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Separation and determination of nitrobenzenes by micellar electrokinetic chromatography and high-performance liquid chromatography

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Abstract

A micellar electrokinetic chromatographic method and a high-performance liquid chromatographic method are proposed for the separation and determination of a mixture of 12 nitrobenzenes and their reduction products, namely 4-nitro-1,2-phenylenediamine, 4-nitro-1,3-phenylenediamine, 2-nitro-1,4-phenylenediamine, 2-nitroaniline, 3-nitroaniline, 4-nitroaniline, 4-amino-2-nitrophenol, 2-amino-5-nitrophenol, 2-amino-4-nitrophenol, 2-nitrophenol, 3-nitrophenol, and 4-nitrophenol. A solution of 50 m*M* sodium dodecyl sulfate and 10% ethanol in 23 m*M* sodium borate buffer was used as the electrophoretic medium. Good resolution could be obtained by the addition of tetrahydrofuran to the liquid chromatographic mobile phase. The retention and migration behavior of the nitrobenzenes are discussed.

Keywords: Mobile phase composition; Buffer composition; Nitrobenzenes; Amines; Nitrophenols

1. Introduction

The nitrobenzenes, which are carcinogic pollutants, are widely used in the dye industry. Commercial oxidative permanent hair dyes commonly contain several kinds of dye intermediates in the range of 0.01 to several percent. There are several types of dye intermediates: aminophenols, polyphenols, nitrobenzenes and their salts [1]. Some of these compounds may present a risk, even at low concentrations [2]. All of the 12 analytes studied here are positional isomers possessing a nitro moiety as well as an amino and/or a hydroxyl moiety. However, they may both be present after synthesis. The use of most nitrobenzenes in skin-care products is limited in several countries due to their highly toxic nature. Various methods have been reported for the determination of nitrobenzenes by gas chromatography (GC) [3,4], high-performance liquid chromatography (HPLC) [5–7] and capillary electrophoresis (CE) [8–11]. With all of the HPLC methods, either an ion-pairing reagent or a column modifier is added to optimize selectivity. In this work, the addition of tetrahydrofuran (THF) was used to enhance selectivity. The 12 nitrobenzenes of this study are listed in Table 1. The isomers of nitroaniline exhibit identical migration behavior and are difficult to separate by micellar electrokinetic chromatography (MEKC), even after adding sodium dodecyl sulfate (SDS). They can be satisfactorily resolved by adding cetyl trimethylammonium chloride (CTAC).

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Table 1
Abbreviations and pK_a values of the studied nitrobenzenes

No.	Abbreviation	Compound name	pK_a
1	oDAN	4-Nitro-1,2-phenylenediamine	2.33[a], 2.45[a]
2	mDAN	4-Nitro-1,3-phenylenediamine	2.33[a], 0.37[a]
3	pDAN	2-Nitro-1,4-phenylendiamine	1.27[a], 4.09[a]
4	oHNA	4-Amino-2-nitrophenol	7.44[a], 3.54[a]
5	mHNA	2-Amino-5-nitrophenol	2.59[a], 8.12[a]
6	pHNA	2-Amino-4-nitrophenol	2.71[a], 8.03[a]
7	oNA	2-Nitroaniline	−0.37[a]
8	mNA	3-Nitroaniline	2.47
9	pNA	4-Nitroaniline	1.0
10	oHN	2-Nitrophenol	7.22[a]
11	mHN	3-Nitrophenol	8.28[a]
12	pHN	4-Nitrophenol	7.15[a]

[a] The numerical value was calculated using the Hammett equation according to Ref. [11].

2. Experimental

2.1. Materials and reagents

The 12 nitrobenzenes listed in Table 1 were purchased from TCI (Tokyo, Japan). All other chemicals were from Merck (Darmstadt, Germany) and were of analytical-reagent grade. Deionized water was purified using a purification system (Barnstead, NANO pure II). Commercial hair dyes were purchased from retail stores.

