Secco painting technique revealed in non-restored Pompeian murals by analytical and imaging techniques

Silvia Pérez-Diez [a,b,*], Francesco Caruso [c,d,*], Elena Frine Nardini [a,e], Martin Stollenwerk [f], Maite Maguregui [d,*]

[a] *Department of Analytical Chemistry, Faculty of Science, University of the Basque Country UPV/EHU, Barrio Sarriena s/n, 48940 Leioa, Spain*
[b] *Department of Restoration, Directorate of Royal Collections, Royal Palace of Madrid, Patrimonio Nacional, Bailén s/n, 28071 Madrid, Spain*
[c] *Department of Art Technology, Swiss Institute for Art Research (SIK-ISEA), Zollikerstrasse 32, 8032 Zurich, Switzerland*
[d] *Department of Analytical Chemistry, Faculty of Pharmacy, University of the Basque Country UPV/EHU, Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain*
[e] *Department of Chemistry "Giacomo Ciamician", University of Bologna, Via Girotto Guaccimanni 42, 48121 Ravenna, Italy*
[f] *Department of Art Documentation, Swiss Institute for Art Research (SIK-ISEA), Zollikerstrasse 32, 8032 Zurich, Switzerland*

ABSTRACT

The fresco technique appears to have been widely used on Roman mural paintings. The close observation of many of such murals suggests, however, the application of a *secco* technique over the fresco background. In this work, for the first time, fragments from Pompeian mural paintings that had never been restored have been investigated by optical microscopy, reflectance transformation imaging, micro-Energy-Dispersive X-Ray Fluorescence, micro-Fourier Transform Infrared Spectroscopy, and Direct Temperature-resolved Mass Spectrometry. A small number of fragments from restored paintings from the same Archaeological Park was also analyzed by the same techniques for the sake of comparison. The results of this multi-analytical approach that involves - for the first time on this type of samples - scientific photographic investigation, point out to the use of a protein-based medium, most probably egg, to execute the *secco* layers. On the other hand, wax was ubiquitous in samples taken from restored mural paintings. This last evidence further highlights the importance of selecting appropriate candidates for the study of original organic binders.

1. Introduction

The predominant technique used in Roman mural paintings seems to have been fresco. However, the application of organic binders to extend the uppermost layers has been discussed by many scholars since the first excavations in the Vesuvian area [1,2].

For instance, in his work, Helbig summarized the state of the art of the chemical analysis of Pompeian wall paintings carried out in the 19th century [3] (by Chaptal in 1809 [4], Davy in 1815 [5], and Geiger and Roux in 1826 [6], among others). Geiger and Roux were the only ones to have detected organic materials purposefully added to the wall paintings. In fact, they found animal glue in the finishing layers and other fatty substances (likely related to milk) in the inner mortar layers, sometimes even mixed with wax. Coincidentally, Pliny the Elder mentions the addition of milk to painted stuccos [7,8].

Based on the lack of detection of any organic substance by Davy, Helbig stated that Pompeian mortars, constituted by lime, marble and sand, did not need an organic binder. In addition, he commented that the pristine appearance of recently unearthed Pompeian wall paintings lacked the glossy sheen given by a wax [3]. Despite this observation, he admitted as valid the detection of wax by Geiger in a cinnabar fragment from a mural painting in Hadrian's Villa [6], where it could have been applied with a *kausis* or *encausticatura* (hot wax painting) process [3,9].

Nonetheless, Mora et al. claimed that, if wax had been used as binder in Pompeian paintings, it would have disappeared due to the thermal impact of the eruption and the painting layers would have completely lost their adherence [8]. Yet, emulsified beeswax has been proposed as the painting technique employed in Roman mural paintings from Ampurias, Cartago Nova, Baelo Claudia, Emerita Augusta, Complutum and Marsala, after Gas Chromatography-Mass Spectrometry (GC–MS) and Fourier Transform Infrared spectroscopy (FTIR) analyses [2,9].

According to Winckelmann (quoted by Requeno in 1787), when paintings from Herculaneum, which had showed figurative scenes over a background, were cleaned with water, the pictorial layer was lost [10].

This could be due to a deterioration of the *secco* binder and a weaker adherence to the wall than the initial attachment between the *secco* and the fresco background or to a too harsh brushing.

In 2012, scientific analyses of samples from the Villa Imperiale in Pompeii revealed the application of paint made with a tempera binder, composed of flour, gums and oil [11]. In a subsequent publication, the analysis of fresco samples from the four painting styles in the House of Marcus Fabius Rufus constituted an interesting case study [12]. Here, no proteinaceous components were isolated. Interestingly, the organic compounds identified were similar throughout the different historical periods, although some quantitative differences were present.

Painting samples from the Villa of the Papyri in Herculaneum were also analyzed by a combination of non-invasive and invasive techniques, and the amino acid profile of one of the samples seemed to fit the one of egg [13]. This hypothesis was also reinforced by the detection of cholesterol. Finally, no resins, waxes or saccharide compounds were identified in the related samples.

On the other hand, the analysis of the murals of the Domus of Octavius Quartio in Pompeii suggests that fresco painting was the only technique used. According to the authors, pigments were mixed with slaked lime and water and applied on moist lime-based plaster, since calcium carbonate was the only binder detected [14].

As can be observed, according to the aforementioned results, various types of binders have been detected in Pompeian mural paintings. Indeed, it is true that the preferred binder could have changed from workshop to workshop and depending on the historical period. In addition, the identification of ancient binders is a challenging task, due to the degradation of the organic compounds, the small sample size, the possible presence of biological contamination, and the contamination from restoration materials. However, certain extraction methods may lead to the selective isolation of some organic compounds, hence biasing the results obtained [2]. Thus, the use of analytical techniques that do not require any extraction represents a more suitable approach.

Pliny himself wrote that encaustic painting could not be applied to walls [15]. However, rests of wax can be found nowadays [16,17]. Such a material was a common one during the preservation works carried out during the 18th, the 19th and even part of the 20th century [1,18]. Abiding by Vitruvius' and Pliny's indication on the use of wax to protect mural paintings, different kinds of organic materials were applied to protect the paintings after excavation [7]. Unfortunately, such wax-based mixtures of organic materials were eventually known to be harmful for the conservation of the wall paintings, since they preclude the transpiration of the walls and darken the surface by absorbing atmospheric pollutants and dust [19,20]. In addition, they cause the yellowing and opacification of the pictorial layer [21,22], the aggravation of salt efflorescences [23], and the enhancement of biocolonization processes [24–28]. Apart from wax, mixtures of organic products have been also employed since the 18th century (e.g., turpentine, lavender spirit, copal gum, amber, elemi gum and sandarac) [29].

