



## DETECTION OF MARTIAN AMINO ACIDS BY CHEMICAL DERIVATIZATION COUPLED TO GAS CHROMATOGRAPHY : IN SITU AND LABORATORY ANALYSIS

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### ABSTRACT

If there is, or ever was, life in our solar system beyond the Earth, Mars is the most likely place to search for. Future space missions will have then to take into account the detection of prebiotic molecules or molecules of biological significance such as amino acids. Techniques of analysis used for returned samples have to be very sensitive and avoid any chemical or biological contamination whereas *in situ* techniques have to be automated, fast and low energy consuming. Several possible methods could be used for *in situ* amino acid analyses on Mars, but gas chromatography would likely be the most suitable. Returned samples could be analyzed by any method in routine laboratory use such as gas chromatography, already successfully performed for analyses of organic matter including amino acids from martian meteorites. The derivatization step, which volatilizes amino acids to perform both *in situ* and laboratory analysis by gas chromatography, is discussed here. © 2001 COSPAR. Published by Elsevier Science Ltd. All rights reserved.

### INTRODUCTION

By analogy with Earth, life may have originated on Mars early in its history. Indeed, Mars may have had an atmosphere similar to that of the early Earth (Kasting and Toon, 1989) and presence of fluvial features provides evidence that liquid water was once present on the martian surface (Carr, 1996).

Detection and identification of organic molecules including those that play a major role in biochemistry give clue to exobiology researches. In the Viking Mission, analyses of martian soil were performed by a pyrolysis-gas chromatograph/ mass spectrometer (PY-GC/MS) (Bieman, 1977). The analytical results did not show any evidence of the presence of organic compounds in surface regolith of Mars (Soffen, 1976). However it does not mean that there is no organic compounds in Mars, since the analysed samples were surface samples, likely to include superoxides (Yen *et al.*, 2000) and peroxides which might destroy any organic compounds there. Future samplings have then to be performed in place without oxidants such as deep underground soils. Any exobiological strategy for investigating if organic molecules are present in martian environments should focus on compounds that (1) are readily synthesised under plausible prebiotic conditions, (2) play a major role in biochemistry and (3) are abundant in carbonaceous meteorites. Organic molecules that meet all these conditions are amino acids. A

central problem in future organic analyses of martian samples is not only identifying and quantifying organic compounds that may be present, but also distinguishing those molecules produced abiotically from those synthesized by extinct or extant life. The most reliable indicator of the biological vs. abiotic origin of organic molecules is molecular homochirality. Terrestrial organisms, with few exceptions (Brückner and Hausch, 1993), use exclusively L-amino acids (the L-enantiomer) in protein biosynthesis. The structural principles on which biomacromolecule activity is based lead to believe that any functional biochemistry must use only one enantiomer of any molecule that possesses a chiral carbon (homochirality). In contrast, all known laboratory abiotic synthetic processes result in racemic mixtures of organic compounds with chiral carbons (Bada and McDonald, 1995). Thus amino acid homochirality provides a possible way of distinguishing between abiotic vs. biotic origins if samples are not submitted to racemization, which biases results. Mars is a cold, dry planet with no known plate tectonic activity and thus is valuable for homochirality analysis (Bada and McDonald, 1995; Cohen and Chyba, 2000). Gas Chromatography / Mass Spectrometry (GC/MS) is an obvious method for molecular organic analysis from a landed martian spacecraft and for laboratory analysis, which has proven to be efficient during the Viking missions and the numerous analyses of meteorites performed with this technique (Kvenvolden *et al.*, 1971; Cronin and Moore, 1971; Cronin and Pizzarello, 1997; Brinton *et al.*, 1998).

We then suggest to use GC in future martian missions for *in situ* (Cabane *et al.*, 2000) and laboratory analyses. However, as many polar compounds, amino acids are non-volatile and need derivatization reactions before GC analysis. We describe here possible derivatization procedures followed by Gas Chromatography separation for both *in situ* and laboratory analyses of amino acids.

