Study of Complex Organic Binding Media Systems on Artworks Applying GC-MS Analysis: Selected Examples from the Kunsthistorisches Museum, Vienna

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Summary: Within the Kunsthistorisches Museum (KHM), Vienna, three off-line GC-MS analytical procedures for the identification of natural organic media have been refined, tested, and validated for the use in investigating original, historic works of art. The analysis of a sample from a Greek terracotta statue suggested that the white ground layer of the polychromy, still partly present today, contained a gum-based binding medium. Furthermore, a procedure for the simultaneous analysis of oils, waxes and resins was applied to the identification of the composition of the varnish layer from the 16th century painting "Lucretia" by Jacopo Negretti (1480–1528). In addition, in a collaboration with the Department for Conservation and Restoration, University of Applied Arts, Vienna, a series of samples from paintings on wooden panels from the Buddhist temple complex in Nako, Himachal Pradesh, India, was investigated for the presence of proteinaceous binding media.

Keywords: art objects; binding media; derivatisation; gas chromatography-mass spectrometry; organic polymers

Introduction

Chemistry plays a fundamental role in the preservation of cultural heritage enabling us to understand the mechanisms that cause objects to deteriorate and so allowing the development of processes aimed at stabilising decay and preventing further degradation. The close and effective interdisciplinary collaboration of chemists, conservation scientists, restorers and art historians is crucial to answer a great variety of questions in connection to preservation. In Austria the Conservation Science Department at the Kunsthistorisches Museum (KHM), Vienna, offers both organic and inorganic in-house analyses of art objects. These are mainly used for studying the collections of the KHM itself, i.e. Ancient Egyptian, Greek and Roman Antiquities, Medieval Art, and splendid masterpieces from the Renaissance and Baroque periods.

Since installing of a gas chromatography-mass spectrometry (GC-MS) system in 2002 three simultaneous GC-MS analytical procedures for the identification of complex organic materials (lipids, resins, waxes, proteins, and gums) have been tested, optimised and applied to the investigation of art objects either from KHM's collections or from external clients.[1,2]

The first procedure for the analysis of polysaccharides is based on an acidic hydrolysis treatment to liberate monosaccharides followed by their transformation into oximes (to eliminate monosaccharides multiple peak production) and their sub-

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sequent derivatisation by acetic anhydride into methyloxime acetates.[1–6]

As a second method a simultaneous analysis of lipids, resins and waxes based on the transesterification of fatty acids from lipid binders – using Meth-Prep II, 0.2 M methanolic (*m*-trifluoromethyl-phenyl)trimethylammonium hydroxide (TFTMAH; Alltech Associates, Inc., Belgium) as a reagent – was performed.[1,2,7–15] The relative intensity ratios of the detected fatty acids were used to identify particular lipids, their state of pre-polymerisation as well as their drying/alteration state. Resinous binding media were analysed by the transesterification of resinous acids (using the same reagent) followed by the identification of particular resins according to their resinous acid methyl esters. Waxes were identified by the presence of a series of long-chained alkanes, alkenes, and esters.

The third analytical procedure for the analysis of proteinaceous materials is based on an acidic hydrolysis to liberate amino acids, followed by the derivatisation by MTBSTFA (N-tert.-Butyl-dimethylsilytl-N-methyltrifluoroacetamide + 1% tert. Butyldimethylchlor- silane) and quantitative determination of the amino acids as their silyl derivatives. [1,2,16–19]

In this paper examples of the application of all the three refined analytical methods to selected art objects are discussed (the full description of the methods' improvements was already published in detail elsewhere [2,15]).

Experimental

GC-MS analysis was performed using a 6890N gas chromatograph connected to a 5973N quadrupole mass spectrometer, manufactured by Agilent Technologies, USA.

