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Determination of aromatic amines at trace levels by ion interaction reagent reversed-phase high-performance liquid chromatography

Analysis of hair dyes and other water-soluble dyes

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method, making use of interaction reagents, was developed for the separation and determination of aromatic amines. Different interaction reagents were used and compared. The use of octylammonium salicylate or octylammonium orthophosphate as the interaction reagent and a reversed-phase spherical 5-μm RP-18 as the stationary phase, with spectrophotometric detection at different wavelengths, gave good results. The separation of up to seven aromatic amines (the three isomers of phenylenediamine, benzylamine, 2-phenylethylamine, 3-phenylpropylamine and aniline) was obtained with sensitivity levels ranging between 50 and 100 ppb. The method was applied to the analysis of water-soluble dyes such as fabric dyes, brown and blond hair dyes, black shoe dye and black fountain-pen ink. It was possible to detect and determine 1,4-phenylenediamine in commercial hair dyes. The results indicate that very large amounts of this amine are present in these products, namely 7320 ppm for brown hair and 598 ppm for blond hair.

INTRODUCTION

The need to develop reliable and sensitive methods for the identification and determination of aromatic amines at trace levels continues to increase in fields such as the environment, food and cosmetics.

Only a few methods have been proposed for the determination of aromatic amines, generally using high-performance liquid chromatography (HPLC), through derivatization precolumn reactions [1–5]. Also flow injection voltammetric methods were employed, with diazotization [6] or bromination [7] reactions. Unfortunately, these methods involve tedious and time-consuming procedures for the preparation of the sample. No example has been found in the literature of the use of the interaction reagent reversed-phase HPLC technique for the identification and separation of aromatic amines. This technique, already employed in this laboratory for the separation of anionic species and amines [8,9], is generally characterized by good resolution and sensitivity. Further, it requires no derivatization, clean-up or pretreatment of the sample.

In this paper the optimum conditions for the determination of amounts of aromatic amines are discussed.

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EXPERIMENTAL

Apparatus

Analyses were carried out with a Merck–Hitachi Lichrograph Model L-6200 chromatograph, equipped with a Merck–Hitachi Model D-2500 Chromato-Integrator and an L-4200 UB–VIS detector.

For pH measurements, a Metrohm 654 pH meter equipped with a combined glass–calomel electrode was employed. A Hitachi 150-20 spectrophotometer was employed for absorptivity evaluation.

Chemicals and reagents

Ultra-pure water from a Milli-Q system (Millipore) was used for the preparation of solutions.

Octylamine of analytical-reagent grade was obtained from Fluka and salicylic acid and all other reagents of analytical-reagent grade from Carlo Erba.

Chromatographic conditions

A Merck Hibar LiChrosorb RP-18 (5 μm) column (250 × 4.0 mm I.D.) was used. Although a Merck LiChrosorb RP-18 (10 μm) column (250 × 4.0 mm I.D.) was also tested it was not employed because it gave too long retention times.

The solutions to be used as eluents, namely octylammonium salicylate and octylammonium orthophosphate, were prepared (as elsewhere described [8–10]) by dissolving a weighed amount of octylamine in ultra-pure water and adjusting the pH of the solutions to 6.4 ± 0.4 by addition of salicylic or orthophosphoric acid. At this pH and taking into account the acid formation constant, octylamine is present in the protonated octylammonium form and the composition of the eluents so prepared is not completely stoichiometric. For the sake of simplicity, however, the eluents used as the mobile phase are referred to henceforth as octylamonium salicylate and octylammonium orthophosphate.

In order to condition the chromatographic system properly, the eluent was allowed to flow through the column until a stable baseline signal was obtained (a minimum of 1 h was necessary). The eluent solutions were freshly prepared every third day.

The reproducibility of measurements was very good for sequential measurements under the same conditions of eluent preparation and column conditioning but slightly lower for different eluent preparations. For the sake of general validity, the average data and reproducibilities listed in Table I were calculated for different preparations.

Between uses, the column was regenerated by passing water–methanol (1:1, v/v) through it. This treatment maintained the column lifetime comparable to that in other chromatographic techniques.

Preparation of samples

The samples of the water-soluble dyes tested for the presence of aromatic amines (namely fabric dye, brown and blond hair dyes, black shoe dye and black fountain-pen ink) were prepared simply by dissolving the samples in ultra-pure water, filtering through a 0.45-μm filter and diluting when necessary with ultra-pure water.

