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Case Report

The Detection of Nicotine in a Late Mayan Period Flask by GCMS and LCMS Methods

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Abstract

Several ancient Mayan vessels from the Kislak Collection of the Library of Congress were examined for the presence of alkaloids by GCMS and LCMS methods. One of these Late Classic period (600 to 900 AD) containers was positively analyzed for the presence of nicotine and its derivatives. The experimental findings were consistent with the hieroglyphic text on the vessel: yo-'OTOT-ti 'u-MAY, translated as 'the home of its/his/her tobacco'. The results are indicative for that it was used as a holder of tobacco leafs.

ABBREVIATIONS

GCMS: Gas Chromatography-Mass Spectrometry; LCMS: Liquid Chromatography-Mass Spectrometry; HILIC: Hydrophilic Interaction Liquid Chromatography; MSMS: Mass Spectrometry/Mass Spectrometry or Tandem Mass Spectrometry

INTRODUCTION

Our knowledge of ancient civilizations, their traditions, and their customs comes from various sources. The understanding of foodstuffs which were used/consumed by ancient people can be accessed from hieroglyphic texts and images, food remains (such as grains), or from chemical analysis of the contents of ancient vessels and food containers. Mass spectrometry has become an important method for analyzing the organic compounds in archaeological objects [1,2]. In particular, this method by itself or in combination with chromatographic techniques [3-5], has been employed in the detection of alkaloids providing solid evidence for the presence of cacao products in Mayan vessels [6,7]. Indigenous to the Americas, tobacco (*Tobacco rustica* and *T. tabacum*) was consumed by smoking, sniffing, chewing, topically, or as additives to food and drink. Nicotine is the signature alkaloid of tobacco. The research presented in the current article was conducted on a vessel held in the Kislak collection of the Library of Congress where it was stored in archival bedding at 67°F. The flask discussed here (catalog #1988.042.00.0010; Figure (1) was made in the Mirador Basin, in southern Campeche, Mexico around 700AD. The style of the pot (Codex style) is well known and limited to a specific time range of the Late Classic period given its decoration [8]. The hieroglyphic text from this vessel (Figure 1) contains a well-known collocation spelling for *yotoot* "its house" [9]. A house/home in Classic Mayan text is referential of constructions that contain something- in this case a clay container designated to hold some product. In this study, GCMS and LCMS analyses of organic compounds retained in the vessel on (Figure 1) were performed to find any chemical signature of what this vessel was used for.

CASE PRESENTATION

The dry residue samples were extracted from the interior of the vessels of interest in the dry laboratory of the Conservation Department of the Library of Congress by scraping internal surfaces. The sample contained a portion of clay from the deteriorating portions of the vessel, lime stone plaster from the eroding building, but the majority was the tobacco sample found wedged in a crevice at the bottom of the vessel. The sample was not charred. The collected residues (~4 g) were manually grounded into thin powder. Methanol - methylene chloride (1:1) mixture was used to extract organic compounds from the powder. The solution was removed and the second extraction was performed by using water. Solvents were evaporated and the samples were re-dissolved in 10 µL of the corresponding solvent before been analyzed by GCMS and LCMS methods.

Out of more than 30 ancient Mayan vessels from the collection of Library of the Congress studied in this work only the flask shown on (Figure 1) showed a well-defined signal due to an alkaloid, nicotine. GCMS analysis showed two nicotine peaks while the nicotine standard produced only one signal (Figure 2). Nicotine is optically active, having two enantiomeric forms. The naturally occurring form of nicotine is levoratory, e.g. the same as the (-)-nicotine standard used in this study. The smaller peak clearly has the pattern of nicotine, and is likely a (+)-enantiomer.

Figure 1 The vessel studied in this work (left) and hieroglyphic texts from front and back of this vessel (right).

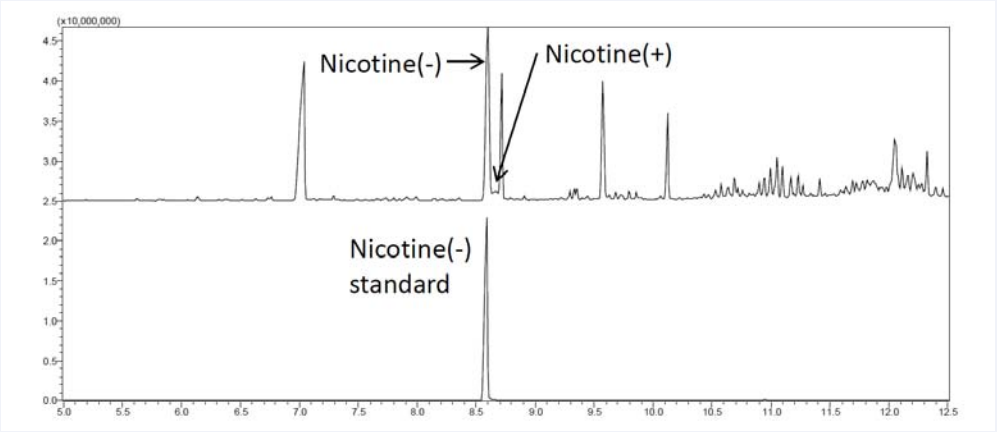

Figure 2 GCMS total ion traces of methanol - methylene chloride extract from the vessel on Figure 1 and of the nicotine standard (bottom).