The Analysis of Polysaccharides

Reference Materials

Reference standards of polysaccharide materials (including plant gums, honey, sugar, starches, and dextrin) were supplied from various sources (for details see Table 1). A series of pigmented mock-ups were prepared in the Conservation Science Department, Kunsthistorisches Museum, by mixing the fresh binder with a finely ground pigment, spreading the mixtures on glass slides, and allowing them to dry in daylight at room temperature. These mock-ups were a part of a three year research project (P15640-N03, ''Chemical analysis of binding media in historic works of art'', supported by the Austrian Science Fund), whereby over 30 different lipid, proteinaceous, resinous, waxy, and polysaccharide binders were each pigmented with around 30 single pigments (see Table 2) and were aged under UV light, controlled temperature and relative humidity in turn.[2,15]

Analytical Procedure

For acidic hydrolysis the solid reference standards and the sample were separately placed in conical Reacti-vials and treated with a 1.2 M trifluoroacetic acid (TFA,

Table 1.
List of reference standards and their suppliers

Supplier	reference standard
Kremer*	•oils: linseed oil, stand oil, poppyseed oil, walnut oil •proteins: casein, rabbit skin glue, bone glue, isinglass, sturgeon glue, fish glue •resins: manila copal, dammar, mastic, sandarac, colophony, shellac, dragon's blood, turpentine, elemi, copaiba balsam •waxes: beeswax, carnauba wax, candellila wax •gums: arabic gum, tragacanth
Sigma-Aldrich*	locust bean gum, karaya gum, ghatti gum, guar gum
local market	egg, starch, honey
stock from the KHM	cherry gum, sturgeon glue

* full address of suppliers is mentioned in the end of the article in Appendix.

Table 2.
List of pigments and binding media used for the mock-ups. Pigmented oils and proteins were prepared between 2001 and 2003, resins, waxes, and gums in 2004 and 2005

pigment	supplier, order number	binding medium	supplier, order number
lead white	Kremer 46000	linseed oil	Kremer 73054
titanium white	Kremer 46280	linseed stand oil	Kremer 73200
chalk	Kremer 58000	poppyseed oil	Kremer 73600
yellow ochre	Kremer 40320	walnut oil	Kremer 73550
naples yellow	Kremer 43130	casein	Kremer 63200
cadmium yellow	Kremer 21100	egg yolk	local market
cinnabar	Kremer 42200	egg	local market
red lead	Kremer 42500	egg white	local market
ultramarine	Kremer 45040	rabbit skin glue, 3%	Kremer 63052
azurite	Kremer 10280	bone glue, 3%	Kremer 63000
smalt	Kremer 10000	isinglass, 4%	Kremer 63100
malachite	Kremer 10300	sturgeon glue	stock from the museum
verdigris	Kremer 44450	fish glue, 6%	Kremer 63550
oxide of chromium	Kremer 44200	gelatin, 3%	Kremer 63040
burnt umber	Kremer 40610	copal resin	Kremer 60190
zinc white	Kremer 46300	dammar	Kremer 60000
baryte yellow	Kremer 43940	mastic	Kremer 60050
lead-tin yellow	Kremer 10100	sandarac	Kremer 60100
cobalt blue	Kremer 45710	colophony	Kremer 60310
cerulean blue	Kremer 45730	shellac	Kremer 60450
prussian blue	Kremer 45210	dragon's blood	Kremer 37000
Indigo	Kremer 36000	gambodge	Kremer 37050
rose madder	Kremer 23610	turpentine	Kremer 70010
bone black	Kremer 47100	elemi	Kremer 62050
orpiment	Kremer 10700	copaiba balsam	Kremer 62100
schweinfurt green	Kremer 10870	bees wax	Kremer 62200
		carnauba wax	Kremer 62300
		candellila wax	Kremer 62300
		gum arabic, 3%	Kremer 63300
		gum tragacanth	Kremer 63400

100 μL). The vials were fitted with a Mininert valve to allow them to be evacuated and purged with nitrogen (repeated twice). The sealed vials were heated to 125 °C for 1 hour and cooled to room temperature. Their content was evaporated to dryness under a gentle stream of nitrogen while warming the vials to 60 °C. Ethanol (40 μL) was added twice, stirred and the content evaporated to dryness to eliminate ambient humidity, which could harm the further derivatisation of monosaccharides.

