ELSEVIER

Journal of Chromatography A, 791 (1997) 221–230

JOURNAL OF CHROMATOGRAPHY A

Determination of aromatic amines by solid-phase microextraction and gas chromatography–mass spectrometry in water samples

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Received 2 December 1996; received in revised form 15 July 1997; accepted 16 July 1997

Abstract

Solid-phase microextraction (SPME) in gas chromatography–mass spectrometry (GC–MS) has been introduced as a rapid and sensitive quantitative method for the detection of some aniline derivatives (*o*-toluidine, *p*-chloroaniline, 2,4-dichloroaniline, 2,5-dichloroaniline, 3,4-dichloroaniline and 3,5-dichloroaniline) in environmental water samples. Many parameters for optimisation of the extractive method, such as linearity, sensitivity, equilibration time, precision, and different operating conditions (pH, salt) have been evaluated. After a comparison of the commercially available SPME fibers, a carbowax–divinylbenzene 65 μm polymeric phase was chosen. Linearity was excellent in the concentration range 0.05–5 μg/l, and the method showed good reproducibility (coefficient of variation of around 5%). The detection limits differ substantially for the various compounds analysed, but all were below any other limit of detection for these compounds in the literature. The addition of salt (sodium chloride) at pH 7.6 significantly improved the amount of analytes extracted by the fiber. Operating under basic conditions (pH 11), we did not observe a better sensitivity of the method. To evaluate its applicability on a real aqueous matrix, various groundwater samples collected in an industrially polluted area north of Milan, Italy, were analysed. © 1997 Elsevier Science B.V.

Keywords: Anilines; Amines, aromatic

1. Introduction

Aromatic amines, such as aniline and its substituted derivatives, are generally dangerous because of their toxicity and carcinogenicity [1,2] or else they can be converted into toxic *N*-nitroso compounds through reactions with nitrosylating agents in the environment [3]. These contaminants may be released as chemical residues of dyestuffs, cosmetics, medicines and rubber manufacture [3–5] and also as by-products of energy technologies such as coal-conversion waste processing [1]. Chlorinated anilines such as *p*-chloroaniline and 3,4-dichloroaniline were also found as degradation products and intermediates of various phenylurea and phenylcarbamate pesticides [1,3]. In view of the importance of these compounds, a rapid and sensitive method of analysis is needed to detect them in the environment.

Aromatic amines have already been analysed in environmental water samples using a variety of analytical techniques such as gas chromatography (GC) coupled with different detectors [3,6–8], high-performance liquid chromatography (HPLC) [10,11], capillary zone electrophoresis (CZE) [12] and ultraviolet spectrophotometry [13]. GC–MS has been recognised as the method of choice in a wide series

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0021-9673/97/$17.00
PII S0021-9673(97)00795-4

of environmental analyses, due to its superiority in selectivity and sensitivity [14,15]. However, in the case of polar compounds, such as aromatic amines [3,7,16], a derivatization step is often required to improve the gas chromatographic properties; other problems stem from the extraction of polar compounds from water samples.

The purpose of this study was to optimise an analytical method for the GC–MS analysis of some aromatic amines in water samples, achieving free amine detection without chemical derivatization and using solid-phase microextraction (SPME) to reduce the sample preparation time and increase the extraction efficiency.

SPME is a fast, simple, solvent-free extraction technique that can be easily automated, reduces analyte loss during extraction and requires only small water samples [8,17–28]. Organic pollutants are extracted by the stationary phase from an aqueous or gaseous matrix until equilibrium is achieved. More details about this technique are given in Section 2.

Parameters for development of the extractive method, such as linearity, sensitivity, equilibration time profile, precision, pH and salt effects, were investigated. The aniline derivatives analysed, presented in Fig. 1, are *o*-toluidine, *p*-chloroaniline, 2,4-dichloroaniline, 2,5-dichloroaniline, 3,4-dichloroaniline and 3,5-dichloroaniline. To our knowledge, the SPME method has been applied to this class of pollutants only in a few cases [8,9].

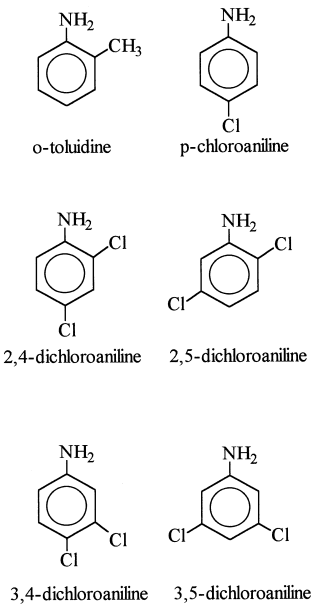

Fig. 1. Aromatic amines investigated in this study.

