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# ***Analysis of organic molecules extracted from Mars analogues and influence of their mineralogy using N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide derivatization coupled with gas chromatography mass spectrometry in preparation for the Sample Analysis at Mars derivatization experiment on the Mars Science Laboratory mission***

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## Abstract

The search for complex organic molecules on Mars, including important biomolecules such as amino acids and carboxylic acids will require a chemical extraction and derivatization step to transform these organic compounds into species that are sufficiently volatile to be detected by gas chromatography mass spectrometry (GCMS). We have developed a one-pot extraction and chemical derivatization protocol using *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) and dimethylformamide (DMF) for the Sample Analysis at Mars (SAM) experiment on the Mars Science Laboratory (MSL). The temperature and duration of the derivatization reaction, pre-concentration of chemical derivatives, and gas chromatographic separation parameters have been optimized under SAM instrument design constraints. MTBSTFA/DMF extraction and derivatization at 300°C for several minutes of a variety of terrestrial Mars analogue materials facilitated the detection of amino acids and carboxylic acids in a surface soil sample collected from the Atacama Desert and a carbonate-rich stromatolite sample from Svalbard. However, the rapid reaction of MTBSTFA with water in several analogue materials that contained high abundances of hydrated minerals and the possible deactivation of derivatized compounds by iron oxides, as detected by XRD/XRF using the CheMin field unit Terra, proved to be highly problematic for the direct extraction of

organics using MTBSTFA. The combination of pyrolysis and two different chemical derivatization methods employed by SAM should enable a wide range of organic compounds to be detected by GCMS if present on Mars.

## 1. Introduction

Mars remains a key target of astrobiological interest since its past environmental conditions are thought to have been more favorable for the emergence of life. Since 2004, several missions to Mars, including the Mars Exploration Rovers Spirit and Opportunity, the Mars Express probe, the Mars Reconnaissance Orbiter, and the Phoenix lander, have provided mineralogical data that indicate a past sustained presence of liquid water on Mars, probably during the first 500 million years of the planet's history (Squyres et al., 2004, Bibring et al., 2006). During this period, Mars was bombarded by asteroids, comets, and their fragments (Cottin et al., 1999, Botta and Bada, 2002, Pizzarello et al., 2006), which would have delivered organic matter, including compounds potentially useful for the emergence of a prebiotic chemistry or even the origin of martian life to the surface of the planet (Chyba and Sagan, 1992).

Even if life never arose on Mars or became extinct, the cooling of Mars and the lack of extensive plate tectonic recycling may have enabled the preservation of molecular evidence of prebiotic and/or biotic activity in ancient sediments (Morrison, 2001). Future *in situ* exploration of the Mars surface will include specific experiments to detect organic molecules that may represent chemical fingerprints of a prebiotic chemistry or biological activity, past or present. While the detection of trace concentrations of the only organic compound, methane, is debated in the martian atmosphere from both orbital and Earth based observations (Formisano et al., 2004, Krasnopolsky et al., 2004, Mumma et al., 2009), more complex nonvolatile organic compounds trapped in the regolith, such as carboxylic acids or amino acids, cannot be detected remotely and will require direct *in situ* measurements of the regolith at the surface.

To date, the only *in situ* experiment devoted to the search for complex organic compounds in the martian regolith was the Gas Chromatograph-Mass Spectrometer (GCMS) experiments of the Viking missions in 1976. In these experiments, several surface samples, collected down to approximately 10 cm depth, were heated to temperatures of up to 500°C, and the gases released were analyzed directly by GCMS. No organic molecules of martian origin were detected at the two different landing sites within the detection limits of the instruments

(Biemann et al., 1976, Biemann et al., 1977). Although, the Viking GCMS instruments did detect chloromethane and dichloromethane at part-per-billion (ppb) levels at both landing sites in the surface regolith samples, it was argued that these chlorohydrocarbons were derived from cleaning solvents used on the instrument hardware (Biemann et al., 1977). Several explanations for the lack of organics in surface materials on Mars have been proposed including the destruction of hydrocarbons by UV and ionizing radiation (Oro and Holzer, 1979, Stoker and Bullock, 1997, Ten Kate et al., 2005, Dartnell et al., 2007, Stalport et al., 2008, Stalport et al., 2009, Stalport et al., 2010) and/or other oxidation processes (Chun et al., 1978, Pang et al., 1982, Yen et al., 2000, Clancy et al., 2004, Encrenaz et al., 2004). Recent *in situ* data obtained from the Northern polar region of Mars by the *Phoenix* mission showing high concentrations of magnesium perchlorate (Hecht et al., 2009), and laboratory thermal volatilization GCMS measurements of Atacama Desert soils containing perchlorate suggest that a significant amount of organic carbon in the martian regolith (up to part-per-million levels) may have been converted to chlorohydrocarbons during high temperature pyrolysis (up to 500°C) due to the possible presence of perchlorates in the soils analyzed by *Viking* (Navarro-Gonzalez et al., 2010). It is also been suggested that significant amounts of non-volatile products such as amino acids and carboxylic acids, would not have been extracted by the Viking pyrolysis procedure or would have been destroyed prior to GCMS detection (Glavin et al., 2001, Benner et al., 2000). Therefore, future GCMS analyses of complex organic compounds on Mars may require lower temperature extraction protocols, such as chemical derivatization, that can transform less volatile and less thermally stable organic compounds into molecules that can be readily detected (Meunier et al., 2007, Buch et al., 2009).

NASA and ESA are planning a series of new robotic missions to Mars and other destinations that will incorporate *in situ* wet chemistry experiments. Chemical derivatization using dimethylformamide-dimethylacetal (DMF-DMA) has already been incorporated into the Cometary Sampling and Composition (COSAC) evolved gas experiment on ESA's Rosetta Lander and will provide amino acid detection and enantiomeric measurements on the surface of comet 67P/Churyumov-Gerasimenko in 2014 (Meierhenrich et al., 2001, Szopa et al., 2003, Goesmann et al., 2007). The use of multiple chemical derivatization agents including MTBSTFA and DMF/DMA are also under consideration for inclusion in the Mars Organic Molecule Analyzer (MOMA) instrument on the 2018 ExoMars rover mission (Buch et al., 2009). The

NASA Mars Science Laboratory (MSL) mission, which is scheduled to land on Mars in early August 2012, is carrying the Sample Analysis at Mars (SAM) instrument suite designed to detect a wide range of chemical biosignatures, organic and inorganic, that could provide evidence of a habitable environment and possibly signs of life (Cabane et al., 2004, Mahaffy et al., 2010). The SAM instrument suite includes a gas chromatograph quadrupole mass spectrometer (GCMS) that will enable the separation and direct analysis of volatile species in the atmosphere and released from solid samples heated up to 1000°C. In addition, SAM will employ a lower temperature ($\leq$ 300°C) chemical extraction and derivatization step using sealed metal cups filled with a mixture of MTBSTFA and DMF that will target less volatile and less-thermally stable organic compounds such as amino acids and carboxylic acids that cannot be readily extracted and detected by high temperature pyrolysis and GCMS analysis alone.

MTBSTFA was originally selected as a derivatizing agent for the SAM wet chemistry experiment since the reaction can occur in a single step (Knapp, 1979), MTBSTFA is less susceptible to hydrolysis compared to other reagents, and it does not require separation of the derivatives prior to GC analysis. In addition, MTBSTFA will rapidly react with a wide range of organic compounds with acidic hydrogen atoms including amino acids, carboxylic acids, nucleobases, primary and secondary amines, alcohols, and amides (Buch et al. 2006). Furthermore, the derivatization yields for pure amino acid and carboxylic acid standards are high and typically in the range of 90-100% (Rodier et al. 2001). SAM also has the ability to extract and detect higher molecular weight organic matter, including fatty acids, by thermochemolysis at temperatures >340°C using tetramethylammonium hydroxide (TMAH). Thermochemolysis was not investigated in this study, but TMAH protocols and experiments have been tested previously using Mars analogue materials from the Atacama Desert (Geffroy-Rodier et al., 2009).

Here we report the first GCMS results of amino and carboxylic acids that were extracted from a suite of terrestrial Mars analogue materials using a derivatization test-bed that approximates the front end extraction capabilities of the SAM flight instrument. MTBSTFA derivatization experiments were not run using the actual SAM flight instrument to avoid contamination of the flight instrument by the analogue materials and derivatization agents themselves. The primary goal of this study was to understand the influence of minerals on the efficiency of MTBSTFA derivatization of amino and carboxylic acids using the SAM one-pot extraction approach. Since SAM flight hardware components were not available for this study, we did not focus on optimization of the derivatization process that will be used on Mars by SAM which will be done on the SAM testbed instrument when it becomes available. The experimental results described here will be used to guide SAM testbed derivatization operations, help formulate a sample selection strategy for the SAM derivatization experiment on Mars, and enable a more accurate interpretation of the *in situ* derivatization GCMS results obtained by SAM.

## 2. Experimental

### 2.1 Analogue Samples and Preparation

Five terrestrial Mars analogue samples, one meteoritic sample and one procedural blank were selected for this study and represent a diverse set of the types of samples that could be encountered on Mars (Table 1). Understanding the potential interferences of different analogue materials on the extraction of organics will permit more accurate interpretations of *in situ* derivatization data obtained by SAM on Mars.

Two soil samples were collected in 2006 from Site #3 (27°20.2'S, 70°42.4'W) in the Atacama Desert in Chile using a sterile metal scoop (personal communication, K. Snook). The surface sample collected at 0-1 cm depth (hereafter, Atacama-01) and a subsurface sample collected from ~10 cm depth (hereafter, Atacama-02) were stored in solvent cleaned Teflon capped glass vials. Although the Atacama Desert region has been characterized as a reasonable Mars analogue due to the extremely dry climate, oxidizing environment, and soil mineralogy (Navarro-Gonzalez et al., 2006), it should be noted that samples analyzed in this study from Site #3 were collected from a coastal region of the Atacama where fog events are much more common than in the more arid core of the Atacama Desert.