This work aims to provide a first approach to the study of the binders used, if any, in selected painting fragments. For this aim, two groups of samples have been investigated. Firstly, non-restored painting fragments recovered in the recent excavations of the House of Marcus Lucretius (Regio IX 3, 5/24) were taken into account. The traceability of these non-restored painting fragments is fundamental, unlike in several scientific publications concerning this topic [12,14,18]. The second group included samples taken from restored wall paintings from the House of Marcus Lucretius, the House of Ariadne (Regio VII 4, 31/51) and the House of the Golden Cupids (Regio VI 16, 7), which were used for comparison and identification of the organic restoration materials used. The painting fragments were first observed under the optical microscope (OM) and by Reflectance Transformation Imaging (RTI), and then analyzed by micro-Energy Dispersive X-Ray Fluorescence (micro-EDXRF) imaging to identify the painting layers that had been applied over the fresco background. RTI is a computer-aided photographic method used to document artworks and samples and we employ it on Pompeian wall painting samples for the first time in this work. For the sake of completeness, RTI has already been applied to the study of historic wall paintings [30] and of ancient graffiti from Herculaneaum [31], as well as for identifying brushstrokes [32]. However, to the best of the authors' knowledge and to date, polynomial texture mapping (as RTI is also often referred to [33]) has never been used on Pompeian wall paintings. The technique consists in photographing an object with varying lighting angles, following the shape of an imaginary (or a real) dome [34,35]. Once the RTI data is processed, the illumination direction can be changed in order to evidence slight texture differences.

Then, the organic material analysis was performed by micro-invasive and non-invasive micro-Fourier Transform Infrared spectroscopy (FTIR, both in transmission and reflectance modes) and Direct Temperature-resolved Mass Spectrometry (DTMS). In line with the outcome of the work by Cuní [2], these techniques offer a direct analysis of the samples without any extraction method that could bias the results [36–38]. Hence, these were chosen for the organic binder analysis. Finally, to the best of our knowledge, this is the first application of DTMS for the study of Pompeian wall paintings.

2. Experimental

2.1. Samples

Several painting fragments, both restored and non-restored, were obtained from different locations of the House of Marcus Lucretius, the House of Ariadne and the House of the Golden Cupids (see Tables S1, S2 and S3, and Fig. S1). Restored samples were taken using a steel scalpel, with the exception made for sample 16A. Fragment 16A had already fallen from the wall when it was selected for analysis, but no information is available about how the sample fell. This could have been due to natural weathering of the wall painting or because of previously induced mechanical stress. Non-restored fragments were directly recovered from the context of the excavations.

2.2. Instrumentation

Pompeian painting fragments were observed under a Zeiss Axio microscope (Carl Zeiss AG, Feldbach, Switzerland), using ×10, ×20, ×50, and ×100 lenses and photographed with a Zeiss AxioCam MRc 1.4 MP camera. Images were acquired in dark field and using episcopic illumination. Fragments were not embedded in any resin and their surface was investigated without any pre-treatment.

RTI was used to observe the surface of the Pompeian painting fragments to distinguish overlying painting layers that could have been applied using a *secco* technique over the fresco background. The camera used for the acquisition of the necessary 48 50-MP pictures was a Canon 5DSr mounting a Canon EF 100m f/2.8L Macro IS USM lens (Canon Schweiz AG, Switzerland). The hardware kit used for the acquisition of the RTI images was purchased from Cultural Heritage Imaging (San Francisco, CA) [39]. The RTI building and viewing Windows and MacOS programs used to visualize fragments were RTIBuilder 2.0.2 and RTI-Viewer 1.1, both by Cultural Heritage Imaging.

Micro-EDXRF and micro-Raman were employed to investigate the elemental and molecular composition of the different painting layers of the mural samples [40].

Micro-XRF maps were acquired using a dual EDXRF spectrometer (M4 TORNADO by Bruker, Bruker Nano Analytics, Bruker Nano GmbH, Berlin, Germany), The X-ray tubes of this instrument are two microfocus side window Rh-tubes powered by low-power HV generators and cooled by air. One of the tubes can work between 10 and 50 kV and between 100 and 600 µA. This tube is connected to a polycapillary X-ray optics that allows to achieve an average lateral resolution of 25 µm at Mo Kα (from 17 µm at 2.3 keV to 32 µm at 18.3 keV). The second X-ray tube is able to operate at a maximum voltage of 50 kV and at a maximum current of 700 µA and the X-ray beam is mechanically collimated at 1

mm. The detection of the fluorescence radiation was achieved using a 30 mm^2 XFlash SDD with an energy resolution of 145 eV at Mn Kα.

The molecular study of the samples was achieved using the inVia confocal Raman microscope (Renishaw, Gloucestershire, UK). The main lens used was the x50 one. An excitation laser of 785 nn with a nominal power of 350 mW was employed for the acquisition of the spectra. The spectra were acquired in the 60–1200 cm^{-1} or 60–3000 cm^{-1} spectral range and accumulated 3, 5, or 10 times for 5–10 s.

A PerkinElmer Frontier FTIR spectrometer coupled with a PerkinElmer Spotlight 400 FTIR microscope (both by PerkinElmer AG, Schwarzenbach, Switzerland) featuring a Mercury Cadmium Telluride detector (MCT) was used for the micro-FTIR analyses of organic binders and restoration products in Pompeian samples. Spectra were acquired from 4000 to 650 cm^{-1} by accumulating 64, 256 or 512 scans, at a spectral resolution of 4 cm^{-1}. Whenever possible, tiny microsamples were compressed in a diamond cell and measurements were performed in transmittance mode. Different areas of the fragments were also analyzed in reflectance mode without sampling. Acquisition and qualitative evaluation of the data were performed with the Spectrum software (version 10 by PerkinElmer) without the use of correction algorithms. The interpretation of the spectra was supported by the use of the SIK-ISEA internal library, mainly based on the IRUG one [41].

Direct temperature-resolved mass spectrometry (DTMS) was performed for the analyses of organic binders and restoration products in Pompeian samples in a DSQII Thermo (Brechbühler AG, Schlieren, Switzerland) quadrupole instrument. Minute microsamples (around 10 µg) of Pompeian fresco fragments were taken with a microscalpel from the fragments, suspended in a droplet of isopropanol and applied to a noble-metal alloy filament, which was then resistively heated at a rate of 5 mA/s up to approximately 1000 mA. Samples were analyzed in electron ionization mode, electron energy of 100 eV, source temperature of 150 °C, and a scan range of 45–1050 Da. The data were analyzed on Xcalibur by Thermo and MZmine 3 software [42], both working on Windows. The interpretation of the mass spectra was achieved with the aid of the SIK-ISEA internal library, mainly based on the NIST one [43].

