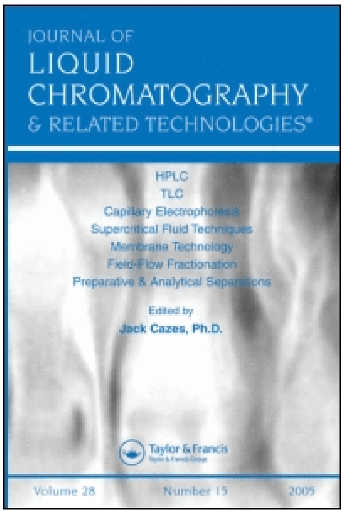

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Identification of Red Natural Dyes in Post-Byzantine Icons by HPLC

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To cite this Article Karapanagiotis, Ioannis , Daniilia, sist. , Tsakalof, Andreas and Chryssoulakis, Yannis(2005) 'Identification of Red Natural Dyes in Post-Byzantine Icons by HPLC', Journal of Liquid Chromatography & Related Technologies, 28: 5, 739 — 749

To link to this Article: DOI: 10.1081/JLC-200048896

URL: http://dx.doi.org/10.1081/JLC-200048896

Journal of Liquid Chromatography & Related Technologies®, 28: 739–749, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200048896

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Abstract: A high performance liquid chromatography (HPLC) methodology, combined with UV-Vis Diode Array Detection, is developed for the separation and identification of five reddish natural dyestuffs: cochineal, madder, lac dye, dragon blood, and brazilwood. The method is used for the identification of organic dyes in extracts originating from five icons, four of which are representative for the post Byzantine era (15th to 19th century) and one is typical for the Byzantine coloring technology (created at the 14th century). The origin and the nature of the coloring content of Mediterranean art objects, created in these historical periods are not well known. Carminic acid, the main active, coloring, ingredient of cochineal was identified in four icons, including the one of the 14th century. Brazilwood was found to be the only organic colorant in one icon of the post Byzantine era. It was also present, in addition to cochineal, in the icon of the Byzantine period. Further discussion, associated with the dyestuff origin, is provided based on the analytical results and the available historical data.

Keywords: HPLC, art analysis, dyestuff, cochineal, brazilwood

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INTRODUCTION

Detailed characterization and identification of the components of archaeological objects attracts an increasing interest to optimize the applied conservation or restoration strategies and to reveal valuable historical data. A challenging part of this characterization, for analytical chemistry, is the identification of organic colorants found in art objects of the cultural heritage.

High Performance Liquid Chromatography with a diode-array-detector (HPLC-DAD) has been successfully applied to the identification of natural organic dyes found in historic yarns, textile fibers,[1–7] printed documents,[8] and paintings,[9,10] proving that HPLC is a powerful tool to detect the components of such organic natural compounds even when they are present in tiny quantities.[11–14] Besides the optimization of the obtained chromatographic separation and the improvement of the detection limits, current investigation deals also with the dyestuff extraction methods developed so far.[15,16] More elaborate analytical methods complying mass spectrometric (MS) detection have been rarely used for natural dyes investigation.[17–20] It has been proven, though, that LC-MS has enhanced analytical capabilities.[21,22]

The goal of this study is to perform natural red dyestuff analysis using HPLC-DAD in samples extracted from icons, representative for the Byzantine and post Byzantine cultures developed in the Mediterranean area from 14th to 19th century. Very little information is available about the origin of the organic pigments, present in art objects of these civilizations. In general, it is known that Byzantine painters utilized mainly inorganic pigments (e.g., red ochre), while in the post Byzantine period, organic dyestuffs were preferably used. Historical information of the tested samples is provided in Table 1.

