Dart-Ms Analysis of Historic Tobacco Pipes to Investigate the Preservation of Nicotine Residues

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Recommended Citation
Torres, Sylvia A. () "Dart-Ms Analysis of Historic Tobacco Pipes to Investigate the Preservation of Nicotine Residues," McNair Scholars Research Journal: Vol. 7: Iss. 1, Article 11.
Available at: http://commons.emich.edu/mcnair/vol7/iss1/11

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DART-MS ANALYSIS OF HISTORIC TOBACCO PIPES TO INVESTIGATE THE PRESERVATION OF NICOTINE RESIDUES

Sylvia A. Torres
Dr. Ruth Ann Armitage, Mentor

ABSTRACT

Direct analysis in real time (DART) mass spectrometry has potential for rapid characterization of residues in and on archaeological materials, often without the need for extraction and sample preparation. These residues may provide insight into people’s behavior in the ancient past, including what they ate and drank and what plants they used. Residues change over time through oxidation, loss of water-soluble components during washing, and contamination from burial and handling. We are investigating how nicotine residues in historic smoking pipes from Historic St. Mary’s City, Maryland, have changed through time. To understand how residues change as they age and decompose, we are studying tobacco pipes that have been excavated and not cleaned, as well as pipes that have been cleaned and processed by the Historic St. Mary City museum. Nicotine and several of its oxidation products are readily observed in replica residues analyzed with DART-MS, even after the ceramic surfaces have been cleaned.

INTRODUCTION

Archaeology is the study of the human past through the artifacts left behind. Usually, these artifacts are hard, durable materials like ceramics and stone tools. In the past 20 or so years, researchers have been interested in the residues adhering to those durable artifacts and how the molecules that make up those residues can inform us about past human behavior. Chemical analysis of residues provides information about what foods people included in their diets, how they processed those foods, and even what medicines and dyes they might have used.1, 2
While archaeological materials are evidence from the past, forensic evidence tells the story of a modern-day crime. One technique that has revolutionized both archaeological and forensic sciences is DNA amplification using the polymerase chain reaction (PCR). PCR has fundamentally changed the way in which forensic evidence is treated. In archaeological sciences, PCR has been used to amplify DNA in a number of different materials, including rock paintings, with questionable success. DNA analysis was also undertaken to study artifacts from the Boston Saloon in Virginia City, Nevada. Remarkably, the user of a clay tobacco pipe stem found at the site was shown to have been female, leading to significant questions about the population of that site.

Many analytical methods can be applied to identifying archaeological residues, most of which include some kind of separation step. Separation is best done with chromatography, in the gas or liquid phase, and often in conjunction with mass spectrometry in order to identify the separated molecules. Gas chromatography-mass spectroscopy (GC-MS) and liquid chromatography-mass spectroscopy (LC-MS) are the most widely applied in archaeological residues. This report focuses on methods used for identification of nicotine or tobacco residues in archaeological samples. Both GC-MS and LC-MS were successfully used to investigate Mayan vessels from the Late Classical period for traces of nicotine.

Rafferty has published several reports on the analysis of smoking pipe residues, primarily using GC-MS with positive results. Tushingham also used GC-MS, with a rigorous approach to investigate tobacco pipe residues. While GC-MS has been shown to be a reliable method for identifying nicotine and its derivatives on archaeological materials, it requires the samples to be extracted and often derivatized, which is both time-consuming and often destructive to the artifacts. Rafferty et al. unsuccessfully attempted to use Raman microscopy to nondestructively study pipe residues, due to Raman’s lack of sensitivity and interferences from other substances. Nondestructive or even noninvasive methods for identifying residues are generally preferred, but few have as yet been proven effective.
Direct analysis in real time (DART) ionization for mass spectrometric analysis was first reported in 2005 and was developed in 2005 as a means of identifying small molecules on surfaces without sample preparation. DART can be used to determine the chemical composition of a sample that is directly introduced to the ion source in less than a minute. DART-MS has been widely adopted in forensic science laboratories because of its ability to produce instantaneous results. In a recent study, DART-MS was used in a sexual assault case to identify non-DNA trace evidence (e.g. lubricant residue, trace fibers).