3. Results and discussion

3.1. Documentation of the painting fragments by OM

Fig. 1 shows the micrographs obtained by OM on non-restored painting fragments. Interestingly, six samples present an underlying yellow layer that is, in some cases, part of the visible decoration (Fig. 1a: 1EE; Fig. 1f: TB003; Fig. 1g: TB005). In three of the fragments, the yellow underlying layer does not seem to be part of the final decoration, at least from what can be observed on the surface of the fragment, although it could appear as main background hue on other parts of the wall to which each fragment belonged (Fig. 1b: 3T; Fig. 1d: 7Y; Fig. 1e: TB002). On the contrary, the underlying layer of sample TB007 is green-colored (see Fig. 1h), probably because green is the background color of the decoration.

Two of the fragments (3T and 7Y, see Fig. 1b and Fig. 1d) show a red cinnabar layer over a green layer constituted by a mixture of green earth and Egyptian blue grains and a final yellow layer underneath, analyzed by Raman spectroscopy in a previous work [40]. Although the overlying red cinnabar layer of sample 3T seems to be better preserved than that of sample 7Y, both could correspond to the same decorative scheme.

The whitish-creamy color of sample 6Rb (see Fig. 1c) was applied on a thin layer of red color. In general, white tones were always the last layer to be painted (TB002, TB005 and TB007, see Fig. 1e, Fig. 1g and Fig. 1h). As regards the blackish layers, they seem to have been painted over the intermediate red layer in all cases studied (1EE, TB002 and TB003, see Fig. 1a, Fig. 1e and Fig. 1f).

The use of a yellow plaster as final rendering over the mortar layers has also been described in the Third Style *predella* of the House of the Golden Cupids, painted with red cinnabar, where the yellow hue does not contribute to the final decoration [44]. This plaster is composed of granular lime with fine orange inclusions of goethite, as revealed by Raman microscopy. It is worth noting that this yellow-tinted plaster has been found in paintings pertaining to different locations of the House of Marcus Lucretius. For instance, in the fragments from the *viridarium*, red
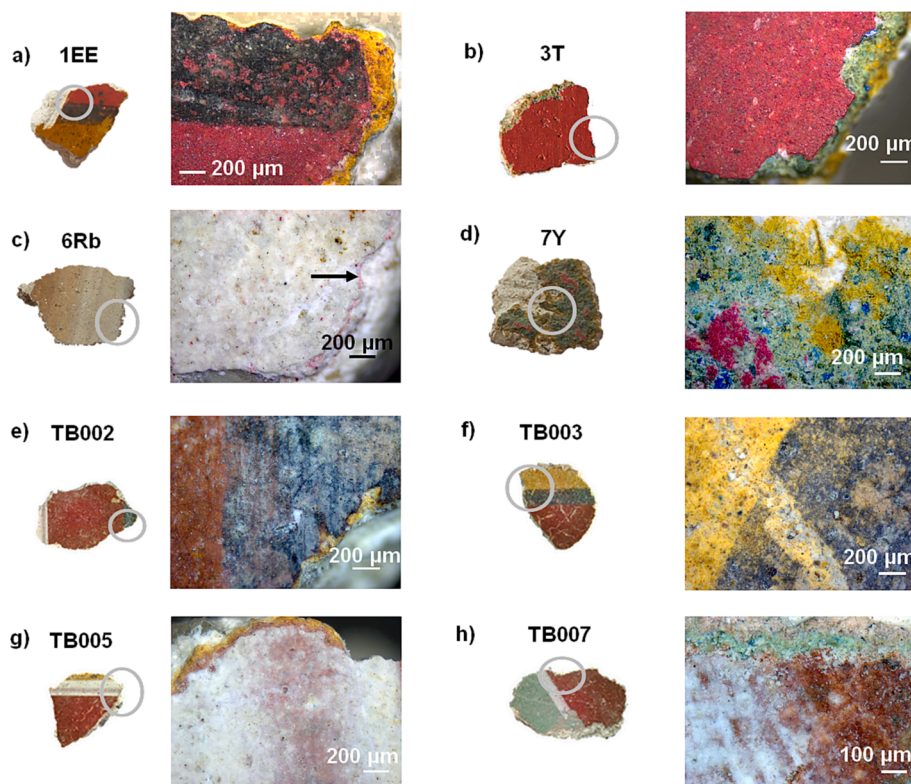

Fig. 1. Optical micrographs of painting fragments of the Second Style recovered from the *viridarium* and the secondary *atrium* of the House of Marcus Lucretius acquired in dark field. The investigated zone of each fragment is highlighted with a circle. a) 1EE: black stripe on a red background, underlying yellow plaster, part of the final decoration; b) 3T: red paint over a green layer and an underlying yellow plaster, probably not part of the final decoration; c) 6Rb: underlying red layer, marked with a black arrow; d) 7Y: red paint over a green layer and an underlying yellow plaster, probably not part of the final decoration; e) TB002: white and black stripes on a red background, underlying yellow plaster, probably not part of the final decoration; f) TB003: black stripe on a red background, underlying yellow plaster, part of the final decoration; g) TB005: white stripe on a red background, underlying yellow plaster, part of the final decoration; h) TB007: white stripe on a red background, underlying green plaster, part of the final decoration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cinnabar was employed, whereas in those found in the secondary *atrium*, originally part of a completely separate house, a red earth was used [45].

The application of tinted plasters over the rest of the mortar layers, usually applied wet-on-wet, has been described as a characteristic of Roman mural painting [8]. Indeed, a pinkish plaster, colored with red ochre, was found underneath the background colors of the upper register of the south wall and the lower register of the west and east walls of the *tablinum* of the House of the Bicentenary (Herculaneum). In other areas of the *tablinum* of the same house, the colors were applied directly on the white–grey mortar. It is believed that this fine technique, involving another layer of plaster of the desired hue, was reserved to the most important areas of the houses (known as primary presentation zones) [46].

3.2. Documentation of the painting fragments by RTI

Fig. 2 shows the RTI images of non-restored painting fragments. The diffuse gain image obtained for sample 6Rb (Fig. 2a) provides the topography of the surface of the painting layer and allows distinguishing the different brushstrokes among the slightly variable tones of the whitish-creamy hue, marked with rectangles in the figure. Moreover, indentations (either due to the painting process itself or to later manipulation) are more evident in the RTI images of fragments BC 18.95 and 33.1.18 (see arrows in Fig. 2b and Fig. 2c) than in the visible images. The RTI image of 33.1.18 (Fig. 2c and S2) also shows how the green layer was painted over a pink and a yellow one, depending on the area subsequently applied over the white background. On top of that, a red layer was painted on some areas.