Mexican cochineal, an insect (Dactylopius coccus Costa) red colorant native to the New World, was imported into Europe after the Spanish conquest and replaced, almost entirely, kermes, which used to be the dominant red colorant of the ancient times.[23] Other, Old World cochineal species are the Polish cochineal (*Pophyrophora polonica* L.) and the Armenian cochineal (Porphyrophora hameli Brandt). All cochineal species appear to have similar composition

Table 1. Icon theme and date of creation

Icon #	Icon theme	Date of creation
1	Mother of God Hodegetria	1835
2	Mother of God Eleoussa and St John the Forerunner	1816–1839
3	Sts John the Evangelist, Nicolas and Protomartyr Stephen	1750–1800
4	Our Lady, the life-giving Spring	1543
5*	Christ Pantokrator Enthroned	1300–1350

*Icon 5 belongs to the Byzantine period (before 1453 AC). Icons 1, 2, 3 and 4 belong to the post Byzantine period.

with carminic acid being, by far, the major coloring material, but is not exactly the same.[2,10,23] Brazilwood, a red vegetable dyestuff, which belongs to the Caesalpinia group, was known before the discovery of South America.[24] When European navigators arrived in the region of today Brazil's coast, they identified trees very similar to those (called brazilwood) used as a source of a red pigment in Asia. It is believed that brazilwood was imported into Europe from the 10th century,[25] or even later, from the 13th century.[26] Main coloring component is brazilein which forms by autoxidation of brazilin.[24,26]

EXPERIMENTAL

Chemicals

Alizarin, purpurin (Sigma-Aldrich Co., USA), and carminic acid (Fluka Chemie, Sigma-Aldrich Co., USA) were used for standard solution preparations and for the development of a preliminary chromatographic method. The latter was further developed to achieve sufficient separation of reference reddish materials of madder, cochineal, brazilwood, dragon blood, and lac dye (Kremer Pigmente, Germany). For liquid chromatography, HPLC grade acetonitrile (Riedel de Haen, Germany), and trifluoroacetic acid, TFA, (Merck, Germany) were utilized. Type I reagent grade water with resistivity up to $18.3\,M\Omega/cm$ and organic content <5 ppb was produced by Barnstead EASYpure water purification system and used for buffer and solution preparations. For the latter HPLC grade methanol (Merck, Germany) was also used. All HPLC solvents were filtered through a 0.2 μm filter prior to use.

Sample Preparation

All reference samples and extracts (less than 1 mg) from icons were treated with a solution mixture of H_2O:MeOH:37% HCl (1:1:2, v/v) for 15 minutes at 100°C in open small tubes. For the extracts this treatment is necessary to isolate the organic dye from its mordant metal. After cooling the solutions were filtered through 0.2 μm nylon syringe filter (Alltech Associates Inc., USA) to remove undissolved particulates. Subsequently, the solutions were evaporated by heating (50–60°C) under gentle nitrogen flow. The dry residues were dissolved in 0.5 mL of a mixture of H_2O:MeOH (1:1, v/v) and submitted for HPLC analysis.

Instrumentation

Reversed phase liquid chromatography (RPLD) was 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 20 μL sample loop and Diode Array Detector UV 6000LP.

The HPLC separation was carried out, in a Kromasil C18 5 μm 250 × 3.2 mm HPLC column (Alltech Assotiates Inc., USA) thermostatted at 40°C, by a gradient elution program that utilizes two solvents: solvent A: H_2O-0.1%TFA and solvent B: CH_3CN-0.1%TFA. The solvent selection originated from previous publications.[5] Gradient elution program: initial 95% A evolved to the final 5% A within time period of 33 min and flow rate 0.6 mL/min were injected in the mobile phase flow.

Xcalibur™ data system (Thermoquest, Manchester, UK) was employed for data acquisition and processing.

RESULTS AND DISCUSSION

In Table 2, retention times (R_t) and corresponding spectral characteristics of the main coloring components of madder, cochineal, brazilwood, dragon blood, and lac dye, detected in Figure 1, are presented. The main coloring components (alizarin, purpurin, and carminic acid) of madder and cochineal can be fully separated, detected, and identified by their UV-Vis spectra. Detection of these components in extracts originating from art objects

Table 2. Chromatographic and spectral characteristics of the investigated reference dyestuffs

