Gas chromatography–mass spectrometry of hexafluoroacetone derivatives: First time utilization of a gaseous phase derivatizing agent for analysis of extraterrestrial amino acids

C. Geffroy-Rodier, A. Buch, R. Sternberg, S. Papot

Journal of Chromatography A

ABSTRACT

Within the perspective of the current and next space missions to Mars (MSL 2011 and Exomars 2016–2018), the detection and enantioselective separation of building blocks such as the amino acids are important subjects which are becoming fundamental for the search for traces of life on the surface and subsurface of Mars. In this work, we have developed and optimized a method adapted to space experimentation to derivatize and analyze amino acids, using hexafluoroacetone as the derivatizing agent. The temperature, duration of the derivative transfer to the analyser, and chromatographic separation parameters have been optimized to meet the instrument design constraints imposed on devices for extraterrestrial experiments. The work presented in this rationale has established that hexafluoroacetone, in addition to its intrinsic qualities, such as the production of light-weight derivatives (no racemization) and great resistance to the drastic operating conditions, has indeed facilitated simple and fast derivatization that appears to be suitable for in situ analysis in space. By using hexafluoroacetone as the derivatizing agent, we successfully identified, 21 amino acids including 12 of the 20 proteinic amino acids without stirring or extraction steps. Ten of these derivatized amino acids were enantioselectively separated. The precision and accuracy measurements for the D/L ratio showed that the proposed method was also suitable for the determination of both enantioselective forms of most of the tested amino acids. The limits of detection obtained were lower than the ppb level of organic molecules detected in Martian meteorites.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

In order to search for traces of life on other planets, it is necessary to analyze extraterrestrial samples for known terrestrial organic biomarkers. Detection of these biomarkers would provide evidence that the sample’s planetarian source has supported some form of life. Terrestrial biomarkers could either be a remainder of life and/or an indication of actual life. Among the several known classes of organic compounds that could be used as possible life indicators, we have chosen the amino acids as these are the monomer building blocks of proteins. While these compounds are not functional biomarkers, the detection of their presence would make them a good complementary diagnostic tool from which biotic or prebiotic activity can be proven.

If an instrument is to be used in a space mission, it will require a number of features: simplicity of construction, small size and weight, low energy/power consumption and most of all, it will be tolerant to high mechanical shock and resistant to vibration. Gas chromatography (GC) coupled with mass spectrometry (MS) instruments fulfills these requirements [1,2]. Unfortunately, GC instruments can only be used to analyze molecules which are volatile and thermally stable. As such, in their unmodified state, few or none of the proposed terrestrial organic biomarkers would be compatible with a GC–MS experiment. However, it has been demonstrated that refractory molecules can be derivatized prior to GC analysis to facilitate their in situ detection by GC–MS [3–5]. So, within the context of the proposed NASA/MSL and the ESA-NASA/Exomars missions, all GC experimentation designed for terrestrial biomarkers detection will include a pre-analysis chemical derivatization procedure [6].

To achieve successful detection, quantitative as well as enantioselective separation of amino acids, the in situ derivatization has to meet a number of requirements. First, for use as a space application, in situ derivatization needs to be sensitive, automated and both minimally energy and time consuming. Secondly, derivatization reactions have to enable detection of enantiomeric excess. Lastly, reaction conditions have to minimize the possibility of generating racemized amino acid products. In these drastic conditions,
target molecule detection is the main goal, whereas quantitation and enantioselective separations are the secondary objectives.

Many derivatizing agents allow enantioseparation of α-amino acids by gas chromatography on several chiral stationary phases [7] but very few fulfill the requirements of working in space [3]. To date, the most popular derivatizing agent used in conjunction with GC–MS experimentation is N-(ter-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA) [3,8–10]. However, MTBSTFA is unsuitable for enantioselective separation of amino acids. In order to separate amino acid enantiopure pairs, other derivatizing procedures have been developed including alkylalkoxy-carboxylate–esterification (alkyl chlorofomate) [3,11–14] and perfluoroacylation/perfluoroesterification [15,16]. N,N-Dimethylformamide-dimethylacetal (DMF-DMA) dependent derivatization has also been proposed as an alternate chiral-based separation technique for amino acid compounds [8,17,18]. However all these reactions need high temperatures or vigorous stirring and therefore lead to partial racemization or numerous by-products. Under the constraints of working in space, the use of a derivatizing agent in gas phase at low temperature without stirring appears to be a valid alternative for in situ chemical treatment.