Although sample 33.1.31 (Fig. 2d) is quite deteriorated, the RTI image allows appreciating certain accretions, probably caused by burial materials, and to assess how the yellow and red painting layers were applied over the background. Accretions are also observed in sample TB006 (Fig. S3a), and the flakiness of the white painting layer is noticeable in fragment TB007 (Fig. S3b).
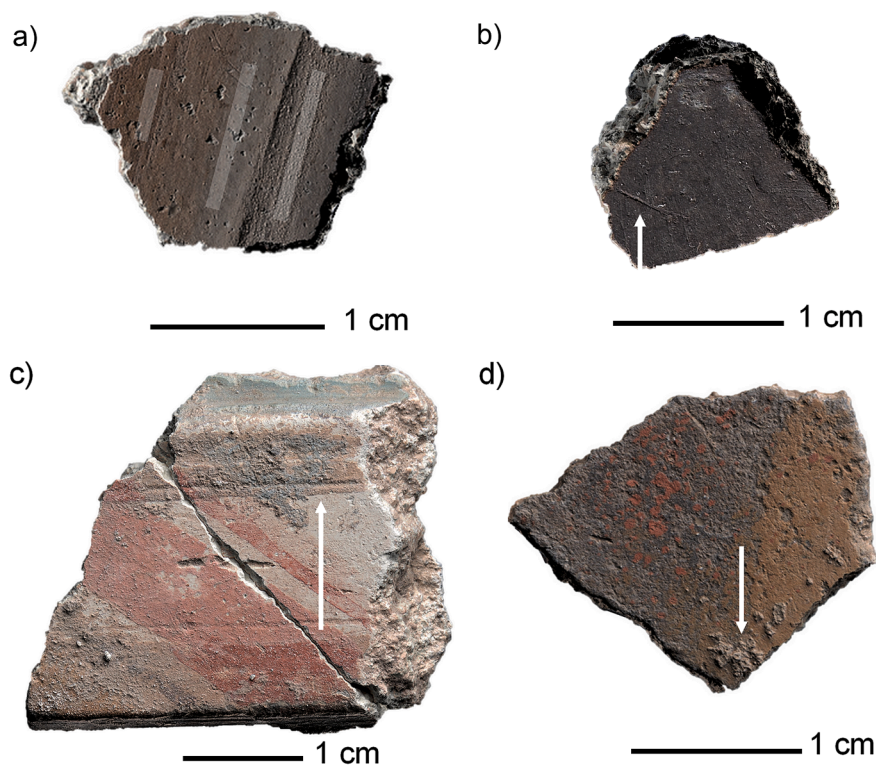
Finally, the black painting layer of M1 (from a restored wall painting in the House of Ariadne, Fig. S3c) seems to have been applied over a reddish plaster. This color is not responsible of the final hue of the area from which the sample was taken, but could correspond to the red borders of the mural (Fig. S4) or to a final rendering of the mortars with a reddish plaster, as in some areas of the *tablinum* of the House of the Bicentenary [46]. In that case, the whole wall (or, at least, the mentioned area) could have first been covered by the reddish plaster and, afterwards, the blackish decoration would have been applied.

Lastly, sample Blue-4 (Fig. S3d) is very thin, unlike most of the fragments presented in Fig. 2 and S3. The RTI image evidences pictorial layer losses consistent with the presence of a non-colored mortar, which nowadays appears greyish due to its weathering. In a previous publication, Egyptian blue was found to have been applied over a mixture of Egyptian blue and green earth (celadonite) in the wall painting from which this fragment was sampled [47]. This evidence indicates that there are at least two pictorial layers superimposed in this mural.

3.3. Micro-EDXRF imaging of non-restored and restored painting fragments

Fig. 3 presents the distribution maps of the main elements detected by micro-EDXRF on selected painting fragments in which two or more painting layers were totally or partially superimposed.

As expected, according to the OM image (Fig. 1a), the black layer of sample 1EE was painted over the red cinnabar layer (Fig. 3a), which is, in turn, painted over the yellow background. The elemental distribution maps of fragment 7Y (Fig. 3b) helped unveil the composition of the pigments used: red cinnabar (Hg map), yellow and green iron-based pigments (Fe and K maps) and dispersed grains of copper-based pigment (Cu map).

Surprisingly, the black layer of sample BC 18.95, composed of carbon black [48], was painted over a previous layer of red cinnabar, as evidenced by the Hg map (Fig. 3c). It would be in fact quite rare for

Fig. 2. RTI images (diffuse gain) of non-restored painting fragments of the Second Style. a) fragment 6Rb: brushstrokes are highlighted with partly transparent white-filled rectangles; b) fragment BC 18.95: the indentation is marked with an arrow; c) fragment 33.1.18: the indentation is marked with an arrow and the superimposition of painting layers is visible; d) fragment 33.1.31: the zone with accretions is marked with an arrow.
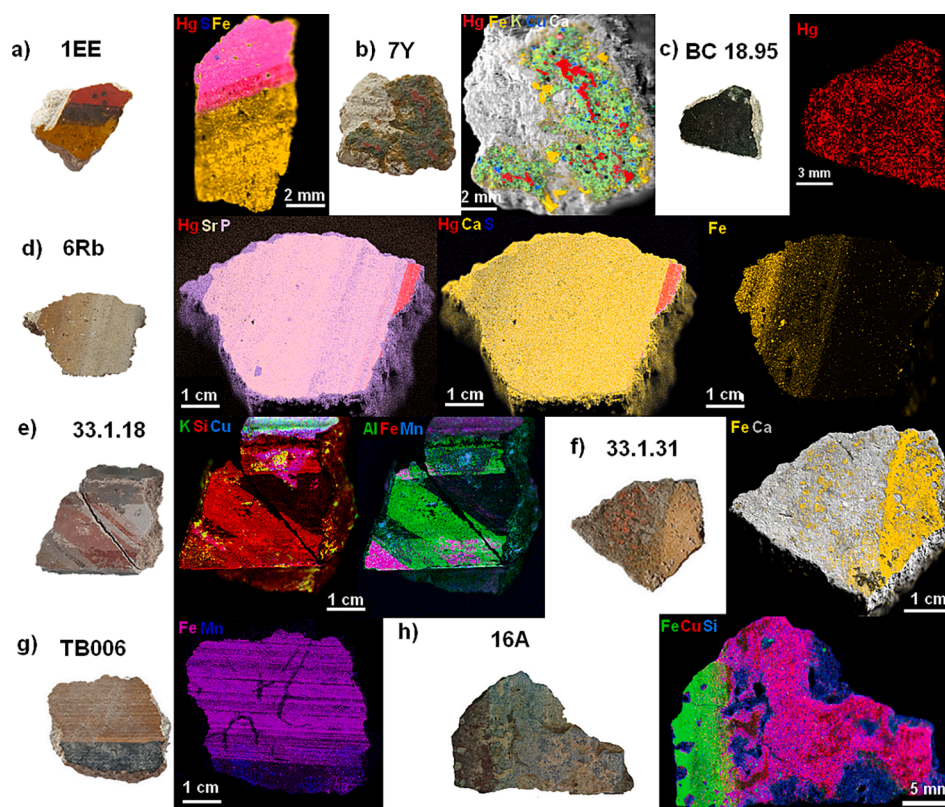
Fig. 3. Selected micro-EDXRF elemental distribution maps acquired on painting fragments of the Second Style (non-restored: a-g) and of the Fourth Style (restored: h).

cinnabar to be part of a reddish plaster (tinted with red ochre in the case of the House of the Bicentenary) [46], due to its high cost [49]. Hence, although the global composition of the Second Style painting is unknown, this fragment could correspond to a black detail painted over a lavish red cinnabar background, and not to a wall painted with red cinnabar completely covered by a black painting layer. In addition, this fragment is an interesting candidate for binding media analysis, not only because it is quite evident that the black tone was applied as an overlying layer, but also due to the information about black pigments given by Vitruvius. In fact, according to the latter, the black pigment was manufactured by burning pitch in a sort of *laconicum* (circular room with a conical ceiling and plastered marble walls). Eventually, it was mixed with either gum or other glutinous materials and used as ink by transcribers and as colored paint by stuccoers [50].