Dyestuff	Coloring component	R_t(min)	Absorbance maxima (nm)	Corresponding peak in fig. 1
Madder	Alizarin	28.1	247, 277, 429	M1
	Purpurin	29.7	255, 293, 381, 479	M2
Cochineal	Carminic acid	14.8	275, 309, 493	C1
Brazil wood	Component 1	20.3	316, 361, 499	B1
	Component 2	21.0	357, 517, 549	B2
	Component 3	25.9	245, 335, 381	B3
	Component 4	11.3	271, 465	B4
Dragon blood	Component 1	24.5	267, 297, 437	D1
Lac dye	Component 1	17.9	285, 489	L1
	Component 2	21.2	285, 381, 491	L2
	Component 3	13.5	221, 285, 381, 467, 489	L3
	Component 4	16.0	217, 285, 381, 463, 489	L4

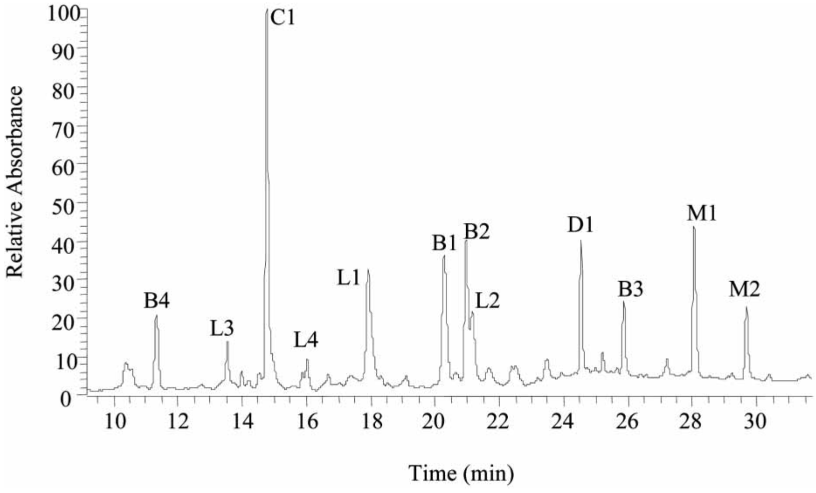

Figure 1. Chromatogram of reference sample mixture. Retention times and UV-Vis absorbance maxima of the detected coloring components are presented in Table 1. PDA: 191–799 nm (full scan detectionfull scan detection).

allows univocal identification of madder and cochineal. The rest of the reference dyestuffs, such as brazilwood, dragon blood, and lac dye were also chromatographically and spectrophotometrically (UV-Vis) characterized, without identifying their constituents because the corresponding standards were not available. Absorbance maxima in Table 2, which correspond to the four lac dye components, appear to be similar and in good agreement with the spectral characteristics of laccaic acid A, B, C, and E, the main coloring components of the lac dye, that can be found in the literature.[22]

Table 3 provides the results of HPLC-DAD analysis of the sample extracts, including retention times and corresponding absorbance maxima. Chromatographic peaks are presented in Figures 2, 3, 4, 5, and 6 for the samples extracted from icons 1, 2, 3, 4, and 5, respectively. Comparison of these with Table 2 results in the identification of the detected dyestuffs, which can be summarized as follows. Carminic acid, the main component of cochineal, was recorded for the samples extracted from icons 1, 2, 3, and 5. Brazilwood was identified in samples extracted from (the older) icons 4 and, in addition to cochineal, 5. No other red dyestuffs were detected with respect to Figure 1. It should be noted here, that both reference materials and sample extracts from icons, were treated by, exactly, the same HCl process, described in the paragraph Sample Preparation. Consequently, a direct comparison of the chromatograms achieved by the analyses of reference materials and extracts is possible, as any effect of the acetic extraction process in the chromatograms should be present in either case.

