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Journal of Chromatography A, 709 (1995) 361–366

JOURNAL OF CHROMATOGRAPHY A

Selective determination of aromatic amines in water samples by capillary zone electrophoresis and solid-phase extraction☆

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First received 8 December 1994; revised manuscript received 1 April 1995

Abstract

The use of a 20:80 (w/w) mixture of keto-derivatized and underivatized poly(styrene–divinylbenzene) copolymer for the selective solid-phase extraction (SPE) of pollutants in environmental water samples is described. In previous paper work, this technique was used for phenol determination with amperometric detection. In this modification, the use of a selective separation technique such as capillary zone electrophoresis (CZE) limits the necessity for obtaining a selective class separation with the extraction procedure. In this case, the same extract as used for phenols was analysed by CZE in phosphate buffer with UV detection, and the cationic species of interest, aromatic amines, were well separated from all the other non-ionic and anionic compounds. Experimental conditions for the use of CZE and the extraction technique are described. Different recoveries of aromatic amines in environmental samples, linearity and detection limits are discussed.

1. Introduction

Substituted anilines and benzidines are widely used in the chemical industry as intermediates in the production of dyes, pesticides, pharmaceuticals, etc.. These compounds are very well known because of their high toxicity and suspected carcinogenicity [1]. Owing to their high solubility in water, aromatic amines can easily permeate through soil and contaminate groundwater.

This paper is based on previous research on the determination of phenolic compounds, also widely used in the chemical industry, in soil and groundwater of a heavily contaminated area.

The previously proposed method for the determination of phenolic compounds [2] was subject to interference due to contemporaneous extraction of compounds with an $ArNH_2$ group, so we sought an analytical method with high selectivity towards this category of substances, based mainly on differences in ionicity of the two classes. The most common technique used for the determination of aromatic amines in environmental samples is gas chromatography coupled with mass spectrometry (GC–MS). However, the use of this technique requires long and cumbersome purification steps before the analy-

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☆ Presented at the *International Ion Chromatography Symposium 1994, Turin, 19–22 September 1994.*

0021-9673/95/$09.50
SSDI 0021-9673(95)00435-1

sis. The high sensitivity of GC–MS is not always needed in investigations of known events and a simple, but highly selective, technique for a rapid throughput would be more convenient.

The use of capillary zone electrophoresis (CZE) allows the group separation of cationic aminocompounds from both neutral and anionic phenolic compounds present in SPE extracts, based on different migration times [3–5]. The same extract can be used to perform the two different analyses without changing the sample preparation steps dramatically.

2. Experimental

2.1. Instrumentation

A CES-1 capillary electrophoresis system equipped with a variable-wavelength spectrometric detector (Dionex, Sunnyvale, CA, USA) operating at 280 nm was used. Data manipulation and the operation of all components in the system were controlled by AI-450 chromatographic software (Dionex) interfaced via an Advanced Computer Interface ACI-2 (Dionex) to a 80486-based computer (Compaq, Milan, Italy). Water samples were extracted using the same glass apparatus as described in a previous paper [2].

2.2. Reagents and standard

Phosphoric acid, sodium dihydrogenphosphate, anhydrous sodium carbonate, anhydrous sodium sulfate and, 1,3-diaminopropane were of analytical-reagent grade (Novachimica, Milan, Italy) and acetonitrile, acetone and methylene chloride were of HPLC grade (Carlo Erba, Milan, Italy).

Standard of all amines were free base reagent grade with the exception of benzidine hydrochloride (Novachimica, Milan, Italy).

All reagent solutions were prepared daily with ultra-pure deionized water (DI water), with conductivity <0.1 μS at 25°C, obtained using a Milli-Q system (Millipore, Milford, MA, USA). A standard solution of each amine (1 g/l) was prepared by dissolution in acetonitrile. Working standard solution of amine mixtures in the concentration range 0.5–100 mg/l were obtained by serial dilution of stock standard solutions with 150 m*M* H_3PO_4.

