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Direct Analysis of Xanthine Stimulants in Archaeological Vessels by laser desorption REMPI

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Abstract

Resonance enhanced multiphoton ionization spectroscopy (REMPI) generates simultaneous vibronic spectroscopy and fragment free mass spectrometry to identify molecules within a complex matrix. We combined laser desorption with REMPI spectroscopy to study organic residues within pottery sherds from Maya vessels (600-900 CE) and Missisippian Mississippian vessels (1100-1200 CE), successfully detecting three molecular markers, caffeine, theobromine and theophylline, associated with the use of cacao. This analytical approach provides a high molecular specificity, based on both wavelength and mass identification. At the same time, the high detection limit allows for direct laser desorption from sherd scrapings, avoiding the need for extracting organic constituents from the sherd matrix.

1. Introduction

Analysis of organic compounds in pottery sherds traditionally relies on the use of infrared spectroscopy[1], separation techniques such as gas chromatography-mass spectrometry (GC-MS)[2,3], high-performance liquid chromatography (HPLC)[4], and liquid chromatography-mass spectrometry (LC-MS)[2,5-7]. Chromatographic techniques, while providing a wealth of information, often cannot be routinely applied to cultural heritage artifacts, e.g. pottery sherds, due to the relatively large sample-size, and sample consumption, required by most GC and LC techniques.[7] Typically, pieces of several cm^3 in size may be needed to yield 500 mg of material for extraction. Fourier transform infrared (FTIR) spectroscopy of pottery samples can provide functional group identification of organic, as well as some inorganic, compounds, but can be challenging since the organic materials often are present as part of an extremely complex mixture.[1] While these techniques provide important information, they often lack the capability to identify unique compounds, or require hundreds of milligrams of sample to do so. Therefore there remains a need for techniques that require less sample, thus causing less physical damage to the object, while maintaining high molecular selectivity. Here we describe a technique for organic tracer molecule analysis for archaeometry, in a specialized form of laser mass spectrometry. A laser-desorption

jet-cooling source is followed by resonance enhanced multiphoton ionization (REMPI) and time-of-flight (TOF) mass spectrometry. This approach combines the *selectivity* of resonant laser spectroscopy with the *sensitivity* of mass spectrometry and is therefore simultaneously highly specific and sensitive[8-16].

As a member of the theobroma genus, the cacao bean (*Theobroma cacao*) originates from a tree confined to within the tropical regions of South America and Mesoamerica[17]. The seeds, or pods, produced by the tree were ground up and mixed with other ingredients (water, maize, honey) to make a drink that had a mild stimulating effect.[18] It was a particularly important cultural icon in Mesoamerican society, and has been consumed by the Maya as early as 600 BCE[18] in addition to being the precursor to modern day chocolate.

Three molecules associated with the cacao bean are caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine) and these have been found in pottery sherds found in the Mesoamerican region.[2,18-22] These compounds are still very common today in stimulant drinks such as coffee, tea, and yerba mate. Serving as natural pest deterrents, these three methylxanthines are found in over 13 orders of plants, comprising well over 100 different plant species and are often used as molecular markers to identify geo-cultural origins of pottery sherds, particularly cacao.[3,23]

Similar to cacao in MesoAmerican culture, people from regions around the American gulf coast prepared a black tea made from the yaupon holly (*I. vomitoria*) as well as the dahoon holly (*I. cassine*). These species contain caffeine and theobromine, but are not believed to contain theophylline.[3,7,24] Further, yerba mate (*I. paraguariensis*), guarana fruit (*P. cupana*), and the yoco vine (*P. yoco*) is widespread in South America and contains caffeine, theobromine and/or theophylline.[23,25-27] Due to the shared occurrence of caffeine, theobromine, and/or theophylline it is clear that analytical tools need to go beyond positive identification of these molecules within complex matrices and need to consider relative occurrence to identify the organic origin of the residue in question.

