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## HPLC of Oxidation Hair Colours

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### Introduction

Several comprehensive thin layer chromatographic (1–5) and some gas chromatographic (3, 4) methods have been described for the identification and determination of oxidation hair colours. Obviously the more recent high performance liquid chromatography (HPLC) offers an analytical tool in this field.

In this work a reversed-phase ion-pair HPLC system separates and determines in one run within an hour quantitatively fifteen important compounds in oxidation hair colours.

The analytical method needs a powerful antioxidant, i. e. ascorbic acid, to prevent oxidation losses during extraction and HPLC determination. Extraction is in fact a simple dilution step with aqueous acetone buffered at pH 9 and followed by filtration. HPLC should be run immediately on the same day. HPLC system was a C-18 reversed-phase column and a mobile phase of aqueous acetonitrile buffered at pH 4.2 and containing octanesulphonic acid as ion-pairing agent.

Sixteen compounds separated by this system are listed in table 1. For convenience, abbreviations mentioned in that table are used throughout this report. Two important compounds ( $\alpha$ -naphthol, hydroquinone) could not be determined by the proposed method.

The method was studied for its reliability. Successive HPLC injections of the same sample have shown variations between 0.13 to 1.2%. Furthermore recovery trials on prepared samples gave yields of 90.3–99.1%. Linearity of the standard curves within the working range show correlation coefficients better than 0.999 for  $n = 5$ . We consider that the proposed method is sufficiently reliable for quantitative purposes.

With the proposed method 39 commercial samples were analysed. Compounds identified by the HPLC determination were confirmed by thin layer chromatography. The satisfactory agreement has shown the reliability of the HPLC method.

Table 1. List of oxidation hair colour compounds of this study

Abbreviation	Chemical name
25TDA	2,5-toluenediamine*
34TDA	3,4-toluenediamine
24TDA	2,4-toluenediamine*
PPD	p-phenylenediamine*
OPD	o-phenylenediamine*
MPD	m-phenylenediamine*
25DAA	2,5-diaminoanisole*
24DAA	2,4-diaminoanisole* (= 4MMPD = 4-methoxy-m-phenylenediamine)
PAP	p-aminophenol*
OAP	o-aminophenol*
MAP	m-aminophenol*
2NPPD	2-nitro-p-phenylenediamine*
4NOPD	4-nitro-o-phenylenediamine*
2A4NP	2-amino-4-nitrophenol*
2A5NP	2-amino-5-nitrophenol*
RES	resorcinol*
AN	$\alpha$ -naphthol* **
HQH	hydroquinone* **

\* Compound listed on the CTFA (Cosmetics Toiletries & Fragrances Association of the USA) comprehensive safety testing programme of 39 oxidation hair colours (7).

\*\* Compounds not determined by the proposed method.

## Experimental

### *General description of the method*

To the sample (hair colour shampoo or lotion) ascorbic acid is added to prevent oxidation losses during extraction and HPLC, and then further diluted with aqueous acetone which is buffered at pH 9. The solution is filtered and as soon as possible (on the same day) analysed by HPLC. Retention data are compared with HPLC data of standard reference mixtures, prepared and also analysed within one day. Identification is made by retention volumes, which are recorded as retention times. It is important to notice that small shifts of retention volumes may be due to the particular matrix in the different samples. In case of doubt a second injection is made with the extract spiked with the compound in question.

In our work an extra checking by TLC was used as a confirmatory check which is not necessary. The quantitative composition in the sample is finally calculated by comparing peak area ratios of samples with those of reference compounds or of standard mixtures.

### *Reagents and apparatus*

#### Reference chemicals

25TDA sulfate (Merck)	PAP (Fluka)
34TDA (Fluka)	2NPPD (Fluka)
25DAA-sulfate (Fluka)	4NOPD (Fluka)
24DAA (Fluka)	2A4NP (Fluka)
OPD (Fluka)	2A5NP (Fluka)
MPD (Fluka)	RES (Merck)
PPD (Fluka)	AN (Merck)
OAP (Fluka)	HQH (Merck)
MAP (Fluka)	

Ascorbic acid (Merck)

Sörensen Buffer solution pH 9:

15 ml 0.1 N HCl + 85 ml 0.05 M sodium borate

Octanesulphonic acid (Merck), ion-pairing agent, sodium salt

Acetonitrile (Chromar; Mallinkrodt)

