

Identification of Natural Organic Dyes Used in Art Objects of the Cultural Heritage of the S.E. Mediterranean Area by High Performance Liquid Chromatography

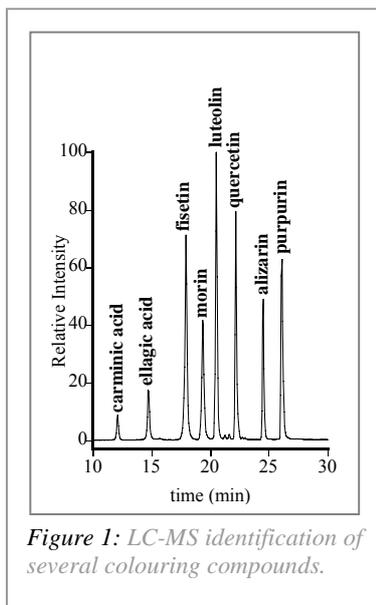
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Introduction

Natural organic dyes have been extensively used in the past for the decoration of objects such as textiles (e.g. carpets, garments, furniture tapestries), papers and figurines and for the creation of paintings/icons and mural paintings. Consequently, developing analytical strategies to identify these materials is very important for art historians, conservators and curators. Furthermore, natural dyes have the advantage that their production causes minimum environmental pollution and has a low risk factor in relation to human health. For all these reasons the optimization of the analytical procedures applied on natural organic dyes has attracted considerable attention. This is proven by the large number of relative scientific investigations that can be found in the literature and also by the number of European projects which deal with the protection and conservation of the cultural heritage. MED-COLOUR-TECH (www.medcolourtech.org) is one of these efforts supported by the European Commission. The project actually focuses on the cultural heritage of the S.E. Mediterranean area.

The goals of this paper are (i) to present some important key points with respect to dyestuff analysis addressed within the framework of MED-COLOUR-TECH and (ii) to discuss the origin of the organic dyes used in art objects of the S.E. Mediterranean area. The latter is based on the conclusions drawn after the examination of a large number of textiles, mural paintings and icons, dated from the 17th century BC (Bronze age) up to the 18th century AC (post Byzantine period).

Several analytical techniques have been employed for the identification of natural organic dyes in art objects. High Performance Liquid Chromatography (HPLC) coupled to a Photodiode Array Detector (PDA) is probably the most common instrumentation, used nowadays, because of its superior detection limits. Recently, however, more elaborate techniques including mass spectrometric (MS) detection appear to be more frequently used. Figure 1, shows the identification of several colouring compounds by LC-MS. The latter has in principle enhanced analytical capabilities compared to HPLC-PDA. However, in the case of natural organic dyes this general principle has not been confirmed yet. Another major advantage of liquid chromatography is its separation capability, which many times is crucial for the identification of minor colouring compounds. In some cases these minor constituents can be used as indices for the identification of a dyestuff source. For example the distinction of cochineal species, Armenian (*Porphyrophora hameli* Brandt), Polish (*Porphyrophora polonica* L.) and Mexican (*Dactylopius coccus* Costa) has been achieved by quantitation of the secondary, minor colouring compounds [1].

Experimental

In the following, the sample preparation procedure, applied prior to HPLC analysis according to the analytical protocol of OADC and the chromatographic instrumentation utilized at the ORMYLIA Art Diagnosis Center (OADC) are described.

Sample preparation

Samples extracted from art objects are examined by a microscope and digital photos are stored in a computer. The colour of the fibers is identified. If threads with different colours are identified attempts are performed to separate them. Then, samples (on the order of 1-2 mg) are weighted and treated with 400µl of a solution mixture of H₂O:MeOH:37% HCl (1:1:2, v/v) for 15 minutes at 100°C in open small tubes to extract organic dye molecules from their mordant metals. After cooling, the solution is evaporated to dryness by heating (60°C) under gentle nitrogen flow. The dry residue is dissolved in 300µl of DMF, except the reddish materials which are dissolved in H₂O:MeOH (1:2). If necessary, further dilution takes place and the sample is finally centrifuged. A volume of 20µl is submitted to HPLC.

