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Gas chromatography/mass spectrometry methods applied for the analysis of a Round Robin sample containing materials present in samples of works of art

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ABSTRACT

The Users' Group for Mass Spectrometry and Chromatography (MaSC) was established as a discussion forum for scientists involved in the study of artworks and cultural artefacts who wish to exchange information regarding sampling strategies and protocols, preparation of samples, chromatographic and mass spectrometric analysis, data treatment and interpretation of results. Comparing protocols and different approaches to the interpretation of results requires the use of standard reference materials and Round Robin tests. To this end, an artificial, complex Round Robin sample was sent to fifteen laboratories specializing in the analysis of cultural materials. Ten of the participants analyzed the sample and reported their results, which were anonymously discussed at the MaSC meeting in Philadelphia, September 2007. In this paper, the results of the confirming GC/MS analysis of the sample by the author are presented and compared with the results of the participants. The confirming analysis was performed with thermally assisted hydrolysis and methylation GC/MS in combination with pyrolysis, and by a combined method for the analysis of proteins and carbohydrates. A number of different instrumental setups and methods were used by the Round Robin participants. The results show that stepwise extraction followed by different types of derivatization is a successful approach for the analysis and identification of complex samples. A disadvantage is the need for relatively large samples, which are not always available in practice. Pyrolysis with or without tetramethylammonium hydroxide (TMAH) or hexamethyldisilazane (HMDS) was used by four of the participants. The results show that thermally assisted hydrolysis and methylation GC/MS in combination with pyrolysis is a powerful technique for the analysis of multi-component samples. The presence of gums and proteins can be indicated. Synthetic resins such as acrylics can also be analyzed in combination with the traditional binders and resins.

1. Introduction

Samples obtained from works of art for instrumental analysis are often small and complex in composition. It is not uncommon to find several organic materials such as a drying oil, natural and synthetic resins, a polysaccharide, a protein, or a wax in a single sample. In order to compare and evaluate different protocols and approaches to interpretation of results from the analysis of such samples, standard reference materials and Round Robin tests are therefore important. In January 2007 an artificial multi-component sample was prepared at the Instituut Collectie Nederland (ICN, Netherlands Institute for Cultural Heritage) by the author. The Round Robin sample was presented as an unknown sample with a complex composition. A sample to be analyzed with all standard protocols and methods used to characterize samples from works of art.

Members of the Users? Group for Mass Spectrometry and Chromatography (MaSC [1]) were invited to participate in the Round Robin test. Samples were send to fifteen laboratories together with a note to inform the participants of the general classes of component which could be present in the sample and advice to powder the sample to homogenize its contents before analysis. Ten of the participants analyzed the sample and submitted reports, and the results were discussed at the MaSC meeting at the Philadelphia Museum of Art, September 2007.

In this paper the preparation of the sample is described, followed by a description of the author's confirming analysis of the sample using thermally assisted hydrolysis and methylation GC/MS in combination with pyrolysis, and a second GC/MS method for the combined analysis of amino acids and carbohydrates. The methods and results of the participants in the Round Robin test are then presented. The setup and advantages of using online derivatization

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pyrolysis-GC/MS for the analysis of complex samples will also be discussed.

2. Background: pyrolysis-GC/MS analysis of organic materials in artworks

The methylating reagent tetramethylammonium hydroxide (TMAH) was initially used for the analysis of fats and oils [2,3] and also aromatic acids [4]. In the early studies, sample material was injected, together with an excess of the reagent, into a hot GC injection port. The reagent gained wider popularity for the technique of thermally assisted hydrolysis and methylation (THM) pyrolysis, in which a sample is pyrolyzed in the presence of the TMAH. This technique has been applied for the analysis of a variety of organic materials including synthetic [5,6] and natural resins [5,7–11], lipids [5,12], waxes [5,13], wood products [5], carbohydrates [5], proteins [5], and dyes [5].

Due to the strongly basic properties of the quaternary ammonium salt, ester bonds are hydrolyzed and ammonium salts are formed at the carboxyl and also hydroxyl groups. With the heat of the GC inlet or pyrolysis unit the salts are thermally decomposed. The carboxyl and hydroxyl groups are methylated and trimethylamine is formed as a side product. The excess of TMAH is thermally decomposed to methanol, dimethyl ether and trimethylamine.

The overall reaction which occurs in the hot injection port or pyrolysis unit can be summarized as:

$$\mathrm{RCOOH} \xrightarrow[\Delta]{(\mathrm{CH_3})_4\mathrm{N^+OH^-}} \mathrm{RCOOCH_3} + (\mathrm{CH_3})_3\mathrm{N} + \mathrm{H_2O}$$

Because the sample is heated with an excess of TMAH, trimethylamine is formed to a large extent. In the past, with the use of packed columns or wide bore glass capillaries, the high column flow could manage the amount of trimethylamine produced. For the narrow bore capillaries used nowadays the amount of trimethylamine formed by the reaction is problematic and must be vented or split off after pyrolysis.

For the THM analysis of samples of works of art a temperature of about 610 °C is often used for a complete and reproducible reaction and an effective fragmentation of the polymer fraction of the sample. The majority of the components of interest formed by the reaction have a boiling point (elution temperature) higher than 240 °C. To prevent condensation of the components in the pyrolysis chamber, the chamber should therefore have a temperature of at least 280 °C.

In our laboratory, samples of unknown composition are always analyzed using THM-GC/MS in combination with Curie-point pyrolysis. The Curie-point pyrolyzer used, a GSG Pyromat, is modified to optimize results when analyzing samples from works of art. The modifications are described in Section 3.

