

MaSC

Users' Group for Mass Spectrometry and Chromatography

MaSC¹ Round Robin 1 (2007) sample
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1. Introduction

Samples from works of art are often small and complex in composition. The presence of drying oils, resins, polysaccharides and waxes as components of a single sample is not uncommon. The type of analysis done to characterise the sample is often prompted by the questions posed by the conservator or curator; Is the binder an oil?; Is it a protein?; What kind of resin is applied as varnish?

To characterise such samples, the MaSC members use a range of different analytical methods and instrumental setups, all used for the same purpose - the qualitative or quantitative analysis of all components present in the sample.

Usually the first step in determining the composition of a sample is the use of Fourier Transform Infrared spectroscopy (FTIR), a non-destructive method which produces information about the kind of compounds present in the sample. The second step is the choice of the appropriate GC/MS derivatization methods for the identification of the sample.

Because the precise composition of the samples is often not known, identification cannot be answered with a simple yes or no. On the other hand, if we did know the composition of the sample, are the methods used for analysis selective and sensitive enough to detect and identify the components in the sample? It was felt that it would be interesting to share and discuss the results obtained using the range of different methods used by MaSC members, by preparing and analysing a Round Robin Sample.

The sample, an artificial sample, was made of compounds which can be present in samples from works of art, like (drying) oils, waxes, natural resins and gums. To enable the application of the full range of analytical methods, the sample was a multi component mixture. The composition was not revealed before hand, but together with the sample a note was included to inform the participants about the possible composition and to advise powdering the sample before analysis.

The sample was sent to fifteen participants who expressed interest in being involved in the first MaSC Round Robin test. Ten were able to analyse the sample and to report the results within the time schedule, and the test sample and results were discussed at the MaSC meeting in September 2007. At this meeting the participants agreed to share the analytical results between those involved with the Round Robin test and submit a report.

This document (also available at the MaSC website) describes the preparation and analysis of the sample and includes a summary of the results obtained by the participants in this experiment.

2. Preparation of the Round Robin test sample

The components selected to be part of the sample were deliberately chosen to complicate the interpretation of the analytical results. Gum Arabic, for instance, also contains a small amount of protein, about 0.5 %, with a high portion of hydroxyproline². Hydroxyproline is also a marker for the presence of collagen (animal glue). Sandarac, a diterpenoid resin, which contains polycommunic acid as the major component was also added³. Some copal resins also contain polycommunic acid, while others contain polyozic acid. Sandarac has a composition different from

the copal resins, but can be misinterpreted as a mixture of copal and colophony.

Preparation of the sample started with two glass plates covered with oil paint. The plates were marked as 'ultramarine blue in linseed oil, Winsor and Newton'. The paint was applied about 25 years ago and formed part of a reference collection. The paint was scraped off the plates and reduced to a powder in a mortar. The powdered paint was sieved and a known amount was used for the Round Robin sample. All of the additional compounds in the sample were added as weighed amounts of powder. Sandarac and mastic resins were added to the sample. The rationale for this addition was that these resins, together with the dried linseed oil, would result in low, mid and high boiling components being detected in a GC/MS analysis if the components in the sample were analysed as methyl esters.

Succinic acid was also added to the mixture, not only to add something puzzling for the interpretation, but also because, when analysed as the dimethyl ester, succinic acid has a low boiling point and will not be detected using a GC method with a high initial temperature. Succinic acid is a marker for Baltic amber³, a fossil tree resin. When analysed Baltic amber yields borneol and components typical of pine resin, including $\Delta 8$ isopimaric acid, abietic acid, and dehydroabietic acid, in addition to succinic acid. However, pure succinic acid and sandarac resin were added to the mixture, rather than Baltic amber. Sandarac has a composition different from the pine resins and the absence of abietic acid in sandarac meant that oxidized pine resin components were not present in the Round Robin sample, despite the apparent marker component for Baltic amber being present.

To explore the use of pyrolysis techniques, B82, an acrylic EA/MMA resin, was added to the Round Robin sample. Of course a polysaccharide and a protein had to be in the sample in order to assess the application of the full range of analytical methods and Gum Arabic and egg white were selected. As mentioned above, Gum Arabic contains a small amount of protein with a high portion of hydroxyproline. The hydroxyproline, typically interpreted as a marker for the presence of animal glue, would therefore potentially complicate the successful identification of the egg white protein component.