RESULTS AND DISCUSSION

Previous work [8–11] indicated the versatility of ion interaction reagent reversed-phase HPLC. The mechanisms that govern retention were discussed and it was pointed out that the mechanism of interaction through which amines are retained involves the formation of ammonium salts (or ions pairs) between the amine injected and the anion of the flowing interaction reagent. The investigated amines are then released and eluted in the same form in which they were retained, a result which can be demonstrated when octylammonium salicylate is used as the mobile phase. Under these conditions, aliphatic amines can be easily detected at 254 nm, even if their absorptivities are almost zero at this wavelength. The observed absorbance is due to the accompanying salicylate anions.

In order to establish the optimum conditions for the trace determination of aromatic amines, a preliminary comparative study was carried out by employing different stationary phase packings and different interaction reagents.

The choice and the comparative use of octylammonium salicylate and octylammonium orthophosphate as the interaction reagents, together with a spherical 5-μm RP-18 as the stationary phase, proved to be of particular interest.

Spectrophotometric detection at different wavelengths was employed; Table I lists the absorptivity values obtained for the investigated amines at 210, 230 and 254 nm.

Good results were obtained in the separation of aromatic amines with the above eluents. Fig. 1 shows the chromatogram obtained by using octylammonium salicylate as the interaction reagent in the separation of a mixture of six aromatic amines, namely 1,4-phenylenediamine (0.50 ppm), 1,3-phenylenediamine (1.50 ppm), 2-phenylethylamine (10.00 ppm), 1,2-phenylenediamine (2.00 ppm), 3-phenylpropylamine (10.00 ppm) and aniline (5.00 ppm) with UV detection at 254 nm. It is worth remembering that at this wavelength both aromatic amines and salicylate anions are characterized by non-zero absorptivities (see Table I).

Fig. 2 shows the chromatogram obtained by using octylammonium orthophosphate as the mobile phase in the separation of a mixture of benzylamine, 2-phenylethylamine, 3-phenylpropylamine and aniline at concentrations of 1.00 ppm each and employing spectrophotometric detection at 210 nm.

TABLE I

ADSORPTIVITY VALUES, ε (l mol^{-1} cm^{-1}), FOR THE INVESTIGATED AMINES AND FOR SALICYLATE EVALUATED AT DIFFERENT WAVELENGTHS

Compound	λ = 210 nm	λ = 230 nm	λ = 254 nm
Aniline	$(4.1 \pm 0.1) \cdot 10^3$		$(2.9 \pm 0.1) \cdot 10^2$
Benzylamine	$(8.4 \pm 0.3) \cdot 10^3$	$(1.1 \pm 0.1) \cdot 10^2$	$(1.7 \pm 0.1) \cdot 10^1$
1,2-Phenylenediamine	$(3.59 \pm 0.09) \cdot 10^4$	$(8.4 \pm 0.1) \cdot 10^3$	$(2.0 \pm 0.1) \cdot 10^3$
1,3-Phenylenediamine	$(3.47 \pm 0.08) \cdot 10^4$	$(1.03 \pm 0.06) \cdot 10^4$	$(2.0 \pm 0.1) \cdot 10^3$
1,4-Phenylenediamine	$(6.5 \pm 0.1) \cdot 10^3$	$(7.0 \pm 0.1) \cdot 10^3$	$(4.4 \pm 0.2) \cdot 10^3$
2-Phenylethylamine	$(5.5 \pm 0.9) \cdot 10^3$		$(2.3 \pm 0.1) \cdot 10^2$
3-Phenylpropylamine	$(7.7 \pm 0.1) \cdot 10^3$		$(2.0 \pm 0.1) \cdot 10^2$
Salicylate			$(3.1 \pm 0.1) \cdot 10^2$