The standards and the sample were then derivatised with a methoxyamine hydrochloride in pyridine mixture (60 μL) and kept at 70 °C for 10 min to form monosaccharide oximes. After cooling acetic anhydride (30 μL) was added and heated to 70 °C for another 10 min. The final methyloxime acetates were extracted in chloroform (100 μL) and 1 μL of the extracted reaction mixture was injected into the GC inlet at a temperature of 240 °C.

Separations were performed on a DB-WAX capillary column (J&W, USA) with 0.25 mm internal diameter, 0.25 μm film thickness, and 30 m length. The temperature of the oven was programmed from 155 °C (1 min) to 235 °C (5 min) at 3 °C min^{-1}. Both the injector and the transfer line temperatures were set to 240 °C. Helium (purity 99.999%) was used as a carrier gas at an inlet pressure of 100 kPa. The MS operating conditions were electron impact ionisation (70 eV), scan rate 1 scan s^{-1} over the range *m/z* 45–550, and source temperature 200 °C. Solvent delay was 15 min. The instrument was tuned daily using the auto tune procedure. Electron ion (EI) mass spectra were measured in the total ion monitoring mode and the peak area (TIC) data were used for quantitative investigations.

The Simultaneous Analysis of Lipids, Resins, and Waxes

Standards and Reference Materials

Fatty acid standards were purchased in analytical grade from Sigma-Aldrich. Individual fatty acid standards were examined in order to obtain the chromatographic retention data and to compile a mass spectra database for the fatty acid methyl esters (FAMEs). Stock solutions of each fatty acid in methanol (1 mmol L^{-1}) were kept at 5 °C. Reference materials of drying oils, natural resins and waxes were obtained from Kremer (Germany) and pigmented mock-ups (see Table 2) prepared and treated according to the above description.

Analytical Procedure

Solid reference materials or original samples (0.2–0.5 mg) were placed in vials with conical inserts and treated with a 0.2 M methanolic solution of Meth-Prep II (30 μL) and 70 μL of a solvent mixture (methanol:toluene, 1:2 *v/v*). Each sealed vial was then heated to 60 °C for 1 h, cooled, and centrifuged. The clear solution was transferred to a new vial, and 1 μL injected into the GC at an inlet temperature of 300 °C.

GC separations were accomplished on a DB-5 MS poly(5% phenyl-95% methylsiloxane) capillary column (J&W, USA) with a 0.25 mm internal diameter, 0.25 μm film thickness, and 30 m length. The temperature of the oven was programmed to 50 °C for 1 min and 320 °C for 12 min at a heating rate of 10 °C min^{-1}. The injector and the transfer line temperatures were set to 300 °C and 275 °C respectively. Solvent delay was 10 min. The MS operating conditions were set as described above.

The Analysis of Proteinaceous Materials

Reference Materials

Reference standards for proteins (egg, casein, and a series of different animal glues) were supplied from various sources (for details see Table 1) and pigmented mock-ups (see Table 2) prepared and treated according to the previous description.

Analytical Procedure

For acidic hydrolysis the solid sample (~1 mg) was placed in a conical Reactivial and treated with a 100 μL hydrochloric acid (6 M), before closing the vial with a Mininert valve, evacuating the vial and purging it with nitrogen (repeated twice). The sealed vial was heated to 105 °C for 24 hours, and cooled to room temperature. Its contents were evaporated to dryness under a gentle stream of nitrogen while warming the vial to 60 °C. High purity water (40 μL) was added, stirred and the contents were again evaporated to dryness. Ethanol (40 μL) was added twice, stirred and the content evaporated to dryness. To minimise contamination by ambient humidity the vials were stored in a sealed dessiccator for 24 h.

The dried sample was processed with a pyridine-pyridine hydrochloride mixture (15 μL) and a silylation reagent (MTBSTFA + 1 % TBDMCS, 30 μL), and kept at 60 °C for 1h. After cooling, the reaction mixture (1 μL) was injected into the GC. Separations were performed on a DB-5 MS poly(5% phenyl-95% methylsiloxane) capillary column (J&W, USA) with 0.25 mm internal diameter, 0.25 μm film thickness and 30 m length. The temperature of the oven was programmed from 80 °C (1min) to 180 °C at 15 °C min^{-1}, and at 10 °C min^{-1} to 320 °C. The injector and the transfer line temperatures were set to 300 °C and 275 °C. Solvent delay was 10 min. The MS operating conditions were as described previously.

