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LC-MS AS ANALYTICAL TECHNIQUE FOR THE IDENTIFICATION OF NATURAL DYES IN HISTORIC TEXTILES*

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Abstract. An analytical protocol for the identification of natural dyes in historic textiles by LC-MS and LC-MS/MS was recently developed for the first time in Romania. The present study discusses the application of this approach in the identification of dyes and biological sources in very low amounts of fibers from textiles in local collections.

Key words: natural dyes, liquid chromatography, mass spectrometry (single stage and tandem), historic textiles.

1. INTRODUCTION

Analysis of natural dyes in historic textiles have kept the attention of scientists for a long time [1, 2, 3]. There are at least three reasons for art historians to ask for dyes identification: (*i*) to get information on where and when an object was created, considering that up to become registered products, only local source of biological material was used (and sometimes comercialisation of natural sources may be well documented, as for example when it is conected to historic events or geographic discoveries); (*ii*) as depending on the use of dyed fibres and structure, some classes of natural dyes being less stable, will decompose faster than others;

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(*iii*) to understand more about the weaving technique, depending on future use of dyed fibers; and (*iv*) to get more information about the object value, as some dyes are more expensive than others.

Liquid chromatography with Diode Array detection (DAD) has been the standard method employed in dye analysis until recent developments of the mass spectrometers as detectors to the LC [4–11]. Most applications of both techniques accurately describe the results obtained for European textiles collections [12–15] and the studies on major and minor components of the natural dyes used in Europe [15–18]. The later is mainly achieved by mass spectrometric detection which is always used complementary to DAD.

An analytical protocol for dye analysis based on the progressive use of LC-MS and LC-MS/MS was recently developed for the first time in Romania [19]. This new approach, which is strongly based on data collected on standard dyes and dyed fibers as well as on the experience gained in recent European projects, aims to characterise and identify the biological sources most commonly used in textile dyeing in objects from local collections.

The present study aims to demonstrate the capabilities of the above-mentioned analytical protocol to identify a large number of biological sources, as well as their combinations, in small amounts of samples from historical textiles. Some case studies were chosen to illustrate the assumed task.

2. EXPERIMENTAL

2.1. SAMPLE PREPARATION AND DATABASE

Tiny samples (about 0.5 cm long) belonging to historic textiles in Romanian collections were analysed. The fibers were obtained from textile restoration workshops and represent small debris which could not be integrated in the objects after conservation.

Samples were heated at 105 ^{o}C for 10 min. with 250 μL solution consisting of hydrochloric acid (37%) / methanol / water (2:1:1, v/v/v). The extract was evaporated to dryness under nitrogen flow at 60 ^{o}C. The residue was taken in 200 μL of a methanol/water 1:1 (v/v) mixture, then centrifuged and the supernatant was transferred to an injection vial. After removal of the coloured solution resulted from hydrochloric extraction, an additional extraction step was included for visual green samples, where the presence of indigoid dyes is checked. 200 μL DMF were added to the coloured fibre and heated at 140^{o}C for 10 minutes. The steps described above were then followed, prior to injection.

A library of references was used for data evaluation. This includes the UV-VIS, single stage and tandem MS data for the dye components discussed. More details on the extraction procedure as well as on the single stage and tandem MS data of dyes in the database could be found in an earlier publication [19].

2.2. LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC-MS)

Experiments were performed on an Agilent 1100 LC equipped with an MS/MS ion trap detector using an ESI ion source, operated under negative mode. Agilent ChemStation software LC incorporating the MSD trap control was used for data acquisition and processing.

LC-MS separation was achieved on a Zorbax C18 column, 150 mm L × 4.6 mm i.d. × 5 μm d.p. Gradient elution was applied to the mobile phase consisting in a mixture of aqueous 0.2% (v/v) formic acid (solvent A) and methanol/acetonitrile (1:1 v/v, as solvent B). The flow rate was set at 0.8 mL/min. Injected volume was 5 μL.