Each plant species has a characteristic concentration of each methylxanthine, which is commonly used to narrow down the species of plant serving as the source of the organic residues in question. The method presented here can positively confirm the presence of methylxanthines with a reasonably high analytical detection limit and may pragmatically identify cacoa residue from pottery samples excavated where cacao and holly species are geolocated. The complete attribution of concentration ratios of the different marker molecules to specific plants is complicated because the distribution and concentration of these molecules in different plants is somewhat contentious given all the variable conditions. Moreover, the extraction dynamics can affect the ratios observed.[25] Previous analysis of pottery from Central America has identified theobromine in residue from the inside of ceramic vessels from Honduras, Guatemala, and Belize dating from 1500 BCE to 480 CE.[18,21,2] The identifications were made by GC/MS and HPLC/MS. Recent research has also suggested the presence of all three molecular markers in sherds found in Northwestern New Mexico and the Central Illinois River Valley, suggesting a previously unknown trade network with MesoAmerican cultures.[7,28,29]

In the following sections we will detail the technique by which we identify these molecular markers in pottery sherds, followed by a first example of the identification of methylxanthine markers in organic residues in Maya and Mississippian pottery sherds.

2. Experimental

2.1 Two-step laser mass spectrometry

Figure 1 schematically shows the experimental setup which has been reported in more detail elsewhere[30]. The sample obtained from the pottery sherd can be either an extract deposited onto a sample bar, or sherd scrapings placed on a sample bar from which we laser desorb directly. The sample bar is mounted in a vacuum chamber, directly in front of a pulsed molecular beam controlled by a piezo cantilever valve [31,32] (4 x 10^{-6} Torr source chamber pressure). Laser desorption provides intact vaporization of large, complex and/or thermally labile molecules. The desorbed molecules are entrained in a pulsed supersonic jet expansion of argon, which provides very efficient cooling of the internal degrees of freedom of the molecules to the order of 10-20 ^{o}K.[33,34] At this point, the cold molecules are gaseous and free of any intermolecular, i.e. matrix, interactions. The cooling makes it possible to perform high-resolution unimolecular spectroscopy while at the same time stabilizing the molecule, permitting its detection at the parent molecular mass. Typical desorption laser fluence is on the order of 10 μJ/cm^2 in 10 ns laser pulses[35-40]. The desorption laser is focused using either a cylindrical lens or a spherical lens, generating desorption spot sizes of 0.50 mm x 3.0 mm or 0.75 mm diameter respectively. Following jet-cooling, the molecular beam is skimmed before being intersected by laser beam(s) and photo-ionized. We implement resonance enhanced multiphoton ionization (REMPI) in two modes, using either one or two colors[41]. The subsequent ions are detected by a reflectron time of flight mass spectrometer (2 x 10^{-6} Torr analyzer pressure, mass resolution m/Δm=500).

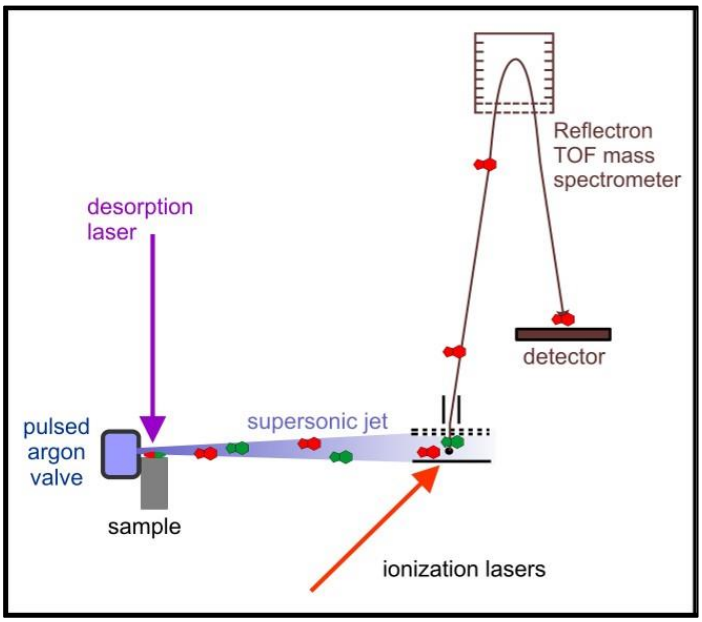

Figure 1: Depiction of the laser desorption jet cooling mass spectrometer where a mixture of isomers (represented by red and green symbols) is laser desorbed in vacuo and entrained in a pulsed Ar molecular beam. Upon entering the ion source tunable REMPI lasers offer spectroscopic selectivity (in this example exciting the "red" isomers) and soft ionization, followed by TOF mass detection.