2.3. Groundwater samples

Water samples were adjusted to pH 6.5–8 and then filtered through a 0.45-μm filter. Before use, the SPE cartridge was activated with sequential application of 5 ml of acetone, 5 ml of acetonitrile and 5 ml of DI water. This cartridge was made by filling an empty commercial SPE cartridge with a 20:80 (w/w) mixture of underivatized and keto-derivatized poly(styrene–divinylbenzene) copolymer prepared as described previously paper [2,6]. Applying nitrogen pressure to the sample reservoir, 1 L of water sample was then passed through the cartridge at a flow-rate of 5 ml/min.

The cartridge was then dried under nitrogen for 5 min and retained compounds were eluted with 2 ml of 150 m*M* H_3PO_4 in water–acetone (80:20). The eluate was concentrated under a flow of nitrogen, diluted to a final volume of 1 ml and then analysed by CZE.

2.4. Soil samples

A 50-g amount of homogeneous soil sample from the dye industry was mixed with 50 g of sodium sulfate–sodium carbonate (2:1), washed with methylene chloride and over dried at 250°C. The mixture was then extracted twice with 50 ml of methylene chloride. The organic extract was collected over anhydrous sodium sulfate in a flask and evaporated to 5 ml with a rotary vacuum evaporator. The solution was mixed with 2 ml of 150 m*M* H_3PO_4, shaken and, after phase separation, the aqueous layer was analysed by CZE.

2.5. CE analysis

Capillary electrophoresis was performed in 65 cm (ca. 60 cm from injection to detection) × 50

