Verification of Electron Beam Irradiation as a Lipid Decontamination Method for Life Detection Instrumentation

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Abstract

Lipids are key targets for current, proposed, and future life detection missions to both rocky and icy worlds. With the high accuracy and low limit of detection (LoD) required by many new and future instruments (sub ˜ppb range), decontamination of life detection hardware, particularly components that handle or process planetary samples in situ, is necessary to prevent false positives. Lipids are a biosignature of interest as they are ubiquitous to all life as we know it, can survive in the geologic record for an order of magnitude longer than any other biomolecule (i.e. billions of years), and are able to form through both biotic and abiotic processes. Traditional NASA contamination control (CC) for life detection instruments primarily focuses on hardware fabrication in sterile cleanroom environments, killing of microbes, and flushing/mechanically removing contaminants off instrument and spacecraft components. However, recent studies suggest that standard cleaning methods are unlikely to sufficiently remove lipid contaminants to meet instrument LoD. These include NASA-approved methods for Planetary Protection (PP) (e.g., dry heat microbial reduction and vapor phase H2O2) and cleanroom decontamination methods (e.g., protective clothing, HEPA air filtration, surface cleaning with detergents/water/isopropyl alcohol, autoclave, ethylene oxide treatment). Effective laboratory standard decontamination methods (ashing at 550° C, chlorinated solvent flushes) are efficient at eliminating lipid contamination, but are often incompatible with sensitive materials required in the construction of life detection instrument components. We will present the results of a study to determine the efficiency of lipid decontamination using a "nontraditional" CC method, Electron Beam Irradiation (EBI), that would be compatible with common major instrument materials used in life detection instrumentation. Percent lipid reduction following EBI application will be compared to percent reduction following treatment with traditional NASA PP methods, using standards from four classes of lipid molecules that are proposed targets of life detection instrumentation.

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OVERVIEW

We studied **Electron Beam Irradiation (EBI)** as a potential decontamination technique for destroying molecular lipid contaminants from the **Extractor for Chemical Analysis of Lipid Biomarkers in Regolith (ExCALiBR)**, our novel life detection instrument. We found EBI was unable to significantly degrade lipids at doses tolerable by instrument materials and should not be implemented for lipid CC. However, resistance to degradation suggests that lipids are an ideal biomarker, and further research is needed to determine longevity in planetary environments experiencing high electron fluxes. (Fig 1,2)

Figure 1: ExCALiBR prototype: our novel, non-aqueous lipid extractor for life detection missions

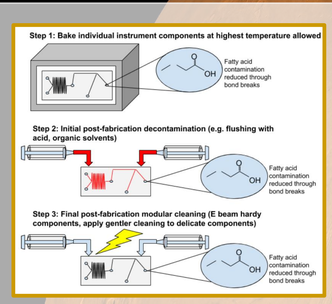

Figure 2: Hypothesized CC plan for ExCALiBR, with EBI as a final, whole-instrument cleaning step

METHODS

1. Selected standards from representative classes of lipid biomarkers, including:
   - **C16:0 saturated fatty acid** (*1-Pentadecanecarboxylic acid*)
   - **C18:1 monounsaturated fatty acid** (*cis-9-Octadecenoic acid*)
   - **C21 saturated alkane** (*Heneicosane*)
   - **C27 saturated tetracyclictriterpene** (*5-α-Cholestane*)
   - **C27 stanol** (*5α-Cholestan-3ϐ-ol*)
2. Prepared samples for irradiation by dissolving lipids in CH_2Cl_2, partitioning aliquots into glass vials, and drying lipids down under pure N_2 (Fig 3)
3. Irradiated lipids at Steri-Tek™ expert sterilization services (Fig 4) under a DualBeam™ processor (*10 MeV, 20 KW linear accelerator*) at doses:
   - **5 kGy, 10 kGy, 25 kGy, 50 kGy, 100 kGy**
4. Prepared samples for analysis by re-dissolving CH_2Cl_2, adding an internal standard, and derivatizing as needed
5. Analyzed irradiated lipids via Gas Chromatography-Mass Spectrometry (*GC-MS*) (Fig 5-9)
   - Quantified percent reduction (Tables 1-5)
   - Identified radiolytic products

LIPID BIOMARKERS

- Required for all life as we know it (*primarily for comprising membranes that protect cells from water*)
- Survive in the terrestrial geologic record for orders of magnitude longer than any other biomarker (*~gyrs*)
- Can form through biotic or abiotic processes
- Found on Earth, in meteorites, and likely on Mars and the moon
- Display origin-diagnostic features [1,2,3,4,5]

RESULTS

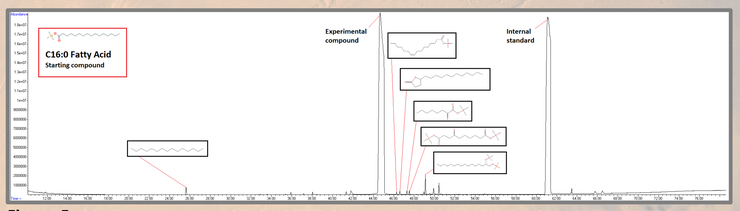

Figure 5: GC-MS chromatogram of C16:0 Fatty Acid standard following 100 kGy of irradiation; tallest peaks are experimental compound and internal standard by abundance, compounds in boxes are radiolytic products

