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An Etruscan ointment from Chiusi (Tuscany, Italy): its chemical characterization

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ABSTRACT

This paper focuses on the chemical characterization of the original contents of an Egyptian origin alabaster *unguentarium*, found in an Etruscan burial in Chiusi (Tuscany, Italy) and dated 150/125–100 B.C. The *unguentarium*, found in an intact tomb belonging to a noble woman of Chiusine society, preserved a good part of its original contents owing to a protective layer of clay. The chemical characterization was carried out using a combination of two analytical procedures based, respectively, on Fourier transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC–MS).
FTIR is a fast fingerprinting tool able to highlight different classes of organic materials. After wet-chemical treatment of the samples, GC–MS was chosen for the recognition of the organic substances and their degradation products.
The analytical approach used allowed us to obtain information on the presence of a mixture of at least three different substances: a vegetable oil, probably moringa, and two plant resins, mastic and pine.

1. Introduction

During the archaeological excavation of an Etruscan necropolis in Chiusi (Tuscany, Italy), at a location known as La Paccianese, a tomb was discovered intact from the Hellenistic era containing an urn with ashes and a cosmetic case belonging to an aristocratic Etruscan woman named *Thana Presnti Plecunia Umranalisa* (Iozzo, 2008) (Fig. 1). This case (*scrinium*) contained a couple of small bronze finger rings, a pair of iron *volsellae* (tweezers for depilation), two objects which may have been combs and an *unguentarium* (Martelli, 2008). At present located in the Archaeological Museum of Chiusi, this *unguentarium* (Fig. 2) is a small vessel made of calcareous alabaster of Egyptian origin. It is dated to the second half, possibly the last quarter of the second century B.C. (Iozzo, 2008). Amazingly, it preserved a good part of its contents, approximately a quarter of its original volume. This exceptional fact is due to the favourable conditions of preservation of the *unguentarium* and due to the favourable circumstances under which the tomb was excavated. The tomb, cut into the arenaceous sediments with an elevated cohesion, preserved its original structure. Owing to the absence of natural disasters which are so frequent in this region, the *scrinium* was not crushed and its contents remained undisturbed. Moreover, the inside of the case was found under a clay layer which deposited throughout time and allowed for the conservation up until now, including the contents within the *unguentarium*. The contents were preserved at the bottom of the small vessel as an amorphous substance: it was a solid, at most 2 cm thick, pale yellow, homogeneous both in colour and morphology, with some blistering of the surface as well as throughout (Fig. 3).

The good preservation of the *unguentarium* contents provided a unique opportunity to chemically study the composition of the ointment used in Etruscan times, in order to obtain information regarding the nature, the source, and the state of preservation of the organic materials and to highlight the presence of exotic substances due to the Egyptian provenance of the *unguentarium*.

In fact, chemical studies are widely recognised as valuable tools to obtain information on food products, raw commodities and amorphous organic substances and materials used in antiquity.

In this study, chemical analyses were performed by both means of Fourier transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC–MS). To detect any possible inorganic components the same material was preliminarily analysed by means of energy dispersive X-ray microanalysis (EDX).

FTIR spectroscopy reveals intermolecular bonds and, in the analysis of organic substances, the presence of specific functional groups. This technique constitutes an expeditious methodology to highlight the characteristic fingerprint of different classes of

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Fig. 1. The urn of *Thana Presnti Plecunia Umranalisa* just when the tomb was open; at the right bottom, the cosmetic case as it was found.

products and to distinguish them from each other. In an archaeological context, FTIR is largely applied in the study of ancient findings of organic nature, as waterproofing products and binder paints (Colombini et al., 2003), glue (Masschelein-Kleiner et al., 1968), medicine (Regert et al., 2001; Serpico and White, 2000), etc.

GC–MS analysis was carried out in order to provide detailed compositional information (molecular level) on mixtures of organic compounds, such as those expected to be found in archaeological residues from cosmetic or medicinal preparations. GC–MS has been successfully applied in the study of archaeological organic residues containing lipids, natural waxes and terpenic resins, pitch and bitumen (Colombini et al., 2005, 2006; Connan, 1999; Evershed, 2000; Modugno et al., 2006; Pollard and Heron, 1996; Serpico and White, 2000) and its importance in the chemical characterization of samples from *unguentaria* has been already demonstrated (Ribechini et al., 2008).

