EXTRACTION OF ORGANIC MOLECULES FROM TERRESTRIAL MATERIAL: QUANTITATIVE YIELDS FROM HEAT AND WATER EXTRACTIONS. L.W. Beegle, W. A. Abbey, A. T. Tsapin., D. Dragoi, and I. Kanik, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr. Pasadena, Ca 91109, Luther.Beegle@jpl.nasa.gov.

Introduction: In the robotic search for life on Mars, different proposed missions will analyze the chemical and biological signatures of life using different platforms. The analysis of samples via analytical instrumentation on the surface of Mars has thus far only been attempted by the two Viking missions. Robotic arms scooped relogith material into a pyrolysis oven attached to a GC/MS. No trace of organic material was found on any of the two different samples at either of the two different landing sites [1]. This null result puts an upper limit on the amount of organics, although the level of detection for each individual molecular species is still debated [2].

Determining the absolute limit of detection for each analytical instrument is essential so that null results can be understood. This includes studying the trade off when using pyrolysis verses liquid solvent extraction to release organic material in terms of extraction efficiencies and the complexity of sample extraction process.

Extraction of organics can be molecules from field samples preformed by a variety of methods. This include utilizing 6N HCl which is common in extraction of organics from meteorites [3,4,5] but that is probably infeasible for robotic exploration due to difficulty of storage and transport. Extraction utilizing H2O is feasible, but could be much less efficient then 6N HCl so that no organics might be detected. If organics exist in very low levels, then removing them in a 1 to 100 level they might never be detected. Methods such as supercritical fluid and Soxhelt extraction, while preformed under laboratory conditions, require bulky hardware and require complex steps which might make them infeasible for inclusion on rover spacecraft.

We will report on the efficiencies of extraction for amino acids for different terrestrial samples. The samples we have begun to study were initially created in aqueous environments and are sedimentary in nature. These were chosen as a starting point because of recent discovery of possible sedimentary river delta near Holden Creator [6] They represent one of the absolute best cases scenarios for finding organic molecules on the Martian surface.

Rock Samples: Four samples we have studied in this work includes a sample of tufa obtained at Mono Lake which possesses two discernable regions. One of the regions is a crusted formation that most likely consists of dead organisms, while the other region does not have this crusted formation. The third sample is a limestone (chalk) sample acquired from Ward's Scientific (#47 E 4663) which was obtained in Oktibbeha County, Mississippi. The fourth sample was collected as part of an expedition in the Mojave Desert.

Each of the samples was crushed using a pestle and mortar and the crushed sample was analyzed. No effort was made to make the sample size uniform, which should be analogous to what happens aboard a space craft such as MSL. We estimated the average size of the particles to be <250 microns, with some chips as large as 1 mm.

Sample 1 is the non-crustal part of the tufa sample obtained at Mono Lake in California, USA while sample 2 is the crustal part of this tufa. Tufa is a travertine (calcium carbonate) deposit formed by precipitation when calcium-rich fresh water flows into carbonate-rich lake water. This process is both chemical and biochemical and frequently occurs in association with algae colonies like those at Mono Lake (i.e. the crust in sample 2).

Sample 3 is a limestone sample acquired from Ward's Scientific (#47 E 4663) which was obtained in Oktibbeha County, Mississippi, USA. This particular variety of limestone, chalk, forms by the accumulation of fine scale organic debris in a marine sedimentary environment. The debris is composed predominantly of microscopic shells and skeletons of sea animals.

Sample 4 was obtained from the Mojave Desert in California, USA. Tremolite is a metamorphic mineral that occurs in both contact and regionally metamorphosed rocks. It commonly occurs when impure dolomites, in association with quartz or silica, experience low grade, thermal metamorphism. Dolomite occurs in marine sedimentary environments as a secondary mineral, formed from ordinary limestone by replacement of Ca with Mg.

Each of these four samples represent a particular time evolution of minerals formed originally in aqueous environments under the influence of biochemical processes. Organics exist naturally created by both biotic and abiotic processes, however amino acids have a chirality that can only be created through biotic processes, and hence makes them a potentially powerful biomarker. Extracting the amino acids while preserving these biomarkers would be the goal of any instrument package.

