IN SITU THERMALLY ASSISTED PYROLYSIS-SILYLATION GC-MS AS TOOL FOR IDENTIFYING ORGANIC COMPOUNDS PRESENT IN ARCHAEOLOGICAL OBJECTS

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ABSTRACT: *The present contribution explores the capability and benefits of the technique of "in situ" thermally assisted pyrolysis-silylation GC-MS using hexamethyldisilazane as derivatisation reagent. This technique has the analytical advantage of having high sensibility and reducing considerably the size of the samples tested. For this reason, it is especially suitable for detecting the presence of organic compounds in objects highly deteriorated such as rock art and buried archaeological remains. The study has been focused on the identification of the binding medium present in several rock paintings found in shelters of the Peruaçu valley (Minas Gerais, Brazil) as well as in the characterization of the adhesive used in the gilding decoration of several glazed tiles from the Takht-e Soleyman palaces (Iran) (13th-15th centuries, Ilkhanian period). The results obtained in the analysis of the series of samples from Peruaçu rock paintings evidenced that a fatty substance of animal origin was used as binding medium of these paintings. On the other hand, linseed oil was identified in the series of samples excised of the glazed ceramics from Takht-e Soleyman. Interestingly, this material has been preserved in an extraordinary low degree of oxidation due to the protective effect of the metallic layer.*

KEYWORDS: binding media, archaeological objects, Py-GC-MS, rock art, ceramic objects, Kaman oil

1. INTRODUCTION

A number of natural products, among them, blood, egg, fats, plant gums, terpenoid resins and drying oils have been used as components of protective finishes and binding media of art objects since ancient times as they are organic products recognized as film-forming materials.

Thus, a number of specialists in ancient cultures and rock art have conjectured on the use of these products as components of binding media of petroglyphs and rock paintings in earlier times, resulting in a positive identification of them in some cases, namely, egg (Rampazzi et al 2002:237; Rampazzi et al 2007: 559), blood (Mori et al, 2006: 344; How, 1970; Loy et al, 1990: 110; Loubser, 1992: 16; Williamson, 2000: 755; Lewis-Williams, 1995: 143), fats (Spades et al 2005: 115; Vázquez et al 2008: 1381; Fiore et al 2008: 3047) and plant gums (Scott, 2002:184). Use of these products as adhesives of all kind of objects in ancient times has also been reported (Prous A. 1992; Kashani, A 1969).

In addition to these specialised studies, organic compounds, in general, have attracted attention of scientists in the fields of art and conservation of heritage. Studies dealing with characterization and photochemical and thermal degradation of organic compounds, used as binding media and varnishes in art objects, have been performed by means of a variety of instrumental techniques (Doménech-Carbó 2008:109).

The present contribution shows the capability and benefits of the technique f *in situ* thermally assisted pyrolysis-silylation GC-MS using hexamethyldisilazane as derivatisation reagent in the analysis of archaeological and rock art samples in which is suspected that some organic compound is present as binding medium or adhesive.

This technique has the analytical advantage of having high sensibility and reducing considerably the size of the samples tested. For this reason, it is especially suitable for detecting the presence of organic compounds in objects highly deteriorated such as rock art and buried archaeological remains.

The study has been focused on the identification of the binding medium present in several rock paintings found in shelters of the Peruaçu valley (Minas Gerais, Brazil) as well as in the characterization of the adhesive used in the gilding decoration of several glazed tiles from the Takht-e Soleyman palaces (Iran) (13th-15th centuries, Ilkhanian period), where it was suspected that gold sheets were fixed by means of an adhesive consisting of drying oil and sandarac resin (Kaman oil). This recipe is nowadays used by craftsmen in this region.

A series of analyses was performed with reference products, artifically aged, that presumably could be used as binding media of rock paintings and with those mentioned in the traditional recipes used in the preparation of the Kaman oil.

The results obtained in the analysis of the series of samples from Peruaçu rock paintings evidenced that a fatty substance of animal origin was used as binding medium of these paintings.

On the other hand, linseed oil was identified in the series of samples excised of the glazed ceramics from Takht-e Soleyman.

