THE IDENTIFICATION OF VEGETABLE OIL FROM OLD OIL-TEMPERA MIXED TECHNIQUE PAINTINGS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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În prezentul studiu a fost utilizată cromatografia de gaze cuplată cu spectrometria de masă pentru analiza microprobelor prelevate dintr-un panou al altarului poliptic de la Proştea Mare, altar pictat pe lemn în tehnică mixtă tempera-ulei, datat şfîrşitul secolului al-XV-lea. Panoul face parte din colecţia Muzeului Naţional Brukenthal din Sibiu. Cromatografia de gaze cuplată cu spectrometria de masă a fost utilizată pentru analiza mediului de pictură (liant pe baza de ulei vegetal) pentru probe care au avut în jur de 1mg, urmărindu-se detecţia esterului metilic al acidului palmitic. Rezultatele servesc la certificarea tehnicii mixte tempera-ulei în care este realizată pictura şi pentru alegerea metodelor corecte de restaurare a lucrării.

In the present study, the gas chromatography coupled with mass spectrometry was used for the analyses of micro-samples taken from "Proştea Mare" polyptych altar panel painting. The investigated painting dates from late 15th century, is painted on wood using the oil – tempera mixed technique and belongs to the Brukenthal National Museum, Sibiu collection. Gas chromatography coupled with mass spectrometry was used to investigate the painting medium (vegetable oil based binders) in samples of around 1 mg by methyl ester of palmitic acid detection. The results serve to certify that the mixed painting technique was employed and also to correctly choose the restoration methods for this particular artwork.

Keyword: easel old oil-tempera paintings samples, fatty acids methyl esters, gas chromatography-mass spectrometry.

1. Introduction

The purpose of any painting analysis (and in general, of any artwork analysis) is the identification of its constituent materials. Based on this knowledge, the restoration principles can be applied when working on the investigated painting or artwork. Such principles include the use of identical or

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similar materials, compatible with the painting technique employed by painter. In this respect, great importance is given to the study of binders.

The binders used today are in many respects the same as the ones used centuries ago. Starting with the ancient era, the first binders utilized by mankind consisted of glue, gum, gelatin and egg. Historical sources cite the use of intermediate layers of oils and resins applied between color layers, starting from the 11th – 12th centuries. This constitutes the mixed oil-tempera technique. Hubert van Eyck (1370-1426) discovers, by chance, the oil painting technique, which would be appropriated by the Italian artists after 1480. Oil gradually replaces egg and becomes the most used binder.

The approach to restoration and the methods used differ based on the artwork choice of binders. Traditionally, for the tempera technique, restoration is performed with egg emulsions, as the binders present are egg – based. The binders lose their cohesion in time; the emulsion acts to restore the color layer cohesion, regenerating the binder layer in which pigments are dispersed.

During the restoration work performed on the "Birth of Jesus" polyptych altar panel from "Proştea Mare" it was observed that egg emulsion restoration could only be successfully applied on some areas. This was true for both the front and the back side of the artwork. Furthermore, the macroscopic aspect of the color layer, as observed after the varnish removal, suggested that the paining was not done in the classical tempera technique. These facts lead to the hypothesis that the panel was painted in the oil – tempera mixed technique. The hypothesis is supported by known historical data, as during the period in which the work was created (late 15th century), the oil–tempera mixed techniques was used experimentally in Europe. During this time frame, the change from tempera to oil painting took place. This corresponds to a change from gothic style to renaissance style in European painting.

Vegetable oils couldn’t be analyzed direct by gas-chromatography because of their low volatility and thermal instability. We need some derivatization for increasing thermal stability and volatility. Probably the most known derivatization is silylation with various reagents like N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), with or without 1% trimethylchlorosilane (TMCS) [1]. But the simplier way is methylation to obtain fatty acids methylesters by acidic conditions [2] or basic conditions [3]. Unfortunately in liquid chromatography mass spectrometry methyl esters do not direct ionize instead of that appear some fragments in non reproductive manner from in-source breakdown [4]

