Derivatization and Detection of FAMEs in Ancient North American Soapstone Artifacts

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Introduction

The presence of fatty acids in ancient pottery can yield important context clues. Since different fatty acids exist in plants and animals, their detection and identification adds information about a societies' dietary habits to include hunting, farming and animal domestication (Evershed et al.,2002). A three step process is being used to identify fatty acids and background matrix in soapstone archeological artifacts retrieved from the Thrash site, a Late Archaic period (3,000-1000B.P,) site in Pike Country, Alabama. Soapstone is a term widely applied to rocks containing talc -$Mg_3Si_4O_{10}(OH)_2$ and other minerals, the properties varying widely (Chidester et al.,1964). The detection of FAMEs (fatty acid methyl esters) can determine the presence of fatty acids left behind by organic matter in the artifacts. Once it is confirmed which fatty acids are in the artifacts,the fatty acids can be correlated to a range of plants and animals that came into an extended period of contact with the artifact.

Methods

Using a method developed by Spade and Russ, the derivation process converts the fatty acids to the FAMEs, making it easier to detect on the GC/MS (gas chromatography/ mass spectroscopy), and identify which original fatty acids were in the artifact.

Overall Method of the Entire Study:

1. The method first started with creating a FAMEs standard, so as to compare the derivation results, and determine what is in the artifact.
2. The next step is to analyze the interior of the quarry soapstone to show the difference of the soapstone in the results and the actual artifacts. At the same time the shards from the pre-ceramic soapstone bowl are analyzed to confirm the method used is effective and can be accurately used on the soapstone pipes.

Experimental Method:

The method, by Spade and Russ, used on the bowl was applied to the original soapstone as well, to keep everything consistent (a control was also processed along with the original soapstone and bowl).

1. The process therefore starts with 0.1 g of analyte with a $C_{9:0}$ fatty acid added as the internal standard.
2. Then three solvents are added in order, starting with 2.0 mL of methanolic HCl, 2.0mL of anhydrous MeOH, and 2.0mL of Hexane.
3. The samples were then sealed and boiling in a water bath of 1.0 hour. Following this, an additional 2.0mL of Hexane was added, and the Hexane layer was removed.
4. The removed layer was filtered through a sodium sulfate column, to then be blown down with N_2 gas. The part of the process, from the additional hexane layer to the N_2 gas, was repeated two more times.
5. However when the solution was blown down for a third time, they are not blown down all the way, only until 0.3mL of solution is remaining.
6. Finally the remaining solution is transferred and analyzed on the GC/MS.

Therefore that is the current point in this project, until the results from the original soapstone and the bowl can be officially obtained, the soapstone pipes will have to wait.

Figure 1: Fatty Acid to Fatty Acid Methyl Ester Process, R and R' are methyl groups

Figure 2: The original soapstone taken from the quarry nearby the sites where the soapstone bowl was found

Figure 3: The artifact from the soapstone bowl that was analyzed in comparison to the original soapstone.

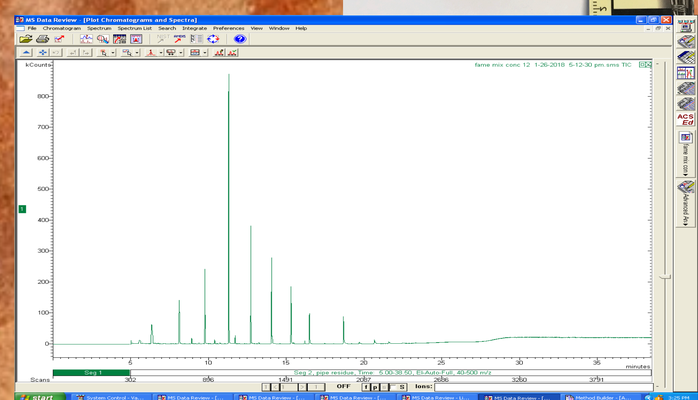

Figure 4: Chromatogram of the FAME standards mixture.
Peaks in order from left to right:
1. Hexanoic FAME, 2. Heptanoic FAME, 3. Octanoic FAME, 4. Nonanoic FAME, 5. Decanoic FAME, 6. Undecanoic FAME, 7. Dodecanoic FAME, 8. Tridecanoic FAME, 9. Pentadecanoic FAME, 10. Hexadecanoic FAME, 11. Heptadecanoic FAME, 12. Octodecanoic FAME, 13. Nonadecanoic FAME

Results

A point should be made that the study is not nearly done and has many more hours required before concrete results come in. So far what can be said is that the derivation of the fatty acids to fatty acid methyl esters (FAME's) without the soapstone involved worked. However due to contamination of the GC/MS along with carryover from one soapstone sample into the next has led to unclear results. By the end of this semester the most likely source of the contamination and the carryover problem has been identified to the instruments' sensitivity.

Conclusion

This study is still in the beginning stages, which reveals the reality of research, that success takes time and rarely happens overnight. What has been discovered during this semester is that GC/MS can be a source of contamination and carryover. Since the issue has been dealt with, the next step of the experiment is to re-analyze the interior of the original soapstone from the quarry and compare it to a re-analysis of the interior of the first sample of the soapstone bowl. Then the remaining interior soapstone bowl samples will be analyzed. The exterior of the bowl samples will be derivatized as well to determine what originated from handling of the artifacts and what originated from the historic use of the bowl. Once the bowl samples are done, then samples from the interior of the soapstone pipes will be extracted and compared to the exterior of the pipes. For the bowl and pipe samples, to have a more accurate understanding of what is detected, standards will be made of the suspect items such as food for the bowl and plant material for the pipes. By the conclusion of this study, it will hopefully reveal not only the eating habits of those from 3,000 years ago, but the smoking habits of those from over a 1,000 years ago.

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