Angewandte Chemie

Recent advances in analytical pyrolysis to investigate organic materials in heritage science

--Manuscript Draft--

Manuscript Number:	201713404R1
Article Type:	Minireview
Corresponding Author:	Maria Perla Colombini University of Pisa Pisa, ITALY
Corresponding Author E-Mail:	maria.perla.colombini@unipi.it
Order of Authors (with Contributor Roles):	Ilaria Degano, Ph.D. (Conceptualization: Equal; Writing – original draft: Lead; Writing – review & editing: Equal)
	Francesca Modugno, Ph.D. (Conceptualization: Equal; Writing – original draft: Lead; Writing – review & editing: Equal)
	Ilaria Bonaduce, Ph.D. (Conceptualization: Equal; Writing – original draft: Lead; Writing – review & editing: Equal)
	Erika Ribechini, Ph.D (Conceptualization: Equal; Writing – original draft: Equal; Writing – review & editing: Supporting)
	Maria Perla Colombini (Conceptualization: Lead; Writing – original draft: Lead; Writing – review & editing: Supporting)
Keywords:	Analytical methods; gas chromatography; Mass Spectrometry; Flash Pyrolysis; heritage science
Abstract:	The molecular characterization of organic materials in samples from artworks and historical objects traditionally entailed qualitative and quantitative analyses by HPLC and GC. Today innovative approaches based on analytical pyrolysis enable samples to be analysed without any chemical pre-treatment. Pyrolysis, which is often considered as a screening technique, shows previously unexplored potential thanks to recent instrumental developments. Organic materials that are macromolecular in nature, or undergo polymerization upon curing and ageing can now be better investigated. Most constituents of paint layers and archaeological organic substances contain major insoluble and chemically non-hydrolysable fractions that are inaccessible to GC or HPLC. To date, molecular scientific investigations of the organic constituents of artworks and historical objects have mostly focused on the minor constituents of the sample. This review presents recent advances in the qualitative and semi-quantitative analyses of organic materials in heritage objects based on analytical pyrolysis coupled with mass spectrometry.
Response to Reviewers:	Dear editor, we revised the paper accordingly to the suggestions and comments raised by the reviewers. Please find a detailed answer to the reviewers' comments below. Reviewer 2: The minireview "Recent advances in analytical pyrolysis investigation of organic materials in heritage science: from qualitative to semiquantitative information" by Ilaria Degano et al presents a very interesting overview of the state-of-the-art in analytical pyrolysis applied to cultural heritage. After an exhaustive introduction, the manuscript has been organized in three sections: 2. Semi-quantitative Py-GC/MS; 3. From DE-MS and DT-MS to EGA-MS; 4. Double-shot, multi-shot, and heart-cut Py-GC/MS. In these sections the main novelties are discussed and illustrated with various case studies. As a general comment, it is evident that the paper is very much focused on proper research and case studies that have been recently published in various journals by the same authors. Some other publications might be included. Answer: As suggested by the reviewer, more publications of other authors were included in the review (……). A case study by the authors was deleted, and a new one

on synthetic paint binders was included.

Some of the case studies are described with too much detail.

Answer: We agree with the reviewer: some case studies were discussed too much in detail. In the revised version of the manuscript, the discussion of the case studies was carefully revised and made more concise when possible.

Abstract: "The review presents the recent advancements in the field of qualitative and semi-quantitative analysis of organic materials in heritage objects based on analytical pyrolysis coupled with GAS CHROMATOGRAPHY AND mass spectrometry"

Answer: The review is focussed on pyrolysis coupled with mass spectrometry, both with and without chromatography in the between. For this reason, we would retain the original sentence.

Lines 14-20 (second column, p. 1): "For most organic materials, however, quantitative or semi-quantitative characterization in addition to qualitative analysis is necessary, because qualitative analysis alone does not allow a precise identification when the same organic molecules are present in multiple materials."
It would be useful to provide an example to illustrate this issue.

Answer: To address this comment the following sentence was added:
For example, the characterisation of proteinaceous binders by GC/MS relies on the analysis of derivatized amino acids, released by the acidic hydrolysis of proteins. The identification of proteinaceous binders is then based on the analysis of the relative amino acid profile, often by statistical tools, i.e. correlation coefficients [3] and principal component analysis [4].

Lines 32-34 (second column, p. 1): "In some cases, they are natural polymers, such as proteins, lignocellulosic polymers, plant gums or fossil and synthetic resins."
Synthetic resins are not natural polymers; it would be better to define these materials as originally macromolecular materials.

Answer: We thank the reviewer for pointing this out. The sentence was corrected as suggested.

Lines 53-57 (second column, p. 1): "These include the possibility of multi-shot pyrolysis steps,[4] of the direct coupling of slow pyrolysis with mass spectrometry as in evolved gas analysis- mass spectrometry (EGA-MS),[5] and of the coupling with tandem mass spectrometry."
Please add a reference for "coupling with tandem mass spectrometry".

Answer: We thank the reviewer for noticing it. The text was amended accordingly.

Lines 5-6 (first column, p. 3): "as demonstrated in the first archaeometric application to Egyptian mummy cartonnages".
The authors are talking about Py-GC/MS techniques. This study is on Py-MS.

Answer: The comment is correct. The paragraph has been rewritten, and in the revised version the application to mummy cartonnages is referred to the as the first application of "analytical pyrolysis"

Lines 6-9 (first column, p. 3): "A novelty in the field is represented by thermal separation probe (TSP) to perform the pyrolysis and sample introduction in the GC injection port, presented by Poulin and Helwig."
The use of TSP is indeed very interesting and very nice results were obtained on the chemical structure of amber. However, in this case the novelty is not on semi-quantitative pyrolysis. I think it would fit better in section 3 or 4.
Lines 48-55 (first column, p. 4): In my opinion this paragraph on the analysis of ambers with Py-(HMDS)-GC/MS is not related to any real novelty in the field of analytical pyrolysis. It's just an application of the classical approach. It might be removed.
Lines 12-30 (first column, p. 5): Apparently, again no real advancement in terms of methodology or use of pyrolysis is related to this study on birch bark tar. It might be removed or the reason of including this study should be clarified.

Answer: Fair comments. The examples were in the wrong part of the text, as they do not refer to semi-quantitative analyses. The description of these examples was shortened and moved to the introduction.

Furthermore, the information that is reported for some case studies might be better resumed. Too much details are given and the main advancements and information on the new developments are not emerging.
This is the case for the Aquazol analyses

Answer: We agree. The description of Aquazol analyses was summarised, highlighting the most essential information.

Lines 39-40 (first column, p. 7): "with a JMS-GCmate (JEOL Ltd.), in combination with Py-GC/MS."
Why do the authors report in this case the model of the instrument ? Is it essential ?
Answer: We agree that mentioning the model of the instrument is not necessary. The information on the instrument model was removed.

Lines 41-42 (first column, p. 7): "Tamborini and coworkers" are very frequently cited …
Maybe in some instances citation of the relevant reference would suffice…

Answer: We agree. The text was modified accordingly.

Line 46 (first column, p. 7): The reference of this case study is lacking here (at the end of the discussion ref 13b is cited but maybe it would be better to anticipate this). Also in this case the discussion might be shortened. Maybe it's not necessary to list all specific aromatic compounds that are evolving …

Answer: We agree. The correct references have been placed in the text, and the description of the example was summarised, retaining the main information only.

Figure 7: the reference is missing in the figure caption

Answer: There is no reference in the figure caption, because it is a new figure, never presented in any publication, although it is an elaboration of the material published in S. Orsini, F. Parlanti, I. Bonaduce, J. Anal. Appl. Pyrolysis 2017, 124, 643 [ref 13a]. Nonetheless, we added the sentence “Figure elaborated from the data presented in [13a].” in the caption

Other comments:
- There are numerous typos and language errors (please check British and American spelling, plural/singular forms of verbs, …)

Answer: We re-read very carefully the text to monitor typos, language errors, and to improve readability. The final version was also proofread by a professional proof reader.

- Some acronyms should be explained (SEC or GPC, SEC-RI, DSC, TGA)

Answer: In the revised version all acronyms were explicated the first time they were used in the text

- Ref 41 would better be cited in the introduction section, for example together with ref 2

Answer: We corrected it as suggested.

Reviewer 3: It is clear that the authors are experts in the theory and use of chromatography. There are many, useful references and detailed descriptions of the applications of pyrolysis to both natural and synthetic polymers in archaeological materials and more modern objects.

The minireview is structured as the journal requests. Although analytical scientists experienced in pyrolysis and chromatography will learn from this minireview, the quantity of technical terms used, often without explanation or definition, makes it unlikely that non-experts will read to the end.

Answer: To address this, all acronyms were explicated the first time they were used in the text. Several references were added dealing with the description of the analytical techniques.

Conservators are unlikely to read and learn from this article due to the technical language. If it had been structured by material instead of by analytical technique, so that the various techniques applied to waterlogged wood or amber or paint had been gathered in one place and compared, instead of being repeated in each section, its value would have been higher. In addition, the coverage of synthetic plastics is weak.

Answer: The structure of the paper was chosen to evidence novel developments of the analytical technique, which do not rely on the type of materials it is applied to, but rather on the use of the technique that is being made, thanks, also, to new instrumental developments. To avoid excessive scattering of examples in the text relating to the same materials and the lack of coverage of synthetic plastics, we removed one example dealing with archaeological wood, and included the following example, dealing with synthetic binding media, in the paragraph dealing with double-shot, multi-shot, and heart-cut Py-GC/MS:

“A similar approach was used to investigate acryl paints[39] and alkyd resins used in artworks.[40] Double-shot was fundamental to study the effect of inorganic pigments in the ageing of modern paints, by highlighting the changes in the relative concentration of the pyrolysis products in the first and second thermal desorption step.[4d] The ratios of oxidization products of CO, CO2, H2O (O) to the deacetylation product (acetic acid A), and to the to the main chain scission product (benzene B), were calculated proving that the values of O/A and O/B for all the aged samples are higher than their unaged counterparts (Figure 10).[40]”

Some of the text on the images is too small to be readable, but perhaps this is only in the proofs.

Answer: We worked on the figures, in order to improve their readability.

	Comments are added to the text. - see PDF file. Answer: The revisions suggested by the Reviewer as annotations on the pdf were used to improve the manuscript, as highlighted in the revised version of the manuscript. Whenever the comments required an answer, they were copied below, and answers are presented point by point. What does analytical pyrolysis mean? Answer: Analytical pyrolysis is the general term used for pyrolysis, when it is used as analytical technique [W. J. Irwin, Analytical pyrolysis—an overview. Journal of Analytical and Applied Pyrolysis 1, 89 (1979/10/01/, 1979).]. It is used as to differentiate the analytical use of pyrolysis from the preparative use, addressed to as “applied pyrolysis” or simply more in general as “pyrolysis”. As the term pyrolysis is more generally used to indicate the name of a process in a larger scale, we would prefer to retain the adjective “analytical” in the text. The reference was added to the text. Could the title be 'Recent advances in pyrolysis in analysis of organic materials in heritage science' Answer: The title was changed into: Recent advances in analytical pyrolysis to investigate organic materials in heritage science. The non-expert does not know what a Gestalt graph is and the small text on the figure is difficult to read. More explanation would help. Answer: “Graphical representation of the semiquantitative molecular profiles” was used instead of Gestalt graph. The figure was taken from the cited paper, so we cannot modify it. We will upload in the best resolution possible. Kemdrick mass defect: please explain Answer: As reported in the reference, “The Kendrick mass scale is defined by setting the mass of CH2 to exactly 14 mass units instead of the IUPAC mass of 14.01565. The observed accurate mass values (numbers given to three decimal places) are then converted to the Kendrick mass (KM) according to the following equation: KM = IUPAC mass x 14 / 14.01565” This was detailed in the text. Moreover, an additional reference was added which clearly explains the usage and utility of using that scale (L. Sleno, The use of mass defect in modern mass spectrometry, J. Mass Spectrom. 47 (2012) 226–236.
Section/Category:	Heritage Science
Additional Information:	
Question	**Response**
Dedication	
Submitted solely to this journal?	Yes
Has there been a previous version?	No
Do you or any of your co-authors have a conflict of interest to declare?	No. The authors declare no conflict of interest.

Frontispice

Click here to access/download

Additional Material - not for peer-review

frontispice.pptx

Recent advances in analytical pyrolysis to investigate organic materials in heritage science

Ilaria Degano, Francesca Modugno, Ilaria Bonaduce, Erika Ribechini and Maria Perla Colombini*

Abstract: The molecular characterization of organic materials in samples from artworks and historical objects traditionally entailed qualitative and quantitative analyses by HPLC and GC. Today innovative approaches based on analytical pyrolysis enable samples to be analysed without any chemical pre-treatment. Pyrolysis, which is often considered as a screening technique, shows previously unexplored potential thanks to recent instrumental developments. Organic materials that are macromolecular in nature, or undergo polymerization upon curing and ageing can now be better investigated. Most constituents of paint layers and archaeological organic substances contain major insoluble and chemically non-hydrolysable fractions that are inaccessible to GC or HPLC. To date, molecular scientific investigations of the organic constituents of artworks and historical objects have mostly focused on the minor constituents of the sample. This review presents recent advances in the qualitative and semi-quantitative analyses of organic materials in heritage objects based on analytical pyrolysis coupled with mass spectrometry.

1. Introduction

Chemical knowledge of the constituent materials of artworks is often required in order to study artistic techniques, identify degradation phenomena, and slow down or mitigate them using scientifically informed conservation approaches.

Knowledge of organic materials present as binders, varnishes or pigments entails determining the molecular profile of complex mixtures of organic molecules.[1] The most effective and commonly used analytical methodologies are based on chromatography and mass spectrometry.[2]

Analytical approaches vary and are used not only for identifying organic materials in polychrome and artistic objects, but also in a wider range of heritage objects such as natural polymers in archaeological objects. In some cases, analyses are based on the search for stable biomarker molecules so as to be able to identify the materials present on the basis of a purely qualitative analysis.[1]

Unfortunately, a qualitative approach can only be exploited in a very limited range of materials that may be present in objects of historical value: terpenoid resins, organic dyes, collagen, and such like. For most organic materials, however, a quantitative or semi-quantitative characterization in addition to a qualitative analysis is necessary, since qualitative analysis alone does not lead to an identification when the same organic molecules are present in multiple materials. For example, the characterisation of proteinaceous binders by GC/MS relies on the analysis of derivatized amino acids, released by the acidic hydrolysis of proteins. The identification of proteinaceous binders is then based on the analysis of the relative amino acid profile, often by statistical tools, i.e. correlation coefficients[3] and principal component analysis[4]. Moreover, qualitative analysis alone does not provide information on most ageing and degradation phenomena.

Thus, the characterization of organic materials in paintings or archaeological samples is generally based on quantitative analyses by high performance liquid chromatography (HPLC) and gas chromatography (GC), which provide an excellent separation and resolution of complex mixtures, as well as low detection limits. Calibrations for quantitative analysis are possible, whenever standards are available.[2a, 2b] Both qualitative and quantitative analyses by GC or HPLC are limited to those analytes that can be made soluble or volatile with adequate sample pre-treatments, including extraction, hydrolysis and derivatisation.