Results and Discussion

Identification of Polysaccharide Binding Media

During the preparation of the new permanent exhibition of the Antiques Collection of the KHM the white ground layer of a terracotta statue, "Sitting Lady with a Grenade" (Tanagra, Greece, 400 BC),

was analysed for the presence of gums as a binding medium (Figure 1a).

The signals detected by GC analysis clearly showed the presence of polysaccharides in the sample investigated. GC retention characteristics and mass spectral fragmentation patterns of these individual signals as well as the chromatographic profiles of various reference polysaccharides from our database were used to further identify the composition of the binding medium.[1,3–6,20] Figures 1b–c summarise the composition of the polysaccharide reference standards and the sample from the terracotta statue. As depicted in Figure 1b, the main components of the

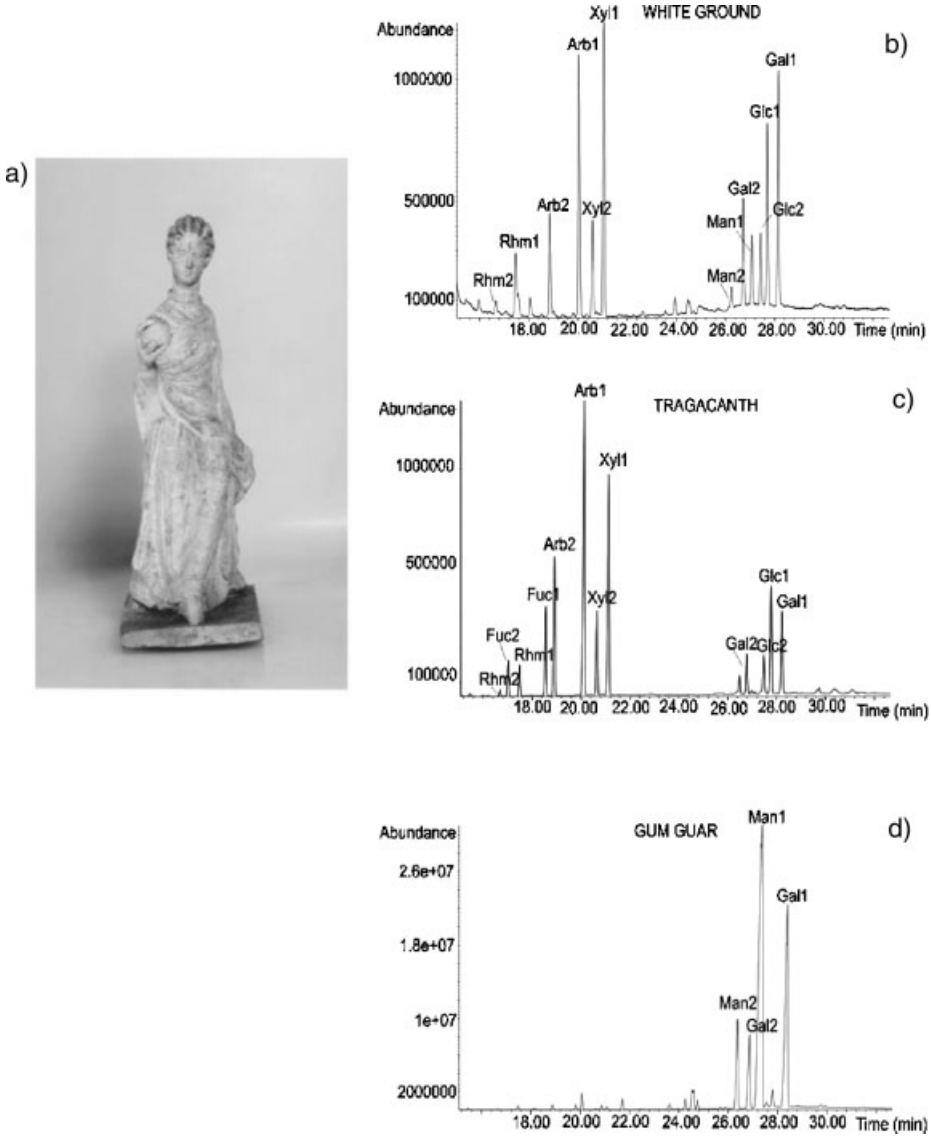

Figure 1.