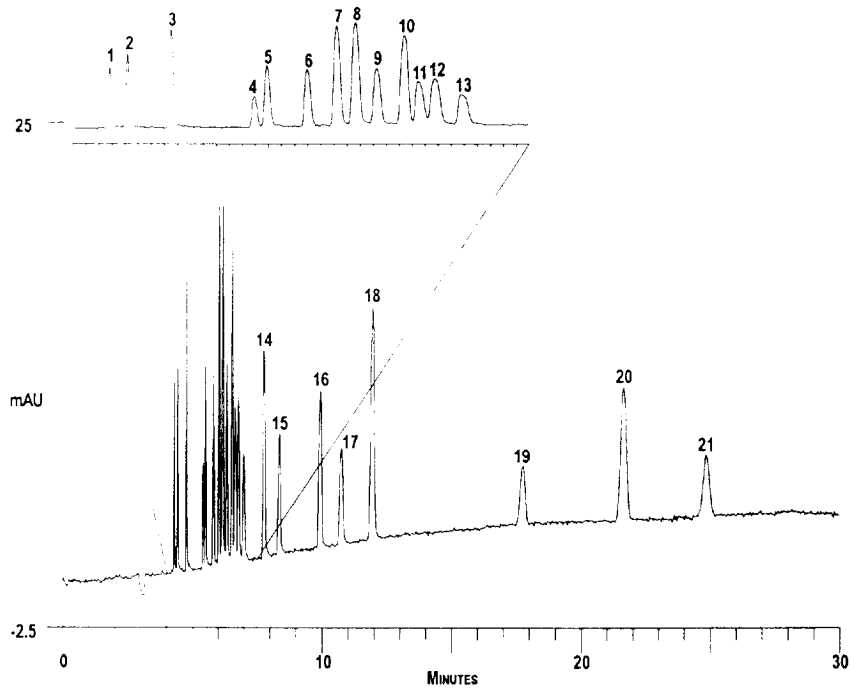

Fig. 1. Electropherogram for a standard solution of aromatic amines. Peaks: 1 = pyridine (35 mg/l); 2 = *p*-phenylenediamine (8 mg/l); 3 = benzidine (16 mg/l); 4 = *o*-toluidine (16 mg/l); 5 = aniline (8 mg/l); 6 = N,N-dimethylaniline (8 mg/l); 7 = *p*-anisidine (8 mg/l); 8 = *p*-chloroaniline (8 mg/l); 9 = *m*-chloroaniline (8 mg/l); 10 = ethylaniline (8 mg/l); 11 = α-naphthylamine (36 mg/l); 12 = diethylaniline (8 mg/l); 13 = N-(1-naphthyl)ethylenediamine (36 mg/l); 14 = 4-aminophenazone (16 mg/l); 15 = *o*-chloroaniline (16 mg/l); 16 = 3,4-dichloroaniline (16 mg/l); 17 = 3,3′-dichlorobenzidine (36 mg/l); 18 = 2-methyl-3-nitroaniline (16 mg/l); 19 = 2,4-dichloroaniline (36 mg/l); 20 = 2,3-dichloroaniline 16 mg/l); 21 = 2,5-dichloroaniline (36 mg/l). Buffer, 50 m*M* NaH_2PO_4–7 m*M* 1,3-diaminopropane (pH 2.35, adjusted with H_3PO_4); capillary, underivatized silica, 65 cm × 50 μm I.D.; applied potential, +30 kV; detection, UV at 280 nm.

μm I.D. fused-silica capillaries. The voltage was +30 kV (constant-voltage mode) and the capillary was cooled by an external forced air flow. Samples were injected by gravity raising the sample to 100 mm for 30 s. All experimental conditions were summarized in Table 1. 1,3-Diaminopropane as a surface modifier [7] was added to 50 m*M* phosphate buffer solution. pH 2.35 was chosen as the best compromise between analysis time and resolution, as shown in the

Table 1
Instrumental conditions used in the determination of aromatic amines by capillary zone electrophoresis

Capillary	Uncoated fused silica, 65 cm × 375 μm O.D. × 50 μm I.D.
Buffer	50 m*M* NaH_2PO_4–7 m*M* 1,3-diaminopropane, adjusted to pH 2.35 (H_3PO_4)
Rising cycle	Destination 6 s; capillary 120 s; source 6 s
Injection	Gravity at 100 mm for 30 s
Temperature control	Air cooling
Applied potential	+30 kV constant voltage
Current	ca. 55 μA
Wavelength	280 nm

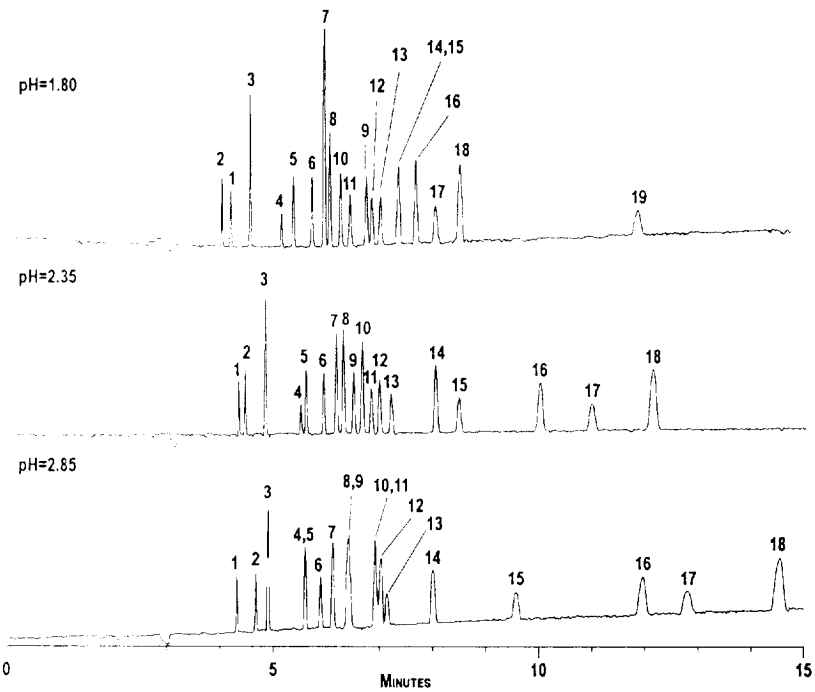

Fig. 2. Migration times of aromatic as a function of pH. Peaks as in Fig. 1. Buffer, 50 m*M* NaH_2PO_4–7 m*M* 1,3-diaminopropane with PH as shown; capillary, underivatized silica, 65 cm × 50 μm I.D.; applied potential, +30 kV; detection, UV at 280 nm.

electropherogram of an amine standard solution shown in Fig. 1. The influence of pH on the mobility of different amines is shown in the electropherograms in Fig. 2.