The aim of this paper is to study the first gaseous derivatizing agent for in situ purposes. To face space constraints the derivatization procedure has to consume low levels of energy, include a one-pot reaction and be sensitive. To enhance detectability, derivatization should introduce groups with high electron affinity, such as halogen atoms leading to high-mass-ions. Indeed, high-mass-ions have greater diagnostic value as they are more specific than low-mass-ions, which can be easily affected by interference from the fragment ions of contaminants such as those due to column bleeding. For this purpose we have chosen to evaluate hexafluoroacetone as a derivatizing agent. In contrast to the drawbacks of using hexafluoroacetone in terrestrial laboratories (gaseous, toxic and leading to very volatile compounds) could provide numerous advantages in space experiments. In fact, gases are easy to handle in space and volatile compounds are easy to transfer to the analyzer. In addition hexafluoroacetone allows the simultaneous derivatization of both the amino and carboxylic groups of amino acids leading to the corresponding 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones (Fig. 1). These five-membered ring derivatives offer several advantages for gas chromatography analysis as they are of essentially high stability and low polarity like 2,2-bis(chlorodifluoromethyl)-4-subst.-1,3-oxazolidin-5-one derivatives [19]. Moreover, it has been reported that 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones can be synthesized in the course of a simple one-step-one pot procedure without racemization of the stereogenic centre of amino acids [20]. As a consequence of the low polarity of most of such derivatives, further treatment with an anhydride or silyl reagent prior to GC experiments is not required. Thus, hexafluoroacetone derivatization appears as a simple and easily automated derivatization procedure that could be incorporated into future in situ space experiments. The use of hexafluoroacetone as a derivatizing agent for amino acids analysis is however poorly described in the literature and to the best of our knowledge no chromatographic separation excluding 7 amino acids has been achieved and no mass spectrometry analysis has been performed [21]. In this study the best analytical conditions for a derivatization reaction for space applications have been determined. With regard to a possible application in the analysis of non-proteinic amino acids, the reaction conditions were also tested on isovaline, amino isobutyric acid, phenyl-glycine, sarcosine, N-ethyl-glycine and N-methyl-leucine. Indeed, many non-proteinic amino acids had been recovered from several carbonaceous chondrites such as Murchison, Orgueil, Murray or Allende [22,23]. Finally, as derivatization in space will be directly conducted on soil samples, we so tested the hexafluoroacetone derivatization on different mineral samples which might hinder the reactions.

2. Experimental

2.1. Chemicals and materials

Eighteen proteinic amino acids, sarcosine, N-ethyl-glycine, 2-amino-isobutyric acid, phenyl-glycine were obtained (98–99% purity) from Sigma–Aldrich (Buchs, Switzerland). Sigma–Aldrich also supplied dichloromethane (DCM) dimethylsulfoxide (DMSO) and hexafluoroacetone (HFA). N-methyl-leucine and isovaline were obtained (97–99% purity) from Acros (Noisy le Grand, France). D-tryptophan (98%) and D,L-isoleusine (99%) were obtained from Alfa Aesar (Ward Hill, USA). Stock solutions of amino acids (10^-3 M) in pure water were kept at 4 °C. Pure helium (99.9995%, Messer, France) was used as the carrier gas for the GC–MS.

2.2. Derivatization process

Hexafluoroacetone has been successfully used as a reagent for the simultaneous protection and activation of 26 amino-, hydroxyl- and mercapto-mono acids in DMSO [20,21] and for α-amino diacids such as aspartic and glutamic acid in DMS [24]. In a quest for spatialization, we omitted the stirring and the extraction steps usually described when hexafluoroacetone is used as the protecting and activating agent [20].