The starting mixture of the Round Robin test sample contained:

	Weight / g	% of total
Dried Linseed oil with ultramarine blue	3.0	33
Paraloid B82	1.7	19
Sandarac	1.0	11
Succinic acid	0.5	5
Mastic	2.0	22
Gum Arabic	0.4	4.5
Egg white (dry)	0.5	5.5

The mixture was stirred into a slurry with some methanol. After evaporation of the methanol the slurry turned into a blue putty. The putty was flattened and left to dry. After drying the sample was crushed into small fragments.

3. GC/MS analysis of the MaSC Round Robin 1(2007) sample

The sample was analysed at the ICN to check the composition and to investigate whether all the components in it could be analysed by the ICN GC/MS methods. Two methods are used at the ICN, one for oils, waxes, resins and (synthetic) polymers and one for the combined analysis of proteins and polysaccharides.

3.1 Analysis of oils, waxes, resins and (synthetic) polymers

At the ICN, samples of unknown composition for GC/MS analysis are always analysed using thermally assisted hydrolysis and methylation GC/MS (thm-GC/MS) in combination with Curie-point pyrolysis. This combination of techniques hydrolyses and methylates (drying) oils and waxes, methylates resin acids and pyrolyses natural and synthetic polymers like copal, shellac, acrylics and polyurethanes⁴. The heat of the pyrolysis wire and the pyrolysis unit also means that neutral components like hydrocarbons and plasticizers are vaporized and can be analyzed. The presence of

proteins and carbohydrates can be indicated by pyrolytic fragments in the chromatogram. For confirmation of their presence and identification, the sample is analysed separately.

Just prior to analysis, samples are made into a suspension with 10 µl of a 2.5 % solution of tetramethylammonium hydroxide (TMAH) in methanol. The suspension is applied to the looped end of a pyrolysis wire by means of a syringe or by dipping the wire into the suspension. After a short period of drying in a stream of slightly warm air (30 °C), the wire is loaded in the unit and pyrolyzed. The pyrolytic fragments are separated and identified with GC/MS.

Apparatus

Modified GSG Pyromat on a Thermo Quest 8000^{top} Gas Chromatograph linked to a Voyager^{plus} Mass Spectrometer.

The Pyromat (configured as a single shot pyrolyzer and without an autosampler) was originally equipped with a removable glass sample holder / pyrolysis chamber to offer the possibility of loading and purging the chamber at room temperature. After purging, the glass pyrolysis chamber could then be placed in the heated zone of the Pyromat.

Using thm-GC/MS analysis in combination with pyrolysis the sample is hydrolysed, methylated and fragmented and most of the components of interest, formed by the reaction, have a boiling point of above 220 °C. To prevent condensation of the components in the pyrolysis chamber, the chamber needs to have a temperature of at least 280 °C which could not be reached during the short time the glass pyrolysis chamber was in the heated zone of the Pyromat prior to pyrolysis.

The pyrolysis chamber was therefore modified by replacing the glass sample holder with a steel tube / glass liner assembly. The steel tube is always in the heated zone of the pyrolysis unit. A probe holding the wire is used for the introduction of the sample. A split exit, directly after the unit, is used to split the sample and to vent the trimethylamine formed by the pyrolytic reaction of the TMAH.

Unit temperature	295 °C
Pyrolysis temperature	625 °C
Pyrolysis duration	6 seconds
Carrier He	constant flow 0.7 ml
Column	SLB5 ms, 20 m id 0.18 mm ft 0.18 µm
Split ratio	1:10
Temperature programme	35 °C (hold 1 min) to 110 °C at 60 °C/min; 14 °C/min to 240 °C at 6 °C/min to 315 °C (hold for 3 min)
Transfer line	250 °C
Source	220 °C
Full scan	m/z 45 - 600
Scan time	0.15 s
Solvent delay	1.2 min

3.2 Analysis of amino acids and sugars

Amino acids and sugars were analysed, in one run, as N,O-acetyl methyl esters after hydrolysis with trifluoroacetic acid of the proteins and the polysaccharides⁵. Hydrolysis was performed with 4 M TFA for 16 hours at 110 °C. After hydrolysis, the acidic fraction was evaporated with a stream of nitrogen and the residue was methylated with 1.5 N methanolic HCl at 90 °C for 2 hours. After evaporation of the methanolic HCl, the residue was acetylated with a mixture of acetonitrile:pyridine:acetic anhydride in a ratio of 50:10:6, at 100 °C for 30 minutes. After derivatization the solution was evaporated to dryness and taken up in acetonitrile.