DART-MS has great potential in applications related to archaeological and other cultural heritage materials. Selvius DeRoo and Armitage reported using DART with high resolution time-of-flight to identify organic dye compounds in cotton textiles without sample preparation. Further work from that group on dyed cotton fibers from a late 19th century treatise showed that DART-MS could be used to identify several different organic colorants. Dyes in historic wool textiles from European tapestries were also readily identified using DART-MS. Of relevance to this project, DART-MS was also used in the identifying food biomarkers on archaeological ceramics that had been exposed to burial and then cleaned.

All analytical methods, particularly those used in forensic science, must produce results that are reliable, consistent, and without systematic errors or bias. Validation is the way that analytical chemists prove a method works as intended and provides results with known accuracy, precision and limits of detection. In the case of identifying archaeological residues on ceramics, the selected analytical technique must be able to identify the molecules of interest on ceramics that are old, contaminated by burial, and often cleaned upon excavation. While it is difficult at best to obtain standard ancient materials that have aged or been processed in known ways, it is possible to make mock-up samples that simulate ancient materials and can then be used to validate a method. With validation, an analytical method lends credibility to the measurements and eliminates the need for repeated analyses with new, untried methodologies. Tushingham’s work made progress on validating the GC-MS analysis of tobacco residues in archaeological samples.
The intent of this study was to use DART-MS to identify nicotine in 17th-century ceramic smoking pipes excavated from St. Mary’s City, Maryland. The samples selected for study were all old and had likely undergone oxidation, had been handled and contaminated in the museum, and had been cleaned with water, possibly removing any water soluble components. The method was validated using mock-up samples of tobacco residue on ceramics that were prepared in the laboratory and subjected to similar conditions. This work will help establish the credibility of using DART-MS for identifying archaeological residues.

**MATERIALS AND METHODS**

**Mock-up Samples**

The mock-up samples were prepared by crumbling dried tobacco leaves on a terra cotta ceramic dish and combusting the leaves in a fume hood. The resulting ash was rubbed onto the ceramic to simulate the residue formed from the compacted burned tobacco in a ceramic pipe. The ceramic dish was broken into three pieces to represent the three different samples that were to be examined. One piece of the ceramic dish was scrubbed with water and a toothbrush to simulate standard field cleaning procedure. The piece was dried briefly in a warm oven at $<100^\circ F$. The second piece was buried in sieved compost soil and stored in a warm oven, to simulate burial conditions. The third piece was left untreated.

In the search for the least destructive and most accurate technique, five different methods were compared. Three of these methods are summarized in Table 1.

<table>
<thead>
<tr>
<th>Name</th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tool utilized</td>
<td>Rotary tool</td>
<td>Cotton Swab</td>
<td>Silicon carbide disk</td>
</tr>
<tr>
<td>Description of sample</td>
<td>Ceramic powder, mixed with methanol</td>
<td>Residue on swab with methanol</td>
<td>Ceramic powder on SiC disk with methanol</td>
</tr>
</tbody>
</table>

Table 1. Summary of methods performed on mock-up samples.
For Method 1, a small rotary tool was used to gently grind off a portion of the surface of each of the mock-up samples for analysis. The sherd powder was mixed with a drop of methanol to make a clay paste that was directly introduced with the closed end of a capillary tube to the DART ion source.

In Method 2, each mock-up sample was swabbed with a cotton swab wetted with methanol. The cotton swab was then introduced to the instrument. The cotton swab method yielded poor results, thus a third method was implemented.

For Method 3, a silicon carbide disk (Pike EasiDiff abrasion disk) was folded in half and lightly abraded against the surface of each sample. The disk was then wetted with methanol after sampling. The silicon carbide disk with methanol was directly introduced to the DART ion source.

