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Communication

Gas Chromatography Fingerprint of Martian Amino Acids before Analysis of Return Samples

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Abstract: Within the perspective of the current and future space missions, the detection and separation of building blocks such as amino acids are important subjects which are becoming fundamental in the search for the origin of life and traces of life in the solar system. In this work, we have developed and optimized a strategy adapted to space experimentation to detect the presence of amino acid-like compounds using gas chromatography coupled to mass spectrometry (GC-MS). Selected derivatization methods meet the instrument design constraints imposed on in situ extraterrestrial experiments. Coupled to a fast selective extraction, GC analysis would be highly efficient for the detection of organic materials. In the future, the corresponding GC-MS TIC could facilitate simple and fast selection of sediments/dust samples onboard GC-MS-equipped rovers for sample return-to-Earth missions.

Keywords: gas chromatography; amino acids; derivatization; extraction; sensor

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1. Introduction

In the quest of interplanetary exploration and the search for indicators of extraterrestrial life, the detection of molecular biosignatures was, and still is, a major objective of astrobiological research missions. Whatever the drastic conditions met in such hostile environments, some organic materials could be preserved due to strong interactions with minerals. Detection of such compounds would provide important clues about the origins of life [1]. Among the several known classes of organic compounds that could be searched for, amino acids, the monomer building blocks of proteins, are the most targeted molecules. In situ robotic missions accompanied by chemical analysis instruments will play a pivotal role in this endeavour [2]. Gas chromatography (GC) coupled with mass spectrometry (MS) is, to date, the sole flight-qualified technique for a molecular level detection. It has already been successfully used in space instrumentation [3–6]. Pyrolysis coupled to GC-MS enabled the detection on of thiophenic, aromatic and aliphatic compounds Mars [7–9].

However, GC instruments can only be used to analyze volatiles that are also thermally stable. The main concern is that few or none of the proposed terrestrial organic molecules would then be compatible with a GC-MS experiment. It has already been previously demonstrated that Martian refractory molecules such as amino acids can be derivatized to facilitate their in situ detection using GC-MS [10–14]. Thus, wet chemistry experiments such as derivatization were dedicated to amino acids' detection on the sample analysis at Mars instrument, on NASA's Curiosity rover.

Derivatization on Mars (i.e, silylation) was successful, as derivatized benzoic acid and ammonia were identified [14]. This amazing result showed the preservation of complex molecules in such an inhospitable environment. It also proved GC-MS to be a highly relevant and robust analytical instrumentation. Despite molecular biosignatures' identification

in numerous extreme environments [15–17], as well as in extraterrestrial meteorites, [18–22], to date none have been detected on Mars.

First, with GC operating conditions among all the targeted silylated amino acids, only alanine and glycine derivatives could have been detectable on the selected column [14]. In addition, the strong adsorption of amino acids on mineral matrices could also result in this lack of detection. No solid–liquid extraction was performed. For future missions, an extraction step followed by a new GC detection strategy would improve the recovery of the molecules, and the interpretation of the chromatograms, respectively [23–25]. Previous in situ ultrasonic extraction strategy was not selected due to the additional device needed [24,25]. In this rationale, a one-pot extraction–derivatization process should avoid this pitfall. The optimized extraction parameters have already been determined [26]. Extraction in water/methanol (1/1, v/v), under focused ultrasonic conditions, would increase the potential to detect extracted amino acids, if present. The heating step to evaporate the extraction solvent would be followed by the release of the derivatizing agent kept in a cup sealed with a specific eutectic, as in the SAM and MOMA experiments [27,28].

Interpretation of in situ chromatograms is also very challenging due to an extremely low signal over noise ratio [29]. For a first overview in future space missions, alternative approaches such as GC-MS treated as sensors, could be developed [30]. We propose to use of the total ion chromatogram (TIC) as a yes/no recognition response pattern to detect the presence of specific chemical compounds. In this view, an achiral versatile column has to be selected. Due to energy constraints for in situ missions, a brief analysis time could be sufficient for a simple recognition response. Samples exhibiting a fingerprint with these sensors will be highly interesting and have to be further studied. This strategy would deeply ease the selection of sampling sites for the future return samples missions.

2. Materials and Methods

2.1. Chemicals

An amino acids standard solution containing L-amino acids at 2.5 µmol/L (except L-cystine at 1.25 µmol/L) in 0.1 N HCl was purchased from Sigma-Aldrich (Steinheim, Germany). It contained L-alanine, glycine, L-valine, L-leucine, L-isoleucine, L-proline, L-methionine, L-serine, L-threonine, L-phenylalanine, L-aspartic acid, L-glutamic acid, L-lysine, L-tyrosine, L-cystine, L-arginine and L-histidine. Sarcosine $\geq$ 97%, β-alanine $\geq$ 99%, 2-aminoisobutyric acid $\geq$ 98%, L-2-aminobutyric acid $\geq$ 99%, L-3-aminoisobutyric acid $\geq$ 97%, L-3-aminobutyric acid $\geq$ 97%, γ-aminobutyric acid $\geq$ 99%, L-Isoserine $\geq$ 98% and L-alloisoleucine $\geq$ 99% lyophilized powders were also purchased from Sigma-Aldrich, while L-homoserine $\geq$ 99%, L-norvaline $\geq$ 99% and L-norleucine $\geq$ 99% from Alfa Aesar (Ward Hill, MA, USA), L-β-leucine $\geq$ 98% were purchased from Chem-Impex (Wood Dale, IL, USA) and L-isovaline $\geq$ 99% from Acros Organics (Geel, Belgium). Individual aqueous solutions were prepared for each amino acid in milliQ water (18.2 MΩ·cm^{-1}), from which a mixture solution containing 31 amino acids was prepared at $5{\cdot}10^{-4}$ mol/L and used throughout the following study.