Apparatus

Thermo Quest 8000^{top} Gas Chromatograph linked to a Voyager^{plus} Mass Spectrometer.

Carrier He	constant flow 0.7 ml
Column	Supelco wax-10, 30 m id 0.2 mm ft 0.2 µm
Splitless injection	1 µl, splitless for 1 minute
Injector temperature	220 °C
Temperature programme	80 °C (hold 2 min) to 210 °C at 50 °C/min; 2 °C/min to 250 °C (hold for 3 min)
Transfer line	220 °C
Source	200 °C
Full scan + SIM	m/z 35 - 600 / SIM
Scan time	0.25 s
Solvent delay	4 min

4. Results and discussion

4.1 Analysis of oils, waxes, resins and (synthetic) polymers

The chromatogram of oils-waxes-resins analysis of the Round Robin sample is shown in two parts. Figure 1 shows the first half of the chromatogram up to and including the fatty acid C18, while Figure 2 shows the second half, from fatty acid C18 until the triterpenoid region.

The identity of the acrylic resin (Paraloid B82) is confirmed by the presence of EA and MMA⁶ in the analysis. Also some iBMA is present, probably as an impurity in this batch of the resin. Glycerol and the methyl esters of the fatty di-acids 2C8, 2C9 and the fatty acids C16 and C18, with a ratio of 1.1, confirm the presence of linseed oil. The palmitic and stearic acid ratio is low for linseed oil, which is normally 1.4 - 1.7³. A small ratio is to be expected in the case of preheated oil, because the boiling point of the free palmitic acid is lower than the boiling point of free stearic acid. Because no information was available about the source and treatment of the oil, the type of oil cannot be indicated.

The dimethyl ester of succinic acid is seen in Figure 1. Succinic acid is often a marker for Baltic amber. In this case succinic acid was added to the sample, but also the absence of borneol, Δ⁸ isopimaric acid and the amount of succinic acid in the analysis, compared to the quantity and the type of diterpenoid components, indicates that amber is not present.

In the region where the internal standard (C13 fatty acid) elutes, small peaks of the so called 'copal markers' are seen. The markers are pyrolytic fragments from the communic and / or ozic acid polymer fraction of copal resins and sandarac⁷. The origin of a copal resin can be determined on the basis of the identity of these markers in combination with the diterpenoid components. For the identification of sandarac it is not the markers but the presence of a hydroxy form of sandaracopimaric acid (m / z 121/346), retention time 13.59 min and an unknown component, probably 12-acetoxysandaracopimaric acid (m / z 121/314), with a retention time of 14.96 that is characteristic.

In the triterpenoid region moronic and oleanonic acid derivatives are present together with other components of the mastic resin and some other triterpenoid impurities.

sample: round robin sample
file: C:\Xcalibur\...rr-1(2007)

GCMSmethod: C:\Xcalibur\methods\tmah-SLB-5ms 20m 0.18 0.18.meth
conditions: py 625°C tmah SLB5ms 20m .18 .18 35-300