2.2. High-performance liquid chromatography

The HPLC apparatus consisted of a Perkin-Elmer 1020 LC plus integrator linked to a Model 235C diode-array detector, a 250B delivery pump and a Rheodyne Model 7161 injector with a 20 μL sample loop. The diode-array detector was set at a wavelength of 250 nm. Separations were performed on a Hypersil BDS C_{18} column (5 μm, 250×4.6 mm I.D.) and elution was carried out with methanol–6% THF and 10 m*M* ammonium dihydrogenphosphate at pH 6 (32:68) at a flow-rate of 1.1 mL/min for the first 3 min. The percentage was increased linearly to 65% in the following 4 min and maintained for a further 5 min. The identity of the separated peaks was assigned by co-chromatography with authentic standards. Quantification was carried out by integration of the peak area.

2.3. Micellar electrokinetic chromatography

The CE apparatus comprised a Spectraphoresis 100 CE system (Thermo Separation Products, Fremont, CA, USA) coupled to a UV detector set at 230 nm. The temperature was controlled with an uncertainty of ±0.5 °C. When the capillary was first used, it was conditioned with 1 *M* NaOH for 4.0 min, and deionized water for 6.0 min. Further equilibration was performed with the running buffer for 5 min before each injection. The migration time was reproducible, with the relative standard deviation varying by less than 2.0% (n=5). The optimum conditions were: a buffer consisting of 50 m*M* SDS and 10% ethanol in 23 m*M* borate, and an electric field strength of 230 V/cm applied on a fused-silica capillary, 75 μm I.D.×375 μm O.D., with a length of 75.0 cm (50.0 cm to detector).

2.4. Sample pretreatment

A 0.05 g (to nearest 0.1 mg) portion of a commercial sample was added to 8–9 mL of blank solution [consisting of 0.15% (w/v) HCl, 0.2% (w/v) sodium sulfite, and 30% (v/v) ethanol] in a 10.00 mL volumetric flask, placed in an ultrasonic apparatus for 5 min for dissolution, and then diluted to the mark with blank solution. The solution was filtered through a 0.45 μm nylon syringe filter before injection into the HPLC and CE system.

3. Results and discussion

3.1. HPLC conditions

A tertiary amine molecule may have three types of potential interaction, hydrophobic, ion-exchange, and hydrogen bonding, with the unendcapped silanols of the reversed phase [12]. The ion-exchange ability will be enhanced when the amino moiety is protonized at low pH. Hydrophobic interactions and hydrogen bonding will still be present even at high pH. The nitrophenols 4-nitrophenol and 2-amino-4-nitrophenol will be absorbed on the column by adding either an ion-pairing reagent or a column modifier. Therefore, we only investigated 10 nitrobenzenes in this HPLC study. Some of the pK_a values listed in Table 1 were calculated using the Hammett equation [13]. The pK_a value of the aminonitrobenzenes ranged from -0.37 to 2.47, and that of the hydroxynitrobenzenes ranged from 7.15 to 8.28. We performed all separations at pH 6, maintained by ammonium dihydrogenphosphate, in order to keep all 10 nitrobenzenes in the nonionic state and maintain peak symmetry. To optimize the efficiency, we tested methanol contents from 20 to 45%, and 32% showed acceptable resolution. Nevertheless, some of the analytes, oHNA/mHNA, oNA/oHN, and oDNA/mDNA, still co-eluted. THF is one of the most widely used dipolar aprotic solvents in LC and CE. It can be used in a diversity of fields because of its physical properties, particular its polarity ($\pi^* = 0.58$), and its relatively weak solvating power for many polar and ionic solutes. THF is therefore a good differentiating solvent, reflected by its small autoprotolysis constant [14]. However, many of the potential benefits to be gained from using THF are not fully realized and, in the majority of cases, the selection of this solvent is largely based on trial and error. This occurs because, often, there is little information available concerning the exact nature of the solute species present and their interaction in this medium. Furthermore, it has no hydrogen-bond donor properties and is a weak hydrogen-bond acceptor. We tested various THF contents from 0 to 6% in 2% increments. The minimum amount added was 4% to separate the isomers oHNA/mHNA, oNA/oHN, and oDAN/mDAN. The chromatogram obtained with 6% THF showed the better resolution, therefore this concentration was chosen. Finally, we used gradient elution to increase the solvent strength and to reduce the run time. The chromatogram obtained is shown in Fig. 1. It was obtained using 32% methanol, pH 6, for the first 3 min, which was increased to 65% in 4 min and maintained for a further 5 min. The chromatogram shows satisfactory reproducibility of the peak area with a relative standard deviation of $<3\%$.