Target compounds were derivatized using the *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA $\geq$ 97%), *N*,*N*-Dimethylformamide (DMF $\geq$ 99.8%) Methyl chloroformate (MCF $\geq$ 99%), *N*,*N*-Dimethylformamide dimethyl acetal (DMF-DMA $\geq$ 95%), Pyridine $\geq$ 99.8%, Methanol (99.8%), Tetramethylammonium hydroxide pentahydrate (TMAH $\geq$ 97%), all of which were purchased from Sigma Aldrich, with the exception of Chloroform $\geq$ 99%, which was obtained from Acros Organics.

2.2. Extraction–Derivatization

Extraction was optimized and validated [26]. Briefly, a 10 min ultrasonic focused extraction in 500 µL water/methanol solution (1/1, v/v) was selected (2 mm microprobe was controlled by a Vibra-Cell ultrasonic processor, Sonics, Newtown, CT, USA, operating at 20 kHz frequency, 80% amplitude, 1 s pulse and 1 s relaxation during 10 min) for a 100 mg sample. The stainless probe entered the derivatization cell to perform focused ultrasounds

extraction. Solvents of extraction were then evaporated to dryness for silylation, whereas other derivatizations were performed in the presence of the extraction solvents (after ultrasonic probe removal). Derivatization procedures were already well-described [11,13,31]. Injection of 0.5 nmol of each amino acids was performed.

2.3. GC-MS Analysis

All analyses were performed on a Thermo Scientific (Waltham, MA, USA) gas chromatograph/mass spectrometer Trace-DSQ II using a HP-5 UI capillary column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). Carrier gas was helium (≥99.9999% purity, Air liquide, Paris, France) at a fixed flow of 1 mL/min and a split of 20:1. The temperature of the SSL injector was 280 °C. The masses were scanned between m/z 50 and m/z 400. The ionization energy was 70 eV. These conditions are similar to those on the SAM and MOMA experiments [14,32].

In order to limit the energy consumption during analysis to a few minutes, an "optimized GC Program" method was put in place. The temperature programming was the following: oven temperature started at 120 °C held for 1 min, then heated at a rate of 60 °C/min up to 300 °C and held for 2 min.

3. Results

While volatile apolar compounds could be identified due to pyrolysis, polar ones needed extraction and derivatization prior to GC separation. Three different chemical reagents are selected for in situ experiments: tetramethylammonium hydroxide (TMAH), *N*,*N*-Dimethylformamide dimethyl acetal (DMF-DMA), and *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide MTBSTFA [27,28]. The first one is dedicated to thermochemolysis, the second to amino acids' detection and the third to maximizing the scope of targets detected using GC-MS [10,11,31]. For future missions, chloroformates are also considered [11,33]. To select future return samples, a simple GC peak detection after the extraction–derivatization steps was developed. An ultrasonic extraction method was already optimized to extract amino acids from solid samples, such as Martian analogues [26]. After evaporation of the extraction solvent ($MeOH/H_2O$), derivatization within the same reactor would enhance the volatility of the targets.

3.1. Methylation: TMAH and DMF-DMA

TMAH is a methylation agent that also facilitates hydrolysis of macromolecules at elevated temperatures. TMAH applications have been demonstrated on a wide variety of sample types and appear suitable even for Martian samples containing perchlorates [31,34]. For future selection of the return samples, this reagent, which is fully compatible with the selected solvents of extraction, would be significant [31]. We propose to perform a methylation at 300 °C on the same extract to detect the presence of free extracted amino acids [35] followed by a thermochemolysis at 600 °C for nucleobases or macromolecules analysis [34]. Thanks to the selected apolar column and thermochemolysis temperatures, the aliphatics and aromatics compounds could also be released and detected in the meantime. However, due to complex chromatograms with high signal over noise, the TIC should only be used as an organic compounds' sensor (Figure 1). Indeed, caution should be exercised in interpreting the presence of native compounds. Dimerization of free amino acids could occur [36] and amino acids other than glycine and alanine may be responsible for the formation of *N*,*N*-dimethylglycine methyl ester and *N*,*N*-dimethylalanine methyl ester [37]. TMAH-resulting products are organic fingerprints, the structure of the native molecules of which we possess no certainty about.