Acetic acid (Merck)

HPLC equipment: Waters Associates (Pump M6000A and detector model 450 with variable wavelength)

Volumetric flasks 25, 100, 200 ml

Aluminium foil

### *HPLC Conditions*

Column: Zorbax ODS (Dupont; a reversed-phase column with octadecylsilane coating) 25 cm x 4.6 mm

Mobile phase: Mix 900 ml water + 100 ml acetonitrile + 6.8 g sodium acetate (3H<sub>2</sub>O)+  
0.432 g octanesulphonic acid, sodium salt. Adjust pH accurately to 4.2 by the aid of a pH-meter after addition of approx. 10 ml acetic acid. Filter through membrane filter of 0.5 µm.

Flow: 1 ml per minute

Detector: UV 280 nm (Absorbance unit full scale 0.1).

Inject 5 µl of extracts or standard solutions.

The recorded retention volumes of the reference compounds under these HPLC conditions are in table 2.

Table 2. HPLC retention volumes recorded from standard reference

HQH	Eluted too fast to allow determination
Ascorbic acid	4.75 ml
PPD	7.08 ml
PAP	7.36 ml
RES	7.95 ml
MAP	8.88 ml
MPD	9.28 ml
25TDA	10.14 ml
25DAA	10.69 ml
OAP	12.81 ml
2NPPD	15.49 ml
OPD	16.01 ml
24DAA	17.82 ml
24TDA	19.10 ml
4NOPD	20.04 ml
2A4NP	21.64 ml
2A5NP	23.38 ml
34TDA	43.53 ml
AN	Not eluted within a reasonable time. Can be eluted with the mobile phase: Water-methanol-Sörensen buffer pH (125 ml + 325 ml + 50 ml), retention volume 7.17 ml.

### Standard solutions

Standard reference solutions of single compounds can also be prepared. The following two standard mixtures, however, are used in most experiments:

Mixture I: Weigh accurately ca. 50 mg of each of the following compounds: 25 DAA-sulfate, OPD, 2A4NP, 2A5NP. Add ca. 100 mg ascorbic acid. Dissolve in 50 ml Sörensen buffer pH 9. Add acetone to a final volume of 100 ml. Wrap container in aluminium foil to protect from light. This solution is stable in its quantitative composition for two days.

An example of a HPLC chromatogram of this mixture is in figure 1.

Mixture II: Use the same procedure for the mixture: OAP, MAP, PAP, 25TDA sulfate, 24DAA, 2NPPD, 4NOPD and RES. See for an example figure 2.

### Extraction of samples

Mix an accurately weighed sample (ca. 3 g) with ca. 100 mg ascorbic acid and 3 ml of a Sörensen buffer solution of pH 9 by means of a glass rod. Transfer the mixture into a 25 ml volumetric flask with the aid of 9 ml of the same buffer solution.



Fig. 1. HPLC standard mixture I  
 1 = Ascorbic acid      4 = 2A4NF  
 2 = 25DAA            5 = 2A5NF  
 3 = OPD

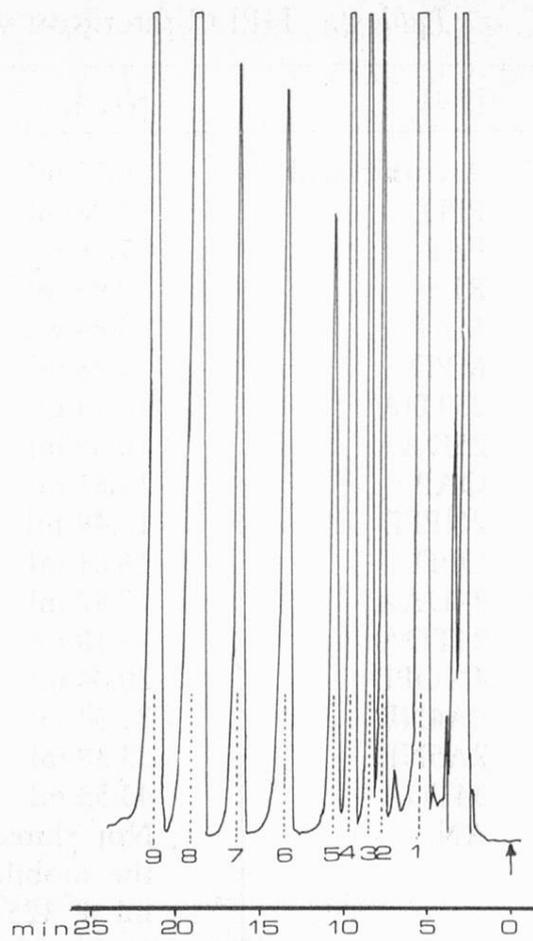


Fig. 2. HPLC standard mixture II  
 1 = Ascorbic acid      6 = OAP  
 2 = PAP                7 = 2NPPD  
 3 = RES                8 = 24DAA  
 4 = MAP               9 = 4NOPD  
 5 = 25TDA