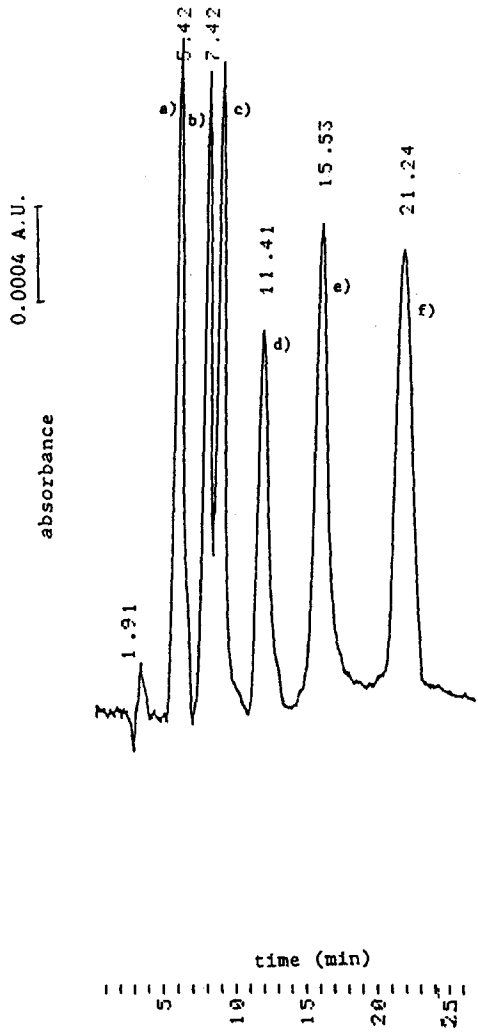

Fig. 1. Separation of a mixture of (a) 1,4-phenylenediamine, (b) 1,3-phenylenediamine, (c) 2-phenylethylamine, (d) 1,2-phenylenediamine, (e) 3-phenylpropylamine and (f) aniline. Stationary phase, Merck Hibar LiChrosorb RP-18, 5 μm; ion interaction reagent, 0.0050 *M* octylammonium salicylate; flow-rate, 0.7 ml/min; injection, 100 μl; detection, UV (254 nm).

Because orthophosphate anions are characterized by zero absorptivity at this wavelength, it follows that the observed absorbance is due entirely to the aromatic amines themselves. Therefore, the detection sensitivity varies as a function of the detection wavelength, which can therefore be chosen as a function of the analyte to be evaluated.

A typical example is shown by the comparison of the three chromatograms in Fig. 3, all concerning the separation of the three isomeric forms of phenylenediamine in a mixture containing 1.00 ppm of each. The three chromatograms were recorded

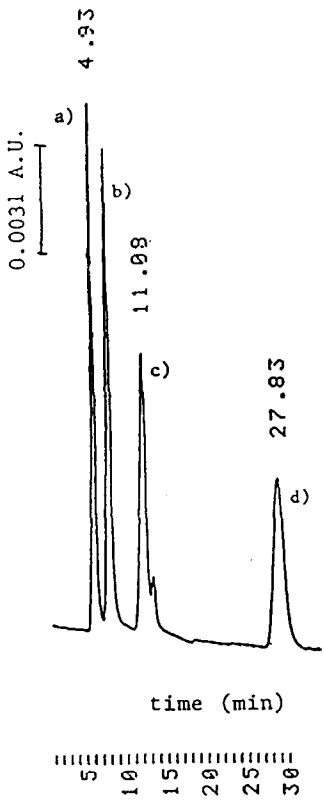

Fig. 2. Separation of a mixture of (a) benzylamine, (b) 2-phenylethylamine, (c) 3-phenylpropylamine and (d) aniline, 1.00 ppm each. Stationary phase, Merck Hibar LiChrosorb RP-18, 5 μm; ion interaction reagent, 0.0050 *M* octylammonium orthophosphate; flow-rate, 0.7 ml/min; injection, 100 μl; detection, UV (210 nm).

under different conditions. In Fig. 3a, octylammonium salicylate was used as the interaction reagent with UV detection at 254 nm. The three amines show comparable detection sensitivities, peak a having a larger area than the others. The observed absorbance is due to the additive contributions of both the salicylate anion and the aromatic ammonium ion. As the contribution from the salicylate anion remains the same, the highest peak area in Fig. 3a is due to 1,4-phenylenediamine, in agreement with the highest absorptivity shown at 254 nm by this analyte (see Table I).

Fig. 3b and c refer to the use octylammonium orthophosphate as the eluent at wavelengths of 210 and 230 nm, respectively. Provided that at these wavelengths no contribution to the absorbance derives from orthophosphate, it can be observed that the sensitivity responses follow the expected absorptivity order (see Table I), namely 1,2- = 1,3- > 1,4-phenylenediamine at 210 nm and 1,3- > 1,2- > 1,4-phenylenediamine at 230 nm.