The in situ derivatization used in our optimized solvent free procedure are described below and further optimization details are given in Section 3.3.1.

2.2.1. Optimized procedure

The amino acid water solution (1 mL, 10^-2 M) was introduced into a 25 mL flask and the water was removed at 60 °C under a N2 flow. The top of the flask was cooled to −78 °C with a dry ice-acetone bath and equipped with a dry ice condenser containing acetone to −78 °C. The gaseous hexafluoroacetone (1 g, 6 mmol) was introduced into the reaction vessel with a gas-tight syringe and maintained in the liquid state without stirring for 30 min. The reaction mixture was then allowed to warm up at room temperature over a period of 30 min. Aliquot of sample was analyzed by GC–MS.

When headspace or SPME transfers were not performed, 1–2 mL of CH2Cl2 was added before warming up to allow liquid injection. In this case proline was poorly recovered (less than 20%) but glutamic acid was fully detected.

The derivatization reaction scheme for the amino acids is illustrated in Fig. 1. For the amino acid samples, the derivatization reaction led to the formation of the corresponding 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-one.

2.3. Gas chromatography mass spectrometric analysis

All experiments were performed using a ThermoScientific Gas Chromatography/Mass Spectrometer Trace-DSQ (GC–MS). For each GC–MS analysis, an autosampler was used to inject into the GC–MS.
Before each liquid injection, the syringe was carefully washed 10 times with dichloromethane, and then again 10 times with methanol to avoid contamination. The DSQ GC/MS instrument was operated in EI (electron impact) and CI (chemical ionization) detection mode with the source temperature set at 280 °C and the ion transfer set at 240 °C. The GC–MS was equipped with a split/splitless (1/50) injector, the temperature of which was set to 200 °C. Gas flow was set at 1 ml/min. Xcalibur Real Time Analysis software controlled data acquisition and GC–MS. Injection of 1 μl was used for liquid analysis. 0.5 ml injection (1 min enrichment delay, 50 ml/min injection speed, 10 ml/vial, 80 °C) was used for headspace analysis and a 15 min sampling on 100 μl PDMs fiber was used in a 10 ml vial with needle speed of 20 mm/s (conditioning 200 °C, 15 min) for SPME analysis.

Four capillary columns were used: one achiral column and three columns dedicated for enantiomeric selection. The achiral column was a BPX5 fused silica capillary column from SGE (5% Phenyl Polysilphenylene-siloxan, 30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness).

For enantiomeric separation a SGE CYDEX capillary column (Chiral-β-dex (heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrine), 25 m length, 0.22 mm internal diameter, and 0.25 μm film thickness), SUPERLCO Gamma DEX TM 120 (20% permethylated γ-cyclodextrine in 35% phenyl/65% dimethylsiloxane phase [0.25 μm film thickness] or CP CHIRAL-L–VAL [0.12 μm film thickness]) from Varian (Middelburg, The Netherlands); both 30 m length, 0.25 mm internal diameter, were used. Maximum operating temperature of the column never exceeded 200 °C.

Chromatographic conditions have been optimized for each column and are discussed in Section 3.2.

To determine the amino acid chromatographic response and unique spectral characteristics, each amino acid was characterized separately on the different columns. Results generated by the mass spectrometer were used to expand the non-existent NIST mass spectral library of hexafluoroacetone amino acid derivatives.

### 3. Results and discussion

#### 3.1. Mass spectra library and structural information on the derivatives

The first step of the study was to synthesize amino acid derivatives at the millimolar level to obtain references to achieve reliable gas chromatographic and mass spectrometric identifications (Table 1).

The interpretation of the described electron-impact mass spectra and the assignment of various ions were based on the knowledge of the fragmentation behaviors of other amino acid derivatives. The assignment of the radical molecular ions M** + was experimentally confirmed by measuring the m/z values of the protonated molecules [M+H]** formed by chemical ionization (methane) mass spectrometry, which exhibited similar fragmentation patterns to the radical molecular ions M**+. Table 1 lists the mass values of the characteristic peaks in the spectra of individual amino acid derivatives. Figs. 2 and 3 describe fragmentation routes.