In the present study, gas chromatography coupled with mass spectrometry analyses were performed on the painting medium (binder) of microsamples belonging to the "Birth of Jesus" polyptych altar panel from "Proştea Mare". The investigated artwork dates from late 15th century, is painted on wood, on both

sides, using the oil – tempera mixed technique and belongs to the Brukenthal National Museum, Sibiu collection (artwork reference number 1518 for the front side and 1535 for the back side). Because the advanced degradation of the paint layer, the restoration of the wood panel became imperative. Six samples from the front side (P 137) and from the back side of the artwork (P 61, P 64, P 142, P 146 and P 147) were collected both during the preliminary investigation which preceded the restoration procedures and during the restoration work. More details concerning these samples can be found in table 1.

Table 1

List of the analyzed samples

Ref. nr.	Sample code	Sampling area
1.	P 61	Red clothing
2.	P 64	Greenish – blue background
3.	P 137	Green clothing
4.	P 142	Green
5.	P 146	White edge
6.	P 147	Blue edge

Gas chromatography coupled with mass spectrometry analysis focused on the identification of oil, in order to confirm the usage of the mixed oil–tempera technique and therefore to lead to the choosing of appropriate restoration methods [5].

2. Experimetal

Methyl palmitate and methyl stearate were used as purchased from Riedel, dry methanol was used as received from Sigma-Aldrich, sodium and dry isooctane were used as received from Merck. Helium (99.999999, purchased from Linde) was used as carrier gas. Gravimetric measurements were performed on an AX 210 Mettler electronic balance. A Heidolph magnetic stirrer and a Towson&Mercer ultrasonic bath were used during laboratory work. Syringe equipped with 0.2 μm disposable cellulose filters were purchased from Schleicher&Schuell.

Sample preparation consisted of mixing the micro sample with 5 mL dry methanol and 1 ml dry isooctane into a volumetric flask, followed by the addition of 100 mg metallic sodium, under constant stirring. The samples were subjected to sonication for 1 minute, in order to disintegrate the solids. As a result of the ensuing reaction, the mixture heated and a part of isooctane evaporated. The mixture was left to stand for 1 hour. The upper layer, containing the dissolved isooctane methyl esters, was separated by filtration and the final solution volume was adjusted to 0.5 mL.

Gas chromatography was performed on a Trace GC Ultra gas chromatograph fitted with an AS 2000 automatic injector and a 10 μL Hamilton syringe, equipped with a TR 5 MS column of 30 m length, 0.25 mm diameter and

0.25 μm film thickness from Thermo. Detection was performed on a Polaris Q ion trap mass spectrometer, all fitted modules being provided by Thermo. Data was processed with the help of the Xcalibur software suite, version 1.3 and for spectral identification was performed by employing the NIST '02 library.

Chromatographic separation was performed at a 1 mL/min helium carrier gas flow rate using the following temperature program as: start at 100 ^{0} C, ramping up to 250 ^{0} C at a 20 degrees/min rate and then isothermal regime up to a final time of 12.5 minutes. Pressure increased from 81 psi at the analysis start up to 165 psi at the finish. Retention times were 7.39 min for methyl palmitate and 8.53 min for methyl stearate and compared against a reference solution containing 0.1 μg/mL of each ester. Injector port was heated to 250 ^{0} C and the injection volume was 5 μl with a split flow of 10 ml/min. The high injection volume coupled with low split flow assured a good sensibility and reliable detection. Electron impact provided the ionization source, at 70 eV. The scanning range was set at 50-350 atomic mass units for 0.32 sec, at 300 V multiplier offset.

3. Results and Discussion

Despite that two esters are very close as structure, methylstearate was rarely detected. This could be explained mainly by composition of linseed oils that contains palmitic acid two-tree times more than stearic acid. Data collection was set up to a speed three spectra/sec. We made constrain to have a correct characterization of peaks with minimum ten points.

Figure 1A contains the chromatogram obtained from the sample P 61 analysis. The main peaks of interest were from 7.39 min corresponds to palmitic acid methyl ester and that from 8.53 corresponds to stearic acid methyl ester. The confirmatory spectrum for chromatographic peak from retention time to 7.39 min is in figure 1 B for methylpalmitate, and in figure 1 C for methylstearate, respectively. Due to high abundance of normalized level ions around 10^6 we could assign with high confidence the presence of both esters in sample P 61. Other peaks became from column bleeding and have the main fragment in spectra to m/z = 73. Where spectra didn't match enough with library search we applied background noise substraction.