3. Experimental

3.1. Preparation of the Round Robin sample

The Round Robin sample was prepared from the following components:

	Weight	% of total
Dried linseed oil with ultramarine blue pigment	3.0 g	33
Paraloid B82 (p(EA/MMA) resin)	1.7 g	19
Sandarac (diterpenoid resin)	1.0 g	11
Succinic acid	0.5 g	5
Mastic (triterpenoid resin)	2.0 g	22
Gum arabic (polysaccharide)	0.4 g	4.5
Egg white (dry) (protein)	0.5 g	5.5

Two glass plates covered with linseed oil paint pigmented with ultramarine blue were used as base for the sample. The paint films were prepared roughly 25 years ago, and the plates marked "ultramarine blue in linseed oil, Winsor and Newton". The paint was scraped off and powdered in a mortar. All additional components in the sample were added as powders in a weighed amount. The mixture was stirred into a slurry with some methanol and spread out to dry, and the dried sample was crushed into small fragments.

3.2. Analysis of the Round Robin test sample

Two methods were used by the author for the confirming analysis of the Round Robin sample: one for oils, waxes, resins and (synthetic) polymers, and one for the combined analysis of proteins and polysaccharides.

3.2.1. Analysis of oils, waxes, resins (synthetic) polymers: modification of the GSG Pyromat

The GSG Pyromat, a single shot hand model, was originally equipped with a removable glass sample holder/pyrolysis chamber to offer the possibility to load and purge the chamber at room temperature. After purging, the pyrolysis chamber was placed in the heated zone of the Pyromat. The injection needle attached to the chamber entered the GC liner through the septum of the GC inlet.

To prevent condensation of high boiling components in the pyrolysis chamber, the chamber has to be at a temperature of at least 280 °C. In the author's experience this temperature could not be reached within the short time the glass pyrolysis chamber was in the heated zone of the Pyromat prior to pyrolysis. Also, the needle attached to the chamber caused severe septum leaks, from the repeated loading and removal of the chamber.

The pyrolysis chamber was therefore modified by replacing the glass pyrolysis chamber with a steel tube/glass liner assembly, with a split exit constructed at the base of the chamber. The modified pyrolysis chamber is always in the heated zone of the pyrolysis unit. The analytical column is directly coupled to the chamber. A probe holding the Curie-point wire is used for the introduction of the sample.

The GSG Pyromat with modified pyrolysis chamber and probe is shown in Figs. 1 and 2 shows the schematic view of the chamber, liner and probe.

3.2.2. Analytical column and pre-column

When using THM pyrolysis with TMAH, gaseous trimethylamine is formed to a large extent. In the author's experience, trimethylamine attacks the stationary phase of the column, causing active spots and degradation of the coating throughout the column. To prolong column life, splitting off the trimethylamine before the analytical column is recommended. A split exit at the pyrolysis unit or the split exit of the GC inlet will remove a large proportion of the trimethylamine, but will also cause loss of sample. A narrow bore column in combination with a highly sensitive mass spectrometer can compensate for this loss of sample information.

Columns from different manufacturers and with different stationary phases have been tested in our laboratory. Standard phenyl/dimethylpolysiloxane columns (DB5 or equivalent) are among those that were degraded by the trimethylamine. Also we have found that the new type of ultralow bleed arylene MS columns (DB5-ms) do not last long. Recently a Supelco SLB^{TM}-5ms column was tested. The column has a stationary phase consisting of a silphenylene polymer in which the phenyl groups are incorporated in the polymer backbone. Due to steric hindering, the coating has been found to withstand the effects of trimethylamine for a longer duration.

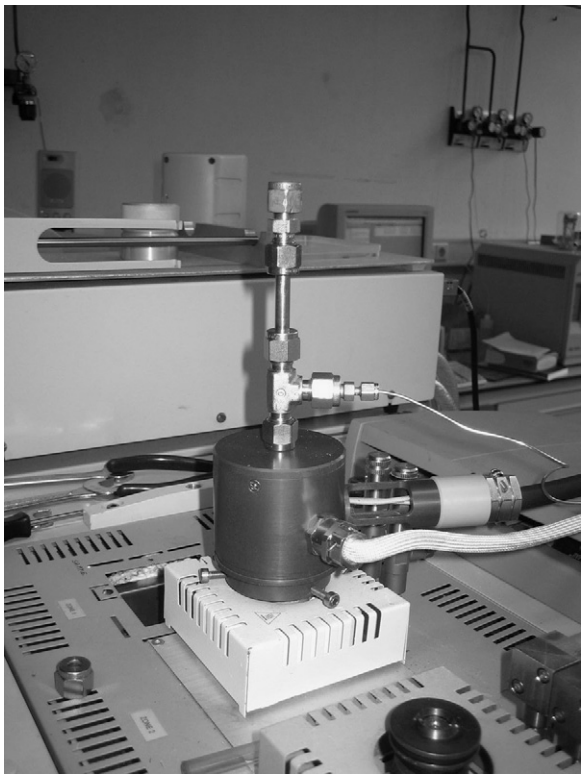

Fig. 1. GSG Pyromat with modified pyrolysis chamber and sample probe.

A SLB^{TM}-5ms column, directly coupled to the pyrolysis chamber, has been successfully used for more than 6 months without showing degradation.

3.2.3. Sample preparation

Just before pyrolysis the sample is made into a suspension with 10 μl of a 2.5% solution of TMAH in methanol (made from Sigma–Aldrich 33,490-1, TMAH 25% in methanol), using a 300 μl Micro Reaction vessel as sample preparation vial. The sample is ground with the reagent using a small glass rod. The suspension is applied to the looped end of the pyrolysis wire by means of a syringe, or by dipping the wire into the suspension. After evaporation of the solvent in a stream of slightly warm air (30 °C), the wire is loaded in the unit and pyrolyzed. The pyrolytic fragments and derivatized components are separated and identified with GC/MS.