3.2. MEKC conditions

As for HPLC, nearly all of the molecules, in spite of their hydroxyl and amino substituents, are in a neutral state at pH 3.5–6.5. Consequently, the pH of the buffer is an important factor for optimization of the separation. As the pH increased from 6.5, the results clearly indicate an enhanced selectivity of hydroxyl nitrobenzenes on the basis of their different

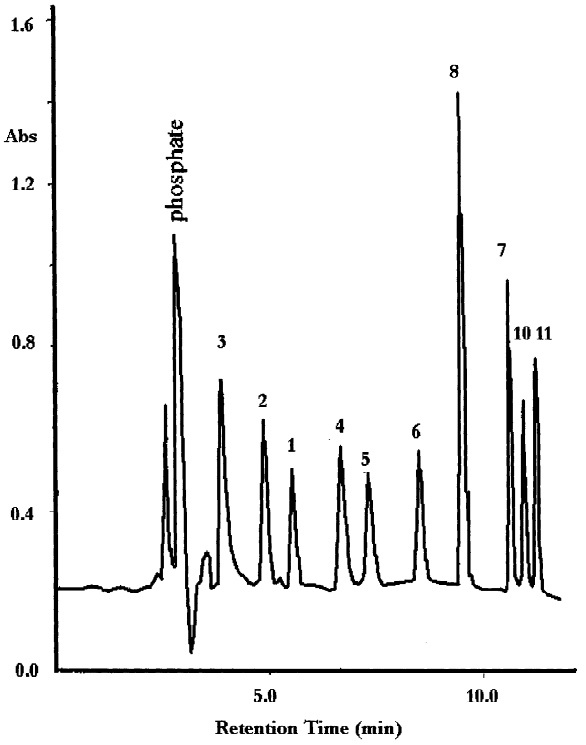

Fig. 1. Chromatogram for the nitrobenzenes. The mobile phase was as described in Section 2.2. The peak numbers are as described in Table 1.

mass/charge ratios. Therefore, a pH of 9.2 was adopted. It was almost impossible to separate the 12 nitrobenzenes by CZE due to their similar (even identical) pK_a values and mass/charge ratios [15,16]. Therefore, we selected MEKC as the separation technique and SDS as the surfactant. We considered the use of a cationic surfactant, such as cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC), or tetradecyltrimethylammonium bromide (TTAB), but the difference in the electrophoretic flow of the analytes was not sufficient to distinguish and identify them all. mNA and pNA still co-migrate under these conditions. Fig. 2 shows the effect of varying the SDS concentration using a 20 m*M* borate buffer system. To modify the buffer system and enhance the solubility of the analytes, an organic modifier, such as acetonitrile, methanol, or ethanol, was added to the aqueous electrolyte in the present work. However, the nitrobenzenes are not completely soluble in methanol and acetonitrile and introduce an unstable background signal. The electroosmotic flow (EOF) decreased with increasing ethanol content. On the other hand, ethanol probably produces the greatest extent of interaction between the analytes and micelles. Fig. 3 shows the influence of ethanol concentration on the electrophoretic mobility of the analytes. We obtained a better resolution of mHNA/mHN/pHNA, 1.5 and 1.3, respectively, by the addition of 10% ethanol. Consequently, optimization was continued by testing the effect of the borate concentration and the applied electric field strength. The electropherogram is shown in Fig. 4, and was obtained using 50 m*M* SDS with 10% ethanol in 23 m*M* borate buffer, and an

