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Identification of natural dyes in archeological Coptic textiles by liquid chromatography with diode array detection

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Abstract

Reversed-phase HPLC with diode-array UV–Vis spectrophotometric detection has been used for identification of natural dyes in extracts from wool and silk fibres from archeological textiles. The examined objects originate from 4th to 12th Century Egypt and belong to the collection of Early Christian Art of the National Museum in Warsaw. Extraction from fibres was carried out with HCl solution containing ethanol or with warm pyridine. As the main individual chemical components of natural dyes, anthraquinone, indigoid and flavonoid dyes including alizarin, purpurin, luteolin, apigenin, carminic acid, ellagic acid, gallic acid, laccaic acids A and B and indigotin were found. For pyridine extracts another mobile phase with an optimized gradient of organic modifier concentration was used. With such an eluent the appearance of double peaks for indigotin and indirubin was eliminated. For acidic extraction of dyes from fibres, ethanol was used. Due to its higher boiling point than methanol it evaporates slower from the extraction solution enabling a more efficient extraction of dyes.

Keywords: Textiles; Archeological analysis; Dyes

1. Introduction

Liquid chromatography methods play an increasingly important role in the chemical investigations of archeological objects. They are especially valuable for the investigation of natural dyes originating from natural biological sources such as plants, insects, and molluscs, which were used for dying fibres and fabrics in the past. Dyes can be found directly in extracts from natural species or are made colored after various chemical pretreatments such as complexation with metals, hydrolysis or oxidation. The chemical identification of natural substances used for dyeing archeological fabrics is of great significance for the conservation and restoration processes. The chemical composition of contemporary extracts from archeological fibres depends on different factors such as the source of natural dye and the dyeing procedure used, on storage conditions through the centuries and

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ageing processes and finally also on extraction conditions.

Since the introduction of HPLC to the investigation of dyes in historical textiles by Wouters [1], several papers on this subject have already been published [2–10]. In the pioneering work on extracts of natural dyes a reversed-phase HPLC on C_{18} columns with a water–methanol eluent containing formic acid and with UV–Vis detection was employed [1]. This method has been applied to extracts from plant roots and insects which were used as a source of red dyestuffs for dyeing textiles. The anthraquinone derivatives present in ancient red dyes were determined after acid hydrolysis. UV–Vis spectra were recorded in a HPLC set-up at stopped flow. The extracts of red dyes from insects were also examined in later work [2]. Due to strong absorption of dyes in the UV–Vis range, in HPLC systems with diode-array detection (DAD), identification of individual chemical components of natural dyes can be based not only on retention times but also on UV–Vis spectra. This was first applied to the identification of sources of some red dyes of insect origin [3].

An improvement in the HPLC separation of dyes was observed when gradient of acetonitrile was employed with constant 0.1% concentration of trifluoroacetic acid [4]. The use of acetonitrile instead of methanol provides a wider observation window in the wavelength range from 200 to 275 nm. Such an eluent that consists of all volatile compounds can be also used in a HPLC system with mass spectrometry detection. HPLC–DAD measurements were employed for investigation of dyes in historical objects [5], and to examine some natural and synthetic dyes [6]. The components of natural dyes non-absorbing UV–Vis radiation were investigated in HPLC–MS systems [7,8].

The solvent extraction of dyes from fibres is usually carried out with a mixture of 3 *M* hydrochloric acid–methanol (1:1, v/v) at boiling temperature, which allows the extraction of anthraquinone and flavonoid dyes. This procedure is inefficient for extraction of indigotin and indirubin, but small amounts of indigotin were extracted in this way. As 6,6′-dibromoindigotin, the main component of real purple, is not soluble under such conditions, for blue or purple fibres, extraction with warm pyridine was recommended [9]. Very little attention has been, so far, focused on the ageing processes of natural dyes in archeological textiles, except studies on changes of flavonoid dyes accelerated by UV irradiation [10].

Although the literature about chemical examination of historical fabrics is quite extensive, very little attention was paid to Coptic textiles. The investigation of the dyes in Coptic textiles based on chemical reaction was pioneered by Pfister as early as in the 1930s [11]. The first HPLC examination of extracts from four Coptic objects from the 3rd to 8th Centuries was reported by Wouters [1]. From these later works [12,13] one can conclude that in different periods of time various compositions of natural dyes were used. The presence of unidentified yellow dye has been also reported [13].

The aim of this study was to identify individual chemical components present in extracts of natural dyes from sample fibres of different colors taken from Coptic textiles from the Early Christian Art Collection in the National Museum in Warsaw [14]. These objects have not been, so far, examined using modern instrumental analytical methods and such a project is to some extent a challenge, as identification should be made in extracts from about 0.5 to 3 mg of fibre sample.