Blank analysis

Blank analyses are performed regularly by injecting 20 µl acetonitrile, on a regular basis as follows: (i) Three times at the beginning of the analytical day and (ii) once after sample (or standard) analysis.

Instrumentation

Reversed phase liquid chromatography (RPLD) is carried out using Thermoquest (Manchester, UK) HPLC system consisted of P4000 quaternary HPLC pump, SCM 3000 vacuum degasser, AS3000 auto

sampler with column oven, Reodyne 7725i Injector with 20µl sample loop and Diode Array Detector UV 6000LP. PDA detection is performed by scanning from 191 to 799 nm with a resolution of 2nm. A Finnigan AQA mass spectrometer, MS (Thermoquest, UK) is utilized, with a negative electrospray ionization (ESI-) on a single quadrupole mass filter to record ion signals. The ESI probe is operated at 400°C and 4kV. The cone voltage is maintained at 20 V. These values can be modified upon tuning. Typical ranges are: For the probe 370 - 420°C and 3.5 - 4.3kV; the cone voltage is set either at 20 V or at 30V. Xcalibur™ data system (Thermoquest, UK) is employed for data acquisition and processing.

Results and discussion

Database of standard compounds

The identification of organic dyes by HPLC-PDA-MS is based on a database that has to be created using standards compounds. Three parameters are then recorded for each eluted compound: (i) retention time, (ii) absorption spectrum and (iii) mass spectrum. Table 1 presents the first two parameters for several colouring ingredients of organic dyes.

HPLC-PDA versus LC-MS: a preliminary study

A major topic of the current literature is the comparison of the recently employed LC-MS over the traditionally used HPLC-PDA. Apparently, the cost of the analysis is inflated whenever LC-MS is utilized. The major question, however, concerns mainly the detection capabilities of LC-MS over the HPLC-DAD. Within the MED-COLOUR-TECH activities a preliminary effort to compare the two detectors has been performed for several compounds which are principle components of

Colouring compound	Retention time	Absorbance maxima (nm)
Ellagic acid	14,6	245, 367
Carminic acid	15,0	275, 309, 493
Laccaic acid A	15,8	225, 285, 491
Fisetin	17,8	221, 247, 359
Morin	19,3	221, 251, 353
Quercetin	20,4	219, 255, 371
Luteolin	20,5	221, 251, 345
Carthamin	20,9	225, 307, 373, 517
Kaempferol	21,5	221, 265, 365
Genistein	21,8	217, 259
Apigenin	21,9	221, 267, 337
Alizarin	22,7	247, 277, 429
Kermesic acid	24,0	271, 309, 489
Purpurin	24,1	253, 293, 479
Flavokermesic acid	24,3	283, 343, 429
Indigotin	24,9	285, 331, 451, 605
Indirubin	25,9	289, 363, 539
6-Bromo indigotin	27,6	287, 341, 597
6-Bromo indirubin	29,2	295, 361, 547
6,6'-Dibromo indigotin	30,8	289, 343, 593
6,6'-Dibromo indirubin	32,5	301, 365, 541

Table 1: Retention times and absorbance maxima for several colouring compounds.

natural organic dyes, found in objects of the cultural heritage. The results are summarized in the following. Detection limits are given in µg/ml. MS was operated in SIM (ESI-) mode to collect molecular ions and spectrophotometric detection (UV-Vis) was acquired at 254nm: Carminic acid, 0.07 (MS) and 0.01 (UV-Vis), ellagic acid 0.04 (MS) and 0.01 (UV-Vis), fisetin 0.01 (MS) and 0.01 (UV-Vis), morin 0.02 (MS) and 0.02 (UV-Vis), luteolin <0.01 (MS) and <0.01 (UV-Vis),

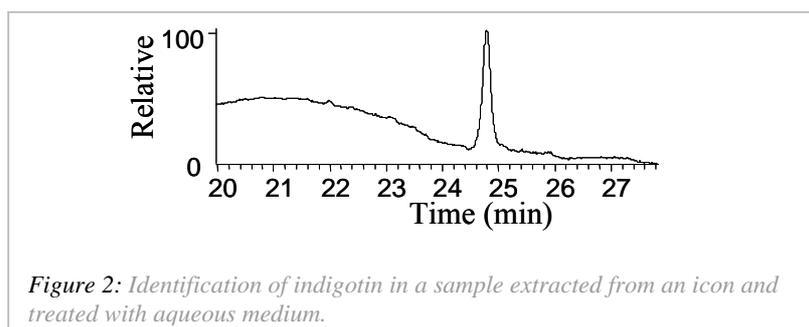
genistein 0.01 (MS) and <0.01 (UV-Vis), alizarin 0.01 (MS) and <0.01 (UV-Vis), purpurin 0.01 (MS) and <0.01 (UV-Vis).