As regards sensitivity, levels of the order of 50 ppb can be achieved. Fig. 4 shows as an example the chromatogram obtained for the injection of 91 pmol of

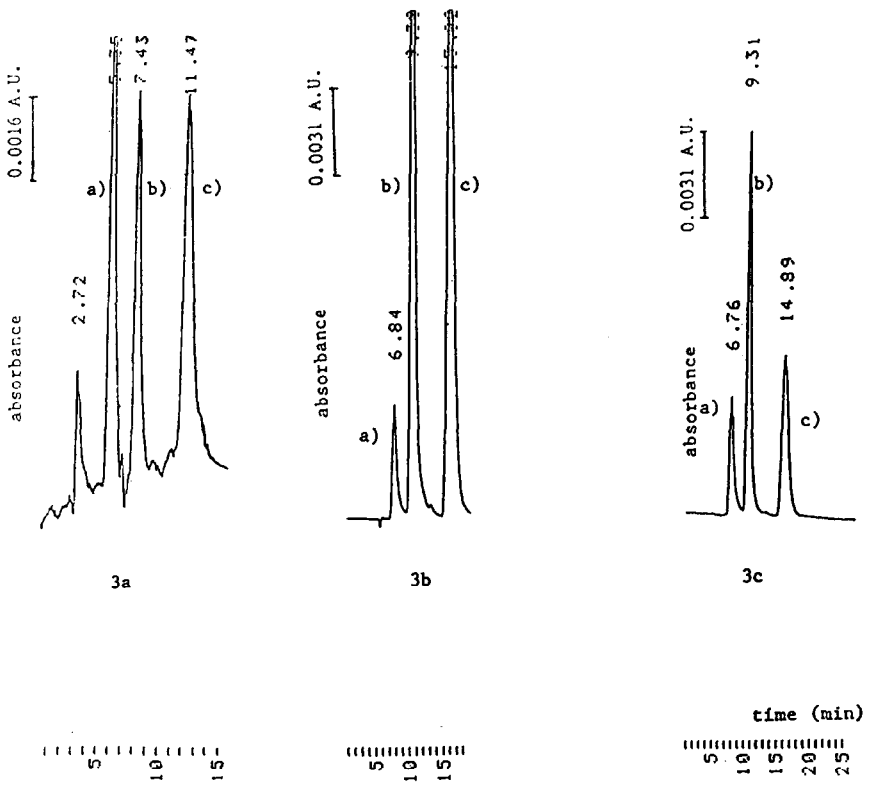

Fig. 3. Separation of a mixture of peak (a) 1,4-phenylenediamine, (b) 1,3-phenylenediamine and (c) 1,2-phenylenediamine, 1.00 ppm each. Stationary phase, Merck Hibar LiChrosorb RP-18, 5 μm; injection, 100 μl. Part (a), ion interaction reagent, 0.0050 *M* octylammonium salicylate; flow-rate, 0.7 ml/min; detection, UV (254 nm). Part (b), ion interaction reagent, 0.0050 *M* octylammonium orthophosphate; flow-rate, 0.7 ml/min; detection, UV (210 nm). Part (c), ion interaction reagent, 0.0050 *M* octylammonium orthophosphate; flow-rate, 0.7 ml/min; detection, UV (230 nm).

1,3-phenylenediamine using octylammonium salicylate as the eluent with UV detection at 210 nm.

The methods described were applied to the analysis of some commercial water-soluble dyes: brown and blond hair dyes, black shoe dye, green fabric dye and black fountain-pen ink.

Whereas the presence of the investigated aromatic amines at concentrations greater than 0.5 ppm can be excluded in shoe and fabric dyes and in ink, significant amounts of 1,4-phenylenediamine were found in the hair dyes. Fig. 5 shows the result of the analysis of a commercial brown hair dye for a sample diluted 1:100 (v/v) and then filtered through 0.45-μm filters. The results obtained for the same sample both with octylammonium salicylate as eluent at 254 nm (Fig. 5a) and with octylammonium orthophosphate at 210 nm (Fig. 5b) are shown. Both clearly indicate the presence of 1,4-phenylenediamine.