A detailed description of the chromatographic and detection conditions was given in an earlier publication [19].

3. RESULTS AND DISCUSSION

3.1. IDENTIFICATION OF FLAVONOID YELLOW DYES FROM *RESEDA LUTEOLA* L. (WELD), *GENISTA TINCTORIA* L (DYER'S BROOM) AND *SERRATULA TINCTORIA* L. (SAWWORT)

In several samples coming from historical textiles the molecular ions of luteolin (m/z = 285) and apigenin (m/z = 269) were detected based on the analysis performed with the mass spectrometer in Full Scan mode followed by extraction of the chromatograms corresponding to dyes in the database.

Luteolin and apigenin are the main dye components in many biological sources, the most common of which are *Reseda luteola* L. (weld), *Genista tinctoria* L (dyer's broom) and *Serratula tinctoria* L. (sawwort) [1–3]. In order to precisely identify the source used, a third dye should be detected in each case: genistein for dyer's broom, chrysoeriol for weld and 3-O-methyl-quercetin for sawwort [16–18]. Being a major compound in dyer's broom, genistein may be easily identified by the present analytical protocol, based on the detection of its molecular ion (m/z = 269). This is the procedure followed by a routine basis and dyer's broom was succesfully detected in a large number of samples [20, 21].

Chrysoeriol is a minor compound in weld [16–23]. In most cases from analysis of historic samples, based on the present analytical protocol, its identification needs further analysis. For a particular case coming from an 17th. c. Oriental carpet, a second injection with a larger injection volume (10 μl) was performed with the mass spectrometer in Full Scan mode and data processing by IEC of the molecular ion of chrysoeriol (m/z = 299). The presence of the minor component was also confirmed by analysis with the mass spectrometer in MRM mode which enabled the detection of chrysoeriol, based on its Product Ion Scan, as compared with that collected from a standard of weld dyed wool (Fig. 1).