Identification of natural dyes on Coptic textiles can be helpful in their dating. For example, kermes (*Kermes vermilio*) started to be widely used in Egypt only after the Arabic invasion, that means in the second half of the 7th Century. So identification of kermes on the textile would mean that it was not dyed before the 7th Century. Results obtained from dye analysis could also help in reconstruction of destroyed textiles with threads dyed with the same natural dyes as the identified ones. That could assure that further aging of the textile would be uniform and there would be no differences between supplements and historical material.

In investigations of natural dyes from archaeological textiles, only qualitative analysis is performed due to the problems connected to the extraction of dye from the fibre, which depends on the kind and quality of dye, as well as the kind of mordant and dyes used. That is why quantitative results obtained for extracts are not directly correlated with what was in fact on the fibre and in the dyeing solution; here, what is essential are the conditions in which dyeing was done, such as temperature, time of dyeing,

presence of mordants and additional substances. Extracts often contain substances that are difficult to identify because of the lack of corresponding standards. Those which were identified as possible components of extracts are often very difficult to obtain—rarely available commercially. Quantitative analysis of only a few components of the extract would not be satisfactory. Additionally, archaeological samples contain many organic and inorganic impurities that are difficult to remove and it is not possible to check if they were removed completely, which increases the error of mass determination. Additional problems occur because of the dyes' aging processes, such as decomposition of dyes and their disappearance during usage, washing of fabrics and so on. If quantitative analysis should lead to the evaluation of the initial mass of dye on the fibre, this would be very valuable information, however because of the above mentioned reasons it is practically impossible. Since identification of the natural dye used is based on the proportion of identified substances, usually for dye analysis a ratio of their peak areas at a given wavelength is given.

2. Experimental

2.1. Instrumentation

Chromatographic measurements were carried out using a Shimadzu HPLC system which consisted of a gradient pump LC-10AT, phase mixer FCV-10AL, diode-array detector SPD-M10A and column thermostat CTO-10AS. Separations were carried out on a C_{18} column Luna 5 μm, 25 cm×4.6 mm (Phenomenex). Chromatographic data processing was carried out using Class-Vp software from Shimadzu. The spectral width of the detection wavelength was 1 nm.

2.2. Reagents

The extraction of dyes from fibres and HPLC measurements were carried out using 25% hydrochloric acid (analytical grade) from Merck (Darmstadt, Germany) and pyridine (analytical grade) from Fluka (Buchs, Switzerland), ethanol (analytical grade) from Polmos (Warsaw, Poland), methanol (super gradient), acetonitrile (super gradient) and tetrahydrofuran (for HPLC) from Lab-Scan (Dublin, Ireland) and trifluoroacetic acid (for spectroscopy) from Merck. HPLC eluents were prepared using Milli-Q deionized water.

The following reagents have been used as references: alizarin, purpurin, carminic acid, ellagic acid, apigenin and gallic acid from Fluka, luteolin from Roth (Karlsruhe, Germany), lawson from Sigma (Steinheim, Germany), weld from G. Weil (Surrey, UK), madder, synthetic indigo, indigo from *Indigofera tinctoria*, lac dye, cochineal and real purple from Kremer (Krakow, Poland). As gifts, indirubin, 6-monobromoindigotin and 6,6′-dibromoindigotin were obtained from Mr. Chris J. Cooksey (Watford, UK, formerly University College London), munjistin from Dr. N.P. Mischenko of the Pacific Institute of Biorganic Chemistry (Vladivostok, Russia), Armenian cochineal from Ms. Ina Vanden Berghe and Dr. Jan Wouters of Koninklijk Institut voor het Kunstpatrimonium (Brussels, Belgium) and kermes form Mr. Andre Verhecken (Mortsel, Belgium) and Mr. Witold Nowik of LRMH (Champs-sur-Marne, France).

2.3. Extraction procedure

A sample of fibre (0.5 to 3.0 mg) was hydrolyzed for 15 min at 100 °C in 400 μl of a mixture of 3 *M* hydrochloric acid–ethanol (1:1). The obtained extract was filtered using 0.45 μm VectaSpin Micro centrifuge filters and evaporated to dryness in a vacuum desiccator, then the obtained solid was dissolved in 200 μl of 50% methanol solution. Non-dissolved residue on the filter was treated with 200 μl of warm pyridine and filtered again. Twenty μl of hydrolysate or pyridine extract were injected into the HPLC set-up.

2.4. HPLC measurements

2.4.1. Gradient program I

Chromatographic separation in hydrolysate was carried out at 40 °C using a linear gradient of acetonitrile (ACN) from 100% A to 100% B in 60 min. Eluent A contained 5% ACN and 0.1% trifluoroacetic acid (TFA) in water. Eluent B contained 0.1% TFA in ACN.