Table 3. Chromatographic and spectral characteristics of the investigated sample extracts originating from corresponding icons

Sample extract	Coloring components detected	Characteristics of the detected coloring components			Original dyestuff used
		R_t (min)	Peak	Absorbance maxima (nm)	
Icon 1	Carminic Acid	14.9	fig. 2	267, 309, 493	Cochineal
Icon 2	Carminic Acid	14.8	fig. 3	267, 309, 493	Cochineal
Icon 3	Carminic Acid	15.1	fig. 4	275, 309, 493	Cochineal
Icon 4	Component 1 of Brazilwood	20.9	fig. 5	451, 495, 515	Brazilwood
	Component 2 of Brazilwood	21.6		495, 515, 549	
Icon 5	Carminic Acid	15.1	fig. 6a	275, 317, 491	Cochineal and Brazilwood
	Component 1 of Brazilwood	20.9	fig. 6b	491, 509	
	Component 2 of Brazilwood	21.5		517, 549	

Quantification of the results is necessary for a more meticulous analysis that could lead to the specification of the exact insect source of the detected cochineals. Unfortunately, the lack of corresponding standards, such as kermesic and flavokermesic acid, restricted the detailed interpretation of the

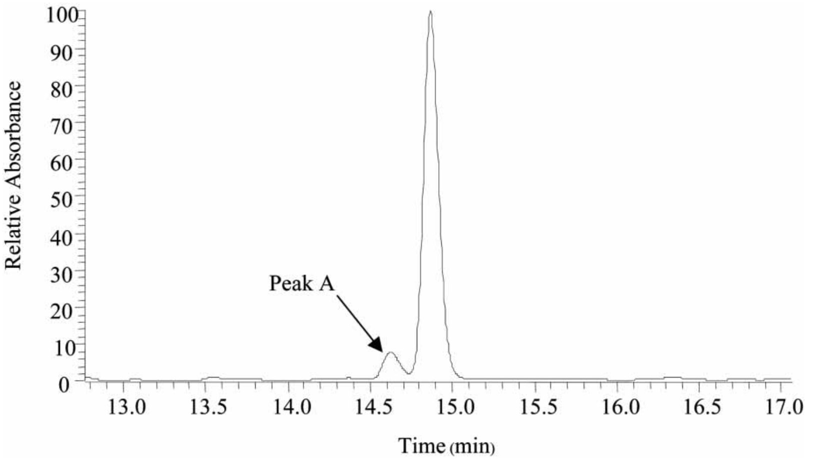

Figure 2. Identification of carminic acid in sample extract of Icon 1. PDA: 300–500 nm.

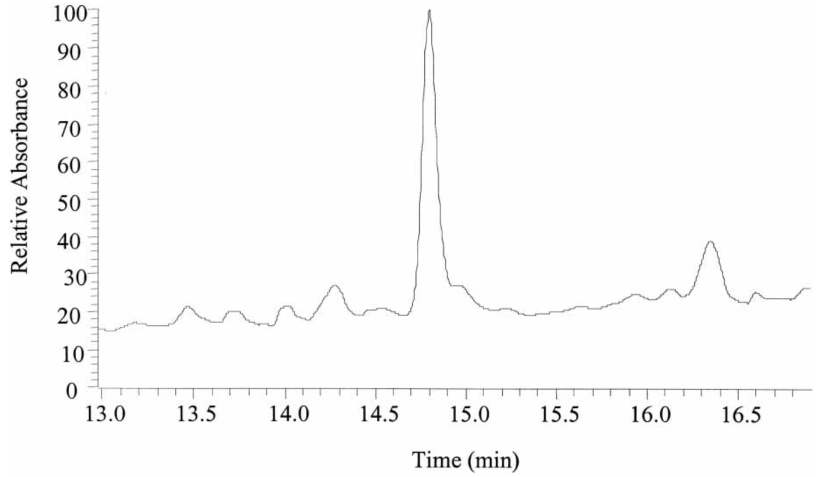

Figure 3. Identification of carminic acid in sample extract of Icon 2. PDA: 300–500 nm.

results to the following speculations. In samples derived from icons 1 and 3 a minor peak, shown as peak A in Figures 2 and 4, respectively, has been recorded just "in front" of the main peak that corresponds to pure carminic acid. The presence of this peak in a considerable amount is representative for Mexican cochineal derived from Dactylopius coccus Costa.[2,4].