We also analyzed a sample of precipitated sediment collected in 2003 from the Rio Tinto "Headwaters Spring A" collection site (hereafter, RioTinto-01). The Rio Tinto sample was collected with stainless steel tongs and stored in a Whirl-Pak polyethylene bag (personal communication, Mary Sue-Bell). Rio Tinto is a highly acidic environment and is another plausible terrestrial Mars analogue because of the presence of jarosite and other sulphate minerals similar to those that have been identified on Mars by the Mars Exploration Rover Opportunity at Meridiani, Planum (Fernández-Remolar et al., 2005, Squyres et al., 2004).

A Mars regolith simulant used in this study, called JSC Mars-1, is a palagonitic tephra (glassy volcanic ash) collected from the Pu'u Nene Cinder Cone on the island of Hawaii (Allen et al., 1998). JSC Mars-1 is a close spectral analog to the bright regions of Mars (Morris et al., 1993), and has a chemical composition that is similar to the soils analyzed by Viking (Clark et al., 1982).

A carbonate-rich stromatolite collected during the 2005 ASTEP Mars Analogue Svalbard Expedition (AMASE) was also included in this study (hereafter, Carbonate-01). Carbonate minerals have been observed from orbit in the Nilli Fossae region on Mars (Ehlmann et al., 2008) and calcium carbonate has recently been identified in the polar regolith by the Phoenix mission (Boynton et al., 2009).

Although not a terrestrial sample, a fragment of the CM2 carbonaceous meteorite Murchison (USNM 6650.2; hereafter Murchison) was also selected for this study as an analogue since it contains a wide variety of soluble organic molecules including amino acids and carboxylic acids of abiotic origin (Cronin et al., 1993), and it has been estimated that material from carbonaceous meteoritic infall could account for between 2 and 29% of the total mass of the martian surface regolith (Flynn and McKay, 1990).

As a control, a fused silica quartz glass powder (FS 120, < 150 μm size fraction, Reade Advanced Materials) that had been heated to 900°C for 7 hours in air to remove organic contamination was processed in parallel with the analogue samples (hereafter Quartz-01). A similar fused silica material, called the Organic Check Material (OCM), will be carried on board the 2011 MSL rover and used as an end-to-end procedural blank to monitor organic contamination from the sample handling system.

All of the glassware and tools used to prepare the analogue and blank samples were pyrolyzed at 500°C in air overnight. The samples (~ 5-10 g each) were crushed and using a ceramic mortar and pestle inside a Class 100 High Efficiency Particulate Air (HEPA) laminar flow hood and the resulting sample powders then passed through a 150 μm stainless steel sieve and homogenized by mixing. The same sample size fraction (< 150 μm) will be delivered by the MSL Sample Acquisition/Sample Processing and Handling (SA/SPaH) directly to the SAM instrument via solid sample inlet funnels on the top of the rover deck. All of the sample powders were stored inside clean Teflon capped glass vials. For this study, aliquots of each powdered sample were carried through a SAM-like derivatization and GCMS analysis to measure the distribution and abundance of amino and carboxylic acids, as well as other compounds that react with MTBSTFA.

## 2.2 Chemicals

A concentrated stock solution ($1 \times 10^{-3}$ M) of 3-fluoro-DL-valine (Fluka, >99% purity, hereafter, 3-FV) was prepared by mixing the amino acid standard in Millipore water (18.2 MΩ, < 5 ppb total organic carbon). 3-FV is used as an internal standard for the SAM derivatization experiment since this synthetic fluorinated amino acid is unlikely to be found on Mars and will not interfere with the detection of any indigenous valine, if present. The derivatization chemical used in this study was *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA, Sigma-Aldrich, 97% purity), a silylating agent that rapidly reacts with amines, carboxylic acids, alcohols, amino acids, sugars, and nucleobases at moderate temperatures (Fig. 1). Dimethylformamide (Pierce, > 99% purity, hereafter, DMF) does not react with MTBSTFA and was used only as a solvent to assist with the extraction of organic compounds from the samples. Pyrene ($C_{16}H_{10}$), a polycyclic aromatic hydrocarbon (Sigma-Aldrich, 100 ng/L in cyclohexane, > 99% purity), also does not react with MTBSTFA, and was used as an internal standard for *in situ* verification of the SAM derivatization experiment and to determine the relative abundance of any derivatized compounds, if present.

## 2.3 SAM Derivatization Experiment

A schematic illustrating the SAM derivatization procedure for Mars is shown in Fig. 2. The SAM instrument contains 74 cups located in two concentric rings inside the Sample Manipulation System (SMS) carousel, nine of which contain solvents needed for chemical derivatization or thermochemolysis. 3-FV is used as an internal derivatization standard inside seven of the cups that are dedicated to the MTBSTFA derivatization experiment (Fig. 3). Since the purpose of the 3-FV is to test the reaction efficiency of MTBSTFA (Fig. 1) under martian conditions, the dried internal 3-FV standard must be isolated from the MTBSTFA until the derivatization experiment is carried out on Mars. To achieve this, the solid 3-FV is located inside a separate foil capped reservoir that is hermetically sealed under vacuum by a pinch off tube (< $10^{-4}$ mbar) and separated from the MTBSTFA and DMF solvents that are present in the outer volume of the SAM derivatization cup (Fig. 3). The total amount of dry 3-FV standard hermetically sealed inside each SAM derivatizaiton cup is ~40 nmol. The sealed internal reservoir prohibits any exposure and reaction of the 3-FV standard with MTBSTFA fluid until the foil caps are punctured and the experiment is carried out *in situ* on Mars. The outer volume of each cup contains ~0.5 ml of a mixture of four times freeze-pump-thaw degassed MTBSTFA/DMF (4:1 by volume), including pyrene (25 nmol) dissolved in solution. Pyrene does not react with MTBSTFA and is used as a second internal standard for the SAM derivatization experiment to determine that the cup was properly punctured and the solvent carried into the GCMS. Prior to receiving sample fines from the MSL Sample Acquisition/Sample Processing and Handling (SA/SPaH) system, both foil caps on a derivatization cup are opened using a puncture pin and the 3-FV internal standard is then exposed to the derivatization solvents.

After foil cap puncture, the cup can be filled with martian regolith or powdered drill fines through the SAM solid sample inlet tube up to a volume of 0.79 cc, the total volume of the outer reservoir of the derivatization cup. Since the sample acquisition system on MSL will deliver ~ 0.05 cc volume aliquots of powder to the SAM derivatization cups, we selected 100 mg as the sample mass for our experiments which assumes a density of the martian regolith of ~ 2 g/cc. After the sample is loaded into the cup, the sample and fluids are heated up to a maximum temperature of 300°C inside the SAM pyrolysis oven for several minutes to initiate derivatization and drive volatile products to the hydrocarbon trap (Carbosieve, Tenax TA (porous 2.6-diphenylene oxide), and glass beads packed inside a glass tube, refer to Fig. 2 and discussion in Section 2.4). Previous experiments by Buch *et al.* (2009) have shown that heating to 300°C for several minutes is needed to desorb amino acids and carboxylic acids bound to the mineral matrix prior to chemical derivatization with MTBSTFA. Helium carrier gas flows at a rate of ~5 x $10^{-2}$ atm.cc/sec from the bottom of the cup through the pyrolysis oven and over the SAM hydrocarbon trap which can be cooled to a temperature of -50°C. The internal pressure inside the SAM pyrolysis oven at ambient temperature under these flow conditions is ~30 mbar. After the derivatization products are transferred from the pyrolysis oven to the hydrocarbon trap, the SAM trap is heated to a maximum of 300°C to flush the derivatized products under helium flow to the inlet of one of six SAM GC columns (MXTU, MXT20, MXT5, MXTCLP, Carbobond, Chirasildex CB) where the compounds are separated using a programmed column ramp. Derivatized compounds eluting from the GC column are then ionized by electron impact and identified by their unique mass fragmentation patterns using the SAM quadrupole mass spectrometer (mass range 2-535 m/z). Quantitation of derivatized 3-FV by the SAM GCMS will indicate the extraction efficiency of the MTBSTFA/DMF reaction on Mars, including evidence for possible side reactions (e.g. MTBSTFA reactions with the mineral matrix or oxidizing materials) that could inhibit the reaction of MTBSTFA with any organics present in the martian sample. For the procedural blank and each analogue sample, we performed multiple (~6 to 10) derivatization experiments and GCMS analyses of the hydrocarbon trap and the average recoveries of the both the pyrene and 3-FV internal standards for each sample were determined.

### 2.4 Derivatization Test-Bed and GCMS Protocol

In order to carry out one-pot MTBSTFA/DMF extraction of organic compounds from the analogue samples using the SAM chemical derivatization approach, a front-end gas processing system was assembled at NASA Goddard Space Flight Center that included a sample reactor oven, gas transfer line, and hydrocarbon trap assembly (Fig. 2). Although the derivatization test-bed used in this study does not include actual SAM flight hardware components (specifically, the flight pyrolysis oven and metal cup assembly), we have made every effort to assemble and operate the derivatization test-bed using materials and experimental conditions (including helium gas flow rates, pressure, temperature, solvent volumes and concentrations, and analogue sample volumes) that are similar to SAM. Additional optimization of the SAM derivatization experiment using a flight-like oven and metal cup assembly will be required when the SAM test-bed instrument becomes available for derivatization testing in 2012.