3. Results and discussion

3.1. Linearity, recovery and retention time stability

Linearity for all amines was verified by determining seven different concentrations of each amine in the range 0.5–100 mg/l. Regression coefficients and characteristics of the calibration plot were calculated. As shown in Table 2, good linearity for all amines was achieved over more than two orders of magnitude of concentration. The limits of detection ranged from 0.06 to 1.8 μg/ml; the limit of detection was calculated according IUPAC guidelines [8]. Recoveries were calculated from "CE clean" surface waters spiked with aromatic amine standards, and for the amines considered were better than 82% except for the most hydrophilic ones, the recovery of which ca. 60% under the same extraction conditions. In Table 3 are summarized the recoveries for representative amines.

In order to limit wall interactions and reduce retention time drift, a surface modifier such as 1,3-diaminopropane at a 7 m*M* concentration was added to the buffer.

3.2. Real samples

CZE was a valuable tool in the determination of cationic species in multi-component samples such as SPE extracts that we used in HPLC with amperometric detection for the determination of phenols. This analysis showed interfences due to aromatic amines, because of the sample origin, while GC–MS was complicated by a very large number of other compounds. Electropherograms b and c in Fig. 3 show a simplified "fingerprint" with few peaks certainly cationic and absorbance characteristics typical of aromatic substances.

Table 2
Parameters of calibration graphs and limits of detection for aromatic amines

Analyte	a	$b \cdot 10^6$	r^2	L.O.D.[a]
Pyridine	-1.679	0.505	0.989	0.65
p-Phenylenediamine	−0.373	0.134	0.997	0.12
Benzidine	−0.399	0.152	0.999	0.17
o-Toluidine	−0.359	0.199	0.998	0.44
Aniline	−0.308	9.801	0.997	0.12
N,N-Dimethylaniline	−0.244	9.507	0.998	0.12
p-Anisidine	−0.107	5.049	0.998	0.07
p-Chloroaniline	−0.145	4.638	0.999	0.06
m-Chloroaniline	−0.234	8.006	0.996	0.11
Ethylaniline	-0.146	4.912	0.999	0.07
α-Naphthylamine	−0.072	0.370	0.989	0.56
Diethylaniline	−0.084	8.003	0.998	0.11
N-(1-Naphthyl)ethylenediamine	−0.566	0.335	0.997	0.59
4-Aminophenazone	−0.477	8.542	0.999	0.18
o-Chloroaniline	−0.527	7.825	0.999	0.33
3,4-Dichloroaniline	−0.334	4.716	0.999	0.26
3,3′-Dichlorobenzidine	−1.331	0.136	0.992	1.5
2-Methyl-3-nitroaniline	-0.472	5.821	0.999	0.24
2,4-Dichloroaniline	0.145	6.275	0.992	1.8
2,3-Dichloroaniline	0.273	2.076	0.995	0.35
2,5-Dichloroaniline	1.627	6.612	0.975	1.6

Amount (mg/l) = $a + b \cdot$ response (area units).
[a] mg/l; L.O.D. calculated according IUPAC. Gravity injection at 100 mm for 30 s.

Identification of some of these peaks was achieved by spiking the sample with aromatic amines and cross-confirmation by GC–MS.

The sensitivity of the method is sufficient to give good accuracy in the determination of macro-contaminants, and further the intrinsic selectivity of the analytical approach helps to simplify the chromatograms. This kind of selectivity allows simpler sample preparation and purification and make this technique suitable for rapid control screening in situations that require evidence of contamination or accidents. On the other hand, the selectivity cannot compensate for the sensitivity, as can be seen in electropherogram a in Fig. 3, where there are no peaks, whereas the same sample analysed by GC–MS showed trace amounts of aromatic amines. As shown in Fig. 3c, for a soil sample, the suitability of the method for the analysis of samples extracted with a liquid–liquid extraction procedure was also verified.