2.4.2. Gradient program II

Chromatographic separation of pyridine extract was carried out at 40 °C at a gradient increase of ACN as follows: isocratic elution with 80% A and 20% B up to 9 min, linear gradient to 100% B up to 15 min, and then 100% B. Eluent A contained water–ACN–tetrahydrofuran (50:45:5). Eluent B was tetrahydrofuran–ACN (5:95). Chromatograms were recorded in the wavelength range from 200 to 700 nm. The ratio of peak areas of identified species was measured at 255 nm. For this wavelength it is possible to see on the chromatogram all peaks of interest and hence to calculate ratios of their peak areas. It is also widely used in the literature devoted to historical dyes' analysis which enables comparison of the obtained results.

3. Results and discussion

The reported investigations were carried out in three stages. Firstly, chromatographic measurements were carried out for purified dyes and natural dyeing substances collected from various sources. Then HPLC data were recorded for extracts of dyes from contemporary fibres, which were obtained from the Conservation Department of the National Museum in Warsaw. Finally, the extracts from fibres, taken from ancient Coptic objects were analyzed. Identification of dyes was based on retention times and their comparison with standards as well as on UV–Vis spectra (see Table 1) recorded for sample extracts and standards. Identity of UV–Vis spectra was confirmed when agreement between their maxima was obtained within ±1 nm.

3.1. HPLC of main components of natural dyes

Chromatographic identification of species in unknown samples requires initial determination of retention times of analytes based on available reference materials. This was carried out with the use of extracts of available natural dyes purchased mostly from Kremer and individual chemical components occurring in natural preparations, which were purchased or obtained as generous gifts from various research laboratories. Chromatograms obtained for aqueous extracts from weld, Armenian cochineal, and lac-dye are shown in Fig. 1A, 1C and 1E, respectively, together with the assignment of peaks corresponding to identified chemical species. As it can be seen especially for extract of weld (Fig. 1A), besides the signal of luteolin there are numerous signals which were not identified in the HPLC–DAD

Table 1
Chromatographic retention times and absorption maxima for examined dyes

Chemical compound	Retention time (min)	Standard deviation	Peak no.	Absorption maxima (nm)	Natural source of compound
Gradient program I					
Gallic acid	6.1	0.07	1	214, 270	Tannins
Laccaic acid B	14.7	0.16	2	286, 491	Lac-dye
Carminic acid	15.8	0.51	3	274, 309, 494	Armenian cochineal
Ellagic acid	17.4	0.12	4	256, 368	Tannins
Laccaic acid A	18.9	0.09	5	285, 492	Lac-dye
Lawson	22.0	0.10		248, 275, 337	Henna
Luteolin	24.6	0.13	6	347, 253, 265	Weld
Apigenin	27.6	0.18	7	336, 266	Weld
Munjistin	30.3	0.12		250, 288, 419	Madder
Alizarin	32.1	0.12	8	251, 278, 428	Madder
Purpurin	35.5	0.10	9	255, 293, 479	Madder
Indigotin	35.9	0.10	10	609	Indigo
Gradient program II					
Indigotin	7.5	0.04	10	609	Indigo
Indirubin	8.5	0.05	11	540	Indigo
6-Bromoindigotin	12.7	0.05		604	Tyrian purple
6,6′-Dibromoindigotin	18.3	0.06		597	Tyrian purple