Interestingly, this material has been preserved in an extraordinary low degree of oxidation due to the protective effect of the metallic layer.

2. EXPERIMENTAL

Materials

Reagents.- Hexamethyldisilazane (purity 99%) (Fluka, Buchs, Switzerland), was used for the *in situ* thermally assisted pyrolysis and silylation–GC-MS analysis of samples.

Reference materials.- Egg and porcine fat from local suppliers (Valencia, Spain), have been used for preparing a series of paint model specimens that simulate the original rock paintings. The pigments used for the preparation of paints were: natural iron oxide red (Fe_2O_3) and ochre yellow ($Fe_2O_3.nH_2O$) pigments from Rio Preto valley (Minas Gerais, Brazil) and natural manganese dioxide pigment (MnO_2) from Amazonia (Brazil).

Composition of them was confirmed by means of scanning electron microscopy-x-ray microanalysis. Cold pressed linseed oil, sandarac and colophony resin were used as reference materials for comparing the results of the samples of the adhesive product used in the Iranian gilded ceramics.

Instrumentation.- Pyrolysis-Gas Chromatography-Mass Spectrometry for the characterization of organic materials was carried out with an integrated system composed of a CDS Pyroprobe 1000 heated filament pyrolyser (Analytical Inc., NY, USA) and a Gas Chromatograph Agilent 6890N (Agilent Technologies, Palo Alto, Ca, USA) equipped with an on-column injection system and coupled with an Agilent 5973N mass spectrometer (Agilent Technologies) and equipped with pyrolysis injection system. Preparation of samples is described elsewhere (Doménech, 2008).

Samples.- Three rock art samples were analyzed that came from the paintings found in the Janelão (black area, ref. 1131, red area, ref. 1222) and the Piolho de Urubu shelter (yellow area, ref. 1116) located in the Peruaçu valley from the region of Minas Gerais (Brazil).

A sample of adhesive excised, by the help of a scalped, from a tile decorated with cobalt blue, turquoise blue and white glaze with gilding (reference 21300a) from the indoor decoration of the Takht-e Soleyman palace was analysed in this study.

Preparation of simulated rock paint specimens artificially aged.- In a series of specimens the egg, animal fat and blood, respectively, were mixed with the appropriate amount of pigments.

Thus, pictorial dispersions 50% (w/w) were obtained and then spread as a thin layers (average thickness, 50-80 μm) on the surface of blocks (10x6x1.5) cm of *bateig azul* stone, a calcarenite rock similar to those occurring in Minas Gerais region. The specimens were stored at room temperature during two months and afterwards analysed.

The paint specimens were artificially light-aged in order to make an approach to the effect of photoageing on the chemical composition of the resin. UV light ageing consisted of irradiating the samples in a Dycometal model QUV-Basic chamber provided with an UVB-313EL UV lamp (Q-Panel Lab Products), which produces mostly short-wave UV light with maximum intensity at ca. 310 nm and is equivalent to a 40 watt fluorescent lamp. Temperature was maintained at constant value of 45ºC. Specimens were exposed to UV light for 720 h.

3. RESULTS AND DISCUSSION

Rock paintings

Figure 1 a,b shows the pyrogram obtained in the sample 1222. The other two samples analyzed exhibited similar pyrograms. It can be seen that long chain fatty acids characteristic of lipids such as palmitic and stearic acid are identified as well as isomers of oleic acid.

Derivatized glycerol was also identified. Interestingly the studied samples exhibited weak peaks corresponding to long chain fatty acids with uneven number of carbons in the chain (C15 and C17).

