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Short communication

High-performance liquid chromatographic determination of some anthraquinone and naphthoquinone dyes occurring in historical textiles

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Abstract

A reversed-phase HPLC method has been developed for identification and quantitation of nine natural quinone dyes and applied to historical textile fibres. A Purospher RP18e column was used with a convex gradient of methanol in a mobile phase of 0.1 *M* aqueous citrate buffer (pH 2.5) and spectrophotometric diode-array detection at 270 nm. For identification of alizarin, purpurin and xanthopurpurin, occurring together in the madder plant, an isocratic method was used with a methanol–0.2 *M* acetate buffer (pH 4.3) (75:25) as the mobile phase. After an acid extraction of textile fibres and the analysis of the extracts, alizarin and purpurin were identified and quantitated in three fibres.

Keywords: Textiles; Anthraquinones; Naphthoquinones; Dyes

1. Introduction

In the study of historical objects of art, the knowledge of the composition of the natural dye mixtures not only assists in their dating and locating their origin but also gives hints on appropriate procedures for restoration works. The dyes studied in the present paper (see Table 1) are various derivatives of anthraquinone and naphthoquinone that were originally obtained from various plant and animal sources. For a survey of the properties of these substances see, e.g., Refs. [1–5].

The analytical chemistry of natural dyes places a great emphasis on identification techniques that involve simple colorimetric tests [6,7] and, primarily, spectroscopic measurements such as infrared (IR) spectroscopy [4,8] or UV–Vis photometry [1,9–14]. Quantitative analyses have widely employed thin-layer chromatography (TLC) because of its simplicity and a low cost [4,15–17] but, of course, with all the limitations in the reliability of the results characteristic for TLC. Reversed-phase high-performance liquid chromatography (RP-HPLC) has yielded good results in analyses of extracts from natural materials, mostly employing mobile phase programming [18–24]. HPLC analysis of dyes in historical textiles [25,26] and of carminic acid as a foodstuff colouration [27,28] has also been described. Isolation methods from natural materials followed by HPLC are treated in Refs. [29,30] and an application of liquid

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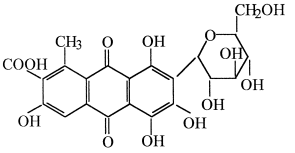
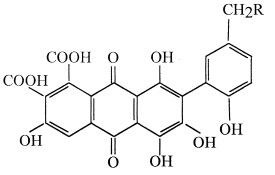
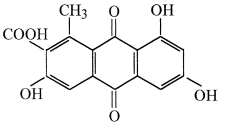
Table 1
The substances studied

Substance	Formula
Alizarin	1,2-Dihydroxy-9,10-anthraquinone
Purpurin	1,2,4-Trihydroxy-9,10-anthraquinone
Xanthopurpurin	1,3-Dihydroxy-9,10-anthraquinone
Lawsone	2-Hydroxy-1,4-naphthoquinone
Juglone	5-Hydroxy-1,4-naphthoquinone
Lapachol	2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone
Emodin	6-Methyl-1,3,8-trihydroxy-9,10-anthraquinone
Carminic acid	
Laccaic acid (a natural mixture of up to five anthraquinone derivatives)	
Form A	R is $-CH_2-NH-CO-CH_3$
Form B	R is $-CH_2-OH$
Form C	R is 
Form D	
Form E	R is $-CH_2-NH_2$

chromatography–mass spectrometry (LC–MS) in Ref. [31]. Normal-phase HPLC was also used [32]. Electrochemical methods have rarely been employed for the purpose [33]. Substantially less attention has so far been paid to electrophoretic methods of determination [34].

This paper deals with a study of HPLC procedures, as the HPLC separations described so far have only involved small groups of dyes, the resolution has often been poor and naphthoquinone dyes have not yet been separated by HPLC.

2. Experimental

2.1. Chemicals

Standard substances of the analytes involved were either purchased (lapachol from Aldrich, purity of 98%, emodin and carminic acid from Fluka with a purity better than 99%), or were isolated at the Microbiological Institute of the Academy of Sciences of the Czech Republic with a purity better than 98% (juglone, lawsone, alizarin, purpurin and xanthopur-

purin). The purity of all these preparations was checked by HPLC.

A laccaic acid substance was provided by Dr. I. Kopecká of the State Institute for the Care of Historic Monuments in Prague. No information was available on its purity and thus the substance was subjected to spectroscopic measurements [^{1}H nuclear magnetic resonance (NMR), electron impact ionisation and fast atom bombardment MS, Fourier transform (FT) IR, UV–Vis] after a HPLC separation. As can be seen from Table 1, the laccaic acid substance may contain up to five anthraquinone derivatives at various ratios, depending on the natural source from which it was extracted. It follows from the spectral analyses of the collected HPLC zones that our substance contained two components, laccaic acids A and B, in a ratio of 4:1.