"Sitting Lady with a Grenade" (terracotta statue, Tanagra, Greece, 400 BC) [1a]. GC-MS chromatograms of the white ground of a dress fold in the front upper torso of the terracotta statue [1b] and reference standards of gum tragacanth and gum guar, respectively [1c, 1d]. Fuc - fucose, Rhm - rhamnose, Arb - arabinose, Xyl - xylose, Man - mannose, Gal - galactose, Glc - glucose, 1,2 – anomers of particular monosaccharide derivative.

sample correspond to the profile of gum tragacanth (Figure 1c) with dominant amounts of arabinose and xylose monosaccharides.

However, presence of galactose and mannose can only be explained by an additional polysaccharide material, probably gum guar (Figure 1d), which mainly contains these two monosaccharides. [5,6,20] In conclusion, the white ground layer contains a mixture of two plant gums, tragacanth and gum guar, in the binding medium.

Simultaneous Identification of Oils, Resins and Waxes

During the restoration of the panel painting ''Lucretia'' (Inv.-No. 3687) by Jacopo Negretti, 1520/1530, the composition of the varnish layer was examined by GC-MS. Two samples from the upper-right part of the painting were taken with a scalpel and analysed for the presence of organic polymer materials.

Both samples indicate the same chromatographic profile as depicted in Figures 2b–c. Besides traces of linseed oil and wax, which could be part of older varnishes but could also originate from paint layers, the varnish is mainly composed of mastic and copaiba balsam. Mastic is identified by a series of triterpenoid resinous acids as their methyl esters, namely methyl moronate and methyl olenonate, and was probably a component of earlier varnishes as well as the one applied during a more recent restoration of the painting. Dominant amounts of kaur-16-en-19-oic acid methyl ester and a series of labdanes, including eperuric acid, labdanic acid, and pinifolic acid (as methyl esters), which are characteristic diterpenoid markers belonging to copaiba balsam, were also observed.

The copaiba balsam most presumably results from a previous restoration treatment. Copaiba balsam is a natural resin produced by trees of the genus *Copaifera*. In the 19th century it was utilised as an additive to oil paints as well as for a certain restoration treatment, i.e. the ''Pettenkofer method'', for regeneration of the old varnishes. The negative effects of copaiba balsam on paintings, i.e. the softening, swelling and darkening of paint layers, was already known when the balsam came into wider use at the end of the nineteenth and the beginning of the twentieth centuries. Nonetheless, the method was only abandoned some decades later.[21] From the restoration records kept in the Kunsthistorisches Museum it was already speculated that the ''Pettenkofer method'' was used in the early 20th century in the painting gallery's restoration workshop. The results shown here are the first evidence of this assumption and support the idea of the ''Pettenkofer method'' applied in the museum.

Identification of Proteinaceous Binding Media

A series of samples from the painted wooden panel ceiling from two temples – Lhakhang Gong-ma and dKar-jung – which are part of the Lot-sa-ba Lha-khang Complex in Nako, a small village in the western Himalayas in India, were investigated in a collaboration with the University of Applied Arts, Vienna.