Add acetone to a volume of 25.0 ml and mix. Filter through filter paper. Wrap container in alu foil to protect from light. This solution, which is ready for the HPLC determination remains stable for two days, but is recommended to perform the HPLC analysis on the same day.

Examples of HPLC chromatograms of commercial samples are given in figures 3–6.

### *Thin layer chromatography*

The TLC check is primarily performed to confirm the HPLC results, and to analyse the two compounds  $\alpha$ -naphthol and hydroquinone which are not detected in the HPLC system. Procedures from previous work (1–5) are used and are not further described in this paper.

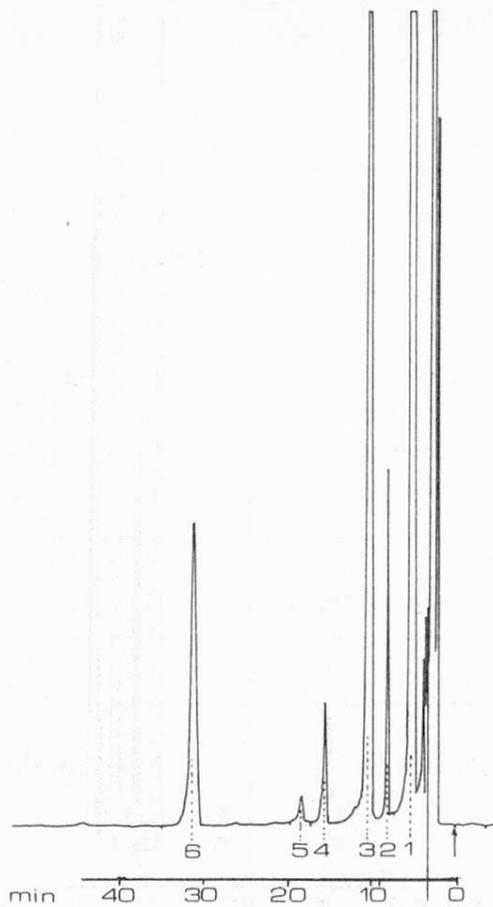


Fig. 3. HPLC sample 15 (dark brown)  
 1 = Ascorbic acid    4 = 2NPPD = 0.03%  
 2 = RES = 0.11%    5 = 24DAA = 0.02%  
 3 = 25TDA = 2.03%    6 = Unknown