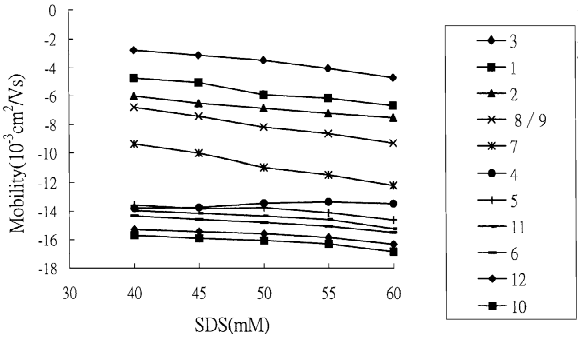

Fig. 2. Influence of various SDS concentrations on the electrophoretic mobility. The peak numbers are as described in Table 1.

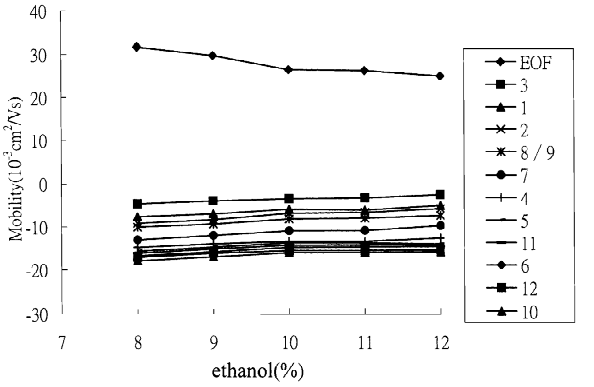

Fig. 3. Influence of ethanol concentration on electrophoretic mobility. The peak numbers are as described in Table 1.

electric field strength of 230 V/cm applied to a capillary of 75 cm×75 μm. We then performed an experiment to verify that micelles still remained in the 10% ethanol electrolyte system. We determined

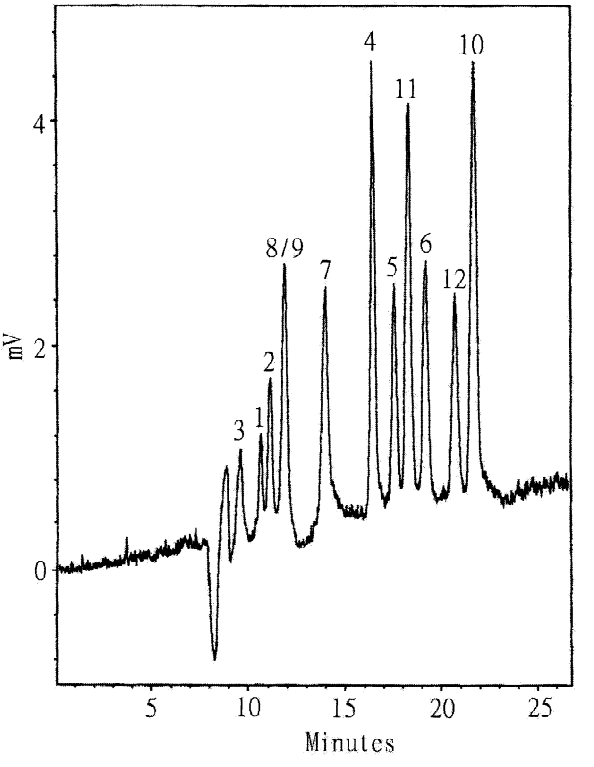

Fig. 4. Electropherogram for the nitrobenzenes. The conditions are described in Section 2.3. The peak numbers are as described in Table 1.