2. Experimental

2.1. Materials

Standard solutions of *o*-toluidine, *p*-chloroaniline, 2,4-dichloroaniline, 2,5-dichloroaniline, 3,4-dichloroaniline and 3,5-dichloroaniline were purchased from Aldrich (Steiheim, Germany), with purities >98%. The SPME holders for manual sampling and the coating fibers were supplied by Supelco (Bellefonte, PA, USA). Groundwater for real sample analysis was collected by USSL 67 (Garbagnate, Milan, Italy) from the Limbiate area, north of Milan, Italy.

2.2. Instrumental analysis

GC–MS analysis was carried out using a Hewlett Packard GC5890-MSD5971. A PTA-5 base-deactivated column (30 m, 0.25 mm I.D., 0.50 μm film thickness) from Supelco and a split/splitless injector were used for all investigations. With this column, the GC–MS analysis of aminic compounds was carried out without derivatization. The injector was maintained at 260°C and the desorption time was 10 min. The linear purge was closed for 5 min during desorption of the analytes from the SPME fiber in the split/splitless injector. The oven temperature program was 60°C for 5 min, 60–250°C at 10°C/min, and then held at 250°C for 3 min. The head pressure was 40 kPa and the detector temperature was 280°C. The GC–MS system was used in the selected ion monitoring (SIM) mode. The selected masses and retention times of each compound are presented in Table 1.

2.3. Solid-phase microextraction (SPME)

This extraction technique comprises two simple steps. First, the fiber is exposed to the aqueous sample for extraction of the analytes by the stationary phase. The fiber is then removed from the

Table 1
Selected ions and retention times

Compound	Retention times (min)	Selected masses (m/z)[a]
o-Toluidine	13.70	106–107
p-Chloroaniline	16.19	127–129
2,4-Dichloroaniline	18.39	161–163
2,5-Dichloroaniline	18.39	161–163
3,5-Dichloroaniline	19.45	161–163
3,4-Dichloroaniline	19.92	161–163

[a]Quantification occurs on the first ion.

solution and introduced into the GC injector where the analytes are thermally desorbed, separated on the column and identified by a detector. The extraction can be carried out by direct immersion of the fiber in the aqueous sample or by exposure to the headspace of the water solution.

The amount of chemicals extracted by the stationary phase at equilibrium, expressed as the number of moles, n, of the analyte on the fiber, is related to the concentration of the analyte in the water sample (C°_{aq}), the distribution constant (K_{fs}) and the volumes of the stationary phase (V_f) and of the sample (V_s), according to Eq. (1) [23,24].

$$n = \frac{K_{fs}V_f C^{\circ}_{aq} V_s}{K_{fs}V_f + V_s} \quad (1)$$

This equation may be simplified if $V_s >> K_{fs}V_f$ and the amount of analyte adsorbed by the polymeric phase is not related to the sample volume, and is described by Eq. (2) [23,25,28].

$$n = K_{fs}V_f C^{\circ}_{aq} \quad (2)$$

The distribution constant, K_{fs}, is an index of the coating affinity for an analyte. The higher the distribution constant relative to a compound, the higher the affinity of this compound for the stationary phase of the fiber. Polar compounds need polar fibers to be extracted efficiently.

In this study, many coating fibers were tested (polyacrylate 85 μm, polydimethylsiloxane 100 μm, polydimethylsiloxane–divinylbenzene 65 μm, carbowax–divinylbenzene 65 μm and an experimental carbon fiber 80 μm), while the entire study was carried out with the carbowax–divinylbenzene coating fibre.

2.4. SPME procedure

Before use, the fibers were conditioned at the recommended temperature, under helium flow, to reduce bleed. The fiber was directly immersed in the water solution for extraction. If not otherwise specified, the extraction time used in this study was 30 min at room temperature, with magnetic stirring. Magnetic stirrers, 12 mm long, were employed. The stirrer speed was set so that the vortex formed in the vial was about 1 cm deep. Vials (10 ml) were filled with 10 ml of mineral water spiked with standard solutions of the aromatic amines or with 10 ml of real groundwater samples. Vials were sealed with aluminium caps with a central hole and PTFE-lined septa that were pierced by the SPME needle. Fresh solutions were prepared for each extraction. Desorption was carried out in the GC injector for 5 min at 260°C. After desorption, the fiber was kept in the glass inlet sleeve for another 5 min to remove memory effects of the compounds.