The entire test-bed assembly was operated inside a chemical fume-hood. This derivatization test-bed was designed to similar specifications as the SAM gas processing system, including the gas path and flow rates from the pyrolysis oven to the hydrocarbon trap, the transfer line dimensions (length and internal diameter) and the chemical composition of the SAM hydrocarbon trap fill material. The glass beads and tube used in the hydrocarbon trap assembly were first conditioned and heated in air at 500°C for 2 hours in a furnace. Prior to each derivatization experiment, the loaded hydrocarbon trap was conditioned overnight at 300°C under helium gas flow (flow rate = 1.6 ml/min) using a commercial pyroprobe instrument (CDS Analytical, Pyroprobe 5200) to remove any hydrocarbon contaminants. The masses and volumes of the 381 micron non porous silica glass beads ($V = 0.32$ cm$^3$, $m = 0.49$ gram), Tenax TA 60/80 mesh (V = 0.32 $cm^3$, m = 0.08 gram), and Carbosieve G 60/80 mesh (V = 0.32 $cm^3$, m = 0.11 gram) used in the test-bed hydrocarbon trap are similar to the volumes of material in the SAM flight hydrocarbon trap (within ±10%). The silica glass beads act as a filter at the front of the hydrocarbon trap and can effectively trap solids at room temperature and higher boiling compounds. Tenax TA has a low affinity for water, but will readily trap medium to high molecular weight volatile organic compounds ($C_6$ to $C_{10}$ and higher) that can be readily released when heated to temperatures of up to 350°C (Cao and Hewitt, 1992). Finally, carbosieve has a very high capacity and breakthrough volume for low boiling point, low molecular weight compounds including $C_2$ to $C_6$ volatile hydrocarbons and noble gases(http://www.sisweb.com/index/referenc/bv-hyd.htm). The MTBSTFA and DMF fluids used in this study as well as a wide range of hydrocarbons and MTBSTFA derivatives extracted from the Mars analogue samples are readily trapped using these materials.

The derivatization test-bed vacuum manifold consists of the following components: i) an active pumping system (Drytel 1025 roughing pump) and a $CO_2$ gas tank to simulate the gas composition and average pressure at the martian surface (~ 6-10 mbar), ii) a helium gas tank with flow restrictor set at 5 x $10^{-2}$ atm.cc/sec to flush and purge derivatization solvents and other gaseous products generated inside the oven during heating to the hydrocarbon trap, and iii) a transfer line heated up to 170°C from the pyrolysis oven to the hydrocarbon trap, the maximum temperature tested on the SAM flight instrument. The hydrocarbon trap itself was maintained at approximately 30°C during the entire derivatization experiment. Most of the plumbing was connected using Swagelok Tube Fittings, however the pyrolysis cell and hydrocarbon trap manifold that were opened between each experiment were sealed using Swagelok metal gasket fittings. When dynamically pumped with no carrier gas flow, the derivatization test-bed vacuum system achieved an internal pressure of < 0.1 mbar.

For each derivatization experiment, the Inconel 625 test-bed reactor cell (used as a proxy for the SAM derivatization cup and oven) was preloaded with 40 nmol 3-FV and 25 nmol of pyrene by pipetting the appropriate volumes of the internal standards into the oven and then evaporating the solutions to dryness under $N_2$ gas flow. A similar total abundance of these internal standards are present in each of the seven SAM flight MTBSTFA derivatization cups. We then added 150 μl of a solution of MTBSTFA/DMF (4:1) directly to the oven containing the dried internal standards. The volume of solvent used in these experiments (150 μl) is lower than the total volume of MTBSTFA/DMF (~ 500 μl) loaded inside the SAM flight cups. We found through testing that volumes of MTBSTFA/DMF in excess of 150 μl can lead to severe oversaturation and degradation of the hydrocarbon trap, as well as clogging of the GC column after subsequent trap heating. As pyrene is dissolved into the MTBSTFA/DMF mixture inside the SAM flight cups, we performed evaporations tests with the setup in order to understandthe evaporation rate of MTBSTFA, DMF, and pyrene. Unlike MTBSTFA and DMF, we observed that the pyrene did not evaporate from the oven during the evaporation step after heating at 75°C for several minutes at 7 mbar, which is not surprising given the very low vapor pressure of pyrene of ~0.12 mbar at 125°C (Smith et al., 1980) and the high boiling point of pyrene (404°C).. For the actual SAM analysis, it will be necessary to evaporate a large fraction of the MTBSTFA/DMF solvent from each cup through the SAM exhaust vent prior to the actual derivatization experiment to avoid solvent oversaturation and possibly damage to the hydrocarbon trap and/or GC columns. After the solvent was added to the reactor cell, approximately 100 mg of each sample was weighed, transferred into the reactor oven and the oven then sealed to the VCR fitting connected to the vacuum manifold (Fig. 2). The reactor oven containing the solid sample was sealed under ~7 mbar $CO_2$ (to simulate martian surface atmospheric pressure in the SAM experiment) using a valve and then heated up to 300°C. After three minutes, the valve of the reactor oven and the helium tank were opened simultaneously to allow helium to flow through the cell. The MTBSTFA/DMF solvent and derivatized volatiles are transferred to the hydrocarbon trap (trap temperature ~30°C). After five minutes, the valve of the hydrocarbon trap is closed and removed from the test-bed manifold and inserted into a commercial pyroprobe system for GCMS analysis.

The pyroprobe was interfaced to a Thermo-Finnigan Trace GC and DSQ quadrupole mass spectrometer (DSQ) for analyses of the derivatized products released from the hydrocarbon trap. Data analysis was performed with The DSQ was operated in quadrupole detection mode (50--550 m/z) with the detector voltage set at 1 kV. The GCMS was equipped with a splitless injector set at a temperature of 300°C. The GC inlet is used in splitless mode to maximize the quantity of derivatized species desorbed from the hydrocarbon trap onto the GC column. For each GCMS analysis, the pyroprobe trap was heated to 300°C for 10 minutes under an optimized helium carrier gas flow of 1.6 ml/minto release the derivatized products and transfer them directly to the inlet of the GC column (Restek RTX 5 MS capillary column, 95%

dimethylpolysiloxane, 5% diphenyl, 30 m, x 0.25 mm x 0.25 μm). The commercial GC column used in all of these experiments was similar to one of the six GC columns used in the SAM instrument (MXT 5). This column is efficient for a wide range of organic molecules such as amines, amino acids, and carboxylic acids. The initial temperature of the column was kept at 140°C to avoid condensation and clogging of the GC column by excess MTBSTFA and DMF from the hydrocarbon trap. After 10 minutes at 140°C, the GC oven was ramped to 300°C at a rate of 10°C/min. The final temperature of the column was maintained at 300°C for 10 minutes. A mass spectrometer solvent delay of 10 minutes from the time of GC injection was included to minimize saturation of the DSQ detector by excess MTBSTFA and DMF.

**2.5 CheMin XRD/XRF Technique**

The CheMin X-Ray Diffraction/X-Ray Fluorescence (XRD/XRF) instrument, can determine the bulk mineralogy of rocks and soil that are provided to it by the MSL SA/SPaH. A detailed description of the CheMin flight instrument and capabilities has been discussed previously (Blake et al., 2010). Several prototype instruments have been built, one of these instruments (Terra) was used for this study. Unlike in CheMin, the XRF capabilities of Terra are limited due to absorption occurs by the beryllium windows of the CCD detector preventing detection below 3 keV. The instrument uses a Co X-ray tube, has a range of 5-55° 2θ and a full width at half maximum of 0.3° 2θ. The CCD detector is sensitive to X-rays between about 2.3 KeV and 8 KeV (Cl Kα to Cu Kα). All samples were received as vials of powder, sieved to < 150 μm grain size. Approximately 65 $mm^3$ aliquots of sample material were poured into the sample holder of the instrument and samples were analyzed continuously for 6 hours, approximating the flux intensity and data collection rates anticipated for MSL. Quantitation of the mineral abundances from XRD patterns by Rietveld refinement and other full pattern fitting techniques are generally accurate to ±10% of the amount present, with the exception of clays and amorphous materials (Bish and Post, 1993, Chipera and Bish, 2002). Detection limits range from 1% for highly crystalline materials, to 5-10% for clays and amorphous materials. Amorphous and disordered minerals such as clay minerals (e.g., smectites) are not quantifiable by Rietveld refinement.

**3. Results and Discussion**

### 3.1 Bulk Mineralogy Measurements

The XRD pattern and XRF spectrum for the Atacama Desert surface soil sample (Atacama-01) is shown below (Fig 4). Similar XRD/XRF measurements were made on the fused silica procedural blank and the other analogue samples selected for this study (data not shown). The mineral abundances in the samples determined from the Terra instrument spectra after Rietveld refinement and fitting are shown in Table 1. XRD data from the FS120 fused silica sample was monotonically amorphous quartz (100% $SiO_2$); no mineral impurities in the sample were detected.

The mineral composition of the two Atacama Desert soil samples analyzed (Atacama-01 and Atacama-02) were similar to previous analyses of soil from the Atacama Desert (Sutter et al., 2007). However, some differences in bulk mineralogy between the surface and subsurface samples were observed (Table 1). The presence of quartz, albite, and anorthite in both samples suggest that their parent rocks were felsic or granitic in composition. The presence of secondary minerals such as calcite suggests that alteration occurred in the presence of groundwater, particularly in the case of the subsurface sample Atacama-02 where calcite is a major constitutent (~30%). Substantially different clays (kaolinite in the surface sample and palygorskite in the subsurface sample) are found in the two soil layers. Since kaolinite is typically a weathering or hydrothermal alteration product of felsic minerals, it follows that it is likely a product of the breakdown of the locally abundant felsic parent rocks. The palygorskite clay found in the lower horizon is present at a much higher abundance (18.5%) than kaolinite at the surface (2.5%) and suggests a different formational or diagenctic environment relative to the upper horizon, possibly a buried soil horizon from an earlier time. Palgorskite clays can occur in a wide range of environments such as marine and lacustrine sediments, soils, paleosols and calcretes. The structure of palygorskite lends itself to adsorption of water and organic molecules (in structural channels in the mineral). This would be a likely place for organic molecules to persist if they had been present in the original environment, and a likely place for organic molecules to be trapped should ground water containing organic compounds have passed through this horizon.

