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High-performance liquid chromatography determination of direct and temporary dyes in natural hair colourings

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Abstract

A simple and reliable HPLC method is described for the simultaneous determination of nine direct and temporary hair dyes in hair colourings containing vegetal extracts. Detection was performed by a diode array detector and two different wavelengths, in the visible range (450 and 650 nm), were used for quantitation. The method does not involve any extraction procedure and it is sufficiently rapid and accurate for routine analyses. The method described was successfully applied to the identification of synthetic organic dyes in 13 direct and temporary hair dyeing formulations commercialized as 'natural'.

Keywords: Hair dyes; Dyes

1. Introduction

Hair dyeing represents an important aspect of cosmetology [1–3]. Its interest has been growing due to the care taken in self appearance by both women and men.

Hair dyeing materials can be classified into three groups, according to the type of dye and its persistence on the hair: temporary, semi-permanent and permanent colours.

The first group, commercialized as 'colour rinses', comprises those preparations that can be removed at the first shampooing. These products use dyes of high molecular mass which are not able to penetrate into the cortex and are deposited on the surface of the hair.

Semi-permanent hair dyes, which resist several shampooings and utilize so-called direct dyes of low molecular mass, show good affinity for hair keratin and are able to penetrate the cortex.

Finally permanent dyes, resistant to external factors, use uncoloured intermediates, which produce the colour 'in situ' by a coupling reaction followed by oxidation.

Dyestuffs used in temporary and semi-permanent hair dyes belong mainly to the following chemical classes: azo compounds, anthraquinones, triphenylmethanes, nitrophenylenediamines, nitroaminophenols and aminoanthraquinones.

The use of synthetic dyes in cosmetics is regulated by different legislations in different countries, stating also the maximum concentrations allowed. There-

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fore, simple analytical methods for routine determination of different dyes used in hair dyeing preparations is of the utmost importance.

Several methods [4–18] for analysing dyestuff materials by TLC [4–7], HPTLC [7], HPLC [8–17] and GLC [18] are available in the literature, but none of them has been applied to the analysis of temporary and semi-permanent dyes in commercial hair dyeing formulations. However, some of the dyes examined in the present study have been previously analysed but the methods used were different from the one described here [7,12,15].

The aim of this research is to set up a method for the identification of such dyes, even in the presence of natural dyes. The investigation, performed on commercial hair dyeing products, has detected the presence of direct synthetic dyes. Our method, therefore, can be used for analysing cosmetics defined as 'natural', that should only contain plant extract dyes.

The method described here, using HPLC, is particularly simple and of practical interest, allowing the determination of direct and temporary dyes present in preparations based on plant extracts. Moreover, the analysis of these compounds, using the method described, does not require a preliminary extraction step, as with other methods described in the literature [12,14,15].

2. Experimental

2.1. Chemicals

Water (Millipore Purification System, MilliQ) and acetonitrile (Lichrosolv, Merck Darmstadt, Germany) were HPLC grade; 1-heptanesulfonic acid sodium salt, 0.005 *M* solution, was prepared by diluting the HPLC-grade PIC B7 reagent (Millipore); ethanol (reagent grade) was obtained from Carlo Erba (Milan, Italy). The reference standards of dyes tested in this work are listed in Table 1, where we reported, when possible, the CI names and numbers, the IUPAC names, other names commonly used, the chemical class and the suppliers.

All standard substances were used as received.

2.2. Equipment

The HPLC apparatus was constituted by a solvent delivery system pump Varian Model 9010 (Mulgrave, Victoria, Australia) equipped with a Rheodyne sampling valve Model 710 (Cotati, CA, USA) and a UV–Vis diode array detector (DAD) Waters Model 994 (Milford, MA, USA). The analytical column was a 60 RP Select B® (Merck, 5 μm, 250×4.0 mm I.D.) with a same packing precolumn (4×4.0 mm I.D.). The gradient elution conditions are given in Table 2. Solvent A was a 0.005 *M* 1-heptanesulfonic acid sodium salt solution and solvent B was acetonitrile. Detection was performed by scanning from 200 to 700 nm with an acquisition speed of 1 s. Quantitation was performed at 450 nm for Disperse Violet 1, Basic Red 76 and Basic Brown 17 and at 630 nm for the other dyes.

2.3. Standard preparation

A stock solution of standards was prepared by accurately weighing about 100 mg of each reference substance into a 100 ml volumetric flask and diluting to volume with water–ethanol (50:50). This solution was stable at +4°C, in the dark, for 1 week. A calibration curve was prepared by injecting five diluted solutions, obtained from stock solution, in the concentration range from 10 to 100 mg/ml. These diluted solutions were freshly prepared.

2.4. Sample preparation

After stirring for about 5 min, 3 g of the hair dye was diluted to 100 ml in a volumetric flask with the same solvent as used for the standards.