Most organic materials in artistic and historical objects are indeed macromolecular in nature. In some cases, they are originally macromolecular materials, such as proteins, lignocellulosic polymers, plant gums or fossil, and synthetic resins. Other materials undergo polymerization upon curing,

---

[*] I. Degano, F. Modugno, I. Bonaduce, E. Ribechini, Prof. M. P. Colombini
Department of Chemistry and Industrial Chemistry
Università di Pisa
via Moruzzi, 13, I-56124 Pisa (Italy)
E-mail: maria.perla.colombini@unipi.it

Laboratory of Chemical Science for the Safeguard of Cultural Heritage (SCIBEC, www.scich.it

such as natural resins and drying oils. Cross-linking also occurs in most materials, as an effect of the exposure to light and air during ageing. In the end, most organic materials in paint layers and archaeological objects contain a major fraction that is insoluble and chemically non-hydrolysable (high molecular weight molecules and polymers, e.g. polymerized siccative oils, cross-linked proteins, synthetic polymers). GC or HPLC alone do not give access this important fraction, thus limiting the analysis of a small, and possibly not fully representative, fraction of the samples.

In order to deal with such limitations, analytical pyrolysis[5] is increasingly being used to characterise natural and synthetic organic materials in the field of cultural heritage. It enables solid microsamples to be directly analysed without pre-treatment. This technique, which is often considered crucial only in the identification of synthetic polymers in modern and contemporary art,[6] has in fact proven successful in the analysis of a wide variety of substances and materials in the field of heritage science,[7] including the study of natural macromolecules in microsamples from precious artistic and archaeological objects.

One of the first applications of analytical pyrolysis was the analysis of Egyptian mummy cartonnages to identify the organic adhesives of objects which were 2000-4000 years old.[8] Pyrolysis coupled with gas chromatography / mass spectrometry (Py-GC/MS) was also successfully used to study birch bark tar, used as adhesive since the Palaeolithic period.[9] The chromatographic profile comprising lupane-type triterpenoids and the pyrolysis products of polymerised suberin and suberan (aliphatic acids, alkanes and alkenes) led to the identification of birch bark tar in the formulation of archaeological adhesives from a wooden spindle recovered in a Gallo-Roman burial (3rd century AD) and from the golden sheath of a Thracian dagger (8th-7th centuries BC) recovered in Bulgaria. Pyrolysis has also been used in the molecular characterisation of fossil resins,[10] entailing the identification of succinite,[10a] also known as Baltic amber, which travelled to Italy through the Amber route already during the 7th century BC.

When analytical pyrolysis is used, the chemical composition of the sample is reconstructed on the basis of the interpretation of the molecular profile of the thermal degradation products of the original components. This analytical strategy involves minimal sample manipulation and no sample pre-treatment, avoiding problems related to contamination, and losses associated with wet-chemical procedures. This makes it particularly attractive in the analysis of artworks and archaeological objects, where sampling needs to be minimised. It also enables the investigation of insoluble and chemically non-hydrolyzable molecular fractions of aged organic materials.[7]

Several natural materials, such as drying oils, proteins, plant resins and polysaccharides, produce polar products upon pyrolysis, containing, among others, amine, hydroxyl and acidic moieties. Such compounds in most cases cannot be analysed by gas chromatography, as they are too polar. To address this, methods for thermally assisted in-situ derivatization have been optimised, generally based on the addition of silylating or methylating reagents in the pyrolysis vessel.[7a, 7d]

Analytical pyrolysis has been enhanced by new technologies and techniques such as the thermal separation probe (TSP) used to perform pyrolysis (maximum temperature allowed 450 °C) and sample introduction in the GC injection port, presented by Poulin and Helwig.[10c, 11] In the first application of TSP in heritage science, the high rate of heating and relative prolonged pyrolysis time (3 minutes), proved ideal for the characterisation of ambers.[10c, 11] Other important instrumental developments include multi-shot pyrolysis steps,[6d, 12] and the direct coupling of pyrolysis with mass spectrometry as in evolved gas analysis–mass spectrometry (EGA-MS).[13]

Although analytical pyrolysis cannot be considered a quantitative technique, due to its difficulty in being adapted to calibration procedures, it still provides semi-quantitative data on a variety of materials in cultural heritage related objects. The development of different instrumental set ups and the application of analytical pyrolysis to a plethora of case studies suggest that semi-quantitative study of the macromolecules in works of art is one of the most promising fields of investigation in heritage science and will likely become a key tool to complement classical chromatographic mass spectrometric approaches.

This review presents the new developments of analytical pyrolysis in heritage science, highlighting a variety of instrumental set-ups and approaches, through a selection of the most novel and recent case studies.

Ilaria Degano is Associate Professor in analytical chemistry at the Department of Chemistry of the University of Pisa. Her research mainly deals with the characterisation of natural and synthetic organic dyes and pigments employed in works of art by chromatography and mass spectrometric techniques. She also studies organic materials such as terpenic resins, proteins, lipids in amorphous archeological residues. She has developed analytical instrumental methodologies for studying complex mixtures of organic materials and understanding ageing pathways.

Francesca Modugno is Associate Professor in Analytical Chemistry at the Department of Chemistry and Industrial Chemistry of University of Pisa. Since 2001 she has carried out research in the field of analytical chemistry applied to heritage science. She teaches analytical chemistry and chemometrics. Her research deals with the application of analytical methods based on chromatography and mass spectrometry to the characterisation and the study of the degradation of organic natural materials in heritage objects, focusing on lipids, resins, synthetic polymers and lignocellulosic polymers.

Ilaria Bonaduce is Associate Professor at the Department of Chemistry and Industrial Chemistry of the University of Pisa. She is currently lecturer in Analytical Chemistry for Cultural Heritage on the Masters degree in Chemistry. Her research focuses on the development and implementation of analytical methods for identifying organic materials in paint samples. Her aim is to reconstruct painting techniques and technologies of the past, and to understand, for example, the degradation of organic material in works of art as an effect of age, the simultaneous presence of other materials, and anthropic factors.

Erika Ribechini is Associate Professor of analytical chemistry at the Department of Chemistry and Industrial Chemistry of the University of Pisa. She teaches analytical chemistry and archaeological chemistry. Her research mainly concerns the development of analytical procedures based on pyrolysis, chromatography and mass spectrometry for the study and characterisation of natural organic substances focusing on those from archaeological findings, works of art and archaeological wooden artefacts.

Maria Perla Colombini is a Full Professor of Analytical Chemistry at the University of Pisa. From 2013-2017, she was also Director of the Institute for Conservation and Valorization of Cultural Heritage of the Italian National Research Council. Her research is related to the development of analytical procedures based on mass spectroscopy and chromatographic techniques for the characterisation of organic materials and their degradation products in works of art and archaeological objects.

2. Semi-quantitative Py-GC/MS

Since its first introduction, the online connection of pyrolysis with gas chromatography has been found to be the best approach to permit the GC characterization of high molecular weight and insoluble polymers.[4] The mass spectrometric identification of pyrolysis products after GC separation can identify polymers based on the detection of monomers, dimers and additives.[6f] Py-GC/MS data interpretation is often based on the identification of molecular markers, such as acryl monomers for acryl polymers,[6c] and hexadecanenitrile and octadecanenitrile for egg yolk,[5a,13] guaiacyl and syringyl units for softwood and hardwood,[14] substituted naphthalenes and phenols for synthetic organic pigments.[14] In selected cases, decisional flow charts can be used to identify species belonging to the same chemical class, such as triarylmethane dyes and pigments.[15]

The high reproducibility of the Py-GC/MS instrumentation has recently led to reliable conclusions from the semi-quantitative information generated by evaluating the relative abundances of different pyrolysis products from a microsample.

The role of this analytical approach has gained momentum as an increasing variety of innovative industrial polymeric products are being developed and introduced in artistic and conservation practices. An example is represented by poly(2-ethyl-oxazoline) Aquazol® as a consolidant, reversible adhesive and paint binder for retouching[16], Py-GC/MS was used in conjunction with TG analysis to understand the molecular interactions between organic pigments and Aquazol® in new formulations of retouch paints containing organic red and artificially aged pigments.[17] In this case, beside the qualitative analysis of the pyrogram, a semi-quantitative approach was adopted based on comparing the relative abundances of five groups of pyrolysis products (low molecular weight molecules (LMW), propionamide and related alkylated structures (P), monomer and related alkylated structures (M), dimer and related alkylated structures (D), trimer and related alkylated structures (T)[17a]) using multivariate principal component analysis (PCA; Figure 1). In addition, the decomposition temperature of the polymer determined by thermogravimetry (TG) was included in the data set.[17a] The differences in the qualitative profiles of pyrolysis products were related to the catalytic action of the inorganic fraction of the alizarin lake-pigment during pyrolysis, to a strong molecular interaction between the polymer and the Irgazin red pigment, and to the photo-oxidation mechanism taking place upon curing.

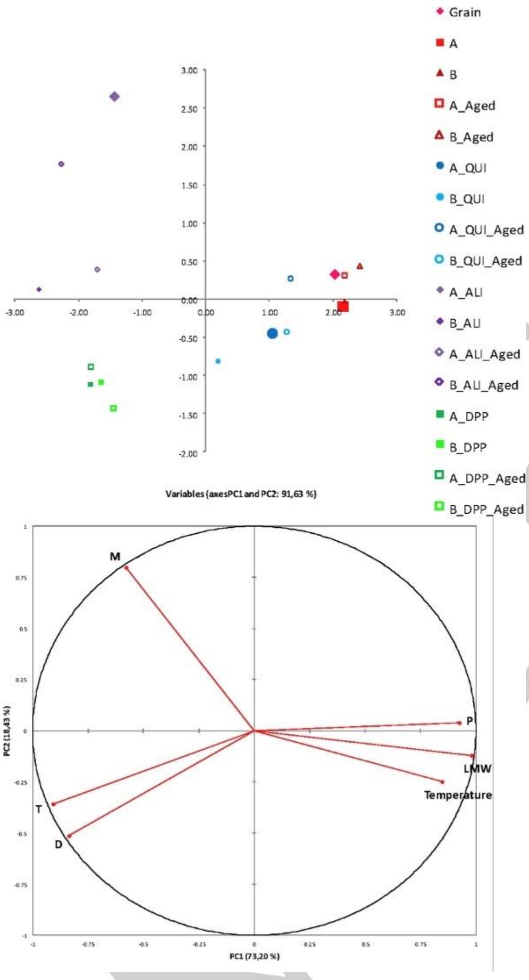


Figure 1. PCA loading plot and scatter plot of the Py-GC/MS and TG data (decomposition temperature of the polymer) of aged and unaged paint layers (two different recipes, named A and B) based on Aquazol as the binder, containing the organic pigments: diketo-pyrrolo-pyrrole (DPP), quinacridone (QUI) and alizarin (ALI).[17a]

Synthetic organic pigments have also been analysed by Py-GC/MS in pure form, before and after artificial ageing. The relative amounts of pyrolysis products in the freshly cast and artificially aged pigments changed, highlighting the photooxidation mechanisms taking place.[14b]

Regarding paint media, pyrolysis has been widely employed for the qualitative identification of proteins, polysaccharides and oil binders, although traditional wet treatments still appear to be preferable in most cases. On the other hand, pyrolysis is the common approach used for the analysis of Asian lacquers, as they contain a major fraction which is polymerised and non-hydrolysable.[18] Identifying the source of the lacquer is challenging due to the sheer number of compounds produced by pyrolysis and to the wide range of materials that have been used in traditional Asian lacquer formulations. To overcome the difficulties in analysing data obtained from Asian lacquers by pyrolysis-gas chromatography–mass spectrometry with thermally assisted hydrolysis and methylation (THM/Py-GC/MS), scientists at the Getty Conservation Institute and conservators at the J. Paul Getty Museum developed a system that was presented at the ‘Recent Advances in Characterizing Asian Lacquers’ (RAdICAL) workshop. The system uses i) the Automated Mass spectral Deconvolution and Identification System (AMDIS), a freeware program developed by NIST, ii) a customized mass spectra library, and iii) a specialized Excel workbook. The system performs automatic calculations in order to display sorted information for each material. THM/Py-GC/MS data are thus compared qualitatively and semi-quantitatively in order to unravel the sample composition. For example, the molecular profiles of urushi (from *Toxicodendron vernicifluum*) and laccol (from *Toxicodendron succedanea*) are compared in a graphical representation of the semiquantitative molecular profiles in Figure 2, highlighting differences in the composition of the two lacquers.[18a]

Urushi has often been used in mixtures with tung oil in oriental art as the resulting film shows enhanced rheological and mechanical properties[19]. The mixture dries more slowly, and the resulting film is less hard than that obtained with pure lacquer, thus facilitating manual polishing to obtain the finishing layers. Tung oil belongs to the group of drying oils that polymerise under exposure to air and light through autoxidation, a well-studied process based on free-radical chain reactions. Urushi contains a major fraction of catechol derivatives.[18b] Curing involves side chain coupling reactions through electrophilic aromatic substitutions, which are initiated by the laccase enzyme, contained in the lacquer. A recent study investigating how the curing of the mixture takes place, highlighted that catechols in urushi act as anti-oxidants for the oil, favouring cross-linking reactions over oxidation. This is demonstrated by a comparison of the pyrograms of a film of tung oil, and a film of a 1:1 mixture of urushi and tung oil (Figure 3). Dicarboxylic acids, which are the final products of oxidation reactions that take place during curing in drying oils, were in fact relatively higher in the film of tung oil than in the mixture. At the same time, short chain saturated and unsaturated fatty acids, which are the pyrolysis products of the cross-linked network of a cured drying oil, were relatively lower in the film of tung oil than the mixture.[18b]

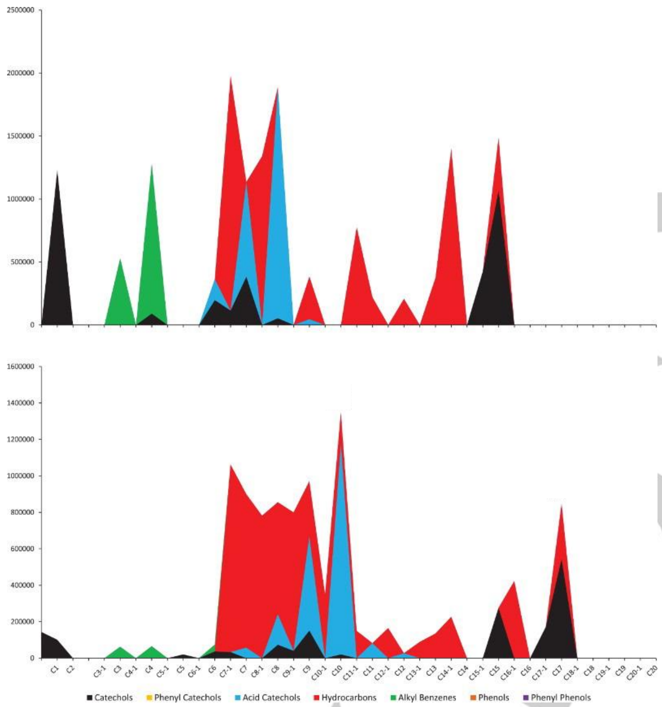


Figure 2. Graphical representation of the semiquantitative molecular profiles for: (top) aged urushi; and (bottom) aged laccol.[18a]