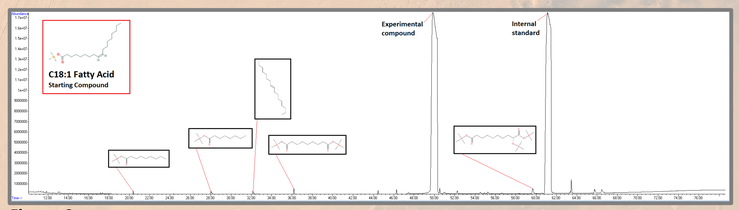

Figure 6: GC-MS chromatogram of C18:1 Fatty Acid standard following 100 kGy of irradiation; tallest peaks are experimental compound and internal standard by abundance, compounds in boxes are radiolytic products

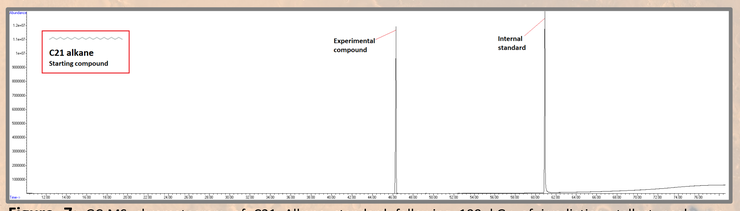

Figure 7: GC-MS chromatogram of C21 Alkane standard following 100 kGy of irradiation; tallest peaks are experimental compound and internal standard by abundance, no significant radiolytic products were resolved

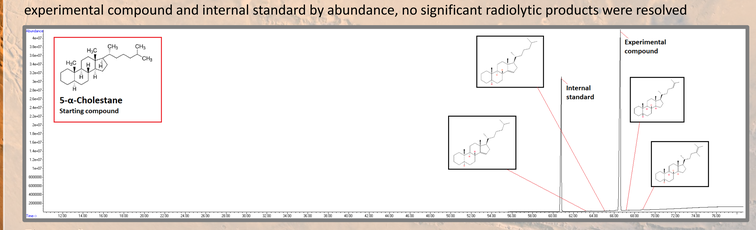

Figure 8: GC-MS chromatogram of C27 5-α-Cholestane standard following 100 kGy of irradiation; tallest peaks are experimental compound and internal standard by abundance, compounds in boxes are radiolytic products

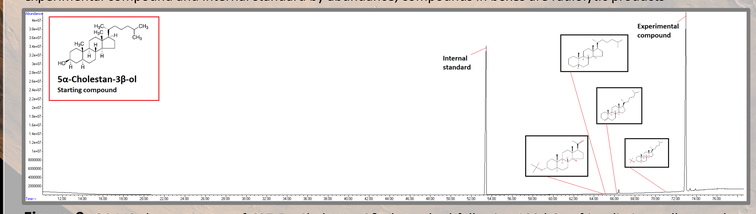

Figure 9: GC-MS chromatogram of C27 5α-Cholestan-3β-ol standard following 100 kGy of irradiation; tallest peaks are experimental compound and internal standard by abundance, compounds in boxes are radiolytic products

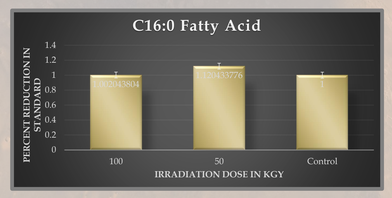

Table 1: Percent reduction in C16:0 Fatty Acid by EBI dose absorbed; no significant degradation observed

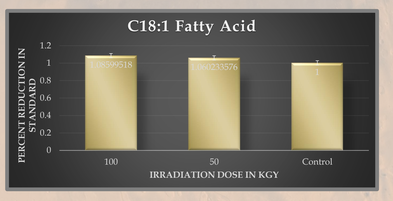

Table 2: Percent reduction in C18:1 Fatty Acid by EBI dose absorbed; no significant degradation observed

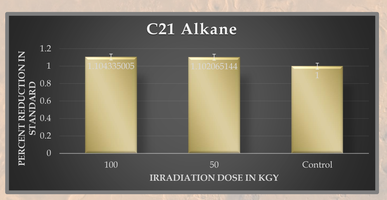

Table 3: Percent reduction in C21 Alkane by EBI dose absorbed; no significant degradation observed

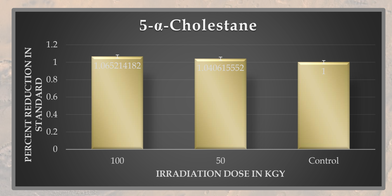

Table 4: Percent reduction in Cholestane by EBI dose absorbed; no significant degradation observed

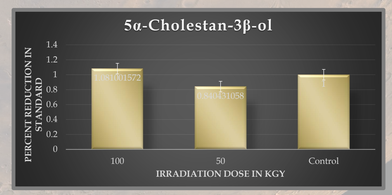

Table 5: Percent reduction in Cholestanol by EBI dose absorbed; no significant degradation observed

DISCUSSION

- No significant lipid degradation observed at doses tested (5 kGy, 10 kGy, 25 kGy, 50 kGY, 100 kGy)
- Small quantities of radiolytic products observed
- Large diversity of radiolytic products observed
- Polycyclic compounds more resistant to breakdown than aliphatics
- Recombination (*particularly in fatty acid compounds*) observed
- Dicarboxylic acids observed following irradiation of monocarboxylic acids (*similar to those found in carbonaceous meteorites, specimens that have experienced high levels of irradiation throughout the solar system's lifetime*)

Figure 3: Steri-Tek EBI facility

Figure 4: Samples pre-irradiation

FUTURE WORK

- Find and verify a contamination control method that will destroy lipids without harming the materials used to construct ExCALiBR
- Further explore lipid longevity under irradiation in simulated Mars, Europa, and Enceladus conditions

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