Fig. 2. The alabaster *unguentarium* brought to light in Chiusi.

Fig. 3. The contents inside the *unguentarium*.

2. The archaeological context

In August 2005, in the course of widening the sand quarry Le Bizzacchere, in the area of La Paccianese, part of a Hellenistic necropolis belonging to the great Etruscan city of Chiusi (*Clevsie-*), one of the most important centres of inland Etruria, was uncovered. The necropolis of La Paccianese belonged, in all probability, to one of the aristocratic clans of Chiusi and, together with similar small necropolis in the vicinity, was part of a network of cemeteries that occupied the north-western area of the Chiusine territory surrounding the lake Il Chiaro. One of the tombs was recovered intact (Iozzo, 2008), its entrance still closed by large terracotta tile inscribed with the name of the deceased (large letters painted in purple–red). This tomb was a wide rectangular niche ("Tomba a nicchiotto"), with a low platform on three sides, with a little more than 1.6 m^2 of floor space and 0.9 m in height. This niche was located in the north wall of the long, very deep *dromos* (entrance corridor) of what originally was a large multi-chambered tomb, unfortunately mostly destroyed at some point in the past. The niche contained an urn with a symbolic handful of the deceased's ashes as

Fig. 4. The urn: on the roof shaped lid, the abbreviated name of the woman is engraved and painted in red.

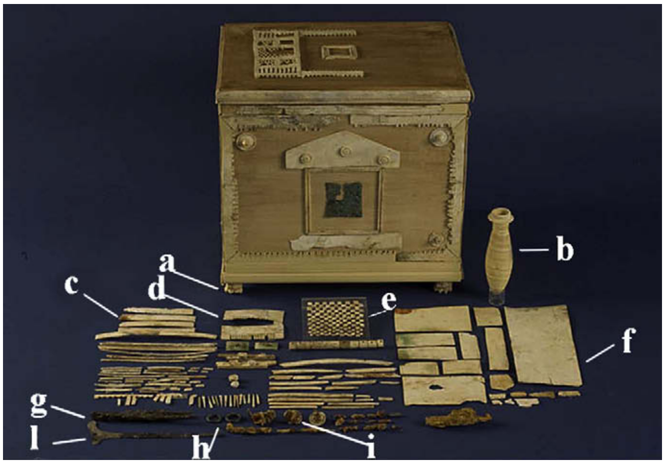

Fig. 5. The cosmetic case with feet carved in bone and shaped as Sirens (a). At the bottom some of the inlaid elements of the box (c, f: bone and ivory strips and plaques; e: a three-dimensional checkerboard; l: a tin decoration; i: bronze buttons) and its content (b: the *unguentarium*; d: (probably) two combs; g: the iron *volsellae* (tweezer for depilation); h: bronze finger rings).

well as the personal belongings of this aristocratic Etruscan woman, *Thana Presnti Plecunia Umranalisa*, whose name we know from the inscription on the urn itself. When she died in the second half of the second century B.C., she was cremated and her remains were put in a small travertine urn, decorated on the front with the head of a female divinity, perhaps *Cel Ati*, the great Etruscan Mother Goddess, flanked by luxuriant foliate elements, a clear reference to the auspice of rebirth and metaphysical regeneration. The roof shaped lid of this urn, which reproduces the shape of a real Etruscan house, is engraved with the abbreviated name of the woman (retrograde and painted in red, Fig. 4), in contrast to the large terracotta tile closing the entrance, on which her full name was written. From the formula of the name we learn that *Thana Plecunia* was the daughter of a lady named *Umranei*, a member of one of the most important aristocratic families of Chiusi.

The only precious object among her grave goods, something which probably also reflected her status, was a rectangular cosmetic case (now under restoration), made of maple wood (*Acer* cf. *campestre*) and embellished by a wide range of applied and inlaid elements of bone, ivory, tin and bronze, such as plain and crenelated plaques and strips, a variety of framing pieces, a panel of three-dimensional checkerboard, buttons and points (Fig. 5). Moreover, the feet of the box were carved in bone in the shape of Sirens. The objects found inside this *scrinium* are a couple of bronze finger rings, a pair of iron *volsellae* (tweezers for depilation), perhaps two combs (this is still being studied) and an alabaster *unguentarium*. More precisely, it is a type of calcareous alabaster that, because of its petrographic characteristics, seems to come from Egypt. This recalls the words of Pliny the Elder, who in *Naturalis Historia* (N.H., XIII, 3) said "*unguenta optime servantur in alabastris*" ("Ointments are best preserved in alabaster").