The mineral composition of the Mars regolith simulant, JSC Mars-1 is similar to previous XRD measurements (Allen et al., 1998, Perko et al., 2006). The JSC Mars-1 sample analyzed in this study was dominated by plagioclase (62% anorthite and 27% augite), with lower abundances of forstetite (~ 9%), and trace abundances (~ 1%) of chromite and ilmentite. We did not detect any magnetite in this JSC Mars-1 sample, although previous XRF measurements of JSC Mars-1 have identified 10-15 wt% magnetite ($Fe_2O_3$). Iron Mossbauer spectroscopy measurements of JSC Mars-1 has shown that the majority of iron (64%) in this sample is present as nanophase ferric oxide particles (Morris et al., 1993, Allen et al., 1998), however this phase was not identified in this study. We did not identify any phyllosilicates in this sample (< 1 wt%) which is consistent with previous analysis (Allen et al., 1998).

The Rio Tinto headwater sample (Rio Tinto-01) is a red-orange colored sample formed at low pH (~ 1) with a mineralogy dominated by hydrated aluminium and iron sulphate minerals including alunogen (40 wt%) and jarosite (36 wt%). Lower abundances (~12 wt% each) of copiapite and amarantite were also identified in this sample. Previous XRD measurements of samples from the Rio Tinto headwaters have revealed similar mineralogies (Fernández-Remolar et al., 2005). This sample is a very good soil analogue for the acid-sulphate chemistry and mineralogy observed by the Mars Exploration Rover Opportunity at Meridiani Planum, Mars (Fernandez-Remolar et al., 2004, Klingelhöfer et al., 2004).

The calcium and magnesium rich carbonate sample collected during the 2005 AMASE Svalbard campaign (Carbonate-01) is a stromatolite. Stromatolites are layered accretionary structures formed in shallow waters by the cementation of sedimentary grains by biofilms of microorganisms. XRD analyses of this sample showed that it consisted predominately of dolomite (99 wt%) with trace amounts of quartz (1 wt%).

Finally, the Murchison meteorite contained a highly diverse distribution of minerals dominated by olivine (forsterite, 43 wt%), serpentine (lizardite, 19 wt%), and pyroxenes (10 wt% augite, 8 wt% diopsite, and 6 wt% enstitite). Lower abundances (< 5 wt%) of chromite, calcite, and tochilinite) were also identified. This meteorite is a fragment of an exceedingly complex extraterrestrial parent body that has a mix of high temperature and low temperature phases and exhibits extensive hydrothermal or aqueous alteration (Lauretta and McSween, 2006). Despite the large number of published studies, very few XRD results have been reported. This is perhaps because of the complexity of the rock and the fine-grained nature of some of the components. It is possible that other fragments of the Murchison meteorite will contain additional minerals not reported here, and there are likely fine-grained phases that are "X-ray amorphous" and not detectable by this technique.

### 3.2 Derivatization and GCMS Analysis of Organic Compounds

The first goal of this study was to verify the ability of the SAM-like derivatization test-bed to efficiently extract and concentrate the two internal standards, 3-FV and pyrene using the oven and the hydrocarbon trap. The experimental sequence discussed in Section 2.4 was optimized by varying both the oven temperature and duration of heating to maximize the recovery of the two internal standards (3-FV and pyrene). The total extraction recoveries of both internal standards placed inside the reaction oven and carried through the entire test-bed derivatization experiment were determined based on the areas of the peaks compared to the areas obtained by direct injection of similar quantities of the standards in the GCMS. From the analysis of fifteen individual experiments using these pure standards in an empty oven, we calculated a maximum extraction recovery of approximately 30±2% for 3-FV and 10±2% for pyrene after heating to 300°C for 3 minutes followed by transfer to the hydrocarbon trap under helium carrier gas flow. We found that higher oven temperatures and longer heating times did not improve the recovery yields for these two internal standards. Based on these findings, subsequent derivatization extraction experiments for the analogue samples were all carried out at 300°C for 3 min.

The low recoveries of the internal standards after one-pot extraction and subsequent trapping of the compounds on the hydrocarbon trap could be due to oversaturation of the hydrocarbon trap with excess MTBSTFA/DMF and the relatively low transfer line temperature of 170°C used. For 3-FV, we believe that the relatively large (150 μl) volume of MTBSTFA/DMF used in the experiments saturates the hydrocarbon trap which inhibits the adsorption of 3-FV on the hydrocarbon trap. For pyrene, the saturation could also explain the low recovery, however we also note that due to the low vapor pressure of pyrene of ~2.2 mbar at 170°C (Smith et al., 1980), a large fraction of pyrene would condense on the interior surfaces of the transfer line from the oven to the hydrocarbon trap which were kept at a temperature of ~ 170°C under He carrier flow at a pressure of 30 mbar for the duration of the experiment. This temperature represents the maximum temperature that the transfer lines can safely be heated to in the SAM instrument. We confirmed in subsequent experiments that much higher transfer line temperatures (≥ 250°C) were required to transfer the remaining pyrene to the hydrocarbon trap under helium carrier gas flow.

GCMS analysis of the hydrocarbon trap after derivatization extraction of the fused silica procedural blank clearly show peaks and mass fragmentation patterns corresponding to the MTBSTFA derivative of 3-FV (retention time ~ 12.4 min) as well as a smaller peak at ~18.2 min corresponding to pyrene. Although the 3-FV standard used in this experiment is chiral (racemic mixture, D/L = 1), we were unable to obtain separate peaks for the D- and L-enantiomers of 3-FV under the conditions employed. Other prominent peaks in the GC chromatogram are clearly observed in Fig. 5 and can be attributed to derivatization artifacts and other unidentified derivatized species that formed from the reaction of MTBSTFA with the hydrocarbon trap material and/or internal metal surfaces of the test-bed setup. A very wide GC peak is also visible at the beginning of the chromatogram (10-12 minutes) as well as others peaks corresponding to polysiloxane fragments from the GC column itself. Due to the extremely high solvent background and the relatively high initial starting temperature of the GC column (140°C), more volatile MTBSTFA derivatives including glycine and alanine will be difficult to detect above background, especially if these amino acids are only present at trace abundances in a martian sample (the detection limit for these amino acids under these conditions is ~1 nmol). However, assuming there are no side reactions with the mineral matrix from a sample on Mars, heavier less volatile amino and carboxylic acids should be identified by GCMS after MTBSTFA chemical derivatization if present in the martian regolith. Finally, we determined that starting the oven ramp at 140°C was important to minimize clogging of the GC column at the inlet with a high flow of solvent from the hydrocarbon trap.

GCMS analysis of the hydrocarbon trap after extraction of the Atacama-01 in Fig. 6 showed the presence of both pyrene and 3-FV which indicated that the MTBSTFA derivatization reaction occurred during the experiment. We also identified two carboxylic acids, hexadecanoic acid and octadecanoic acid (steric acid) based on their mass fragmentation patterns that matched the fragmentation of their MTBSTFA derivatives from standards we injected for comparison and the NIST spectral library. These two carboxylic acids have been previously identified in a different Atacama Desert soil sample using one-pot MTBSTFA derivatization and GCMS analysis (Buch et al., 2009). However, Buch *et al.* reported a much wider range of MTBSTFA derivatized compounds in different Atacama Desert soils including 9 carboxylic acids, 2 hydroxy acids, and 3 amino acids (glycine, alanine, and valine) at concentrations ranging from 0.5 to 9 nmol per gram. This is likely due to non-SAM like extraction methods and GCMS conditions such as a pre-extraction sonication step using water and isopropanol or a thermal desorption step prior to MTBSTFA.

In contrast to the surface sample, we were unable to identify any amino acids or carboxylic acids originating from Atacama-02. Although pyrene and some 3-FV was detected in the extract, the absolute intensity of the 3-FV peak was twelve times smaller than observed in the extract from Atacama-01 which indicates that the derivatization was inhibited in the subsurface soil. Since Atacama-02 contains a much higher abundance of hydrated minerals (Sutter et al., 2007), water bound in the palygorskite clay may have reacted preferentially with the MTBSTFA or hydrolyzed the labeled organics. Thus, reaction of MTBSTFA with free or bound water presented in the clays in the Atacama-02 sample could explain the reduced intensity of the derivatized 3-FV peak in GCMS analysis of the Atacama-02 sample.

We were unable to detect the 3-FV internal standard or any other derivatized organic compounds in JSC Mars-1 and Rio Tinto-01 samples (Fig. 7 and Fig. 8). However, we did detect the internal standard pyrene in both samples which suggests that any organics of similar or lower volatility extracted from the samples should have been collected on the hydrocarbon trap during the experiment and detected by GCMS. The lack of amino acids extracted from the JSC Mars-1 analogue is surprising since a wide range of amino acids have previously been found in this sample with concentrations ranging from ~2 to 35 parts-per-million as measured by high performance liquid chromatography (Garry et al., 2006). JSC Mars-1 contains no evidence for hydrated minerals, but iron oxide minerals such as $FeTiO_3$ are present (Table 1) (Allen et al., 1998) which could inhibit derivatization or deactivate derivatized species at 300°C. Deactivation of the MTBSTFA could result in a reverse chemical reaction producing lower volatility compounds that cannot be extracted from the sample under the conditions employed..

