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THE USE OF LC-MS IN THE IDENTIFICATION OF NATURAL DYES IN THE EPITAPHIOS FROM SUCEVIȚA MONASTERY (15th CENTURY)

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Identification of dyes in historic textiles may bring useful information about the period and area an object was created if we consider that, before being commercially available, biological sources were only used locally. It may also reveal information about the manufacturing technique and contribute to the textiles conservation.
Based on the experience accumulated in the last years, an analytical protocol for the identification of natural dyes in historic textiles by LC-MS was developed. This new approach is based on the progressive use of MS or MS/MS features to identify and confirm the individual dyes extracted from low amounts of fibers.
In the present study the results obtained by applying this analytical protocol to the identification of dyes in a 15th c. epithaphios from Sucevița Monastery, Roumania are presented and discussed, as compared with those obtained on similar embroideries in Romanian collections previously reported within the same research group.[1-3]

INTRODUCTION

Subject of many publications, religious embroideries in Roumanian museums and monasteries have been until recently only studied according to their religious, historic and artistic importance.[4-8] Due to development of analytical instrumentation in Romanian laboratories several studies on scientific investigation of materials in embroideries have been reported in the last years.[1-3,9,10]

Considered as an important tool in choosing the most appropriate conservation strategy as well as a powerful instrument in obtaining information on where, when and how a textile object was created,[11-15] analysis of natural dyes in historic and artistic textiles were performed in European and North American laboratories even from the early 60's. The protocols dedicated to the identification of dyes evolved together with the development of new analytical techniques and are nowadays mainly based on HPLC-DAD, adapted from the method published by Wouters.[16-21] This method have been also applied in the detection of dyes and biological sources in textiles from Romanian collections.[1-3,19] Valuable results have been obtained on religious embroideries from the National Art Museum of Romania and Putna Monastery.[3]

In the last years mass spectrometric detection has been increasingly used worldwide in dyes separation and identification,[22-26] several applications being dedicated to natural dyes in textiles of historical and artistic interest.[27-38]

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An analytical protocol for identification and characterization of natural dyes in historic textiles by LC-MS and LC-MS/MS was developed.[39] This new approach comes to fill an absence, as no method for dyes characterization and identification had existed in the Roumanian museum network before. The analytical protocol proposed is based on the progressive use of MS or MS/MS features to identify and confirm the individual dyes extracted from low amounts of fibers.

In the present work the results obtained by applying this analytical protocol to the identification of dyes in a 15th c. epithaphios from Sucevița Monastery, Roumania are presented and discussed, as compared with those obtained on similar embroideries in Roumanian collections previously reported within the same research group.

The epitaphios from Sucevița (code 304/1967) (Figure 1)

The epitathios presenting the "Lamentation" is part of the Liturgical embroideries and veils collection in the Sucevița Monastery Museum. The piece - whose author is anonymous - is worked in the Moldavian School style and belonged to "St. Nicholas" Church in Rădăuți. It is worked with gilded silver thread and colored silk on a red atlas background, on a red damask support, doubled with linen canvas.[40-42] The piece is 164cm long and 126cm wide.