2.5. Analytical method validation

Known amounts of the reference substances were added to a hair dye that did not present any chromatographic peak at the same retention times of the standards. This 'artificial' hair dye was used to evaluate the accuracy and precision of the method.

2.6. System performance

All system performance calculations were based

Table 1
Charachteristics of the reference standards

Dye	C.I. name	C.I no.	IUPAC name	Commercial names	Chemical class	Supplier
1	Basic Blue 26	44045	Methanaminium,*N*-[4-[[4-(dimethylamino)phenyl][4-(phenylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-*N*-methyl-,chloride	Victoria Blue B	Triarylmethane	Aldrich
2	Basic Blue 99	56059	Benzenaminium,3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphthalenyl)amino]-*N*,*N*,*N*-trimethyl-,chloride	Arianor Steel Blue 306004	Aminoketone	Williams
3	Basic Red 76	12245	2-Naphthalenaminium,8-[(2-methoxyphenyl)azo]-7-hydroxy-*N*,*N*,*N*-trimethyl-,chloride	Arianor Madder Red 306003	Monoazo	Williams
4	Basic Brown 17	12251	2-Naphthalenaminium,8-[(4-amino-2-nitrophenyl)azo]-7-hydroxy-*N*,*N*,*N*-trimethyl-,chloride	Arianor Sienna Brown 306001	Monoazo	Williams
5	Disperse Blue 1	64500	1,4,5,8-Tetramino-9,10-anthraquinone	SolventBlue 18	Anthraquinone	Aldrich
6	Disperse Blue 3	61505	1-Methylamino-4-β-hydroxyethylamino-9,10-anthraquinone (mixture of methyl derivatives)	Acetate Brilliant Blue 4 B	Anthraquinone	Aldrich
7	Disperse Violet 1	61100	1,4-Diaminoanthraquinone	Acetate Fast Red Violet 2 R	Anthraquinone	Aldrich
8			N^1,N^4,N^4-Tris-(β-hydroxyethyl)-1,4-diamino-2-nitrobenzene		Diamino nitrobenzene	Bayer
9			Complex of (chromium 2-amino-4-nitrophenol-7-acetylamino-β-naphtol and 2-amino-1-oxybenzene-4-sullfamido-β-naftol)/anthraquinone dye	Melange acid black 139/anthraquinone dye		Bayer[a]

[a]Commercial mixtures of several components.

on five replicate injections of the central point of the calibration curve. The system was deemed to be suitable when the relative standard deviation of the same peak area response did not exceed 5%, and the resolution between Disperse Blue 1 and Disperse Blue 3 peaks was not less than 1.5.

Table 2
Profile of the concentration gradient used for the analytical separation

Time (min)	% Pic B7	% CH_3CN
0	70	30
10	20	80
20	20	80
25	70	30
30	70	30

2.7. Identification of compounds and calculations

The identification was achieved by comparing the retention times and the spectral data (obtained by DAD) with those of the corresponding standard obtained under the same conditions. The comparison of the spectral data was performed by a suitable algorithm of the detector software. Purity of the chromatographic peaks was also assessed by the same software using the algorithm ‘peak purity’. Calculations were performed by the external standard method; the peak area value of each analyte was

Table 3
Chromatographic parameters of the nine dyes

Dye	Detection limit (μg/ml)	Chromatographic precision (R.S.D. %)	R_t (min)	K
Basic Blue 26	3	4.5	27.0	10.25
Basic Blue 99	5	5.6	9.4	2.92
Basic Red 76	1	1.0	13.7	4.71
Basic Brown 17	1	1.2	13.5	4.62
Disperse Blue 1	3	4.2	8.9	2.71
Disperse Blue 3	3 (as sum of the single components)	3.5	8.4	2.50
			11.9	3.96
			15.5	5.46
Disperse Violet 1	2	3.9	11.9	3.96
N^1,*N4*,N^4-Tris-(β-hydroxyethyl)-1,4-diamino-2-nitrobenzene	1	3.0	3.4	0.42
Melange acid black 139/anthraquinone dye	1 (as sum of the single components)	1.6	11.3	3.71
			12.5	4.21

substituted in the corresponding equation of the calibration curve and the concentration of the sample solution, expressed as mg/ml, was obtained.

3. Results

Under the conditions described the nine dyes showed the retention times and the capacity factors reported in Table 3. When the dye represents a commercial mixture of several components, R_t and K are reported for each of these. Table 3 shows also the chromatographic precision and detection limit for each dye. Chromatographic precision, expressed as relative standard deviation, was calculated by injecting six replicates of the central point of the calibration curve. The detection limit was calculated by the formula:

$$3\ \text{S.D.}/b$$

where S.D. is the standard deviation, calculated by injecting six replicates of the lowest concentration solution of the calibration curve, and b is the value of the calibration curve slope. This formula is one of the possible algorithms for calculating the detection limit defined as the analyte amount that gives a signal-to-noise ratio = 3, and it is adopted by several scientific and professional associations (ACGIH, NIOSH).