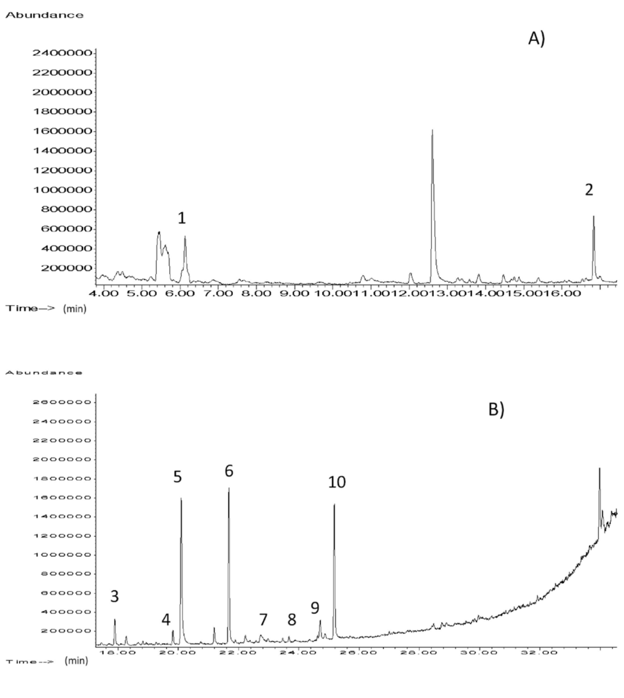

Figure 1. Pyrogram of sample 1222: a) from 0 to 17 min; b) from 17 min until the end of the pyrogram

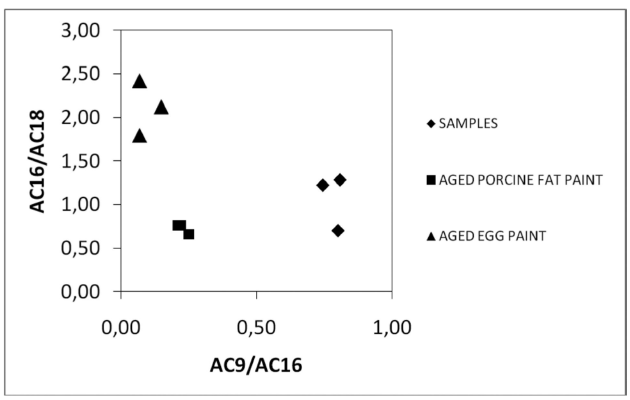

Figure 2. Peak area ratios Anonanedioic acid/Ahexadecanoic acid (AC9/AC16) versus Ahexadecanoic acid/Aoctadecanoic acid (AC16/AC18) obtained in the series of samples, aged porcine pain and aged egg paint

Peak number	Retention time (min)	Compound	Sample 1116	Sample 1131	Sample 1222	Aged egg paint	Aged porcine fat paint
1	3.01	Glycerol	x	x	x	x	x
2	4.33	Heptanoic acid, TMS ester	-	-	-	-	x
3	5.82	Octanoic acid, TMS ester	-	-	-	-	x
4	6.30	Tridecene*	-	-	-	-	x
5	6.45	Tridecane	-	-	-	-	x
6	7.64	Nonanoic acid, TMS ester	-	-	-	x	x
7	8.24	Tetradecene*	-	-	-	-	x
8	8.40	Tetradecane	-	-	-	-	x
9	9.65	Decanoic acid	-	-	-	-	x
10	10.34	Pentadecene*	-	-	-	-	x
11	10.50	Pentadecane	-	-	-	-	x
12	10.79	Hexanedioic acid, TMS ester	-	-	-	-	x
13	11.60	Undecenoic acid, TMS ester	-	-	-	-	x
14	12.49	Hexadecene*	-	-	-	-	x
15	12.91	Heptanedioic acid, TMS ester	-	-	-	-	x
16	13.82	Dodecanoic acid, TMS ester	-	-	-	-	x
17	14.77	Heptadecane	-	-	-	-	x
18	14.94	Octanedioic acid, TMS ester	-	-	-	-	x
19	15.90	Tridecanoic acid, TMS ester	-	-	-	-	x
20	16.98	Nonanedioic acid, TMS ester	x	x	x	x	x
21	17.03	Decanedioic acid, TMS ester	-	-	-	-	x
22	17.77	Tetradecenoic acid, TMS ester*	-	-	-	-	x
23	17.91	Tetradecanoic acid, TMS ester	x	x	x	x	x
24	19.83	Pentadecanoic acid, TMS ester	x	x	x		x
25	21.30	9-trans hexadecenoic acid, TMS ester	x	x	x	-	x
26	21.88	Hexadecanoic acid, TMS ester	x	x	x	x	x
27	23.48	Heptadecanoic acid, TMS ester	x	x	x	-	x
28	24.76	11-cis octadecenoic acid, TMS ester	x	x	x	x	x
29	24.88	11-trans octadecenoic acid, TMS ester	x	x	x	x	x
30	25.34	Octadecanoic acid, TMS ester	x	x	x	x	x
31	30.41	2-Monopalmitin, monoTMS ester	-	-	-	x	x
32	30.89	2-Monopalmitin, diTMS ester	-	-	-	x	x
33	33.67	2-Monostearin, diTMS ester	-	-	-	x	x