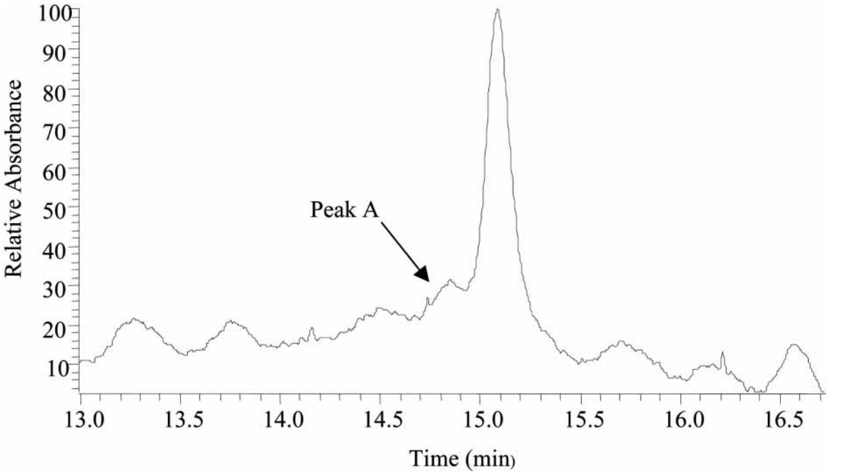

Figure 4. Identification of carminic acid in sample extract of Icon 3. PDA: 300–500 nm.

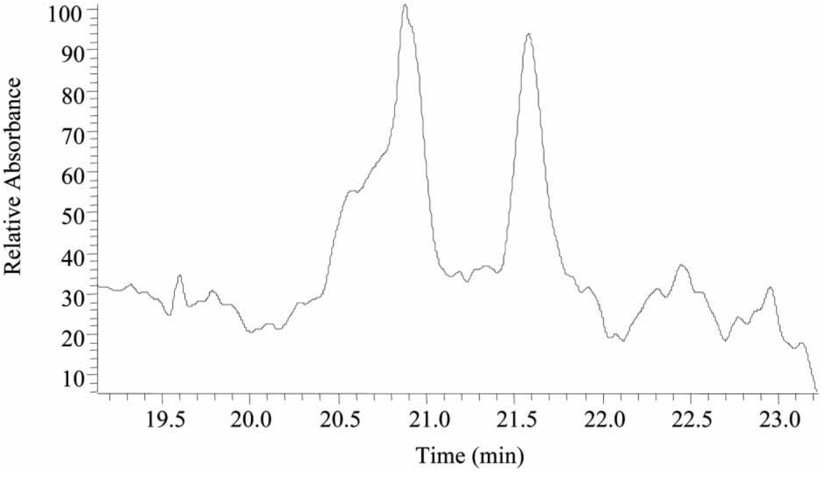

Figure 5. Identification of brazilwood in sample extract of Icon 4. PDA: 300–500 nm.

This observation can lead to the conclusion that icons 1 and 3 were created after the discovery of the New World, which is in agreement with the historical information presented in Table 1. Peak A was not recorded for the extracts of icons 2 and 5, according to Figures 3 and 6a and the acquired absorption spectra corresponding to peak A. This should be expected for icon 5, created before the discovery of the New World, as Old World cochineal insects, such as Polish and Armenian cochineal, contain hardly the component which refers to peak A.[2,4] For icon 2, the absence of peak A is also possible. However, no clear conclusion can be drawn, as the tested sample appeared to include very small dyestuff quantity leading to low absorption signal, which raises uncertainties regarding the exact provenance of the detected cochineal.

Brazilwood was identified in icons 4 and 5. The latter, a typical example from the Byzantine painting art, has been apparently created before the discovery of the South America, verifying the argument that this pigment was well known before the discovery of the New World. Brazilwood has been identified in western European textiles dating from the 13th century.[25] Figure 6 suggests that the pigment was also known to Byzantine, a Mediterranean empire between Asia (the source of brazilwood) and western Europe. Except from brazilwood, cochineal was also detected in icon 5 leading to the conclusion that, in this case, a dye mixture was originally used by the creator. It should be noted here, that the above conclusions associated with the coloring technology, clarified via HPLC analyses, are based on the assumption that icons were not treated after their original creation in ways that could affect their initial coloring content (e.g., overpainted). No historical evidence for such treatment was available.