2.2 REMPI

Resonance enhanced two-photon ionization, combines optical spectroscopy with mass spectrometry. This dramatically enhances the specificity for selected compounds and allows for distinction of structural isomers, tautomers and enantiomers.[42] A tunable laser provides resonant vibronic excitation of the jet-cooled molecules to a low level intermediate electronic state. Subsequently, another photon ionizes the excited molecule either from the same laser pulse (1-color REMPI) or from an overlapped second laser if additional energy is needed (2-color REMPI). In the case of methylxanthine both photons originate from the doubled output of a Lumonics HD-300 tunable dye laser (spectral line width ≈ 0.04 cm^{-1}, pulse energy $\approx$ 0.3-0.7 mJ in 8 ns pulses).

Scanning the wavelength of the first photon while monitoring a specific ion mass generates a mass selected excitation spectrum, or REMPI spectrum. When the REMPI spectrum of a given compound is known, the excitation laser can be tuned to a specific resonance in order to selectively ionize it. Since the sample has been decoupled from matrix interactions in the laser desorption process, REMPI is matrix and concentration independent. This single molecule resonant absorption is a "soft" ionization method with molecular identification based on the wavelength specific signal of the parent ion and no fragmentation pattern disambiguation is necessary. Resonant ionization not only selects for a specific compound; it can also select for specific isomers. We have demonstrated that with this technique we can detect compounds at the femtomol level and in favorable cases down to the 100 attomol level.[9] We can further improve REMPI sensitivity by two color ionization in which the excitation and ionization steps are performed at different wavelengths. Typically, the absorption cross sections for the first and second step are of the order of 10^{-17} cm^2 and 10^{-19} cm^2, respectively. To maintain optimum selectivity it is undesirable to significantly saturate the first step, forcing us to use a laser fluence that is low by two orders of magnitude from what would maximize the second (ionization) step. Therefore, we can improve overall detection limits without sacrificing selectivity if we employ a different wavelength for the second step at higher laser fluence. We have demonstrated this principle for perylene, obtaining an overall 0.25 photoionization efficiency resulting in a 30 femtogram detection limit.[9]

The combination of laser spectroscopy and mass spectrometry provides analytical information in two dimensions: wavelength and mass. Generally spectroscopic resolution, which is typically fractions of wave numbers is several orders of magnitude higher than the mass resolution that can be obtained in conventional mass spectrometry.[42] To fully capitalize on these advantages it is necessary that the spectroscopy of the analyte molecule is known in a predetermined spectral library. We can find a needle in a haystack, provided we know what the needle looks like.

2.3 Detection Limit

We previously reported data for a series of test samples with different concentrations of vanillic acid, a marker for peonidin in grape wine[43]. There the ion signal was linear with concentration ($r = 0.9994$), allowing (a) quantitative measurements when using internal standards and (b) establishment of a lower limit of detection. The latter will differ from compound to compound because it depends on the ionization efficiency. In the case of vanillic acid with one color ionization at 289.192 nm we obtained a detection limit at S/N = 3 of 60 picograms per laser shot. To put this limit in perspective, 250 pg of vanillic acid corresponds to a few microliters of modern wine and indeed we have detected vanillic acid in a 5 μL droplet of wine. Even if only 0.1% of the original peonidin content of the wine can be recovered and converted to vanillic acid, we would still only need a milliliter of residue, from for example an amphora, to be able to detect it. For the

current study of methylated xanthines, a detection limit of 4 picograms per 10 laser shot average was determined. Theophylline ion signal desorbed from graphite substrate at quantities of 0.05, 0.5, 5, and 50 ng detected by 1C REMPI @ 280.71 nm fit a linear regression of the form $\log y = m \log x + b$, where m is slope, x is concentration, b a fitting constant, and y is signal. Extrapolating this fit down to the signal limit, corresponding to the background signal of desorbed blank graphite sample, provided the limit of detection with a S/N of 3. This fit is shown in Figure S.1. If additional detection sensitivity is required 2C REMPI is used. The ionization wavelength of 308 nm was found to maximize ionization efficiency and minimize fragmentation of the analytes, increasing signal by at least a factor of two (shown in S.2). Substrate does have an effect on detection efficiency and we have tested graphite bars, gold plated bars, stainless steel pegs, and double sided tape. Of these, graphite bars provide the greatest detection sensitivity but they suffer from sample carryover due to graphite's ability to readily absorb either organic sample or organic solvated sample. We used new graphite bars to negate the possibility of sample contamination between runs, which we also controlled for by analysis of blanks.