operator: hvk

RT: 1.14 - 11.92 SM: 3B

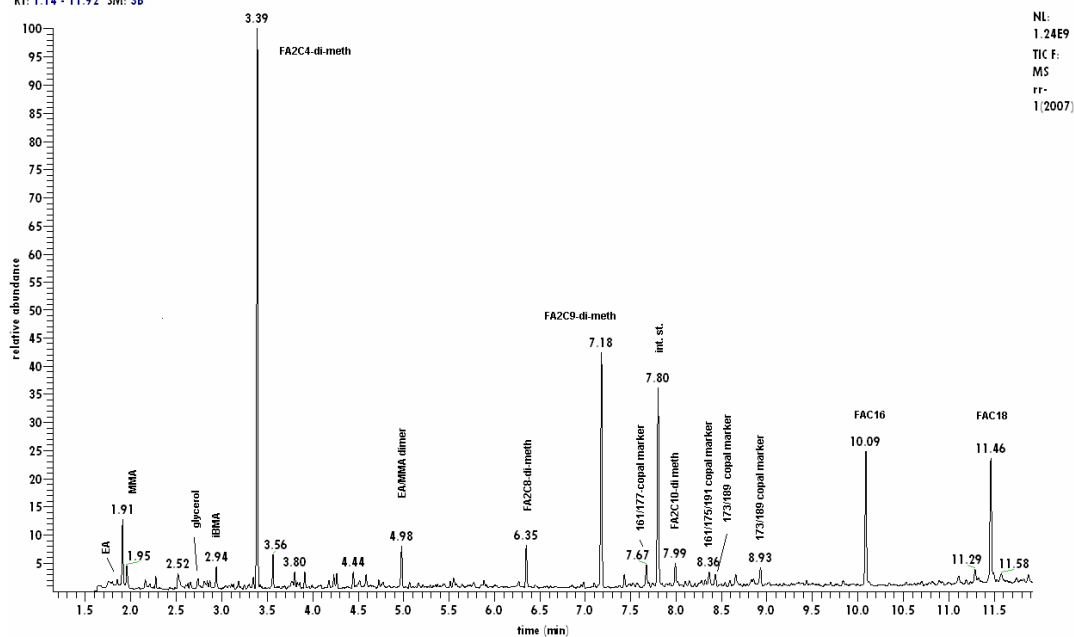


Figure 1: thm-pyrolysis analysis of the Round Robin sample, part 1

sample: round robin sample
file: C:\Xcalibur\...rr-1(2007)

GCMSmethod: C:\Xcalibur\methods\tmah-SLB-5ms 20m 0.18 0.18.meth
conditions: py 625°C tmah SLB5ms 20m .18 .18 35-300

operator: hvk

RT: 11.03 - 28.92 SM: 3B

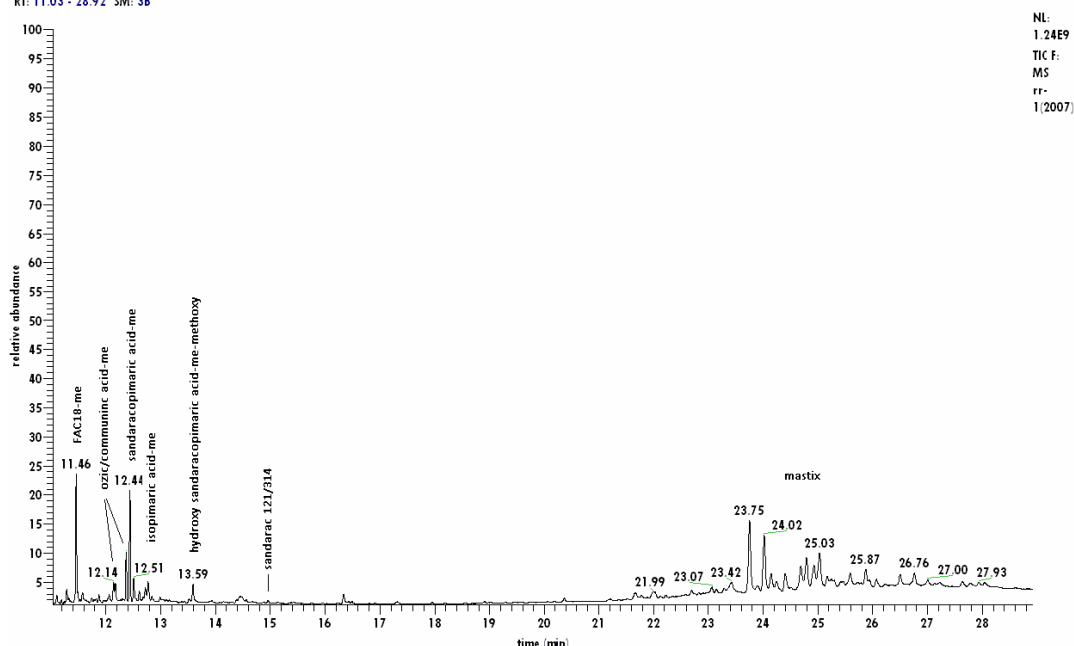


Figure 2: thm-pyrolysis analysis of the Round Robin sample, part 2

4.2 Analysis of amino acids and sugars

Amino acids and carbohydrates were analysed, in one run, as N,O-acetyl methyl esters after hydrolysis with 4 M trifluoroacetic acid (TFA) of the proteins and the polysaccharides. The method is used as a qualitative method; the proteins are identified by correlating the stable amino acids² with those of reference proteins and mixtures of proteins and carbohydrates. The carbohydrates are identified by comparing the presence and peak area of the derivatives with reference gums and

sugars. The simultaneous hydrolysis of the proteins and the polysaccharides is a compromise between an efficient hydrolysis of the proteins and an acceptable loss of the carbohydrates. The sample size and the possible presence of both proteins and carbohydrates in a sample is the basis for the acceptance of the compromise.