Clay buildings of the Nako Temples are decorated indoors with early Buddhist mural paintings and polychromed clay sculptures. The investigation on the painting techniques and the materials (pigments, binding media) used for the painted panel ceilings has been carried out in the frame of the international Nako Research and Preservation Project.[22,23] The stratigraphic survey demonstrated that a preparation clay layer is directly applied onto the wooden surface, followed by a white ground based on gypsum on which different paint layers are executed.[24] Preliminary micro-chemical tests have proven the presence of proteinaceous binding media in the preparation clay layer as well as in the gypsum ground.[24] Also in the paint layers the presence of proteins was confirmed, which was in contrast with the generally accepted opinion, that the binding medium of the paint layers is based on polysaccharides (possibly apricot gum).[25] Proteins,

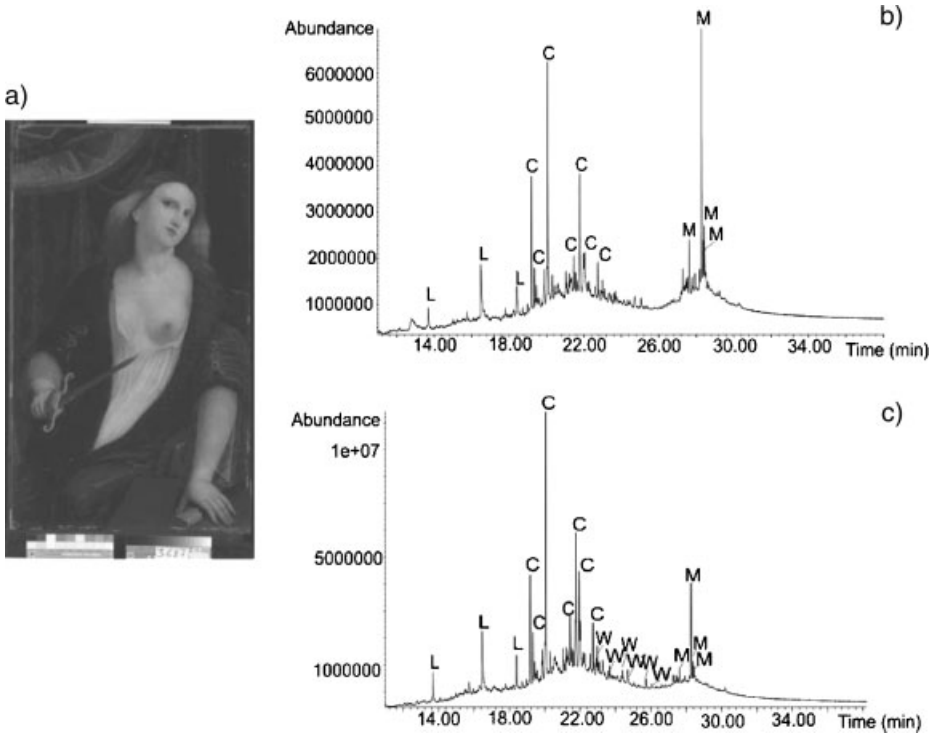

Figure 2.
"Lucretia" (oil on wood, Inv.-No. 3687) by Jacopo Negretti (Palma il Vecchio), 1520/1530 [2a]. GC-MS chromatograms of two samples of a varnish layer taken from the upper-right edge of the painting [2b] and varnish layer from the dark brown background next to the face of Lucretia [2c], respectively. L - linseed oil components, C - copaiba balsam components, W - beeswax components, M - mastic components.

more precisely animal glue, were also found as a binding medium of Nako mural paintings in previous research.[26] The goal of the present GC-MS analysis of the Nako samples from the wooden panels was to clarify the situation. The results are summarised in Table 3 and Figure 3.

As an example the composition of sample 3 is depicted in Figure 3b. The presence of hydroxyproline suggests that animal glue was used as a binding medium in the ground layer, moreover, the dominant amounts of glycine and proline and also the presence of serine shows that rabbit

Table 3.
Description of the samples from wooden panels

Sample number	Sample code	Sample location	Layer	Identified protein
1	160S1	wooden panel removed from the ceiling, now stored in the storeroom, panel 160	gypsum ground	glue (?)[a)]
2	KJ:BA/2/9	dKar-jung, bay A, section 2, panel 9	gypsum ground + paint layers	glue
3	LG: bracket 2S3	Lhakhang gong-ma, bracket n°2	gypsum ground	glue
4	LG: bracket 2S3	Lhakhang gong-ma, bracket n°2	clay layer	glue
5	LG:BA/4/21	Lhakhang gong-ma. bay A, section 4, panel 21	paint layers	glue

[a)] There is a certain doubt in the identification of the binding media composition, because it was not possible to give unambiguous assignments.