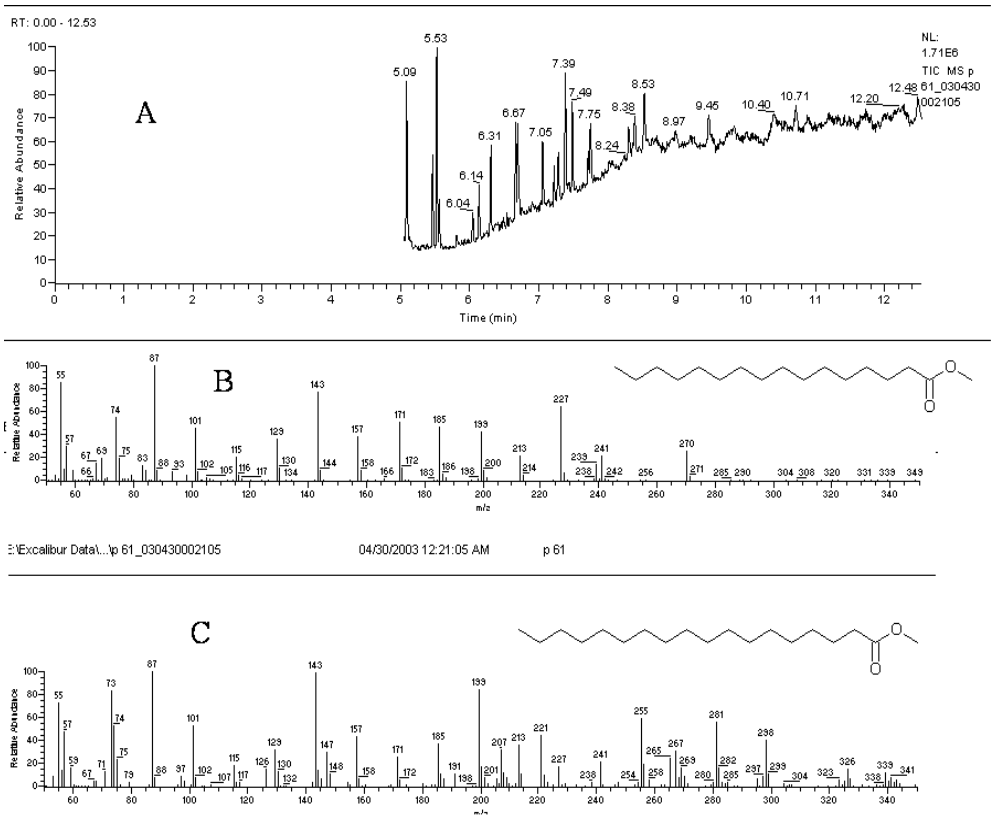

Fig.1. Analyses of sample P 61. Fig. 1A the chromatogram, Fig.1B idenfication of methylpalmitate at retention time 7.39 min.,Fig. 1C idenfication of methylstearate at retention time 8.35 min.

Only for some samples results were negative as is summarized in table 2. In Fig. 2A is presented the chromatogram acquired after injection of solution from sample P 147. We can see very well that is almost a flat baseline chromatogram. However we can see some relevant structural information to 7.39 min. corresponding to methylpalmitate (Fig. 2B). This assured a confident detection of palmitic acid methyl ester in analyzed sample in situation that was present in traces. This is one of the most unfavorable case with a low score match following target search from library after applying noise substraction and chromatographic peak hadn't ten points. In case of information under retention time to 8.53 min. the spectrum did not fit very well to methylstearate even after of noise substraction (Fig. 2C).