## IN SITU ANALYSES

The *in situ* analysis is a challenging task regarding the numerous difficulties induced by space constraints. Derivatization processes, often used in laboratories, have never been performed in space and an automatic derivatization system compatible with space constraints has still to be developed. Thus, various derivatization procedures have to be tested and the one that will best fit scientific constraints and spacecraft procedure requirements (automation, short time and energy consumption) will be selected. This study is conducted on capillary columns that comply with space requirements: vibration, radiation and thermal cycles at vacuum condition, from -50°C to 200°C simulating usual space mission conditions met during the transfer flight (Nieman *et al.*, 1997). According to previous analyses of amino acids present in carbonaceous meteorites (Kvenvolden *et al.*, 1971; Cronin and Moore, 1971) concentrations of target molecules range from ppb to ppm. Assuming a similar range in concentration for amino acids in martian soil and martian meteorites the limit of detection of any technique used has to be at least the ppb level. Then, for a 100 mg sample subpicomole level is almost necessary for amino acids detection.

One-step derivatization reactions are of tremendous interest in term of *in situ* analysis as they are fast and low energy consuming. For example, chloroformates and ammonium salts allow the instantaneous derivatization of amino acids at respectively room temperature (Abe *et al.*, 1996) and 300°C (Amijee and Wells, 1994) with a possible enantiomeric separation. However, derivatization with chloroformates needs vigorous shaking that requires additional hardware and thus could increase the size, weight, and mechanical complexity of the GC/MS system. Ammonium salts derivatization needs further studies as reproducibility is far from easy to achieve (Amijee and Wells, 1994). In terms of automated and spacecraft derivatization system, the use of silylating agents could be planned even though enantiomeric separation is not yet achieved. For example with N-methyl-N-(*tert*-butyldimethylsilyl)-trifluoroacetamide, derivatization of amino acids is performed in 30 minutes at 70°C (McKenzie *et al.*, 1987). Figure 1 shows the chromatogram of silylated derivatives of biological and methyl-amino acids we obtained on an apolar phase [100% dimethylpolysiloxane]. This approach offers subpicomole limit of detection for histidine and femtomole limit of detection for the other amino acids (limits correspond to concentration of about 0.1 ppm and 1 ppb respectively). Then, with further improvements, several techniques could fit all our

requirements. However final derivatisation procedure will be selected after studying the automation of extraction of the amino acids from inorganic matrices.

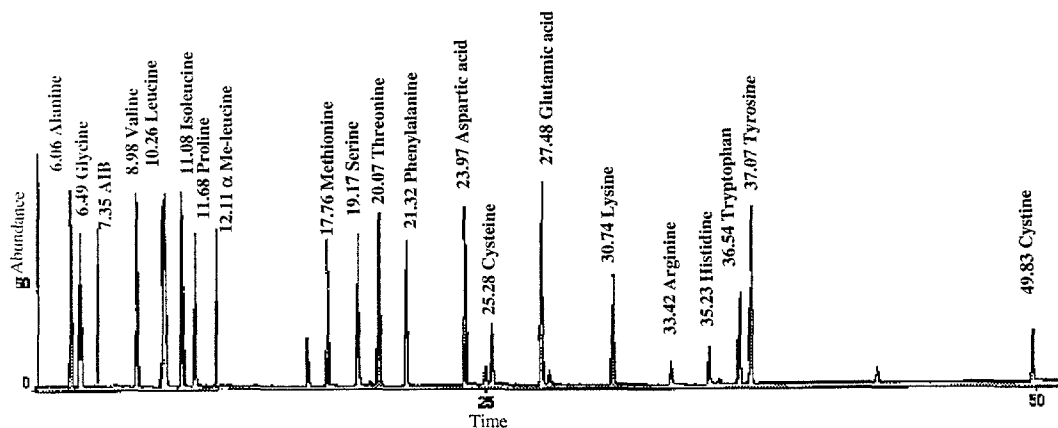


Fig. 1. Gas chromatogram of an amino acids standard mixture (100 mM each). A 15 m x 0.25 mm CPSIL 5 CB fused-silica WCOT column (Chrompack), operated in split mode (1 :100) was programmed at 3°C/min from 120°C to 270°C with an inlet internal helium pressure of 19.5 kPa.