2.4 Sample preparation

We purchased standards of theobromine, theophylline and caffeine from Sigma-Aldrich and used them without further purification. Standards are directly applied to graphite sample bars as a thin solid layer. The spectra for standards were collected using separate graphite bars to ensure each spectrum is free of any other standards.

We analyzed pottery samples directly from the pottery material and from extracts when concentration was necessary. The extracts are made by using a 3:1 mixture of acetone and water. Approximately 400 mg of ground pottery is added to 5 ml of solution and allowed to sit at room temperature for 72 hours. The supernatant liquid is then filtered by a Whatman 13 mm GD/X disposable filter, polypropylene filter media with polypropylene housing, 0.45 mm pore size. The extract is then concentrated by gentle heating (25-35 ˚C) under dry nitrogen flow. The extracts are concentrated approximately 5 fold then deposited drop-wise on the ends of 0.75 mm diameter disposable stainless steel pegs mounted to the sample bar. Gentle heating (35-45 ˚C) is used to speed up evaporation of solvent. The dried, concentrated extracts are then immediately inserted into the instrument for analysis. Direct desorption analysis of the samples is done by applying small amounts (0.5-1.0 mg) of either ground or surface pieces of pottery material to double-sided tape mounted on gold sample bars. A new, clean disposable razor blade was used to scrape sherd fragments directly onto the tape for each sherd tested. Direct desorption does not lend itself to samples in need of concentration, however, it does allow for the most immediate and unaltered analysis of a sample's composition, avoiding possible unintended chemical rearrangements that can occur in an extraction, condensation, or solvated separation step. Direct analysis also makes it possible to separately sample different parts, for example to compare surface versus inside areas.

3.1 Results

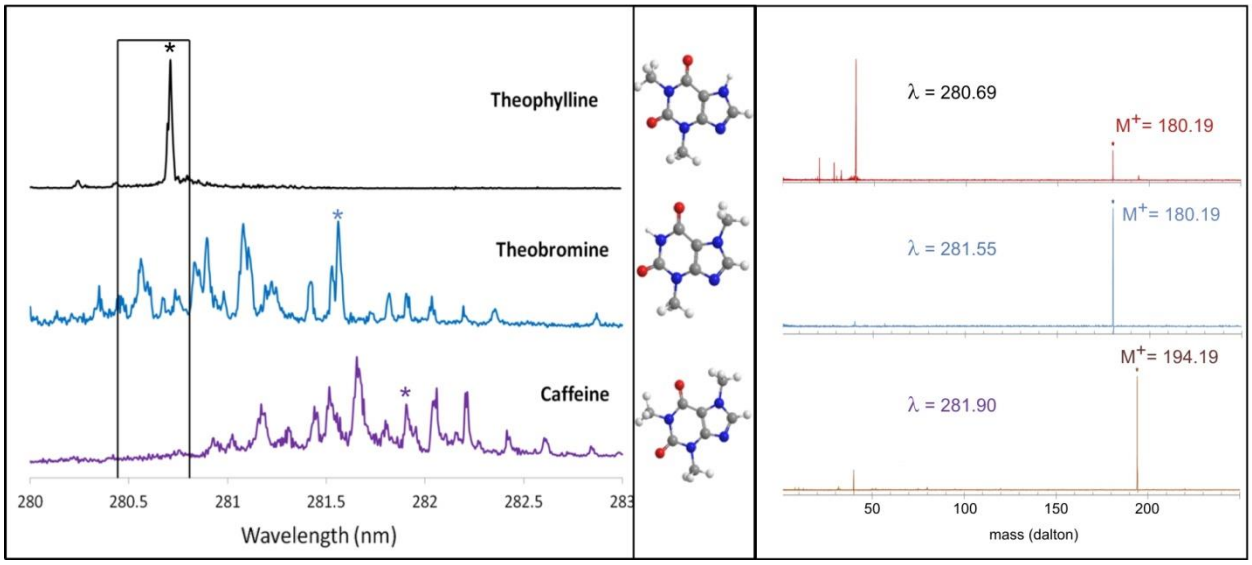

Figure 2: Spectra of methylxanthine standards. Left panel: REMPI spectra recorded on the parent mass, indicated in the right panel. Black box marks the region scanned for the pottery sherds. Right panel: mass spectra recorded at the indicated resonant wavelength, marked for each compound with an asterisk in the left panel. *Y*-axis for both panels in arbitrary units of ion intensity.