Table 2

Analyses results

Ref. nr.	Sample code	Sample size µg	Methylpalmitate detection	Methylstearate detection
1.	61	2490	Positive, good score from library search greater than 70 %	Positive, good score from library search greater than 30 %
2.	64	920	Positive, good score from library search greater than 60 %	Not detected
3.	137	4810	Positive, good score from library search greater than 10 %, after noise substraction	Not detected
4.	142	8510	Not detected	Not detected
5.	146	3130	Positive, good score from library search greater than 30 %	Not detected
6.	147	8060	Positive, poor detection, weak score from library search greater than 4 % , after noise substraction	Not detected

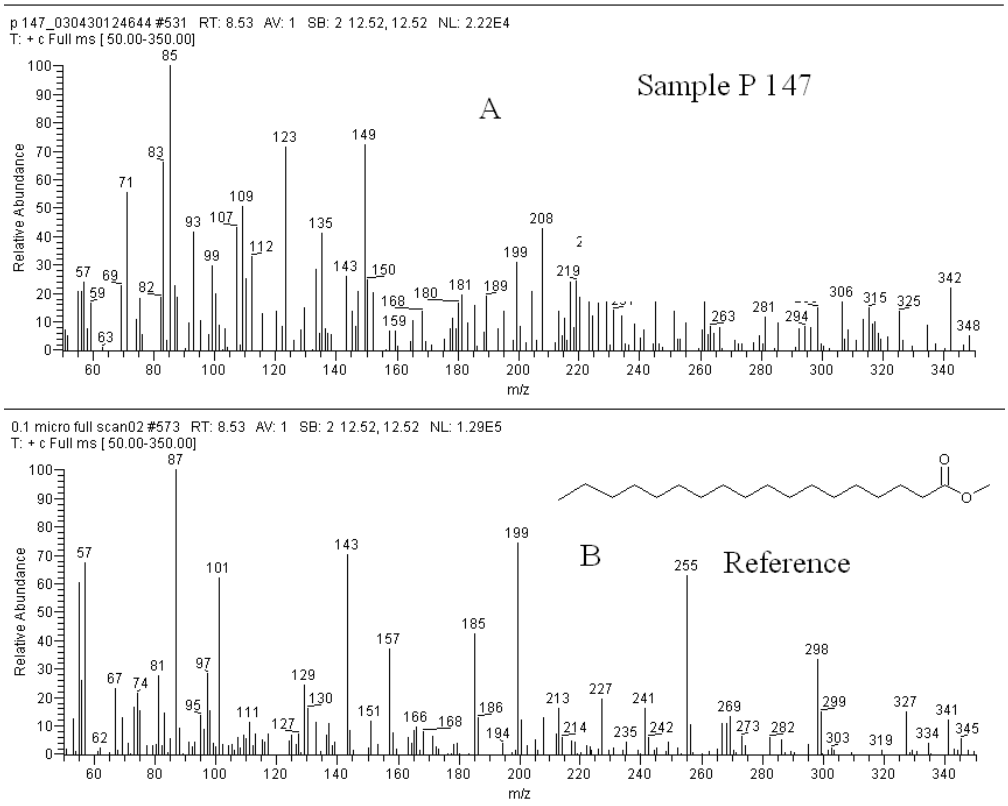

Fig. 2. Analysis of the sample P 147. Fig. 2A the chromatogram, Fig. 2B difficult idenfication of methylpalmitate at retention time 7.39 min., Fig. 2C impossible identification of methylstearate at retention time 8.35 min.

By a carefully comparison between spectral information from the same retention time at 8.53 of methyl stearate spectrum from sample P 147 (Fig. 3A) and from reference solution (Fig. 3B) we can observe that normalized level for sample spectrum is ten times lower than to that from reference. We couldn't observe characteristic mass peaks for methylstearate like m/z = 298, 255, 199, 143, 87. Spectrum from Fig. 3A is noise background so that we concluded that the sample P 147 did not contain methylstearate.

The hypothesis that the "Birth of Jesus" panel is painted in the oil – tempera mixed technique was confirmed by the results of gas chromatography coupled with mass spectrometry
analysis on the P 61, P 64, P 137, P 146 and P 147 micro samples. The presence of acid methylpalmitate and methystearate in the analyzed samples confirms the presence of vegetable oil and thus of the mixed painting technique used to create the artwork.