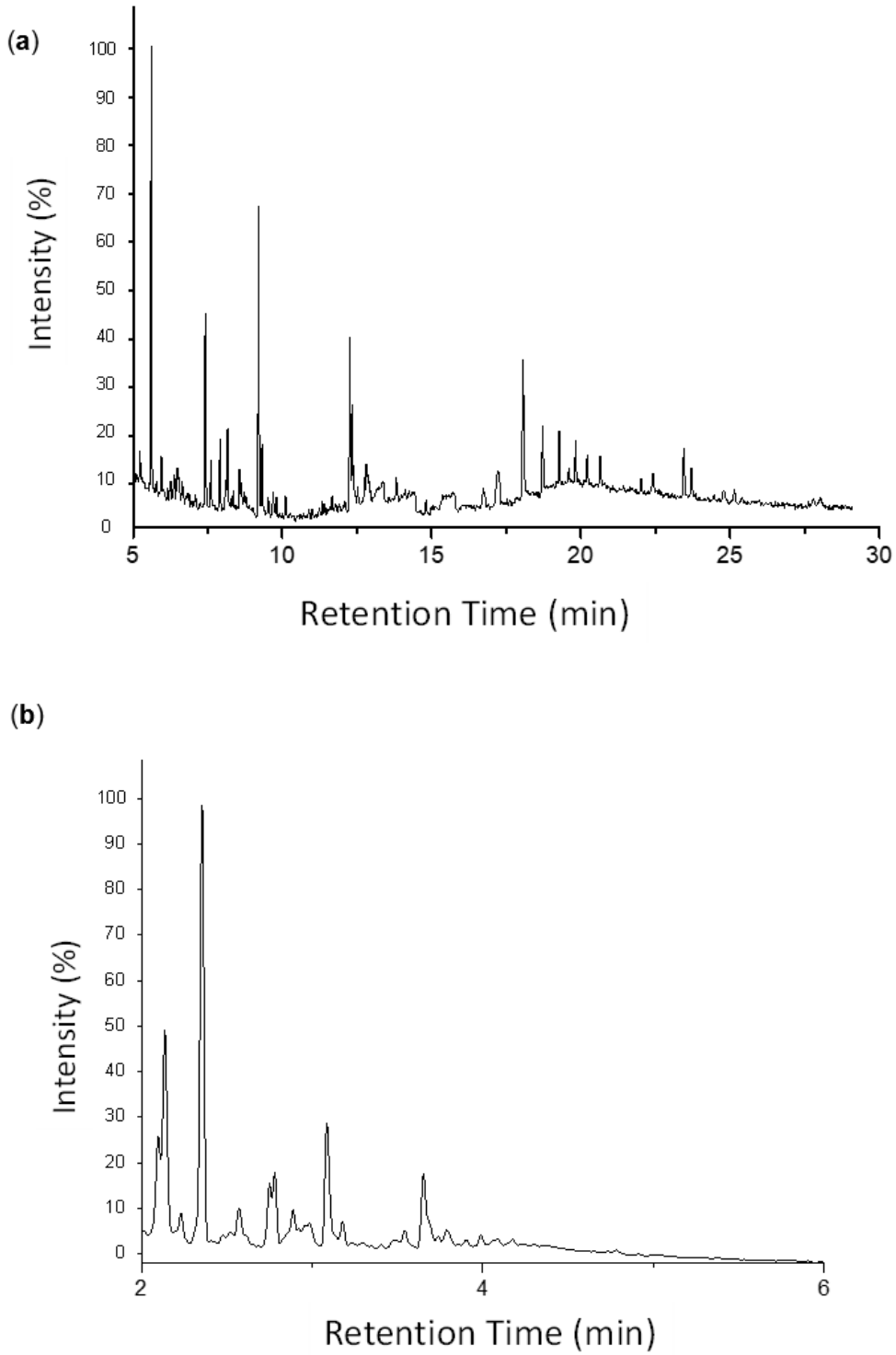

Figure 1. TIC of 31 amino acids derivatized online at 300 °C using a TMAH/MeOH (25% w/v) solution. (**a**). Separation was achieved by setting oven temperature at 70 °C for 2 min, then heating it up to 260 °C at a rate of 10 °C/min, followed by holding the temperature for 8 min. (**b**). Separation was performed using the "short optimized" GC-MS program.

Figure 1 shows the resulting TIC from free amino acids after methylation at 300 °C (3 min) regarding laboratory GC temperature program (Figure 1a) and optimized GC temperature program for space experiments (Figure 1b). Many by-products of methylated organics of interest interfere in the total ion current chromatogram of the resultant derivatives. The optimized TIC provides evidence of organics' presence in less than 6 min; thus,

it is effective to select relevant samples for further analyses. If the structure of the native molecules is needed, DMF-DMA, another methylation reagent, should be used [10,13].

Thanks to the "short optimized" GC program, amino acids from glycine to tyrosine could be detected in less than 6 min (Figure 2) with derivatives characteristic of the native molecules [11]. In a similar manner to TMAH derivatization, DMF-DMA allowed the detection of a full fingerprint of the analysed sample, indicating the presence of amino acids.