Table. 1. Retention time and main compounds identified in the studied samples and in the UV aged paints bound with egg and porcine fat

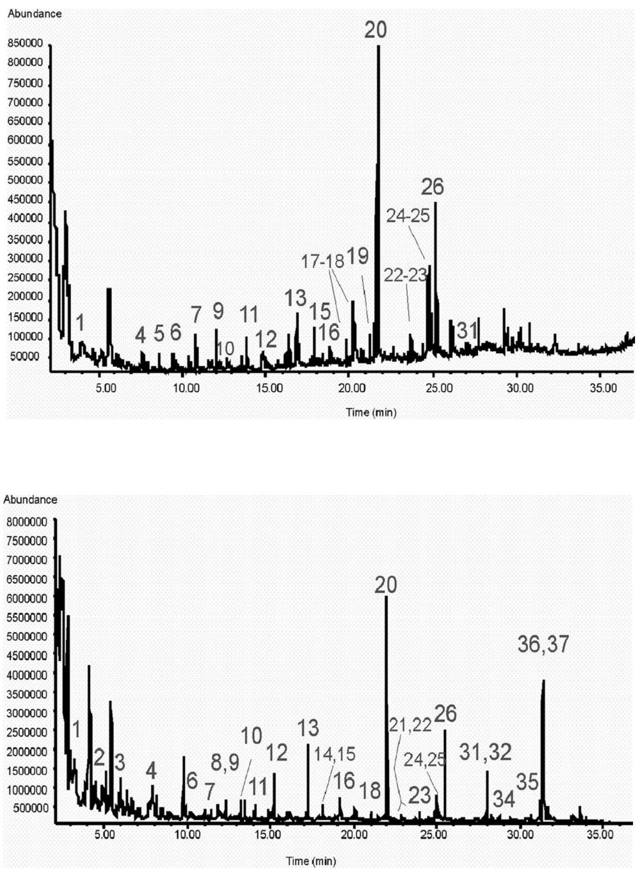

Figure 3. a) Pyrogram obtained in a sample from the studied ceramic fragment; b) pyrogram obtained in the dried Kaman oil

These two last compounds are also present in lipid products of animal origin such as egg or porcine/bovine fats as it is also shown in table 1, where are summarized the compounds identified in the studied samples together with the compounds identified in the UV aged paints bound with egg and porcine fat. Peak area ratios $A_{nonanedioic\ acid}/A_{hexadecanoic\ acid}$ (A_{C9}/A_{C16}) versus $A_{hexadecanoic\ acid}/A_{octadecanoic\ acid}$ (A_{C16}/A_{C18}) from the samples and the reference specimens have been compared as it is illustrated in Figure 2.

Values of A_{C9}/A_{C16} exhibited by the series of samples are higher than those obtained in the artificially aged egg and fat paints, suggesting that the natural ageing underwent by the rock paints has been more intense than the UV light exposition applied to the specimens simulating rock paintings and it has promoted, at greater extent, the oxidative chain scission reactions.

On the other hand, Figure 2 also shows that the peak area ratio $A_{hexadecanoic\ acid}/A_{octadecanoic\ acid}$ values obtained in the series of samples are closer to these from the aged porcine fat paint than to those from the aged egg paint.

Absence of marker compounds specific for proteins is sustaining the hypothesis of the use of an animal fat as binding medium of these rock paintings.

Animal fats are frequently found as organic residues fixed to archaeological ceramic bodies. Animal lipids consist mainly of triacylglycerols containing long chain fatty acids.

