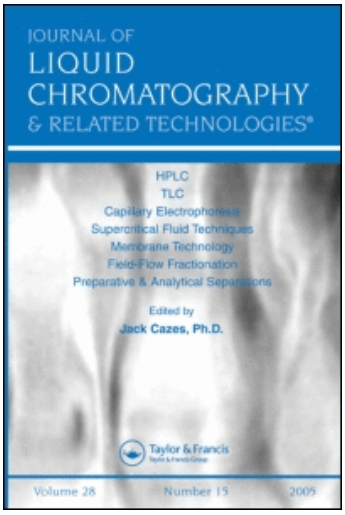

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Identification of Natural and Early Synthetic Textile Dyes with HPLC and UV/Vis-Spectroscopy by Diode Array Detection

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IDENTIFICATION OF NATURAL AND EARLY SYNTHETIC TEXTILE DYES WITH HPLC AND UV/VIS-SPECTROSCOPY BY DIODE ARRAY DETECTION

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ABSTRACT

The separation and identification of complex mixtures of natural and synthetic textile dyes was investigated using HPLC with diode array detection. Separation was carried out on a reversed phase column with acetonitrile-phosphoric acid gradient elution. The results show that the anthraquinones from madder root and the insect dye cocheneal present in ancient red dyes can easily be distinguished from azo-dyes present in later textile fibres. They further show that for an analysis of the numerous flavones and flavonoles, constituting most of the yellow natural dyestuffs, the combination of HPLC with on-line optical spectroscopy is particularly useful. Even when retention times are identical - as for instance for quercetin and luteolin - the ratio of a mixture can still be estimated by evaluating the ratio-chromatogram.

INTRODUCTION

The application of chemical analysis to archaeological objects has a long tradition. In contrast to the investigation of inorganic pigments organic materials like dyestuffs have only been analysed quite recently (1-3,5-7). The identification of dyes used for carpets and other textiles can assist in elucidating their place of origin and time of production. The ancient dyestuffs originate from extracts of plants and insects but during

the nineteenth century synthetic dyestuffs were introduced adding new problems to the difficult task of analysis.

In this report, the separation and identification of a number of natural and synthetic dyes frequently found in carpets and flat weaves is described. These dyes include anthraquinones, flavones, flavonoles, indigo, as well as some azo-dyes and indigodisulfonic acid. HPLC with diode array detection was used, the latter being of particular value for analysis of dyes.

METHOD

A Merck-Hitachi gradient pump L6000 was used with a Waters 990 diode array detector(optical resolution most often 5nm). Separation was carried out on Lichrosorb RP 8 (7μm) in a 250mm x 8mm column, the injection volume being 10 μl. Two different gradients were applied depending on the polarity of the dyes:

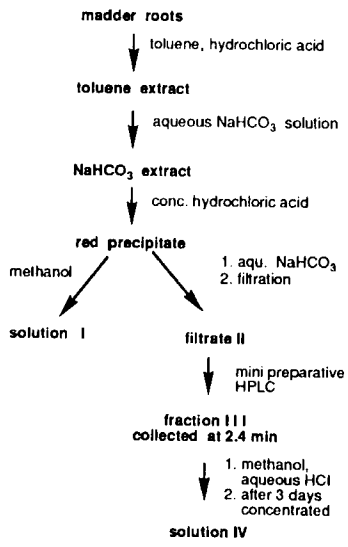
Gradient A for most synthetic dyes and Cochineal: From 10% to 30% increase of acetonitrile within 10 minutes, then constant in diluted phosphoric acid (pH 3.5).

Gradient B for the less polar natural and synthetic dyes : From 30% to 80% increase of acetonitrile within 10 minutes, then constant in diluted phosphoric acid (pH 2.8).

Concentrated sulfuric acid (after 15 min diluted with water and acetonitril), hydrochloric acid/methanol or ammonia were used in order to remove the dyestuffs from the fibre according to Schweppe (1).

MATERIALS

SCHEME 1
Extraction of madder roots



Madder roots were obtained from Anatolia, the commercially available substances from Aldrich, cochineal from Mann-Naturfarbstoffe (D 6719 Lautersheim).

Isolation of pseudopurpurin

The modified isolation procedure of Hill (4) is summarized in *Scheme I.* Madder roots were pulverized and agitated with a mixture of 10% hydrochloric acid and toluene. The organic phase was separated and extracted with an aqueous solution of sodium hydrogen carbonate, resulting in a deep purple-colored water extract. On addition of concentrated hydrochloric acid a red precipitate developed, still containing purpurin and alizarin. For a further purification the red residue was extracted again with a diluted solution of sodium hydrogen carbonate.