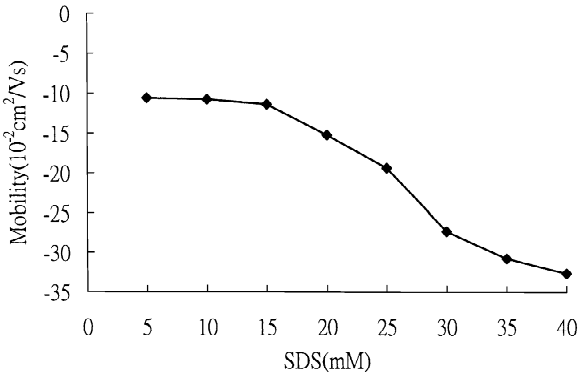

Fig. 5. Electrophoretic mobility of 4-nitro-1,2-phenylenediamine (oDAN) in various SDS concentrations.

the changes in mobility of a neutral molecule, oDAN, in various concentrations of SDS, as if Ref. [17]. The result is shown in Fig. 5. The characteristic point was obtained near 15 m*M*.

We were unable to achieve complete separation of nitroaniline isomers even using SDS, as reported in a previous study [18]. Thus we considered instead the use of CTAC, a cationic surfactant, to alter the hydrophobic interactions between nitroanilines and micelles. Fig. 6 shows the electropherogram of the nitroanilines obtained using 50 m*M* CTAC instead of SDS. A reproducibility of $<5\%$ of the relative standard deviation was obtained with five determi-

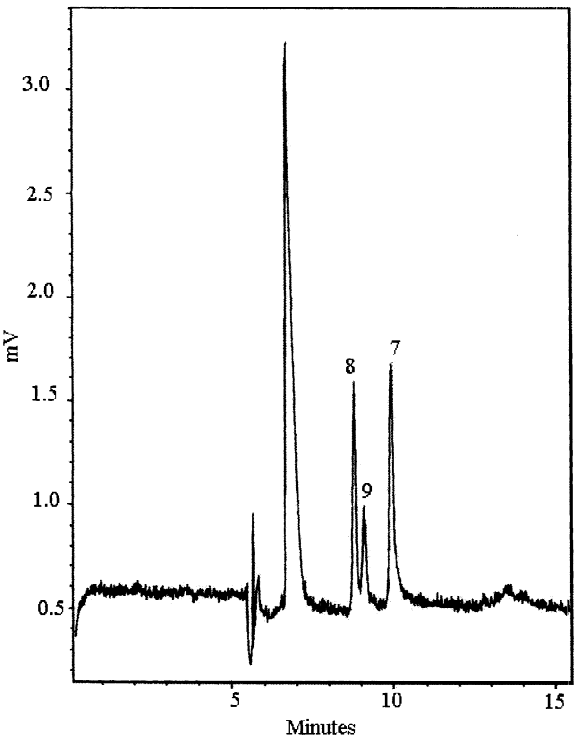

Fig. 6. Electropherogram of the nitroanilines. Conditions: 50 m*M* CTAC and 10% ethanol in 23 m*M* borate and an applied electric field strength of 230 V/cm on a fused-silica capillary of 75 cm×75.0 μm (effective length 50.0 cm). The peak numbers are as described in Table 1.