The other chemicals used were of the analytical grade, obtained from Merck or Lachema, Brno, Czech Republic, and were employed as received. Deionized water (Milli-Q) was used throughout.

2.2. Instruments

The HPLC measurements were carried out on a Crystal chromatograph (ATI Unicam), with a Crystal 200 gradient pump, a Crystal 250 diode-array UV–Vis detector, a CSI 6150 vacuum degasser (Cambridge Scientific Instruments) and a Rheodyne Model 7125 injector with a 5-μl sample loop.

A Purospher RP18e analytical column (Merck, 5 μm, 125×4 mm, specific surface area of 500 m^2/g, a specific pore volume of 0.95 ml/g, a pore diameter of 8 nm and a metal content below 5 ppm) and a RP 18e guard column (Merck, 5 μm, 4×4 mm) were used.

The other instrumentation included a Varian, 400 MHz, NMR spectrometer (the measurements were carried out by Mr. J. Kroutil of our Faculty), a Finnigan MAT INCOS 50 mass spectrometer (the measurements were performed by Dr. M. Štícha of our Faculty) and a Nicolet 740 FT-IR spectrometer with a DSP 670 data station and an Omnic 3.1 software (for the measurements we are grateful to Dr. M. Novotná, Institute of Chemical Technology, Prague, Czech Republic).

2.3. Extraction procedure for textile fibres

The procedure was based in part on that described in Ref. [25]. The fibre was weighed (a maximum of 5 mg) and extracted for 10 min in a heated ground-glass joint tube with a reflux by a HCl–water–methanol mixture (2:1:1) of a maximum volume of 400 μl. The tube was then rapidly cooled in water and its contents were centrifuged in order to separate the fibre residues. The supernatant was then injected into the HPLC separation system.

3. Results and discussion

3.1. Separation system

In view of the character of the test compounds (see Table 1), chemically-bonded reversed-phase columns were selected and tested for separations of the analytes. Standard, silica-based reversed-phase columns could not be used for the purpose because the analytes, especially laccaic and carminic acid, interacted very strongly with the stationary phase support. The same was found for a poly(styrene–divinylbenzene)-based C_{18} stationary phase.

A Purospher C_{18} endcapped stationary phase with a very low metal content was found suitable. The retention factors were smaller than 10 for all the analytes, and the peaks were symmetrical (A_s=0.97).

The selection of the mobile phase was influenced by the fact that a relatively high content of an organic modifier is required to dissolve the analytes. When using acetonitrile (ACN) mixed with water, the test substances elute within a range of the retention factors of 0.5 to 10 at acetonitrile contents of 45 to 55% (v/v), except for laccaic acids A and B and carminic acid which elute with the mobile phase front. The latter substances are more polar compared to the other analytes and thus formic acid was added to the mobile phase to suppress their dissociation. All the substances could be eluted with a mobile phase of ACN–water–HCOOH (25:72:3, v/v/v) with a pH of 2.2. However, this pH may cause damage to the stationary phase and, moreover, xanthopurpurin and purpurin are not adequately separated. As alizarin, purpurin and xanthopurpurin

often occur simultaneously (e.g., in extracts from *Rubia tinctorum*) it was considered useful to divide the separation into two steps, namely, (1) the separation of alizarin, purpurin and xanthopurpurin, and (2) that of alizarin, lawsone, juglone, lapachol, emodin, laccaic acids A and B and carminic acid.

As the first group is not adequately separated with ACN as the organic modifier, methanol was used instead together with an addition of formic acid to suppress the analyte dissociation. A baseline separation of the three compounds was attained within an acceptable time in a mobile phase consisting of methanol–0.2 *M* aqueous acetate buffer, pH 4.3 (3:1). The resolution of the alizarin and purpurin peaks is 1.5 and those of purpurin and xanthopurpurin equals 1.2; the largest retention time (xanthopurpurin) is 10.6 min and the RSD ($n=5$) for the retention time does not exceed 0.4%. Asymmetry factors range between 0.99 and 1.02.

The analyte group No. 2 could not be satisfactorily separated isocratically. Methanol was found to be a suitable organic modifier with a citrate aqueous buffer of a pH not exceeding 2.5. Acceptable resolution values can be attained at methanol contents between 33 and 40% (v/v). However, only the early eluting analytes, laccaic acid B, carminic acid, laccaic acid A and lawsone can be eluted at reasonably short retention times.