A quantitative approach in the interpretation of pyrolysis data has also been exploited to evaluate the preservation state of archaeological wooden objects and shipwrecks.[20] Evaluating the loss and the chemical alteration of lignocellulosic polymers (cellulose, hemicellulose and lignin) is fundamental in assessing the stability of archaeological wooden artefacts and in selecting the difficult and critical conservation treatments for large objects as shipwrecks.[21] The pyrolysis profile generated in the Py-GC/MS analysis of archaeological wood is extremely complex and more than 120 pyrolysis products have been identified in the literature. The holocellulose vs lignin ratio, which is widely recognised as the most important parameter to evaluate the level of decay of waterlogged archaeological wood, can be reliably estimated by Py-GC/MS, with dramatically lower sample amounts and analysis times than those of traditional methods based on wet chemistry.[20b, 22]

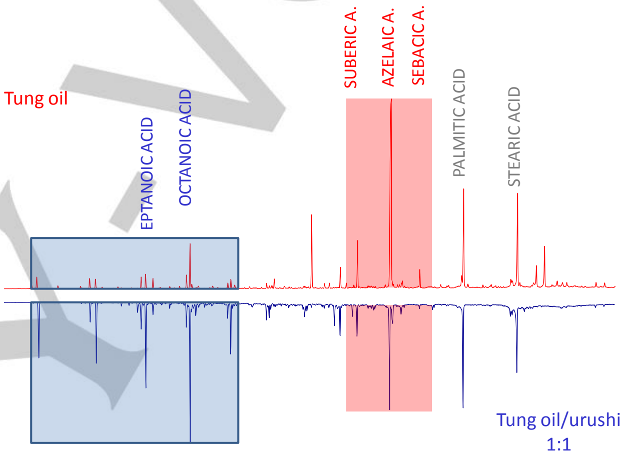


Figure 3. Comparison of the pyrolytic profiles of silyl esters of monocarboxylic and dicarboxylic fatty acids (obtained as the extracted ion chromatogram of *m/z* 129). Top: tung oil alone; bottom: mixture of tung oil and urushi 1:1.

Py-GC/MS semi-quantitative approaches can also be used to characterised shipwreck wood samples.[20d, 23] Traoré and co-workers proposed three quantitative indices as a proxy for “Shipwreck Wood Preservation Indexes” to reflect the preservation of polysaccharides, guaiacyl lignin in softwoods and syringyl lignin in hardwoods.[20d] Due to the intrinsic limitations of such indices, mainly related to the different possible chemical, physical and biological causes and alteration pathways influencing the degradation of wet archaeological wood, their application in conservation practices is far from being established. Nonetheless, semi-quantitative evaluations by Py-GC/MS of degraded wood are playing an increasingly valuable role in understanding the degradation processes of such complex bio-archaeological material.

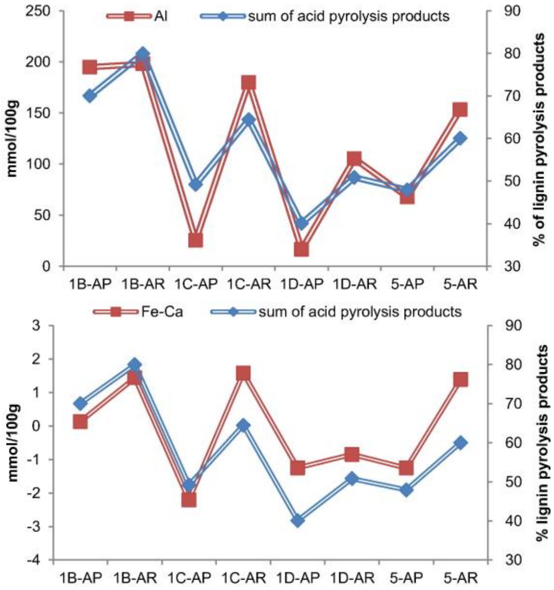


Figure 4. Comparison of the sum of the abundances of acidic lignin pyrolysis products with Al content and with Fe content minus Ca content, in archaeological wood samples from the Oseberg Viking collection.[23a]

Analytical pyrolysis was exploited to investigate the degradation of archaeological wood in the presence of unstable inorganic components, by investigating the collection of Oseberg Viking finds.[24] The artefacts were treated with alum, and contained significant amounts of iron. The amount of specific pyrolysis products had a positive and significant correlation index with the concentration of metals present in the archaeological wood samples, and in particular with Al and Fe, quantified by inductively coupled plasma mass spectrometry (ICP-MS) (Figure 4). Comparing the amounts of specific classes of pyrolysis products of lignin and polysaccharides in archaeological wood highlighted specific degradation pathways, and revealed the role of inorganic components as degradation factors.[23]

3. From DE-MS and DTMS to EGA-MS

The study of alterations occurring in heritage objects resulting from depolymerization or cross-linking is still an enormous analytical challenge, even after the improvements achieved by analytical pyrolysis interfaced with gas chromatography and mass spectrometry. The most frequently used techniques used for polymer characterization are size-exclusion chromatography (SEC) or gel permeation chromatography (GPC), and thermogravimetric analyses.

However, SEC and GPC do not provide satisfactory results, since they are limited to the sample soluble fraction. Moreover, any modification of the sample – including solubilization – can alter the results of the analyses. Finally, the accurate evaluation of the molecular weight can only be achieved if suitable materials are available for calibration.

Tirat and co-workers proved that mass spectrometric techniques applied to interpret the results in conjunction with the data obtained by SEC, lead to interesting results.[25]

On the other hand, thermogravimetric analyses often suffer from the lack of information of the species formed during the temperature scan. Several studies have been performed by TG-FTIR analysis in the field of heritage science to overcome this limitation. [26] Despite this, the interpretation of the FTIR spectra of the evolved species can be complex, and often benefits from the aid of mass spectrometry, as demonstrated in the characterization of pine pitch containing beeswax as an additive, or in studying the effects of pigments on paint materials. [26]

When data obtained by thermal analysis are integrated with those achieved by mass spectrometric systems, more satisfactory results are obtained, since a combination of mass spectrometry with thermal analyses provides profiles of the evolved/pyrolyzed species in relation to temperature increase.[6f]

The range of possible applications of analytical pyrolysis and the type of information that can be gained from a single instrumental run has been widened by using pyrolysers in a different mode, i.e. by ramping the temperature from ambient to thermodesorption temperatures and finally to pyrolysis conditions, combined with mass spectrometric detection (with or without a gas chromatographic separation in between). In this type of set-up, the sample size is reduced both with respect to classical thermal analyses (differential scanning calorimetry – DSC and TG) and SEC/GPC and is within the range of 10-100 micrograms usually employed for analytical pyrolysis.

The main set-ups for the coupling of thermal analysis and mass spectrometry used for heritage science studies are Direct Exposure or Direct Temperature MS (DE- or DTMS) and Evolved Gas Analysis MS (EGA-MS). No sample pre-treatment is required for either technique.

In DE-MS and DTMS, the solid sample (or its dispersion in a suitable volatile solvent) is applied onto a probe filament and directly introduced into the ion source of the mass spectrometer.

The filament is then heated under vacuum by a programmed current, resulting in desorption/pyrolysis. DTMS has been used primarily for qualitative analyses, especially for modern and contemporary artworks.[27] However it is highly reproducible and thus suitable for semi-quantitative analysis, as demonstrated by number of studies that have applied PCA to cluster groups in large datasets.[28] DTMS is mainly employed with instruments with nominal mass resolution. Recently, high resolution mass spectrometry has also been exploited as the detection system [29] combining the results obtained with DTMS with Kendrick mass defect analysis to characterise a series of reference paint materials. The Kendrick mass scale is defined by setting the mass of CH_2 to exactly 14 mass units instead of the IUPAC mass of 14.01565. The observed accurate mass values (numbers given to three decimal places) are then converted to the Kendrick mass (KM) according to the following equation: KM = IUPAC mass x 14 / 14.01565[30]. The authors[29] also present the results of the analysis of a sample collected from the painting “Saul and David” by Rembrandt van Rijn (17th century, Mauritshuis, The Hague), showing the great potential of direct temperature-resolved mass spectrometry for the analysis of very small and complex paint samples.

In a comprehensive study performed by Kokkori and co-workers,[31] THM/Py-GC/MS, DTMS and Electron Spray Ionisation-MS (ESI-MS) have been used to characterize the organic fraction of early 20th century oil-based enamel paints. Both reference materials and case studies are presented. As expected, formulations and processing technology influenced the physical and drying properties of oil-based enamel paints. In DTMS, differentiation is mainly achieved between low molecular weight (volatile) organic species, which are evaporated and detected early in the analytical temperature program and cross-linked, or macromolecular components that are pyrolysed and analysed later. The data obtained by DTMS matched and complemented those achieved by Py and ESI-MS.

In EGA-MS experiments, as in DTMS, mass spectra are obtained at each measuring point in the curve, so that an average mass spectrum can be calculated over the entire analysis time. Selected ion curves are also useful for monitoring the evolution of specific classes of volatile components or single compounds by extracting their characteristic m/z.[3] In EGA-MS however, temperature ramps are significantly slower, and desorption/pyrolysis takes place in a separate unit, connected via an inert column to the mass spectrometer.

Although the EGA system is a relatively new approach, it has already been used to investigate highly chemically complex materials such as synthetic polymers and lignocellulosic materials.[32] This technique provides information both on the thermochemistry and thermo-complexity (EGA) of a sample along with detailed compositional data and compound distribution (MS).

To date relatively few studies have applied EGA-MS to cultural heritage related materials, which nonetheless has proved its promising performance regarding the understanding of complex issues in the field.

Shilling and Learner applied DTMS to synthetic materials in the late 1990s,[6c] and more recently applied EGA to plastics, proving it capable of identifying a wide range of polymers and additives, and of assessing the effects of polymer ageing.[3] They tested the use of mass spectral libraries with EGA data, both comparing the unknown spectra with an in-house library and with a NIST library. The results of the EGA analyses were compared with those obtained by thermogravimetry, highlighting the correspondence of the weight losses of the plastics revealed by TGA and the thermal degradation products detected in the EGA-MS set-up (see Figure 5).

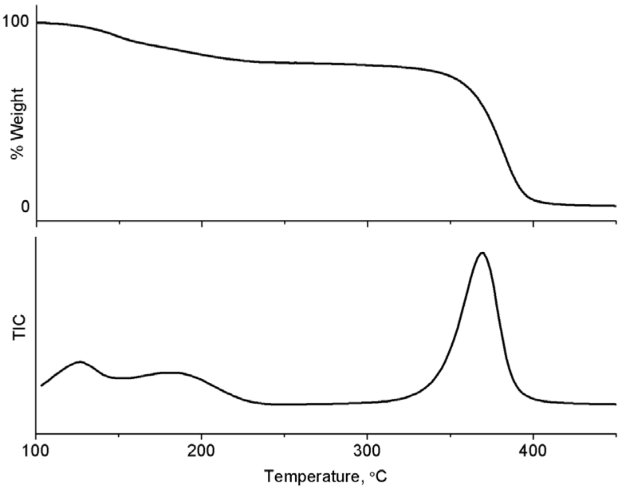


Figure 5. Direct comparison of thermogravimetric analysis and EGA. Results of thermogravimetry (top) and evolved gas analysis (bottom) from a sample of cellulose acetate provided by Goodfellow Cambridge Ltd. [3] ©2011 J. Paul Getty Trust.

After gaining experience in Py-GC/MS, Tamburini and co-workers used EGA-MS to investigate lignin oxidation and depolymerization in archaeological wood .[32c] The reference compounds and materials were first characterized by DE-MS and Py-GC/MS to create a suitable mass spectra database. The materials were then analysed by a dedicated EGA-MS method. The thermograms obtained were interpreted by comparing the Extracted ion thermograms for significant masses, revealing variations in the cellulose, holocellulose and lignin contents. In order to evaluate the parameters usually employed in the study of archaeological wood, indices were calculated based on the different intensities of the m/z signals in the thermograms. The results confirmed the semi-quantitative potential of EGA-MS. In addition, the application of EGA highlighted different zones of thermal degradation, which more traditional pyrolysis methods would not have been able to detect.

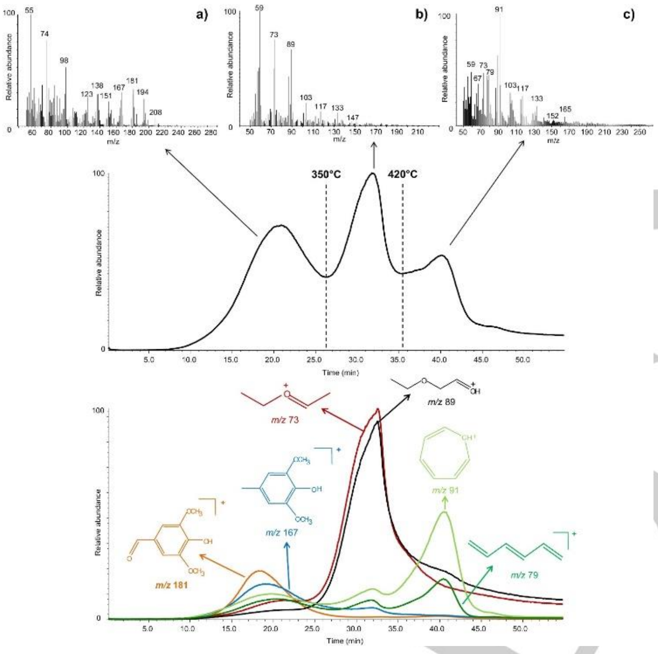


Figure 6. Total ion thermogram obtained by EGA-MS analysis of a sample from the Roman ship Lyon2, discovered in the Saône River (France), and treated with a solution of PEG 4000 and disodium sebacate, and overall mass spectra obtained in the ranges a) 10–26 min, b) 27–34 min and c) 35–45 min. Extract ion thermograms are reported of evolved gas during the thermal degradation and the molecular formula of selected ions. The first thermal degradation region (200-350 °C, a) corresponds to the thermal degradation of wood, with fragment ions with m/z 123, 137, 138, 151, 167, 181, 194, 208, produced in the pyrolysis of lignin, and fragment ion with m/z 98 deriving from furan and pyran structures produced during the pyrolysis of wood polysaccharides. The second thermal degradation region (350-420 °C, b) corresponds to PEG degradation. The third thermal degradation region (420-500 °C, c) shows fragment ions derived from PEG and disodium sebacate.[20c]

EGA-MS analysis of archaeological wood has been invaluable in investigating extremely complex systems, such as archaeological shipwrecks treated with polyethylenglicol based consolidants and disodium sebacate as a buffering/chelating agent (Figure 6).[20c] The different materials in various composite samples had a different stability towards thermal degradation, which was exploited for EGA separation, with almost no overlap between the thermal evolution of wood and polyethylene glycol (PEG). In addition, by comparing different samples, the thermal degradation ranges (in terms of temperature intervals) of wood and PEG were related to the different states of degradation of the two materials.