All HFA amino acid derivatives exhibit low molecular mass base peak within the range of the mass spectrometer instruments used for Mars Science Laboratory space mission (2–535 u) [25].

All HFA derivatives show a similar, characteristic fragmentation pattern. A peak is present in all mass spectra at m/z = 69, corresponding to the ion CF3+. Other ions present in mass spectra are detected and can be attributed to a C–C–C cleavage of the carbonyl group with consecutive loss of CO2 (m/z = M − 44), or from internal cleavage at various points within the molecular ion itself.

Fig. 4 shows the electron impact mass spectrum (EI-MS) of norvaline HFA derivative as a representative of the first amino acid group (N-Me-alanine, alanine, N-Me-glycine, N-Et-glycine, leucine, phenylglycine, proline, methionine, norvaline). Two fragmentation routes (a and b) are possible through the loss of CF3 or F followed by rupture of a C–C–O cleavage of the carbonyl group with consecutive loss of CO (Fig. 2). Path a, exhibiting a more stable ion m/z = M − 69 [M–CF3]** compared to M − 19 [M–F]**, is preferred, except for methionine. Ions at m/z = M − 44 [M–CO2]** and m/z = M − 113 [M–CF3CO2]** are also present.

Fig. 5 shows the EI-MS of the valine HFA derivative as a representative of the second amino acid group (valine, isoleucine, serine and threonine). All the compounds exhibited a substituent on the α C of the chiral carbon. The loss of the side chain is then favored by hydrogen migration originating from the substituted carbon to produce the main peak m/z = 223. Another fragment process involving a double hydrogen rearrangement (“McLafferty + 1” rearrangement) is likely to be responsible for the formation of m/z = 205 and daughter ion m/z = 177.

Fig. 6 shows EI-MS of the phenylalanine HFA derivative as a representative of the last group. Amino acid derivatives with either an aromatic (Ar) or either heterocyclic group in the side chain
Fig. 2. Two fragmentation routes (a and b) of HFA derivative of norvaline.

(histidine, phenylalanine, tyrosine, tryptophan) give a base peak due to the side chain \([\text{ArCH}_2]^+\). The EI-MS of the hexafluoroacetone derivatives of non-\(\alpha\)-amino acids (AlBA, isovaline, 2-methylleucine) exhibited prominent ions resulting from the loss of the side chain at \(m/z = 236\) \([\text{M} - \text{R}]^+\). For both group \(\text{M}^{+*}\), \([\text{M} - \text{CF}_3]^+\) and \([\text{M} - \text{CF}_3-\text{CO}]^+\) are present as minor peaks.

Glutamic acid exhibited prominent peaks corresponding to the loss of two \(\text{CF}_3\) followed by rupture of a \(\alpha\)-C–O cleavage of the carbonyl groups with consecutive loss of two CO molecules \([\text{M} - 2\text{CF}_3-2\text{CO}]^+\) and \([\text{M} - (\text{CF}_3-\text{CO})_2]^+\). Other fragments are given in Fig. 3.

3.2. GC optimization

The chromatographic separation was optimized by selecting temperature program conditions to obtain the best separation of the amino acid derivatives of greatest exobiological interest [19]. The main factors tested to determine experimental settings were split ratio, initial column temperature, and subsequent temperature profile; these parameters being considered as the most critical for analyte separation. All of the results were obtained with gaseous optimized procedure. In this section liquid injection was chosen to overcome derivatives transfer problems and focus on chromatographic separations.

3.2.1. Achiral column

Gas chromatographic separation on achiral column can be achieved in less than 40 min (Fig. 7). The following parameters were adopted: the split ratio was set to 50, and the temperature profile consisted of a starting temperature of 40 °C held for the duration of 5 min, a linear gradient increasing 5 °C/min, and a 1-min temperature plateau held at the maximum temperature of 200 °C.