We previously reported the detailed REMPI spectra of all three molecular markers[44], identifying unique resonances for the selective ionization of each of the markers. The left hand side of Figure 2 shows the REMPI spectra obtained from each standard. The wavelength range marked by a black box indicates the part of the spectra used for subsequent sample analysis. In order to optimize conditions for each target methylxanthine, we chose a wavelength correlating to a strong REMPI transition and unique to each to perform optically-selected mass spectrometry. Figure 2 shows mass spectra obtained at the resonant ionization wavelengths indicated in the figure with asterisks, clearly determining the parent mass with virtually no fragmentation. The peak at mass 40 is from the argon carrier gas of the molecular beam. When comparing theobromine and theophylline signal directly from the same sample we scanned the wavelength range indicated by the black box. This small part of the spectrum contains distinct and spectrally well separated peaks of each of these compounds.

We examined samples from three different archaeological sites:

(1) A Puerto Escondido vessel extract which has previously tested positive for theobromine by HPLC/MS.[1]

(2) Thirteen base sherds of unique Late Classic period (c. 600-900 CE) Maya vessels from the El Pilar area, located on the border of Guatemala and Belize.

(3) Seven sherds from the early Mississippian period (c. 1100-1200 CE) vessels, located from the Central Illinois River Valley in Fulton County Illinois.

[1] Provided by Dr. Patrick McGovern from the Molecular Archaeology lab at the University of Pennsylvania Museum.

3.2 Puerto Escondido Extract

We analyzed extracts provided by Dr. McGovern from theobromine using 2C-REMPI (resonant excitation at 281.55 nm, ionization at 308.00 nm). We detected theobromine in each of these extracts confirming previously published findings.[2]

3.3 Maya Sherds

Thirteen samples from different ranked Maya archaeological dig sites were analyzed. Rank houses may be associated with different social strata in different areas of the Maya settlement.[45] The data from the analysis of extract revealed some levels of all three methylxanthines in each sherd, excluding sherd one from a small rank house, which tested negative for all three markers. The sherds from the Small Rank and High Rank House show much lower levels of theobromine than theophylline, with caffeine being the most abundant of the three markers. Samples from a median rank house and from a small center exhibit more theobromine relative to the other samples.

In order to investigate the potential of directly desorbing from sherd material, we examined scrapings of the sherds for methylxanthines. The large amount of material required for extraction (≈ 400 mg) often prevents any analysis of pottery sherds, as many of these items are essentially priceless. In addition, solubility differences can impact the rate at which each respective molecule is extracted from the sherd matrix. Figure 3 shows REMPI spectra, collected at m/z 180, obtained directly off scrapings from two different sherds, a cylindrical vessel from a minor center and a pedestal base vessel from a median rank house. For comparison Figure 3 shows pure control samples of theobromine and theophylline which have been scaled in relative intensity to represent equimolar quantities. It is clear that the sherd samples exhibit different theobromine to theophylline residue ratios. This observation suggests a different history for these vessels. The presence of both dimethylxanthine isomers with a relatively high theobromine abundance is a positive indication of cacao present in the cylindrical but not the pedestal base vessel.