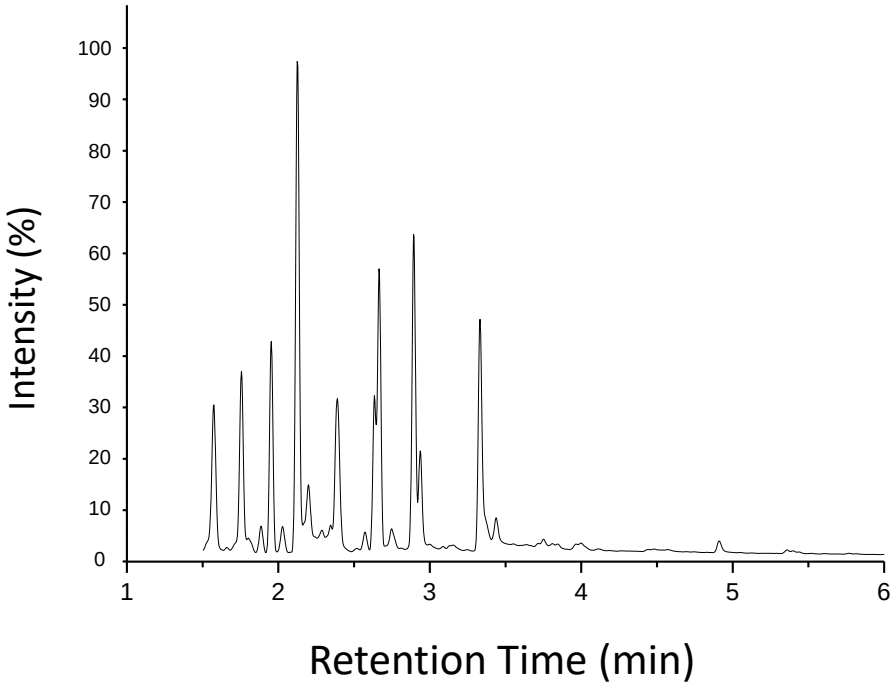

Figure 2. TIC of 31 amino acid derivatized with DMF-DMA and analyzed using the "short optimized" GC-MS program.

3.2. Silylation: MTBSTFA

MTBSTFA, a silylation reagent, is suitable for carboxylic acids, amino acids, nucleobases, amines, and alcohols. Regarding the extraction procedure, only amino acids and nucleobases could be detected by GC-MS after silylation. In laboratory analysis, no coelution occurred and a 31-target chromatogram is fully resolved (Figure 3a). In current space conditions, the TIC was less informative but allowed the detection of main compounds in a narrower time window (from alanine to tyrosine, Figure 3b). Identification of the 31 amino acids was, however, possible thanks to mass fragmentograms. With our "short optimized" GC conditions, a 6 min response was achieved (Figure 4). Despite many coelutions, it enabled the validation of the presence of targeted molecules.

To delve further into the data analysis, mass fragmentograms could also be used (Figures 5–8) with a highly specific mass fragmentation even for coeluting compounds.

For future space missions, a new derivatizing agent could also be considered, such as alkyl chloroformates.