The proposed method is simple and fast and its selectivity limits interferences from similar but differently charged compounds in complex matrices so that a complex procedure for sample preparation is not required. Conversely, it does not meet the sensitivity limits required by the

Table 3
Recovery of some representative aromatic amines from surface water samples by using SPE

Analyte	Recovery (%)[a]	R.S.A. (%)
Benzidine	47	6.0
Aniline	58	4.1
p-Anisidine	58	3.7
m-Chloroaniline	91	2.5
Diethylaniline	89	1.8
o-Chloroaniline	87	2.2
3,4-Dichloroaniline	87	1.4
3,3′-Dichlorobenzidine	82	2.4
2-Methyl-3-nitroaniline	97	1.1

[a] Surface water samples spiked with 20 μg/ml of amine; mean results ($n = 3$).

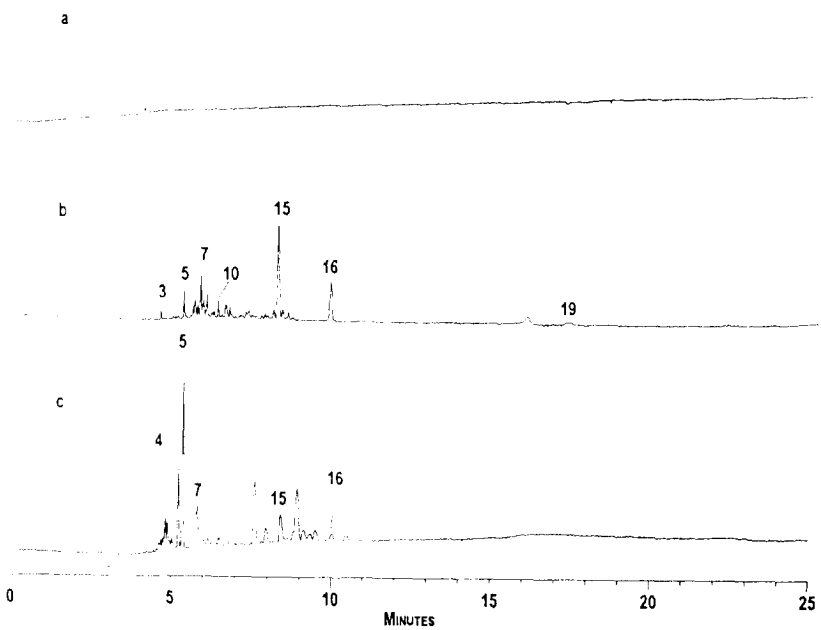

Fig. 3. Electropherograms of real samples after 1000-fold SPE preconcentration. (a) Tap water. (b) First layer groundwater. Peaks: 3 = benzidine (2.7 μg/l); 5 = aniline (1.8 μg/l); 7 = *p*-anisidine (1.5 μg/l); 10 = ethylaniline (0.5 μg/l); 15 = *o*-chloroaniline (9.9 μg/l); 16 = 3,4-dichloroaniline (2.9 μg/l); 19 = 2,4-dichloroaniline (1.1 μg/l). (c) Soil sample from industrial plant. Peaks: 4 = *o*-toluidine (600 μg/kg); 5 = aniline (801 μg/kg); 7 = *p*-anisidine (11.2 μg/kg); 15 = *o*-chloroaniline (15.2 μg/kg); 16 = 3,4-dichloroaniline (1.8 μg/kg). Sample preparation as described. Buffer, 50 m*M* NaH_2PO_4–7 m*M* 1,3-diaminopropane (pH 2.35, adjusted with H_3PO_4); capillary: underivatized silica, 65 cm × 50 μm I.D.; applied potential, +30 kV; detection, UV at 280 nm.

inherent toxicity and danger of the compounds concerned. This method could be useful in those situations of evident contamination or accidents, and when it is necessary to monitor rapidly a site of pollution in order to establish the borders of the contaminated areas.

Acknowledgement

The authors thank Mary Jo Wojtusik for valuable assistance with this project and for helpful discussions.

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