



# A multi-analytical approach applied to the archaeological residues in Iberian glasses. Earliest evidences on the consumption of fermented beverages in votive rituals



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## ABSTRACT

One of the analytical difficulties that may appear in the analytical process of the archaeological materials is related to the complexity of matrices. In this sense, the abundant content of lipids in archaeological degraded material makes the analysis of polyphenols much more problematic. In addition, phenolic compounds with a low-molecular weight might be present at trace level or even not be present in ancient artefacts. In this study, the advantages of gas chromatography–mass spectrometry in selected ion monitoring mode (GC–MS–SIM) for the analysis of wine biomarkers, tartaric and cinnamic acids, preserved in archaeological potsherd found in a votive deposit from the Iberian period in Granada (Spain) are presented. This will contribute to a greater understanding of the consumption of fermented beverages offered in votive rituals in Southern Spain during the Iberian period. To our knowledge, this is the first time that GC–MS–SIM technique is used to identify low concentrations of these polyphenolic acids in archaeological residues. In addition, the GC–MS–SCAN and gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS) analysis revealed the presence of animal fats and vegetable oils mixed with wax and conifer resin preserved in the Iberian glasses. The correlation between organic compounds identified in the residues (short and odd-chain fatty acids (C9:0 to C12:0, C17:0 and C19:0), palmitic, stearic, azelaic, oleic, lignoceric and dehydroabietic acids and long-chain odd-numbered alkanes) and its possible origin are also discussed.

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## 1. Introduction

The application of analytical chemistry to archaeology yields key insights into the past human culture and economic activities. Different approaches have been used to this end, but the study of organic residues preserved in archaeological ceramic containers has been one of the primary focus of interest of this interdisciplinary research field over the last decades [1–9].

The organic components from the commodities processed or stored in pottery vessels during their lifetime of use (e.g., cooking, transport or storage) are released and absorbed into the porous surface of unglazed vessels. Under favorable conditions, complex mixtures of organic compounds and their degradation products may survive over considerable archaeological time periods. Chemical characterization of these organic residues can provide information on many aspects of past human activities including ancient diet and culinary traditions, political and economic organization, trade and treatment of human diseases, among other things [2]. In addition, this characterization can provide information regarding certain ritual practices such as ceremonies involving

votive deposits, which is the focus of this study. The deposit of votive offerings appears to be an important strategy for strengthening community identity and was one of the most significant ways of communicating with divinities. These ceremonies were sacrificial rituals in which objects were thrown at the bottom of a pit, where they were left as an offering and normally buried in the ground.

Because of the chemical complexity of these types of residues and the low concentrations of organic compounds, chromatographic methods should be used. Gas chromatography–mass spectrometry (GC–MS) [1], Pyrolysis–Gas Chromatography–Mass Spectrometry (Py–GC–MS) [10], Thermally Assisted Hydrolysis and Methylation Gas Chromatography–Mass Spectrometry (THM–GC–MS) [11], High Temperature Gas Chromatography–Mass Spectrometry (HT–GC–MS) [5], Liquid Chromatography–Mass Spectrometry (LC–MS) [12], (LC–MS–MS) [3, 13], Direct Infusion Electrospray Ionization–Mass Spectrometry (ESI–MS) [14,15] and isotopic analysis (GC–C–IRMS) [16,17] have been used for the determination of organic residues.

Most of the previous studies focus on the characterization of ancient lipids from animal and vegetable origin, including dairy products [18], animal fats [6], beeswax [14], and di- and triterpenoids [19], retained within pores of unglazed pottery. Although the composition of mixtures

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of lipids is subject to leaching and to microbial and chemical degradation, the characteristic fatty acids and the degraded compounds used as biomarkers can be preserved in potsherds for centuries. Nevertheless, the detection of residues of wine is far more complicated. The survival of small phenolic carboxylic acids (derived from the polyhydroxy aromatic components found in grapes) in archaeological residues is not guaranteed. Therefore, the characterization of wine and other fermented beverages still represents an analytical challenge.

In the search for wine residues, markers such as tartaric acid, syringic acid, succinic acid, malic acid, citric acid and lactic acid have been found [3,11,13] in order to detect very small amounts of residues and the low concentration of the surviving polyphenols, a highly sensitive analytical technique is required, while keeping in mind the fact that usually the pieces of ancient pottery are precious artefacts.

Liquid and gas chromatography in tandem with mass spectrometry even involving a thermally assisted hydrolysis and methylation (GC-MS, THM-GC-MS, LC-MS-MS) [5,11,12] and direct infusion electrospray ionization mass spectrometry (ESI-MS) [15] have been used for the analysis of tartaric and cinnamic acids in archaeological wine residues. In addition to conventional GC-MS in full SCAN mode, GC-MS-SIM can be used to identify specific compounds at low concentrations, therefore enhancing the sensitivity of the analyses. Despite the fact that the determination of phenols and polyphenols in wine samples by GC-MS in SIM mode (or SCAN) is an analytical routine measure, to our knowledge, before the present paper, there is just one study that uses the SIM mode to analyze archaeological samples [14]. However, that study focuses on the determination of fatty acids, monoesters, monohydroxyesters, and diesters but it does not include wine biomarkers.

The use of GC-MS- full SCAN mode was unable to identify these polyphenolic acids in studied samples. Nevertheless, in this paper, an application of GC-MS using SIM mode for the identification of low concentrations of wine biomarkers in archaeological Iberian ceramic was applied. Tartaric and cinnamic acids at trace levels were identified. This will contribute to a better understanding of the consumption of fermented beverages offered in votive rituals in Southern Spain during the Iberian period. In particular, we focus on one votive deposit dated circa 370–360 BCE found in an excavation site adjacent to *Zacatín* street in Granada, a land that was once the outskirts of the Iberian city of Iliberis (Granada, Spain).