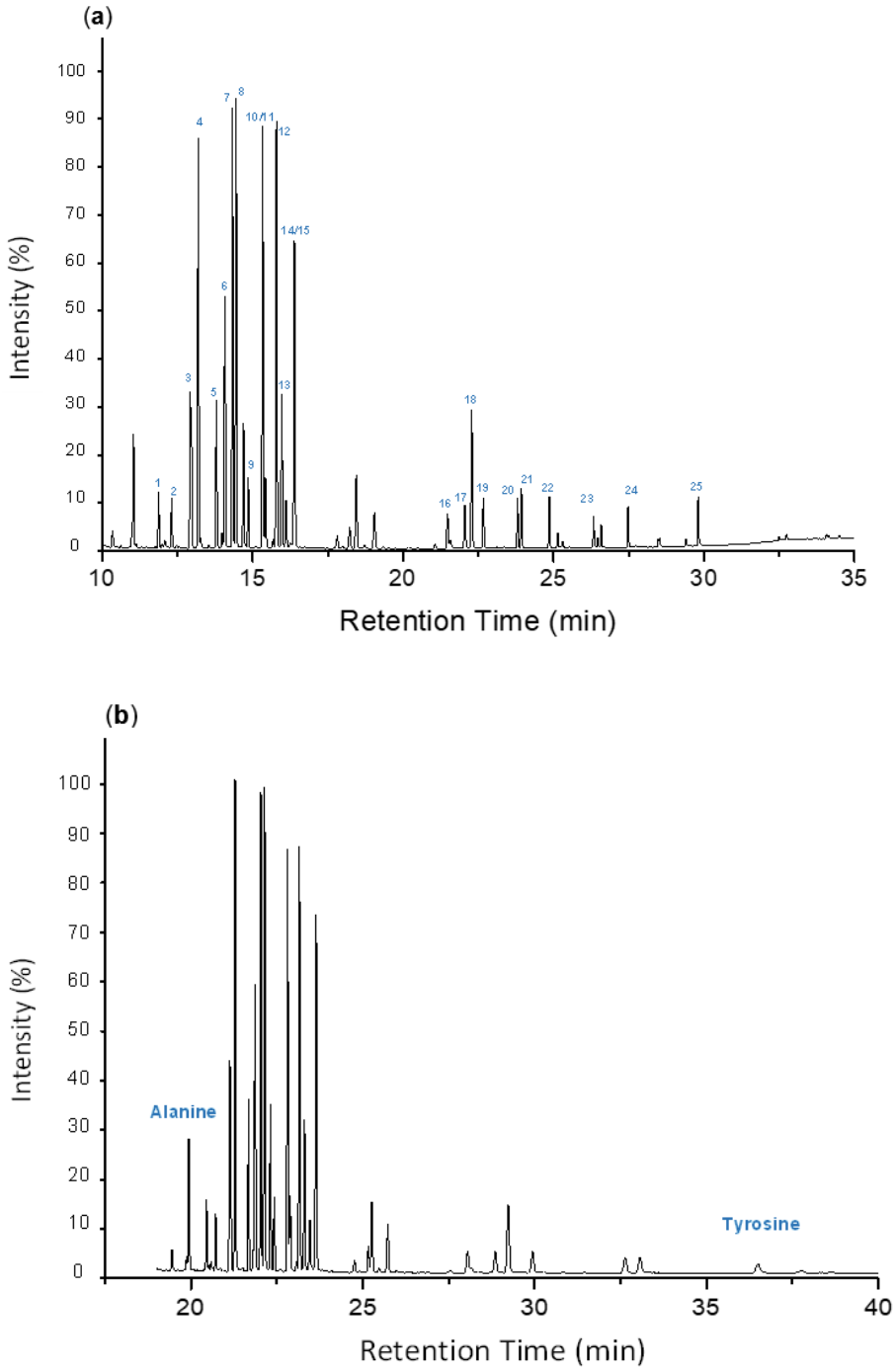

Figure 3. Totally resolved TIC of 31 amino acids derivatized with MTBSTFA separated using: (**a**). A laboratory temperature program: Oven temperature was set at 90 °C raised up to 180 °C at a rate of 6 °C/min, and then held for 5 min. Afterwards, temperature was raised up to 300 °C at a rate of 10 °C/min, and then held for 8 min. 1. Alanine; 2. Glycine; 3. 2-Aminoisobutyric acid, Sarcosine; 4. 2-Aminobutyric acid; 5. β-Alanine; 6. 3-Aminobutyric acid, 3-Aminoisobutyric acid, Valine; 7. Norvaline; 8. Isovaline; 9. Leucine; 10. Alloisoleucine; 11. Isoleucine; 12. Norleucine; 13. ɣ-Aminobutyric acid; 14. Proline; 15. β-Leucine; 16. Methionine; 17. Serine; 18. Isoserine; 19. Threonine; 20. Phenylalanine; 21. Homoserine; 22. Aspartic acid; 23. Glutamic acid; 24. Lysine; 25. Tyrosine. (**b**). SAM GC-MS separation program validated for in situ missions. Oven temperature was set at 34 °C for 6 min, then heated up to 185 °C at a rate of 10 °C/min, and held for 20 min.

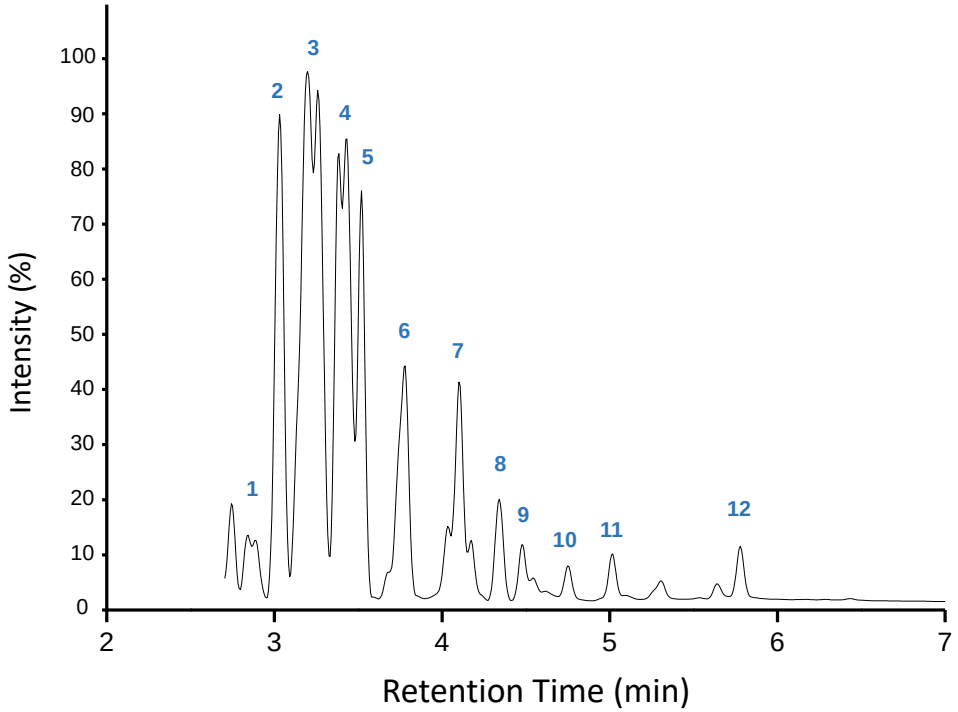

Figure 4. TIC of 31 amino acids derivatized with MTBSTFA using the "short optimized" GC-MS method program. 1. Alanine, glycine; 2. 2-Aminoisobutyric acid, Sarcosine; 3. β-Alanine, 2-Aminobutyric acid, 3-Aminobutyric acid, 3-Aminoisobutyric acid, valine, norvaline; 4. Isovaline, leucine, isoleucine, allo-isoleucine, norleucine, γ-Aminobutyric acid, 5. Proline, 6. β-Leucine; 7. Methionine, serine, isoserine, threonine; 8. Phenylalanine, homoserine; 9. Aspartic acid, 10. Glutamic acid, 11. Lysine; 12. Tyrosine.