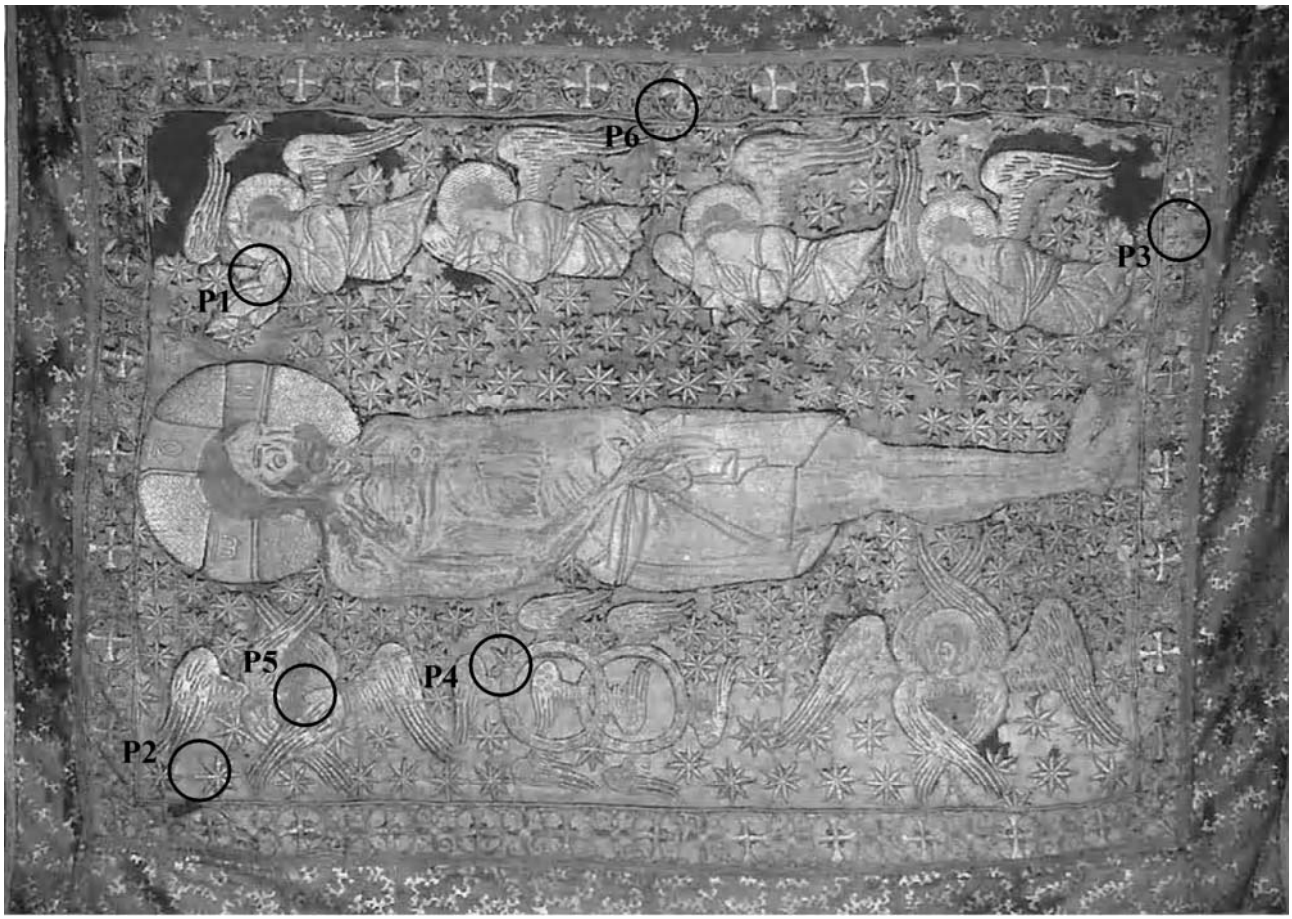

Fig. 1 – The Epitaphios from Sucevița Monastrey, 15th century and samples location
(samples were received from the Textile Conservation Workshop in the National Complex "Bucovina", Suceava).

EXPERIMENTAL

Reference materials

Standard natural compounds selected according to their frequency of use in textiles from Europe were characterized and the results were used as reference for the unknowns. Luteolin, apigenin, genistein, kaempferol, fisetin, izorhamnetin, rhamnetin, carminic acid, laccaic acid A and ellagic acid were available in pure form from Fluka (Redox Lab Supplies Com, Roumania) while alizarin, purpurin and emodine from Sigma-Aldrich Chemie GmbH (Taufkierchen, Germany).

Standard dyed wool fibers (weld, redwood, Cochineal, indigo), kermes dyed fiber from the Helmut Schweppe collection and berries dyed wool were prepared within projects[43,44] or obtained as gifts from various institutions and specialists (see section Acknowledgements).

Solvents

Methanol and acetonitrile were gradient grade from Merck KgaA (Darmstadt, Germany). Formic acid (98-100%) suprapure grade and hydrochloric acid (37%) used in the preparation of the mobile phase and during sample preparation were also from Merck. Water for chromatography (resistivity minimum 18.2 MΩ and TOC maximum 30 ppb) was produced within the laboratory by means of a TKA Lab HP 6UV/UF instrument and used during experiments.