Fig. 3. Fragmentation of HFA derivative of glutamic acid.
Table 2 gives characteristics of the proteinic amino acid separation. Four replicates were analyzed. The amount of each amino acid injected was 0.2–2 nmol. The within-day and between-day precisions were determined by repeated analysis of four quality control samples on the same day and on 5 different days. All standard deviations were inferior to 10%.

For each proteinic amino acid tested, we determined the experimental limit of detection (signal over noise >3) to be around 0.5–6.5 pmol injected. This LOD range is consistent with the concentration found in micrometeorites or meteorites ([18] and references herein). We assume this data to be accurate as the method detection limit MDL, given for eight samples (Student’s \( t = 2.998 \)), is close to LOD. Basically a solution of the analyte that was five times the estimated detection limit (LOD) was analyzed four times to determine the standard deviation of the data set. The method detection limit (MDL) is calculated according to the formula:

\[
\text{MDL} = \text{Student's } t \text{ value } \times \text{ the standard deviation}
\]

### 3.2.2. Chiral columns

The optimum GC parameters such as split ratio, initial column temperature, and subsequent temperature profile, were determined for each chiral column.
The experimental limit of detection (signal over noise >3) was determined to be around 2.5–20 pmol injected for each proteinic amino acid tested.

No chiral separation was achieved on the Chirasil-L-Val column.

On the β-cyclodextrine capillary column, GC parameters were as follow: split 50, from 40 °C (10 min) to 200 °C at 5 °C/min. Among the 11 chiral proteinic amino acids analyzed, seven enantiomeric pairs could be separated under these operating conditions with a constant elution order with the exception of proline since the levogyre form always eluted first (Table 3). Most of these derivatives (8 out of 11) were strongly retained – with retention time longer than 30 min – displaying, in general, highest resolution for the compounds retained the longest (Table 4). The resolution of each enantiomer pairs Rₘ was evaluated for each amino acid. Four enantiomeric pairs displayed resolution values (Rₘ) higher than 1.5: norvaline, isoleucine, threonine and tyrosine. The three other pairs showed lower resolution (0.7 ≤ Rₘ ≤ 1.5). No enantioselective separation was obtained for leucine, methionine and phenylalanine. Tryptophan was not detected.

The optimized GC separation parameters of the γ-cyclodextrine capillary column were as follows: split 50, from 60 °C (5 min) to 200 °C at 5 °C/min. Among the 11 chiral proteinic amino acids analyzed, eight enantiomeric pairs could be separated within these operating conditions with a constant elution order since the levogyre form always eluted first. All of the derivatives analyzed were eluted with retention times shorter than 30 min (Fig. 8). Coelution of the levogyre leucine and norvaline enantiomers occurred only when the selected ion monitoring (SIM) acquisition mode was used with the proper of m/z values specific for the studied amino acids (m/z = 182 and 168). Six enantiomeric pairs displayed resolution values Rₘ higher than 1.5: norvaline, leucine, isoleucine, threonine, methionine and tryptophan. The two other pairs showed lower resolution (0.5 ≤ Rₘ ≤ 1.5). Proline, phenylalanine and tyrosine were detected but no enantioselective separation was obtained (Table 4).

Chiral separations were equivalent in terms of resolution and number of separated enantiomers. Elution on the γ-cyclodextrine capillary column is faster but coelution of leucine and norvaline occurred, so, two cyclodextrine columns have to be used.