Archaeological Samples

Three 17th-century pipe bowls were obtained from Historic St. Mary’s City, Maryland. Samples were selected by Silas Hurry, curator of the museum at Historic St. Mary’s City, using a “double blind” approach; the researchers were not informed about the criteria for sample selection. First, to keep place static, all the pipes were recovered from the same site in St. Mary’s City, 18 ST1-13, the Leonard Calvert site. Second, to keep material static, the samples were all marked specimens by the same, known pipemaker. The “EB” mark represents Edward Bird, an English pipemaker who immigrated to Holland by 1630 and produced large quantities of pipes for the export market. All of the specimens were used, broken, and discarded. However, the three pipes represented different amounts of processing and curation, as described in Table 2.

Two additional methods were developed for the archaeological samples; these are summarized in Table 3. Method 4 involved using a sterile scalpel blade to remove a small portion of the inside of the pipe bowl. The sherd powder was introduced to the DART ion source using a capillary tube moistened with methanol. Method 5 utilized a solvent extraction using ethyl acetate (ultra-residue analysis grade), based on the procedure
Table 2. Archaeological pipe samples.

<table>
<thead>
<tr>
<th>Artifact identification number</th>
<th>Excavation date</th>
<th>Processing</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>18ST1-13-1759K/AB</td>
<td>2011</td>
<td>Washed in water with a toothbrush and air dried</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>18ST1-13-1759K/AA</td>
<td>2011</td>
<td>Soil removed from bowl dry manually, no further washing or processing</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>18ST1-13-1283/AF</td>
<td>Early 1980s</td>
<td>Washed and air dried, labeled and cataloged, curated since recovery</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
</tbody>
</table>

described by Tushingham (ref). The pipes were placed in separate clean beakers, covered with ethyl acetate, and sonicated for 60 minutes. The resulting solution was transferred to labeled test tubes and placed under a fume hood to evaporate the solvent to dryness. The resulting residue was reconstituted with 100
microliters of clean ethyl acetate. This solution was introduced to the DART ion source using the closed end of a capillary tube.

<table>
<thead>
<tr>
<th>Name</th>
<th>Method 4</th>
<th>Method 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tool utilized</td>
<td>Sterile scalpel</td>
<td>Solvent extraction</td>
</tr>
<tr>
<td>Description of sample</td>
<td>Ceramic powder with methanol</td>
<td>Reconstituted dried extraction solvent</td>
</tr>
</tbody>
</table>

Table 3. Summary of methods performed on archaeological samples.

Sample Analysis

The samples were analyzed using a DART ion source (IonSense, Saugus, MA) with a JEOL AccuTOF mass spectrometer (JEOL USA, Peabody, MA). All spectra were collected in positive ionization mode. The samples were directly exposed to the DART gas stream of ionized helium gas at 350 °C. Orifice 1 was set to 30 V to minimize fragmentation of the ions produced at 120 °C. Orifice 2 and the ring lens voltage were both held at 5 V. The DART grid voltage was kept at the default value of +240 V. The mass spectrometer RF ion guide (“peaks voltage”) was set to 1500 V to maximize sensitivity in the mass range of interest. Calibration was done during each acquisition using PEG-600 in methanol. The mass resolving power was approximately 6000 over this region.

RESULTS AND DISCUSSION

Nicotine and its oxidation products, as well as three other tobacco-related compounds are shown in Table 4. These compounds were observed in various concentrations on the ceramic samples, always as MH⁺ ions due to the addition of a proton in positive ion DART.

Mock-up samples

Method 1, using the rotary tool, was the most destructive technique to the mock-up samples, but traces of nicotine and its derivatives were readily identified. Figure 1. shows the spectrum obtained from applying Method 1 to the untreated mock-up
sample. While there are clear signals for nicotine and two of its derivatives, the significant damage incurred to the mock-up led us to use Method 2, which was less destructive.