Table 2
Quantitative results

	HPLC				MEKC			
	Linear range (μg/mL)	Linearity ($\times10^{-6}$)	Correlation coefficient	Detection limit (μg/mL)	Linear range (μg/mL)	Linearity ($\times10^{-3}$)	Correlation coefficient	Detection limit (μg/mL)
oDAN	0.70–10.0	2.43C + 0.09	0.9996	0.60	3.00–70.0	1.03C + 0.28	0.9926	0.40
mDAN	1.00–15.0	4.50C + 1.51	0.9993	0.70	1.00–40.0	1.70C + 0.01	0.9904	0.40
pDAN	1.00–15.0	7.79C + 0.39	0.9993	0.30	0.70–50.0	1.83C + 0.03	0.9946	0.10
oHNA	0.70–10.0	2.60C + 0.09	0.9996	0.80	0.50–40.0	5.40C + 1.35	0.9930	0.40
mHNA	1.00–10.0	2.83C + 0.37	0.9991	0.30	0.50–40.0	3.43C + 0.34	0.9969	0.30
pHNA	–	–	–	–	0.30–60.0	4.16C + 0.19	0.9961	0.20
oNA	0.70–10.0	3.36C + 0.27	0.9992	0.80	0.70–60.0	4.35C + 0.08	0.9909	0.30
mNA	0.70–10.0	6.63C + 0.34	0.9993	0.60	–	–	–	–
pNA	1.00–10.0	2.59C + 0.70	0.9993	0.30	–	–	–	–
oHN	1.00–10.0	2.06C + 2.17	0.9994	0.60	0.30–30.0	8.68C + 0.28	0.9918	0.30
mHN	1.00–10.0	2.11C + 2.44	0.9998	0.80	0.30–30.0	6.34C + 1.97	0.9942	0.40
pHN	–	–	–	–	0.70–40.0	2.50C + 0.21	0.9957	0.70

Table 3
Results of the analysis of real samples by standard addition

	Recovery (%)					Linearity ($\times 10^{-6}$)					Correlation coefficient				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
HPLC															
oDAN	91.8	94.1	96.9	96.0	94.3	2.16C+2.25	3.87C+2.12	2.33C+2.46	2.25C+2.58	2.22C+2.52	0.9990	0.9992	0.9996	0.9992	0.9994
mDAN	92.3	96.6	93.5	95.6	96.4	4.27C+1.26	2.21C+2.41	3.88C+2.19	3.97C+1.81	4.25C+1.49	0.9991	0.9991	0.9996	0.9994	0.9997
pDAN	94.2	90.0	92.7	95.1	94.0	6.68C+1.85	5.83C+2.44	6.75C+1.54	6.89C+1.33	6.50C+2.10	0.9990	0.9991	0.9997	0.9990	0.9994
oHNA	90.7	92.8	94.7	93.7	91.9	1.95C+3.35	2.46C+2.10	2.32C+2.78	2.28C+2.84	2.25C+2.69	0.9992	0.9994	0.9999	0.9994	0.9995
mHNA	91.5	94.8	99.6	94.6	95.7	2.48C+0.59	2.69C+0.29	2.37C+1.31	2.43C+0.87	2.55C+0.64	0.9996	0.9999	0.9991	0.9990	0.9991