Using a multi-column GC system in space exploration missions is necessary because of the complexity of environments to be analyzed. Most space GC–MS instruments are equipped with several columns mounted in parallel. The GC–MS of the

---

**Table 3**

Retention times (min) of HFA amino acid derivatives and their corresponding enantiomers on chiral columns.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>β-Cyclodextrine</th>
<th>γ-Cyclodextrine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>D</td>
</tr>
<tr>
<td>Alanine</td>
<td>23.83</td>
<td>24.52</td>
</tr>
<tr>
<td>Glycine</td>
<td>31.25</td>
<td>nd</td>
</tr>
<tr>
<td>Valine</td>
<td>38.72</td>
<td>39.10</td>
</tr>
<tr>
<td>Norvaline</td>
<td>42.27</td>
<td>42.58</td>
</tr>
<tr>
<td>Leucine</td>
<td>45.74</td>
<td>48.90</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>48.77</td>
<td>49.92</td>
</tr>
<tr>
<td>Proline</td>
<td>33.41</td>
<td>33.28</td>
</tr>
<tr>
<td>Threonine</td>
<td>67.65</td>
<td>67.93</td>
</tr>
<tr>
<td>Methionine</td>
<td>79.11</td>
<td>20.47</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>86.68</td>
<td>141.47</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>127.29</td>
<td>nd</td>
</tr>
</tbody>
</table>

**Table 4**

Enantiomeric resolution factors Rₘ on the two chiral columns of HFA derivatives.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>β-Cyclodextrine</th>
<th>γ-Cyclodextrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>1.05</td>
<td>0.50</td>
</tr>
<tr>
<td>Proline</td>
<td>0.70</td>
<td>0.00</td>
</tr>
<tr>
<td>Valine</td>
<td>1.11</td>
<td>0.67</td>
</tr>
<tr>
<td>Norvaline</td>
<td>1.83</td>
<td>2.12</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.85</td>
<td>1.90</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.23</td>
<td>1.91</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.00</td>
<td>1.92</td>
</tr>
<tr>
<td>Serine</td>
<td>1.42</td>
<td>2.20</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>14.90</td>
<td>0.00</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>nd</td>
<td>1.52</td>
</tr>
</tbody>
</table>

---

Fig. 6. El-MS of HFA phenylalanine derivative.

Fig. 7. Total ion current of HFA derivatives on BPX5 column. Split ratio was set to 50, temperature gradient: 40 °C (5 min), 5 °C/min, 200 °C (1 min) and He constant flow rate 1 ml/min, solvent transfer.
Cassini–Huygens mission (1997–2004) which explored the atmosphere of Titan used three columns [26]. The Cometary Sampling and Composition experiment (COSAC) of the Philae Lander of the Rosetta mission (2004–2014) is equipped with eight capillary columns, three using chiral stationary phases [27], and the Mars Science Laboratory (MSL) mission which has just been launched (on November 25, 2011) has six capillary columns on board, one of which use a chiral stationary phase [28]. The benefits of using several columns with stationary phases of different nature and polarity, are not only to widen the scope of the molecules to be analyzed, but also to allow better identification by cross checking the responses in each case of coelution.

Therefore if we consider the combination of the two chiral stationary phases studied, 10 pairs of amino acids (alanine, valine, norvaline, leucine, isoleucine, proline, threonine, methionine, tyrosine, tryptophan) among the most relevant from the point of view of exobiology can be separated (Fig. 9). In addition if we consider the eight protein amino acids found in martian meteorites (glycine, alanine, valine, leucine, isoleucine, proline, aspartic acid, glutamic acid) ([22] and references herein), six are detected and five enantiomers are separated of the seven chiral targeted. Compared to dimethylformamide-dimethylacetel used in the Mars Organic Molecule Analyzer (MOMA) instrument of the Exomars space mission, which also allows separation of 10 enantiomers (threonine, serine, alanine, valine, proline, aspartic acid, asparagine, cysteine, phenylalanine and tyrosine) [18], hexafluoroacetone could be an alternative or a complementary derivatization reagent.

A mixture of four D/L amino acids was analyzed on β-cyclodextrine capillary column: D/L-alanine, D/L-valine, D/L-norvaline and D/L-proline. The within-day and between-day precisions were determined by repeated analysis of five quality control samples on the same day and on 5 different days. The data given in Table 5 indicate that the method exhibits standard deviations inferior to 2%. The main advantages of this column are the good resolution in less than 30 min and low standard deviations of the D/L ratio measurement (<2%).