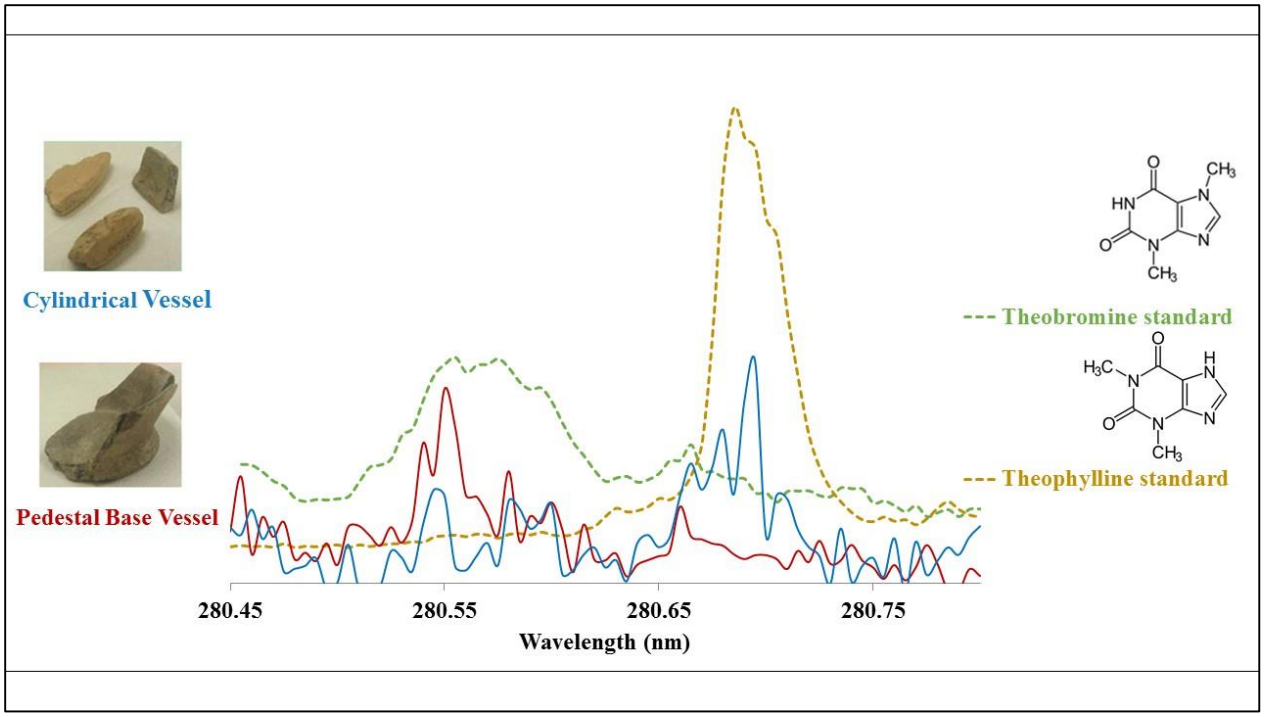

Figure 3: REMPI spectra of two different vessels (cylindrical vessel, blue trace, and pedestal vessel, red trace), performed directly on sherd material. Dotted lines are REMPI spectra of pure standards of theobromine (green trace) and theophylline (yellow trace). Standard spectra have been scaled to represent equimolar intensities (y-axis is ion signal in arbitrary units).

3.4. Mississippi Sherds

We analyzed seven sherds from what is present day Fulton County, Illinois, collected from bluff tops on the western side of the Central Illinois River Valley flood plain. The sherds originated from a Mississippian culture, and date back to between 1100-1200 CE. We analyzed these samples using the same procedures established with the Maya sherds, but only using direct desorption from sherd material. All seven of these samples showed a strong presence of caffeine and theophylline, while six displayed the presence of some theobromine. The presence of all three methylxanthenes from these Northern American findings suggest the presence of cacao residue. The presence of theophylline argues against "black" beverage, which would be based on holly native to the area. This conclusion would be in agreement with two recent research studies suggesting the presence of cacao residue in vessels obtained north of Mesoamerica and possibly indicating a previously unknown trade network.[7,29]

4. Conclusion

The attribution of the geo-cultural origins of pottery sherds is a very complex task, requiring a deep understanding of numerous factors that can affect the presence of certain organic residues. Some of these factors are unavoidable, e.g. physical and biological environmental impacts, solubility differences leading to different leaching rates over time, various clays impacting the affinity for long term storage of organic molecules, but others are manageable. For example, the

initial washing and storage process can be controlled. To preserve water-soluble markers, it can be helpful to avoid the use of water. To minimize the risk of cross contamination sherds can be collected in individual containers. The metabolic n-demethylation of these methylxanthines by soil bacteria can be identified by the products paraxanthine and 7-methylxanthine[28,46], molecules well suited to REMPI analysis, which we have shown previously in publishing the REMPI spectrum of 7-methylxanthine.[44] The ubiquity of the compounds used as biomarkers in museum, laboratory, and storage spaces can be controlled for by the use of blanks both during analysis and in predictive in-situ monitoring of an object's journey from excavation to storage.[28]

We present a new method for analysis of molecular markers of stimulant containing beverages. Pottery sherds from both Central and North America tested positive for all three xanthine alkaloids: caffeine, theobromine and theophylline. Although the method is not yet quantitative, relative amounts of caffeine to theobromine or caffeine to theophylline are consistent throughout the data set at their respective resonant wavelengths for the direct desorption method. This method is more sensitive than previous methods by identifying all three xanthine stimulants in multiple samples, requiring much smaller sample sizes. The three major advantages of this technique are; reduced sample sizes needed for positive identification, the ability to directly analyze samples in complex matrices such as clay from pottery without extraction, and simultaneous positive identification both my mass and spectral signature. We are undertaking a systematic study of a larger set of pottery samples to evaluate if it will be possible to derive conclusions about their use from this type of measurement.