The *unguentarium* has an elongated ovoid shape, resting on a flat bottom so small that it barely allows the object to stand upright on its own. It has a cylindrical mouth with a wide flange below in order to prevent drips. In all likelihood, the *unguentarium* had a cap that was not preserved, probably made of some perishable material. It may be classified as Colivicchi's type 1.2 (Colivicchi, 2001) and dated between the second half or last quarter of the second century and the first half of the first century B.C., about 150/125 to 100 B.C. This is confirmed by the epigraphy, since the letter forms themselves are datable towards the end of the second century B.C., about 120–100 B.C.

This type of vessel, made of alabaster of supposed Egyptian origin[1] (by stylistic feature it seems from Memphis), whose characteristics allowed the widespread exportation of such delicate aromatics; as a matter of fact such vessels, generally named *alabastra*, are well documented in the Mediterranean basin, in central Italy and also in northern Etruria; among all the examples, the one from Chiusi gives us rare and valuable evidence: part of its contents.

The choice of such valued and exotic personal objects to accompany *Thana Plecunia* into the Afterlife probably commemorated an important moment in the life of this aristocratic woman, namely, her wedding. Marriage marked her passage to a new social status and surely was celebrated with a new matrimonial toilette and rituals which began with ceremonial baths and beauty treatments.

3. Experimental

Some contents of the *unguentarium*, approximately 10 mg, were collected with a scalpel under a stereoscopic microscope (Nikon SMZ800) and deposited in a glass vessel that preserved it until it was analysed. In order to achieve the chemical characterization of the material, sub-samples were obtained and submitted to different analytical techniques, namely, Fourier transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC–MS). Moreover, preliminarily, one sub-sample was analysed by energy dispersive X-ray microanalysis (EDX) to detect any possible inorganic component.

3.1. EDX analysis

Analysis of a small sample was carried out using an FEI Company Quanta 200 scanning electron microscope coupled with EDX-DX4 with sUTW+ energy dispersion detector. Operation conditions adopted were: acceleration voltage 25 kV, filament current 40 μA.

3.2. FTIR analysis

The homogenized sample was analysed as follows: one sub-sample ($\leq$1 mg) as it was; one more sub-sample ($\leq$2 mg) was extracted three times: first using 2 ml of ethanol, then with 2 ml of acetone and, finally, with the same volume of chloroform, in order to eventually separate fractions of different polarity. The first sub-sample and the dried extracts were analysed as KBr micropellets by means of an FTIR Spectrum One-Extended Range instrument (Perkin Elmer) with a CsI-TGS detector. Transmittance % was collected in the range of 4000–300 cm^{-1} with 4 cm^{-1} resolution. The peak assignment was based on comparisons with analysed reference substances, with infrared spectra reported in the literature, and also on interpretation of the spectra. The utilised solvents were Merck (Darmstadt, Germany) reagents for analysis, whereas KBr was a BDH Spectrosol (England) reagent for infrared spectroscopy.

[1] The *unguentarium* was carved in a piece of carbonatic stone (EDX confirms the presence of only Ca with impurities of Fe and Si) with a structure concentric-zonate, characterized by bands of different coloration and opacity: from white to amber and from translucent to opaque. These morphological and compositional features resemble those of Egyptian calcareous alabaster whereas the same Italian stone (from: Iano in Tuscany, Monte Circeo in Latium, Latronico in Lucania) has generally a massive non-zonate structure and/or is homogeneously white and has a higher content of Si, Mg, Fe impurities. In antiquity, the mostly used Italian alabaster was of gypsum nature (Gansike, 1994; Harrel, 1992).