As chirality determination strongly depends on the derivatization procedure, we investigated the probability of generating racemized amino acids. Each amino acid derivative (in its L-form only) presenting an enantioselective separation, was injected onto the GC–column and no racemization was observed.

3.3. Space operating conditions

3.3.1. Derivatization optimization

To analyze a wide scope of molecules we first used DMSO as a solvent and in these experimental conditions no derivative of diacid compounds were obtained (glutamic acid was detected when using gaseous procedure).

To study the derivatization process we performed the reaction at the sub-millimolar level (100–250 μmol) as described in the literature [20]. These experiments were taken as references for yield achievement when performing process optimization.

Briefly, amino acid (50 μmol/mL) was stirred in 1.5 mL of dimethylsulfoxide (DMSO) under an atmosphere of hexafluoroacetone in a flask equipped with a dry ice condenser containing acetone at −78 °C. After 2 h at room temperature the reaction was completed. DMSO and HFA hydrate were removed by extraction with 20 mL of a biphasic system of dichloromethane/water (1/1). The organic layer was then evaporated to dryness. The resulting crude product was diluted in 500 μL of dichloromethane and 1 μL of the sample was injected into the GC–MS for analysis. The hydroxyl groups present in serine, threonine and tyrosine were not further esterified. Twenty-one HFA products were obtained in up to 90% yield. HFA derivatives of lysine and asparagine were not detected.

To approach the conditions for in situ analysis of extraterrestrial environments and the expected amino acids concentration in
Table 5
D/L ratio% error and precision of HFA derivative separation on β-cyclodextrine capillary column.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>D/L (n = 5)</th>
<th>% error</th>
<th>aWithin-day precision (n = 10)</th>
<th>bBetween-day precision (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD (%)</td>
<td></td>
<td>SD (%)</td>
<td>SD (%)</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.32</td>
<td>0.08</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Valine</td>
<td>0.32</td>
<td>0.2</td>
<td>0.22</td>
<td>0.01</td>
</tr>
<tr>
<td>Norvaline</td>
<td>0.05</td>
<td>0.8</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Proline</td>
<td>0.02</td>
<td>0.1</td>
<td>0.79</td>
<td>0.16</td>
</tr>
</tbody>
</table>

meteorites, lower amount of amino acids have to be derivatized, we need to suppress the extraction and stirring steps.

First we succeeded in derivatizing from 100 µmol to less than 0.1 µmol of amino acid without stirring during the derivatization step and without decreasing the reaction yield.

To be closer to space operating conditions which require less energy and less complexity a “one pot/one step” sample processing method without extraction was also developed. We evaluated the procedure when the extraction in dichloromethane was omitted. Direct injection of DMSO did not give good resolution (LOD increases up to 50%) independently of whatever chromatographic column was used.

The investigation was pursued without solvent. Gaseous hexafluoroacetone was introduced directly with the amino acid sample. In this experiment, the reaction flask was cooled down at −78 °C in order to condense hexafluoroacetone. The latter was maintained in the liquid state without stirring for 30 min and the reaction mixture was then allowed to warm up at room temperature. In this case proline was recovered with less than 20% yield but detection of other amino acids was achieved with more than 80% yield compared with the previous process. Glutamic acid, not successfully derivatized in DMSO procedure, was also detected.

The result demonstrates that hexafluoroacetone successfully derivatizes amino acids at the micromolar level without solvent (Fig. 8, 1–10 µmol of each amino acid).