Sample preparation

Individual samples (0.5 cm length, weighing about 1 mg) available from the back of the object during restoration (see Figure 1 for position) were provided by the Textile Conservation Workshop in the National Museal Complex "Bucovina", Suceava. Samples were heated at 105 ^{o}C in 250 µL solution hydrochloric acid / methanol / water (2:1:1, v/v/v) for 10 minutes. The mixture was then evaporated to dryness under gentle nitrogen flow, at 60 ^{o}C. The residue was then redissolved in 200 µL methanol/water mixture 1:1 (v/v), centrifuged at 12000 rpm for 5 minutes and the supernatant was transferred in an injection vial.

A second extraction step was used for visual blue and green sampes. For this, after hydrochloric extraction, the thread was transferred in another vial and 200 µl DMF were added. After 10 minutes at 140^{o}C the solution was injected directly in the LC.

Equipments

Experiments were achieved on a system built up from Agilent series 1100 modules (Agilent Technology, Waldbronn, Germany) as following: degasser (G1379A); quaternary pump (G1311A); thermostated autosampler (G1329A); column thermostat (G1316A); diode array detector (G1315 A). Detection was made through a MS/MS ion trap detector (G2445D-SL series) using an ESI ion source, operated under negative mode. The control of the chromatographic system and data acquisition has been achieved with the Agilent ChemStation software LC 3D version 10.02 incorporating the MSD trap control, version 5.2 from Brucker Daltronics (Bremen, Germany).

Chromatographic separation

Chromatographic separation was achieved on a Zorbax C18 column, 150 mm length, 4.6 mm i.d. and 5 µm particle size, thermostated at 40 ^{o}C. The mobile phase consists in a mixture of aqueous 0.2% (v/v) formic acid (solvent A) and methanol/acetonitrile (1:1, v/v as solvent B). Gradient elution was applied, by using the following profile: at 0 min, 15% solvent B; from min 0 to 5, linear increase to 25% solvent B; from min 5 to 10, constant at 55% solvent B; from min 10 to 16, linear increase to 100% solvent B; from min 16 to 18, constant at 100% solvent B; step jump at 15% solvent B, with a minimum 4 min re-equilibration period between runs (post-time). The flow rate was set at 0.8 mL/min.

Detection

For convenience, DAD detection was serially placed between the column and the MS ion source. UV-Vis spectra were permanently acquired over the 200-800 nm range with a frequency of 0.03 min and a resolution of 2 nm. Five wavelengths were simultaneously monitored to generate chromatograms (255, 275, 295, 420 and 490 nm).

MS detection was made in the negative ion monitoring mode, indicated by literature [38,45] to produce increased sensitivity for both flavonoids and anthraquinones. The ESI operational parameters were: drying gas temperature 350 ^{o}C; drying gas flow rate 12 L/min; nebulising gas pressure 65 psi; capillary high voltage 2484 V. The ion trap was using a maximum accumulation time of 300 ms, and a total charge accumulation (ICC) of 30000. The multiplier voltage was set at 2000 V and the dynode potential at 7 kV. When working in the MS/MS mode, the spectral width was of 4 a.m.u. and the collisional induced dissociation amplitude was 1.6 V.

RESULTS AND DISCUSSION

Results were achieved by LC/MS and LC/MS/MS, as compared with data previously obtained on standard dyes and dyed fibres, according to an analytical protocol presented in detail in an earlier publication .[39] A simplified image of the progressive use of mass spectrometric detection is given in Figure 2.

Dyes were identified based on their molecular ions and other ions produced in the ionisation source, while retention was also considered. Scan product ion was also used in some cases for unambigous identification. The most probable biological sources were also estimated, based on the detected dyes.

Kermesic and flavokermesic acids (m/z=329 a.m.u. and 313 a.m.u., respectively) were identified in a red silk thread from the damask embroidery support (sample code P1). Confirmation was reinforced by extracting specific m/z values coresponding to fragments produced through decarboxylation in the source (m/z 285 for kermesic acid and 269 for the flavokermesic acid) (Figure 3). Kermesic and flavokermesic acids are the main dye components in *Kermes vermilio* (kermes), the most expensive source of red in the second part of the 15th century.[46]

Quercitin and rhamnetin (m/z=301 a.m.u. and 315 a.m.u., respectively) were identified in a visual ochre thread used to reinforce the embroidery frame (sample code P2). Their detection was confirmed by the scan product ions, obtained with the mass spectrometer working in MRM mode, resulting data being compared with those obtained on standards. (Figure 4) The two flavonoids are main dyes sources of berries collected from *Rhamnus sp.,* biological source also identified in other embroideries studied in the same research group.[47]