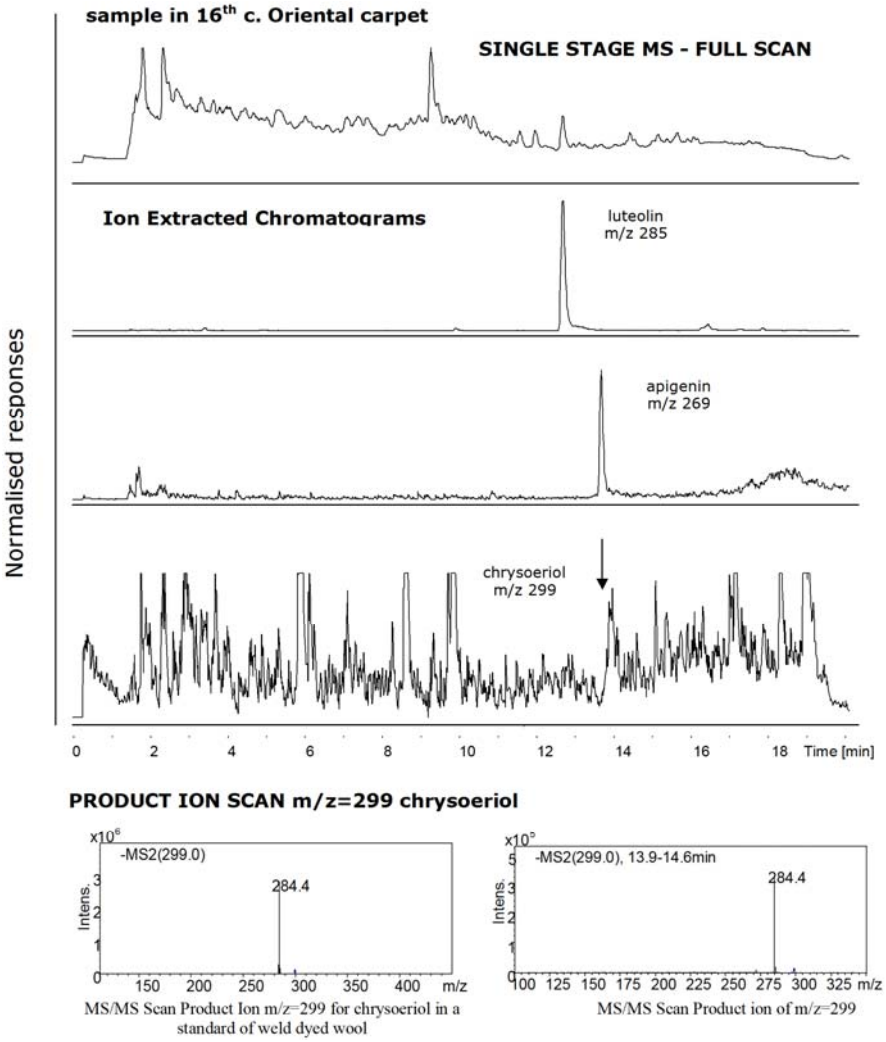

Fig. 1 – Flow chart diagram supporting the result obtained by MSD for a sample in a 16th c. Oriental carpet where *Reseda luteola* L. (weld) was identified.

In another case, coming from a 19th c. kilim, luteolin and apigenin were detected in the absence of genistein and chrysoeriol. However, a compound eluting between luteolin and apigenin and having a molecular ion of m/z = 315 was present. Further analysis was performed with the mass spectrometer working in MRM mode to obtain the Product ion scan of the unknown dye. The compound was identifed as being 3-O-methyl-quercetin, based on the comparisson with the fragmentation spectra of the respective dye from a sawwort dyed wool, as presented in literature data [17].

It was thus concluded based on the detection of luteolin, apigenin and 3-O-methyl-quercetin that *Serratula tinctoria* L. (sawwort) was responsible for dyeing in the wool sample from the 19th c. kilim.

3.2. DETECTION OF INDIGOTIN IN A BLUE SAMPLE FROM A 19TH C. KILIM

Routine analysis performed on fibres having different visual colours showed that blue fibers preserve their colour (and green fibers become blue) after the hydrochloric extraction step according to the standard acid hydrolysis procedure. A second extraction step with DMF was thus introduced, as described in the experimental section.

This method was succesfully applied to identify indigotin in a visual blue sample from a 19th c. kilim. Analysis was performed with the mass spectrometer working in Single Ion Monitoring Mode, the molecular ion of indigotin (m/z = 261) being monitored. Indigotin may originate from *Indigofera* species (indigo) or from *Isatis tinctoria* L (woad), no analytical method to establish which of the two sources was used being discovered so far.

Analysis performed with the mass spectrometer in SIM mode enabled detection of indigotin in a visual blue historic sample.

3.3. IDENTIFICATION OF DYES IN A RED SILK THREAD FROM A 16TH. C. DOCUMENT

The dyes used in a red silk thread which bounds the seal to the parchment in a 16th c. document were investigated by LC-MS. Analysis performed with the mass spectrometer working in Full Scan mode processed by IEC of the molecular ions (and other ions produced in the ionization source) of dyes in the database enabled detection of alizarin, purpurin and flavokermesic acid (Fig. 2).

Alizarin (m/z = 239) and purpurin (m/z = 255 and 277) are the main anthraquinones in madder (*Rubia tinctorum* L.). The presence of minor anthraquinone compounds, such as anthragallol (m/z = 255), munjistin (m/z = 239 and 283) and xanthopurpurin (m/z = 239) was also determined based on the same procedure, confirming the use of madder. If the identification is not clear due to the small value of the S/N ratio (as in the case of anthragallol), the confirmation can be achieved using the SIM/MID modes. Although, as shown in the examples above, a second injection is sometimes need in order to confirm the use of a certain biological source, in this case, madder was undoubtefully identified, due to the detection of a high number of dyes from the same source.