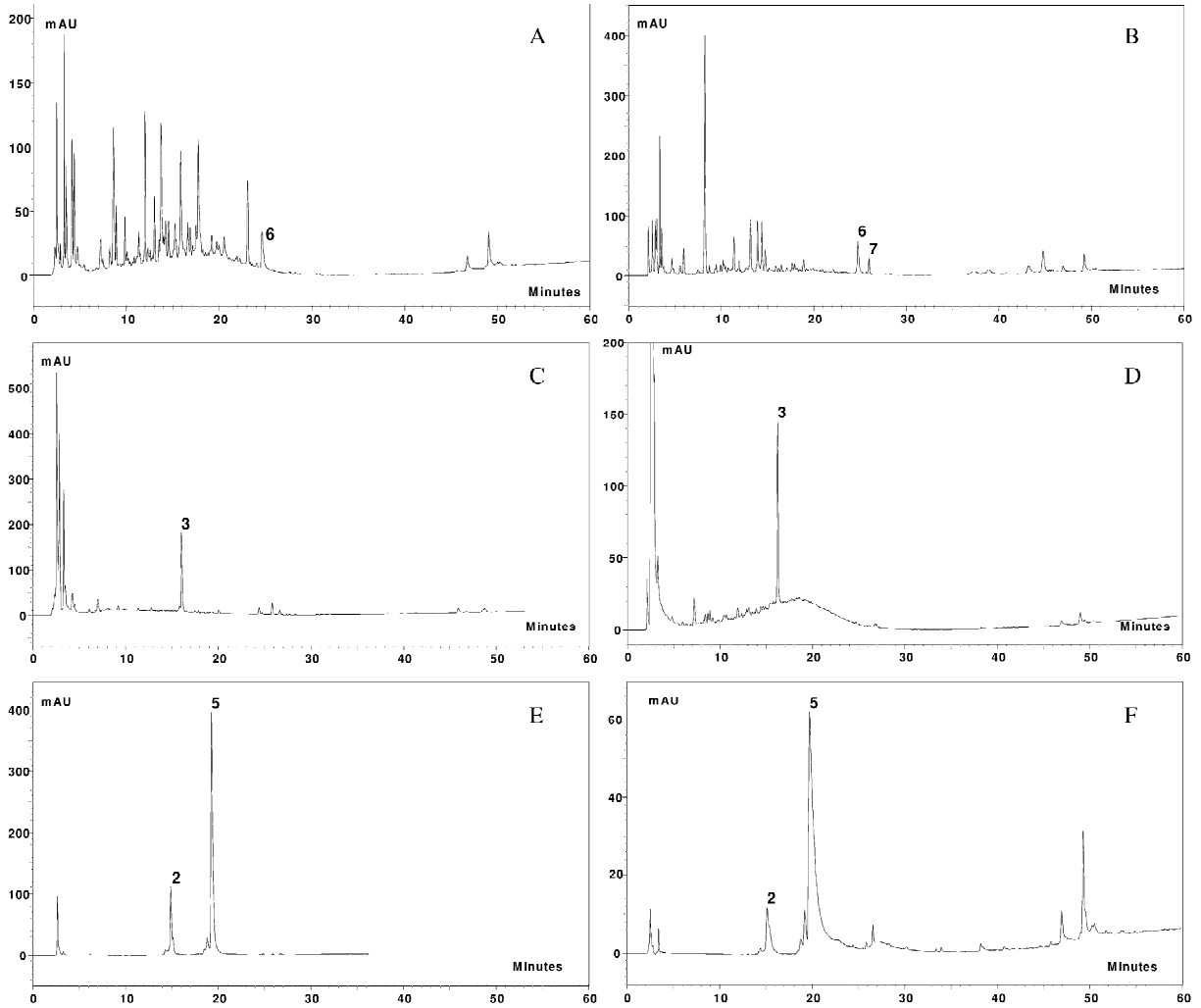

Fig. 1. Chromatograms of aqueous extract of weld purchased from G. Weil (A), acid hydrolysate from aqueous extract of weld purchased from G. Weil (B), aqueous extract of Armenian cochineal (C), acid hydrolysate of wool fiber dyed with Armenian cochineal (D), aqueous extract of lac-dye (E), acid hydrolysate of wool fibre dyed with lac-dye (F). Chromatograms obtained with gradient program I at 255 nm.

system. The extract of weld after hydrolysis (Fig. 1B) in conditions used for extraction from natural fibres exhibits additionally a signal of apigenin and a different pattern of unidentified signals. This means that numerous species present in the extract of weld dye might be decomposed during the extraction procedure from fibres. This can be predominantly ascribed to the presence of heterosides of luteolin and their transformation to respective aglycone structures under acid treatment during extraction from fibres. This is also a reason for their greater retention time on a hydrophobic stationary phase after acid treatment.

A different picture was found for extracts from Armenian cochineal and lac-dye. They contain mostly the main dyeing reagents identified with the use of available reference materials. The complete list of examined dyeing substances that may be present in natural dyes used in Coptic textiles is shown in Table 1, together with their retention times in the chromatographic conditions used and spectrophotometric data. The maxima of absorption in the UV–Vis range

are in good agreement with the earlier data of Wouters [1] and Halpine [4]. Table 1 also shows natural dyes, where given individual species can be found.

3.2. HPLC of extracts from contemporary dyed fibres

Due to a very limited availability of fibres from archeological objects the optimization of the solvent extraction procedure was carried out using wool fibres contemporarily dyed with available natural dyes. In the finally accepted procedure the acid hydrolysis extraction used by Halpine [4] was adopted with a change of methanol to ethanol, that resulted in a marked increase in efficiency of extraction.