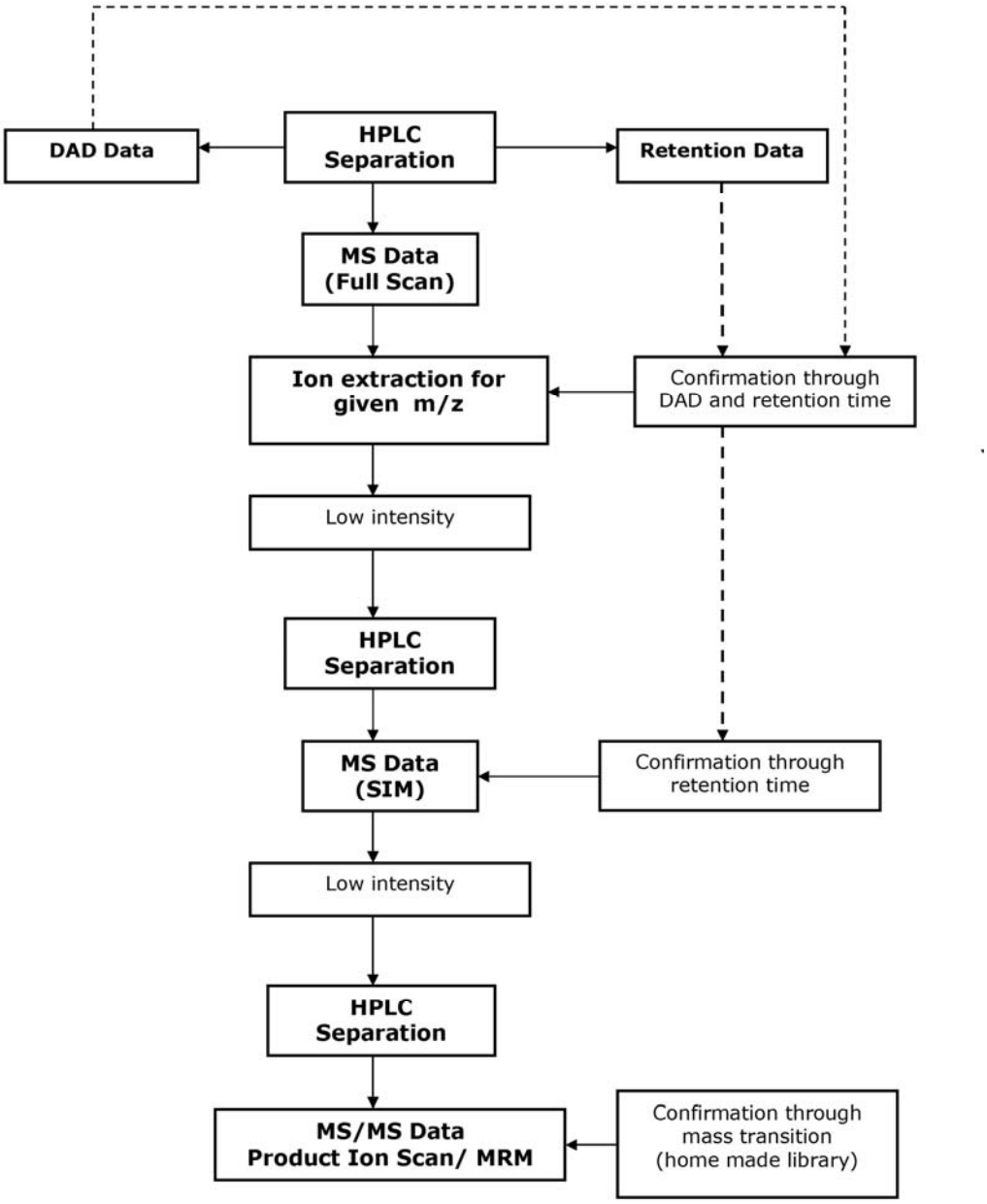

Fig. 2 – A simplified image of the progressive use of mass spectrometric detection in the analytical protocol used in the analysis of the present samples.

Table 1

Sample description (code, colour, fibre function) and the results (dye components and biological sources) obtained by LC/MS and LC/MS/MS analysis on silk threads from the Epitahios from Sucevița (15th century).