EGA-MS also proved promising in studying oriental lacquers, complementing the information obtained by traditional pyrolysis-based methods. The first applications of evolved gas analysis to urushi lacquers were performed by Niimura with Py-GC/MS.[33] EGA, in combination with conventional pyrolysis, highlighted the formation of a thermally stable copolymer between the oriental lacquer and tung oil, when the two materials were applied in mixture.[18b]

Py-GC/MS in combination with EGA-MS can also be used to study the proteinaceous materials used as paint binders.[13a, 34] In a recent study, paint reconstructions and samples from different historical periods (2nd century BC - 20th century AD) and geographical origins, collected from paintings and archaeological findings, were investigated with analytical pyrolysis[13a]. Data indicated that egg, casein and animal glue can be identified and distinguished in a sample of unknown composition using analytical pyrolysis based on the detection of diketopyperazines (DKPs): cyclo(Pro-Ile) and cyclo(Pro-Ala) were the most abundant in casein, cyclo(Pro-Val) and cyclo(Pro-Ile) in egg white, and cyclo(Pro-Gly), cyclo(Pro-Hyp) and diketodipyrrole were the most abundant DKPs found in collagen[13a, 35]. Aromatic and heteroaromatic compounds are also produced by the pyrolysis of proteins, showing different molecular patterns,[13a] which are produced by the thermal decomposition of amino acid lateral chains, and by the pyrolysis of the residual material remaining after condensation reactions, pyrolytic scissions and cyclization reactions.[34] Differences in the pyrolytic profiles however, disappear with ageing, leading to very similar pyrolytic profiles for extremely degraded samples, irrespective of the nature of the proteins originally present.[13a] The older and more degraded the sample, the smaller the relative amounts of DKPs produced by pyrolysis. In addition, the higher the temperature at which the evolution of nitrogen-containing aromatic compounds occur, the smaller the number of nitrogen-containing compounds that are detected in the pyrograms, and the higher the relative amount of non-specific polycyclic aromatic hydrocarbons produced by pyrolysis (Figure 7).[13a] This indicates that proteins tend to become more thermally stable with ageing, which suggests that extensive intramolecular and intermolecular aggregation and covalent cross-linking occur over time. Interestingly, egg yolk, which historically was used as a paint binder, seems to show a different behaviour, as its pyrolytic marker compounds - hexadecanonitrile and octadecanonitrile - are significantly more stable than DKPs.[32a]

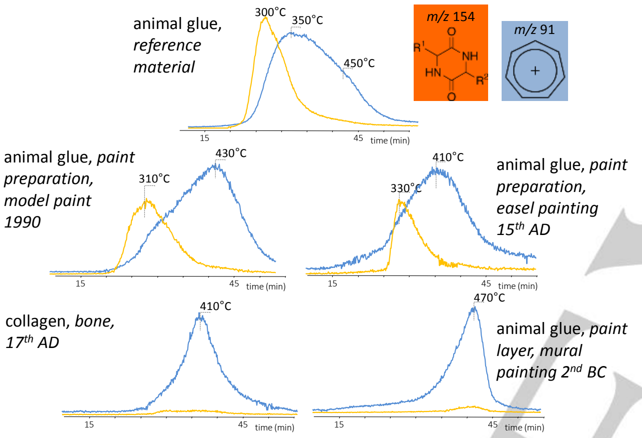
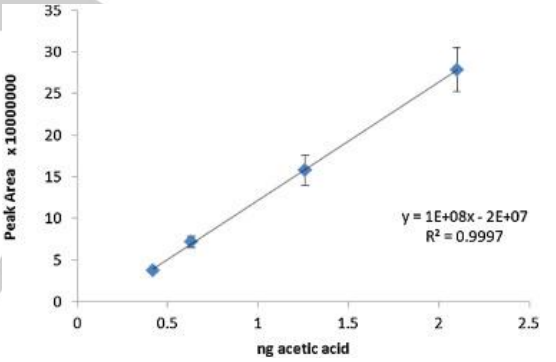


Figure 7. EGA-MS of proteinaceous materials in artistic and archaeological samples: comparison of the extracted ion thermogram of ion with *m/z* 154 (orange highlights the evolution of DKPs) and ion with *m/z* 91 (blue, to display the evolution of aromatic compounds containing a benzyl unit). Figure elaborated from the data presented in [13a].

Although most papers present the semi-quantitation of analytes by pyrolysis techniques, EGA-MS is also suitable for absolute quantitation. Samide and Smith[36] developed a method to quickly evaluate the off-gassing of acetic acid from construction materials used in museums. Acetic acid is a common pollutant in museum environments,[37] and its off-gassing from wood products and some polymers commonly employed as conservation or display materials represent a threat to collections. The amount of acetic acid and other corrosive substances is commonly evaluated with the Oddy test.[38] This involves placing the material for 28 days at 60 °C, in a sealed borosilicate glass tube containing a small amount of water and three high purity metal coupons: lead, silver, and copper. Based on the level of corrosion on the coupons, the material is deemed either as suitable for permanent use, temporary use, or as unsuitable. The method presented in [38] allows the quantitative determination of acetic acid evolved from a sample of known dimensions to be performed using evolved gas analysis coupled to GC/MS. The EGA approach appears to be a rapid, sensitive and viable alternative to more traditional methods based on Solid Phase Micro Extraction (SPME)-GC/MS or Thermal Desorption (TD)-GC/MS after adsorption on Tenax tubes. The sample is heated up at 115 °C for 30 seconds, evolved gases are trapped by a cryotrap, and then analysed by GC/MS. Figure 8 reports the calibration curves and some of the data obtained through external calibration. The study shows how EGA-GC/MS can be a valid, fast and efficient approach for screening materials used in museum environments. It highlights changes in polymer formulations, and can be used for quantitative measures, and also supports the selection of the most appropriate construction materials for museums and galleries.



	Amount (ng/mg)	Percent error
Cellulose acetate	5.3 ± 0.2	4.7
Poly(vinyl acetate)	0.0826 ± 0.0001	0.13
Jade 403 (TALAS)	0.0462 ± 0.0006	1.2
Jade 403 (Light Impressions)	0.059 ± 0.002	2.6
Titebond II, freshly cured	5.0 ± 0.5	9.1
Elmer's school glue, aged	0.21 ± 0.01	5.3

Figure 8. Calibration curve for acetic acid using a selected-ion chromatogram (SIC,60 amu) and table presenting the average quantity (n=3) of acetic acid evolved from 12 mg of various polymer samples under EGA conditions.[36]

4. Double-shot, multi-shot, and heart-cut Py-GC/MS

The analysis of samples by EGA-MS highlighted that when dealing with macromolecular materials, performing a Py-GC/MS analysis at a high temperature might hinder important information related to the most volatile fractions of the sample. Systems have been developed that perform subsequent pyrolyses at different temperatures on the same sample. These techniques are known as heart-cut pyrolysis or multi-shot pyrolysis. The instrumental set up enables two (or more) desorption and/or pyrolysis steps to be performed at specifically selected temperatures. The products of each step are focused, possibly by exploiting a cryotrap system, and separately analysed by GC/MS. Figure 9 shows the thermal program used in the double shot Py-GC/MS.[39]

In the double shot Py-GC/MS, two pyrograms are obtained, which are useful when studying the photochemical degradation of polyvinyl acetate paints used in artworks, by a combination of a thermal desorption step (100-250 °C with cryotrap) for the analysis of volatile surfactants, and a pyrolysis step (600 °C) of the polymeric binder. Differences in the amounts of plasticizers and degradation products between the unaged and aged paint layers are revealed, and specific ratios between the abundances of the observed pyrolysis products are identified as oxidation parameters and are related to the effects of different pigments on photo-oxidation reactions.[12]

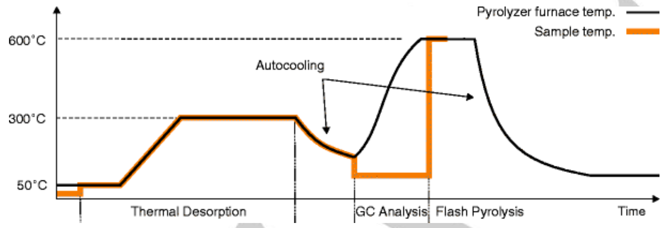


Figure 9. Heating curve of the double-shot technique for Py-GC/MS.[39]

A similar approach was used to investigate acryl paints[39] and alkyd resins used in artworks.[40] The double-shot was fundamental in studying the effects of inorganic pigments in the ageing of modern paints, by highlighting the changes in the relative concentration of the pyrolysis products in the first and second thermal desorption steps.[6d] The ratios of the oxidation products of CO, CO_2, H_2O (O) to the deacetylation product (acetic acid A), and to the main chain scission product (benzene B), were calculated by proving that the O/A and O/B values for all the aged samples are higher than their unaged counterparts (Figure 10), indicating the influence of the pigments in the ageing of the paint.[40]

Double shot pyrolysis has also helped to determine the molecular composition of dammar resin.[41] The combined used of double shot pyrolysis with TGA as well as flow injection analysis with electrospray ionization mass spectrometry, and GC/MS, highlighted that the raw resin was mainly comprised of a fraction of free terpenoids (63%). In addition, there was an oligomeric fraction (30%), containing the same terpenoids found in the free fraction bonded through ester bonds.

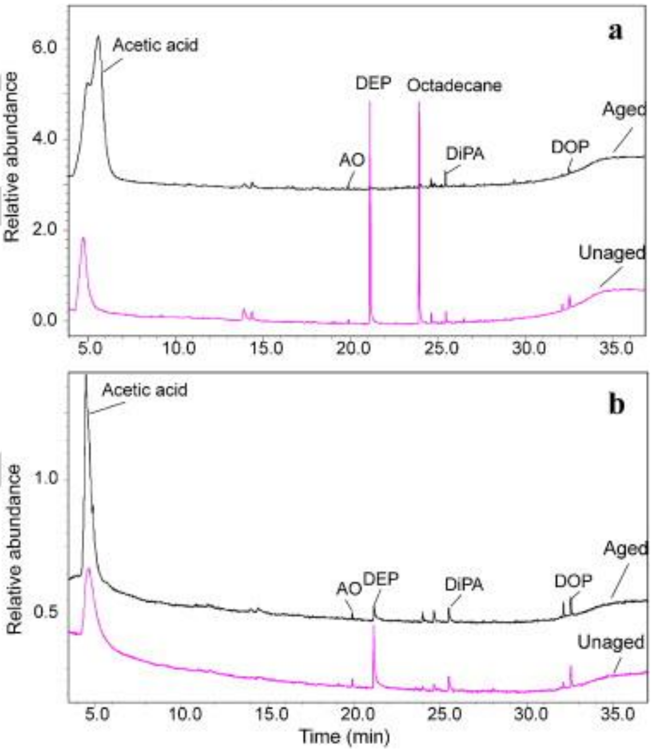


Figure 10. Pyrograms obtained by Py–GC/MS with the double shot technique in the first step-thermal desorption of unaged/aged samples of (a) golden paint PVAc cobalt blue, (b) golden PVAc paint titanium white; AO, antioxidant KB (butylated hydroxytoluene); DEP, diethyl phthalate; DiPA, *N*,*N*-diethyl-*N′*-phenylethylene diamine, DOP, di-*n*-octyl phthalate.

5. Summary and Outlook

Recent advances in analytical pyrolysis in the field of heritage science highlighted the added value of the possible information provided by the semi-quantitation of organic materials. Starting with classical analytical pyrolysis-gas chromatography, this review has detailed some of the developments in coupling thermal treatments with mass spectrometric detection and new perspectives based on multi-shot pyrolysis-gas chromatography. The main novelty is the unconventional application of analytical pyrolysis, which is now a golden standard for obtaining information on wood and synthetic polymers. Analytical pyrolysis has also provided valuable information on other organic materials in paintings and archaeological residues, thus allowing unprecedented access to their insoluble and chemically non-hydrolysable molecular fractions.

Future challenges entail the wider application of multi-shot approaches, which have already proved promising in tackling a detailed characterisation of synthetic polymers and which have not yet been applied to studying the different fractions evolving at different temperatures. Pyrolysis has been combined with 2D gas chromatography for studying fuels, however in the field of heritage science it has only been tested on handmade paper.[42] It thus has the potential for further applications, possibly coupled with multi-shot pyrolysis or EGA analysis.

Acknowledgements

The authors are very grateful to all their colleagues in the Laboratory of Chemical Science for the Safeguard of Cultural Heritage, and in particular Alessia Andreotti, Jacopo La Nasa, Anna Lluveras-Tenorio, Jeannette Łucejko, Sibilla Orsini and Diego Tamburini.

Keywords: Analytical pyrolysis • Gas chromatography • Mass spectrometry • Flash Pyrolysis • Heritage Science

[1] *Organic Mass Spectrometry in Art and Archaeology*, John Wiley & Sons, Chichester, **2009**.

[2] a) I. Bonaduce, E. Ribechini, F. Modugno, M. P. Colombini, *Topics in Current Chemistry* **2016**, *374*, 6; b) I. Degano, J. La Nasa, *Topics in Current Chemistry* **2016**, *374*, 20; c) C. D. Calvano, I. D. van der Werf, F. Palmisano, L. Sabbatini, *Analytical and Bioanalytical Chemistry* **2016**, *408*, 6957-6981; d) S. Dallongeville, N. Garnier, C. Rolando, C. Tokarski, *Chemical Reviews* **2016**, *116*, 2-79.

[3] M. Schilling, T. Learner, in *16th ICOM Committee for Conservation Triennial Meeting*, Lisbon, **2011**.

[4] C. J. Wolf, M. A. Grayson, D. L. Fanter, *Analytical Chemistry* **1980**, *52*, 348A-358A.

[5] W. J. Irwin, *Journal of Analytical and Applied Pyrolysis* **1979**, *1*, 3-25.

[6] a) J. La Nasa, S. Orsini, I. Degano, A. Rava, F. Modugno, M. P. Colombini, *Microchemical Journal* **2016**, *124*, 940-948; b) M. Lazzari, A. Ledo-Suárez, T. López, D. Scalarone, M. A. López-Quintela, *Analytical and Bioanalytical Chemistry* **2011**, *399*, 2939-2948; c) T. Learner, *Analysis of Modern Paints*, Getty Conservation Institute, Los Angeles, **2004**; d) V. Pintus, S. Wei, M. Schreiner, *Microchemical Journal* **2016**, *124*, 949-961; e) M. Schilling, M. Bouchard, H. Khanjian, T. Learner, A. Phenix, R. Rivenc, *Accounts of Chemical Research* **2010**, *43*, 888-896; f) S. Tsuge, H. Ohtani, C. Watanabe, *Pyrolysis - GC/MS Data Book of Synthetic Polymers*, Elsevier, Oxford, **2011**.