3.2.4. Apparatus and method

The apparatus used is a modified GSG Pyromat, as described above, used with a Thermo Quest 8000^{top} Gas Chromatograph and Voyager^{plus} Mass Spectrometer. The pyrolysis unit is attached to the GC inlet with the chamber directly coupled to the column so that the GC inlet is effectively used as heated interface. The temperature of the pyrolysis unit is set at 295 °C, with a pyrolysis temperature of 625 °C and duration of 6 s. The temperature of the GC inlet is 310 °C. The split ratio is 15:1; after one minute the split exit is closed with a magnetic valve controlled by the GC/MS software. A Supelco SLB^{TM}-5ms column with a length of 20 m, internal diameter 0.18 mm and film thickness 0.18 μm is used, with helium as the carrier gas, constant flow 0.9 ml/min. The oven temperature is programmed from 35 °C, held for 1 min, and raised with a rate of 60 °C/min until 110 °C, then with a rate of 14 °C/min until 240 °C, and finally with a rate of 6 °C/min until 315 °C, held for 3 min. The temperature of the MS transfer line is set at 250 °C, the temperature of the ion source at 220 °C. MS data is collected in EI mode from m/z 45 to 600, at 70 eV electron energy, with a scan time of 0.15 s. The solvent delay is 1.2 min. Xcalibur 1.4 software is used for data acquisition and processing.

3.3. Analysis of amino acids and carbohydrates

In our laboratory, amino acids and carbohydrates are analyzed in one run as *N*,*O*-acetyl methyl esters. The applied method is still in development, and was presented at the MaSC meeting, September 2007 [14]. In summary, hydrolysis of the sample is performed in a 100 μl Micro Reaction vessel with 100 μl of 4 M trifluoroacetic acid (TFA), sealed under a nitrogen atmosphere, for 16 h at 110 °C. After hydrolysis the content is evaporated to total dryness under a stream of nitrogen while warming the vial to 50 °C. Two portions of 50 μl of methanol are added and evaporated. Methanolysis of the carbohydrates and methylation of the amino acids is then performed with 20 μl 1.5 N methanolic HCl at 90 °C for 2 h. After evaporation of the methanolic HCl at 50 °C, 50 μl of methanol is added and evaporated to remove traces of hydrochloric acid. The residue is acetylated with 50 μl of a mixture of acetonitrile:pyridine:acetic anhydride in a ratio of 50:10:6, for 30 min at 100 °C. After derivatization the solution is blown to dryness and reconstituted with 50 μl acetonitrile. Norleucine and sorbitol are used as internal standards.

An amino acid standard solution (Sigma AAS-18), mixed with a solution containing hydroxyproline, glucose and arabinose in the same concentration as the amino acids in the standard solution, was used to calculate the amino acid content of the analyzed sample.

3.3.1. Apparatus and method

The apparatus used for amino acid and carbohydrate analysis is a Thermo Quest 8000^{top} Gas Chromatograph and Voyager^{plus} Mass Spectrometer. The column is a Supelco Wax-10, length 30 m, internal diameter 0.2 mm and film thickness 0.2 μm. Helium is used as the carrier gas, constant flow 0.8 ml/min. Injection of the sample is done in splitless mode, splitless for 1 min. The GC inlet temperature is 220 °C. The oven temperature is programmed from 80 °C, held for 2 min, raised with a rate of 50 °C/min until 210 °C and then with a rate of 2 °C/min until 250 °C, held for 3 min. The temperature of the MS transfer line is 220 °C, the ion source is set at 200 °C. MS data is

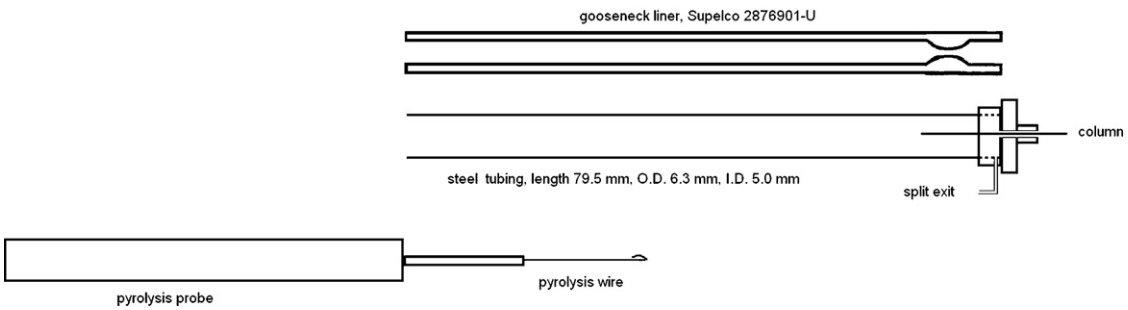

Fig. 2. Schematic view of the pyrolysis chamber, liner and probe.

collected in EI mode from m/z 35 to 600, at 70 eV electron energy, scan time 0.25 s. The solvent delay is set to 4 min.

Xcalibur 1.4 software is used for data acquisition and processing.