Performing HPLC measurements for extracts from contemporary dyed fibres may also be helpful in estimation of the effect of acid hydrolysis on the composition of the extracted dye. Among the examples shown in Fig. 1, extracts from fibres dyed with Armenian cochineal and lac-dye do not show a different composition than the aqueous extract of dye, where weld aqueous extract after additional hydrolysis has shown essential differences as discussed above. This indicates that hydrolyzed extracts should be used as reference rather than raw aqueous extracts of natural dyes, which was recommended by other authors [15].

This is also true for the detection of tannin compounds from a fibre, which may be performed by detecting gallic or elagic acid in the acidic hydrolysate. They might have been used as mordants or as colorants when they were applied as complexes with metals. Comparison of chromatograms recorded for hydrolysates from contemporary fibres treated with 40% gallic acid solution and fibres treated with 40% solution of ferric sulfate and then dyed with 40% gallic acid solution (to obtain gray–blue color), revealed about 60-fold decrease of the signal for gallic acid for the latter extract. A similar observation was made for an analogous pair of samples when 20% solutions of gallic acid and ferric sulfate were employed. This leads to the conclusion that for fibres where ferric mordants and tannins were used, the presence of the latter may not be detected. The detection is not certain for some ferro-gallic complexes.

For hydrolysates from certain dyes a quick ageing process was observed, which suggests the necessity of performing HPLC measurements immediately after obtaining the extract. Aging was observed for an extract from thread dyed with Armenian cochineal. After a few days of storage the sample changed color. Also for several extracts from archeological threads, precipitation was observed within 24 h from preparation. Indigotin detected in freshly prepared extracts was no longer seen after longer (e.g. 1 week) storage.

In the case of the majority of extracts obtained as a result of acid hydrolysis of dyed wool fibres, both dyed contemporary (Fig. 1D) or archeological objects (Figs. 3B, 4A and 4B), a broad elevation of the baseline between 10 and 30 min was observed. This elevation is especially pronounced in the UV range and interferes in identification of species eluted in this period of time. This signal was also observed for hydrolysates of non-dyed wool but not for non-dyed silk or for extracts from Coptic silk fibre (Fig. 3A); it was assigned to some product of acid hydrolysis of wool. The range and shape of this elevation is not identical for all fibres and probably depends on the kind of wool. However this ‘hump’ is observed only in the UV region, whereas dyes absorb in the visible region. Background correction was sufficient for identification of dyes on the basis of their spectra. Peaks at 47 and 49 min observed for aqueous extracts of natural dyes, for extracts of non-dyed fibres as well as for extracts from dyed fibres have been assigned as system peaks of unknown origin. Although purpurin and indigotin have very similar retention times, significant differences in their absorption maxima (479 nm for purpurin and 609 nm for indigotin) enables their simultaneous identification.

A difference between an extract from indigo dye and an extract from a fibre of contemporary dyed wool has been found also for pyridine extraction in the case of *Indigofera tinctoria* (Fig. 2). In a dye extract both indigotin and indirubin were found, whereas in an extract from dyed wool indigotin was present only. Additionally, in the HPLC of pyridine extracts for eluents without tetrahydrofuran, double peaks were observed for each analyte. This can be

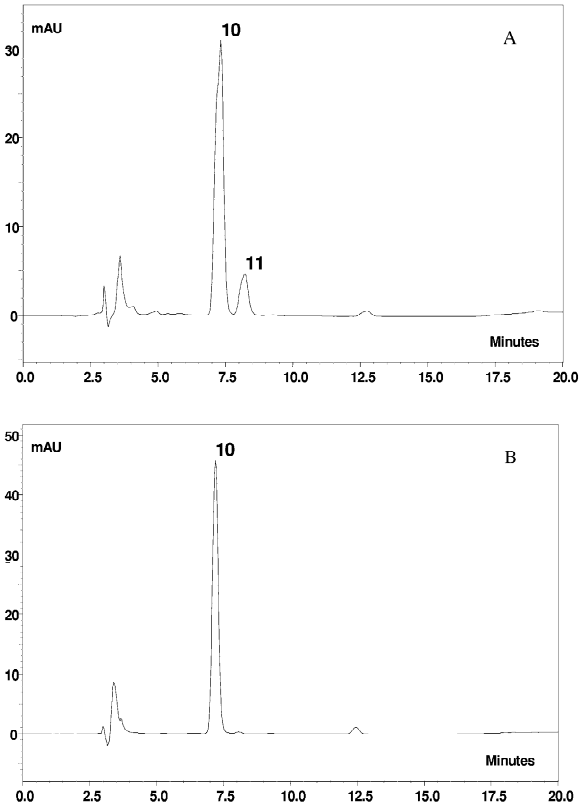

Fig. 2. Chromatograms of pyridine extracts of *Indigofera tinctoria* (A) and of pyridine extract of wool fibre dyed with the same dye (B). Both chromatograms obtained with gradient program II at 550 nm.

explained by retention of pyridine on the column, which interferes in the partition of analytes between stationary and mobile phases. A similar disturbance of the peak purity in HPLC of pyridine extracts can be found in chromatograms published earlier [10]. Addition of THF to the eluent eliminates this effect.