Acknowledgements

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(1) Shillito, L. M.; Almond, M. J.; Wicks, K.; Marshall, L. J. R.; Matthews, W. *Spectrochim Acta A* **2009**, *72*, 120-125.
(2) Henderson, J. S.; Joyce, R. A.; Hall, G. R.; Hurst, W. J.; McGovern, P. E. *P Natl Acad Sci USA* **2007**, *104*, 18937-18940.
(3) Reber, E. A.; Kerr, M. T. *J Archaeol Sci* **2012**, *39*, 2312-2319.
(4) Naik, J. P. *J Agr Food Chem* **2001**, *49*, 3579-3583.
(5) Guasch-Jane, M. R.; Ibern-Gomez, M.; Andres-Lacueva, C.; Jauregui, O.; Lamuela-Raventos, R. M. *Anal Chem* **2004**, *76*, 1672-1677.
(6) Mottram, H. R.; Dudd, S. N.; Lawrence, G. J.; Stott, A. W.; Evershed, R. P. *J Chromatogr A* **1999**, *833*, 209-221.
(7) Crown, P. L.; Gu, J.; Hurst, W. J.; Ward, T. J.; Bravenec, A. D.; Ali, S.; Kebert, L.; Berch, M.; Redman, E.; Lyons, P. D.; Merewether, J.; Phillips, D. A.; Reed, L. S.; Woodson, K. *Proceedings of the National Academy of Sciences* **2015**, *112*, 11436-11442.
(8) Arrowsmith, P.; de Vries, M. S.; Hunziker, H. E.; Wendt, H. R. *Applied Physics B* **1988**, *46*, 165-173.
(9) Meijer, G.; de Vries, M. S.; Hunziker, H. E.; Wendt, H. R. *Applied Physics B* **1990**, *51*, 395-403.
(10) Nir, E.; Hunziker, H. E.; de Vries, M. S. *Anal Chem* **1999**, *71*, 1674-1678.
(11) de Vries, M. S.; Elloway, D. J.; Wendt, H. R.; Hunziker, H. E. *Review of Scientific Instruments.* **1992**, *63*, 3321-3325.