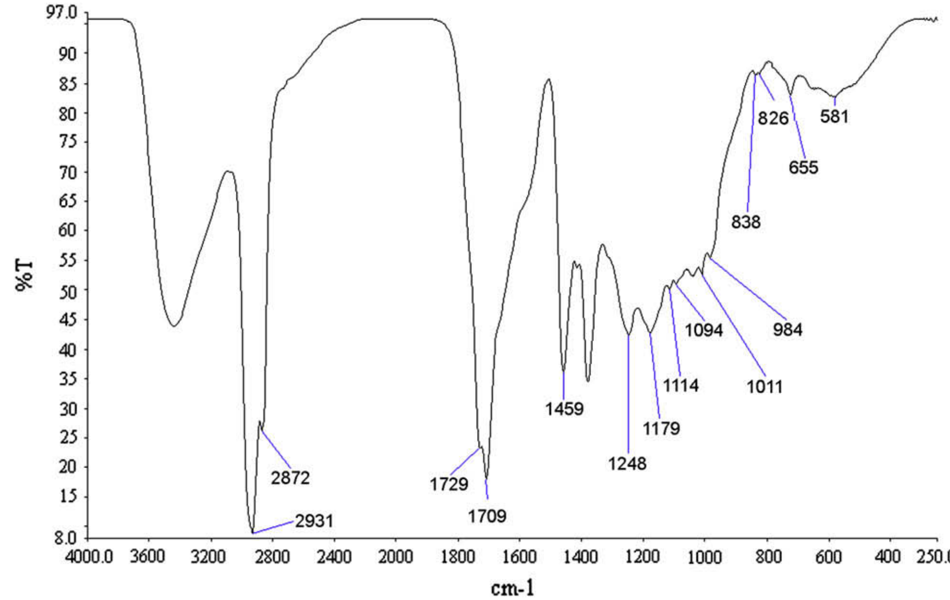

Fig. 6. FTIR spectrum of the contents, such it was, of the *unguentarium*.

3.3. GC–MS analysis

The adopted analytical procedure involves the following steps:

- sample (1.5 mg) was hydrolysed using 10% hydroalcoholic KOH (3 h, 60 °C);
- neutral organic components were extracted three times with *n*-hexane (the combined extracts made up the neutral fraction) and, after acidification, the acidic organic components were extracted from the residual solution with diethyl ether (the combined extracts made up the acidic fraction);
- aliquots of both fractions were derivatised with N,O-bis (trimethyl)silyltrifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane, using *iso*octane as the solvent;
- 2 μl of the resulting solutions were analysed by GC–MS using hexadecane (IS_1) and tridecanoic acid (IS_2) as internal standards.

The instrumentation consisted of a Trace GC gas chromatograph (ThermoFinnigan, USA) equipped with a PTV injection port, and a mass spectrometric detector based on an ion trap analyser (ThermoFinnigan Polaris Q). The PTV injector was in the "CT splitless with surge" mode at 280 °C with a surge pressure of 100 kPa and mass spectrometer parameters were: electronic impact ionisation (70 eV), ion source temperature 230 °C, scan range m/z 50–650, interface temperature 280 °C. Chromatographic separation was performed on an HP-5MS chemically bonded fused silica capillary column (Hewlett Packard), with stationary phase

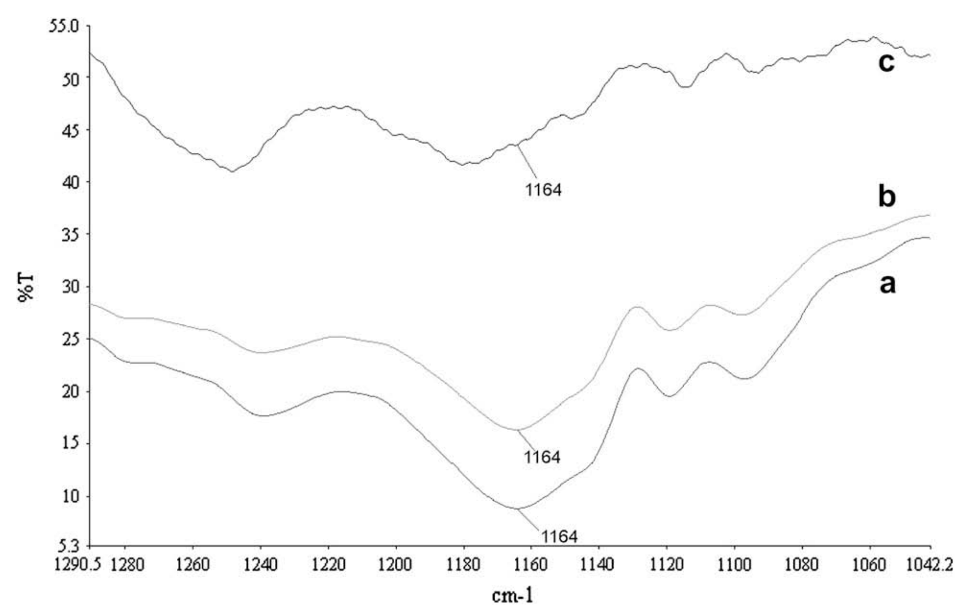

Fig. 7. Section of olive (a) and moringa (b) oils reference spectra *vs* deconvoluted FTIR spectrum of the *unguentarium* contents (c).