<table>
<thead>
<tr>
<th>Name and formula</th>
<th>Exact molecular mass, Da</th>
<th>MH(^+) ion mass, Da</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine, C(<em>{10})H(</em>{14})N(_2)</td>
<td>162.116</td>
<td>163.124</td>
<td><img src="image" alt="Structure of Nicotine" /></td>
</tr>
<tr>
<td>Nicotine-1-n-oxide, C(<em>{10})H(</em>{14})N(_2)O</td>
<td>178.111</td>
<td>179.118</td>
<td><img src="image" alt="Structure of Nicotine-1-n-oxide" /></td>
</tr>
<tr>
<td>Nicotine-1,10-di-n-oxide, C(<em>{10})H(</em>{14})N(_2)O(_2)</td>
<td>194.106</td>
<td>195.113</td>
<td><img src="image" alt="Structure of Nicotine-1,10-di-n-oxide" /></td>
</tr>
<tr>
<td>Cotinine, C(<em>{10})H(</em>{12})N(_2)O</td>
<td>176.095</td>
<td>177.103</td>
<td><img src="image" alt="Structure of Cotinine" /></td>
</tr>
<tr>
<td>Myosmine, C(<em>9)H(</em>{10})N(_2)</td>
<td>146.084</td>
<td>147.092</td>
<td><img src="image" alt="Structure of Myosmine" /></td>
</tr>
<tr>
<td>Anatabine, C(<em>{10})H(</em>{12})N(_2)</td>
<td>160.100</td>
<td>161.108</td>
<td><img src="image" alt="Structure of Anatabine" /></td>
</tr>
</tbody>
</table>

*Table 4. Compounds of interest on smoking pipes.*

Method 2, with the cotton swab, yielded results that were not reproducible. Figure 2. shows two different swabs from the untreated mock-up sample. In the first run (2A), nicotine and a few derivatives were readily identified, but in the second run (2B) no trace of nicotine was observed. This could be due to thermal breakdown of the cellulose making up the cotton swab; we believe the numerous ions produced from the cotton swamped out the
nicotine signal in this case. The high-resolution mass spectrometer is particularly important, as demonstrated by these spectra. In Figure 2B, a peak is present at m/z 163.076. However, this does not correspond to nicotine but is likely due to contamination from phthalates, ubiquitous plasticizers that are often observed. The peak at m/z 149.058 is also common to phthalates and supports this conclusion.

Figure 2A. Mass spectra for the same mock-up sample with Method 2. Note that nicotine is not identified in 2B.
Of the different sampling techniques, Method 3, utilizing the abrasive disks wetted with methanol, seemed to produce the most promising results. The abrasive disks were easy to use and relatively nondestructive to the samples. The addition of methanol improved the signal, probably by increasing the amount of nicotine released from the sample where it was ionized. Nicotine and its derivatives were quickly identified on the mock-up samples using Method 3, as shown in Figure 3. In Figure 3A., the spectrum shows nicotine and several derivatives using Method 3 on the untreated burned tobacco mock-up. Figure 4B. shows that washing the mock-up with water appears to remove nicotine, which was not detected, though other derivatives were readily identified. In Figure 4C, nicotine was just detectable in the buried mock-up, along with other derivatives. The abrasive disk sampling in Method 3 proved to be easy and minimally destructive, while providing sufficient material for identifying nicotine and its derivatives on the mock-up samples. Method 3 was then implemented on the archaeological samples from St. Mary’s City, Maryland.

Figure 2A. Mass spectra for the same mock-up sample with Method 2. Note that nicotine is not identified in 2B.
Figure 3. Mass spectra of (A) untreated burned tobacco mock-up, (B) scrubbed burned tobacco mock-up, and (C) buried burned tobacco mock-up.
Archaeological samples

When Method 3, using the abrasive disks, was applied to the actual samples, it yielded poor results. The results were not reproducible, and no tobacco residue could be reliably identified on these samples. Method 4, using a sterile scalpel blade, was then implemented to remove some of the interior of the historic pipe bowls. The resulting powder was introduced into the DART-MS source using a capillary tube moistened with a few microliters of methanol. Two of the pipes showed no clear signal at m/z 163.134 for nicotine +H ion, or a peak for any of the other nicotine derivatives. Figures 4. and 5. illustrate this, showing the total ion “chromatogram” (on top, not indicating any separation, but total signal as a function of time) and a selected ion “chromatogram” (SIC, on the bottom) for the protonated nicotine signal at m/z 163.134. The lack of a clear peak above the baseline in the SIC in Figures 4. and 5. shows that no nicotine was detected. For sample 1759K/AA, this could be due to the washing step the pipe underwent after