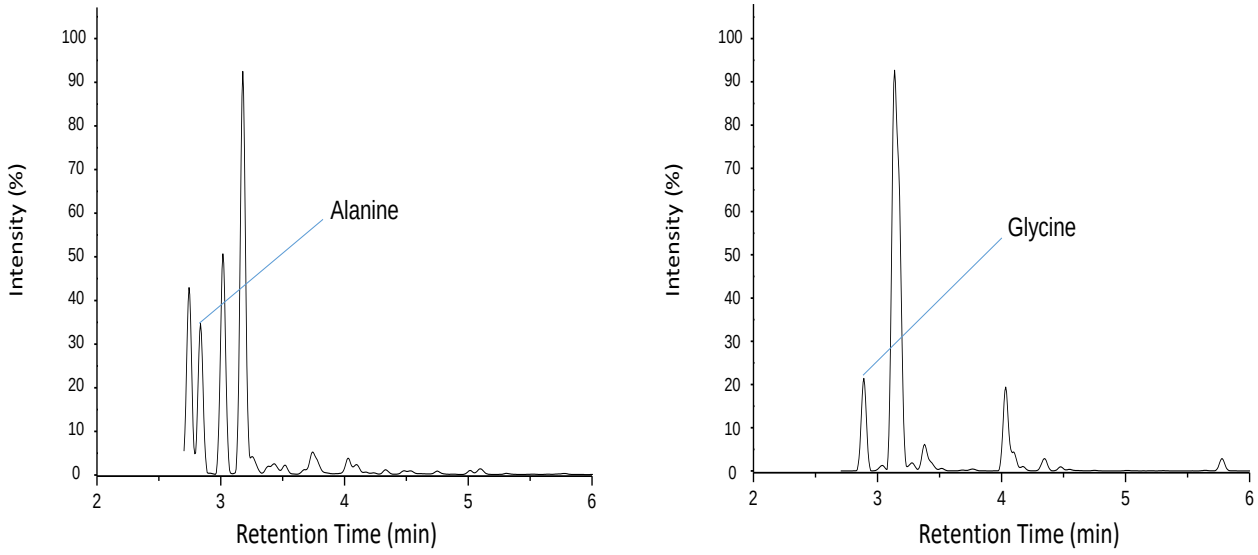

Figure 5. Mass fragmentograms of two compounds: alanine and glycine extracted from peak 1 at 2.87 min using specific mass fragments (m/z 158 and m/z 218, respectively).

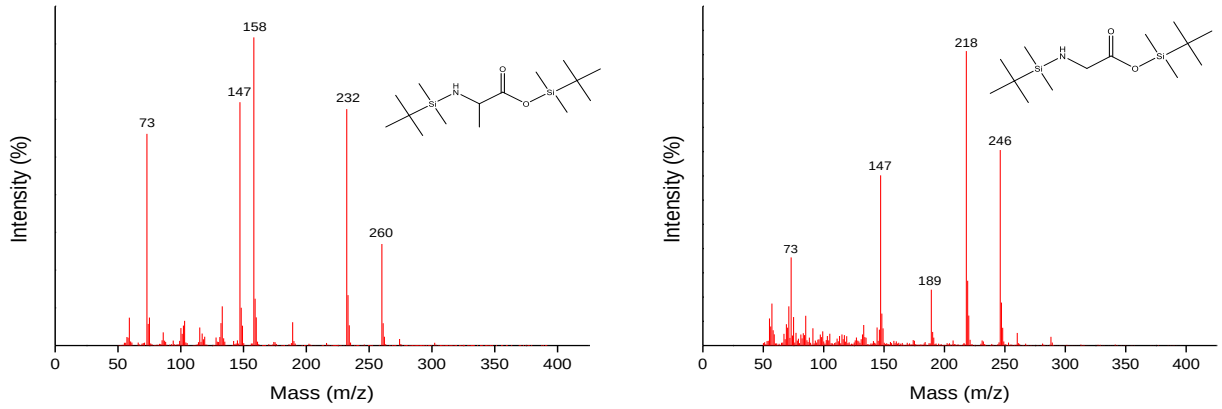

Figure 6. Mass spectra of coeluting derivatized alanine and glycine.