## LABORATORY ANALYSES

A complete and precise evaluation of the inventory of organic compounds that may be present on Mars will require returned samples, especially if preliminary *in situ* analyses yield any positive results. Procedures chosen in both analyses are distinct because of the different constraints undergone but in any cases results will be complementary. Future Martian samples returned to Earth could be analyzed by any suitable existing analytical technique. However, there are limitations in returned sample analyses. The main limitation of organic analyses of samples returned from Mars will be the problem of terrestrial contamination. Even the best and most sensitive analytical methodologies used today must deal with terrestrial contaminants that limit the detection of extraterrestrial organic compounds. Thus, in order to detect trace levels of amino acids in samples of Mars returned to Earth, contamination from terrestrial amino acids and other interfering compounds would need to be greatly reduced. We selected a two-step chemical derivatization process (esterification of the acidic group followed by trifluoroacetylation of the amino group) often used, during the last 30 years, to transform the non volatile amino acids into volatile N-TFA, prior to the separation of amino acid enantiomers on chiral columns (Cronin and Pizzarello, 1997). This technique allows the detection at the femtomole level of the main amino acids found in Martian meteorites thanks to special devoted vials of reaction reducing surface contact of the amino acids solution with laboratory atmosphere: lost of amino acids during evaporation and contamination are then lowered compared to the traditional two-step derivatisation process. The enantiomeric separation focused on dialkyls amino acids and biological amino acids found in carbonaceous meteorites. The separation of two dialkyl amino acids, Isovaline D and L and α-aminoisobutyric acid (AIB), has been performed simultaneously with several biological amino acids (figure2). A chiral column was used (Column FS Lipodex E) to perform the proper separation of isovaline and AIB (limit of detection for AIB and isovaline are respectively 0.9 and 2.7 femtomoles).

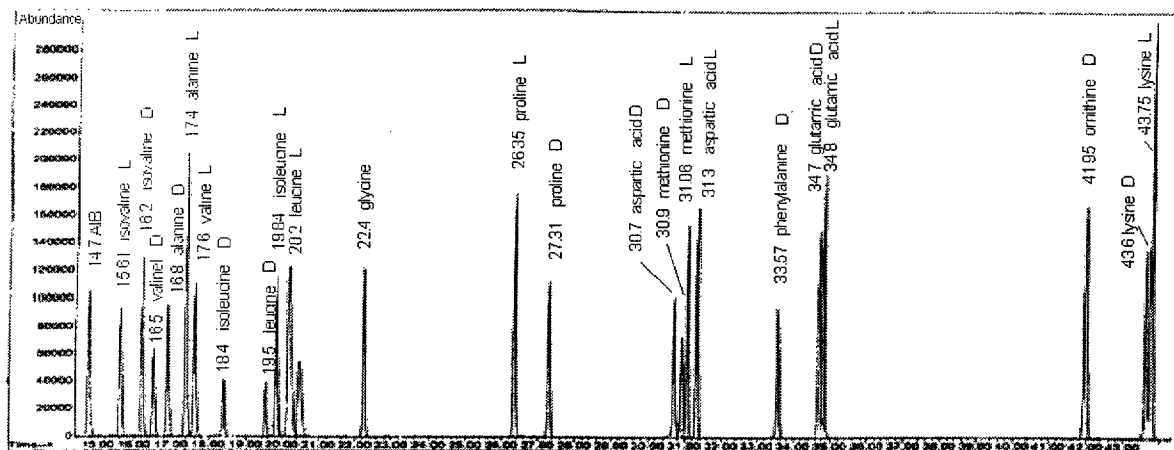


Fig. 2. Gas chromatogram of an amino acids standard mixture: A 50 m x 0.25 mm Column FS Lipodex E [Octakis-(2,6-di-o-pentyl-3-o-butyryl)- $\gamma$ -cyclodextrine], (Macherey Nagel) operated in splitless mode ) was programmed at 3°C/min from 60°C (5min) to 185 °C with an inlet pressure of 57.3 kPa.

Even though this procedure of derivatisation is sensitive and allows enantiomeric separation, improvements of procedures and instruments in chromatographic analysis are still in progress. For example, analysis of amino acids by capillary electrophoresis and laser-induced fluorescence is achieved at picomolar concentration (Vandenabeele-Trambouze *et al.*, 2000).

## CONCLUSION

Gas chromatography is a powerful analytical instrument for exobiology science and extraterrestrial environment exploration. We propose two different techniques of derivatisation to detect amino acids in Mars soil respectively aimed at laboratory and *in situ* analyses. The two procedures allow detection and identification of amino acids, with enantiomeric separation for laboratory analysis, at the femtomole level. Developments are however in progress to achieve enantiomeric separation for *in situ* analysis and lower limit of detection for laboratory analysis.

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