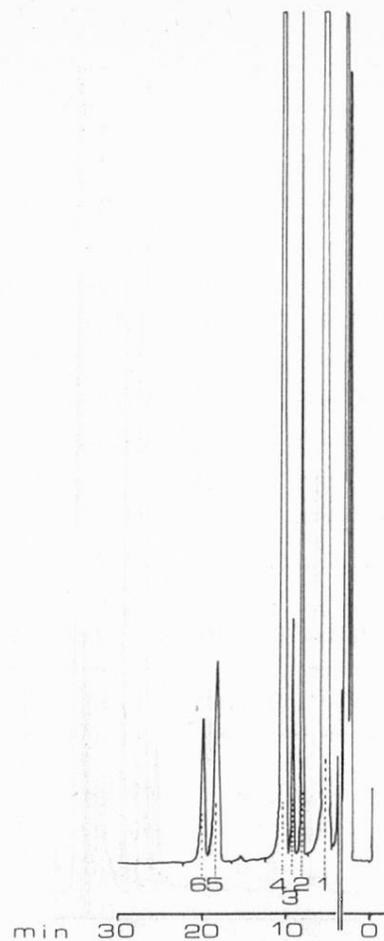


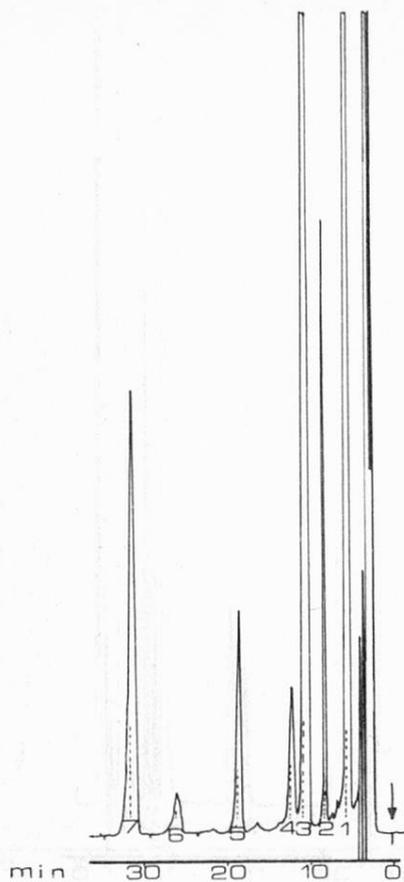
Fig. 4. HPLC sample 16 (black)  
 1 = Ascorbic acid    4 = 25TDA = 2.29%  
 2 = RES = 0.34%    5 = 24DAA = 0.24%  
 3 = MPD = 0.10%    6 = 4NOPD = 0.06%

### *Model samples for recovery trials*

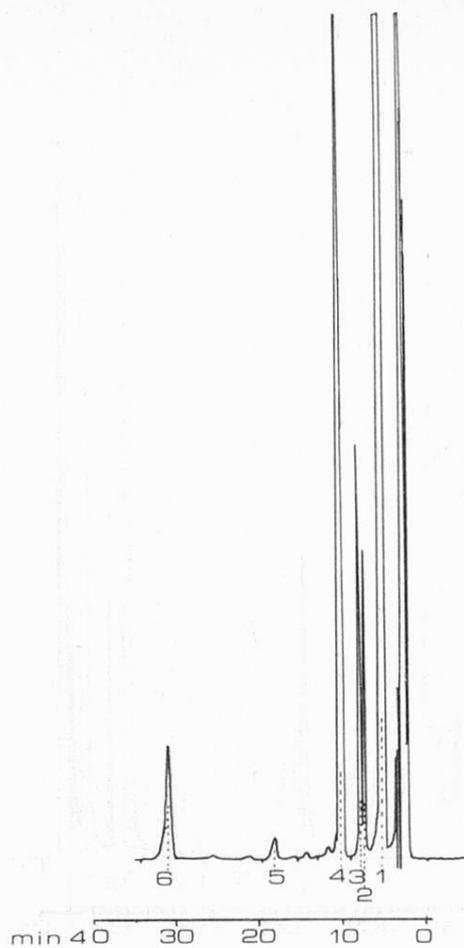
A colour base is prepared according to *Kass* (6).

Mix: 18 g oleic acid  
 14 g isopropanol  
 4 g methylcellosolve  
 2 g propylene glycol  
 3 g sod. lauryl sulfate  
 0.2 g EDTA

Add sufficient conc. ammonia (25%) to obtain a pH 9.5. Add water to make a volume of 100 ml. Measure 100 ml of the above colour base and add ca. 500 mg ascorbic acid and accurately weighed amounts of the reference compounds under consideration (amounts between 50 and 1500 mg of each compound). Dilute with water to a final volume of 200 ml. Mix until dissolved and wrap in alu foil to protect from light. Analyse sample within two days.



*Fig. 5.* HPLC sample 20 (black)  
 1 = Ascorbic acid    5 = 24DAA = 0.20 %  
 2 = RES = 0.2%    6 = Unknown  
 3 = 25TDA = 2.93%    7 = Unknown  
 4 = Unknown



*Fig. 6.* HPLC sample 21 (middle brown)  
 1 = Ascorbic acid    4 = 25TDA = 1.34%  
 2 = PAP = 0.16%    5 = 24DAA = 0.02%  
 3 = RES = 0.17%    6 = Unknown

## Results and discussion

We first checked the reliability of the proposed method. Repeatability was checked on successive injections of a mixture and on analyzing several standard mixtures. In this way variation coefficients can be calculated for the different compounds (table 3). We may conclude that less than 1% variations were observed.