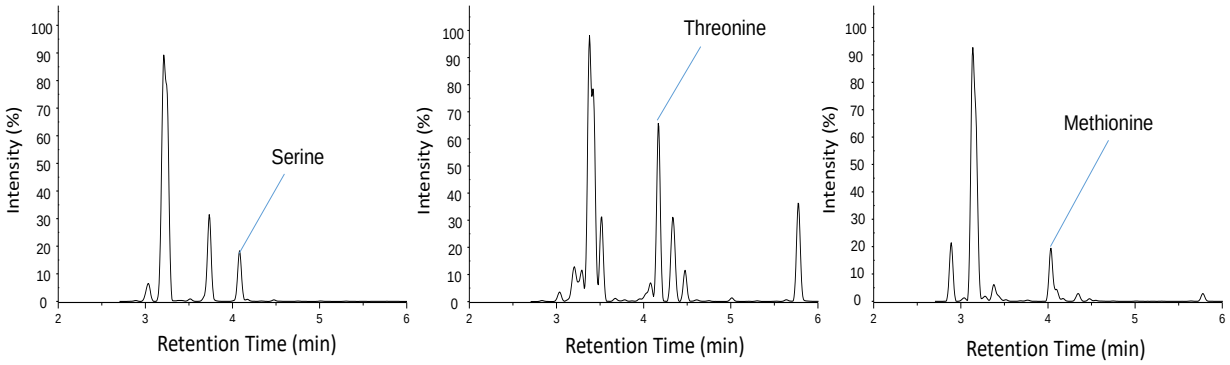

Figure 7. Mass fragmentograms of three compounds serine, threonine and methionine extracted from peak 7 at 4.18 min using specific mass fragments (*m*/*z* 362, 303 and 218, respectively).

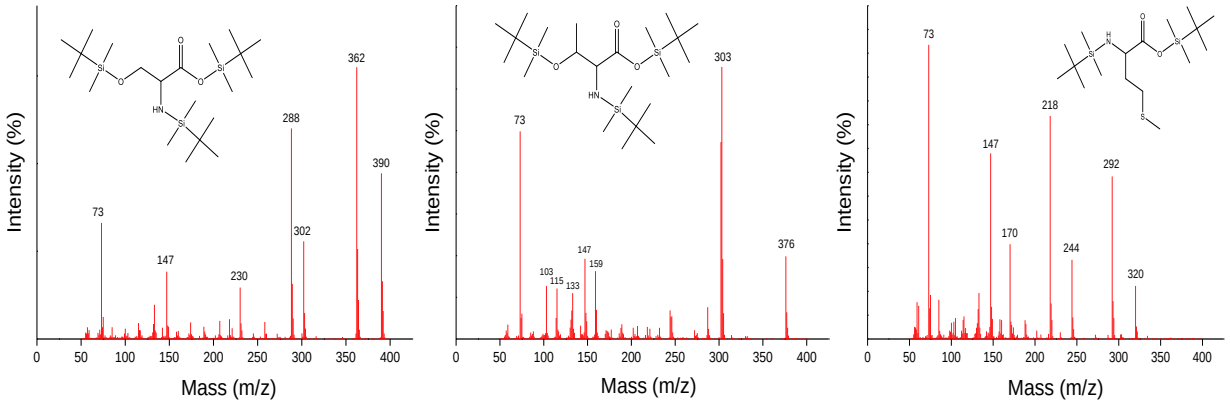

Figure 8. Mass spectra of coeluting derivatized serine, threonine and methionine.

3.3. Esterification: MCF-MeOH

Alkyl chloroformates are also suitable for space analysis since the strong agitation needed is now afforded by our ultrasonic extraction device. After MeOH/water extraction, methyl chloroformate—MeOH—derivatization was performed without previous evaporation of the extraction solvent (Figure 9).

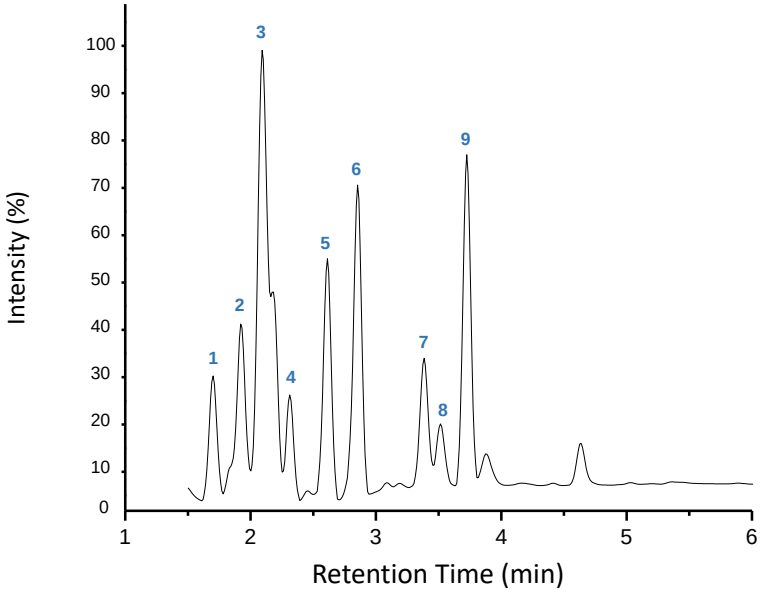

Figure 9. TIC of 31 amino acids derivatized using MCF-MeOH and analyzed using the "short optimized" GC-MS program. 1. Alanine, glycine; 2. β-Alanine, 2-Aminobutyric acid, 3-Aminobutyric acid, 3-Aminoisobutyric acid; 3. Proline, valine, norvaline, isoleucine, allo-isoleucine, leucine, threonine, β-Leucine, homoserine; 4. Aspartic acid; 5. Methionine; 6. Phenylalanine; 7. Lysine; 8. Histidine; 9. Tyrosine.