Rio Tinto contain evidence of a variety of microorganisms (Gónzalez-Toril et al., 2003), and abundant lipids (Fernández-Remolar et al., 2005) and microcapillary electrophoresis analyses of subcritical water extracts from Rio Tinto-01 was observed to have a variety of amino acids present at part-per-million (ppm) concentrations [Stockton et al. 2010]. Given the high abundances of amino acids and lipids present in the sample and the lack of detection of the 3-FV internal standard, we believe that the MTBSTFA reagent probably reacted with the iron and aluminium rich hydrated sulfate minerals during the extraction experiment in preference to the organics known to be present.

The Murchison meteorite contains a wide variety of extraterrestrial organic matter, including both amino acids and carboxylic acids at great than ppm concentrations that are readily extracted and detected using standard GCMS analysis protocols (Cronin and Chang, 1993). However, the low intensity of 3-FV and the lack of any other identifiable derivatized amino acids or carboxylic acids in Murchison (Fig 10) suggest that the MTBSTFA reaction with these compounds is being inhibited by the mineral matrix or possibly non-volatile macromolecular organic matter. We believe that the most likely source of interference is the hydrated magnesium silicates (e.g. lizardite) in the Murchison sample that act to deactivate the MTBSTFA reagent. The other peaks in the chromatogram were detected in Quartz blank and are polysiloxanes from the GC column bleed and unidentified products resulting MTBSTFA reacting with the hydrocarbon trap.

The most promising results obtained in this study were from Carbonate-01. GCMS analysis of this sample after extraction revealed a strong 3-FV peak indicating that MTBSTFA derivatization occurred (Fig. 9). Although we were unable to identify any carboxylic acids in the sample, we were able to identify the MTBSTFA derivatives of two common protein amino acids, leucine and proline based on our own database and the NIST library. Since these amino acids were not detected in any of the procedural blanks, these amino acids were extracted from the stromatolite sample itself and are likely derived from biological organic matter in the sample. Other common protein amino acids such as glycine and alanine are also likely present in the stromatolite sample, however these amino acids are more volatile and are not easily detected under these conditions since they elute with the MTBSTFA and DMF solvents. At least two other peaks corresponding to derivatized compounds (though not in our data base neither in the NIST library) were detected in the sample and not the blank. Unlike many of the other analogue samples tested in this study, Carbonate-01 is a nearly pure dolomite and lacks hydrated minerals and iron oxides that are present in the other samples. Therefore, for carbonate rich samples, our results suggest that MTBSTFA will not be inhibited by the mineral matrix and will readily react with free amino and carboxylic acids present in the sample.

## 4. Conclusion

Here we report on the first analyses of a suite of Mars analogue samples using the MTBSTFA extraction protocol being developed for the SAM instrument under "flight-like" experimental conditions. The results obtained from this study showed that the extraction and detection of both amino acids and carboxylic acids using a SAM-like instrument protocol is possible for some analogue materials. The most promising result we obtained was the GCMS detection of two derivatized protein amino acids, leucine and proline, that were extracted from the carbonate-rich stromatolite sample using the "one-pot" MTBSTFA derivatization procedure and preconcentration using a SAM-like hydrocarbon trap. Carbonate minerals, such as those recently detected on Mars by the Phoenix lander and the Mars Reconnaissance Orbiter may represent the best targets for MTBTSFA extraction of organic compounds using the SAM instrument.

In contrast to the carbonate stromatolite sample, we found that reactions of the MTBSTFA derivatization solvent with the mineral matrix during the one-pot derivatization extraction was highly problematic, and based on the extraction efficiency of the 3-FV internal standard, the extraction of amino acids and carboxylic acids from the analogue samples was clearly inhibited. For some samples, no indigenous amino and carboxylic acids could be identified by GCMS after the samples were extracted in MTBSTFA and DMF at 300°C for several minutes, even though these samples are known to contain high concentrations of soluble organic compounds. We believe that the most probable inhibitor to the MTBSTFA derivatization reaction is the presence of hydrated minerals and/or iron oxides in the analogue samples. Additional derivatization testing on pure minerals will be required to further understand these effects.

The influence of the mineral matrix and chemical composition on derivatization, especially the presence of hydrated minerals and oxides in martian samples, will likely be a major constraint in the ability for SAM to detect amino and carboxylic acids using MTBSTFA. Additional testing will be required to understand if the derivatization extraction efficiency can be improved using a multi-step procedure where the sample is first heated to high temperatures to extract and concentrate organic compounds on the hydrocarbon trap, followed by exposure of the hydrocarbon trap directly to MTBSTFA in a second step. Although a large fraction of the amino and carboxylic acids originally present in the sample could be destroyed during the first heating step, this approach would avoid direct exposure of the rock sample with MTBSTFA. SAM also contains two cups with a second derivatization agent, tetramethylammonium hydroxide (TMAH). Thermochemolysis using TMAH is much more resistant to the presence of water and would be a good alternative for the extraction of organic compounds in samples containing abundant hydrated minerals. The results from this martian analogue study provide an important framework for the sample selection and organic compound detection strategy for the SAM derivatization experiment on the 2011 Mars Science Laboratory (MSL) mission as well as ESA's current ROSETTA mission scheduled to arrive at comet 67P/Churyumov-Gerasimenko mission in 2014 which includes a similar derivatization experiment as part of the Cometary Sampling and Composition experiment (COSAC).

## 6. Tables and Figures

Table 1: Description of the samples analyzed in this study including the organic compounds identified and bulk rock compositions.

| Sample | Description | Internal Standards Detected (percent recovery) | Organics Detected (concentration) | Mineralogy Deduced (wt %) |
|---|---|---|---|---|
| Quartz-01 | Procedural blank, fused silica (FS 120) | 3-FV (30%), Pyrene (10%) | None | 100% Quartz ($SiO_2$) |
| Atacama-01 | Atacama Desert surface soil sample, Chile (0-1 cm depth) | 3-FV (24%), Pyrene (11%) | Hexadecanoic acid (0.05 nmol/g) octadecanoic acid (0.04 nmol/g), several unidentified compounds | 37.0% Quartz ($SiO_2$)<br>4.1%Calcite ($CaCO_3$)<br>27.2% Albite ($NaAlSi_3O_8$)<br>24.5% Anorthite ($CaAl_2Si_2O_8$)<br>4.7% Hematite ($Fe_2O_3$)<br>2.5% Kaolinite ($Al_2Si_2O_5(OH)_4$) |
| Atacama-02 | Atacama Desert subsurface soil sample, Chile (10 cm depth) | 3-FV (2%), Pyrene (9%) | None | 21.0% Quartz ($SiO_2$)<br>29.7% Calcite ($CaCO_3$)<br>30.3% Albite ($NaAlSi_3O_8$)<br>18.5% Palygorskite clay ((Mg, $Al)_2Si_4O_{10}(OH)\cdot 4H_2O$)<br>0.5% Hematite ($Fe_2O_3$ |
| JSC Mars-1 | Mars regolith simulant, Mauna Kea, Hawaii, USA | Pyrene (10%) | None | 62.1% Anorthite ($CaAl_2Si_2O_8$)<br>Augite 27.3% ($(Ca,Mg,Fe)_2(Si,Al)_2O_6$)<br>8.8% Forstetite ($MgSiO_4$)<br>1.0% Chromite ($FeCr_2O_4$)<br>0.9% Ilmenite ($FeTiO_3$) |
| Rio Tinto-01 | Rio Tinto | Pyrene (8%) | None | 36.2% Jarosite ($KFe_3(SO_4)_2(OH)_6$)<br>11.6% Copiapite ($Fe_5[OH(SO_4)_3]_2\cdot 20(H_2O)$)<br>40.1% Alunogen ($Al_2(SO_4)_3\cdot 17(H_2O)$)<br>12.0% Amarantite ($FeSO_4OH\cdot 3(H_2O)$) |
| Carbonate-01 | Carbonate rich stromatolite from Svalbard | 3-FV (27%), Pyrene (10%) | Leucine (0.02 nmol/g), proline (0.01 nmol/g), several unidentified compounds | 99.0% Dolomite ($CaMg(CO_3)_2$)<br>1.0% Quartz ($SiO_2$) |
| USNM 6650.2 | Murchison Meteorite | 3-FV (1%), Pyrene (8%) | None | 43.3% Forstetite ($MgSiO_4$)<br>19.2% Lizardite ($Mg_3Si_2O_5(OH_4)$)<br>9.5% Augite ((Ca, Mg, Fe)2(Si, $Al)_2O_6$)<br>7.5% Diopside ($Ca,MgSiO_6$)<br>5.7% Enstatite ($MgSiO_3$)<br>3.5% Chromite ($FeCr_2O_4$)<br>4.1% Calcite ($CaCO_3$)<br>4.9% Tochilinite ($Fe_{5-6}(Mg,Fe)_5(OH)_{10}S_6$) |

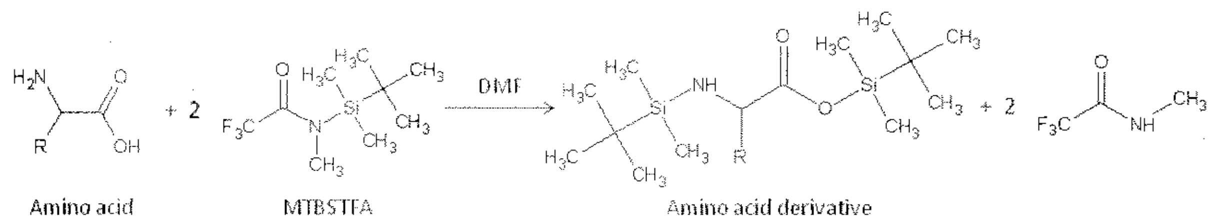


**Figure 1**: Scheme of the derivatization reaction between a generic amino acid and MTBSTFA. A similar reaction will occur for carboxylic acids. DMF is used as the solvent.