oNA	91.4	91.2	95.7	95.0	95.3	3.25C+2.73	2.77C+4.32	3.07C+3.72	2.82C+4.25	2.93C+4.02	0.9993	0.9998	0.9995	0.9996	0.9993
mNA	95.3	92.8	93.9	91.2	91.6	5.76C+7.62	6.29C+5.77	6.17C+6.37	6.21C+5.64	6.28C+5.57	0.9993	0.9990	0.9993	0.9992	0.9993
pNA	90.2	97.8	91.2	95.4	90.6	2.14C+0.99	2.45C+0.80	2.25C+0.80	2.37C+0.66	2.28C+0.72	0.9990	0.9991	0.9991	0.9995	0.9995
oHN	97.8	96.8	92.7	98.3	97.0	1.85C+2.45	2.27C+1.24	1.84C+2.13	1.97C+2.20	1.91C+2.26	0.9991	0.9996	0.9995	0.9993	0.9992
mHN	93.3	93.8	97.5	95.7	96.4	1.94C+2.21	1.77C+2.74	2.08C+2.22	1.80C+2.80	1.87C+2.69	0.9991	0.9992	0.9995	0.9991	0.9992
MEKC						Linearity ($\times 10^{-3}$)									
oDAN	93.7	97.5	94.5	96.0	94.4	1.85C+1.38	2.00C+1.27	1.86C+1.45	5.88C+1.47	1.87C+1.39	0.9902	0.9963	0.9939	0.9978	0.9989
mDAN	92.8	93.5	93.5	98.0	95.9	3.03C+1.89	3.03C+1.96	3.07C+1.83	3.29C+1.79	3.39C+1.29	0.9947	0.9991	0.9963	0.9981	0.9936
pDAN	92.8	92.2	91.2	95.1	92.6	3.21C+2.30	3.25C+2.15	3.45C+1.50	3.54C+1.71	3.26C+2.15	0.9939	0.9973	0.9948	0.9931	0.9973
oHNA	91.6	93.4	92.8	94.1	92.3	1.03C+5.21	1.09C+4.31	1.01C+5.88	1.01C+6.38	1.02C+5.62	0.9972	0.9973	0.9945	0.9927	0.9948
mHNA	90.9	93.2	98.1	93.3	94.9	6.98C+1.66	6.83C+2.36	5.84C+2.73	6.28C+4.06	6.61C+3.32	0.9994	0.9931	0.9932	0.9960	0.9938
pHNA	96.1	97.4	93.9	90.3	91.1	8.13C+3.77	8.18C+4.02	8.03C+3.48	8.32C+2.03	8.54C+1.65	0.9948	0.9959	0.9970	0.9951	0.9952
oNA	90.3	90.2	94.2	95.0	94.9	7.53C+4.77	7.93C+3.77	8.35C+3.78	7.90C+5.06	8.26C+4.15	0.9999	0.9991	0.9948	0.9964	0.9973
oHN	96.2	97.1	93.1	97.9	97.7	1.57C+1.09	1.62C+1.09	1.61C+8.66	1.74C+7.72	1.70C+8.72	0.9984	0.9965	0.9963	0.9986	0.9962
mHN	94.3	94.3	96.6	95.7	96.2	1.22C+7.37	1.24C+4.02	1.34C+4.95	1.17C+8.90	1.21C+8.03	0.9900	0.9959	0.9917	0.9952	0.9941
pHN	93.7	94.2	93.0	90.7	93.7	4.83C+2.25	5.31C+1.08	4.96C+1.79	5.25C+1.08	4.67C+2.54	0.9987	0.9985	0.9986	0.9958	0.9939