[7] a) I. Bonaduce, A. Andreotti, in *Organic Mass Spectrometry in Art and Archaeology* (Eds.: M. P. Colombini, F. Modugno), John Wiley & Sons, Chichester, **2009**, pp. 303-325; b) M. P. Colombini, A. Andreotti, I. Bonaduce, F. Modugno, E. Ribechini, *Accounts of Chemical Research* **2010**, *43*, 715-727; c) M. T. Doménech-Carbó, *Analytica Chimica Acta* **2008**, *621*, 109-139; d) D. Scalarone, O. Chiantore, in *Organic Mass Spectrometry in Art and Archaeology* (Eds.: M. P. Colombini, F. Modugno), John Wiley & Sons, Chichester, **2009**, pp. 327-361.

[8] M. M. Wright, B. B. Wheals, *Journal of Analytical and Applied Pyrolysis* **1987**, *11*, 195-211.

[9] E. Ribechini, M. Bacchiocchi, T. Deviese, M. P. Colombini, *Journal of Analytical and Applied Pyrolysis* **2011**, *91*, 219-223.

[10] a) M. P. Colombini, E. Ribechini, M. Rocchi, P. Selleri, *Heritage Science* **2013**, *1*, 6; b) T. Devièse, E. Ribechini, D. Castex, B. Stuart, M. Regert, M. P. Colombini, *Microchemical Journal* **2017**, *133*, 49-59; c) J. Poulin, K. Helwig, *Analytical Chemistry* **2014**, *86*, 7428-7435; d) I. D. van der Werf, D. Fico, G. E. De Benedetto, L. Sabbatini, *Microchemical Journal* **2016**, *125*, 85-96.

[11] J. Poulin, K. Helwig, *Organic Geochemistry* **2015**, *86*, 94-106.

[12] S. Wei, V. Pintus, M. Schreiner, *Journal of Analytical and Applied Pyrolysis* **2012**, *97*, 158-163.

[13] a) S. Orsini, F. Parlanti, I. Bonaduce, *Journal of Analytical and Applied Pyrolysis* **2017**, *124*, 643-657; b) D. Tamburini, J. J. Łucejko, F. Modugno, M. P. Colombini, *Journal of Analytical and Applied Pyrolysis* **2016**, *122*, 429-441.

[14] a) G. Germinario, S. Garrappa, V. D'Ambrosio, I. D. van der Werf, L. Sabbatini, *Analytical and Bioanalytical Chemistry* **2018**, *410*, 1079-1094; b) E. Ghelardi, I. Degano, M. P. Colombini, J. Mazurek, M. Schilling, H. Khanjian, T. Learner, *Dyes and Pigments* **2015**, *123*, 396-403.

[15] G. Germinario, E. C. L. Rigante, I. D. van der Werf, L. Sabbatini, *Journal of Analytical and Applied Pyrolysis* **2017**, *127*, 229-239.

[16] T. Chamberlin, N. Madison, *Vol. 4,001,160*, **1997**.

[17] a) J. La Nasa, F. Di Marco, L. Bernazzani, C. Duce, A. Spepi, V. Ubaldi, I. Degano, S. Orsini, S. Legnaioli, M. R. Tiné, D. De Luca, F. Modugno, *Polymer Degradation and Stability* **2017**, *144*, 508-519; b) S. Orsini, J.

La Nasa, F. Modugno, M. P. Colombini, *Journal of Analytical and Applied Pyrolysis* **2013**, *104*, 218-225.

[18] a) M. R. Schilling, A. Heginbotham, H. van Keulen, M. Szelewski, *Studies in Conservation* **2016**, *61*, 3-27; b) D. Tamburini, D. Sardi, A. Spepi, C. Duce, M. R. Tinè, M. P. Colombini, I. Bonaduce, *Polymer Degradation and Stability* **2016**, *134*, 251-264.

[19] S. Wei, G. Song, Y. He, *Journal of Archaeological Science* **2015**, *59*, 211-218.

[20] a) J. J. Łucejko, M. Mattonai, M. Zborowska, C. Cartwright, D. Tamburini, G. Cofta, F. Modugno, M. P. Colombini, E. Ribechini, *Microchemical Journal* **2018**; b) J. J. Łucejko, M. Zborowska, F. Modugno, M. P. Colombini, W. Prądzyński, *Analytica Chimica Acta* **2012**, *745*, 70-77; c) D. Tamburini, J. J. ſucejko, E. Ribechini, M. P. Colombini, *Journal of Analytical and Applied Pyrolysis* **2016**, *118*, 249-258; d) M. Traoré, J. Kaal, A. Martínez Cortizas, *Journal of Analytical and Applied Pyrolysis* **2017**, *126*, 1-13; e) S. A. Stout, J. J. Boon, *Organic Geochemistry* **1994**, *21*, 953-970.

[21] J. J. Łucejko, F. Modugno, E. Ribechini, D. Tamburini, M. P. Colombini, *Applied Spectroscopy Reviews* **2015**, *50*, 584-625.

[22] D. Tamburini, J. J. Łucejko, F. Modugno, M. P. Colombini, *International Biodeterioration & Biodegradation* **2014**, *86*, 142-149.

[23] a) S. Braovac, D. Tamburini, J. J. Łucejko, C. McQueen, H. Kutzke, M. P. Colombini, *Microchemical Journal* **2016**, *124*, 368-379; b) D. Tamburini, J. J. Łucejko, M. Zborowska, F. Modugno, W. Prądzyński, M. P. Colombini, *Journal of Analytical and Applied Pyrolysis* **2015**, *115*, 7-15.

[24] C. M. A. McQueen, D. Tamburini, J. J. Łucejko, S. Braovac, F. Gambineri, F. Modugno, M. P. Colombini, H. Kutzke, *Microchemical Journal* **2017**, *132*, 119-129.

[25] S. Tirat, I. Degano, J.-P. Echard, A. Lattuati-Derieux, A. Lluveras-Tenorio, A. Marie, S. Serfaty, J.-Y. Le Huerou, *Microchemical Journal* **2016**, *126*, 200-213.

[26] a) C. Duce, E. Bramanti, L. Ghezzi, L. Bernazzani, I. Bonaduce, M. P. Colombini, A. Spepi, S. Biagi, M. R. Tine, *Dalton Transactions* **2013**, *42*, 5975-5984; b) C. Duce, L. Ghezzi, M. Onor, I. Bonaduce, M. P. Colombini, M. R. Tine', E. Bramanti, *Analytical and Bioanalytical Chemistry* **2012**, *402*, 2183-2193; c) C. Duce, S. Orsini, A. Spepi, M. P. Colombini, M. R. Tiné, E. Ribechini, *Journal of Analytical and Applied Pyrolysis* **2015**, *111*, 254-264; d) M. R. Tiné, C. Duce, in *Nanotechnologies and nanomaterials for Diagnostic, Conservation and Restoration of Cultural Heritage. Advanced methods and materials.* (Eds.: G. Lazzara, R. Fakhrullin), Elsevier, **2018**.

[27] a) S. Lomax, M. Schilling, T. Learner, in *Modern paints uncovered*, Getty Conservation Institute, Tate Modern, London, **2007**; b) K. J. v. d. Berg, J. J. Boon, I. Pastorova, L. F. M. Spetter, *Journal of Mass Spectrometry* **2000**, *35*, 512-533.

[28] F. Modugno, E. Ribechini, M. P. Colombini, *Rapid Communications in Mass Spectrometry* **2006**, *20*, 1787-1800.

[29] A. van Loon, W. Genuit, C. Pottasch, S. Smelt, P. Noble, *Microchemical Journal* **2016**, *126*, 406-414.

[30] L. Sleno, *Journal of Mass Spectrometry* **2012**, *47*, 226-236.

[31] M. Kokkori, K. Sutherland, J. Boon, F. Casadio, M. Vermeulen, *Heritage Science* **2015**, *3*, 30.

[32] a) S. Sotiropoulou, J. Mazurek, M. Schilling, A. Lluveras-Tenorio, S. Prati, G. Sciutto, R. Mazzeo, M. P. Colombini, *Microchemical Journal* **2018**; b) R. Stacey, J. Dyer, J. Mazurek, M. Schilling, A. Lluveras-Tenorio, M. P. Colombini, S. Sotiropoulou, S. Prati, G. Sciutto, R. Mazzeo, F. Rosi, C. Miliani, L. Cartechini, M. Sablier, M. Küppers, *Microchemical Journal* **2018**; c) D. Tamburini, J. J. Łucejko, E. Ribechini, M. P. Colombini, *Journal of Mass Spectrometry* **2015**, *50*, ii-ii.

[33] N. Niimura, *Thermochimica Acta* **2012**, *532*, 164-167.

[34] S. Orsini, C. Duce, I. Bonaduce, *Journal of Analytical and Applied Pyrolysis* **2018**.

[35] A. Adamiano, D. Fabbri, G. Falini, M. Giovanna Belcastro, *Journal of Analytical and Applied Pyrolysis* **2013**, *100*, 173-180.

[36] M. J. Samide, G. D. Smith, *Journal of Chromatography A* **2015**, *1426*, 201-208.

[37] P. Hatchfield, *Pollutants in the Museum Environment*, Archetype Publications Limited, London, **2002**.

[38] D. Thickett, L. Lee, *British Museum Occasional Paper* **2004**, *111*.

[39] V. Pintus, M. Schreiner, *Analytical and Bioanalytical Chemistry* **2011**, *399*, 2961-2976.

[40] S. Wei, V. Pintus, M. Schreiner, *Journal of Analytical and Applied Pyrolysis* **2013**, *104*, 441-447.

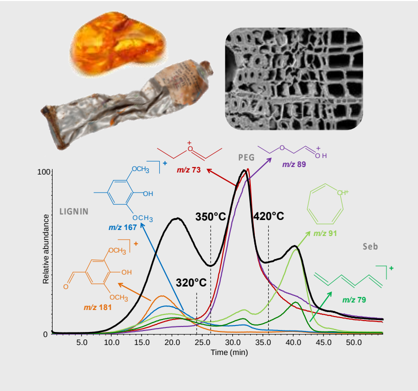
[41] I. Bonaduce, F. Di Girolamo, I. Corsi, I. Degano, M. R. Tinè, M. P. Colombini, *Journal of Natural Products* **2016**, *79*, 845-856.

[42] a) B. Han, J. Vial, M. Inaba, M. Sablier, *Journal of Analytical and Applied Pyrolysis* **2017**, *127*, 150-158; b) B. Han, G. Daheur, M. Sablier, *Journal of Analytical and Applied Pyrolysis* **2016**, *122*, 458-467.

Entry for the Table of Contents

MINIREVIEW

Analytical pyrolysis is featuring now previously unexplored potentialities, thanks to recent instrumental developments. In this review we present recent advances in the heritage field enabling the investigation of several organic materials in artistic and historical objects that are macromolecular in nature, or which undergo polymerization upon curing and ageing, by strategies based on analytical pyrolysis coupled with mass spectrometry.