Table 1
Diagnostic vibrational group frequencies (from Fig. 4) in the FTIR spectrum of the Etruscan ointment.

Assignment	Comment	Range (cm^{-1})	Substance
ν(C–H)	CH_3, CH_2 asymmetric	2931	Diterpenic resin
	CH_3, CH_2 symmetric	2872	Triterpenic resin
	CH_2 symmetric	2858	Acyl lipid
ν(C=O)	Ester	1729	Acyl lipid
	Acid	1709	Triterpenic resin
$\delta(CH_2)$, $\delta(CH_3)$	CH_2 scissoring, CH_3 asymmetric	1459	Diterpenic, triterpenic resin
Fingerprint region	C–O bands	1248	Triterpenic resin
		1179	Diterpenic resin
		1164	Acyl lipid
		1114	Triterpenic resin
		1094	Acyl lipid
		1011	Triterpenic resin
		984	Diterpenic resin
	C–H bands	838–826	Triterpenic resin
		724	Acyl lipid
		655	Diterpenic resin
		581	Triterpenic resin

5%phenyl–95%methylpolysiloxane, and dimensions 0.25 mm i.d., 0.25 μm film thickness, 30 m length, connected to a 2 m long deactivated fused silica capillary pre-column (0.32 mm i.d.). The gas chromatographic conditions were as follows: initial temperature 80 °C, 2 min isothermal, then ramped at 10 °C/min up to 200 °C, 4 min isothermal, then ramped at 6 °C/min up to 280 °C, 40 min isothermal. The carrier gas was He (purity 99.9995%), at a constant flow rate of 1.2 ml/min.

Mass peak assignment was based on comparisons with analysed reference compounds and materials, with library mass spectra (NIST 1.7) and with mass spectra reported in the literature, and also on interpretation of mass spectra.

All the utilised solvents were Carlo Erba (Milan, Italy) pesticide analysis grade, and were used without further purification. Hexadecane (internal standard, IS_1), and N,O-bis(trimethyl)silyltrifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane, were purchased from Sigma Aldrich (Milan, Italy).

4. Results and discussion

No other elements but C, O and occasionally Ca traces, presumably coming from the alabaster, were detected by EDX elemental analysis confirming the organic nature of the contents of the *unguentarium*.

The FTIR analysis of the material, analysed as it was, gave the spectrum reported in Fig. 6.

Comparing it with reference spectra and literature data (Derrick et al., 1999), it is possible to distinguish characteristic signals pertaining to three kinds of organic materials: a diterpenic material, (2931, 984, 655 cm^{-1}), a triterpenic material (1709, 1248, 1114, 1011, 838–826, 581 cm^{-1}), and an acyl lipid material (2858, 1729, 1164 – the first and latter evidenced in the deconvoluted spectrum, see Fig. 7 – 1094, 724 cm^{-1}). Moreover, the spectrum presents a series of signals common to diterpenic and triterpenic resins (2872, 1459 cm^{-1}). The diagnostic bands are reported in Table 1.

The extracts in ethanol and acetone did not give any further information. Taking into account that the extraction of the sample with both the alcohol and acetone left a residue and that the latter was completely solubilised by chloroform, the spectrum resulting from the treatment with this last solvent gave new evident signals (Fig. 8). The new bands ranging in 1665–1572 cm^{-1}region can be attributed to different conjugated carbonylic functions, probably due to the ageing process.

GC–MS analyses performed on the acidic and neutral fractions revealed a wide array of molecules indicating a very complex chemical composition and the presence of several materials admixed. Fig. 9 shows the chromatograms of the acidic and neutral fractions of the sample.