Decarboxylation of pseudopurpurin

To obtain a pseudopurpurin free of any purpurin it was purified by preparative HPLC (same conditions as for analytical work). For its decarboxylation the fraction was treated three hours in the dark with a methanolic solution of concentrated hydrochloric acid. After evaporation of the solvents the residue was redissolved in a little methanol (solution IV) and analysed.

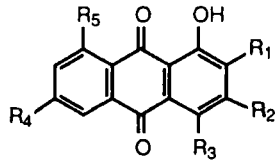
RESULTS AND DISCUSSION

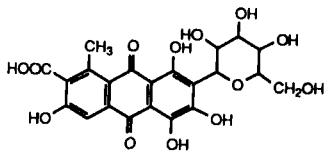
Anthraquinones

The red dyestuffs used for oriental carpets are extracts from madder roots and insects. They are all anthraquinones. The anthraquinones like the flavones and flavonoles dicussed later are fixed to woolen fibres by mordants. For analysis the dyes are hydrolysed and removed by acids (1). The retention times in HPLC and the optical absorption maxima are compiled in *Table 1*.

TABLE 1

Retention times and absorption maxima of some anthraquinones.



Compound	R_1	R_2	R_3	R4	R5	Ret.Time [min] (Gradient)	λ_{max} [nm] *= shoulder
Alizarin	OH	H	H	H	H	8.0 (B)	250, 280 ,330, 430
Purpurin	OH	H	OH	H	H	9.0 (B)	255, 292 *,480,515 *
Pseudopurpurin	OH	COOH	OH	H	H	2.5 (B)	255, 285 *,490,525 *
Xanthopurpurin?	H	OH	H	H	H	6.8 (B)	245, 280, 335, 415
Alizarin-2-methylether	OCH3	H	H	H	H	9.8 (B)	249,281,335,425
not identified						10.7(B)	257, 295, 424
not identified						5.8 (B)	260*, 275, 469*, 490
Emodin	H	OH	H	CH_3	OH	9.8 (B)	220,253,265,286, 438
Carminic acid						1.9 (A)	278,310,490,530*

From the madder extract (solution I in *Scheme 1*) six components were separated in the chromatogram *(Figure 1)*. Peak 4 and 5 were easily identified as alizarin and purpurin respectively by comparing with standards. They agree both in retention time and uv/vis-spectrum *(Figure 2a)*. Peak 1 is ascribed to pseudopurpurin, whose spectrum is 1 in *Figure 2a*. There are three more components, peaks 2, 3 and 6, which are red dyes (spectrum 2, 3 and 6 of *Figure2b*), but they are not yet identified. On the basis of its optical absorption spectrum 2 is tentatively ascribed to xanthopurpurin (8).

Pseudopurpurin is an important component in madder dyes, but is not available commercially. Some difficulties were encountered in the identification of pseudopurpurin, since the madder extraction method yielded mixtures containing several components exhibiting spectra in the expected wavelength region. After precipitation of pseudopurpurin and smaller amounts of some other compounds with concentrated hydrochloric acid, filtrate II (*Scheme 1*) was obtained by treating the red

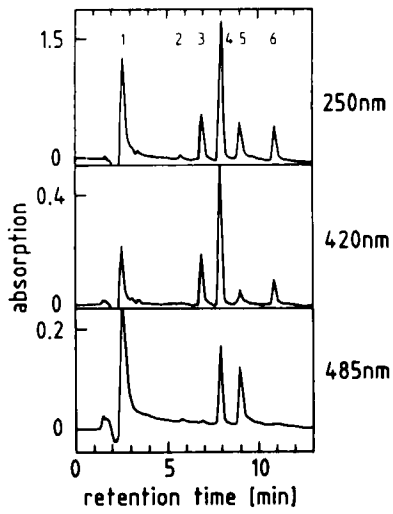


Figure 1 Chromatogram of madder extract (solution I)