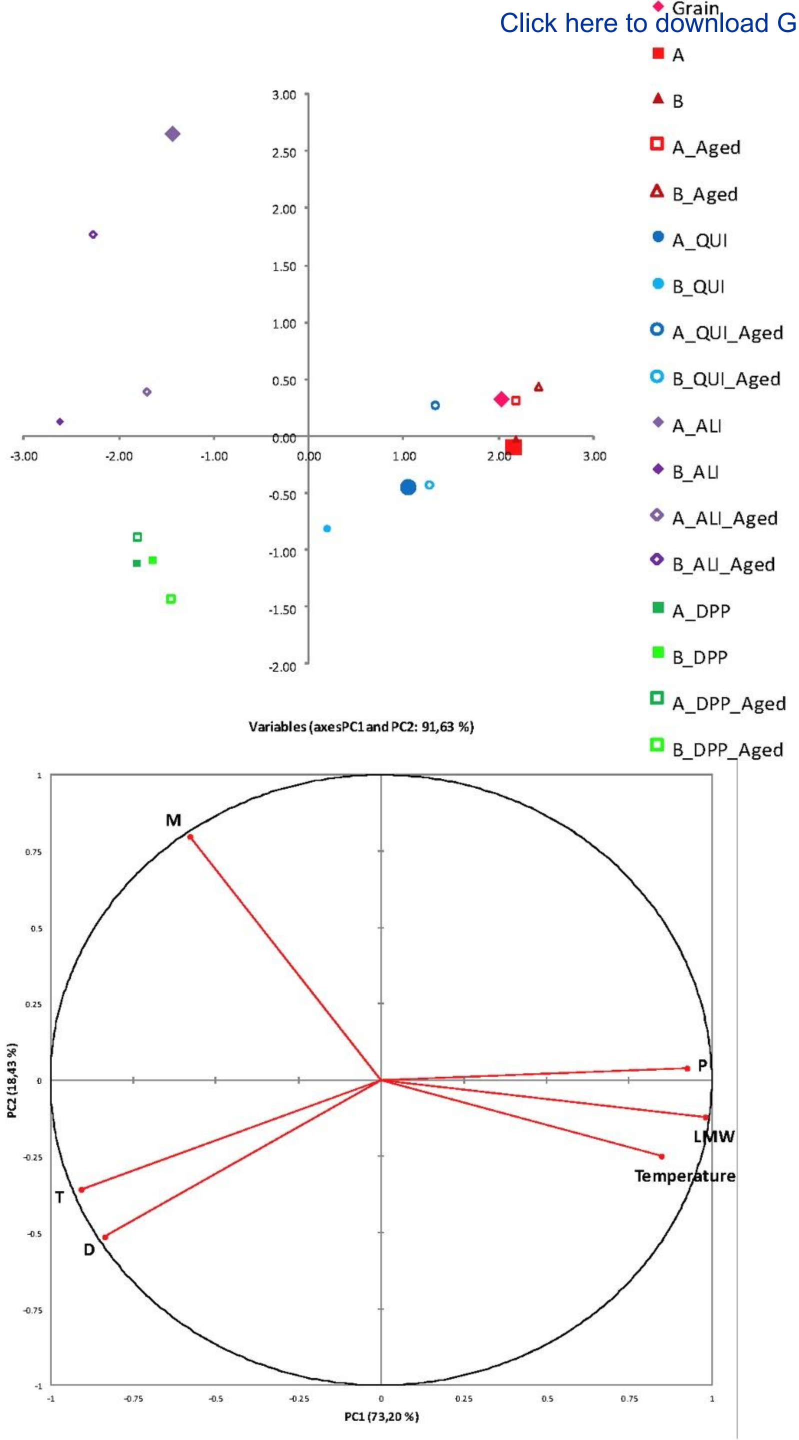


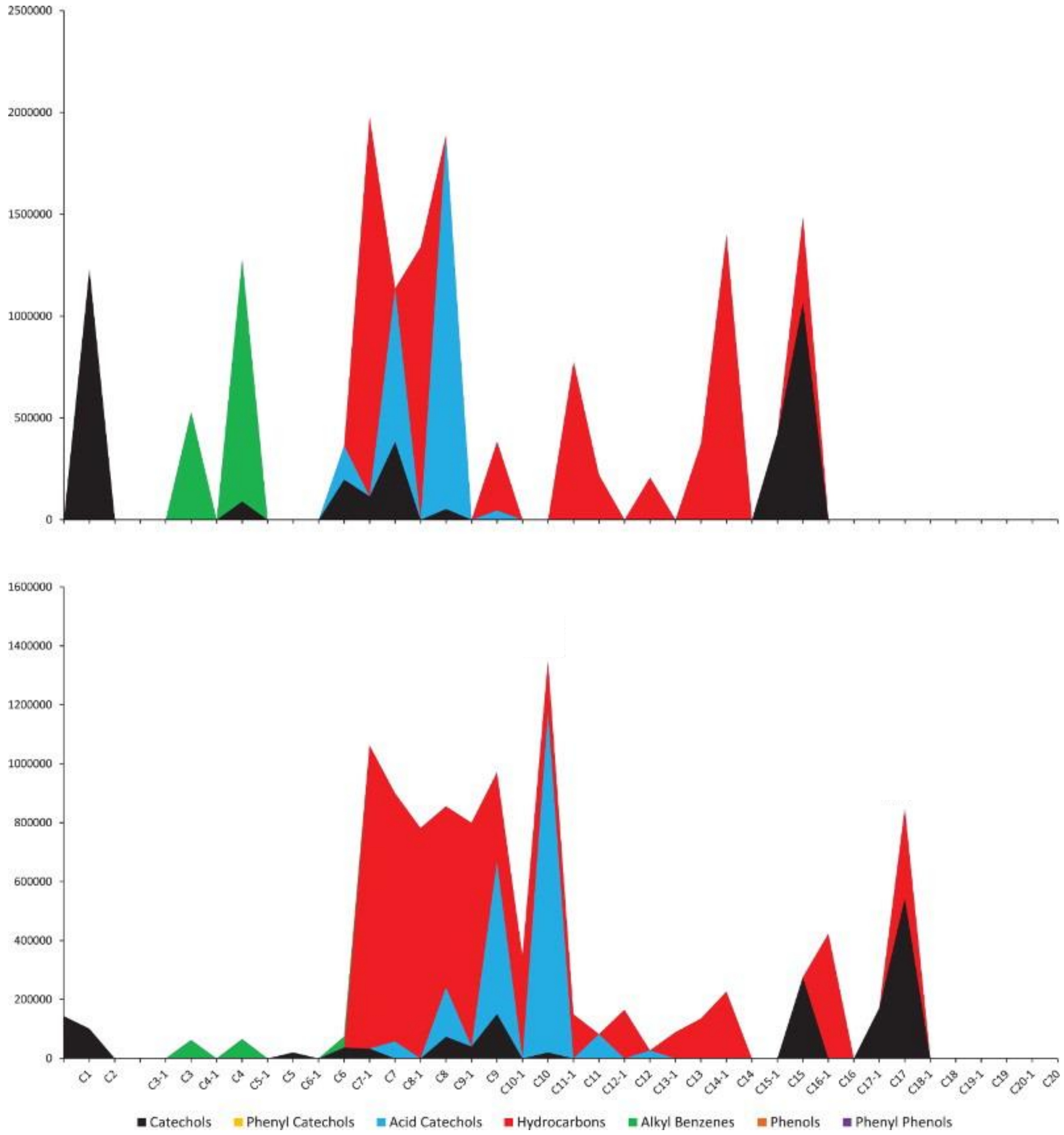
*Ilaria Degano, Francesca Modugno, Ilaria Bonaduce, Erika Ribechini and Maria Perla Colombini**

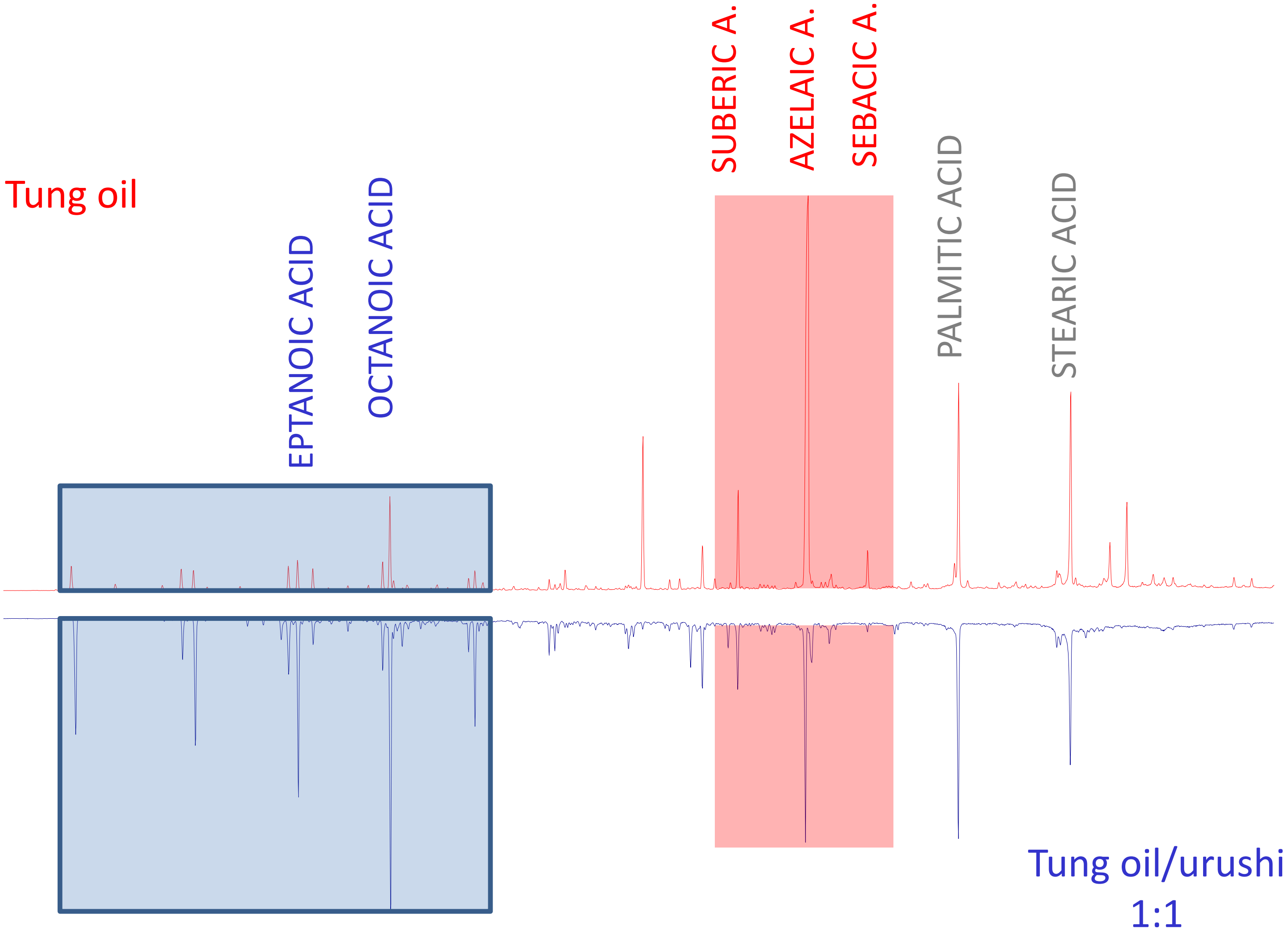
Page No. – Page No.

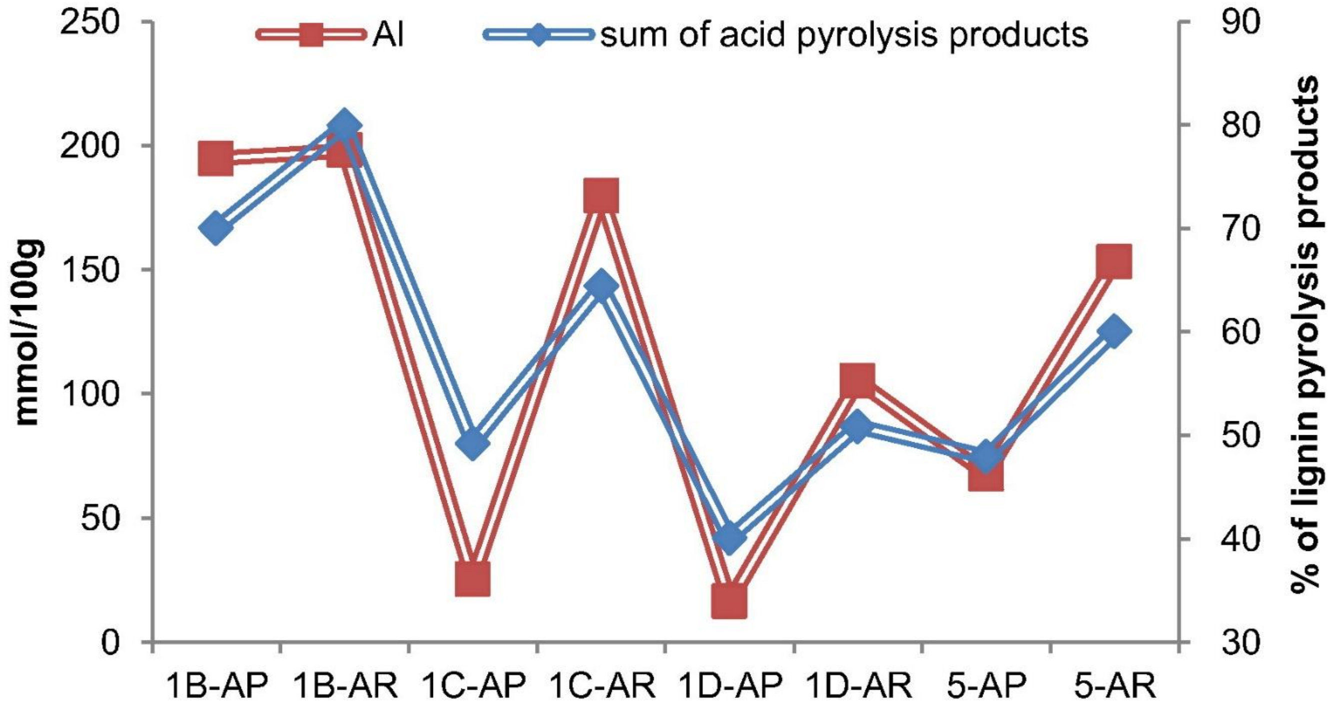
Recent advances in analytical pyrolysis to investigate organic materials in heritage science