In the acidic fraction three classes of compounds were highlighted, namely triterpenes, diterpenes and lipids, with a major abundance of the lipid fraction. Four triterpenoid acids were detected, namely, moronic, oleanonic, masticadienonic and *iso*-masticadienonic acids and in addition, appreciable quantities of diterpenoid acids, dehydroabietic and 7-oxo-dehydroabietic were also present. Moreover, in the neutral fraction, among various triterpenoid compounds, nor-β-amyrone and nor-olean-17-en-3-one were recognised. The above cited diterpenoid and triterpenoid molecules indicate that a Pinaceae resin and a mastic resin were

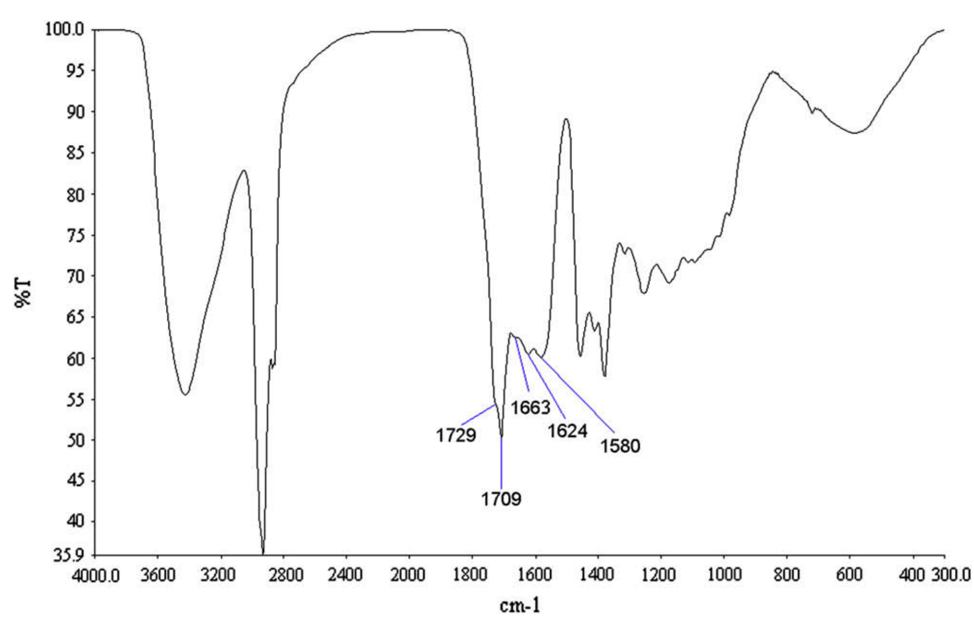

Fig. 8. FTIR spectrum of the chloroform extract of the *unguentarium* contents.