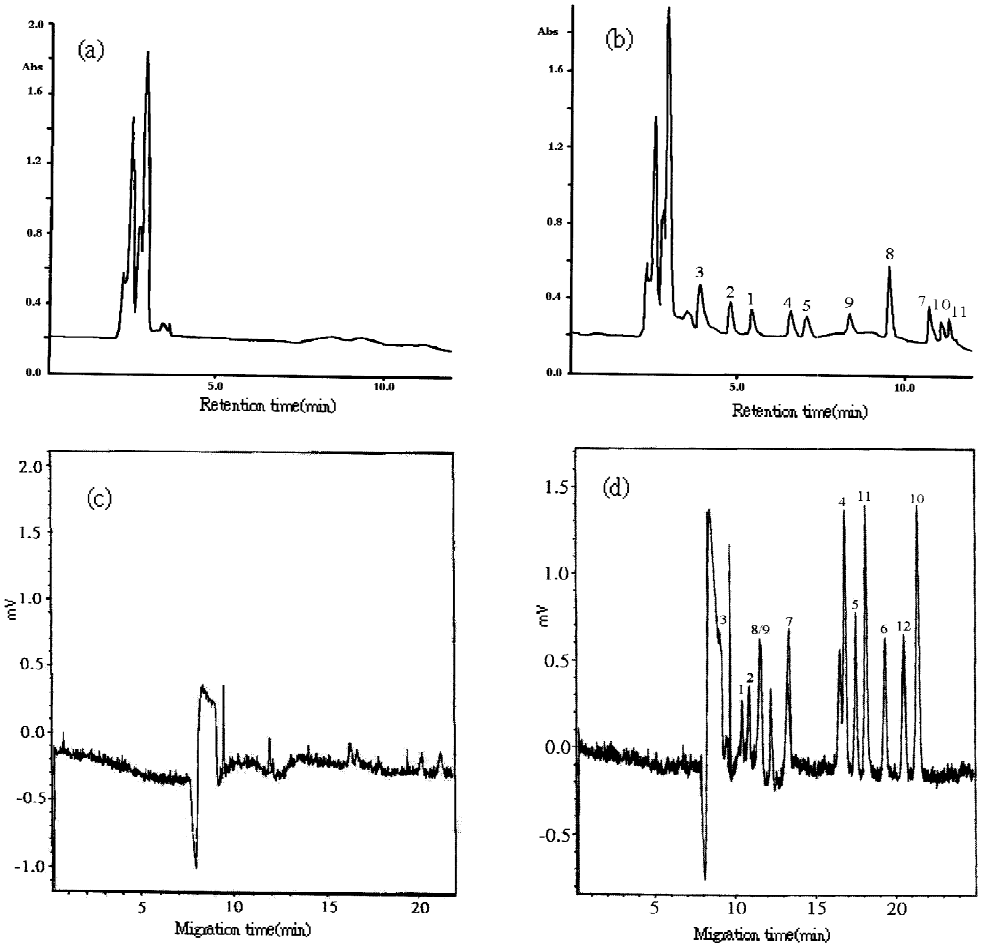

Fig. 7. Chromatograms of hair dye product S1 obtained by (a) direct analysis and (b) standard addition, and electropherograms obtained by (c) direct analysis and (d) standard addition. The conditions were as described in Sections 2.2 and 2.3.

nations. Some of the nitrobenzenes, DANs and NAs, are in a neutral state at pH 9.2. In this case, the migration order of these nitrobenzenes can be explained on the basis of their polarity. There is greater mobility with increasing polarity and with decreasing hydrophobic interactions. On the other hand, the hydroxyl moiety of HNA and HN should be partially or totally ionized at pH 9.2. However, the mass/charge ratio may play a significant role in the separation order of the HNA and HN isomers.

3.3. Quantitative results and analysis of hair dyes

HPLC and MEKC were used to study a linear range of nitrobenzenes by a series of injections of a standard mixture containing various concentrations. The linearity, correlation coefficients and detection limits are shown in Table 2. Calculation of the detection limits was based on IUPAC with a signal-to-noise ratio of 3.

HPLC and MEKC were applied to the assay of five commercial hair dye products by direct analysis and standard addition of 1.0, 3.0, 5.0, 7.0, and 9.0 ppm. The analytical results are shown in Table 3. The chromatograms and electropherograms are presented in Fig. 7. The recoveries, calculated from the standard addition results, were in the range from 90.0 to 99.6% with a relative standard deviation of

<4.0%. The levels measured indicate that all five samples contained less than 0.2%, although several peaks are present in Fig. 7c. These were verified as being impurities by comparison with the chromatogram obtained by standard addition. On the other hand, no nitrobenzenes were found by HPLC.

4. Conclusion

HPLC can be used for the separation of nitrobenzenes by adding THF without the addition of a surfactant or a column modifier. A shorter access time (12 min) and better reproducibility were obtained. With MEKC, detection limits of 0.1 to 0.7 μg/mL, a comprehensive linear range of 0.3 to 60 μg/mL, and the avoidance of column degradation can be obtained. The separation of nitroaniline isomers was achieved by using CTAC as a micelle in MEKC. The novel result obtained in this work was the first separation of nitrobenzenes with hydroxyl and/or amino substituents. These methods can be applied to the assay of a real sample with acceptable accuracy and precision.

Acknowledgements

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