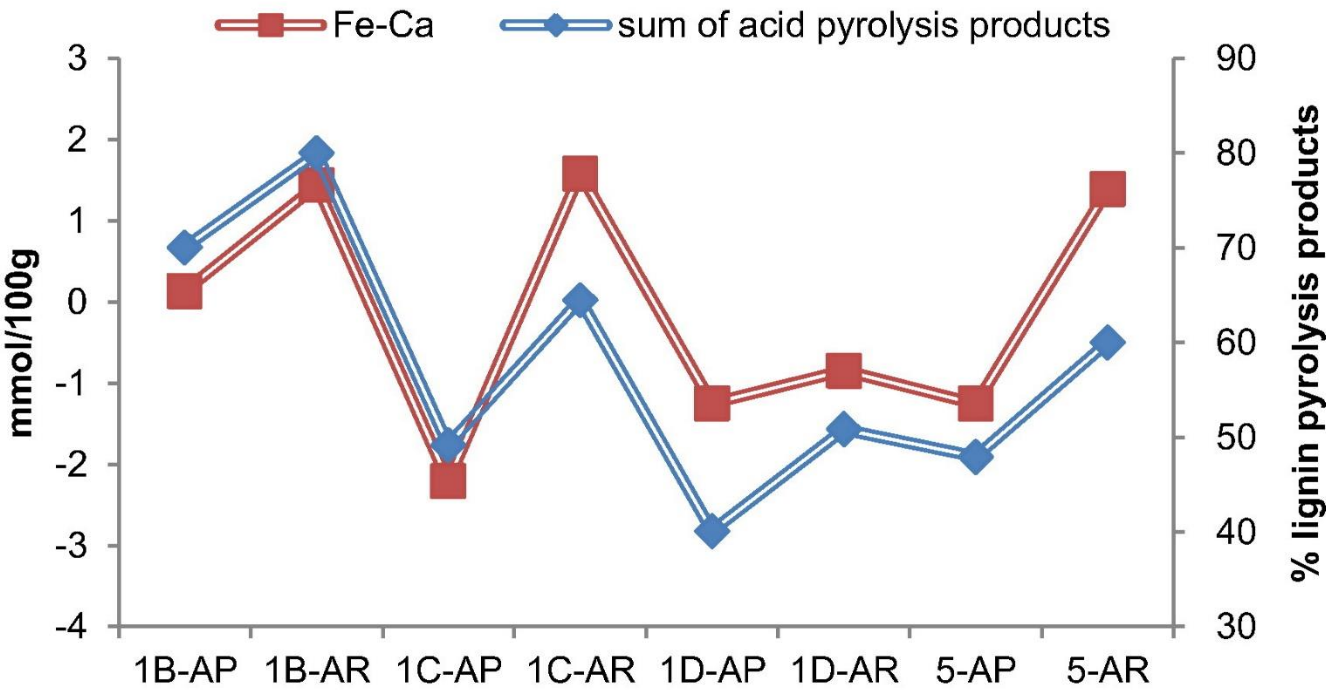
Figures

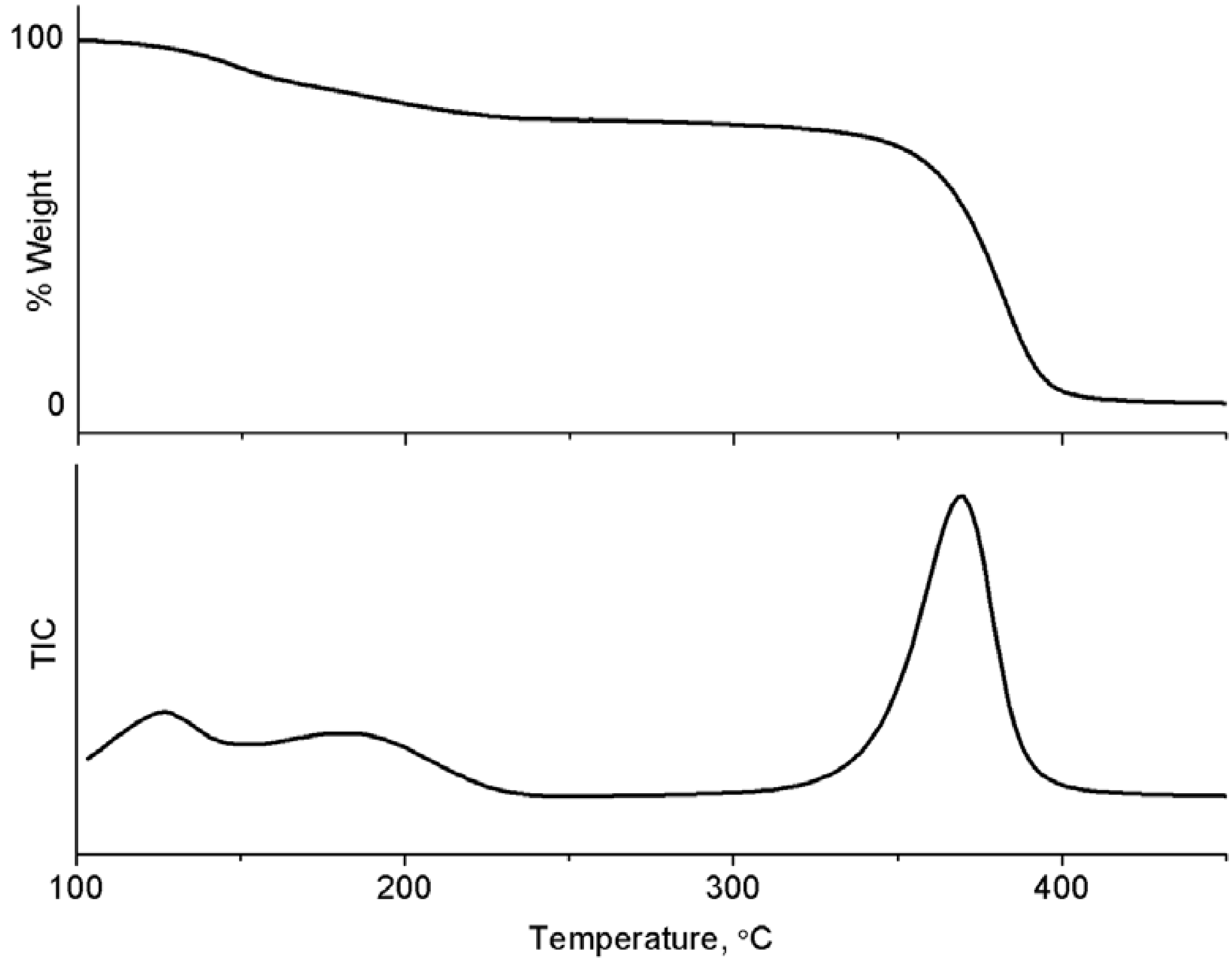

Grain
A
B
A_Aged
B_Aged
A_QUI
B_QUI
A_QUI_Aged
B_QUI_Aged
A_ALI
B_ALI
A_ALI_Aged
B_ALI_Aged
A_DPP
B_DPP
A_DPP_Aged
B_DPP_Aged
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00
-3.00
-2.00
-1.00
0.00
1.00
2.00
3.00
Variables (axes PC1 and PC2: 91,63 %)
M
P
LMW
Temperature
T
D
PC2 (18,43 %)
PC1 (73,20 %)
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1

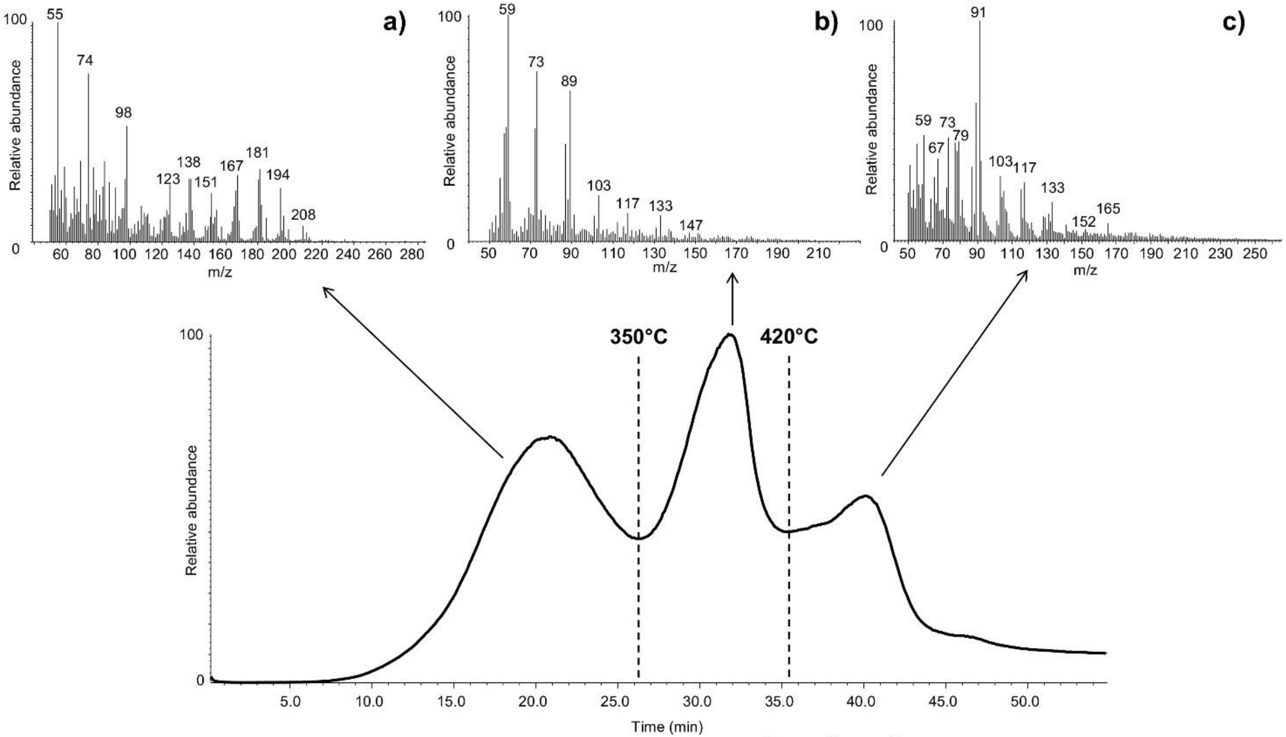

2500000
2000000
1500000
1000000
500000
0
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
C1
C2
C3-1
C3
C4-1
C4
C5-1
C5
C6-1
C6
C7-1
C7
C8-1
C8
C9-1
C9
C10-1
C10
C11-1
C11
C12-1
C12
C13-1
C13
C14-1
C14
C15-1
C15
C16-1
C16
C17-1
C17
C18-1
C18
C19-1
C19
C20-1
C20
Catechols
Phenyl Catechols
Acid Catechols
Hydrocarbons
Alkyl Benzenes
Phenols
Phenyl Phenols

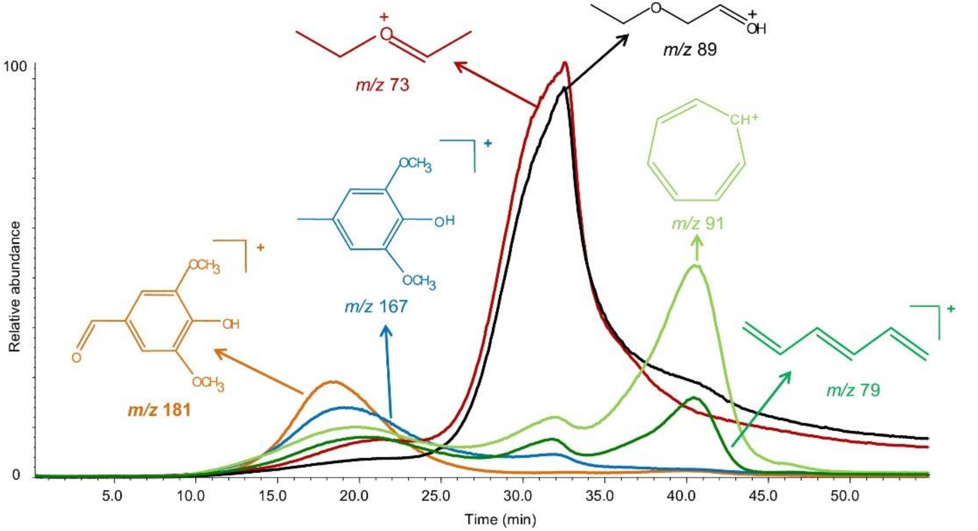

Tung oil
EPTANOIC ACID
OCTANOIC ACID
SUBERIC A.
AZELAIC A.
SEBACIC A.
PALMITIC ACID
STEARIC ACID
Tung oil/urushi
1:1


Al
sum of acid pyrolysis products
mmol/100g
% of lignin pyrolysis products
250
200
150
100
50
0
90
80
70
60
50
40
30
1B-AP
1B-AR
1C-AP
1C-AR
1D-AP
1D-AR
5-AP
5-AR

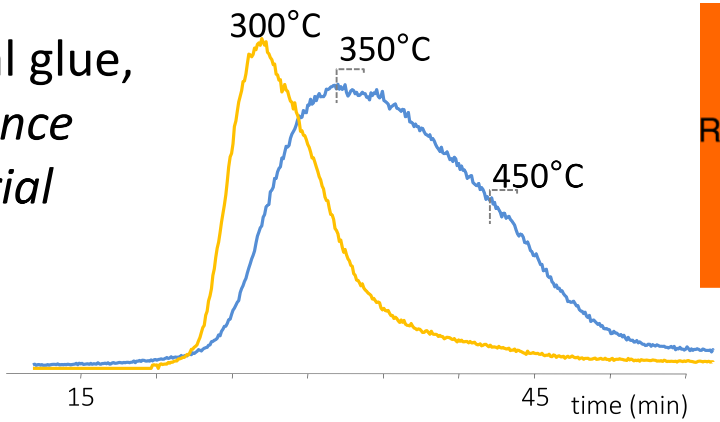

Fe-Ca
sum of acid pyrolysis products
mmol/100g
% lignin pyrolysis products
3
2
1
0
-1
-2
-3
-4
90
80
70
60
50
40
30
1B-AP
1B-AR
1C-AP
1C-AR
1D-AP
1D-AR
5-AP
5-AR

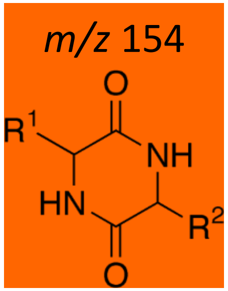

100
0
% Weight
TIC
100
200
300
400
Temperature, °C

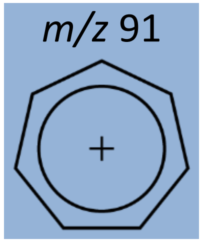

a)
b)
c)
Relative abundance
m/z
100
0
55
74
98
123
138
151
167
181
194
208
60 80 100 120 140 160 180 200 220 240 260 280
59
73
89
103
117
133
147
50 70 90 110 130 150 170 190 210
91
59
67
73
79
103
117
133
152
165
50 70 90 110 130 150 170 190 210 230 250
350°C
420°C
Time (min)
5.0 10.0 15.0 20.0 25.0 30.0 35.0 40.0 45.0 50.0


m/z 89
m/z 73
m/z 91
m/z 167
m/z 181
m/z 79
OCH3
OH
CH+
Relative abundance
100
0
Time (min)
5.0 10.0 15.0 20.0 25.0 30.0 35.0 40.0 45.0 50.0

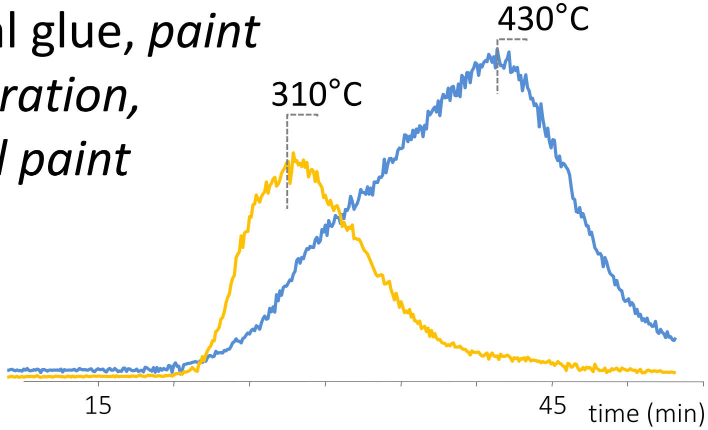
animal glue, *reference material*

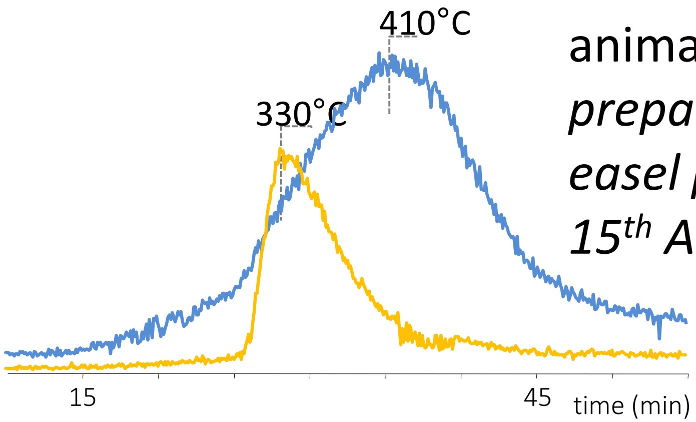






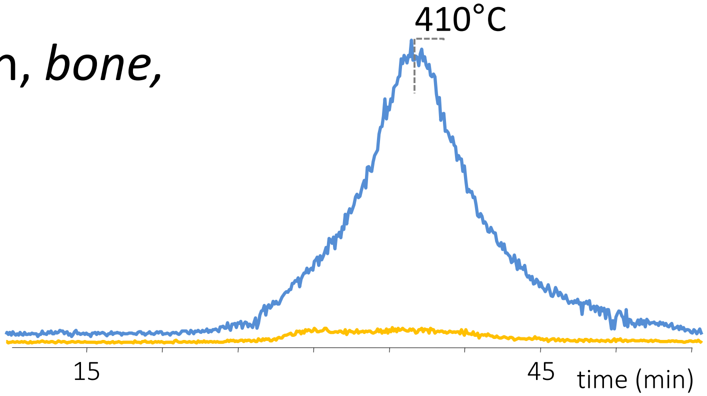
animal glue, *paint preparation, model paint 1990*