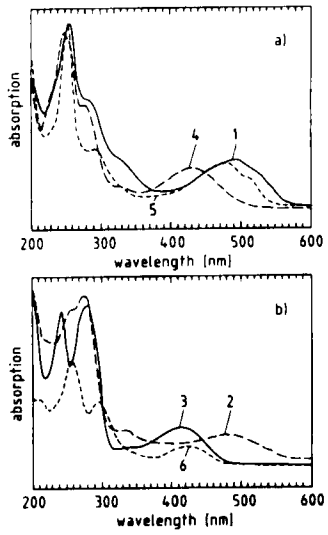
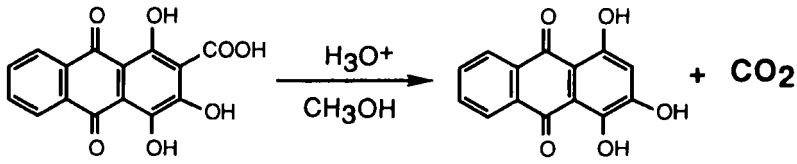


Figure 2 Spectra of the components of chromatogram Figure1

1 pseudo-purpurin; 3 xanthopurpurin?; 4 alizarin; 5 purpurin; 2 and 6 not yet identified



SCHEME 2. Decarboxylation of psudopurpurin to purpurin

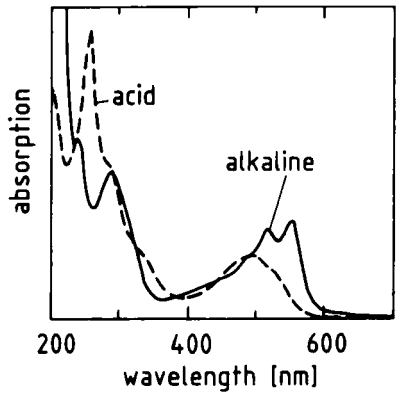


Figure 3 Spectra of pseudopurpurin in acid and alkaline solution.

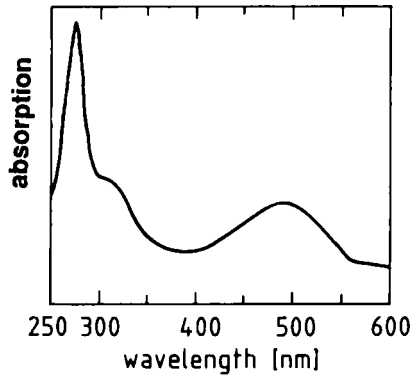


Figure 4 Spectrum of carminic acid.

precipitate with a solution of sodium hydrogen carbonate. The attribution of pseudopurpurin was based on spectra (spectrum 1 of *Figure 2a* and *Figure 3*) retention time and decarboxylation to purpurin *(Scheme 2)*. In order to obtain pseudopurpurin free of purpurin preparative HPLC of filtrate II was undertaken leading to fraction III, *Scheme 1*). The spectra of both pseudopurpurin in an acidified solution of acetonitrile and in an alkaline solution are shown in *Figure 3* . The spectra are in fact very similar to those of purpurin. Thus, pseudopurpurin can only be distinguished by its much shorter retention time. A shorter retention time is to be expected because of its higher polarity.

Pseudopurpurin can be decarboxylated by acids leading to purpurin, and indeed after treatment of fraction III (*Scheme 1*) with concentrated hydrochloric acid in methanol to give solution IV, the peak attributed to pseudopurpurin had disappeared, while the purpurin peak appeared.

Emodin - a yellow dye - is contained in rhamnus petiolaris and was used in Anatolia together with indigodisulfonic acid for green dyeing (5).

Carminic acid is the coloring red dye of cochineal, which is an insect dye. Its spectrum is shown in fig. 4.

Flavones and flavonoles

Most of the natural yellow dyes are mixtures of flavones and flavonoles which are contained in numerous plants (5,7). *Figure 5* shows a chromatogram of a mixture of five yellow dyes. The retention times listed in *Table 2* indicate that except for quercetin and luteolin the dyes are well separated.