In good agreement with the hint given by the microscopic analysis (Fig. 1c), Hg and S maps of sample 6Rb confirm that there is a red cinnabar layer under the white overlying layer on the upper right corner. Besides, the distributions of Sr, Ca and P probably indicate that different white pigments (based on calcium carbonate) were used on the sample. Warmer hues of such white pigments were achieved by the gradual addition of an iron-based pigments, as the Fe map indicates (Fig. 3d).

Various painting layers overlap in fragment 33.1.18, as previously suggested by the RTI results (Fig. 2c and S2). Apart from iron oxides, mixed with manganese oxides (see the overlap of the Fe and Mn maps in pink in Fig. 3e), Egyptian blue and green earth (see the overlap of the Cu and Si maps in pink, and the K and Si maps in yellow in Fig. 3e) have been used. What is especially remarkable is that the pink hue has not been obtained by mixing red iron earth and calcite, but probably thanks to an organic pigment precipitated on a matrix of aluminosilicates [51–53]. This hypothesis is based on the superimposition of the Ti maps with the shown Al and Si maps. The presence of these elements indicates the probable occurrence of an aluminosilicate clay as the inorganic mordant of the lake pigment [51], and the lack of overlap of any map of

other element that could explain the pinkish color (e.g., Fe) with the mentioned Al, Ti and Si maps.

The painting layers of sample 33.1.31, mainly composed of yellow and red ochre (see Fig. 3f), are quite deteriorated, revealing an unpigmented mortar underneath.

Again, the micro-EDXRF imaging study of fragment TB006 (Fig. 3g) has unveiled the partial superimposition of the black layer, rich in Mn (probably pyrolusite and not carbon black), over the iron earth layer, since the Fe distribution is not completely coincident with the visual image. However, in the case of sample 16A (Fig. 3h), the superimposition of the red (green Fe distribution map in Fig. 3h) and blue (overlap of the Si and Cu distribution maps in pink in Fig. 3h) painting layers is minimal.

In this section, Fe has been selected as distinctive element of yellow and red iron-based pigments in fragments 1EE, 7Y, 6Rb, 33.1.18, 33.1.31 and TB006, whose distribution map is not perfectly coincident with that of Si, characteristic of clays, usually found as a component of yellow and red earth. On the contrary, based on Raman measurements and the EDXRF maps presented in Fig. S5 (fragment 16A), the pigments used in these fragments seem to be quite pure examples of goethite and hematite.

Lastly, it is relevant to remark that many fragments present various pictorial layers (see Table S4), thinly applied over a tinted plaster or directly on the mortar, that represent interesting case studies since they were likely painted using a *secco* technique.

3.4. Micro-FTIR analyses of restored and non-restored painting samples

Micro-FTIR measurements were conducted on non-restored painting fragments and on samples from previously restored paintings. The summary of the main compounds detected on the restored fragments and the non-restored ones is given in Table 1.

The spectra acquired in transmittance mode were more convenient

Table 1

Main species detected in non-restored and restored painting samples by micro-FTIR, both in transmittance (T) and reflectance (R) modes. Question marks indicate probable attributions.

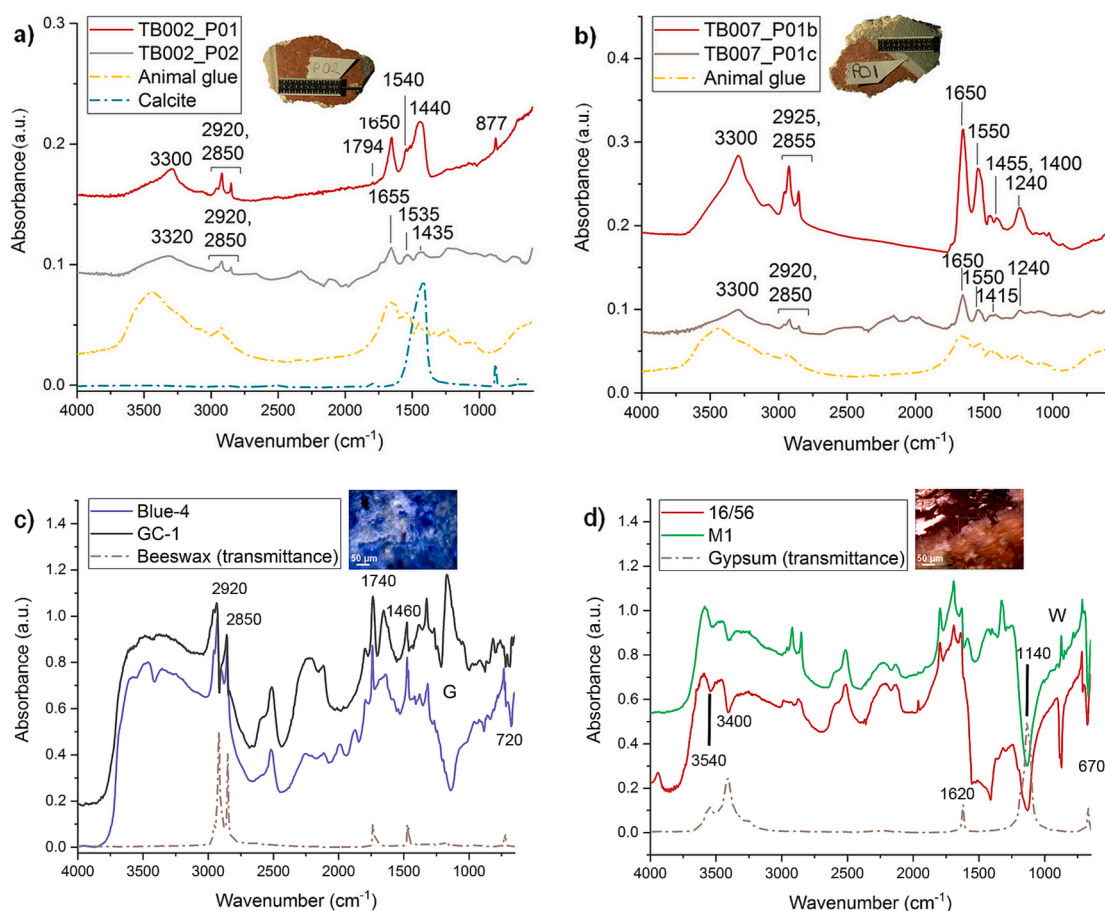| Fragments		Proteins		Gums		Beeswax		Oil		Calcite		Aragonite		Gypsum		Kaolinite		Ca-oxalate		Egyptian blue		Celadonite	
		T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R
Non-restored fragments	1EE		?								✓												
	33.1.18		?							✓	✓					✓	✓			✓			
	33.1.31		?								✓						✓						
	3T		?								✓												✓
	6Rb		?								✓						✓						
	7Y		?								✓		✓										✓
	BC 18.95				?					✓	✓			✓									
	TB001		?								✓												
	TB002	✓	?							✓	✓												
	TB003		?							✓	✓												
	TB004										✓									✓			✓
	TB005							✓		✓	✓												
	TB006										✓												
	TB007	✓	?					✓		✓	✓									✓		✓	✓
Restored fragments	1/2				?						✓				✓			✓					
	1/3				?	✓					✓							✓					
	16/56					✓					✓				✓								
	Blue-4					✓					✓			✓	✓			✓		✓		✓	
	GC-1					✓					✓				✓								
	GC-2					✓			?		✓												
	M1					✓					✓				✓								
	M2					✓					✓				✓								