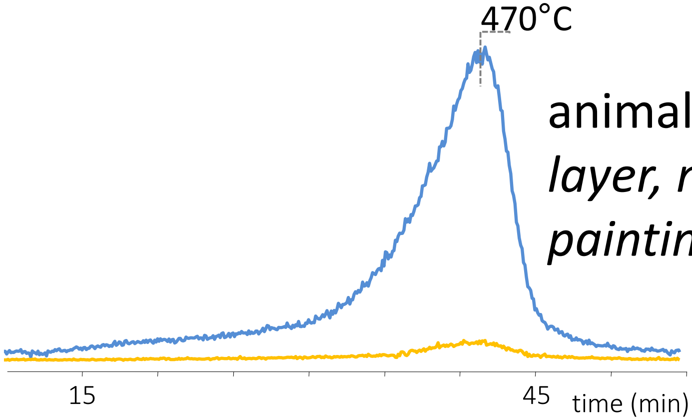




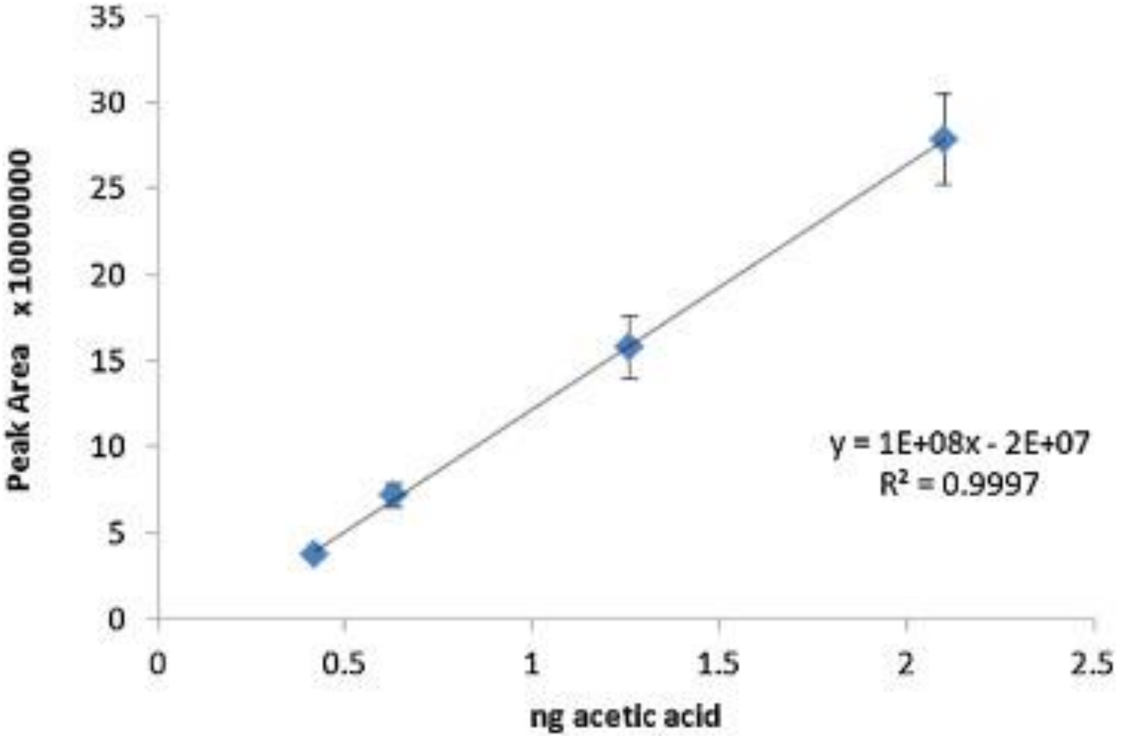
animal glue, *paint preparation, easel painting 15th AD*

collagen, *bone, 17th AD*

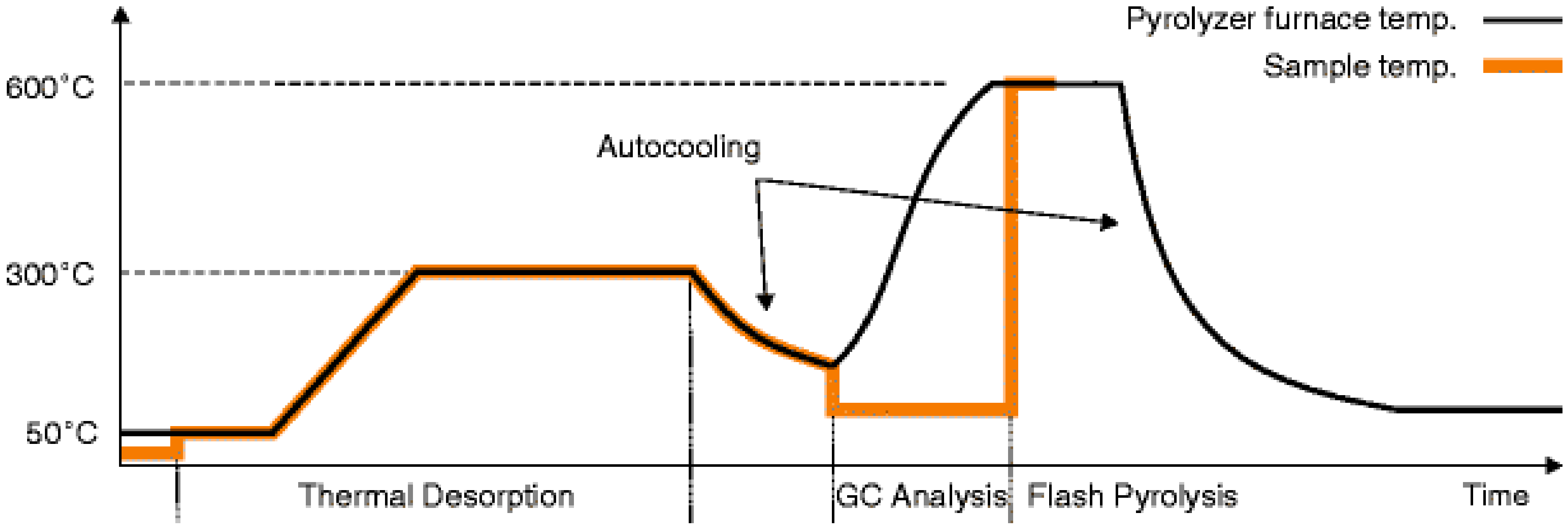


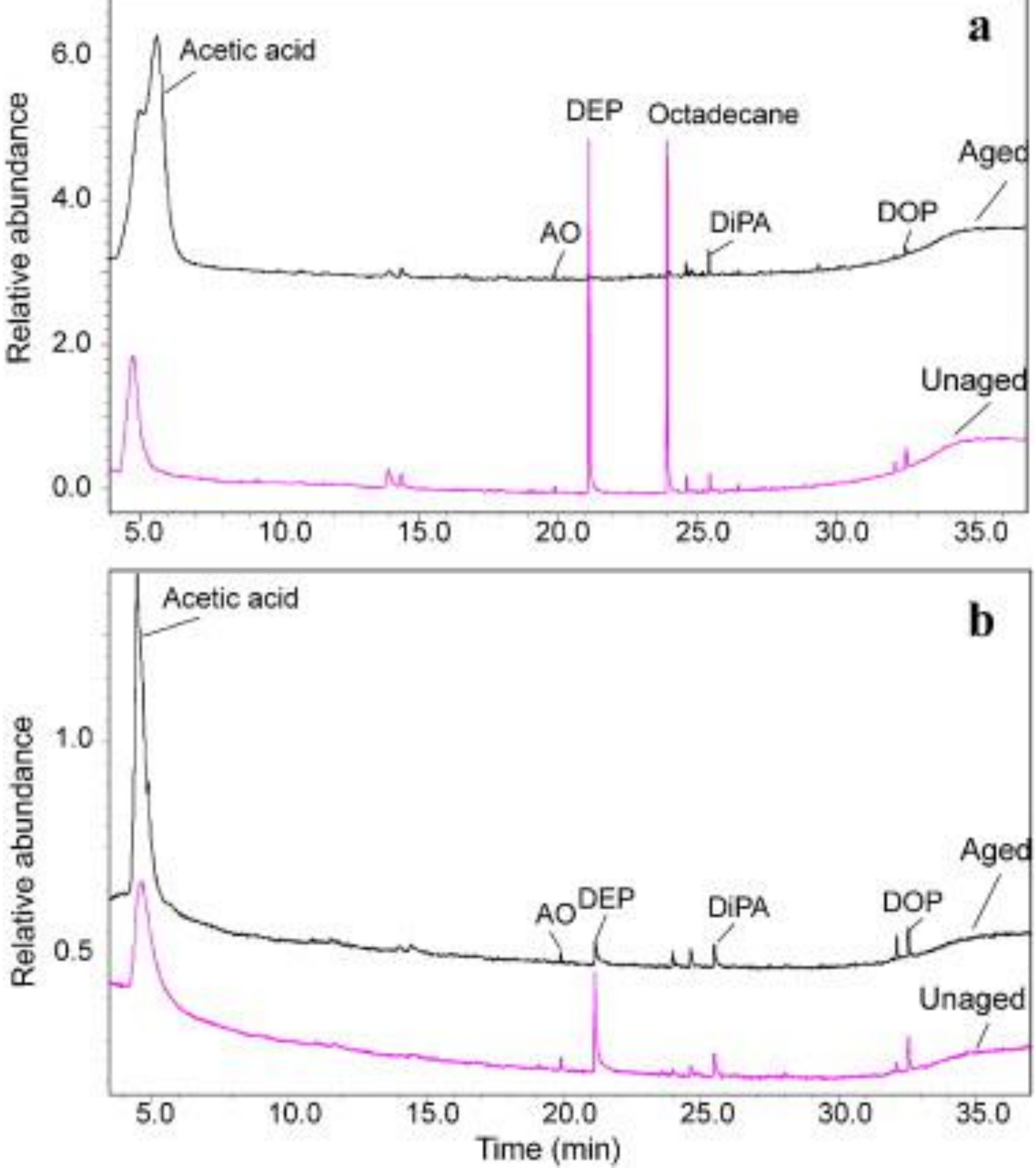


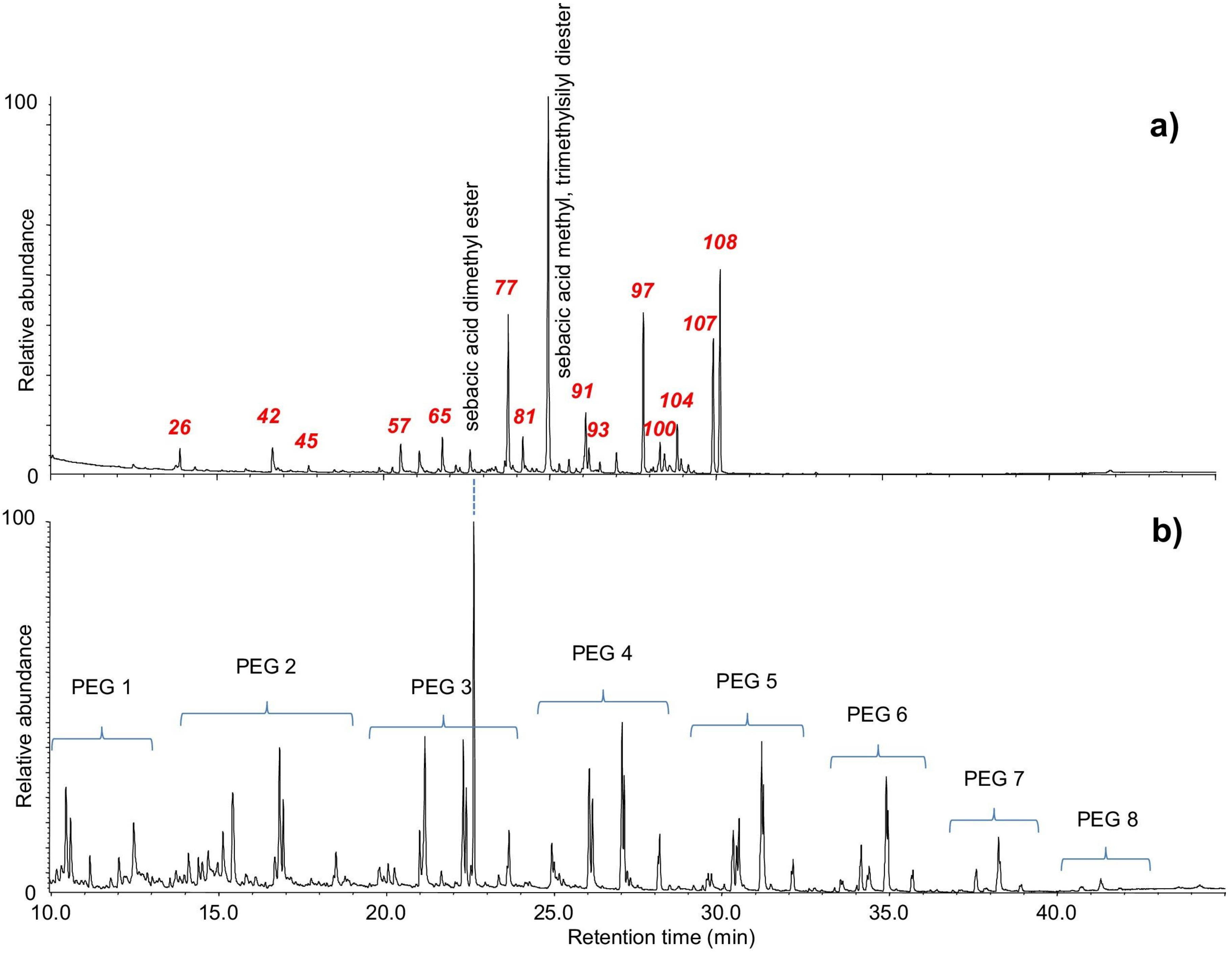
animal glue, *paint layer, mural painting 2nd BC*



	Amount (ng/mg)	Percent error
Cellulose acetate	5.3 ± 0.2	4.7
Poly(vinyl acetate)	0.0826 ± 0.0001	0.13
Jade 403 (TALAS)	0.0462 ± 0.0006	1.2
Jade 403 (Light Impressions)	0.059 ± 0.002	2.6
Titebond II, freshly cured	5.0 ± 0.5	9.1
Elmer's school glue, aged	0.21 ± 0.01	5.3


Pyrolyzer furnace temp.
Sample temp.
600°C
300°C
50°C
Autocooling
Thermal Desorption
GC Analysis
Flash Pyrolysis
Time


a
Relative abundance
6.0
4.0
2.0
0.0
Acetic acid
DEP
Octadecane
AO
DiPA
DOP
Aged
Unaged
5.0
10.0
15.0
20.0
25.0
30.0
35.0
b
Relative abundance
1.0
0.5
Acetic acid
AO
DEP
DiPA
DOP
Aged
Unaged
5.0
10.0
15.0
20.0
25.0
30.0
35.0
Time (min)


a)
b)
Relative abundance
100
0
sebacic acid dimethyl ester
sebacic acid methyl, trimethylsilyl diester
26
42
45
57
65
77
81
91
93
97
100
104
107
108
PEG 1
PEG 2
PEG 3
PEG 4
PEG 5
PEG 6
PEG 7
PEG 8
10.0
15.0
20.0
25.0
30.0
35.0
40.0
Retention time (min)