4. Results and discussion

4.1. Composition of the Round Robin sample

As described in the Experimental section, the Round Robin sample was made from Paraloid B82 acrylic resin (a copolymer of ethyl acrylate and methyl methacrylate, p(EA/MMA)), sandarac, mastic, linseed oil with ultramarine pigment, egg white, gum arabic and succinic acid. The compounds selected for the sample were chosen in part to complicate the interpretation of the analytical results. Gum arabic, for instance, contains a small amount of protein (about 0.5%), with a high proportion of hydroxyproline [16]. Hydroxyproline is also a marker for the presence of collagen (animal glue). The protein in the gum arabic will therefore influence the identification of the protein in the sample, in this case egg white.

Sandarac, a diterpenoid resin derived from Tetraclinis articulata (North Africa), contains polycommunic acid as the major component [17], together with minor amounts of sandaracopimaric acid, 12 acetoxy sandaracopimaric acid and totarol [17]. Copal resins also contain poly communic or poly ozic acid [8,17]. Sandarac has a composition different from the copal resins, but can potentially be misinterpreted, for example, as a mixture of copal and colophony.

Succinic acid was added to the mixture, not only to add something puzzling for the interpretation of the composition, but also, when analyzed as its dimethyl ester, the compound has a low elution temperature and may not be detected with a GC method using a relatively high initial temperature or long solvent delay.

A modern synthetic resin, Paraloid B82 was added, along with the polysaccharide (gum arabic) and protein (egg white), to necessitate the application of a variety of analytical protocols.

For the confirming analysis by the author, the sample was first analyzed by Py-GC/MS to check if all the organic components in it could be analyzed, and then by a GC/MS method for the combined analysis of amino acids and carbohydrates.

4.2. Thermally assisted hydrolysis and methylation GC/MS

The chromatogram for the oil/wax/resin analysis of the Round Robin sample is shown in two parts: Fig. 3 shows the first half of the chromatogram until methyl stearate (FA-C18) and Fig. 4 shows the second half, from FA-C18 until the triterpenoid resin region.

The identification of components in the sample was based on data and information gained from literature and from the analysis of fresh and aged reference materials and samples of works of art. The interpretation must take into account that the composition of fresh materials can differ significantly from aged materials. Often a specific combination of compounds has to be present for an unambiguous identification, although a quick indication for the presence of a compound in the analyzed sample can be obtained by plotting extracted ion chromatograms.

A list of characteristic marker compounds used in the interpretation of the Round Robin sample, with m/z values of abundant and/or significant ions, and elution temperatures relative to the fatty acids and the di- and triterpenoid resins, is presented in Appendix A.

The identity of the acrylic resin (Paraloid B82) was affirmed by the presence of EA and MMA [15] in the analysis. Some iBMA (*iso*-butyl metacrylate) was also present, probably as an impurity in this batch of the resin.

The presence of linseed oil was confirmed by the presence of glycerol (detected as the di- and trimethyl ether), methyl esters of the dicarboxylic acids suberic (FA-2C8) and azelaic acid (FA-2C9), and the fatty acids palmitic (FA-C16) and stearic acid, with a ratio of 1.1. The palmitic to stearic acid ratio is low for linseed oil, which is typically in the range of 1.4–1.7 [17]. A ratio below 1.4 is to be expected in the case of a boiled oil, because the boiling point of the free palmitic acid is lower then that of free stearic acid. However, since no information was available about the preparation and han-