Fig. 4. a) FTIR spectrum acquired in transmittance mode on microsamples taken from the white (P01) and the black stripes (P02) painted on fragment TB002, compared with the reference spectra of animal glue and calcite; b) FTIR spectra acquired in transmittance mode on a microsample (P01) taken from white stripe painted on fragment TB007, compared with the reference spectrum of animal glue; c) FTIR spectrum acquired in reflectance mode on sample Blue-4 (see measurement area in the micrograph) and GC-1, compared with the reference spectrum of aged beeswax. Note the main gypsum band, marked with G, at around 1170 cm^{-1}; d) FTIR spectrum acquired in reflectance mode on sample 16/56 (see measurement area in the micrograph) and M1, compared with the reference spectrum of gypsum. Note the wax bands, marked with W, at 730–720 cm^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for a direct comparison of the FTIR signals, since negative and derivative-like bands are prone to appear in reflectance mode, thus complicating the interpretation [54,55].

Fig. 4 presents the FTIR spectra acquired in transmittance mode on microsamples taken from non-restored fragments (TB002_P01, TB002_P02, and TB007_P01). The amide I band of proteins can be seen at 1650–1655 cm^{-1} in both samples taken from TB002 (see Fig. 4a), although the strong absorption of the 1440 cm^{-1} calcite band partly precludes the observation of the typical stair-step pattern of the rest of the amide bands, evident in the spectra of TB007 (Fig. 4b). In this last, the amide I (1650 cm^{-1}), II (1550 cm^{-1}) and III (1455 cm^{-1}) bands are clearly noticeable [56]. Animal glue has been selected as reference proteinaceous compound for comparison (IRUG reference spectrum: IPR00002).

The main corpus of micro-FTIR results is composed, however, of reflectance spectra acquired on the surface of the fragments without microsampling. Also with this spectral mode, bands attributable to proteinaceous materials were found in many of the non-restored fragments. Fig. S6a presents some of the abovementioned FTIR spectra acquired on Pompeian samples as an example. The bands at 1640 and 1515 cm^{-1}, close to those expected for isinglass (IRUG reference spectrum: IPR00013), an example of a protein-based binder, also acquired in reflectance mode, are often distorted by the derivative-like band at 1440 cm^{-1} of calcite, whose transmittance spectrum is also shown in Fig. 4a (IRUG reference spectrum: IMP00115).

The FTIR reflectance spectrum of BC 18.95, constituted by a black painting layer over a red cinnabar layer, was more similar to the reflectance spectra of polysaccharide gums or oleo-gum resins, such as myrrh gum (IRUG reference spectrum: INR00227), shown as an example in Fig. S6b, than to the reflectance spectrum of a protein. The 1660–1625 cm^{-1} bands could correspond to the deformation band of intramolecularly bound water and/or to a carboxyl stretching [56].

Apart from a proteinaceous material and calcite, characteristic bands of green earth are observable in all the FTIR spectra of Fig. S6a. This is consistent with the observations from the micrograph of sample 3T in Fig. S7a – as an example – since all measured areas corresponded to green painting layers. Green earth has been detected in other spectra (see Fig. S7a), based on the bands at 3600, 3550, 3530, 1120, 1075, 975, 960, 840, 800 and 685 cm^{-1} (IRUG reference spectrum: IMP00164) next to other pigments, such as Egyptian blue (see Fig. S7b), characterized by the bands at 1450, 1250, 1160, 1060, 1010, 755, 670, 615 and 595 cm^{-1} (IRUG reference spectrum: IMP00005).

In addition, kaolinite has been identified as part of the composition of the inorganic binder of the pink lake pigment used in sample 33.1.18 (see Fig. S8) thanks to its bands at 3700, 3670, 3655, 3620, 1120, 1030, 1010, 915, 800, 755 and 700 cm^{-1} (IRUG reference spectrum: IMP00179).

The FTIR spectra of the samples of restored mural paintings showed, on the contrary, the extensive occurrence of bands related with the presence of a restoration wax, probably beeswax (see Fig. 4c), according to the bands at 2955, 2920, 2850, 1740, 1475, 1465, 1175, 730 and 720 cm^{-1} (IRUG reference spectrum: IWX00007). This was not found on non-restored fragments. Apart from that, due to the deterioration of these in-situ preserved paintings, weathering products of the calcitic mortar have also been identified, such as gypsum (see Fig. 4d), based on its bands at 3555, 3490, 3410, 3245, 1685, 1620, 1135 and 670 cm^{-1} (IRUG reference spectrum: IMP00105).

Further organic products have been detected in restored samples. Fig. S9a presents the FTIR spectrum of sample 1/3, in which both tragacanth gum (bands at 3440, 2930, 1750, 1635, 1445, 1375, 1330, 1245, 1080 and 1040 cm^{-1}) and beeswax (IWX00007) are detected. On the other hand, sample GC-2 (a varnish) may be composed of a drying oil or other polyester product. The FTIR spectrum of sample GC-2 is shown in Fig. S9b and compared with the transmittance spectrum of walnut oil (IRUG reference spectrum: IOF00049, bands at 3010, 2930, 2855, 1745, 1650, 1465, 1380, 1240, 1165, 1100 and 720 cm^{-1}).