The results suggest that, in general, MS and UV-Vis detection limits do not have pronounced differences and can be considered as comparable, except for carminic and ellagic acid for which spectrophotometric detection is clearly better. Similarly, UV-Vis signals are slightly better (than MS signals) for genistein, alizarin and purpurin. We note, however that the operating conditions of Mass Spectrometry were not fully optimized for each of the investigated compounds but were adjusted for a standard solution mixture (figure 1) according to the values which are given at the experimental section.

Solubility of indigoids and HPLC detection

In analytical chemistry a key point for the successful identification of a compound contained in an unknown sample is the appropriate preparation of the sample. For the identification of dyestuffs in samples extracted from artworks the sample preparation procedure followed by OADC is presented in the paragraph entitled “sample preparation”, at the experimental section. The first step of the described procedure includes sample treatment with a solution mixture of H₂O:MeOH:37% HCl (1:1:2, v/v). However, this procedure is not recommended for dyes which contain indigoid compounds, such as indigotin, indirubin, brominated indigotins and brominated indirubins (table 1) because they are insoluble in typical solvents such as H₂O and MeOH. For indigoids the use of other solvents such as DMF, DMSO and pyridine has been recommended [2,3]. The “insolubility” of indigoids is well known to researchers and has been discussed in detail. This “insolubility”, however, could be a major difficulty because in some cases indigoids are contained in mixtures with organic pigments for which the HCl treatment is necessary. In that

case indigoids could not be detected, because of their insolubility in aqueous media. The superior detection limit of HPLC, however, overcomes this problem. Figure 2 shows the identification of indigotin (HPLC-PDA) in a sample extracted from a post Byzantine icon, upon HCl treatment. Despite indigotin's insolubility, traces of the compound can be detected by HPLC.



Identified dyes in artworks of the S.E. Mediterranean area

Table 2 summarizes the organic dyes identified in textiles, icons and wall paintings of the S.E Mediterranean area. Possible dyestuff sources are also provided. Madder was found in all kinds of art objects. Cochineal was found to be the most common organic dye found in Byzantine and post Byzantine icons and has been also detected in samples extracted from textiles. Similarly, redwood and indigo or woad (these two blue dyes cannot be distinguished by analytical means) were found in icons and textiles. Along with the reddish lac dye, several yellow organic dyes were found in textiles as follows: weld, dyer's broom and young fustic. Finally, Tyrian Purple known also as Royal Purple has been detected in wall paintings of the Bronze Age [4].

Acknowledgements

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Dyestuff	Colour	Source of dyestuff
Dyestuffs of animal origin		
Cochineal	Red	<i>Porphyrophora polonica</i> L. <i>Porphyrophora hameli</i> Brandt <i>Dactylopius coccus</i> Costa
Lac dye	Red	<i>Kerria lacca</i> , Kerr
Tyrian Purple	Purple	<i>Bolinus brandaris</i> L. <i>Hexaplex trunculus</i> L. <i>Stramonita haemastoma</i>
Dyestuffs of plant origin		
Madder	Red	<i>Rubia tinctorum</i> L. <i>Rubia peregrina</i> L.
Redwood (Brazilwood, Sappanwood)	Red	<i>Caesalpinia</i> trees
Weld	Yellow	<i>Reseda luteola</i> L.
Young Fustic	Yellow	<i>Cotinus coggygria</i> Scop.
Dyer's broom	Yellow	<i>Genista tinctoria</i> L.
Indigo	Blue	<i>Indigofera tinctoria</i> L.
Woad	Blue	<i>Isatis tinctoria</i> L.

Table 2: Dyestuffs identified in works of art such as icons, textiles and mural paintings of the S.E. Mediterranean area.

References

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