For the other substances, a methanol gradient elution was employed. The optimisation led to the following general elution scheme: 3 min isocratically at 40% (v/v) methanol, followed by a convex increase to 95% methanol within 15 min. We have found that this convex increase in methanol can be described by the equation: % MeOH=ln ($A_0+A_1t+A_2t^2+A_3t^3+A_4t^4+A_5t^5$), where $A_0=-1.607$, $A_1=1.264$, $A_2=-3.707$, $A_3=5.117$, $A_4=-3.423$, $A_5=9.422$. The separation then took only ca. 12 min (Fig. 1) with a good resolution. Purpurin and xanthopurpurin do not appear in Fig. 1 but they are eluted together at a time of 11.1 min and thus do not interfere with the other analytes.

3.2. Detection

A diode-array detector is convenient for sepa-

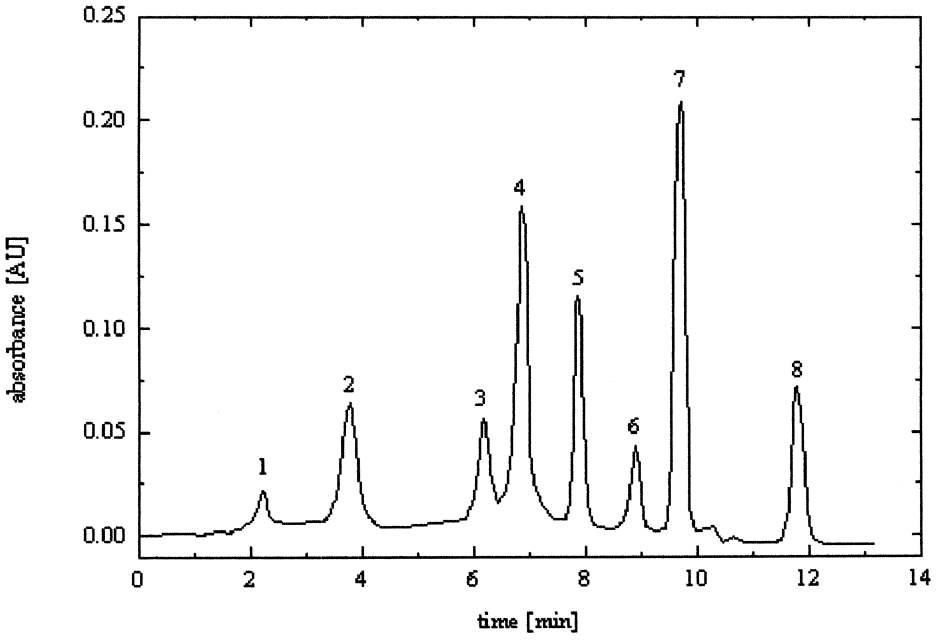


Fig. 1. Separation of laccaic acid B (1), carminic acid (2), laccaic acid A (3), lawsone (4), juglone (5), alizarin (6), lapachol (7) and emodin (8). Elution: 3 min isocratically at 40% (v/v) MeOH in 0.1 *M* aqueous citrate buffer of pH 2.5, then an exponential increase of MeOH (see Section 3.1) to 95% within 15 min. UV detection at 270 nm, flow-rate 0.6 ml/min, Purospher RP18e column.

rations of these substances as their UV–Vis spectra are characteristic and facilitate their identification. If a single-wavelength measurement is employed, then a compromise must be taken, as lapachol, lawsone, carminic acid and laccaic acids A and B absorb most intensely around 280 nm, whereas juglone, alizarin and emodin exhibit the strongest absorption at ca. 260 nm. The limits of detection obtained are similar, ranging from 0.6 ng (xanthopurpurin – isocratic separation at 254 nm) to 12 ng (carminic acid – gradient separation at 270 nm).

The concentration calibration plots are linear (the r^2 values were always better than 0.99) in the whole concentration range studied; the lower LDR (concentration corresponding to a signal equal to three times the peak-to-peak noise) limit equals the detection limit and the upper limit is determined by the solubility of the dye in the mobile phase (between $4 \cdot 10^{-4}$ and $1 \cdot 10^{-3}$ mol/l). There is no significant difference between the measurement of the peak area and the peak height. The repeatability is satisfactory, as typical RSDs ($n=6$) amount to 3.3% for the isocratic and 6.6% for the gradient elution separation.

3.3. Application to historical textiles

Using the extraction procedure described in Experimental, the separation procedures were applied to identification of the dyes used in the 17th century tapestry fibres kindly provided by Dr. I. Kopecká of the State Institute for the Care of Historic Monuments and to determination of their relative contents. To test the stability of the dyes during heating in strongly acidic media, the recoveries were determined first for the standard substances. The recoveries always exceeded 70%, except for laccaic acids A and B and juglone, where, after 10 min extraction, the peak of juglone completely disappeared and the peaks of laccaic acids A and B decreased to 57 and 53% respectively. Therefore, laccaic acids should be extracted for a time not longer than 5 min and juglone for only 2 to 2.5 min.