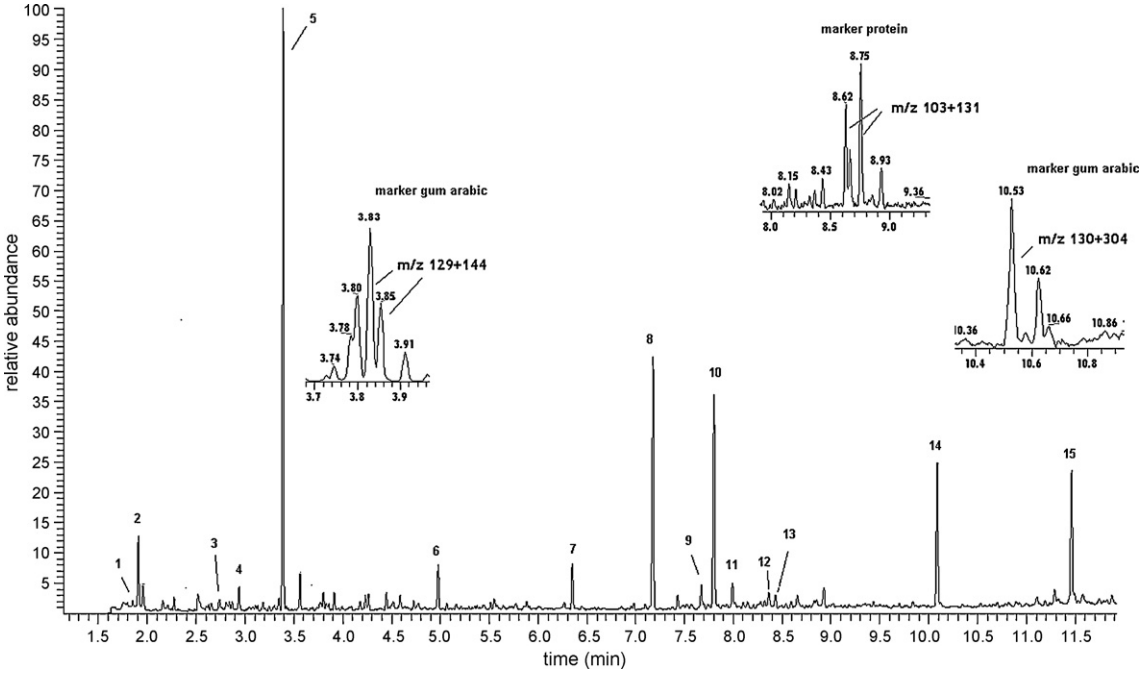

Fig. 3. THM-pyrolysis-GC/MS analysis of the Round Robin sample, part 1: 1 = ethyl acrylate (EA); 2 = methyl methacrylate (MMA); 3 = glycerol; 4 = *iso*-butyl methacrylate; 5 = succinic acid; 6 = EA/MMA dimer; 7 = suberic acid; 8 = azelaic acid; 9 = sandarac marker (236); 10 = internal standard, tridecanoic acid; 11 = sebacic acid; 12 = sandarac marker m/z 161–175–191–250; 13 = sandarac marker; 14 = palmitic acid; 15 = stearic acid. All acidic/alcoholic compounds detected as methyl esters/ethers. Inserts in the chromatogram show extracted ion chromatograms of pyrolytic fragments of gum arabic and protein.

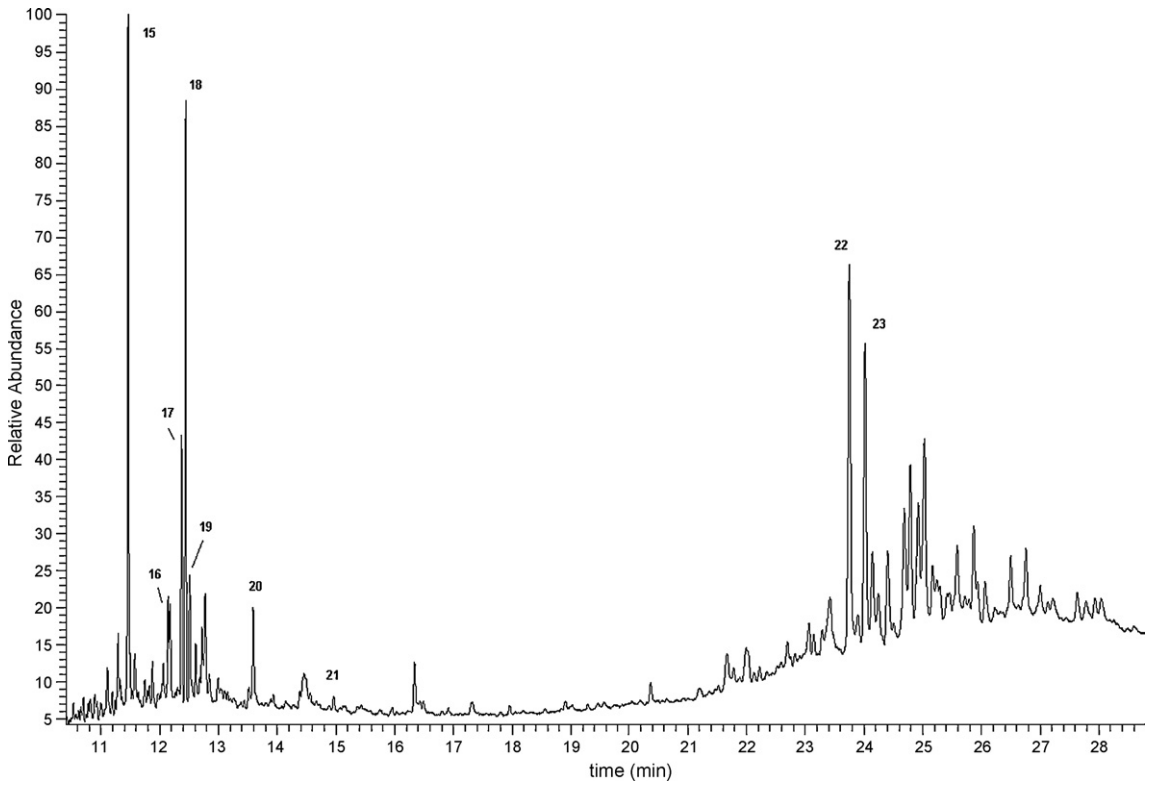

Fig. 4. THM-pyrolysis-GC/MS analysis of the Round Robin sample, part 2: 15 = stearic acid; 16 = ozic acid–communic acid; 17 = ozic acid–communic acid; 18 = sandaracopimaric acid; 19 = isopimaric acid; 20 = hydroxy sandaracopimaric acid; 21 = 12 acetoxy sandaracopimaric acid; 22 = moronic acid; 23 = oleanonic acid. All acidic/alcoholic compounds detected as methyl esters/ethers.

dling of the linseed oil, the low palmitic to stearic ratio cannot be explained with certainty.

The dimethyl ester of succinic acid is detected early in the chromatogram. Succinic acid is a marker for 'Baltic amber', a fossil pine tree resin. In addition to succinic acid, Baltic amber contains borneol and pine resin components such as $\Delta 8$ isopimaric acid, abietic acid, and dehydroabietic acid [17], which were not observed in the Round Robin sample.