Similarly to MTBSTFA, fragmentograms can be used to differentiate between multiple coeluted compounds (Figure 10) with a unique mass fragmentation for each derivative (Figure 11).

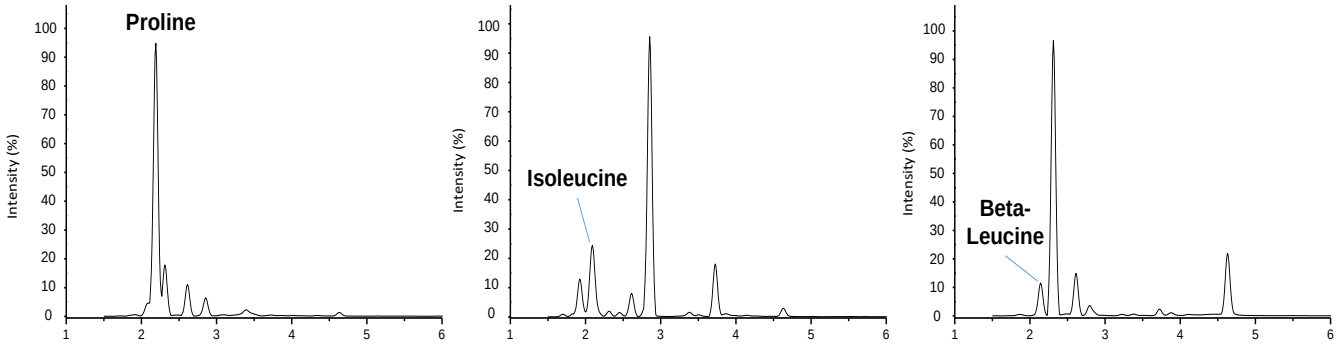

Figure 10. Mass fragmentograms of three compounds: proline, isoleucine and beta-leucine extracted from coelution peak 3 at 2.14 min using specific mass fragments (m/z 128, 144 and 160, respectively).

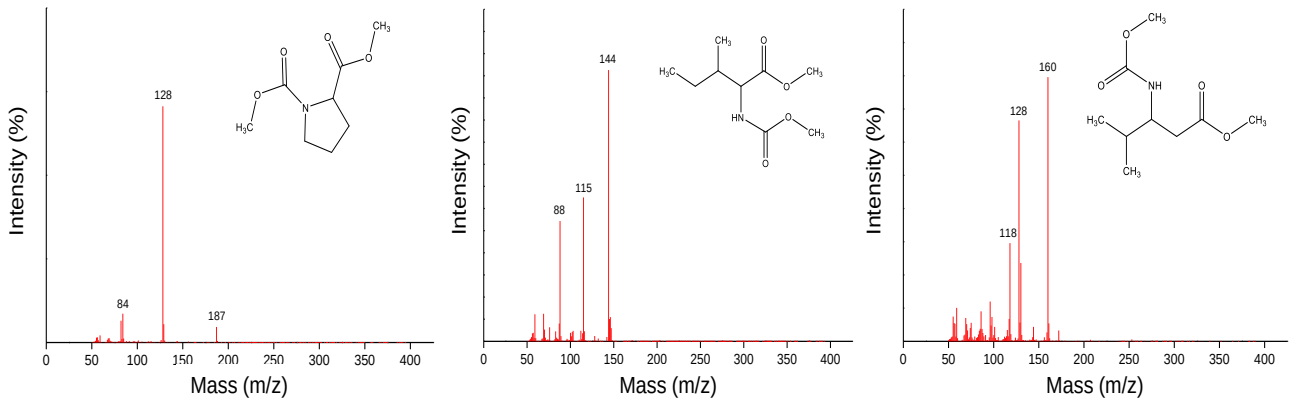

Figure 11. Mass spectra of derivatized proline, isoleucine and beta-leucine.

If selected for future missions, methyl chloroformate treatment could also be coupled with automatic data handling to be more informative. Indeed, chemometric simplified data treatment already developed for alkyl chloroformates is especially helpful in handling the low quality data recovered from space [38].

4. Conclusions

In summary, we have designed a GC analysis program for fast selection of future sediments/dust samples in return-to-Earth missions. Our integrative strategy enables both screening and targeted analysis of free amino acid-like molecules. By crossing the data from derivatization methods, our strategy can circumvent low quality data of space instrumentations. This strategy could also be implemented with the lighter thermal conductivity detector (TCD). TCD detection is less sensitive but would beneficiate from increased signals of coeluted compounds. Therefore, we believe that, coupled to signal processing, our workflow represents an important progress in returned samples from planets and comets.

Author Contributions: Conceptualization, C.G.-R. and R.T.; validation, R.F. and R.T.; formal analysis, R.F.; writing—original draft preparation, R.T.; writing—review and editing, C.G.-R.; visualization, B.G., F.B. and P.P.; supervision, G.R. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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