Figure 4. Total ion chromatogram (top) and selected ion chromatogram for m/z 163.134 (bottom) for pipe sample 1759K/AA.
excavation. For sample 1759K/AB, it is possible that the nicotine is below the limit of detection, and that the soil adhering to the sample is obscuring the nicotine signal. Surprisingly, only sample 1283/AF (Figure 6.), the sample excavated nearly 30

**Figure 5.** Total ion chromatogram (top) and selected ion chromatogram for m/z 163.134 (bottom) for pipe sample 1759K/AB.

**Figure 6.** Total ion chromatogram (top) and selected ion chromatogram for m/z 163.134 (bottom) for pipe sample 1283/AF using the sterile scalpel method.
Figure 7. Mass spectra for the St. Mary’s City pipe samples using solvent extraction: (A), solvent blank; (B) 1283/AF; (C) 1789 K/AA; (D) 1789 K/AB.
years ago that was washed and handled, showed a clear signal for nicotine as the MH$^+$ ion.

In an effort to clarify why the sample that was least expected to show the presence of nicotine did so, we implemented Method 6, a solvent extraction using ethyl acetate. This method was not visually destructive to the archaeological samples, but likely removed all traces of residues from the samples, rendering them useless for further study. While this was not ideal, it was deemed necessary to evaluate DART-MS as a viable method for identifying nicotine residues. Method 6 was carried out as described in the Methods and Materials section. No nicotine was observed in the samples prepared in this manner, although some of the oxidation products of nicotine were. Figure 7 shows the mass spectra for the solvent extraction procedure. Figure 7A shows the mass spectrum for the method blank (an empty beaker processed in the same manner as the pipe bowls), and two possible markers of nicotine were observed. This indicates that the presence of these two compounds is not indicative of a smoking pipe residue and should be discounted in the solvent extract spectra for the archaeological samples. In sample 1759 K/AA (Figure 7B) only nornicotine was observed in the extract. Figure 7C shows that in sample 1759 K/AB three additional markers of tobacco were observed, but nicotine was not. Sample 1283/AF previously showed the presence of nicotine using the scalpel method, but with solvent extraction only a few oxidation products were observed (Figure 7D). Oxidation products that were not observed using the scalpel method were suddenly observed using solvent extraction. Compared to Methods 1-5, solvent extraction proved to be the most reliable for identifying nicotine oxidation products on archaeological samples.

CONCLUSIONS

The archaeological sample that was washed prior to analysis (1759K/AB) showed no evidence of nicotine or its derivatives, except when the sample was extracted with ethyl acetate. One of the pipes (1759K/AA), that was clearly not washed,
showed no evidence of tobacco residue in the initial tests, but a trace of nornicotine was observed in the extract. This nornicotine can be discounted, as it is also present in the method blank. Only the one sample (1283/AF) showed nicotine present in the scalpel-sampled material, though nicotine was not observed in the extract. Further studies are needed with the mock-up samples to explain the loss of nicotine and to clarify why the sampling methods are not reproducible. Although we were unable to validate DART-MS as a reliable method for identifying residues in archaeological materials, this work marks significant progress toward that end. All of the tested methods were relatively nondestructive. The DART-MS results, while not always reproducible, were obtained in just a few minutes of analysis time with limited sample preparation, unlike GC-MS or LC-MS that require lengthy analysis times and extensive sample preparation.

ACKNOWLEDGMENTS

The author gratefully acknowledges Mr. Silas Hurry, Historic St. Mary’s City, for providing archaeological materials and information for this project. This work was supported by NSF MRI-R² award #0959621 and the McNair Scholars Program at Eastern Michigan University. Additional support was provided by the EMU Chemistry Department, the EMU Provost’s Office Faculty Research Fellowship and Research Support Award, and the Sellers Fund.

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