After hydrolysis the amino acids and the sugars were methylated and acetylated. Oximation of the carbohydrates is not included in the method and thus the carbohydrates show up as multiple isomer peaks. Also fatty acids, as methyl esters and acetylated glycerol can be detected by this method.

Figure 3 shows the first part of the chromatogram up to the retention time of 14 minutes, while Figure 4 shows the retention time region from 14 to 25 minutes. The analysis is done in full scan mode and the stable amino acid, glycerol, the fatty acids and the derivatives of the sugars are indicated in the chromatogram.

Gum Arabic contains a small amount of protein, about 0.5 %, with a high portion of hydroxyproline. The amino acids originating from the gum are visible in the chromatogram; hydroxyproline is detected in the slope of the phenylalanine peak.

The protein in the Round Robin test sample was identified by correlating the peak area of the stable amino acids with a correlation database². The Round Robin test sample contains Gum Arabic and egg white in a ratio of 45:55. Due to the contribution of the amino acids of the gum to those of the egg white, the correlation coefficient of the stable amino acids in the sample with a reference egg white was only 0.600. The best correlation of the stable amino acids was with a mixture of 1% animal glue with 99% whole egg (0.960). However, by applying a combined derivatization and GC/MS method for proteins and carbohydrates, misinterpretation can be avoided.

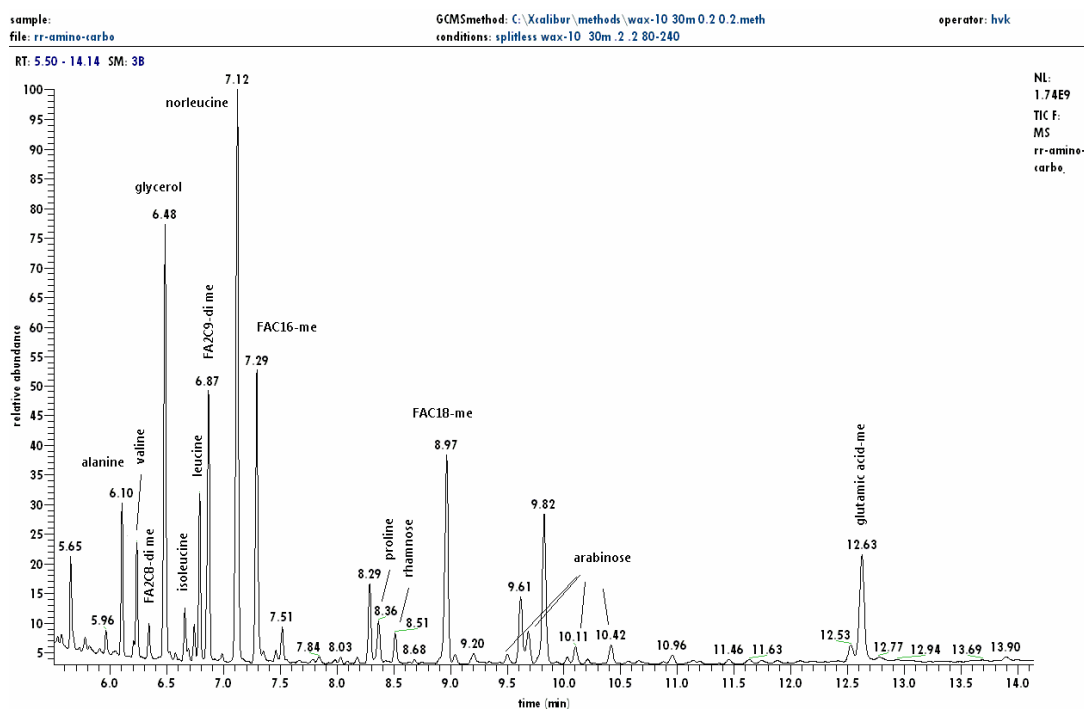


Figure 3: amino acids and carbohydrates analysis of the Round Robin sample, part 1