Linearity was tested for every standard generally in the range of 10–100 mg/ml and we typically obtained a correlation coefficient (R^2) between 0.995

Table 4
Characteristics of recovery and precision of the whole analytical method

Dye	Recovery (%)	Precision (R.S.D. %)
Basic Blue 26	96.8	4.9
Basic Blue 99	99.5	3.6
Basic Red 76	102.4	5.5
Basic Brown 17	100.9	7.3
Disperse Blue 1	100.5	4.2
Disperse Blue 3	99.2	5.4
Disperse Violet 1	98.5	6.0
N^1,N^4,N^4-Tris-(β-hydroxyethyl)-1,4-diamino-2-nitrobenzene	101.8	3.9
Melange acid black 139/antraquinone dye	97.7	6.5

Table 5
Amounts (wt.%) of synthetic dyes in hair colouring formulations commercialized as 'natural'

Dye	4538	4576	4581	4656
Disperse Blue 1	0.26	—	—	0.29
Disperse Blue 3	0.23	—	0.79	—
Disperse Violet 1	0.08	—	0.16	0.22
N^1,N^4,N^4-Tris-(β-hydroxyethyl)-1,4-diamino-2-nitrobenzene	1.63	0.12	0.30	0.29

and 1.000, with the exception of Disperse Violet 1 ($R^2 = 0.991$).

Recovery and precision of the whole analytical procedure were tested with an artificial hair dye. For the matrix, a commercial preparation was used which did not exhibit any peak at the retention times of the nine standards. This 'blank' hair dye was spiked with known amounts of the standards at about 1% concentration and was analyzed, including the sample preparation procedure, six times. Recovery was expressed, for each component, as the mean percentage ratio between the measured amounts and the actual ones; precision was expressed as relative standard deviation of the results obtained in the recovery test. Table 4 shows the obtained results. The proposed method was applied to 13 commercial hair dyes. Four of these were shown to contain at least one of the tested substances and the results are shown in Table 5.

4. Discussion

The chromatograms corresponding to the sample labelled 4538, containing four of the nine standards, are reported in Fig. 1a (450 nm) and Fig. 1b (630 nm). Identification of the peaks was accomplished by comparing the retention times with those of the standards and by comparing the UV–Vis spectra collected at the apex of the chromatographic peaks with those of the standard recorded under the same conditions.

The match quality was always higher than 95%, and peak purity ranged from 93.8 to 97.7% for each component at the same R_t of the standards. For the peak at 11.9 min in the chromatographic profile, shown in Fig. 1, there were some problems. The Disperse Violet 1 peak is not well separated from the peak of the second component of Disperse Blue 3 (see Table 3).

In this case match quality is very poor and peak purity lower than 90.0%. In fact the UV–Vis spectrum collected at 11.9 min (Fig. 2c) is the resultant of the overlay of those of the two corresponding standards (Fig. 2a, Fig. 2b). Even if the chromato-

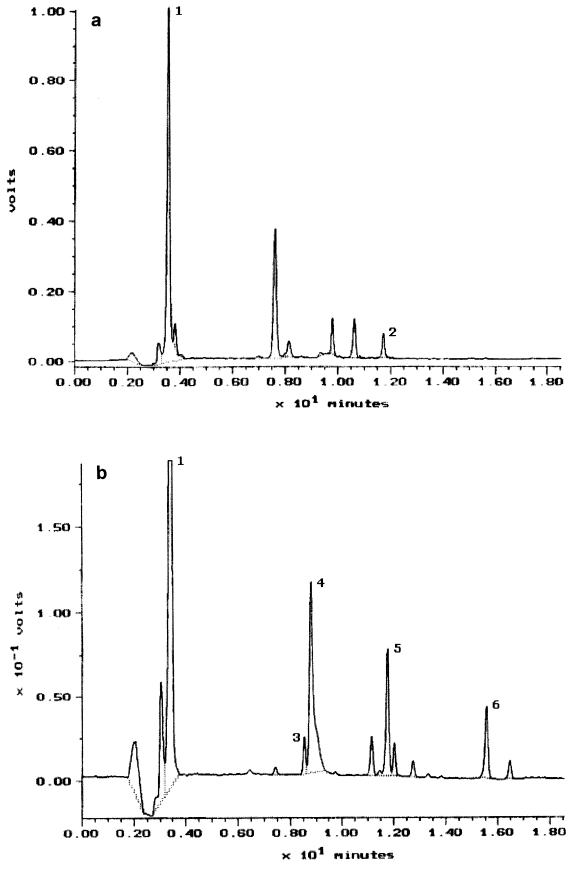

Fig. 1. Chromatographic profiles of hair dye sample 4538 at 450 (a) and 630 nm (b). Peak 1, N^1,N^4,N^4-tris-(β-hydroxyethyl)-1,4-diamino-2-nitrobenzene; peak 2, Disperse Violet 1; peaks 3, 5 and 6, Disperse Blue 3 (three components); peak 4, Disperse Blue 1.