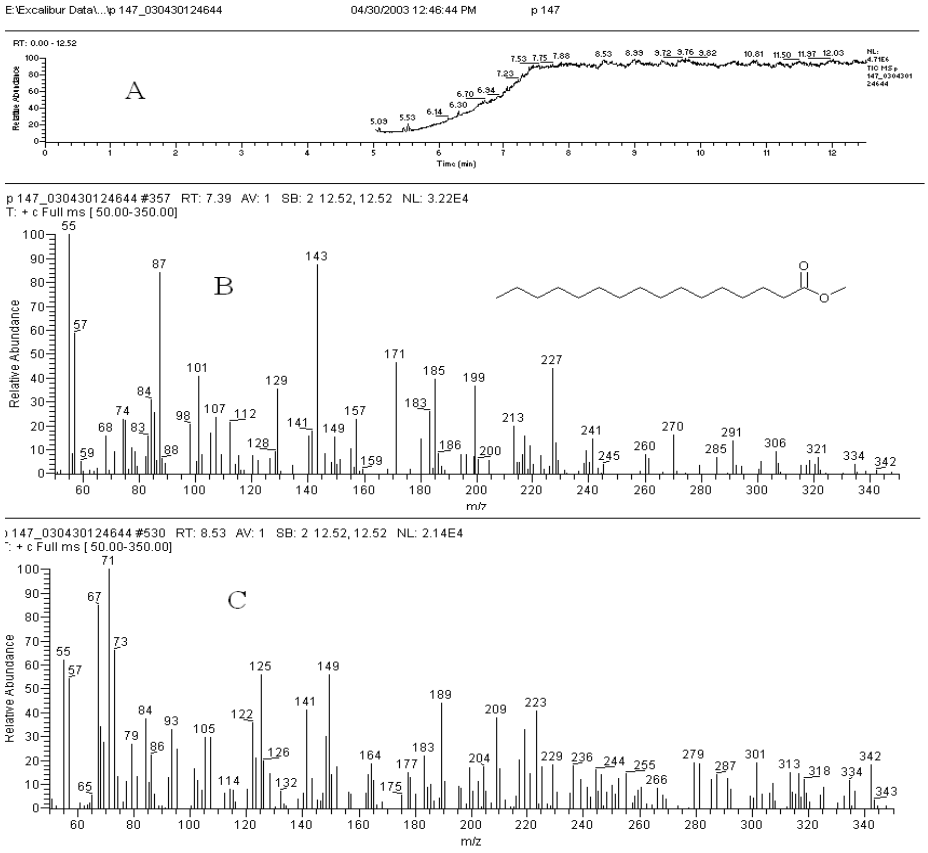

Fig. 3. Comparison of structural information from the chromatographic peak at 8.53 min.;
Fig. 3A, information from chromatogram corresponding to the sample P 147;
Fig. 3B, a information from chromatogram corresponding to the reference sample.

Both esters were detected in the P 61 sample. The library search confidence score for these compounds was 70% and 30% for the palmitic and stearic derivative, respectively. In the case of P 64, a 60% score was achieved for the methylpalmitate, but no methylstearate could be identified. The confidence scores are a good descriptor of compound concentration in the analyzed samples.

Negative results for the methylstearate were obtained for the P 137, P 142, P 146 and P 147 samples. In the case of P 142 no methylpalmitate was identified. For all the same samples, the methylpalmitate scores were positive, but lower than those obtained for the P 61 and P 64 samples.

The library search scores varied between 10%, for P 137 after both background noise, and 30% for P 146. In the case of P 147 a very low score of 4% was obtained.

4. Conclusions

Gas chromatography coupled with mass spectrometry results are not influenced by the sample mass, as good detection was achieved in low mass samples, while a high mass sample (P 142) did not yield positive results. The compound identification score is correlated with the oil quantity present in the samples. From the experimental data it can be inferred that the vegetable oil is not the main binder of the artwork, as the oil quantity found is lower than the expected value for a painting created using only oil as binder.

It can be concluded that the investigated panel was created using the mixed, oil–tempera technique. Furthermore, the present study verifies that the proposed method of investigation has proven to be suitable for the goal of this paper.

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