3.3. HPLC extracts from Coptic textile

Sixteen samples of mostly wool fibres from Coptic textiles have been examined using optimized acid hydrolysis or pyridine extraction and HPLC–DAD analysis. The peak absorbance values obtained at 255 nm were used for determination of the ratio of identified dyes for each extract (Table 2). This wavelength allows to obtain sufficient sensitivity of detection for all determined compounds. Fig. 3A shows a chromatogram obtained for an extract from red silk fibre and Figs. 3B, 4A and B show example chromatograms recorded for extracts from red, orange and green wool fibres, respectively.

In the examined archeological samples, the presence of natural dyes such as madder, weld, lac-dye, indigo (of unknown origin) and Armenian cochineal was found. In an extract from a silk fibre also tannins have been found, which are known to be used as a weighting factor for silk.

In 10 of 16 samples only alizarin and purpurin have been found in proportions from 94:6 for brown fibre (sample 12) to 29:71 for red fibre (sample 9). It cannot be concluded definitely whether the examined fabrics were dyed only with madder (*Rubia tinctorum*) or with a mixture of madder and wild madder (*Rubia peregrina*). According to the literature data [16], there is no alizarin in extracts from wild madder, but it can be found in hydrolysates from threads dyed with wild madder in amounts from 7 to 9%. Hydrolysates of threads dyed with common madder contain from 14 to 50% of alizarin. The amount of purpurin for both species is similar: 19–43% for wild madder and 12–42% for common madder. The amount of rubiadin was proposed as an indicator of wild madder, in which this compound can be found in amounts around 17–24%. Common madder contains only around 1% of rubiadin. In analysis of most of the Coptic samples it was assumed that thread was dyed with common madder, when the amount of alizarin found in the extract was more than 10%. Because of the lack of standards of rubiadin, its content was not determined in the extracts. That is why in cases of samples 6 and 11 it was not possible to determine which madder was used for thread dyeing.

The histogram in Fig. 5 shows proportions of alizarin and purpurin found in different samples. The obtained data indicate that there is no correlation between relative amounts of both species in extracts and the color of the fibre examined. At similar proportions of both species, the fibre can show different colors such as red or orange, but the same color was found also for samples with very different proportions of both species. Different colors of threads having the same ratios of purpurin and alizarin identified in extracts probably result from different concentrations of dyes and kind of mordants used.

Table 2
Natural source and identified main components in Coptic textiles

No.	Data (centuries); probable mordants [17]	Color	Fibre	Identified dyes; ratio between identified main components, all integrated at 255 nm
1	AD 7th–9th; Fe	Yellow	Wool	Madder (*Rubia tinctorum*); 51 alizarin, 49 purpurin
2	Data unknown; Fe, Al	Orange	Wool	Madder (*Rubia tinctorum*); 69 alizarin, 31 purpurin
3	AD 7th–9th; Fe, Al	Orange	Wool	Madder (*Rubia tinctorum*); 39 alizarin, 61 purpurin
4	AD 6th; mordants not investigated	Orange	Wool	Madder (*Rubia tinctorum*), weld (*Reseda luteola*); 44 alizarin, 43 luteolin, 9 purpurin, 4 apigenin
5	AD 7th–9th; Fe	Red	Wool	Madder (*Rubia tinctorum*); 80 alizarin, 20 purpurin
6	Data unknown; Al	Red	Silk	Armenian cochineal (*Porphyrophora hamelii*), lac-lac (*Laccifer lacca*), madder (*Rubia tinctorum*) or wild madder (*Rubia peregrina*), weld (*Reseda luteola*), tannins; 41 carminic acid, 21 ellagic acid, 13 laccaic acid A, 11 purpurin, 5 luteolin, 4 alizarin, 2 gallic acid, 2 laccaic acid B, 1 apigenin
7	Data unknown; Al	Red	Wool	Madder (*Rubia tinctorum*); 60 alizarin, 40 purpurin
8	Data unknown; Fe, Al	Red	Wool	Madder (*Rubia tinctorum*); 67 alizarin, 33 purpurin
9	AD 7th–9th; Al	Red	Wool	Madder (*Rubia tinctorum*); 29 alizarin, 71 purpurin
10	AD 7th–9th; no mordants	Red	Wool	Madder (*Rubia tinctorum*); 65 purpurin, 35 alizarin
11	AD 4th; Fe, Al	Brown	Wool	Madder (*Rubia tinctorum*) or wild madder (*Rubia peregrina*), indigo; 90 purpurin, 10 alizarin, indigotin (*detected in pyridine extract*)
12	AD 6th; mordants not investigated	Brown	Wool	Madder (*Rubia tinctorum*); 94 alizarin, 6 purpurin
13	AD 3rd–5th; mordants not investigated	Beige	Wool	Madder (*Rubia tinctorum*); 43 alizarin, 57 purpurin
14	AD 7th–9th; Al	Green	Wool	Weld (*Reseda luteola*), madder (*Rubia tinctorum*), indigo; 75 luteolin, 16 alizarin, 9 indigotin
15	AD 7th–9th; no mordants	Green	Wool	Madder (*Rubia tinctorum*), indigo; 61 indigotin, 39 alizarin,
16	AD 6th; mordants not investigated	Green	wool	Weld (*Reseda luteola*), madder (*Rubia tinctorum*), indigo; 76 luteolin, 24 alizarin, indigotin (*detected in pyridine extract*)