Sample code and colour	Function	Dye components detected	Biological source - scientific and common name
P1 red	damask, embroidery support	kermesic acid, flavokermesic acid	*Kermes vermilio (*kermes)
P2 ochre	frame embroidery, reinforcing thread	quercitin, rhamnetine, ellagic acid (-)	*Rhamnus sp.* (berries) and traces of tannins
P3 green	embroidery thread	luteolin, apigenin, chrysoeriol (-) ellagic acid (-), indigotin (-)	*Reseda luteola L. (*weld*),* traces of tannins and indigoid

Table 1 (continued)

P4 yellow	silk core metallic thread	luteolin, apigenin, chrysoeriol (-)	*Reseda luteola L. (*weld*)*
P5 ochre	embroidery support, warp	luteolin (-), ellagic acid (-), "srw"* (-)	traces of luteolin based dye (weld or eq.), tannins and *Caesalpinia sp*. (redwood)
P6 blue	embroidery thread	indigotin	indigoid

Note: dyes marked with (-) were detected in traces; * - "srw" stands for a marker compound used for redwood dyeings, according to literature.[49,50]

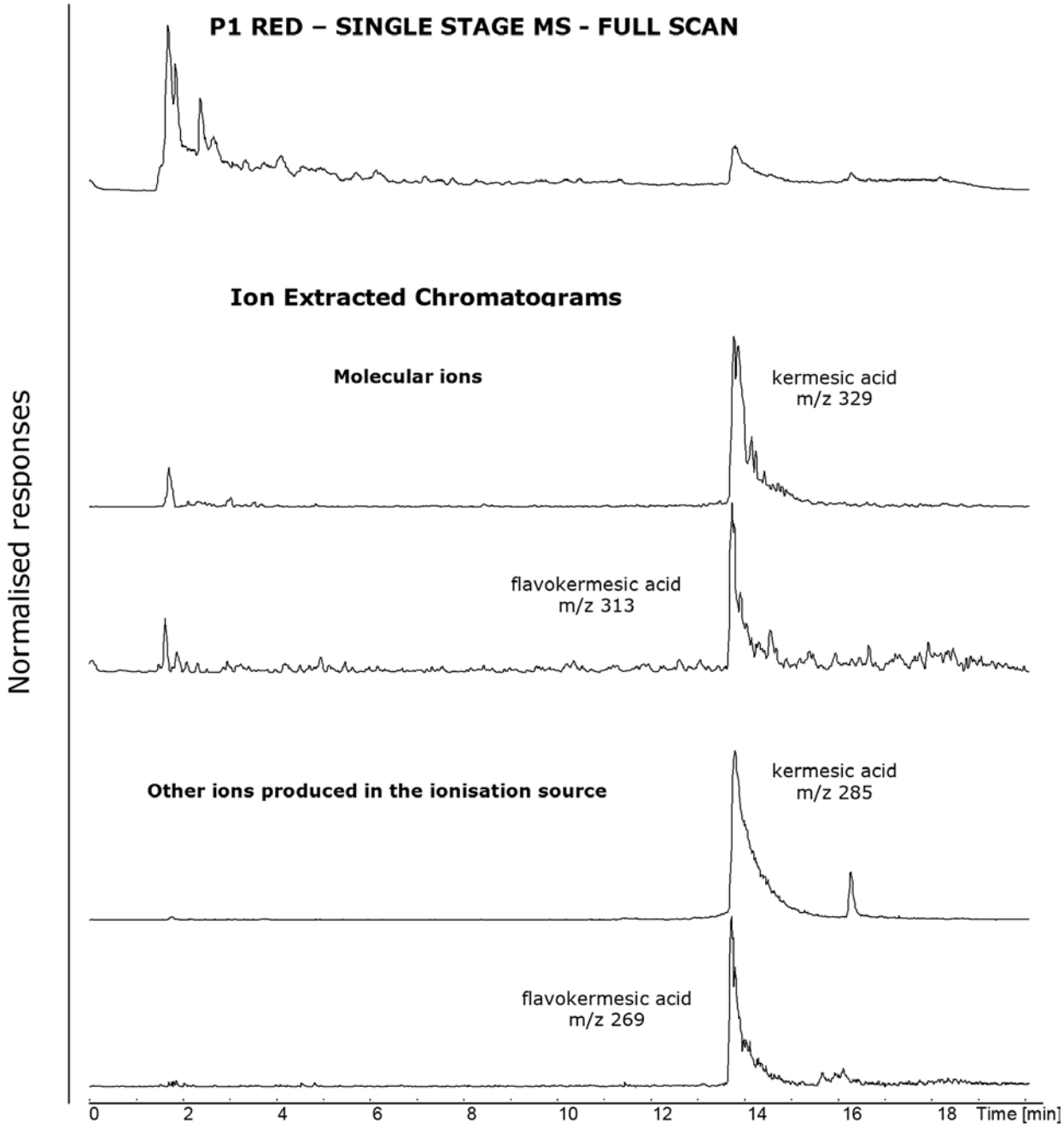

Fig. 3 – Illustrative chromatograms supporting identification of dyes in sample P1. From top to bottom chromatograms resulting from single stage MS in full scan (FS) mode and ion extracted chromatograms (IEC) according to molecular ions m/z values obtained for the target compounds; at the bottom ion extracted chromatograms (IEC) according to other ions produced in the ionisation source according to.[39]