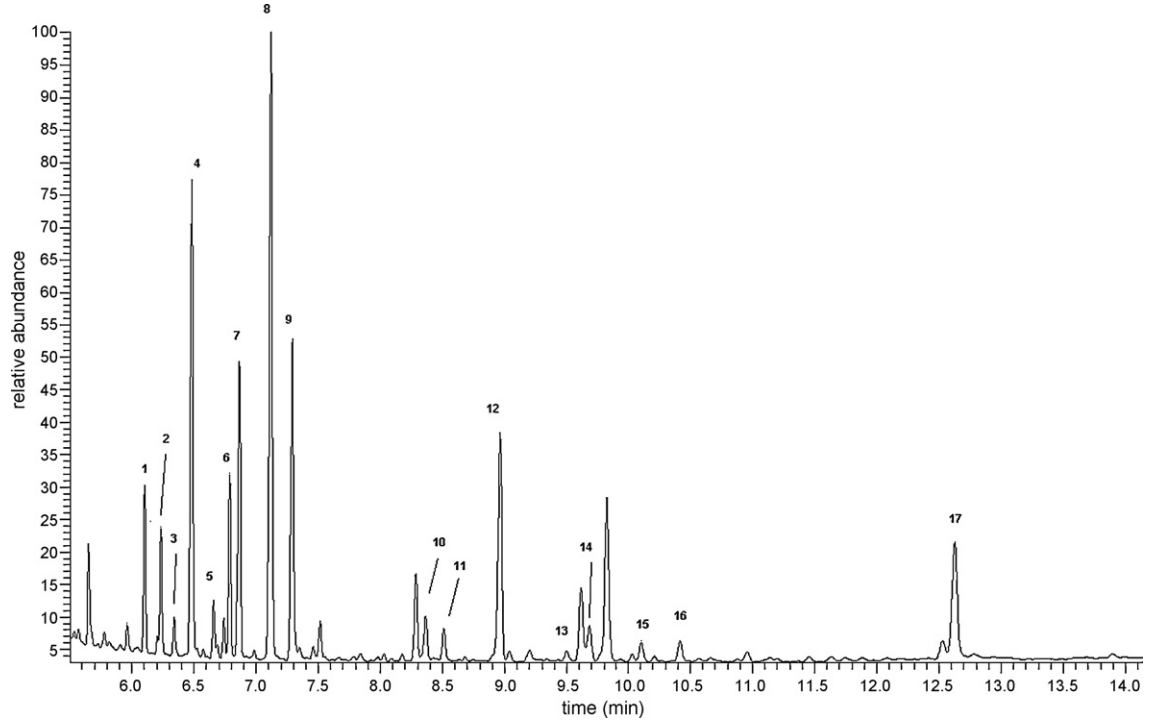

Fig. 5. Amino acids and carbohydrates, detected as *N*,*O*-acetyl methyl esters, in the Round Robin sample, part 1: 1 = alanine; 2 = valine; 3 = FA-2C8; 4 = glycerol; 5 = isoleucine; 6 = leucine; 7 = FA-2C9; 8 = norleucine (internal standard); 9 = FA-C16; 10 = proline; 11 = rhamnose; 12 = FA-C18; 13,14,15,16 = arabinose; 17 = glutamic acid.

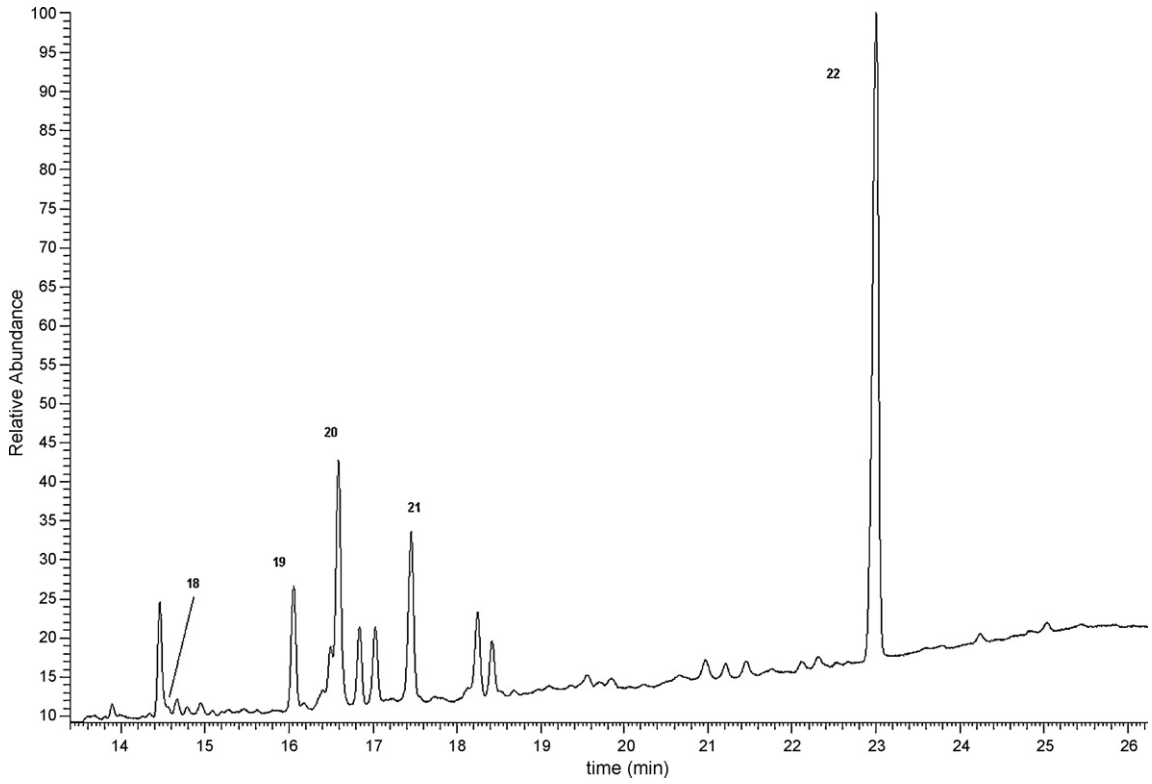

Fig. 6. Amino acids and carbohydrates, detected as *N*,*O*-acetyl methyl esters, in the Round Robin sample, part 2: 18 = hydroxyproline; 19–21 = galactose; 22 = sorbitol (internal standard).