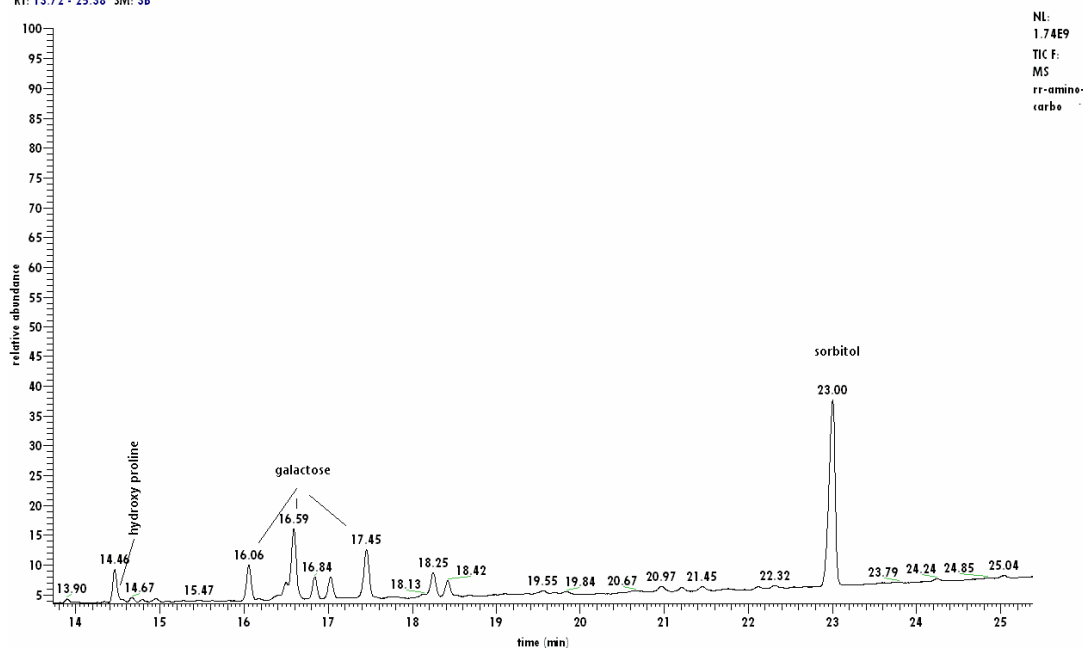


Figure 4: amino acids and carbohydrates analysis of the Round Robin sample, part 2

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

5.1 Introduction

The first Round Robin sample, a multi component mixture, was intended to be an unknown sample, to be analysed with all the standard methods normally used by the participants to analyse samples from works of art. Because the materials in the sample were deliberately chosen for their overlapping composition to complicate the interpretation the composition, identification of the components in the sample was a matter of interpretation of the analytical results. Right or wrong interpretation of the results was not the issue and analysis of the sample was not meant as a competition. The reports containing the applied methods, the results of the methods and the interpretation of the results were accepted in confidence and will not be shared with all the MaSC members. Sharing methods and results between the participants was the main goal of the first MaSC Round Robin.

5.2 Methods

The reports submitted by the participants show that usually the first step in determining the composition of a sample was the use of Fourier Transform Infrared spectroscopy (FTIR) as a non destructive check of the composition of the sample or as a first step in the standard approach of analysing unknown samples. The second step was the choice of the appropriate GC/MS derivatization methods for the identification of the 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 the analysis of carbohydrates.

Six of the participants used FTIR as the first step in the standard approach of analysing unknown samples. The presence of the synthetic and natural resins, oil, pigment, protein and carbohydrate was noted by many, but not all, of those who used FTIR. SEM-EDS was used by one participant to identify the pigment.

FTIR and py-GC/MS, with or without online derivatization with TMAH, were used by one of the participants to determine the composition of the sample, resulting in a successful identification of all of the materials in the sample. Py-GC/MS and stepwise extraction, followed by different types of derivatization and analysis, was applied by one of the participants, also with a successful identification of the materials in the sample.

For the analysis of oils, waxes and resins, transesterification with (m-trifluoromethylphenyl trimethylammonium hydroxide, (TMTFTH) Methprep^{II}), was used by seven of the participants, two used py-GC/MS in combination with TMAH and one used py-GC/MS in combination with hexamethyldisilazane (HMDS) and transesterification with trimethyl sulfonium hydroxide (TMSH).

