Chemical Evidence for Cacao-based Beverages in Ancient Central American Vessels Using HPLC and LC-MS.

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Introduction

Cacao was used in a variety of ceremonial beverages in many pre-Columbian Central American cultures[1]. The cacao may have been mixed with other plant derived stimulant substances. Published studies indicate that traces of cacao and other additives can be identified through analysis of three chemical markers indicative of cacao: **theobromine**, **theophylline**, and **caffeine**[1, 2, 3]. The M. C. Carlos Museum contains a large collection of ceramic vases displaying iconography (such as shamanic transformations[4]) and physical characteristics (cylindrical and globular-footed shapes[5]) associated with such cultural rituals. High-Performance Liquid Chromatography (HPLC) is a technique used to separate molecules in a solution by passing it through a dense, solid column with a high pressure solvent. The separated molecules are identified by measuring their UV absorbance and the time it takes for them to pass through the column. Liquid Chromatography – Mass Spectrometry (LC-MS) expands on HPLC by collecting mass spectra at each point in the chromatogram. The goal of this project is to systematically sample and analyze residues found in pots from the Carlos Museum's Americas collection using HPLC and confirm the peaks' identities through LC-MS. This information will be invaluable to the Carlos Museum because it will ground these works of art to religious, cultural, and social applications in pre-Columbian Central America.

Figure 1. Globular Vessel with Thick Deposits, 1990.11.138

Figure 2. Tripod Vessel with Bird Head, Costa Rica or Nicaragua. Papagayo Polychrome. Period VI, 1000-1300 CE. Ceramic. 1991.4.513

Figure 3. Cylindrical Vessel with Processional Figures, Honduras, 550-600 CE, 1990.11.139

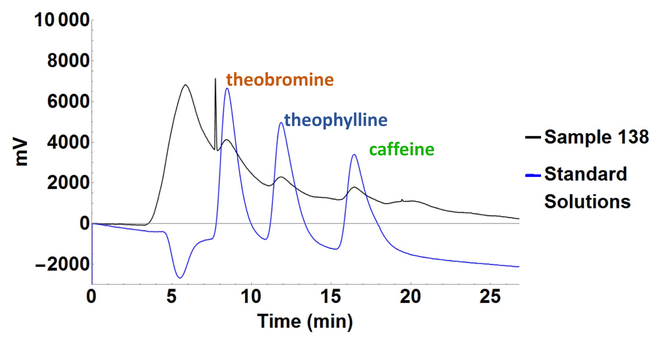
Methods

1. Develop and optimize various HPLC solvent conditions and parameters allowing for the complete and distinct separation of known caffeine, theobromine, and theophylline standards.
   i. Optimal separation found using a 4:1 water-methanol solvent (pH = 2.63 lowered with glacial acetic acid) at a 0.1mL/min flow rate. A 10µL injection volume was used with a C18, 2.7 um, 150 x 2.1 mm column.
2. Systematically document and sample the interior deposits (by scraping with a scalpel) from the Carlos Museum's collection of Central American vessels. Prepare the deposit sample for analysis with HPLC by filtering out solid debris and extracting the molecules in question.
3. Inject each sample through the HPLC column and deduce the presence of caffeine, theobromine, theophylline, and/or other unknown molecules using the chromatogram.
4. Analyze samples of interest with optimized conditions using LC-MS to confirm the peaks' identity.
5. Catalogue which vases are most likely to have contained a cacao based beverage and develop connections between each vase's location of origin, shape, and iconography to its presence of cacao.

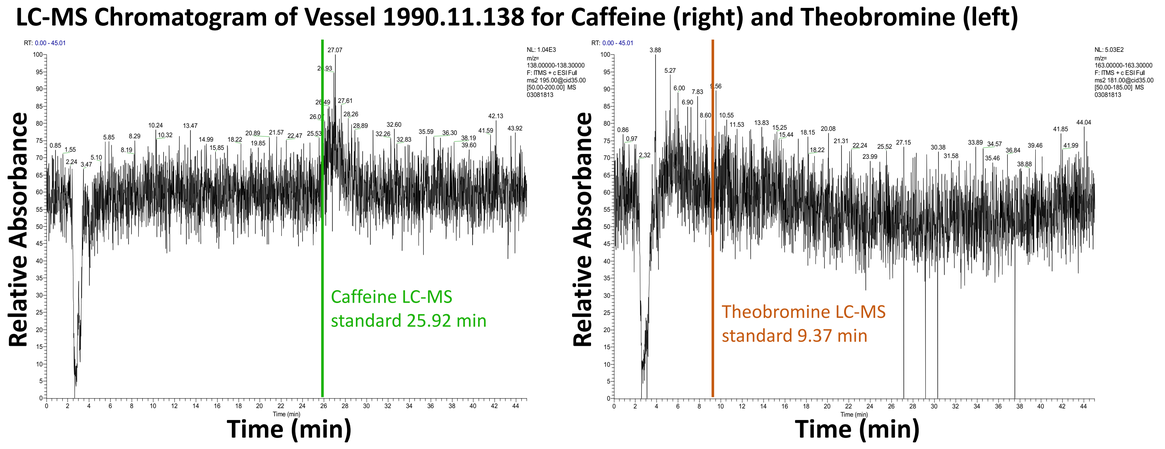
Results and Discussion

*UV detector recorded absorbance at 254nm and 274nm. Chromatograms at 254nm are shown.

HPLC Chromatogram of Vessel 1990.11.138 Compared to Methylxanthine Standard Solution



LC-MS Chromatogram of Vessel 1990.11.138 for Caffeine (right) and Theobromine (left)



Data spotlight for Vessel 1990.11.138 (Figure 1)

HPLC

The chromatogram for Vessel 138 (left), matches closely with the theobromine, theophylline, and caffeine standard solution thus suggesting the presence of cacao.

LC-MS

For vessel 138, the detection of caffeine (right; 138 m/z fragment) is shown to emerge at 26.93 min matching with the standard. Theobromine (left; 168 m/z) is undetectable above the baseline. Theophylline (not shown) is also undetectable above the baseline.

Conclusion

Optimal separation of the methylxanthine standard solution was accomplished using a 4:1 water-methanol solvent (pH = 2.63 lowered with glacial acetic acid) at a 0.1 mL/min flow rate. A 10 µL injection volume was used with a C18, 2.7 µm, 150 x 2.1 mm column. Samples from 24 vessels in the Carlos Museum's Americas collection were collected for analysis using this set of conditions. HPLC analysis indicated at least nine vessels, including vessel 1990.11.138 discussed above, to be of interest for containing theobromine, theophylline, and caffeine. A preliminary LC-MS overview of all vessel samples was inconclusive to confirm the presence of theobromine or theophylline; yet three samples indicated the presence of caffeine at low sample concentration. This project is therefore unable to confirm the identification of cacao in these vessels. Repeating the LC-MS analysis with increased sample concentrations (achieved through modifications to the sample preparation) can be done to further investigate these results and will reveal information about the function and cultural context of these vessels.

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