As the mixtures contained different concentrations of constituents, the linearity of the results within certain working concentrations can be calculated and expressed as a correlation coefficient. Linearity within the working range is remarkably good (see table 3). Recovery trials were made on three model hair colour shampoos. We found 90 to 95% recoveries for the different compounds, including the most important ingredient 25TDA and RES (table 4).

We consider that the HPLC method is reliable, simple in operation and elegant in performance; it should therefore be recommended for further development into

Table 3. Reliability of the HPLC method

Compound	Variation coefficient of $n$ successive injections of the same solution	Linearity of obtained data in the working concentrations (mg/100 ml) $c$ expressed as correlation coefficient $r$
25TDA-sulfate	$n = 4$ 0.94%	$c = 20-103$ $r = 0.9985$
25DAA-sulfate	$n = 4$ 1.19%	$c = 8-40$ $r = 0.9997$
PPD	$n = 4$ 0.60%	$c = 10-50$ $r = 0.9997$
OAP	$n = 5$ 0.86%	$c = 12-62$ $r = 0.9981$
MAP	$n = 4$ 0.64%	$c = 15-77$ $r = 0.9996$
PAP	$n = 6$ 0.65%	$c = 12-60$ $r = 0.9991$
2NPPD	$n = 6$ 0.56%	$c = 8-42$ $r = 0.9995$
RES	$n = 4$ 0.13%	$c = 9-45$ $r = 0.9998$

Table 4. Recovery trials with three model hair colours shampoos

	Added	Recovery (separate trials)
Shampoo I	25TDA-sulfate 125.2 mg	97.4% 97.0%
	2NPPD 65.7 mg	96.9% 99.1%
	OAP 106.1 mg	96.4% 97.6%
	MAP 94.9 mg	90.3% 90.3%
	PAP 108.1 mg	95.6% 95.1%
	RES 86.6 mg	90.1% 89.3%
	Shampoo II	25TDA-sulfate 445.2 mg
2NPPD 254.7 mg		98.0% 98.3%
OAP 282.5 mg		96.3% 98.9%
MAP 294.7 mg		93.8% 94.0%
PAP 284.6 mg		92.8% 92.6%
RES 290.0 mg		91.5% 91.9%
Shampoo III		25TDA-sulfate 1328.8 mg

official methods for quantitative determination of regulated compounds in oxidation hair colours. The method was tried on 39 commercial samples from different manufacturers\* purchased in January 1980. The darker shades of black, brown and red have been selected for this survey. The results are summarized in table 5. As mentioned earlier, HPLC results were confirmed by TLC analysis. Additional information on the presence of  $\alpha$ -naphthol and hydroquinone was also obtained from the TLC work and included in table 5. TLC also revealed the presence of direct colours in several samples (table 5, under «direct colours»). It is not

\* Indola, Nowa, l'Oreal, Roux, Schwarzkopf, Therachemie, Vendra, Wella.

Table 5. Composition of commercial hair colours purchased in January 1980  
Sample\* No.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
25TDA	5.03	1.10	0.15	0.10	2.77	1.25	1.06	2.55	1.34	0.49	2.60	0.96	0.18	—	2.03	2.29	0.04	0.35	0.23	2.93	25TDA
34TDA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	34TDA
24TDA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	24TDA
OPD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	OPD
MPD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.10	—	—	—	—	MPD
PPD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	PPD
25DAA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	25DAA
24DAA	0.47	—	—	—	0.07	1.42	—	—	—	—	—	—	—	—	0.02	0.24	—	0.01	—	0.20	24DAA
OAP	0.30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	OAP
MAP	0.61	0.11	—	—	0.17	—	—	0.09	—	—	—	0.03	0.02	—	—	—	—	—	—	—	MAP
PAP	—	0.37	—	0.33	0.27	—	—	0.05	—	—	—	—	—	—	—	—	—	—	0.11	—	PAP
2NPPD	—	0.48	—	0.30	—	—	—	—	—	—	—	0.02	—	0.43	0.03	—	0.26	—	0.69	—	2NPPD
4NOPD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.06	—	—	—	—	4NOPD
2A4NP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.13	—	2A4NP
2A5NP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03	—	2A5NP
RES	0.29	0.29	—	—	0.41	0.12	—	0.44	—	—	—	0.43	0.06	—	0.11	0.34	0.02	0.13	0.05	0.22	RES
HQH***	—	0.05	—	—	0.10	—	0.30	0.20	—	—	—	—	—	—	—	—	—	—	—	—	HQH***
AN***	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03	0.03	—	—	AN***
Un- known**	—	—	1	—	—	—	1	2	—	—	1	—	—	1	1	—	—	1	1	3	Un- known**
Direct col- lours***	—	—	3	2	1	1	4	—	—	—	—	—	—	—	1	1	—	—	—	—	Direct col- lours***