From *Table 2* it is seen that some spectra are very similar to each other as for instance quercetin and rhamnetin but then the retention times are quite different. A mixture of quercetin and luteolin may cause difficulties in the identification with one-wavelength-detection, but the absorption maxima of both compounds $\lambda_{max(quercetin)}$ and $\lambda_{max(luteolin)}$ are sufficiently different (*Figure 6*), so that the ratio R of the absorptions at these two wavelengths $R = E_{\lambda max(quercetin)} / E_{\lambda max(luteolin)}$ enables a clear decision which of the dyes is present. The insert in *Figure 5* shows the computer calculated ratio-chromatograms for the pure compounds with R-values of 0.6 for pure luteolin and 1.5 for

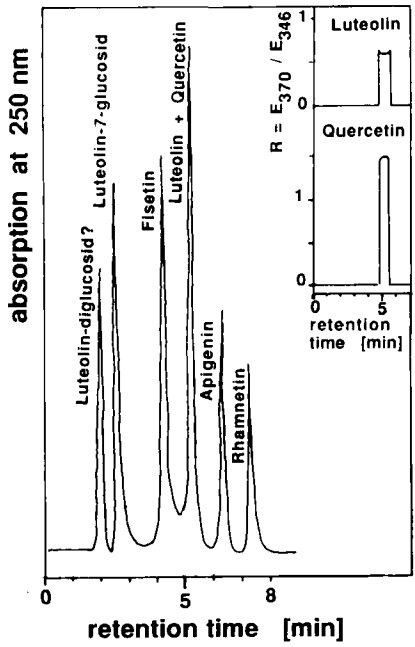


Figure 5 Chromatogram of a mixture of flavones and flavonoles. **Insert:** Ratiochromatogram of pure luteolin and pure quercetin solutions, respectively. (Ratio R = $E_{\lambda max}$ (quercetin) / $E_{\lambda max}$ (luteolin) = E_{370nm} / E_{346nm} as a function of time).

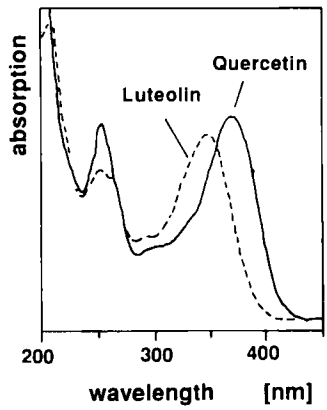
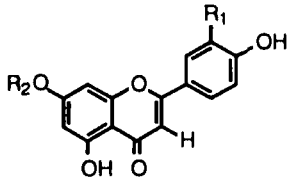


Figure 6 Spectrum of quercetin and luteolin.

TABLE 2

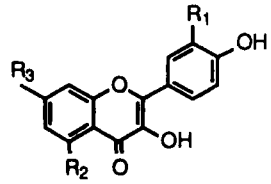
Retention times and absorption maxima of some flavones and flavonones.

Flavones



Dye	R_1	R_2	Retention Time [min] (gradient)	λ_{max} [nm]
Luteolin	OH	H	5.0 (B)	257, 348
Luteolin-7-glucosid	OH	Glucosyl	2.5 (B)	257, 348
Apigenin	H	H	6.2 (B)	268, 338

Flavonoles



Dye	R1	R2	R3	Ret.time [min] (gradient)	λ_{max} [nm]
Fisetin	OH	H	OH	4.1 (B)	248, 360
Quercetin	OH	OH	OH	5.0 (B)	257, 370
Rutin*	OH	OH	OH	1.7 (B),10.3(A)	257, 370
Rhamnetin	OCH3	OH	OH	7.0 (B)	257, 370

* = Quercetin-3-glucosid

quercetin. In the case of a mixture of both dyes the fraction may be estimated from the R-value somewhere between 0.6 and 1.5.

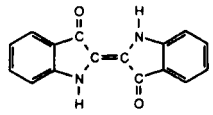
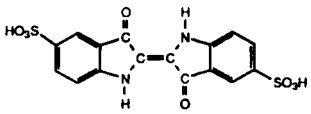
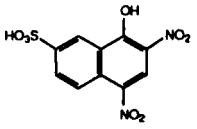
Indigo, indigodisulfonic acid and naphthol yellow

Indigo has been used for dying for several thousand years and synthetic indigo as well as indigodisulfonic acid and naphthol yellow, water soluble dyes, were introduced in the 19 century. Their retention times and spectral properties are listed in *Table 3*.

