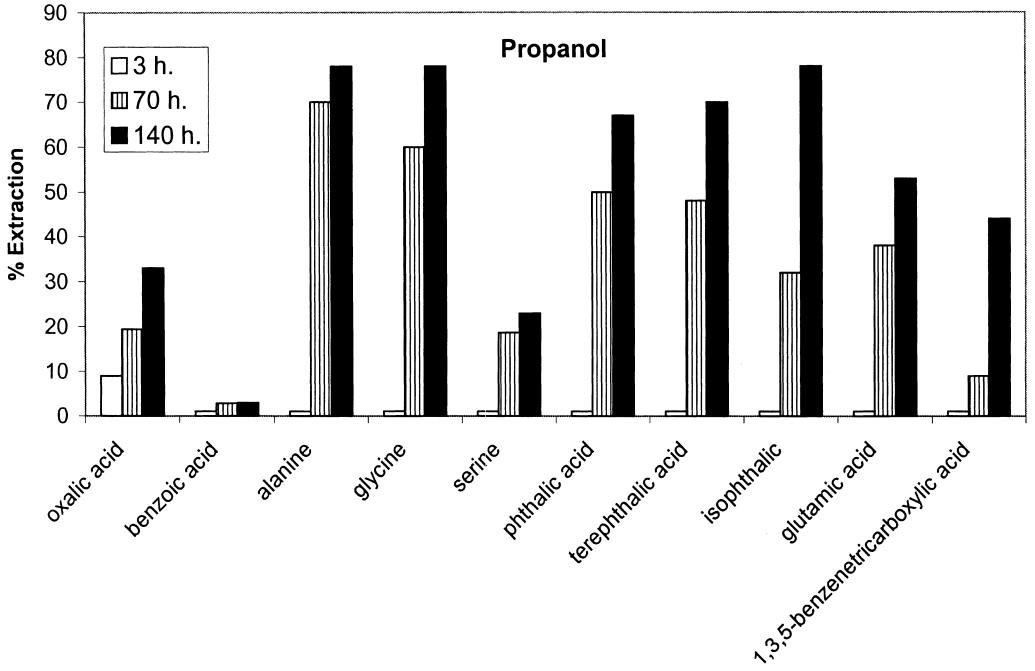

Fig. 5. Effect of the extraction time on the recoveries of organic acids with propanol.

benzoic acid and 1,2,3-benzenetricarboxylic acid. Its higher extraction efficiency for amino acids compared to ethanol is probably due to its longer alkyl chain which increases the amino acids solubility. Fig. 5 illustrates the effect of time on the yield of extraction. The best recoveries ranging from 20 to 80% are obtained for an extraction time of 140 h.

Although amino acids are generally analysed from hot water or acid hydrolysed extracts [14], aqueous solvents cannot be used for in situ analysis on Mars. It is therefore interesting to compare the extraction capacity of hot water with that of the other organic solvents. The results listed in Table 3 show that even through high extraction power permits quantitative analysis of amino acids, the recovery is low for carboxylic acids of high hydrophobic character such as benzoic acid. This can be explained by the low solubility of this compound in water.

With propanol (Table 3), the extraction yield for amino acids in soil is almost as good as that of

Table 3
Comparison of the extraction yields with propanol and water

Compounds	Melting point (°C)	Extraction yield with water (%)	Extraction yield ratio propanol:water
Oxalic acid	190	13	2.5
Benzoic acid	121–123	2	1.0
Alanine	272–275	90	0.9
Glycine	245	92	0.9
Serine	240	85	0.3
Glutamic acid	194	61	0.9
Phthalic acid	200–205	82	0.9
Terephthalic acid	300	60	1.3
Isophthalic acid	341–343	81	1.0
1,3,5-Benzenetricarboxylic acid	380	29	1.5

water, except for serine which has a more hydrophilic character. Moreover, for carboxylic acids, the extraction yield with propanol is better than with water. Such a result shows that propanol is a good solvent for extracting organic acids except benzoic acid, but this is also the case for water.

In order to perform the extraction of all targeted compounds, benzoic acid included, preliminary studies were carried out using a propanol–ethyl acetate solvent mixture. We have observed that mixed with ethyl acetate (1:1, v/v), propanol extracts benzoic acid as well as the other compounds, with a maximum extraction yield of 28%. A mixture of organic solvents is probably a solution for improving the extraction yield of the less soluble compounds in propanol.

3.4. Detection limit

We studied the detection limits obtained for trace amounts of the target compounds in soil after performing the whole sample analysis process, including extraction by propanol, derivatization and GC–MS detection. These analyses were carried out by preparing standard soils with decreasing amounts of organic acids (10^{-7} to $10-^{10}$ nmol g^{-1}). The level at which the organic acids were detected in 1 g of solid sample is reported in Table 4. The detection limit is probably lower considering that the presence of the targeted compounds at half of this concentration was not detected after extraction with propanol. These values are about 10 times larger than the detection limit given for derivatization and GC–MS analysis [12]. This is due to the extraction process as the solvent is not able to extract the totality of the organics when adsorbed at trace levels in the soil.