As mentioned above, several recipes, some based on complex mixtures of organic products, were used to protect the mural paintings of Pompeii [29]. More concretely, it is well documented that, around 1850, the *triclinium* of the House of Marcus Lucretius, from which sample 16/56 was taken, served as a case study for various essays with different protective coatings, formulated by Sebastiano Cipolla, a Neapolitan artist, and Pietro Paolo Trapani, the honorary superintendent of Pompeii. Interestingly, both were allowed to apply their own recipe to a part of the decorated murals of the *triclinium*. Later on, the then director of Museo Borbonico, Domenico Spinelli, applied another treatment on the walls of the same room [7]. This short summary of protective materials used on the House of Marcus Lucretius is an example of the superimposition of protective layers based on organic compounds throughout more than 170 years.

3.5. DTMS analyses of non-restored and restored painting fragments

The main peaks observed in the mass spectra of microsamples taken from mural painting fragments, both restored and non-restored, are presented in Table 2, where the parent peaks are highlighted in bold and the chemically related *m/z* appear in brackets in order of intensity.

It is interesting to note that almost all of the non-restored microsamples gave rise to mass spectra in which peaks related to proteinaceous material were observable, with 84–85 as main *m/z*. For instance, Stankiewicz and co-authors found *m/z* 83 to be the main peak of a pyrrolidinopiperazine derivative, with other peaks at *m/z* 110, 70 and 98, and the molecular ion at *m/z* 154 [57]. In addition, *m/z* 84 and 55 were also the most intense peaks in a mass spectrum of egg white obtained at 305 °C, next to *m/z* 70, 98, 138, 154, 111, 125, 165 and 186 [58]. These fragment ions are due to nitrogen-bearing cyclic products of amino acids, known as 2,5-diketopiperazines (DKPs, *m/z* 70, 111, 125, 154, 196). More concretely, ions at *m/z* 70 and 154 are considered to be markers of diketopiperazines derived from proline [58], whereas ion at *m/z* 84 is related with pyroglutamic acid, the pyrolysis product of

Table 2

Main peaks obtained from the mass spectra of microsamples taken from non-restored and restored painting fragments. Fragment values in bold refer to parent peaks, whereas the fragment values in brackets refer to peaks chemically related to the most intense ones.

Non-restored samples	Main peaks detected in the mass spectra (*m/z*)
1EE	**1.06 min: 206** (2 0 2), **386** (382, 368, 371, 353), 119, 80, 163, 325
	1.70 min: 84 (111, 55, 97), 132
3T	**1.33 min: 202**, 64
6Rb	**1.34 min: 202**, 64
	1.75 min: 85 (58, 70, 97, 138)
BC 18.95	**1.32 min: 202**, 64
	1.38 min: 98 (56, 82, 106, 119), 202, 64, **354**
33.1.18	**1.91 min: 84** (56, 110, 97, 70, 126)
TB001	**1.74 min: 97** (111, 64, 85, 58, 71, 126, 139, 153, 165), **367** (301, 299, 352, 379, 384, 382, 386, 269, 247, 229, 192, 174)
	2.05 min: 55 (125, 111, 96, 83, 70, 139, 152, 168)
TB002	**1.85 min: 85** (59, 64, 70, 97, 125, 110), **367** (247, 299, 286, 276)
TB003	**1.75 min: 85** (98, 70, 112), **369** (371, 368, 137, 386, 342, 271, 262)
TB004	**2.18 min: 85** (55, 97, 133, 64, 69, 125, 110), **369** (367, 353, 299, 287, 136)
TB005	**1.16 min: 368** (386, 382, 384, 369, 368, 367, 353), 59, 236
TB007	**1.84 min: 84** (59, 133, 97, 110), **369** (367, 354, 301, 247, 261, 229)
	2.13 min: 55 (119, 70, 84, 95, 110)
Restored samples	**Main peaks detected in the mass spectra (*m/z*)**
Blue-4	**1.14 min: 257** (256, 648, 621, 676, 593)
GC-1	**1.15 min: 257** (649, 705, 622, 593, 367)
GC-2	**1.17 min: 257** (256, 649, 677, 621, 705, 593, 368)

glutamic acid [59,60]. The mass spectra of 1EE and a casein standard, from the internal database of the Swiss Institute for Art Research (SIK-ISEA), are compared in Fig. 5a.

According to the literature, fragment ions at m/z 85 and 98 could also be present due to a pyrolysis product of hexose sugars, whereas ions at m/z 110, 112 and 126 could be assigned to 2-furylmethyl-ketone, 3-methyl-2-hydroxy-cyclopentenone and a fragment ion of anhydrosugars, respectively [61,62]. However, in that case study (gum arabic), the base peak was m/z 126, and not 85. Hence, the peaks observed in the mass spectra of the microsamples of Pompeian paintings and summarized in Table 2 are more likely due to a proteinaceous binder.

In addition, ions possibly related to cholesterol (e.g., m/z 367, 369, 386) were observed in the microsamples obtained from fragments 1EE (see Fig. 5b), TB001, TB002, TB003, TB004, TB005 and TB007. The attributions of each peak, based on a publication by van den Brink et al.

[63], are summarized in Table S5, while the thermogram of sample 1EE is presented in Fig. 5c.

Therefore, when both fragments related with nitrogen-bearing cyclic products of amino acids and cholesterol, next to their fragment ions, are observed in the mass spectra of the same microsamples, egg tempera could be proposed as the *secco* binder used.

In the measurements of samples from fragments 1EE, 3T, 6Rb, BC 18.95, very intense peaks at m/z 202 and 64 were observed (Fig. S10), which correspond to Hg and a dimer of S [64]. As expected, those peaks were not present in the rest of the samples, in which red cinnabar was not used as a pigment.