Fibres of different colours (red, brown and green) were selected for the analysis. If the gradient elution procedure after 10 min extraction indicated the presence of alizarin, then the isocratic procedure was further used to separate alizarin, purpurin and xanthopurpurin. Finally, the fibres were subjected to shorter extraction procedures (2 and 5 min) to test for the presence of juglone and laccaic acids.

A typical gradient elution chromatogram of the extract of one of the fibres is given in Fig. 2. A prominent peak of alizarin can be seen in the chromatogram, together with three other peaks. When the isocratic procedure was applied to the extract, then peak 3 in Fig. 2 was identified as purpurin. Peaks 1 and 4, present in both the chromatograms, cannot be identified, as they correspond to substances other than any of our standards. Their UV–Vis spectra suggest that they may be derivatives of anthraquinone occurring in extracts from *Rubiaceae* plant species, e.g., rubiadin, pseudopurpurin, lucidin, munjistin, etc.

An example of quantitative analysis of three fibres is given in Table 2. As alizarin predominates, it is probable that the dye was an extract of *Rubia tinctorum*, as the other members of the species, e.g., *Rubia pelegrina* or *Rubia munjista* contain other predominating substances. The presence and amount of alizarin and purpurin indicates that the same species of *Rubiaceae* was used for dyeing of this textile material.

4. Conclusions

It can be concluded that the HPLC method proposed yields a reliable means of both qualitative and quantitative analysis of dyes in historical textiles. For unambiguous identification of the components it is necessary to have the appropriate standard substances. Preliminary experiments in capillary electrophoresis are promising and this method is estimated to be a useful and possibly simpler alternative in these analyses.

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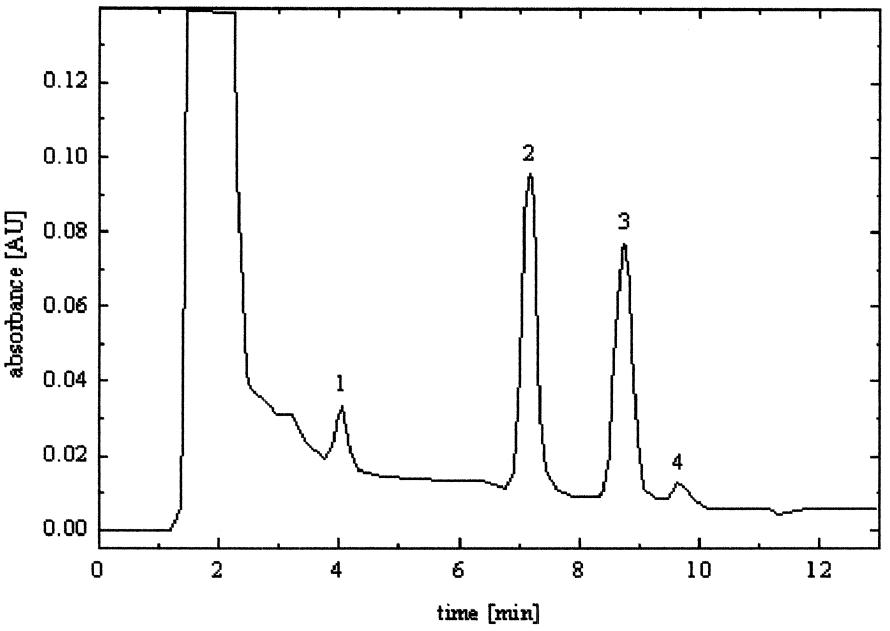

Fig. 2. An isocratic chromatogram of the extract of fibre No. 1058-3. Peak 2 corresponds to alizarin, peak 3 is purpurin. Conditions of analysis: Purospher RP18e column, mobile phase MeOH–0.2 mol/l acetate buffer, pH 4.3 (75:25), flow-rate 0.5 ml/min, 5 μl sample, UV detection at 245 nm. Peaks 1 and 4 were not identified.

Table 2
Quantitation of dyes in fibre extracts (for the experimental conditions see the text)[a]

Fibre No.	Amount of alizarin on a fibre[b] (μg/mg)	Amount of purpurin on a fibre[b] (μg/mg)	Content of the extract components (%)	
			Alizarin	Purpurin
1058-3	2.21	1.44	54	34
758-4	3.01	2.39	47	36
1069-5	2.62	1.88	52	36

[a] The percentage contents were obtained by internal normalisation.
[b] RSD values range between 0.52 and 1.32%.

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