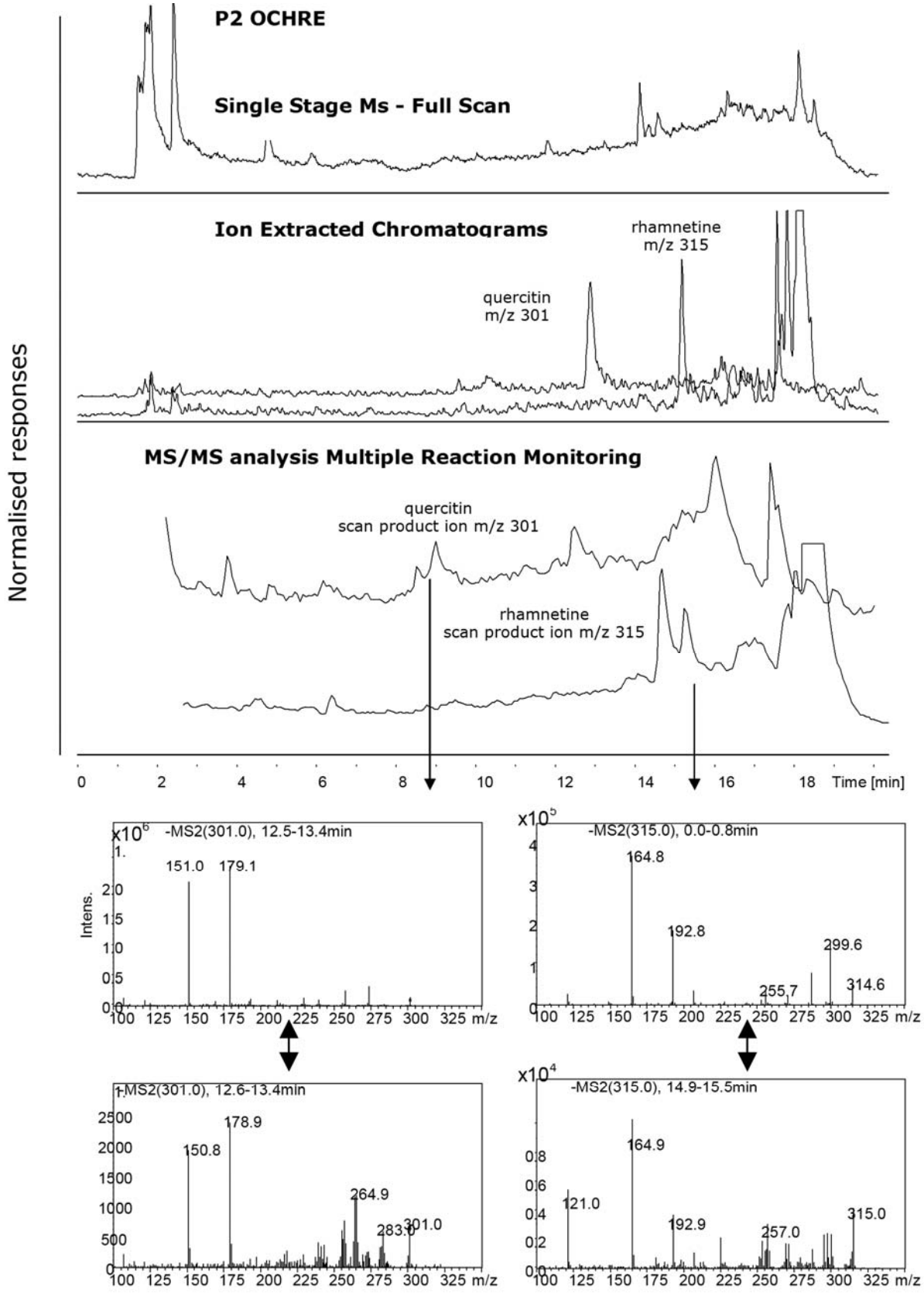

Fig. 4 – Illustrative chromatograms supporting identification of dyes in sample P2. From top to bottom chromatograms resulting from single stage MS in full scan (FS) mode and ion extracted chromatograms (IEC) according to molecular ions m/z values obtained for the target compounds; the lowest figures illustrate the product ion mass spectra for quercitin and rhamnetin, obtained with the mass spectrometer in Multiple Reaction Monitoring (MS/MS) as compared with reference spectra.

Luteolin and apigenin (m/z=285 a.m.u. and 269 a.m.u., respectively) were identified in the green embroidery thread used for decoration (sample code P3) and in a yellow silk core of metallic thread (sample code P4). The two flavonoid dyes may be found in several vegetal sources from which *Reseda luteola L.* (weld) is the most widely used in textile dyeing. Crysoeriol (luteolin 3-methy-ether) is a minor compound which could be found in weld, together with luteolin and apigenin. The identification of crysoeriol (m/z=299 a.m.u.) confirms the use of weld in both samples. As already mentioned, weld is one of the widest used sources of yellow, being identified in textiles dated since Antiquity.

Indigotin (m/z=261 a.m.u.) was also identified in the green thread mentioned before (sample code P3) as well as in a blue embroidery thread (sample code P6). Indigotin may originate either from the European *Isatis tinctoria L.* (woad) or from the *Indigofera* species, but no analytical method was reported so far to identify with certaincy which of the two sources was used.

Ellagic acid (m/z=301 a.m.u.) was identified in a thread used as warp in the embroidey support (sample code P5), suggesting the use of tannins. Traces were also detected in another ochre sample (sample code P2) for which berries were identified as the main source. The identification is reinforced by the detection of m/z=257a.m.u. which corresponds to the decarboxylation of elagic acid, according to the mechanism presented elsewhere.[39] Tannins may be found in a large number of biological sources, including *Quercus* species, and may be used for dyeing or in silk weighting.[48]