Azo-dyes

Azo-dyes were introduced for dying carpets towards the end of the last century. Of the large number of azo dyes commercially available only a few early dyes are considered here. Most of the dyes contain one or more groups of sulfonic acid. Due to their high polarity they elute fast when gradient B is applied. If a spectrum is not taken from these early peaks, they could easily be misinterpreted as belonging to the injected

TABLE 3
Retention times and absorption maxima of indigo, indigo-5,5`-disulfonic acid and naphthol yellow

Dye		Retention Time [min] (gradient)	λ_{max} [nm]
	Indigo	8.3 (B)	243, 285, 603
	Indigo-5,5`-disulfonic acid, (Natural blue 2)	1.1 (A)	253, 288, 611
	Naphthol-yellow (acid yellow 1)	1.5 (A)	390, 437

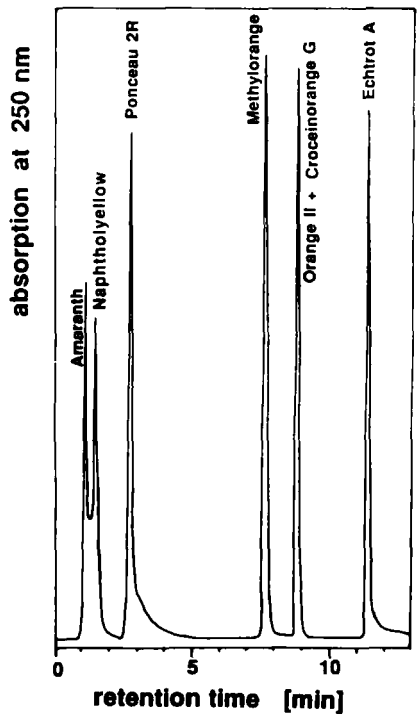
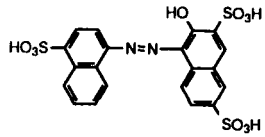
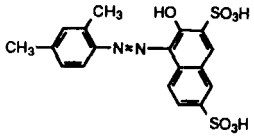
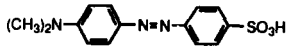
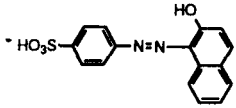
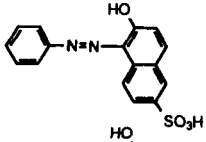
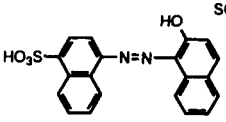
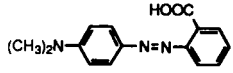


Figure 7 Chromatogram of a mixture of some synthetic dyes.

solvent. When the spectrum indicates the presence of a dye, a second run using gradient A is required. The resulting chromatogram of a mixture of seven early synthetic dyes is shown in *Figure 7*, the retention times and optical absorption maxima are gathered in *Table 4*.

The retention times depend very much on the pH of the phosphoric acid. This is shown in *Figure 8*. Since the retention times of all dyes decrease in a similar fashion with increasing pH (except for the amaranth, which elutes near the dead time), it is not the change in the acid - base - equilibria of the dyes but rather the change in the elution strength of the eluent which causes this drastic effect. Since some dyes tail at a low pH of, say, 2.8, a pH of 3.5 is preferred.

TABLE 4
Retention times and absorption maxima of some Azo-dyes

Dye		Retention Time [min] (gradient)	λ_{max} [nm] * = shoulder
	Amaranth (acid red 27)	1.1 (A)	215,240*, 522
	Ponceau 2R (acid red 26)	2.8 (A)	220, 330, 510, 530*
	Methylorange	7.7 (A)	270, 455
	Orange II (acid orange 7)	8.9 (A)	228,260*, 310, 485, 505*
	Crocein-orange G (acid orange12)	8.9 (A)	237,315, 405*, 485, 505*
	Echtrot A (Rocelline, acid red 88)	11.4 (A)	218, 290, 515
	Methylred	11.0 (B)	288, 500

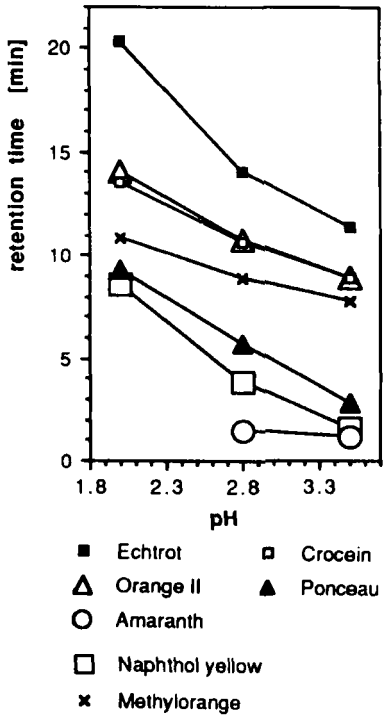


Figure 8 Retention times (gradientA) of some synthetic dyes as a function of the pH - value of the phosphoric acid in the eluent.

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