# The ExCALiBR Instrument for Lipid-Based Life Detection on Mars

M.B. Wilhelm[1], A.J. Ricco[1,2], D.K. Buckner[3,4,1], T. Boone[5,1], M. Chin[1], J.L. Eigenbrode[6], L. Jahnke[1], R. Williams[7,6], M. J. Anderson[5,1], T. Hoac[5,1], A. Rademacher[5,1], T. Chinn[1], A. Southard[8,6], X. Li[7,6], S. Wisnosky[9,5], A.J. Williams[3];
[1]NASA Ames Research Center, Moffett Field, CA 94035 (marybeth.wilhelm@nasa.gov), [2]Stanford University, [3]University of Florida, [4]Blue Marble Space Institute of Science, [5]Axient Corp., [6]NASA Goddard Space Flight Center, [7]University of Maryland, [8]Universities Space Research Association, [9]University of Miami

**Introduction:** Lipids are key molecular targets to search for evidence of life, as they can reveal information about life processes and their chemical origins (Georgiou and Deamer, 2014). Essential for terrestrial life and likely required for putative extraterrestrial organisms, lipids make up the membranes that separate cell contents from an external environment. They are well preserved in the geologic record, some recording the activity of organisms that lived over a billion years ago (Eigenbrode, 2008), an order of magnitude longer than other types of molecular biosignatures. Lipids are also synthesized abiotically, making up ~60% of soluble organics detected in carbonaceous chondrite meteorites (Sephton, 2005). However, there are numerous challenges to the successful detection of relevant lipids *in situ*, including interference by inorganics, molecular complexity of the lipids, limitations on their solubility, and low lipid abundance.

**Lipid Extraction Techniques:** Laboratory lipid characterization techniques are well established and used successfully in terrestrial laboratories for ~70 years (e.g., Bligh and Dyer, 1959). The efficacy of these techniques at overcoming the abovementioned challenges and the breadth of published data from a diverse range of sample ages and mineralogies make them attractive for spaceflight applications. Our literature review of lipid extraction techniques (*see AbSciCon 2022 abstracts from Buckner et al., and Wisnosky et al., for more information*) reveal solvent-based techniques are most commonly used to extract lipids from geologic samples, leveraged in ~83% (1308/1574) of samples reviewed and varying by solvent choice, apparatus, temperature, pressure, ultrasonic energy.

**The ExCALiBR Breadboard:** Our team of organic geochemists and chemical, mechanical, and electrical engineers with expertise in spaceflight microfluidics has adapted traditional wet chemistry techniques for soils to a TRL-4 breadboard called ExCALiBR (Extractor for Chemical Analysis of Lipid Biomarkers in Regolith). ExCALiBR (**Fig. 1**) will enable lipid detection with coupled mass spectrometry by (1) conserving origin-diagnostic lipid structures and patterns by maintaining them in the liquid phase, using the organic solvents required for optimal lipid extraction, and delivering them to an analytical system unadulterated; (2) reducing signal interference by extracting lipids from mineral matrices and filtering out minerals, which are known to interfere with sample analysis (Sephton et al., 2014; Royle et al., 2018); (3) increasing the signal by concentrating lipids by > 1000x to ensure detection even in organic-lean samples; and (4) maintaining a sample flow path with contamination below analytical instrument LoD.

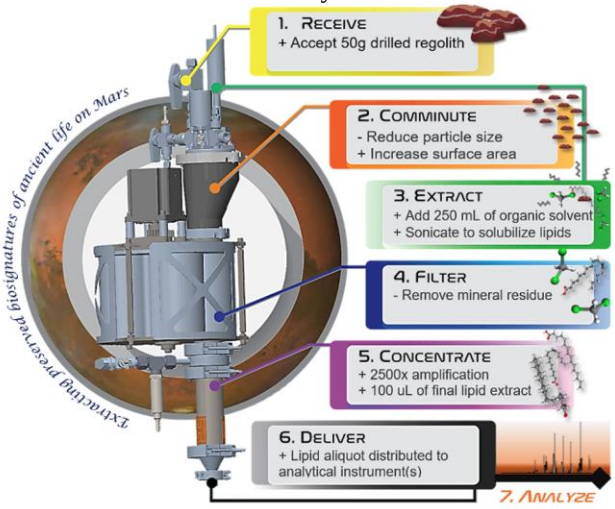


**Figure 1.** *ExCALiBR integrates four sample processing capabilities: comminution, extraction via organic solvents and sonication, filtration of mineral residue, and concentration of lipid extract to 100 microliters. ExCALiBR-generated lipid extracts can be analyzed by numerous analytical systems (e.g., GC-MS, LD-MS, Raman).*

The TRL-4 ExCALiBR breadboard (**Fig. 2**) is being validated with Mars analog samples varying in age and mineralogy as well as abiotic carbonaceous chondrite meteorites.

**Figure 2.** *The integrated TRL-4 ExCALiBR breadboard.*

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