Finally, wax was identified in the mass spectra of the microsamples of the restored fragments (Table 2 and Fig. 5d, see thermogram of sample GC-2 in Fig. 5e), according to the base peak at m/z 257 (palmitic acid ion), next to other peaks at m/z 256, 649, 677, 621, 593, 705 and 368, whose attribution, published by Ferreira et al., is summarized in
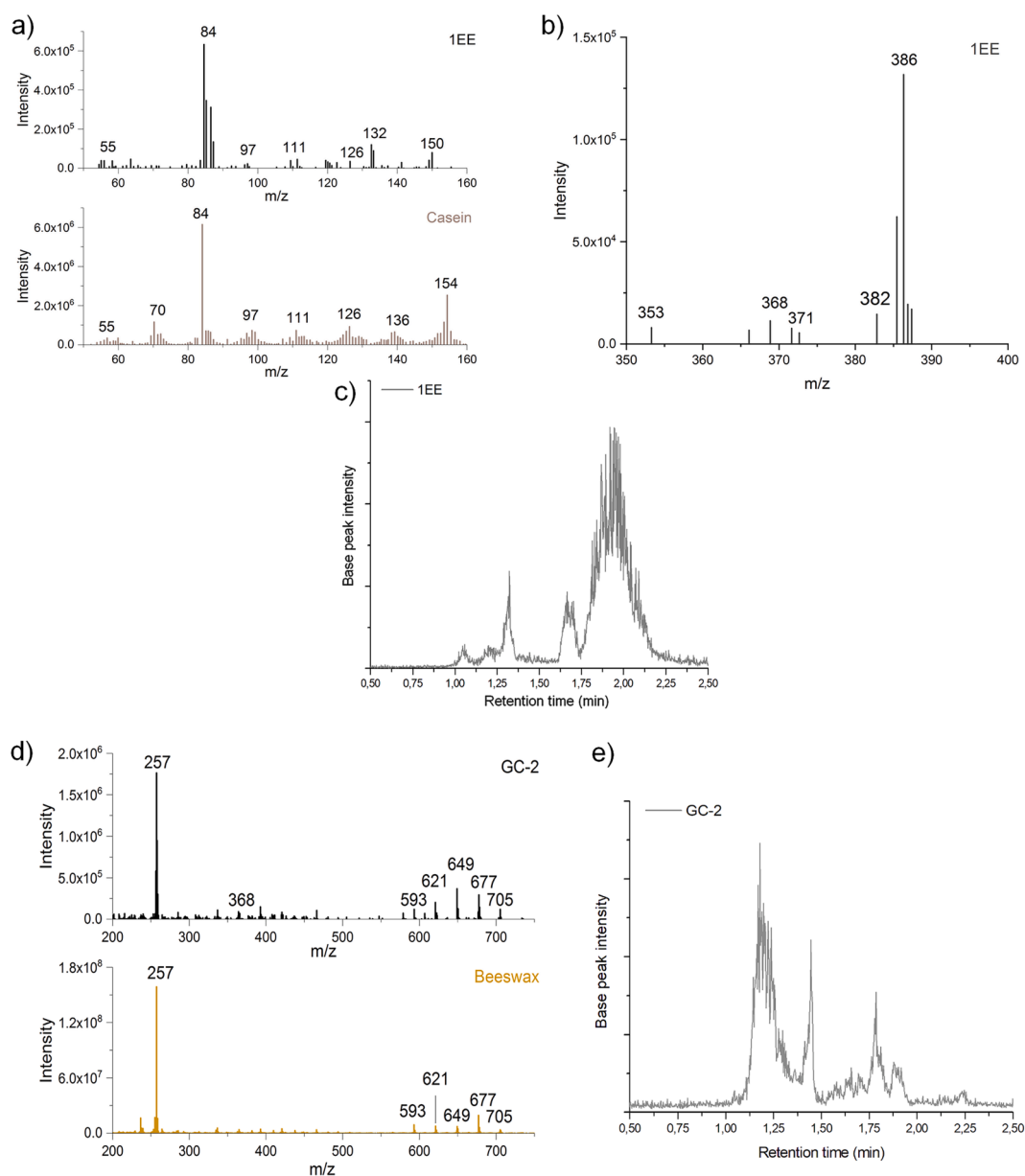

Fig. 5. a) DTMS spectrum of the microsample taken from fragment 1EE, showing peaks possibly related to the pyrolysis of proteinaceous material, and comparison with the DTMS spectrum of a casein standard; b) DTMS spectrum of microsample 1EE in which the molecular ion of cholesterol is visible at m/z 386, next to other fragment ions; c) thermogram of fragment 1EE; d) DTMS spectrum of the microsample taken from fragment GC-2, showing the characteristic m/z peaks of a wax, and comparison with the DTMS spectrum of a beeswax standard; e) thermogram of sample GC-2.

Table S6 [63,65].

Summarizing the DTMS results, the mass spectra of seven non-restored fragments (1EE, 6Rb, 33.1.18, TB002, TB003, TB004 and TB007; with *m/z* 84–85 as the main fragment) are compatible with the presence of proteins. The mass spectra of five of them specifically point out to the presence of egg (1EE, TB002, TB003, TB004 and TB007, additional fragments at *m/z* 367, 369, and 386, these last related to cholesterol). The mass spectra of the three restored fragments studied (Blue-4, GC-1, GC-2) stand out for the detection of wax (*m/z* 257), not observable in the case of non-restored fragments.

4. Conclusions

An examination of non-restored mural painting fragments through OM, RTI, micro-EDXRF imaging and micro-Raman has been relevant to assess the occurrence of overlying painting layers, likely applied using a *secco* technique over the fresco background. This superimposition of layers would have remained unnoticed in certain fragments, had not these examination techniques been used. The results from the non-destructive techniques served as a guide for microsampling, both for the FTIR analyses in transmittance mode and the DTMS measurements, without the need of selecting an extraction method that could bias the results.

It is worth remarking that the fact that these painting fragments had not been restored in the past is quite unique, since many of the results from the corpus of scientific publications were obtained from samples taken from restored paintings. This implies that, in this research, the detected organic materials are part of the original stratigraphy of the paintings and that no contamination with modern restoration materials has likely taken place. Moreover, since samples from restored paintings were also available to us, a comparison between both types of case studies is offered in this work. Further investigations should rely on non-restored decontextualized fragments coming from new excavations, both from the Vesuvian area and other Roman sites, to ensure there is no contribution of modern organic products that could bias binder analysis.

Thanks to micro-FTIR measurements, a proteinaceous material was identified in the transmittance spectra acquired on microsamples from two non-restored painting fragments. Afterwards, the reflectance spectra obtained on the surface of almost every non-restored painting fragment supported the previously obtained data. Regarding restored fragments, wax was detected in the spectra, as expected according to the restoration records of Pompeian mural paintings.

In the same line, fragment ions likely related with protein and cholesterol pyrolysates were detected in the DTMS spectra of the microsamples taken from non-restored fragments. Signals corresponding to elemental Hg and a S-dimer were also observed in the mass spectra of those samples in which cinnabar had been previously identified as red pigment. In good agreement with the FTIR results, the DTMS spectra of restored microsamples showed the corresponding peaks of a natural wax.

In conclusion, according to the results here presented, a proteinaceous material could have been used as organic binder of *secco* layers the Pompeian mural paintings considered in this work. However, additional studies, possibly using gas chromatography-mass spectrometry, with or without pyrolysis ((Py)-GC–MS), or liquid chromatography-mass spectrometry (LC-MS) are needed to further corroborate this hypothesis, and to determine the type of protein employed with a higher level of confidence.

CRediT authorship contribution statement

Silvia Pérez-Diez: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Francesco Caruso:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Visualization, Supervision. **Elena Frine Nardini:** Validation, Formal analysis, Investigation. **Martin Stollenwerk:** Methodology, Validation, Investigation, Visualization. **Maite Maguregui:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2023.109365.

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