Flavokermesic acid was also detected in the same sample, based on the presence of the molecular ion (m/z = 313) and reinforced by the detection of the fragment produced through decarboxylation in the ionisation source (m/z = 269), as

demonstrated in a previous publication [19]. Flavokermesic acid (also called laccaic acid E) is the minor compound in *Kermes vermilio* P. (where the major compound is kermesic acid), in Cochineal species (major dye is carminic acid) and *Kerria lacca* Ker (major compound being laccaic acid A).

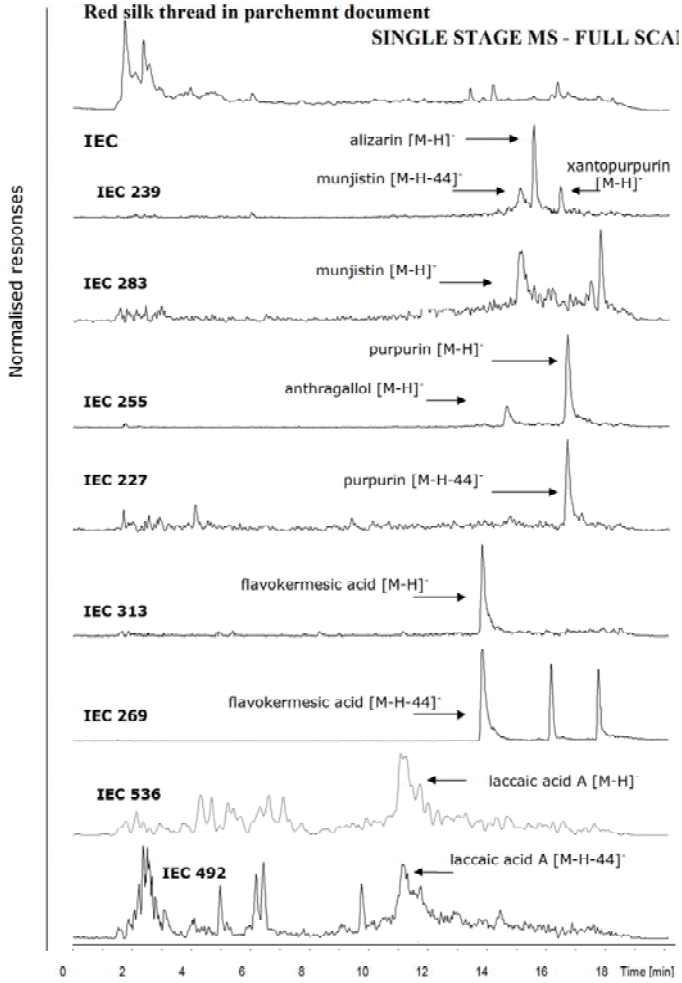

Fig. 2 – Flow chart diagram supporting the result obtained by MSD for a red silk thread sample from a parchment document where *Rubia tinctorum* L. (madder) and *Kerria lacca* Ker (lac dye) were identified.

In the present sample none of the major dyes mentioned was detected. Experiments performed on a standard of *Kerria lacca* Ker (lac dye) dyed wool showed that only flavokermesic acid may be identified based on the detection of the molecular ion in ESI(-). Further experiments performed on a mixture of similar quantities of standard dyes, also containing a standard of laccaic acid A, as well as

on a standard of laccaic acid A injected directly in a mass spectrometer, showed that the life time of the molecular ion of laccaic acid A (m/z = 536) is very short (due to reactions in the source) which limits its detection in lac dye dyed fibres (Fig. 3).