The more important saturated fatty acids present in animal fats are the dodecanoic (lauric) (C12:0), tetradecanoic (myristic) (C14:0), hexadecanoic) palmitic (C16:0), and octadecanoic (stearic) (C18:0) acids. It can also be found the unsaturated hexadecenoic (palmitoleic) (C16:1), octadecanoic (oleic) (C18:1), octadecadienoic (linoleic) (C18:2) and octadecatrienoic (linolenic) (C18:3) acids.

Peak number	Retention time (min)	Compound	Sample 21300 EX	Aged Kaman oil	Fresh Kaman oil
1	3,00	Glycerol, tris-TMS eter	v	v	v
2	4.44	Heptanoic acid, TMS ester	-	v	v
3	5,79	Octanoic acid, TMS ester	-	v	v
4	7,66	Nonanoic acid, TMS ester	v	v	v
5	8.63	Pentanedioic acid, diTMS ester	v	-	-
6	9.68	Decanoic acid, TMS ester	v	v	v
7	10.83	Hexanedioic acid, diTMS ester	v	v	-
8	12.00	Undecenoic acid, TMS ester	-	v	v
9	12.06	Undecanoic acid, TMS ester	v	v	v
10	12,77	Heptanedioic acid, diTMS ester	v	v	-
11	13.87	Dodecanoic acid, TMS ester	v	v	v
12	14,90	Octanedioic acid, diTMS ester	v	v	v
13	16.86	Nonanedioic acid, diTMS ester	v	v	v
14	17.74	Tetradecenoic acid, TMS ester	-	v	v
15	17.92	Tetradecanoic acid, TMS ester	v	v	v
16	18.86	Decanedioic acid, TMS ester	v	v	v
17	19.86	Pentadecanoic acid, TMS ester	v	-	-
18	20.77	Undecandioic acid, diTMS ester	v	v	-
19	21.22	Hexadecenoic acid, TMS ester	v	-	v
20	21.69	Hexadecanoic acid, TMS ester*	v	v	v
21	22.57	Dodecandioic acid, diTMS ester	-	v	-
22	23.50	Heptadecanoic acid, TMS ester	v	v	v
23	23.69	Octadecanol, TMS eter	v	v	v
24	24.65	Octadecadienoic acid, TMS ester	v	v	v
25	24.74	Octadecenoic acid, TMS ester	v	v	v
26	25.20	Octadecanoic acid, TMS ester	v	v	v
27	26.30	Pimaric acid, TMS ester	-	-	v
28	26.57	Sandaracopimaric acid, TMS ester	-	-	v
29	26.84	Isopimaric acid, TMS ester	-	-	v
30	27.12	Laevopimaric/Palustric, TMS ester	-	-	v
31	27.69	DHA acid, TMS ester	v	v	v
32	28.00	Diisooctyl adipate	-	v	v
33	28.10	Abietic acid, TMS ester	-	-	v
34	28.30	Eicosanoic acid, TMS ester	-	v	v
35	30.70	2-Monopalmitin, TMS eter	-	v	v
36	31.15	7-oxo-DHA acid, TMS ester	-	v	v
37	31.36	Heneicosanoic acid, TMS ester	-	v	v

Table. 2. Retention time and composition of the main components identified in the pyrograms of the archaeological sample and aged and fresh reference samples of Kaman oil

Presence of small amounts of pentadecanoic acid (C15:0) and heptadecanoic (margaric) acid (C17:0) is also characteristic of animal fats as these fatty acids are not present in vegetal oils (Vázquez et al. 2008: 1381).

Lipids are sensitive to chemical and biological processes including hydrolytic degradation that results in the release of fatty acids from triacylglycerols and β-oxidation processes leading to the loss of unsaturated acids and the formation of short chain mono and diacids.

Due to higher solubility in percolating ground water of short- and medium-chain fatty acids arising from oxidation processes it should not be expected to find it in archaeological samples coming from shelters.

Thus, degraded lipids present in archaeological samples are characterized by high concentrations of palmitic (C16:0), and stearic (C18:0) acids and oleic acid (C18:1).