Sandarac contains about 70% of polycommunic acid [17]. The polycommunic acid is pyrolyzed into characteristic markers [8], consisting of different types of hydrogenated and substituted naphthalene carboxylic acid. In the region of the internal standard (tridecanoic acid), small peaks corresponding to these markers were detected. The presence of a hydroxy form of sandaracopimaric acid [8] (significant ions *m*/*z* 121–346) and 12 acetoxy sandaracopimaric acid (significant ions *m*/*z* 121–314) was also important for the confirmation of sandarac.

In the later portion of the chromatogram (Fig. 4) the triterpene species moronic and oleanonic acid are detected (as their methyl esters), together with peaks for other derivatives of mastic components and some non-mastic, triterpenoid compounds possibly present as impurities in the reference material used for the test sample. Pyrolytic fragments of the gum arabic and the protein are detectable as small peaks in the baseline. Inserts in the chromatogram in Fig. 3 show the extracted ion chromatograms for these components.

4.3. Analysis of amino acids and carbohydrates

In the second GC/MS analysis, for amino acids and carbohydrates, proteins were identified by correlating the stable amino acid [16] content with the amino acid content of reference proteins and mixtures of proteins and polysaccharides, collected in a correlation database.

Stable amino acids – that is, amino acids not affected by the aging of proteinaceous binding media present in samples of works of art – are alanine (*m*/*z* used for quantitation: 86), glycine (72), valine (72), leucine (86), isoleucine (86), proline (70) and hydroxyproline (68).

Carbohydrates were identified by comparing the derivatives detected with those of reference gums and sugars. Fatty acids (as methyl esters) and acetylated glycerol can also be detected by the method.

Fig. 5 shows the first half of the chromatogram, until retention time 14 min, and Fig. 6 shows the second half from retention time 14 to 25 min.

The analysis was performed in full scan mode. The peaks for the stable amino acids, glycerol, fatty acids, the internal standards and the component sugars of gum arabic are indicated in the chromatogram. Gum arabic is identified by the presence of rhamnose (*m*/*z* used for quantitation: 157), arabinose (128) and galactose (115). Although the amount of protein originating from the gum arabic is relatively small (gum arabic contains about 0.5% of protein), the amino acids are nonetheless detectable in the analysis, with hydroxyproline eluting as a shoulder of the phenylalanine peak.

The Round Robin sample contains gum arabic and egg white in a ratio of 45:55. Due to the contribution of the amino acids of the gum, the correlation coefficient of the stable amino acids in the Round Robin test sample with a reference egg white was only 0.600. The best correlation was obtained with a mixture of 1% animal glue and 99% whole egg (0.960). A combined derivatization and GC/MS method allowing the detection of both proteins and carbohydrates is therefore important to avoid misinterpretation of a mixture containing both types of material.

5. Methods and results of the participants of the MaSC Round Robin test

Sharing methods and results between the participants was the main goal of the Round Robin test. The full reports containing the applied methods, results and interpretation of the data were shared between the ten participants. Some general observations on their methods and results are presented below.

Table 1
Analytical techniques/protocols and number of times used by participants to analyze the Round Robin sample.

Analytical technique/method	Number of times used
Overall composition	
FTIR	6
Synthetic resins	
Py-GC/MS	4
Pigment	
SEM-EDS	1
Oils/waxes/resins	
Transesterification, MethprepII	7
Transesterifacation, TMSH	1
Py-GC/MS, TMAH	2
Py-GC/MS, HMDS	1
Silylation after extraction	3
Proteins	
Silylation MTBSTFA (+TMDMCS)	4
Amino Acid Analyzer	1
Py-GC/MS	1
Carbohydrates	
Methanolysis-silylation	1
Hydrolysis-oximation-acetylation	3

5.1. Methods used by the Round Robin participants

The analytical techniques and methods used by participants are summarized in Table 1.

The reports submitted by the participants show that Fourier transform infrared spectroscopy (FTIR) is often used as a non-destructive check of the composition of the sample or as a first step in the standard approach of analysing unknown samples.

Six of the participants used FTIR, as shown in Table 1. In general the presence of the synthetic and natural resins, oil and pigment was noted, and in some cases specific identifications were made using this technique. The presence of the protein and carbohydrate was not always observed in every case where FTIR was used. Scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) was used once to identify the pigment.

The second step was the choice of GC/MS methods for the identification of the organic components in the sample. Often three different methods were used: one for the analysis of the oils, waxes and resins, one for the analysis of proteins, and one for carbohydrates.

For the analysis of oils, waxes and resins, transesterification with (*m*-trifluoromethylphenyl)-trimethylammonium hydroxide (available commercially as TMTFTH or "MethprepII" (Alltech)), was used by seven of the participants. Transesterification with trimethyl sulfonium hydroxide (TMSH) was used once.

Py-GC/MS in combination with TMAH was used in total by two participants; and Py-GC/MS in combination with hexamethyldisilazane (HMDS) once.

Silylation of extractable compounds and materials was used for the identification of free fatty acids, waxes and resins by three of the participants.

Transesterification with MethprepII or TMSH is a simple and effective way to analyze oils, waxes and natural resins. Sample preparation takes a couple of minutes, and no extra equipment is needed for the sample introduction.

The injection of the sample/reagent solution is done in split or splitless mode in a hot injector. One of the disadvantages of the use of MethprepII is the co-elution of reagent peaks with components with a low elution temperature. In addition, the analysis of synthetic resins such as acrylics is not possible by these hot injector transesterification techniques.