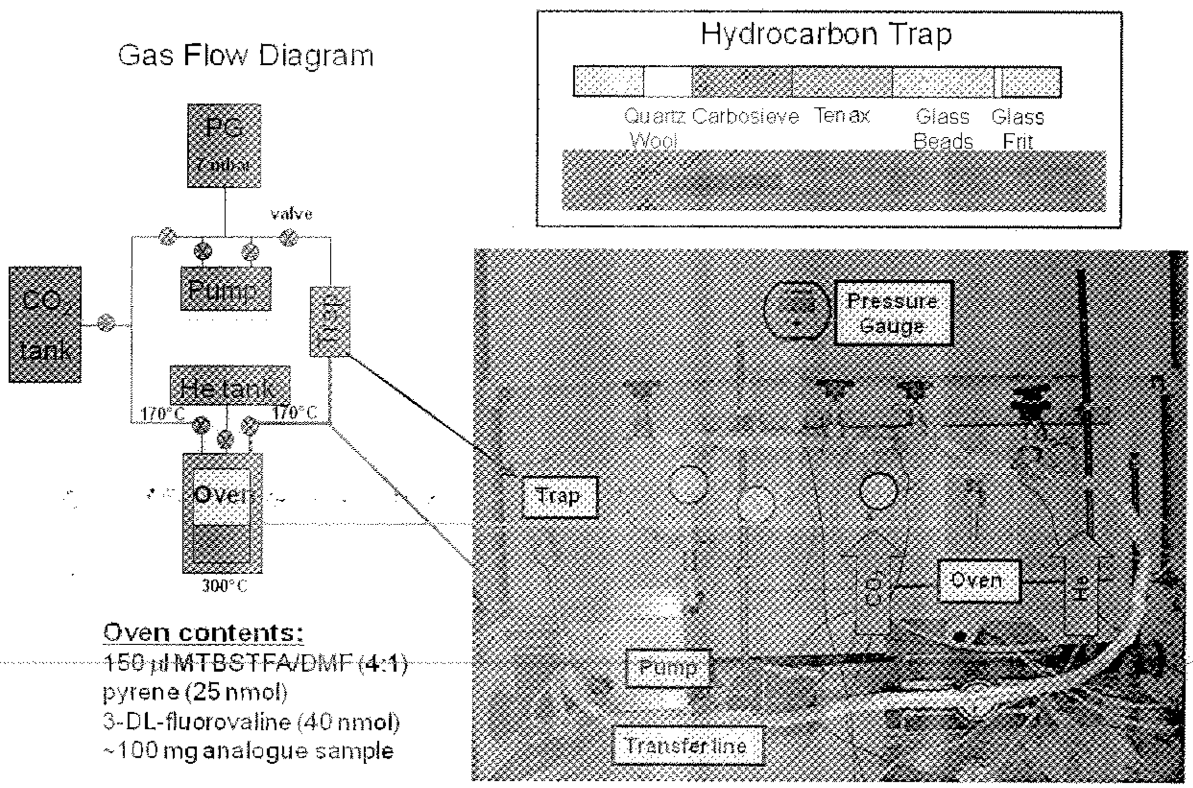


**Figure 2:** Flow diagram and photo of the experimental setup used in this study to extract organic compounds from Mars analogue materials and concentrate the MTBSTFA derivatization products on a SAM-like hydrocarbon trap for GCMS analyses (commercial pyrolysis GCMS instrument not shown).

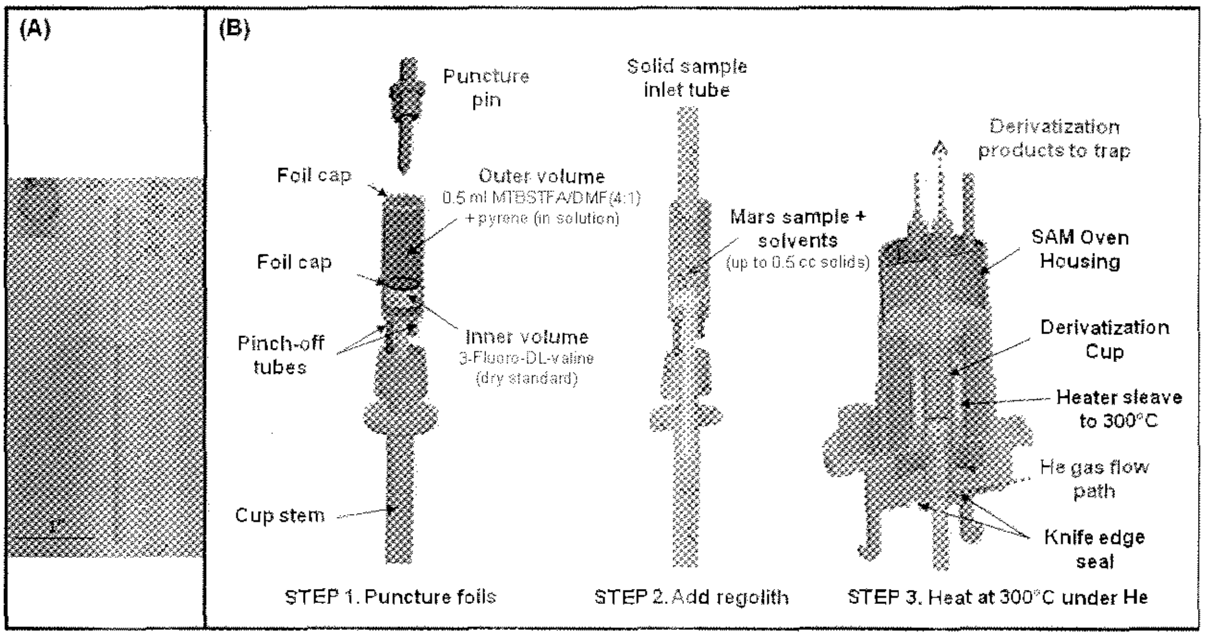


**Figure 3:** Photo of one of the SAM derivatization solvent filled cups (A) and a diagram of the metal derivatization cup and pyrolysis oven illustrating the interior contents of the cup and the extraction process for the SAM derivatization experiment on Mars (B).

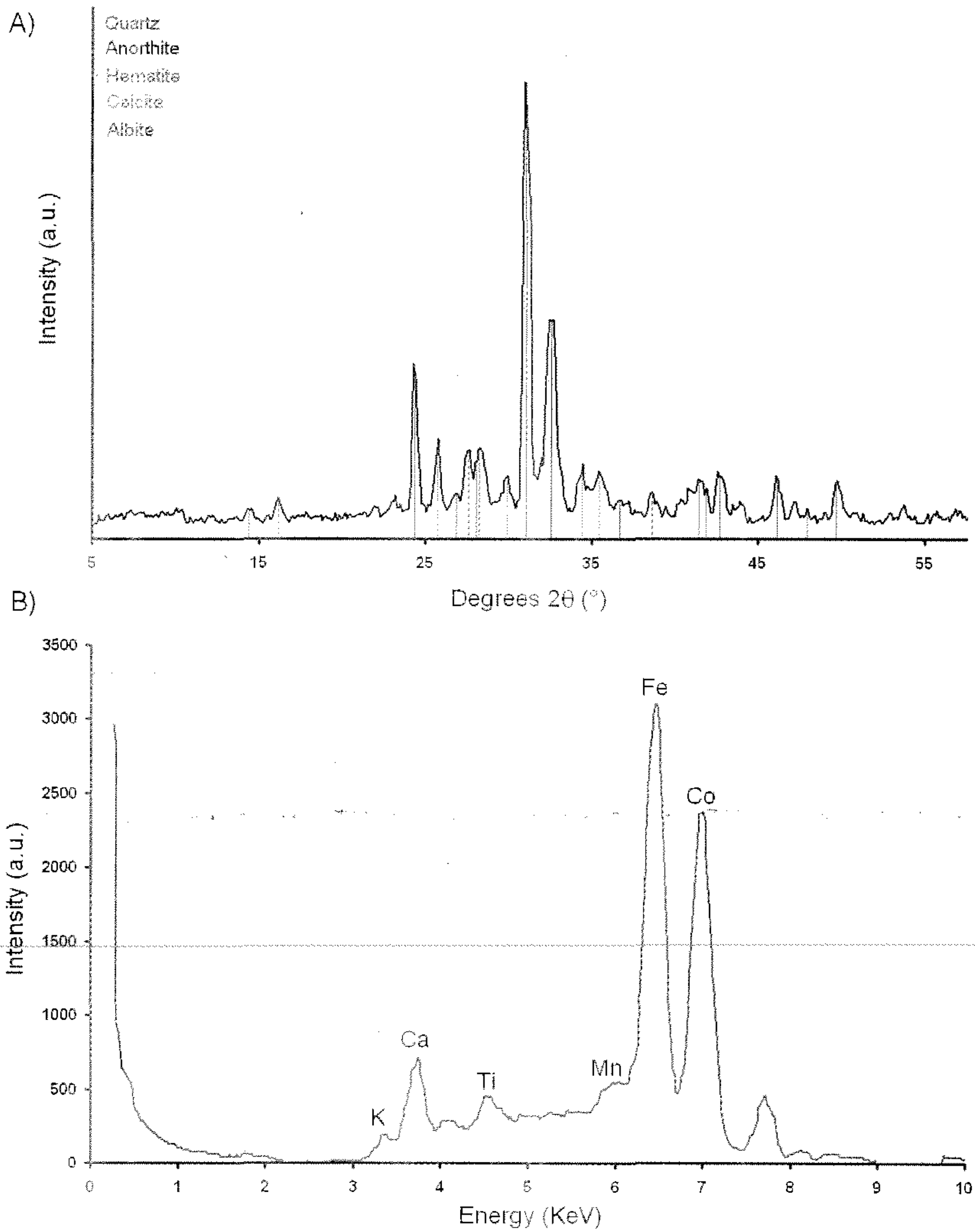


**Figure 4:** (A) XRD pattern, with quantitative analysis by Rietveld refinement, for the Atacama Desert surface soil sample (Atacama-01). Colored markers at the bottom of the plot show the

positions of the peaks for each mineral. (B) Elemental composition determined from the X-ray fluorescence spectrum obtained by summing a total 100 frames of the X-ray photons detected by the CCD. A brief discussion of the patterns and the quantitative mineral abundances for each sample is provided in the text.