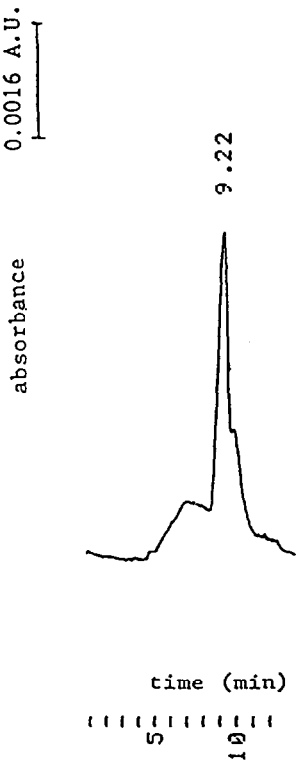

Fig. 4. Injection of 100 μl of a 0.10 mg l^{-1} solution of 1,3-phenylenediamine. Stationary phase, Merck Hibar LiChrosorb RP-18, 5 μm; ion interaction reagent, 0.0050 *M* octylammonium orthophosphate; flow-rate, 0.7 ml/min; detection, UV (210 nm).

Owing to the good linearity of the plot of peak area *versus* standard concentration, it was possible to carry out a quantitative evaluation by employing the standard additions method. Amounts of 7320 ± 8 ppm of 1,4-phenylenediamine were determined for a brown hair dye and about 598 ± 7 ppm for blond hair dye.

1,4-Phenylenediamine is widely used as an ingredient in oxidative hair-dyeing formulations. It is not included in the list of carcinogenic amines, but recent papers have reported its toxic effects [4] and percutaneous absorption during hair-dyeing procedures employing a commercial product [12].

In conclusion, the proposed method permits the reliable, sensitive and rapid determination of aromatic amines in real samples and does not require any sample derivatization or work-up procedures.

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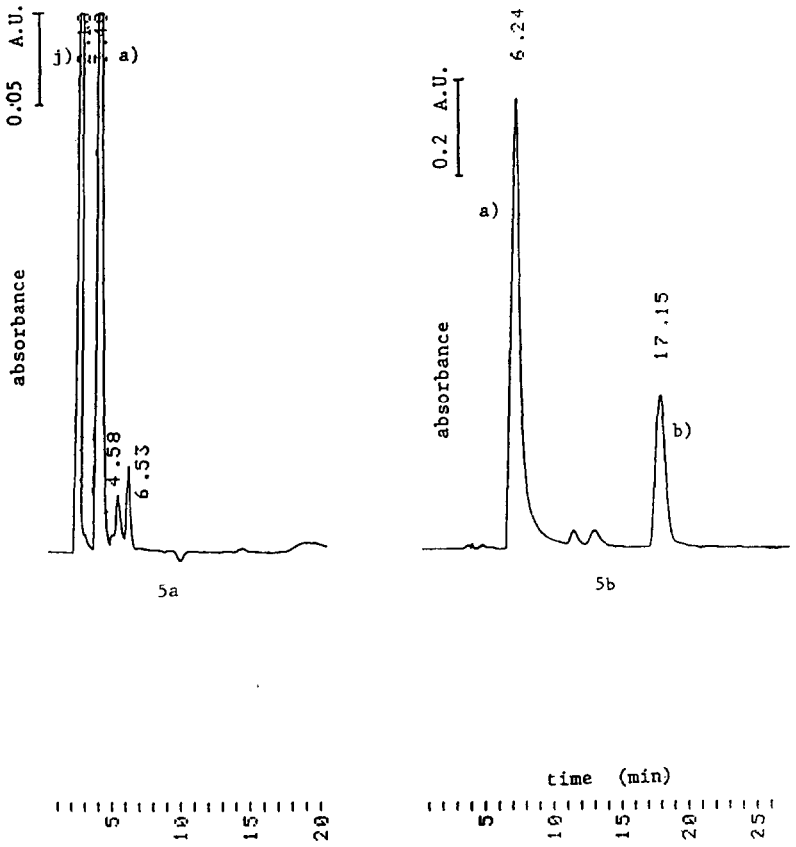

Fig. 5. Analysis of brown hair dye. Stationary phase, Merck Hibar LiChrosorb RP-18, 5 μm; injection, 100 μl. Part (a), ion interaction reagent, 0.0050 *M* octylammonium salicylate; flow-rate, 1.00 ml/min; detection, UV (254 nm). Part (b), ion interaction reagent, 0.0050 *M* octylammonium orthophosphate; flow-rate, 0.7 ml/min; detection, UV (210 nm). Peaks: (j) injection peak; (a) 1,4-phenylenediamine; (b) unidentified.

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