(12) Mahajan, T. B.; Plows, F. L.; Gillette, J. S.; Zare, R. N.; Logan, G. A. *J Am Soc Mass Spectr* **2001**, *12*, 989-1001.
(13) Gillette, J. S.; Ghosh, U.; Mahajan, T. B.; Zare, R. N.; Luthy, R. G. *Israel J Chem* **2001**, *41*, 105-110.
(14) Mahajan, T. B.; Ghosh, U.; Zare, R. N.; Luthy, R. G. *Int J Mass Spectrom* **2001**, *212*, 41-48.
(15) Boesl, U.; Zimmermann, R.; Weickhardt, C.; Lenoir, D.; Schramm, K. W.; Kettrup, A.; Schlag, E. W. *Chemosphere* **1994**, *29*, 1429-1440.
(16) Hafner, K.; Zimmermann, R.; Rohwer, E. R.; Dorfner, R.; Kettrup, A. *Anal. Chem.* **2001**, *73*, 4171-4180.
(17) Ogata, N. *Lowland Maya Area: Three Millennia at the Human-Wildland Interface* **2003**, 415-438.
(18) Hurst, W. J.; Tarka, S. M.; Powis, T. G.; Valdez, F.; Hester, T. R. *Nature* **2002**, *418*, 289-290.
(19) Brunetto, M. a. d. R.; Gutiérrez, L.; Delgado, Y.; Gallignani, M.; Zambrano, A.; Gómez, Á.; Ramos, G.; Romero, C. *Food Chemistry* **2007**, *100*, 459-467.
(20) Hall, G. D.; Tarka, S. M.; Hurst, W. J.; Stuart, D.; Richard, E. W. A. *American Antiquity* **1990**, *55*, 138-143.
(21) Hurst, W. J.; Martin, R. A.; Tarka, S. M.; Hall, G. D. *J Chromatogr* **1989**, *466*, 279-289.
(22) Lo Coco, F.; Lanuzza, F.; Micali, G.; Cappellano, G. *Journal of Chromatographic Science* **2007**, *45*, 273-275.
(23) Ashihara, H.; Kato, M.; Crozier, A. In *Methylxanthines*; Springer Berlin Heidelberg: Berlin, Heidelberg, 2011, pp 11-31.
(24) Edwards, A. L.; Bennett, B. C. *Econ Bot* **2005**, *59*, 275-285.
(25) Saldaña, M. D. A.; Mohamed, R. S.; Baer, M. G.; Mazzafera, P. *J Agr Food Chem* **1999**, *47*, 3804-3808.
(26) Meinhart, A. D.; Bizzotto, C. S.; Ballus, C. A.; Rybka, A. C. P.; Sobrinho, M. R.; Cerro-Quintana, R. S.; Teixeira, J.; Godoy, H. T. *J Agr Food Chem* **2010**, *58*, 2188-2193.
(27) Schimpl, F. C.; da Silva, J. F.; Gonçalves, J. F. d. C.; Mazzafera, P. *Journal of Ethnopharmacology* **2013**, *150*, 14-31.
(28) Washburn, D. K.; Washburn, W. N.; Shipkova, P. A.; Pelleymounter, M. A. *J Archaeol Sci* **2014**, *50*, 191-207.
(29) Washburn, D. K.; Washburn, W. N.; Shipkova, P. A. *J Archaeol Sci* **2011**, *38*, 1634-1640.
(30) Meijer, G.; Devries, M. S.; Hunziker, H. E.; Wendt, H. R. *Applied Physics B-Photophysics and Laser Chemistry* **1990**, *51*, 395-403.
(31) Meng, C. S.; Janssen, M. H. M. *Rev Sci Instrum* **2015**, *86*.
(32) Irimia, D.; Kortekaas, R.; Janssen, M. H. M. *Phys Chem Chem Phys* **2009**, *11*, 3958-3966.
(33) Li, L.; Lubman, D. M. *Rev Sci Instrum* **1988**, *59*, 557-561.
(34) Weyssenhoff, H. V.; Selzle, H. L.; Schlag, E. W. *Zeitschrift fur Naturforschung, Teil A* **1985**, *40a*, 674-676.
(35) Meijer, G.; de Vries, M. S.; Hunziker, H. E.; Wendt, H. R. *Journal of Physical Chemistry.* **1990**, *94*, 4394-4396.
(36) Anex, D. S.; de Vries, M. S.; Knebelkamp, A.; Bargon, J.; Wendt, H. R.; Hunziker, H. E. *International Journal of Mass Spectrometry and Ion Processes.* **1994**, *131*, 319-334.
(37) Nir, E.; Grace, L. I.; Brauer, B.; de Vries, M. S. *Journal of the American Chemical Society* **1999**, *121*, 4896-4897.
(38) Cohen, R.; Nir, E.; Grace, L. I.; Brauer, B.; de Vries, M. S. *Journal of Physical Chemistry A* **2000**, *104*, 6351-6355.
(39) Nir, E.; Imhof, P.; Kleinermanns, K.; de Vries, M. S. *Journal of the American Chemical Society* **2000**, *122*, 8091-8092.
(40) Nir, E.; Muller, M.; Grace, L. I.; de Vries, M. S. *Chem Phys Lett* **2002**, *355*, 59-64.
(41) Zandee, L.; Bernstein, R. B. *Journal-of-Chemical-Physics.* **1979**, *70*, 2574-2575.
(42) Imasaka, T.; Moore, D. S.; Vo-Dinh, T. *Pure Appl Chem* **2003**, *75*, 975-998.

(43) Callahan, M. P.; Gengeliczki, Z.; de Vries, M. S. *Anal Chem* **2008**, *80*, 2199-2203.
(44) Callahan, M. P.; Gengeliczki, Z.; Svadlenak, N.; Valdes, H.; Hobza, P.; de Vries, M. S. *Phys Chem Chem Phys* **2008**, *10*, 2819-2826.
(45) Ford, A.; Fedick, S. *Journal of Field Archaeology* **1992**, *19*, 35-49.
(46) Summers, R. M.; Louie, T. M.; Yu, C. L.; Subramanian, M. *Microbiology* **2011**, *157*, 583-592.