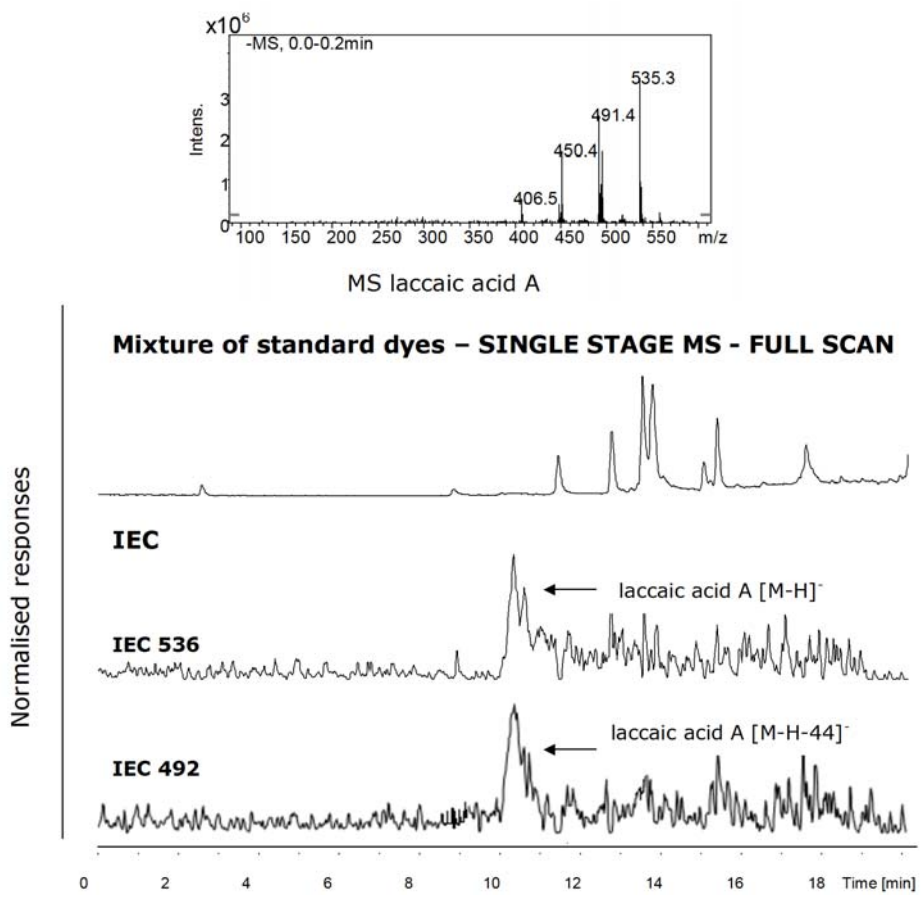

Fig. 3 – (top) MS spectra illustrating the molecular ion and fragments produced in the ionization source for a standard of laccaic acid A; (bottom) chromatograms obtained in Single Stage MS for a mixture of standards, also containing laccaic acid A; the IEC obtained for the molecular ion of laccaic acid A (m/z = 536) and the ion produced by its decarboxylation in the ionization source (m/z = 492).

However, the detection of flavokermesic acid in the absence of carminic and kermesic acid would suggest the use of *Kerria lacca* Ker (lac dye). This hypothesis can be confirmed by a second injection of the red silk thread sample analysed above, this time an increased volume (10 µl) being injected, which allowed the detection of the molecular ion of laccaic acid A as well as the ion produced by the decarboxylation process (Fig. 2).

It may be thus concluded that 7 dyes were detected in the red silk sample from the 16th c. document, suggesting the use of two biological sources: *Rubia tinctorum* L.(madder) and *Kerria lacca* Ker (lac dye). These results are in agreement with data previously reported for religious embroideries from the same period [24].

3. CONCLUSION

From the case studies discussed it may be concluded that the LC-MS based analytical protocol elaborated is a powerful tool in the identification of natural dyes in historic textiles. Several dyes and biological sources were detected by the progressive use of the mass spectrometer from the the Single stage MS (Full Scan and SIM) to MS/MS Product Ion Scan and Multiple Reaction Monitoring modes. Identification of dyes and biological sources is strongly based on data collected on standard dyes and dyed fibers.

Attribution of the biological sources most probably used requires strong literature research and sufficient knowledge on the natural sources used for dyeing in the history of mankind.

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