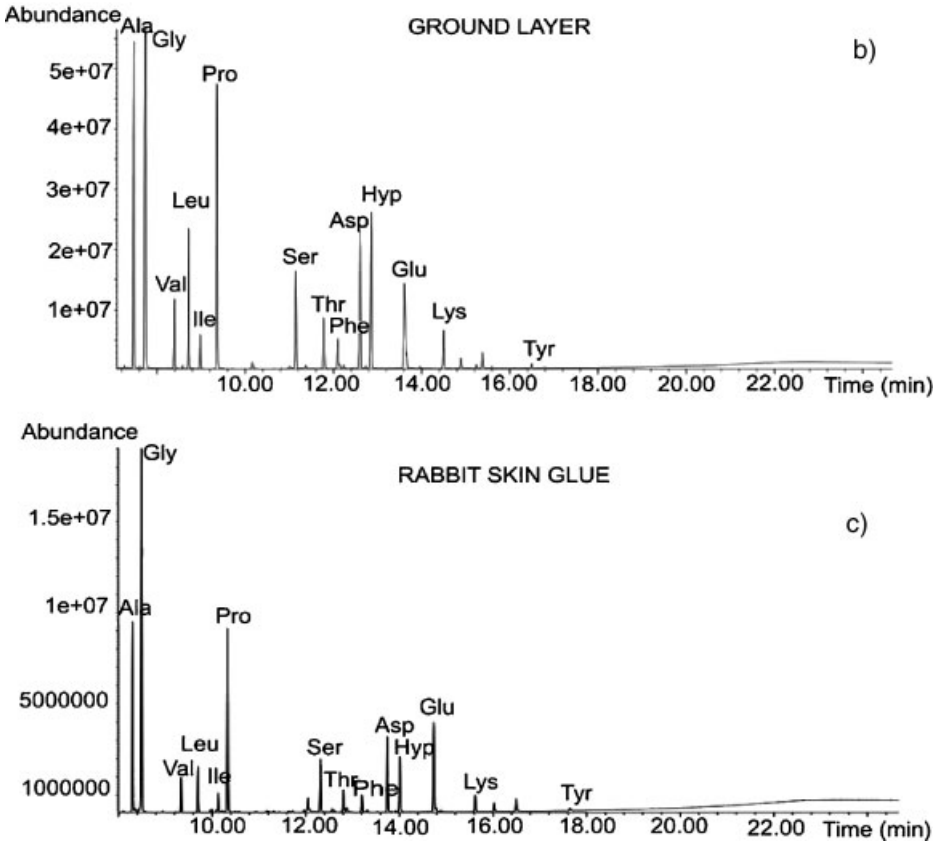

Figure 3.
Painting on wooden panels and bracket (Lhakhang gong-ma, bracket n[a] 2) [3a]. Chromatograms of the ground layer from sample 3 [3b] and a rabbit skin glue reference standard [3c]. Ala - alanine, Gly - glycine, Val - valine, Leu - leucine, Ile - isoleucine, Pro - proline, Ser - serine, Thr - threonine, Phe - phenylalanine, Asp - aspartic acid, Hyp - hydroxy- proline, Glu - glutamic acid, Lys - lysine, Tyr - tyrosine.

skin glue was probably applied as a binder. The particular proteinaceous binding medium was identified according to the amino acids relative percentage and the amino acids ratios.[17,18]

Conclusion

The GC-MS system in the Conservation Science Department of the Kunsthistorisches Museum has proven to be a useful instrumental tool in extending research activities to the field of organic material analysis. This analytical method is able to separate and identify complex mixtures of organic polymers according to specific marker components, which do not change with time. Therefore, it offers important information on the organic content of art objects. Such information is highly valued by the Museum's restorers and curators dealing with objects hundreds or even thousands of years old.

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Appendix

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