Comment: Transesterification with Methprep or TMSH is a simple and effective way to analyse oils, waxes and natural resins. Sample preparation takes a couple of minutes; no extra equipment is needed for the sample introduction. The injection of the sample / reagent solution is done in split mode in a hot injector. One of the disadvantages of the use of Methprep is the co elution of the reagent peaks with components with a low elution temperature.

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

Five of the participants used py-GC/MS or py-GC/MS with TMAH or HMDS to identify the type of acrylic resin.

Proteins were analysed as MTBSTFA(-TBDMCS) derivatives after hydrolysis with 6 N HCl by four of the participants. Because the triglycerides of oils and egg yolk are also hydrolysed by the hydrochloric acid, fatty acids and glycerol can be analysed by the same method. An Amino Acid Analyser (AAA) was used by one participant.

Apparently the analysis of gums and sugars is not a part of a standard routine in analysing unknown samples. Methanolysis of the gum followed by silylation was used by one participant; hydrolysis with trifluoroacetic acid followed by oximation and acetylation was used by two participants.

The analytical techniques and the frequency of use by participants are presented in Table 1.

Analytical technique / method	Frequency of use by participants
Overall composition	
FTIR	6
Synthetic polymers	
Py-GC/MS	5
Pigments	
SEM-EDS	1
Oils, waxes and resins	
Transesterification, Methprep ^{II}	7
Transesterification, 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

Table 1. Analytical techniques and the use of the technique by the participants to analyse the composition of the Round Robin test Sample.

5.3 Results and discussion

The Round Robin test sample was composed of B82 acrylic resin, sandarac, mastic, linseed oil (with ultramarine blue pigment), egg white, Gum Arabic and succinic acid. The reports showed that all

the participants made use of some kind of method for the analysis of oils, waxes and resins. The identification of the oil and the mastic was unambiguous, while the identification of sandarac proved more troublesome; four participants interpreted the combined presence of succinic acid and the sandarac components as indicating the presence of Baltic amber, or into a possible presence of Baltic amber together with the 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 mentioned six times; the ultramarine blue pigment seven times.

Six of the participant used FTIR to identify or indicate the presence of (egg) protein or polysaccharides. Egg white and Gum Arabic are a minor part of the sample and their presence was not always noticed. Also not all the participants run a GC/MS protein and carbohydrates analysis as a part of a standard routine.

GC/MS analysis of the proteins was performed five times, resulting in the identification of egg white three times and egg twice. An Amino Acid Analyser and py-GC/MS were used once, both giving a positive identification.

A GC/MS carbohydrate analysis was performed four times, resulting three times in a positive identification of the gum. The possible presence of a gum / polysaccharide was mentioned two times, on the basis of FTIR analysis.

The materials identified in the sample and the frequency of reporting, are presented in Table 2.

Compound	Frequency of identification
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

Table 2. Identified materials in the Round Robin test sample and the frequency of identification, based on analysis by the participants.

6. Conclusion

The Round Robin test sample was an artificial sample, with no information available about the provenance or the composition. Experience and knowledge of the scientist and all kinds of instrumental setups had to be used for the identification of the materials in the sample. Interpretation of the composition of the sample was done on the basis of the identified components. The Round Robin test sample was a complex sample; the materials in the sample were deliberately chosen to complicate the interpretation of the composition. Although in principle all samples of works of art are unknown and can be complex in composition, the results of the participants show that analysis and correct identification of all the materials in a sample is not common practice.

Stepwise extraction, followed by different types of derivatization and instrumental setups is a successful approach for the analysis and identification of complex samples. For stepwise extraction, relatively big samples are needed, which are not always available. To analyse polymers like acrylics and urethanes, pyrolysis equipment is essential.

Pyrolysis, with TMAH or HMDS, is used by four of the participants. Thermally assisted hydrolysis and methylation GC/MS in combination with pyrolysis, also used for the screening of the sample, is a powerful technique for the analysis of single and multi-component samples. The presence of gums and proteins can be indicated using this approach and it also allows synthetic resins like acrylics and urethanes to be analysed in combination with the traditional binders and resins.

Acknowledgements

I would like to thank those who participated in the MaSC Round Robin test, devoting time and effort to analysing the sample and reporting the results including a description of the methods used. I would also like to thank them for their trust in taking part in the Round Robin, their permission to share the results (including the methods used) among the participants and the use of the anonymous results for this report.

Literature

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