A marker compound for redwood dyeings, called "srw" by Wouters and "type C by Nowik" ,[49,50] was detected in the embroidery support (warp, sample code P4),which suggests the use of *Caesalpinia sp.* (redwood). The dye components in this species which initially have a visual red-violet colour are very light sensitive, so that in historic textiles this source is mostly associated with pink or ochre-pink-yellow fibres. Considering its poor light-fastness, it was mostly used in the hidden parts of the textiles. This is confirmed by its detection in the embroidery support - warp.

All the biological sources identified were in use in the 15th century and were also detected in the embroideries from Putna Monastery in the same period. In an earlier publication dedicated to the study of red dyes in 15th-18th century textiles from Putna Monastery it was demonstrated that the more valuable a textile was intended the most expensive dyes were used.[3] The use of kermes in the red silk support in the epitaphios from Sucevita confirms the high value of the embroidery and places it at the same level with in the Grave cover of Princess Maria of Mangop and the Cover lectern/61 , the only two other objects in which this very valuable source was detected until now.

CONCLUSION

The performed analysis proved the usefulness of dyes analysis in providing interesting data, complementary to those obtained by artistic and stylistic criteria, which may enrich the existing information in artistic, historical and archaeological textiles. Liquid chromatography with mass spectrometric detection proved once more to represent a valuable tool for the identification of dyes in historic textiles. A deep knowledge of the biological sources and the use of standard dyes and standard dyed fibers play a decisive role in building adequate databases required for the unambiguous identification of the vegetal and animal sources used for dyeing.

The high value of the Epitaphios from Sucevița (15th century) was certified by the detection of kermes, the most expensive source of red in the second half of the 15th century.

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