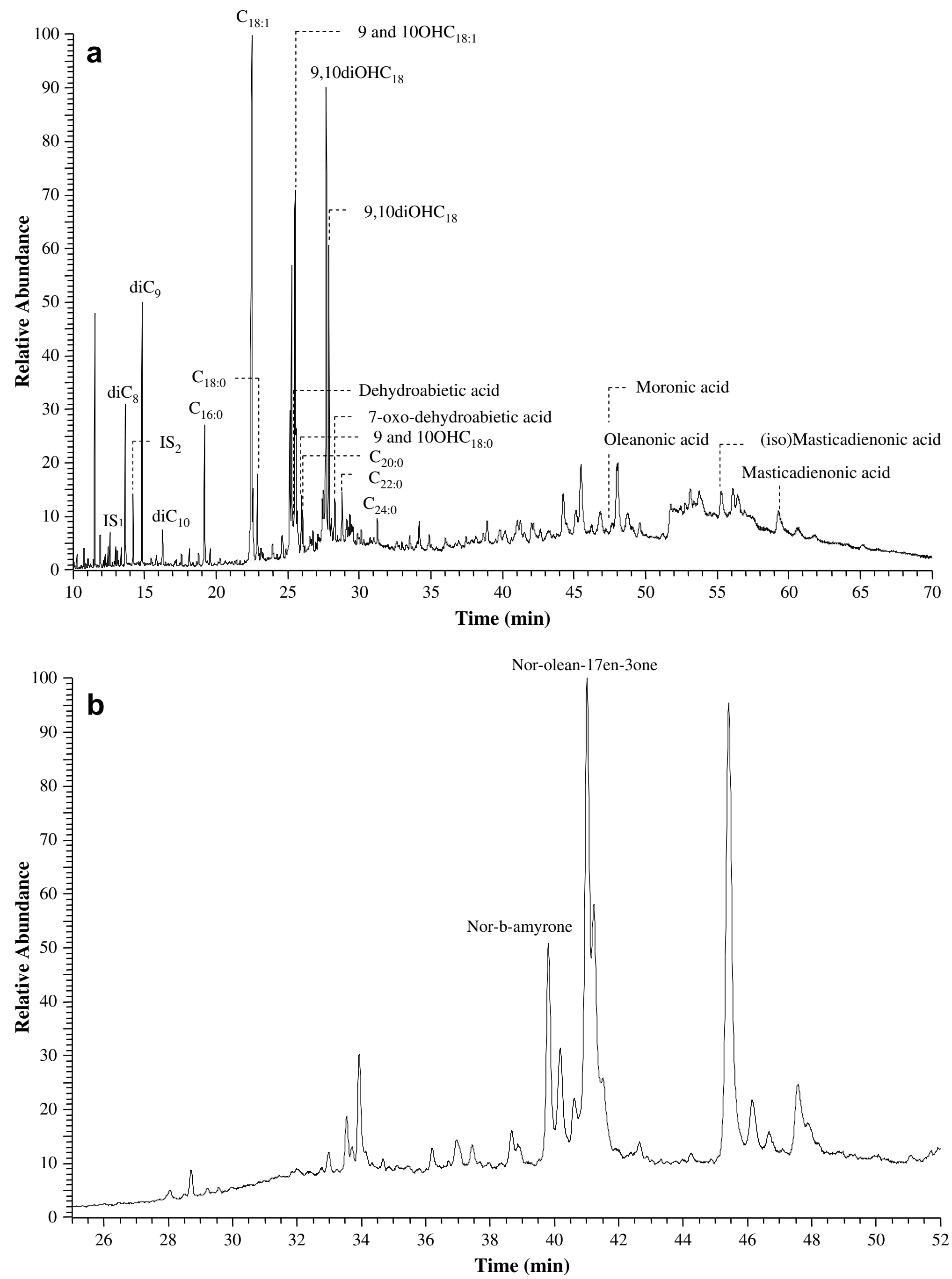

Fig. 9. TIC of the trimethylsilylated a) acid fraction and b) neutral fraction of the sample collected from the *unguentarium* obtained by GC–MS. $A_{X:Y}$ are fatty acids of chain length *x* and degree of unsaturation *y*; diA_X are α,ω-dicarboxylic fatty acids of chain length *x*; $XOHA_X$ are hydroxy fatty acids of chain length *x* and with hydroxy group at position *X*; $9,10OHA_{18:0}$ are 9,10-dihydroxyoctadecanoic acids; IS_1 and IS_2 are the *n*-hexadecane and tridecanoic acid internal standards.

used in the realization of the ointment (Colombini et al., 2003, 2006; Ribechini, 2006).

As far as the lipid component is concerned, it mainly consists of:

- a series of linear monocarboxylic even numbered saturated fatty acids ranging from 12 to 24 carbon atoms with hexadecanoic acid (palmitic) as the most prominent;
- a series of α,ω-dicarboxylic acids, ranging from 8 up to 10 carbon atoms, with nonanedioic acid (azelaic) as the main constituent of this group;
- mono and dihydroxycarboxylic acids with 18 carbon atoms, namely, 9,10-dihydroxyoctadecanoic acid (present as a pair of threo-erythro isomers), 9-hydroxyoctadecanoic acid, 10-hydroxyoctadecanoic acid, 9-hydroxyoctadecenoic acid and 10-hydroxyoctadecenoic acid;
- oleic acid, being the major compound of the lipid fraction, as monounsaturated fatty acids.

The fatty acid profile detected in GC–MS, suggests that the lipid was an oil of vegetable origin (Colombini et al., 2005; Ribechini, 2006). Due to the complex molecular patterns obtained, it is rather difficult to establish its botanical origin. In any case, the presence of long-chain saturated fatty acids which easily survive ageing (eicosanoic acid, docosanoic acid and tetracosanoic acid), may be related to the use of moringa oil (Ribechini, 2006; Serpico and White, 2000). In fact, among vegetable oils (olive, almond, balanos, castor, coconut, linseed, moringa, palm, poppy, radish, safflower and sesame oils) which was well known by ancient Mediterranean people, moringa oil is the only one which contains about 8–12% of long-chain saturated fatty acids together with high abundance of oleic acid (70–75%) (Ribechini, 2006; Serpico and White, 2000). Anyway, it is not possible to exclude that a mixture of vegetable oils was prepared.