\* Expressed in % (m/m)

\*\* Number of unidentified HPLC peaks

\*\*\* Found by TCL; HQH and AN by visual estimation of spots; amount of spots for the direct colours

## Sample\* No.

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39		
25TDA	1.34	0.14	—	—	0.04	0.80	0.26	1.03	1.23	—	—	1.46	1.35	1.44	1.65	3.11	2.62	0.10	0.02		25TDA
34TDA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		34TDA
24TDA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		24TDA
OPD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		OPD
MPD	—	—	—	—	—	—	—	0.03	0.21	—	—	—	—	—	—	—	—	—	—		MPD
PPD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		PPD
25DAA	—	—	—	—	2.15	—	—	—	—	—	—	—	—	—	—	—	—	—	—		25DAA
24DAA	0.02	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—	—	—		24DAA
OAP	—	—	—	—	—	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—		OAP
MAP	—	0.25	—	—	0.05	0.02	—	—	—	—	—	0.06	0.12	0.09	—	0.48	0.31	—	—		MAP
PAP	0.16	—	—	—	—	—	—	0.15	—	0.45	1.15	0.02	—	—	—	—	—	0.33	0.66		PAP
2NPPD	—	0.07	0.33	0.37	0.01	—	0.55	—	—	—	—	—	0.40	—	0.51	—	—	0.30	0.51		2NPPD
4NOPD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		4NOPD
2A4NP	—	—	—	0.78	0.06	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.19	2A4NP
2A5NP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2A5NP
RES	0.17	0.18	—	—	0.04	0.17	0.05	0.13	0.34	—	—	0.66	0.18	0.41	—	0.75	0.76	—	0.03		RES
HQH***	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	HQH***
AN***	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03		AN***
Un- known**	1	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	1	—	—		Un- known**
Direct col- ours***	—	—	—	3	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—		Direct col- ours***

\* Expressed in % (m/m)

\*\* Number of unidentified HPLC peaks

\*\*\* Found by TLC; HQH and AN by visual estimation of spots; amount of spots for the direct colours

surprising that HPLC of the extracts has detected unknown compounds which absorb light at 280 nm. In 11 samples the following unidentified peaks were recorded: 6, 12, 19, 22, 25, 30 (6 samples\*), 36, 46, 76 ml retention volume. Examples of HPLC-chromatograms are the figures 3–6. The results in table 5 are very informative for the frequency of use and the practical levels of concentration of the 18 important functional compounds in to-day's permanent hair colours. In short we can conclude: Phenylenediamines: no PPD (oldest known oxidation hair colour) and no OPD were found in the samples. The meta isomer (MPD) was found in 3 samples.

Toluenediamines: 25 TDA is unquestionably the most important ingredient. The highest level was for the back colour no. 1: 5.03%. The 24TDA, a toxicologically suspected ingredient (7), was absent in all samples. Diaminoanisoles: In one sample (no. 25) the 25DAA was the principle ingredient. No other samples contain this ingredient. The 24DAA, a toxicologically suspected compound (7) (which is sometimes coded as 4MMPD = 4-methoxy-m-phenylenediamine), seems to be an important ingredient which was found in 9 samples. In sample no. 1 the highest level of 0.47% was found. The HPLC method is particularly suitable for samples containing 25TDA and 24DAA. Even small ratios (1%) of 24DAA to 25TDA could be analysed by the proposed method. Figure 3 (of sample 15) clearly shows this ability.

Aminophenols: This is an important class of compounds, which were found in more than half of the samples. Of the 3 isomers, singles or a combination of two (ortho + meta; meta + para; but no ortho + para) were detected in samples.

Nitro compounds: By far the most important compound is 2NPPD which is found in every mahogany-red tint. The highest level was 0.69% (no. 19). The other nitro compounds are far less important (4NOPD, 2A4NP).

Phenols: Resorcinol (at levels of less than 1%) was found in 3 of the 4 samples. HQH is less important and seems to be used only by a few manufacturers. AN was found in 3 samples.