In yellow, orange and green fibres the presence of yellow dye can be expected. In earlier studies on Coptic textiles the only yellow dye found was weld (*Reseda luteola*), and it was also identified in orange sample 4 (Fig. 4A), red fibre 6 and two green samples 14 (see Fig. 4B) and 16.

Surprising results were obtained for yellow fibre sample 1, in which only alizarin and purpurin have been identified and no yellow dye was present, however, the signal magnitude was 10 times smaller than for the same amount of orange fibre sample 2 taken for extraction, which could be an explanation.

The examples of orange fibres from Coptic textiles with main components identified as madder (as samples 3 and 2) have been already reported in the literature. On the other hand, no example of yellow or green fibre was described where luteolin, the main component of weld, was not identified. In extracts from green wool sample 15, luteolin has not been found.

The presence of indigotin has been detected in extracts of three green fiber samples 14, 15 and 16 and one brown fibre 11. In two cases (samples 11 and 16) indigotin has not been found in chromatograms of hydrolysate, however a blue residue after acid hydrolysis indicated the presence of this dye, which was confirmed by HPLC of pyridine extract.

The most interesting results have been obtained for the only examined silk fibre sample 6 (see chromatogram in Fig. 3A). The presence of gallic and ellagic acids in the acid hydrolysis extract indicates the use of tannins in the dyeing process, probably as organic mordants or weighting components. To date, simultaneous presence of carminic acid and laccaic acids has not been reported in extracts from Coptic textiles.

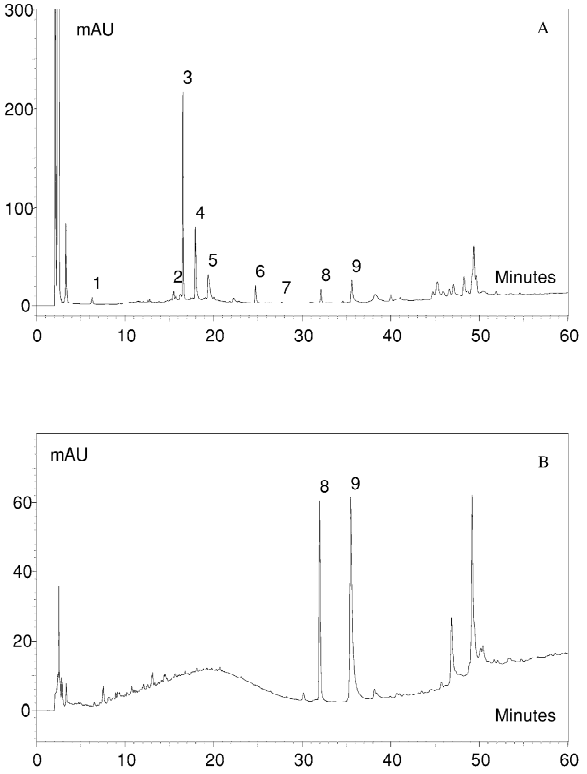

Fig. 3. Chromatograms of acid hydrolysates from Coptic textiles: red silk fibre (sample 6) (A), red wool fibre (sample 10) (B). Chromatograms obtained with gradient program I at 255 nm.