3.3.2. Transfer of derivatives

To prepare future experiments in pilot reactor we studied the transfer properties of derivatives from the reactor to the GC column. To mimic in situ transfer to GC, we have optimized headspace transfer. To determine the optimal transfer conditions, the influence of temperature and duration of heating on the efficiency of recovery of the HFA derivatives recovery was studied. Results were compared with direct liquid injections of the HFA derivatives in the split/splitless injector of the GC–MS. The obtained results show that similar trends exist as a function of varied temperature or heating period for most of the amino acids studied. Tyrosine and tryptophan were not recovered. Fig. 10 illustrates the results obtained for this experiment. 30 min at 80 °C were found to be the best parameters for the recovery of more than 80% of 10 analytes (alanine, glycine, valine, norvaline, isovaline, isoleucine, leucine, threonine, serine and methionine). In these conditions, about 50% of phenylalanine and 80% of proline derivatives were lost. Lower temperature transfers did not enable detection of phenylalanine. Higher temperatures did not enable better recoveries. Recoveries of phenylalanine and proline were respectively improved by 20 and 10% when the transferred sample was focused on poly(dimethylsiloxane) PDMS fiber prior to GC separation. These results demonstrate that hexafluoroacetone derivatives transfer is possible. Further studies need to be conducted on a pilot reactor to determine the best parameters.

3.3.3. Mineral matrixes effect

With regards to space constraints, treatment of extraterrestrial samples has to be a one pot processing method. Extraction, derivatization and transfer are likely to be conducted in the same reactor. The mineral matrix of soil samples are not removed prior to derivatization and thus could interfere with the derivatization agent, decreasing the yield of reaction or inhibiting the reagent. To study the influence of a mineral matrix on the hexafluoroacetone we first studied mineral silica (with or without carbonates) matrices.

To estimate the derivatization efficiency on this mineral matrix, given amounts of amino acids were first added to standard sand, free of organic material. Although the concentrations (100 ppm) in the solid matrix are higher than those expected to be present in the Martian soil, this standard sample allowed us to evaluate the optimized procedure, within controlled and reproducible conditions. Quantitative recoveries of each amino acid were achieved.

No influence of simple silica mineral matrix on the derivatization reaction was observed.

The whole procedure (derivatization, SPME transfer and separation) was then evaluated on 50 mg of Atacama subsurface soil (chili) and 10.7 mg Mars regolith stimulant JSC Mars-1 (Hawai) samples, supposed to be one of the best Mars surface analogue samples [29]. JSC Mars-1 sample was dominated by plagioclase (80%), whereas Atacama subsurface soil also contained 20% of calcite.

Samples were spiked with 100 ppm of a 17 amino acids mixture and derivatized without solvent. Fig. 11 shows no main differences between mixture derivatized with no mineral matrix and sample derivatized on Atacama soil. Quantitative recovery was achieved for each amino acid except for proline (25% loss) and the total loss of glutamic acid. It must be noted that a better recovery of phenylalanine was even obtained with the mineral matrix. The same trends were observed for the JSC Mars-1 sample but coelution of leucine (quantitatively recovered) and an unidentified compound prevented from their TIC quantititation. Some hydrocarbons and one tetra naphthalene compound present in the mineral matrix where also detected.
The mineral matrices studied did not prevent derivatization occurring and even facilitated the reaction between amino acids and hexafluoroacetone. The matrix might increase accessibility to amino acids by trapping liquid hexafluoroacetone.

4. Conclusion

In this paper, we have shown that in situ hexafluoroacetone derivatization can be achieved, using an automatisable procedure. This optimized formation of low mass derivatives can be achieved in an hour, at low temperature without stirring and solvent, and within the mass range of new space mass spectrometer. The precision and detection limit measured were in the range of other derivatization processes adapted to in situ analyses. It is important to note that no formation of any by products or racemization occurred. Thus, 21 amino acids were separated and 10 α-amino acid enantiomers were separated (5 of which were detected in the Murchinson meteorite) when using two complementary beta- and gamma-cyclodextrine chiral stationary phases.

We propose that these findings can already give a clue to the chirality of the detected molecules especially as the derivatizing procedure occurred with a gaseous reagent. This result is of tremendous importance as eliminates the need for liquid storage and handling which is difficult in environments with low gravity. Hexafluoroacetone derivatization without solvent would be the easiest chemical reaction to perform in extraterrestrial environments.

Given the potential applicability of the derivatization reaction for space experimentation, HFA derivatization seems very promising for future in situ analyses, like the planned Exomars Martian mission.

Acknowledgement

This work was supported by a research and technology grant from the Centre National d’Etudes Spatiales (CNES).

References