## Postscript

### *GC/MS identification of unknown HPLC peak*

The unknown compound appearing at retention volume of 30 ml, which occurred in 6 samples, was isolated by HPLC from sample 15 (see figure 3, peak no. 6).

The obtained HPLC fraction was extracted with ethylacetate filtered over anhydrous sodium sulfate and finally evaporated to dryness under reduced pressure.

\*See under Postscript.

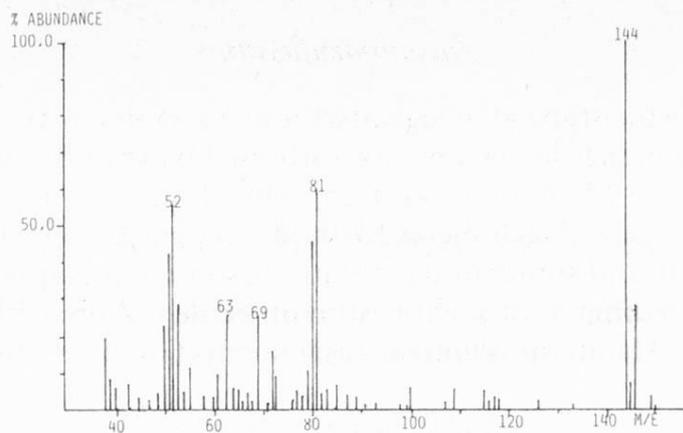


Fig. 7. Mass spectrum of isolated compound (4-chlororesorcinol) from HPLC fraction corresponding to unknown peak 6 (figure 3) of sample 15 (retention volume 30 ml)

The residue was dissolved in 0.3 ethylacetate and examined by GC/MS (Finnigan 1020, computerized), which made a library search on the obtained MS spectrum (see figure 7). A possible compound was 4-chlororesorcinol.

A reference solution of this compound was examined by GC/MS and identical retention time and spectrum were obtained.

The HPLC retention volume of the reference solution was also similar.

Conclusion could be made that the unknown substance was 4-chlororesorcinol.

Finally the 6 samples with this compound were determined by the proposed HPLC on this substance.

The results were:

Sample 3	absent	Sample 19	0.05%
Sample 15	0.20%	Sample 20	0.20%
Sample 18	0.07%	Sample 21	0.08%

### Acknowledgements

We wish to express our thanks to Miss *J. M. B. Geverinck*, who did the careful TLC checking of all the samples; to *A. M. de Roos*, director of the Food Inspection Service of Enschede for his encouragement of this study.

### Summary

A reversed-phase ion-pair HPLC method to identify and to determine the principal compounds in oxidation hair colour shampoos and lotions is described. The method is simple, fast and sufficiently reliable. Octanesulphonic acid is added as ion-pair agent. Sixteen of the most important compounds can be identified and determined simultaneously in one run.  $\alpha$ -naphthol and hydroquinone, however, are not determined by this HPLC system. A market survey of 39 hair colour shampoos was made with the proposed method and the results discussed.

## Zusammenfassung

Es wird eine Hochleistungs-Flüssigkeitschromatographiemethode (Ionenpaar, Umkehrphase) zur Bestimmung der Hauptwirkstoffe in Oxidationshaarfärbeschampoos und Lotions beschrieben. Die Methode ist einfach, schnell und zuverlässig. Oktansulfonsäure dient als Ionenpaar-Reagenz. Nach dieser Methode werden 16 der wichtigsten Oxidationsfärbemittel identifiziert und simultan quantitativ bestimmt.  $\alpha$ -Naphthol und Hydrochinon können unter diesen Bedingungen nicht bestimmt werden. Zum Schluß werden die Analysergebnisse von 39 Handelsprodukten zusammengefaßt und diskutiert.

## Résumé

Une méthode de chromatographie liquide haute performance (HPLC), utilisant la technique des « paires d'ions » en phase inversée, est décrite pour la détermination des arylamines et phénols oxydables dans les teintures capillaires et shampoings colorants. Elle est simple, rapide et de fiabilité satisfaisante. L'acide octanesulfonique est utilisé comme réactif pour la formation des « paires d'ions ». Seize composants parmi les plus importants peuvent être identifiés et dosés simultanément. Le naphthol-1 et l'hydroquinone ne peuvent pas être déterminés dans les mêmes conditions. Les résultats de l'examen de 39 shampoings colorants du marché sont présentés et discutés.

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