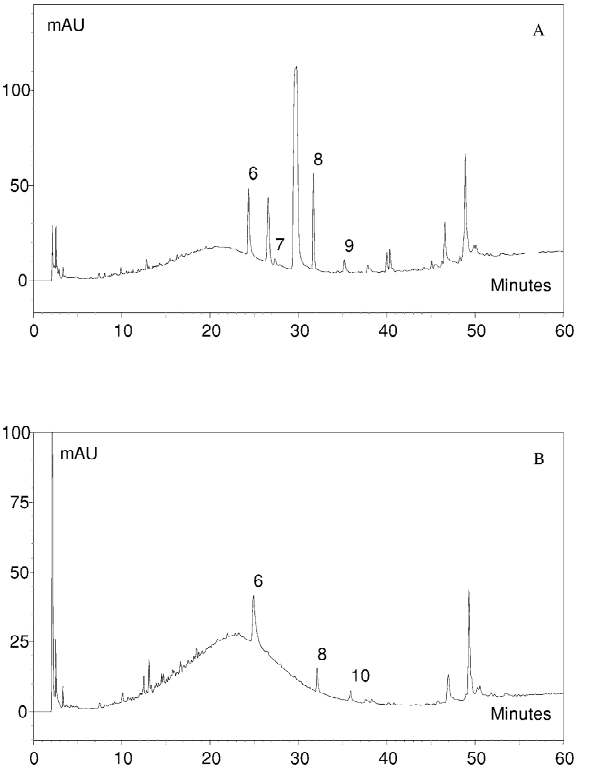

Fig. 4. Chromatograms of acid hydrolysates from Coptic textiles: orange wool fibre (sample 4) (A), green wool fibre (sample 14) (B). Chromatograms obtained with gradient program I at 255 nm.

4. Conclusions

For a variety of different dyes, wider than in the previous work, the suitability of the use of RP-HPLC for identification of dyes in archeological textiles has been shown. As a result of this study the efficiency of two-step extraction from fibres has been improved and for several cases it was shown as more appropriate to use as reference hydrolyzed standard samples instead of standards which were not hydrolyzed in acidic conditions. Spectrophotometric and chromatographic identification of particular chemical species allows the limited identification of natural materials used for dyeing textiles in ancient times.

Because of the special eluent used for analysis of pyridine extracts, problems with double peaks for indigotin and indirubin were eliminated. In further studies another eluent should be optimized for analysis of hydrolysates in order to obtain better separation of purpurin and indigotin. It was impossible to achieve that on the column used with the eluent described above. For elongation of extraction time, ethanol instead of methanol was used. However, it would be interesting to perform more studies on the yield of extraction of dyes from threads. With small amounts of sample available (sometimes even around 0.5 mg) it would be of great importance for identification of compounds present in very small concentrations. Determination of rubiadin would be helpful in differentiation between a wild madder and common madder.

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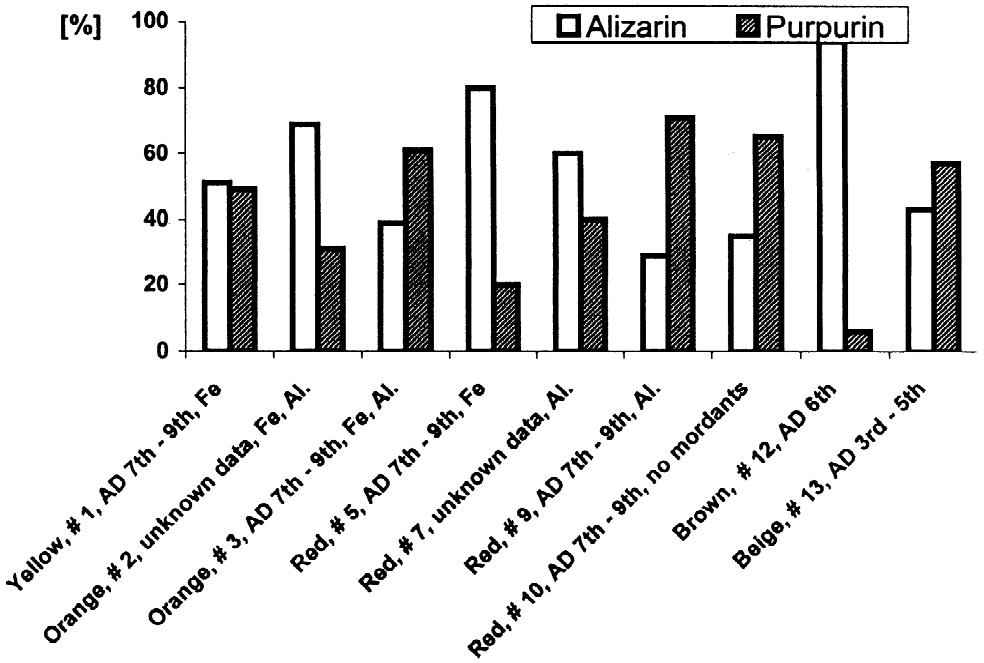

Fig. 5. Histogram showing relative peak area at 255 nm of alizarin and purpurin in samples of fibre of different color.

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