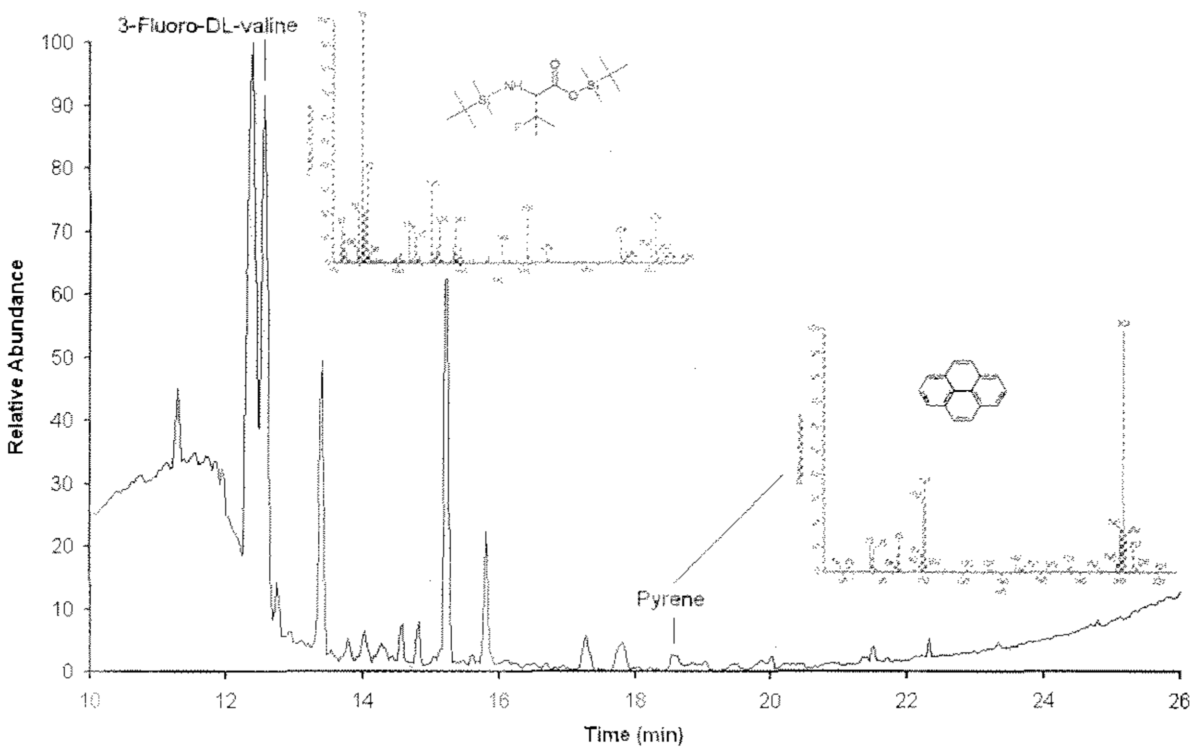


**Figure** 5: GCMS analysis of the hydrocarbon trap after derivatization of the fused silica procedural blank (Quartz-01) with 150 μl of MTBSTFA-DMF at 300°C for 3 minutes. The others peaks are also present in the blank (MTBSTFA/DMF only, no 3-FV) and are polysiloxanes or reaction products from the hydrocarbon trap. Top and right: mass spectrum of 3-FV derivative and pyrene extracted from sample.

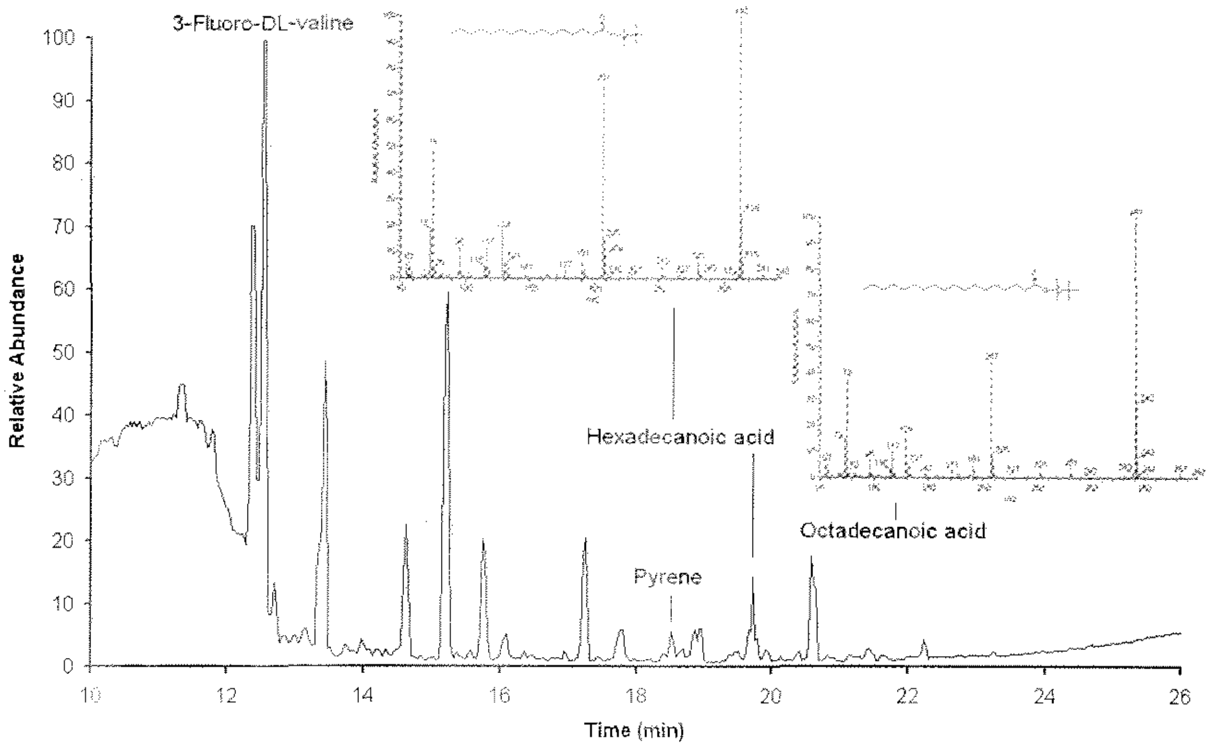


**Figure 6**: GCMS chromatograms of the hydrocarbon trap after one-pot extraction and derivatization of the Atacama Desert surface (Atacama-01) soil at 300°C. Top and right: mass fragmentation pattern of octadecanoic acid and hexadecanoic acid derivatives extracted from sample. Fragmentation patterns for MTBSTFA/DMF, 3-FV and pyrene not shown.

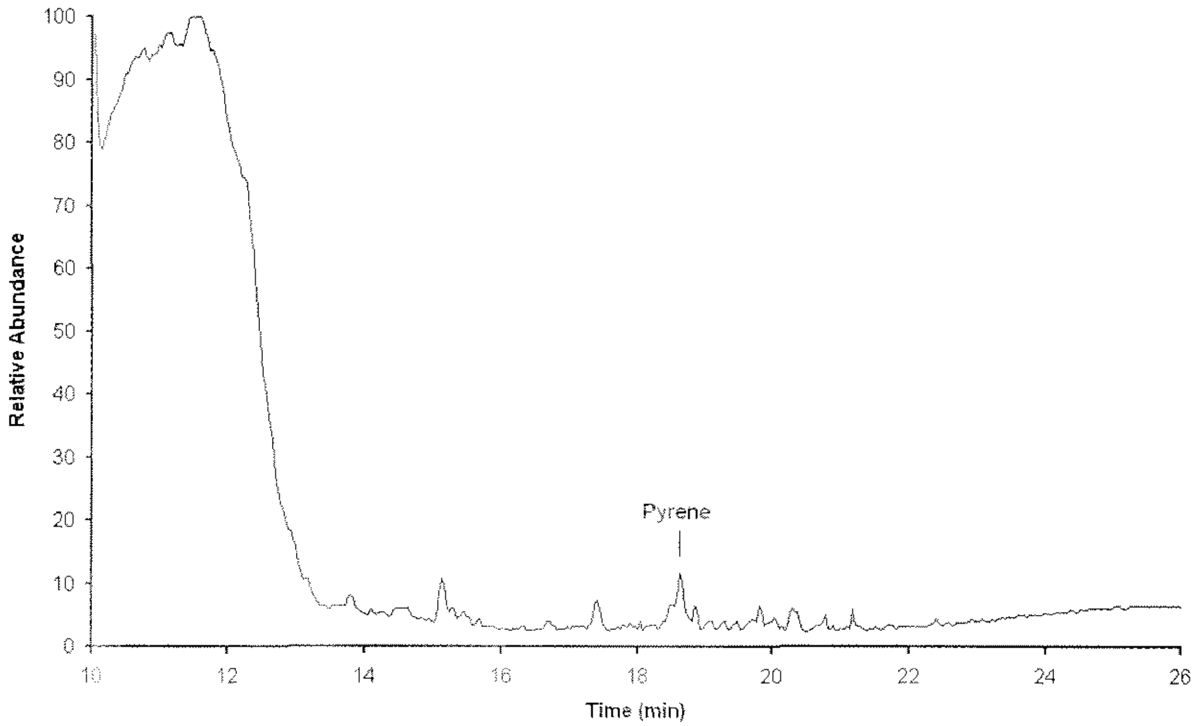


**Figure** 7: GCMS analyses of the hydrocarbon trap after extraction and derivatization of JSC Mars 1 at 300°C. The only compound that could be identified by mass fragmentation pattern in these samples was pyrene. Mass fragmentation pattern data for pyrene not shown.