Proteins were derivatized using MTBSTFA/TBDMCS (*N*-[*tert*-butyldimethylsilyl]-*N*-methyltrifluoroacetimide with *tert*-butyldimethylchlorosilane) after hydrolysis with 6 N hydrochloric acid by four of the participants. Because the triglycerides of oils and egg yolk are also hydrolyzed by the HCl, fatty acids and glycerol can be analyzed by the same method. Py-GC/MS and an Amino Acid Analyzer was used once.

The analysis of gums and sugars appears not to be part of a standard routine in analysing unknown samples in all laboratories. Methanolysis of the gum followed by silylation was used once; hydrolysis with trifluoroacetic acid followed by oximation and acetylation was used twice.

Py-GC/MS was used by four of the participants for the identification of the synthetic resin.

5.2. Materials identified by the Round Robin participants

All participants used a combined method for the analysis of oils, waxes and resins. The identification of the oil and the mastic was unambiguous. The identification of sandarac was more troublesome: four participants interpreted the succinic acid along with sandarac components as Baltic amber, or as an indication of the presence of Baltic amber together with sandarac. One participant identified the sandarac as a copal. Three of the participants mentioned the succinic acid just as it was, as a (probable) addition to the sample.

The presence of B82 acrylic resin was determined six times; the ultramarine blue pigment seven times.

Egg white and gum arabic are a minor part of the sample. Six of the participants used FTIR to indicate the presence of (egg) protein or polysaccharide, but the presence of the protein and the carbohydrate was not always noticed. Also not all the participants run a GC/MS amino acid and carbohydrate analysis as a part of a standard routine.

GC/MS analysis of the amino acid composition of proteins was performed five times, resulting in the indication of egg white three times and of egg (not specifying white/yolk or whole egg) twice. An Amino Acid Analyzer and Py-GC/MS were used once with a positive identification.

GC/MS carbohydrate analysis was performed four times, resulting thrice in a positive identification of gum arabic.

One of the participants used Py-GC/MS, with and without online derivatization with TMAH, to determine the composition of the sample, with a correct identification of all of the materials present.

Stepwise extraction, followed by different types of derivatization and GC/MS analysis, was applied by one of the participants also with a correct identification of the materials in the sample.

The materials identified in the sample, and the number of times reported, are summarized in Table 2.

Table 2
Materials identified in the Round Robin test sample and the number of times reported by the test participants.

Compound	Number of times identified
Acrylic resin (EA/MMA)	6
Succinic acid	3
Baltic amber (possible)	4
Copal	1
Sandarac	7
Mastic	10
Linseed oil	10
Ultramarine blue	7
Gum arabic	3
Gum (possible)	2
Egg white	4
Egg	4

6. Conclusion

The type of analysis performed to determine the presence of a compound in a sample from a work of art is often decided in response to a specific question: is the binder an oil? Is a protein present? What kind of resin is applied as varnish? By the MaSC members different analytical methods and instrumental setups are used for the same purpose: qualitative or quantitative analysis of components of interest present in the sample. The small sample size typically available demands proven analytical methods with a reliable result. Identification of the composition of a sample is a matter of interpretation of the analytical results and the coherence of the results with the provenance of the sample.

The Round Robin test sample was therefore challenging since it was an artificial sample, with no information available about its provenance or the possible composition. The test sample was complex, with materials deliberately chosen to complicate the interpretation the composition. Experience and knowledge of the scientist and a variety of instrumental setups had to be used for the identification of the materials in the sample.

Stepwise extraction, followed by different types of derivatization, is a successful approach for the analysis and identification of complex samples. For stepwise extraction, however, relatively large samples are needed, which are not always available. For the analysis of polymers such as acrylics, pyrolysis equipment is essential.

Thermally assisted hydrolysis and methylation GC/MS in combination with pyrolysis, also used for the confirming analysis of the sample, has been demonstrated to be a powerful technique for the analysis of multi-component samples. The presence of gums and proteins can be indicated, and synthetic resins can be analyzed in combination with the traditional binders and resins.

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Appendix A

Characteristic ions (m/z) used to check the presence of marker compounds in the Round Robin sample. Analysis performed using pyrolysis-GC/MS with TMAH: carboxylic and hydroxyl groups converted to methyl ester and methyl ether groups, respectively.

m/z	Compound
Drying oil	
59	glutaric acid (FA-2C5)
114	adipic acid (FA-2C6)
115	pimelic acid (FA-2C7)
138	suberic acid (FA-2C8)
152	azelaic acid (FA-2C9)
125	sebacic acid (FA-2C10)
74–270	palmitic acid (FA-C16)
74–298	stearic acid (FA-C18)
Succinic acid	
115	succinic acid
Sandarac	
121–316	sandaracopimaric acid
121–346	hydroxy sandaracopimaric acid
121–314	sandaracopimaric acid, 12-acetoxy
189–285	totarol/ferruginol
Mastic	
189	moronic acid
203	oleanolic acid
219	oxidized triterpene
Proteins	
127–183	marker, eluting before FA-2C8
127–142	marker, eluting before FA-2C6
103–131–160	marker, eluting after FA-2C10
103–131–175	marker, eluting after FA-2C10
98–157	glutamic acid, eluting before FA-2C8
126–158	hydroxyproline
128	proline
Acrylics	
69–100	methyl methacrylate (MMA)
56–69–87	*iso*-butyl methacrylate (iBMA)
55–99	ethyl acrylate (EA)
Gum Arabic	
45–101	pyrolysis product, eluting before FA-2C4
55–129–144	pyrolysis product, eluting after FA-2C4
130–304	pyrolysis product, eluting after FA-C16
101–143–175	pyrolysis product, eluting after FA-C18

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