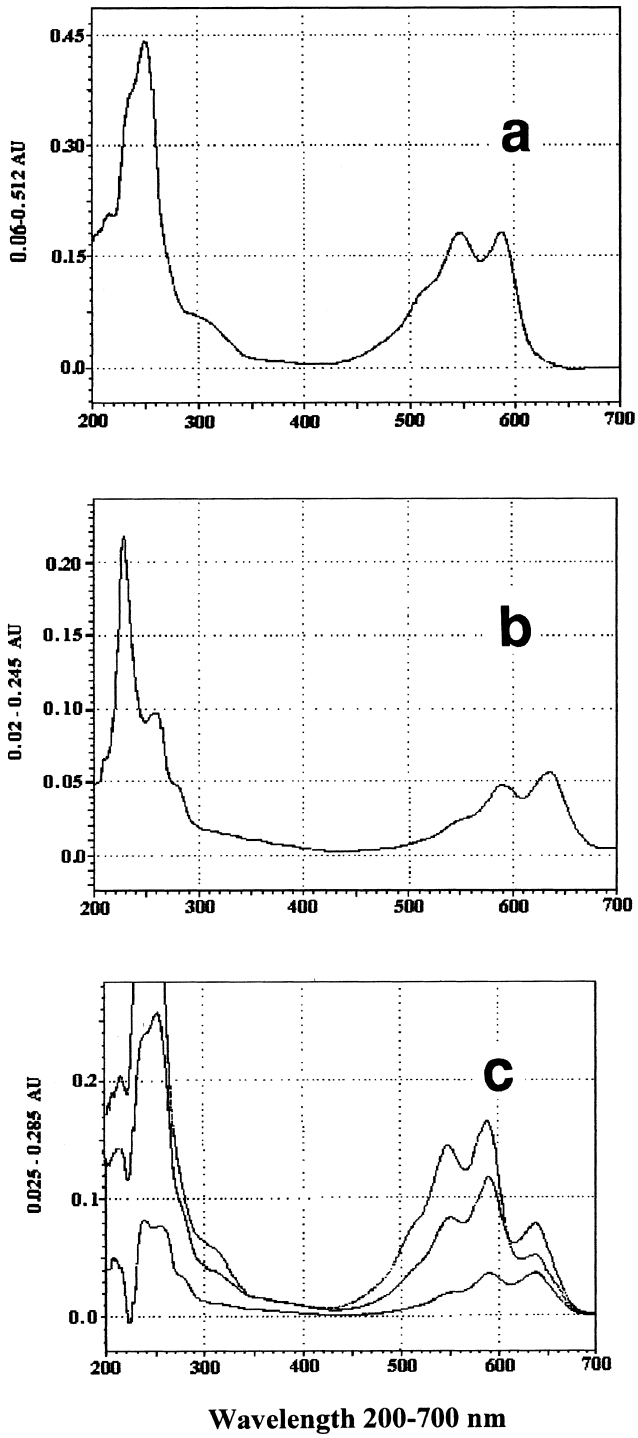

Fig. 2. UV–Vis spectra of standard Disperse Violet 1 (a) and standard Disperse Blue 3 (b). Spectral purity of the chromatographic peak at the retention time of Disperse Blue 3 and Disperse Violet 1 in hair dye sample 4538 (c).

graphic separation was not sufficient, the choice of the two wavelengths made the quantitation possible and correct. Disperse Blue 3 does not absorb at 450 nm, and Disperse Violet 1 was determined at this wavelength without interferences. The absorption of Disperse Violet 1 at 630 nm is about half that at 450 nm. This value is then subtracted at the area registered at 11.9 min, and the remaining area is assigned to Disperse Blue 3. Using this manual calculation we obtained recovery and precision for Disperse Violet 1 and Disperse Blue 3 sufficient for routine analyses as shown in Table 4.

Under the described chromatographic and preparation conditions we tested some crude vegetable extracts and their most important relative functional components: (1) Henna (*Lawsonia inermis*) extract and Lawsone; (2) Chamomile (*Matricaria Chamomilla*) extract and Apigenine; (3) Nut-hull (*Juglans regia*) extract and Juglone; (4) Natural Indigo.

In all these cases we found no peaks at the retention times of the nine tested substances, and thus no inteferences could arise from the presence of the declared natural components in the hair dyes.

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