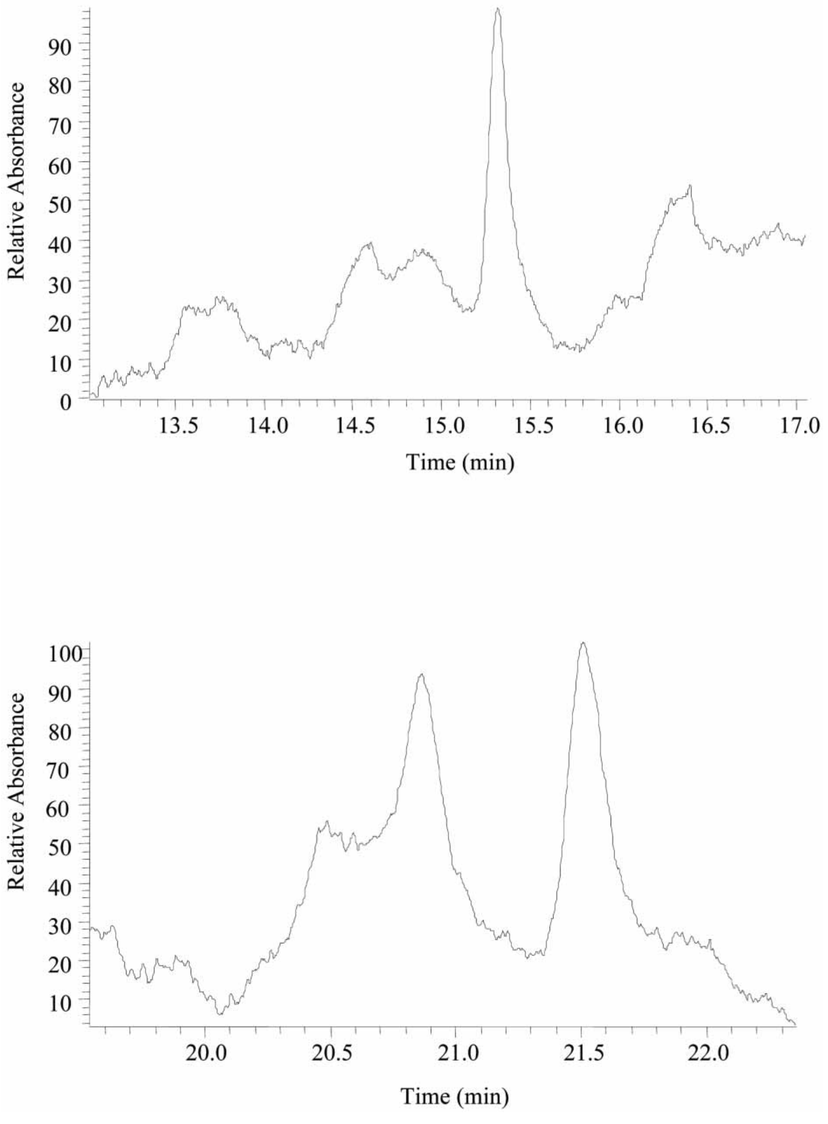

Figure 6. Identification of (a) carminic acid and (b) brazilwood in sample extract of Icon 5. PDA: 300–500 nm.

CONCLUSION

RPLC-DAD was used to analyze, the organic colorants of samples (less than 1mg) extracted from five Byzantine and post-Byzantine portable icons, which were created in the Southeast Mediterranean area from the 14th to 19th century. The developed method is able to analyze and detect natural reddish dyestuffs such as madder, cochineal, brazilwood, dragon blood, and lac dye. From these, only cochineal and brazilwood were detected in sample extracts. Mexican cochineal was speculated as the most probable source for extracts originating from two post-Byzantine icons. Brazilwood was found, in addition to cochineal, in an icon created before the discovery of the New World and was identified as the exclusive organic colorant of a post Byzantine icon extract. As cochineal and brazilwood are common colorants for historic paintings and art objects of Western Europe, the relationships and the interactions between the Southeast Mediterranean art and the Western Renaissance in the field of the coloring technology, becomes an interesting subject for further investigation.

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Received October 10, 2004
Accepted November 9, 2004
Manuscript 6510