On the other hand, lack of arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) acids has been previously used as an evidence of the character intentionally anthropic of this material present in the paint layer such as their inclusion as binders during the process of paint preparation rather than the result of taphonomic agents (Fiore et al 2008: 3047).

4. GILDING ADHESIVE OF TAKHT-E SOLEYMAN CERAMICS

Analysis of a series of fragments ceramics from Takht-e Soleyman palace has been previously reported by the authors (Osete-Cortina et al 2010: 319).

In this study linseed oil was identified as main component of the adhesive used for fixing the thin gold sheet to the glaze.

Interestingly, this material has been preserved in an extraordinary low degree of oxidation due to the protective effect of the metallic layer.

Results obtained in the samples analyzed have been compared with those from Kaman oil prepared according to the old recipes by mixing linseed oil and pinaceae resin and they are summarized in Table 2.

Figure 3-a shows the pyrogram obtained in a sample from the studied ceramic fragment whereas the pyrogram obtained in the dried Kaman oil is shown in Figure. 3-b.

It is interesting to note that the archaeological sample exhibited a higher content in the linoleic unsaturated long chain fatty acid (C18:2) than the reference sample.

This result, as previously mentioned, is due to the protective effect of the gold sheet that avoids the atmospheric oxidative action. On the other hand, only a small amount of dehydroabietic acid was found in the archaeological sample analyzed, which is indicative of an addition of a diterpenoid resin for the elaboration of the organic material used for attaching the gold sheet.

Other degradation products in a higher state of oxidation, such as 7-oxo-dehidroabietic acid, frequently formed during the ageing of diterpenoid resins, were not detected in a significant proportion, which is in agreement with the assumption that the adhesive has not reach a great degree of oxidation.

5. CONCLUSIONS

Py-GC-MS has probed to be an excellent tool for analyzing organic compounds when they are present as minor compounds or at trace level in archaeological remains such as rock paintings and ceramics.

Presence of an animal fat as binding medium used in the rock paintings of Peruaçu valley has been sustained in the absence of protein markers and the presence of characteristic long chain fatty acids with even and uneven number of C atoms in the chain.

Adhesive used in the ceramic fragments from the Takht-e Soleyman palace is mainly composed of a drying oil, probably, linseed oil with small additions of a pinaceae resin. The high content of linoleic and oleic acids from the drying oil as well as the absence of the highest oxidation products of the original abietane compounds from the pinaceae resin is attributed to the protective effect of the gold sheet.

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Versión española

TITLE: ***Pirólisis-sililación "in situ" (Py-Gc-Ms) como técnica para la identificación de compuestos orgánicos presentes en objetos arqueológicos***

RESUMEN: *El presente trabajo explora la capacidad y ventajas que aporta la Pirólisis-sililación GC-MS empleando HMDS como reactivo derivatizante. Esta técnica presenta la ventaja de su elevada sensibilidad y la considerable reducción del tamaño de muestra que se require para el análisis. Por este motivo, es especialmente adecuada para la detección de compuestos orgánicos en objetos que se encuentran en un avanzado estado de deterioro tal y como es el caso del arte rupestre y los restos arqueológicos. El estudio se centra en la identificación del aglutinante presente en diferentes pinturas ruprestres encontradas en los abrigos del Valle de Peruaçu (minas Gerais, Brasil) así como la caracterización del adhesivo empleado en la decoración con lámina de oro de varios azulejos vidriados procedentes de los palacios de Takht-e Soleyman (Iran) (siglos XIII-XV, periodo Iljanato). Los resultados obtenidos en el análisis de las muestras de las pinturas rupestres de Peruaçu evidencian que se empleó una sustancia lipídica de origen animal como aglutinante de la pintura. Por otro lado, en las muestras extraídas de los azulejos de Takht-e Soleyman se identificó aceite de linaza. Destacar, que este material se ha conservado en un estado de oxidación extraordinariamente bajo debido al efecto protectivo de la lamina de oro.*

PALABRAS CLAVE: *aglutinante, objetos arqueológicos, Py-GC-MS, arte rupestre, objetos cerámicos, aceite de Kaman*