The concentrations of organic acids detected in Martian meteorites are also given in Table 4. Except for the Murchison meteorite, our propanol extraction method is not sensitive enough to detect the targeted compounds. This shows the need to further optimize the extraction procedure.

3.5. Ultrasonic solvent extraction

The analysis duration is a major concern for an in situ analysis. An extraction time ranging from 70 to

Table 4
Detection at trace levels of the key organic compounds using the propanol extraction method. Comparison with the levels detected in meteorites

Organic acid	Trace level detected per gram of solid (nmol)	Meteorites	Concentration (nmol g^{-1})	Ref.
Oxalic acid	9	–	–	–
Benzoic acid	0.7	–	–	–
Alanine	6	Nakhla	0.36	[23]
		Nile Delta	0.05	[23]
		Murchison	12.9	[4]
Glycine	5	Nakhla	0.5	[23]
		Nile Delta	6.5	[23]
		Murchison	28.1	[4]
Serine	0.7	–	0.2	[23]
		–	1.2	[23]
Phthalic acid	5	–	–	–
Terephthalic acid	5	–	–	–
Glutamic acid	5	Nakhla	0.01	[23]
		Nile Delta	0.6	[23]
		Murchison	4.6	[4]
Isophthalic acid	5	–	–	–
1,3,5-Benzene-tricarboxylic acid	6	–	–	–

140 h is too long for an in situ experiment. It is not only a problem of analysis time, but also of energy consumption. Therefore, the procedure described above is not compatible with the constraints of space instrumentation. In order to accelerate the extraction step, we have performed ultrasonic extraction from soil as suggested by previous studies [24–26], using a laboratory device.

Many references are pointing out that this technique is a very efficient way to significantly increase the recovery. Babic [24] reported that the use of sonication allows one to accelerate Soxhlet extraction from 24 h to 15 min. Martens et al. [17] used repeated extractions with sonification to improve the recovery of ATP from soil. The advantage of using an ultrasonic device is thus to increase both the rate and the yield of extraction.

The results with ultrasonic assisted extraction and propanol as solvent are given in Fig. 6. The extraction duration has been reduced to 15 min at 60 °C. Compared to the previous extraction procedure, the extraction yields are larger for all targeted compounds. The use of sonication has considerably reduced the extraction time and makes it compatible with the use for a space experiment. These are preliminary results; various experiments at different sonication times and temperatures are now needed to optimize the extraction method. This will be performed on more representative samples such as Martian soil analogues.

4. Conclusion

The first objective of this study was to find an appropriate solvent, excluding water, for an in situ quantitative extraction of compounds of exobiological interest in the Martian soil. These targeted compounds are expected to be present in extraterrestrial environments such as the Martian soil. The results of this study carried out on standard soil samples indicate that propanol can be used to extract with a high recovery yield, both amino and carboxylic acids from a solid matrix. Sonication greatly reduces the extraction time and makes this method compatible with space constraints. The next step currently in progress in our laboratory, is the development of an automated miniaturized reactor, where both the extraction and the derivatization processes can take place.

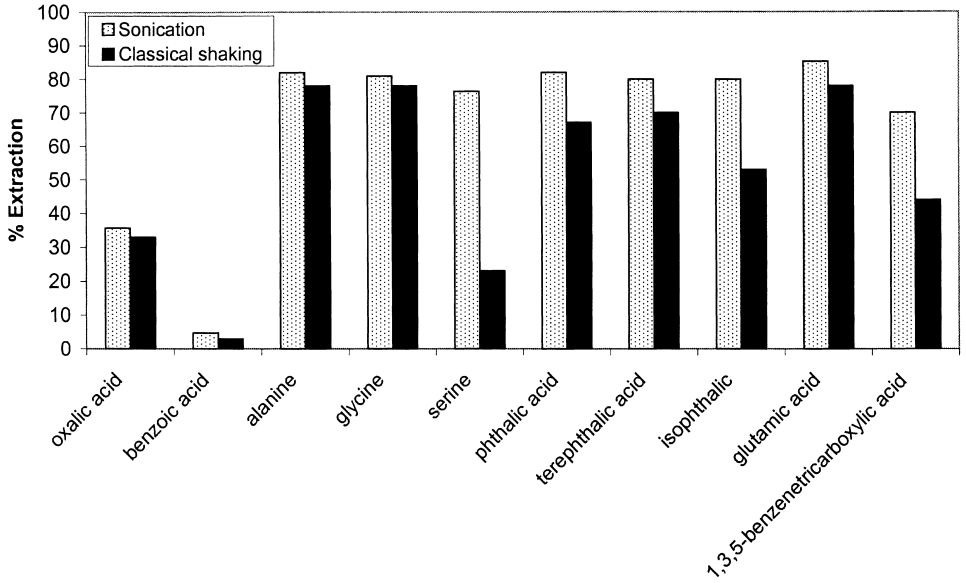

Fig. 6. Comparison of organic acids recoveries with propanol extraction by sonication (60 °C, 15 min) and classical shaking (60 °C, 140 h).

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