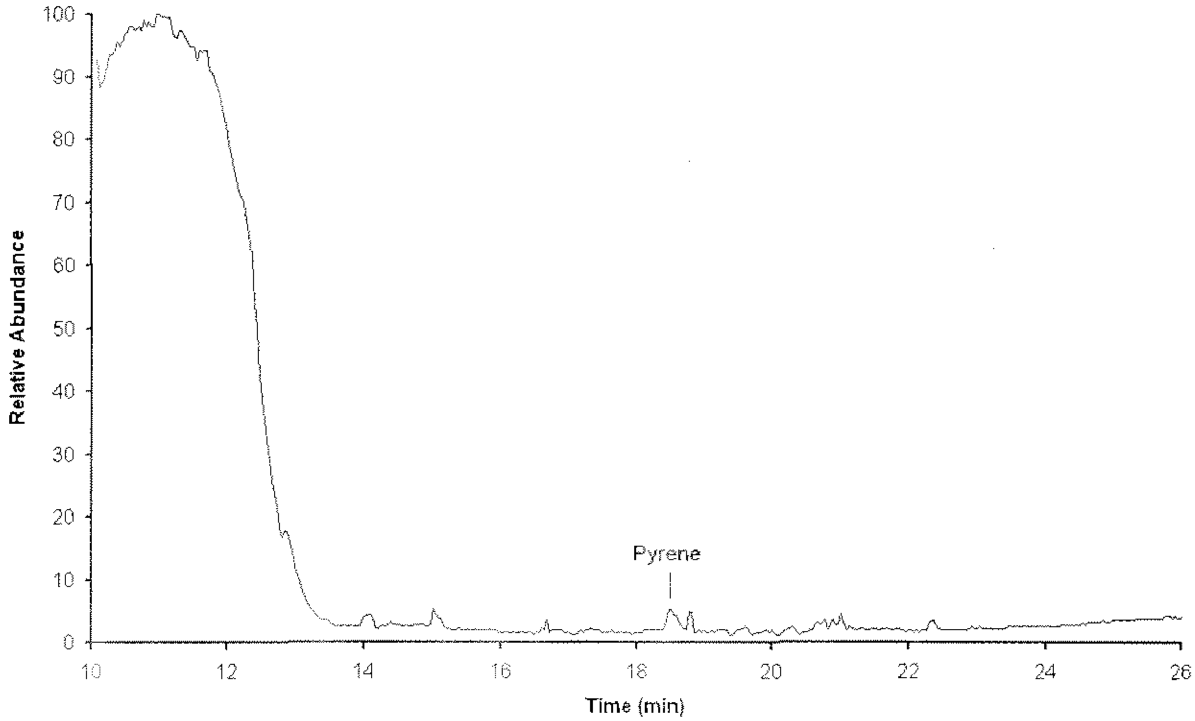


**Figure 8**: GCMS analyses of the hydrocarbon trap after extraction and derivatization of the Rio Tinto (Rio Tinto-01) headwaters sample at 300°C. The only compound that could be identified by mass fragmentation pattern in these samples was pyrene. Mass fragmentation pattern data for pyrene not shown.

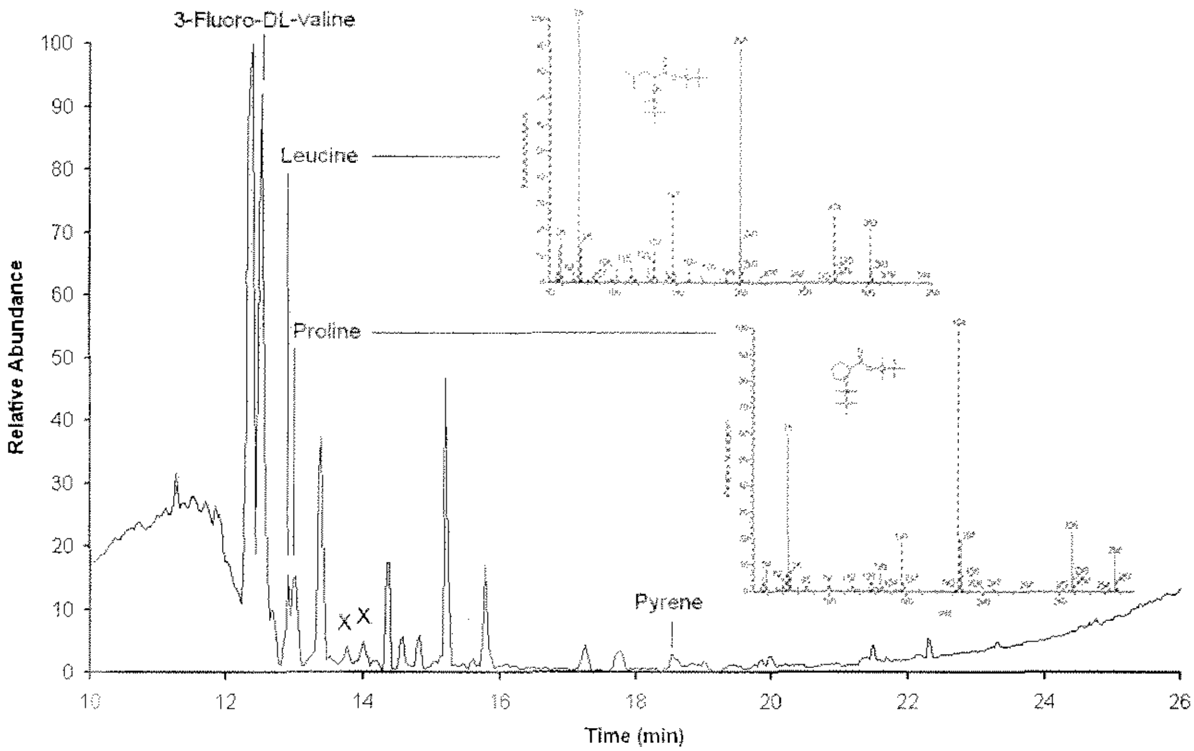


**Figure 9**: GCMS analysis of the hydrocarbon trap after MTBSTFA extraction and derivatization of the carbonate-rich stromatolite (Carbonate-01) sample at 300°C. Right: mass fragmentation pattern of corresponding to the MTBSTFA derivatives of leucine and proline. Mass fragmentation pattern of pyrene and 3-FV not shown. The peaks with a "X" mark are unidentified compounds not present in the procedural blank.

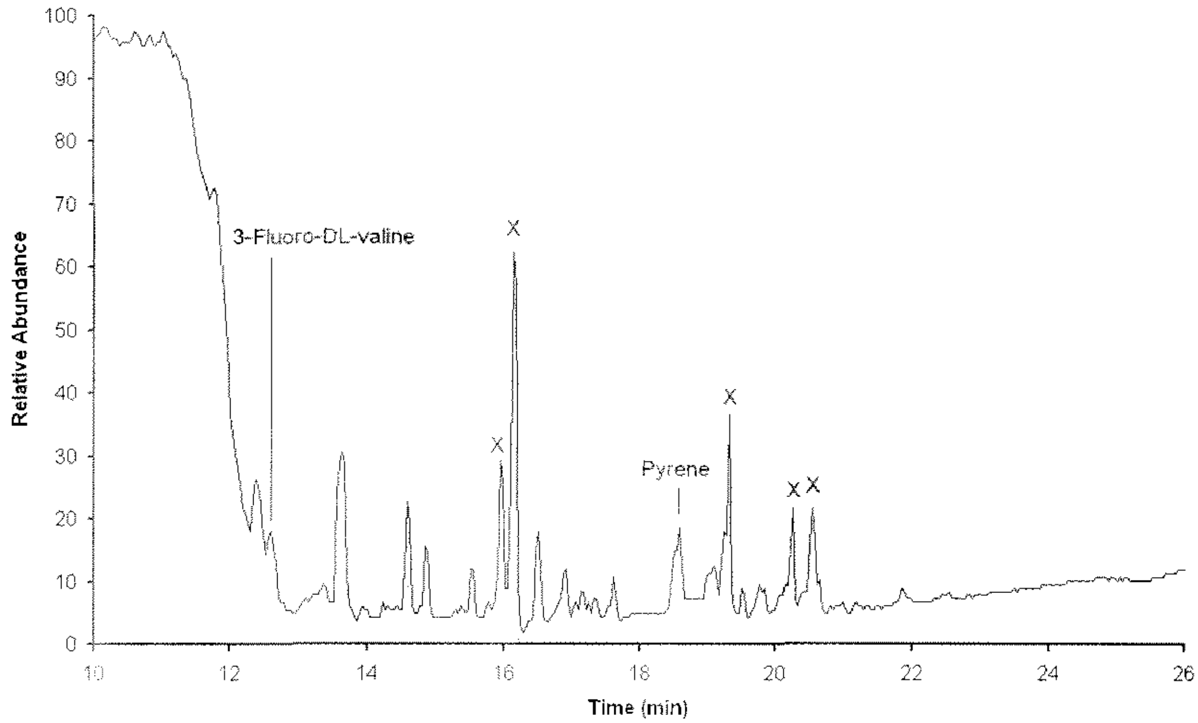


**Figure 9**: GCMS analyses of the hydrocarbon trap after extraction and derivatization of the Murchison meteorite (USNM 6650.2) at 300°C. Mass fragmentation pattern of pyrene and 3-FV not shown. The peaks with a "X" mark are unidentified compounds not present in the procedural blank.

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