The presence of oleic acid in high abundance is quite surprising. In fact, in archaeological environments, unsaturated, especially polyunsaturated, fatty acids easily undergo oxidation processes localized at the double bonds via radical reactions with the inclusion of oxygen in the acyl chain, carbon–carbon bond cleavage, and the formation of lower molecular weight species such as α,ω-dicarboxylic acids. The fact that the sample contains quite high levels of oleic acid seems to indicate that the oxidation of the organic material has not yet been completed, highlighting a low degree of oxidation. This phenomenon may be related to the sealing of the *unguentarium* by a filling of clayish earth which prevented contact with oxygen and slowed down the oxidation processes. This hypothesis is reinforced by the presence of mono and dihydroxycarboxylic acids which are considered as the intermediates of the oxidation reaction which transforms oleic acid into azelaic acid. It is clear that the degree of oxidation and the kind of oxidation products are strictly dependent on the amount and the nature of unsaturations present in the original materials. Moreover, the natural degradation processes of lipids could be accelerated or modified if the material was exposed to oxidizing conditions such as high temperatures or UV radiation. This means that the speed of degradation reaction and the nature of degradation products are strictly correlated to the composition of the original material, the use of container and the burial conditions.

5. Conclusions

The recent excavation of an Etruscan necropolis in the countryside of Chiusi brought to light an intact tomb from the Hellenistic era, belonging to a lady of noble rank, *Thana Presnti Plecunia Umranalisa*. The circumstances surrounding this find, such as the stability of the tomb structure which prevented its collapse, in addition to the clay layer deposited inside the tomb which slowed down to some extent the degradation of the collected materials, allowed for the preservation of *Thana*'s cosmetic case, which contained an *unguentarium* of calcareous alabaster that retained most of its original contents. This discovery is of the highest importance because it furnishes us with a product actually used in Etruscan times, moreover one found during a scientific excavation. A chemical characterization was then carried out on the material found in the *unguentarium*. The analytical techniques used in analysis were energy dispersive X-ray microanalysis (EDX), Fourier transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC–MS). These analyses made it possible to verify that the contents of the alabaster *unguentarium* (*alabastron*) of Chiusi are completely of organic nature and consist of a mixture of substances of a lipid and resinous nature. The two natural resins that were identified were the pine resin, exudated from Pinaceae, and the mastic resin, from Anacardiaceae trees. The results obtained indicate that the lipid was a vegetable oil, probably moringa oil, already known to and used by Egyptians and Greeks to produce ointments and perfumes (Forbes, 1955). Moringa trees are native to Sudan and Egypt (Serpico and White, 2000) and they were not attested in Italy. For this reason and due to the Egyptian origin of the *unguentarium*, it is possible to suppose that the ointment already prepared was imported in Etruria.

Usually, oils were employed as vehicles for perfumed substances, *sucus* for Pliny (N.H., XIII, 7), and in their formulations they were thickened with the addition of resins which could act as fixatives (something like mordant for dyeing) (Forbes, 1955; Plinius 77 A.D.). In same cases oils and resins could be scented themselves. Several sources of oil were well known at the time of the *unguentarium* from Chiusi, including linseed (*Linum usitatissimum*), safflower (*Carthamus tinctorius*), sesame (*Sesamum indicum*), balanos (*Balanites aegyptiaca*), castor (*Ricinus communis*), and olive (*Olea europaea*) oils; moreover, moringa oil, called also myrobalan oil (Forbes, 1955) was available. Pliny the Elder talks of myrobalan tree and of the perfumed oil that it produces (N.H., XXII, 46). The same myrobalan oil is reported as an ingredient of a particular recipe of a "regal perfume" realized for the king of Parthes (N.H., XXIII, 2). In any case, due to the therapeutic characteristics properties (Fahey, 2005) of this oil, it cannot be excluded that the mixture discovered in Chiusi was prepared for medical purposes only, rather than as a cosmetic ointment.

The contents of the *scrinium*, therefore, evidence *Thana*'s attention to her personal care and hygiene, in all its aspects, from the hygienic–cosmetic (including the toilette and make-up), to the art of personal adornment (hair dressing, apparel and jewels). In addition, the imported Egyptian *unguentarium* and its exotic ointment, attest to the high social rank to which *Thana Plecunia* and her family belonged.

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