Heat extraction: For heat extraction of organics from the aforementioned samples we used a small volume 2.1 cm^3 Knudsen cell (KC) which 100 mg of samples were placed. The cell was then heated to 100°C,

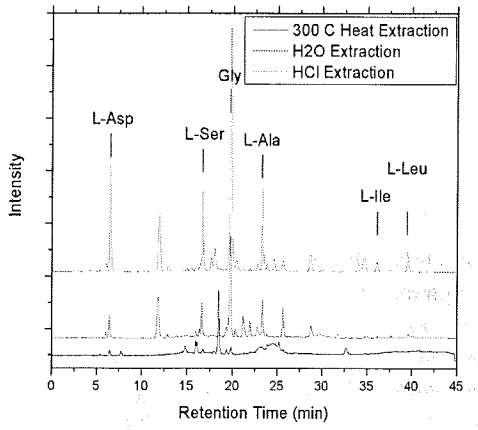

Fig 1- Heat (*Bottom*), H2O (*middle*), and HCl (*Top*) extraction of Tufa (without crust) found at Mono Lake Ca. ***NOTE:*** The Intensity of H2O, and HCl was reduced by 10.

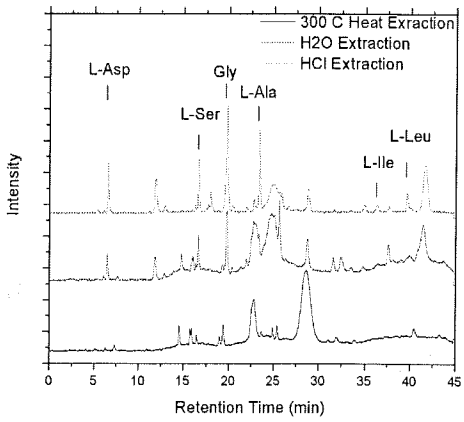

Fig 2- Heat (*Bottom*), H2O (*middle*), and HCl (*Top*) extraction of the Tremolite mineral found in the Mojave Desert. The intensities of each sample were the same.

200°C, and 300°C and kept there for 1 hour. Al foil was placed above the KC and held at room temperature which froze the sample on its surface. The samples were then dissolved in H2O and introduced into the HPLC.

Liquid Extraction: Liquid extractions of amino acids and other organics were done for all four samples using both 6N HCl and ultra high purity H2O. 100 mg (dry weight) of each sample was placed in a sterilized screw cap glass tube (baked at 500°C overnight) and treated with 1 mL of 6N HCl. A duplicate set was treated with 1 mL of ultra pure H2O (deionized and filter sterilized).

HPLC Analysis: In order to remove any potential instrument bias from the analysis of samples, all samples were analyzed on the same HPLC, with the same settings applied. Amino acid analyses were carried out by high pressure liquid chromatography (HPLC) separation of fluorescent diastereomeric derivatives [4,7]. The derivatives separated and identified by reverse-phase HPLC using a C_{18} column (Phenomenex) with 50 mM sodium acetate and methanol as the solvent system. Eluting amino acid derivatives were detected using a fluorescence detector, with $ë_{ex}$ = 340 nm and $ë_{em}$ = 450 nm. Aspartic acid standards were used to calibrate retention times and detector response.

Conclusions: Figure 1 shows the comparison between heat (bottom) H2O (middle) and HCl (top) with the latter 2 reduced in intensity by an order of magnitude. As was expected, the HCl and H2O extraction returned far more peaks in the HPLC spectra which was expected. In addition the number of potentially identifiable peaks also increased, which would result in a more complete understanding of the molecular make-up of the samples studied.

The metamorphic mineral found in the Mojave desert (Fig. 2) while, showing the same increase in number of species present, does not have the same increase in intensity of the peak intensities. Several amino acids, Aspartic acid (Asp), Glycine (gly) etc., have substantially increased peak intensities with the solvent extraction over heat extraction. For many species, including Serine (ser) and Isoleucine (Ile). There is no detectable trace of these molecules in the heat only sample. This could be a direct result either from not-sublimating the molecules, or due to de-carboxilication of the species, which is a known result of heating amino acids [8].

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