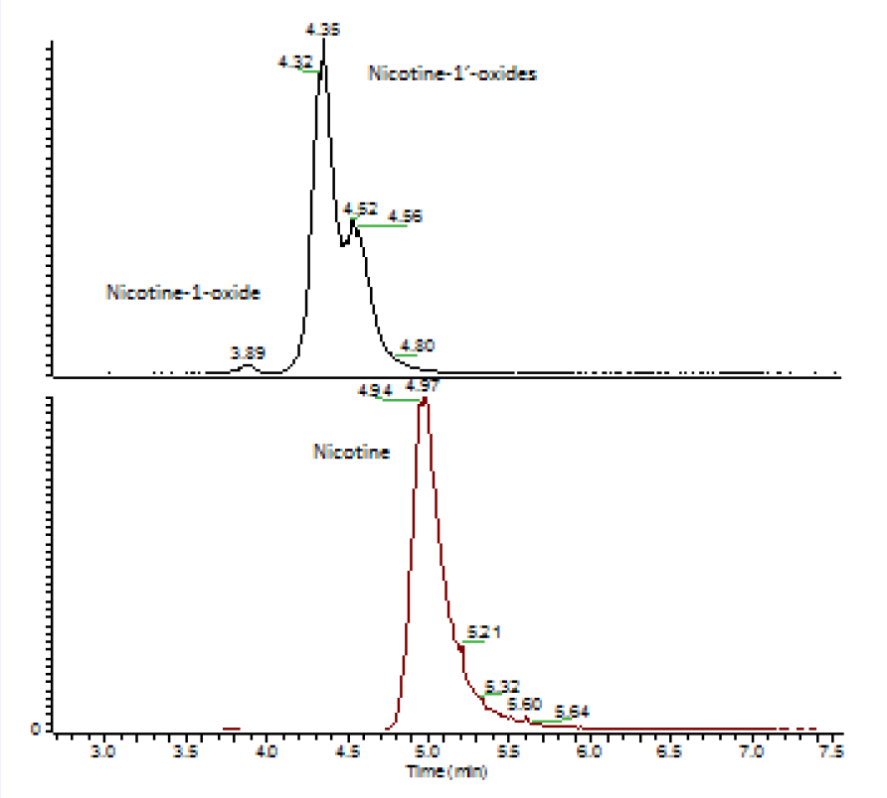

Figure 3 Selected ion chromatograms for peaks at m/z 179 (top) and m/z 163 (bottom).

The estimated area ratio between (-)- and (+)-enantiomer is < 1:200, which is consistent with the naturally observed in tobacco leafs ratio of <0.5%.

The combined extract from the Maya container was also analyzed by LCMS using a HILIC column (Figure 3). The major component identified by electro spray ionization was nicotine which was detected at *m/z* 163. The retention time and MSMS spectrum were exactly the same as for the nicotine standard. Another important group of products detected by LCMS appeared at *m/z* 179. Accurate mass measurements allowed assigning $C_{10}H_{15}N_2O$ compositions to all three components, and suggested that the corresponding neutral molecules were isomeric nicotine oxides. Based on the dissociation pathways, ions at *m/z* 179 were assigned to (protonated) nicotine-1-oxide and two nicotine-1'-oxides. No cotinine, which is a product of thermal oxidation of nicotine, was found. The estimated ratio between nicotine and the sum of two nicotine-1'-oxides was *ca.* 9:1 and the peak area corresponding to nicotine-1-oxide was ~0.005 of that of nicotine-1'-oxide(s).

DISCUSSION

The use of tobacco by ancient Mayan civilization is well documented. Textual evidence written on the pottery is often considered a prime indicator of contents or of an intended purpose, but the actual usage of a container could be changed, altered, or falsely represented making actual function of the vessel difficult to discern. There are several issues which may limit the success of chemical analysis on ancient residues, such as removal of the content by water, effect of soil and bacteria, the usage of containers changed over time, and others. As a result, the recovery of original content of food residues becomes a very difficult task. The most compelling evidence for a container's purpose and usage is when hieroglyphic text or suggestive iconography on a vessel of interest is consistent with the chemical analysis findings. Another challenge in chemical analysis of archaeological objects is of false positives. For example, it was reported that human bones can absorb nicotine even if occasional smoking takes place in the place of their storage [10]. During our study of archaeological objects from the Kislak collection and from *Proyecto Calakmul*, more than 30 various Mayan vessels were analyzed. Some of them were originating from the same burial site as the flask described in this paper. The only positive identification of nicotine was observed for the *y-otoot 'u-may* vessel indicating that external nicotine contamination was not the case.

Nicotine oxidation is a natural process and the detection of its mono-oxides can be easily attributed to processes such as bacterial degradation/oxidation. No other common metabolites of nicotine, such as cotinine, have been detected from the vessel under study. Their presence is usually associated with smoking and/or nicotine metabolism. The GCMS experiments showed a possibility of the presence of (+)-R-nicotine enantiomer with its relative abundance been at the level, which is characteristic to natural content in tobacco leafs. Higher ratios between (+)-R- and (-)-R-isomers are associated with pyrolytic process such as smoking [11,12]. All these observations are consistent with the intended usage of the flask as a storage of tobacco leafs, "house of tobacco", which is indicated by the hieroglyphic text. It is unlikely that the flask has been used as an ash tray or for any other purpose involving thermal treating the tobacco (leafs).

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