## 2. Materials and methods

### 2.1. Chemicals and reagents

Dichloromethane and methanol (analytical grade) purchased from Fluka (St. Louis, MO, USA) were used as extraction solvents. Toluene (analytical grade), Meth-Prep II (m-trifluoromethylphenyl trimethylammonium hydroxide) and BSTFA (*N,O*-bistrifluoroacetamide) were selected as the solvent and reagents respectively for the derivatization process prior to GC analysis (Meth-Prep II for SCAN mode and BSTFA for SIM mode). Both compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). In order to prevent degradation and obtain high levels of reproducibility, Meth-Prep II and BSTFA were stored at  $-4\text{ }^{\circ}\text{C}$ .

Analytical grade standards of fatty acids (C10, C12, C13, C14, C15, C16, C16:1, C18, C18:1, C18:2, C19, C20, C22, C26, C30) and Cinnamic acid, Azelaic acid, Suberic acid, Adipic acid, Tartaric acid, Syringic acid, Cholesterol and  $\beta$ -Sitosterol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fatty acid C13 was used as an Internal Standard. Individual standard solutions of compounds ( $1000\text{ mg}\cdot\text{mL}^{-1}$ ) were prepared in methanol and stored at  $20\text{ }^{\circ}\text{C}$ . These solutions were prepared fresh monthly. Working standard mixtures were prepared by diluting the individual stock solution in methanol. They were stored at  $4\text{ }^{\circ}\text{C}$  and prepared fresh weekly. All solutions were stored in dark glass bottles to prevent photodegradation.

### 2.2. Instrumentation

#### 2.2.1. Gas chromatography–mass spectrometry (GC-MS)

An Agilent 6890 N series GC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an automatic injector (model 7683) and automatic sample tray (model 7683) was used. The capillary column was an HP-5 (5% Phenylsiloxane  $30\text{ m}\times 0.25\text{ mm}$ ,  $0.25\text{ }\mu\text{m}$  of particle size). In addition, an Agilent 5973 N MS detector (Agilent Technologies, Palo Alto, CA, USA) was coupled to the chromatograph. Regarding mass spectrometer, the detector consisted of electron impact ionization source ( $70\text{ eV}$ ) as an ionization source and a quadrupole as an analyzer, respectively. Table 1 shows the parameters used for the GC-MS analysis. Each parameter was previously optimized [8]. Peak assignments were performed on the basis of the analysis of available standard compounds and by comparing their mass spectra to those from the Wiley mass spectral database.

#### 2.2.2. Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS)

The GC-C-IRMS equipment included a Thermo Trace GC ultra and Thermo Delta V Advantage for IRMS detection (Thermo Fisher Scientific, Waltham, MA). ConFlo III was the interface and the reactor temperature (Cu-Ni-Pt) was set at  $940\text{ }^{\circ}\text{C}$ . The mass spectrometer source pressure was  $1.9\times 10^{-6}\text{ mBar}$ . A HP-1 column ( $30\text{ m}\times 0.25\text{ mm}$  ID  $\times 0.25\text{ }\mu\text{m}$ ) was used for assessing chromatographic conditions. The carrier gas was helium and the GC oven temperature programming  $60\text{ }^{\circ}\text{C}$  (1 min) to  $320\text{ }^{\circ}\text{C}$  at  $6\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$  for 20 min. Carbon isotope ratios are reported in the standard delta notation relative to the Pee Dee Belemnite (PDB) standard. The results were expressed as  $\delta^{13}\text{C}\text{ (}\text{‰}) = [(\text{R}_{\text{sample}} - \text{R}_{\text{standard}}) / \text{R}_{\text{standard}}]$ , where R is  $^{13}\text{C}/^{12}\text{C}$  in per mil. Replicate samples of reference  $\text{CO}_2$  gas are introduced at the beginning and end of each analysis for standardization purposes. To obtain accurate and reproducible  $\delta^{13}\text{C}$  values, triplicate injections were performed for each sample.

#### 2.2.3. Archaeological samples

The ceramic vessels studied here were found during an archaeological excavation in 1999, in a plot of land adjacent to *Zacatín* street in the historic center of Granada. The artefacts were found in a pit that was dug into the Darro river bank. Around 2400 years ago, a series of objects, possibly part of an offering devoted to the Darro river divinity, were literally thrown at the bottom of the pit probably after a ritual feast, after which all the objects used in the feast were abandoned inside the pit that was dug for that purpose. The fact that the deposit was found in a pit located far from the pre-Roman necropolises of Iliberis rules out a funerary origin of the deposit. The proximity of the deposit to the Darro river, however, suggests a sacrificial ritual devoted to the protector deity of the city, the Darro river.

The objects recovered from the pit included a decorated bone plate, weaving accessories (spindle whorls), metal pieces, necklace beads, ostrich egg-shell fragments, perfume containers of polychrome glass and a large collection of clay vessels of various origins that were all used in the ritual, but with different functions. For example, amphorae from Punic Carthage (now Tunisia) and Phoenicia and plates made in *Seks* (Almuñecar, Spain) and *Gadir* (Cadiz, Spain), drinking cups from Athens (Greece), and finally a set of dishes and vases made by local inhabitants of Iliberis were found in the deposit. All this material allowed us to date the ritual at 370–360 BCE. Among all the vessels found, there is a Greek *kylix* depicting a red figure on a black background (Attic red-figure painting) and some red-painted plates with an internal shoulder (Fig. 1). This set of artefacts was sampled to determine the role they played in the ritual.

#### 2.2.4. Sampling procedure

The organic residues from 5 fragments of the *kylix* cup (samples 026, 033, 048, 050, 060) and 5 of plates (samples 201, 202, 205, 218 and 227)

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