3. Results

Standard solutions of the aromatic amines in methanol were used in scan mode to obtain the mass spectra and to set up the chromatographic conditions. Fig. 2 shows the total ion GC–MS chromatogram of pure water samples spiked with standard solutions of the aromatic amines. The two isomers, 2,4- and 2,5-dichloroaniline, coelute as one peak, with the column and the chromatographic conditions employed. Since each isomer contributes equally to the peak area, as demonstrated by preliminary separate injections of their standard solutions, we proceeded with simultaneous detection of these two compounds.

Five fibers coated with different polymeric phases (polyacrylate 85 μm, polydimethylsiloxane 100 μm, polydimethylsiloxane–divinylbenzene 65 μm, carbowax–divinylbenzene 65 μm and an experimental carbon fiber 80 μm) were compared in order to choose the stationary phase with the best affinity for these compounds. Their different behaviours were studied for the extraction of 10 μg/l spiked water solutions and the results are presented in Fig. 3. Although the fiber coated with polydimethylsilox-

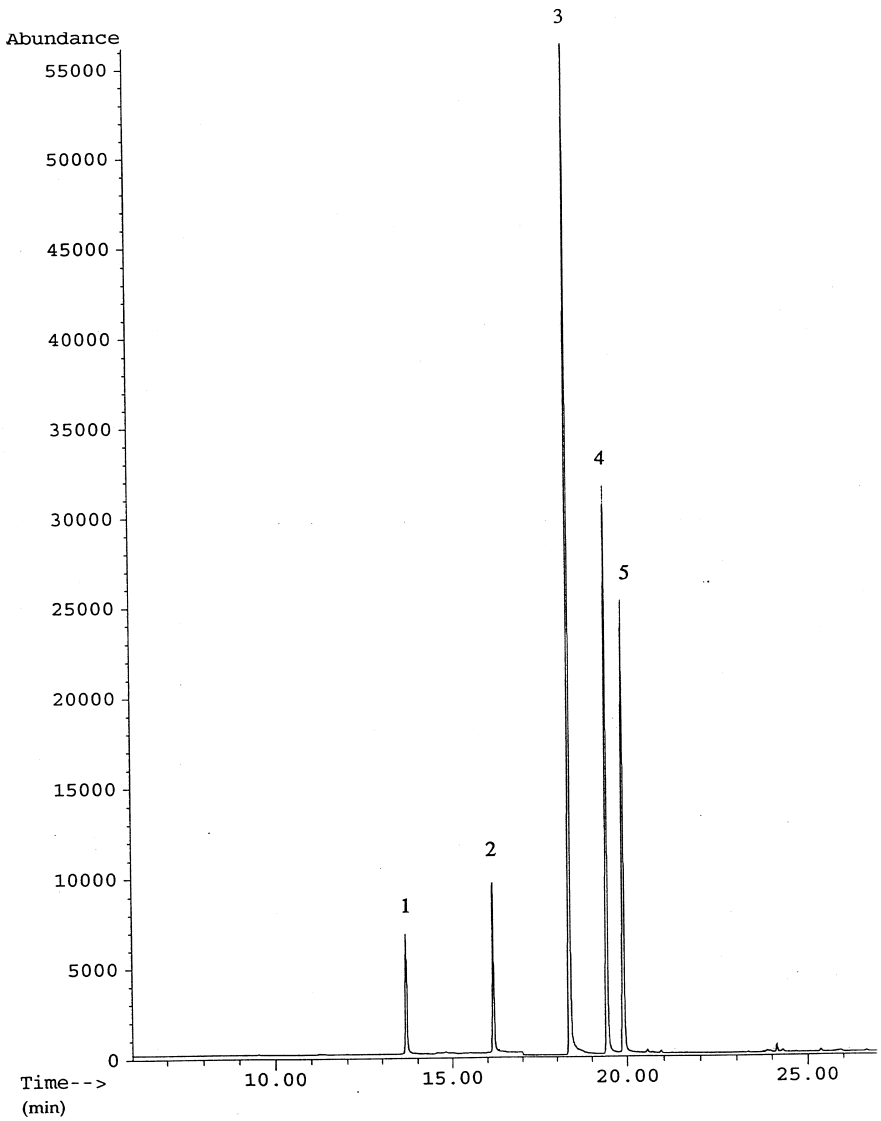

Fig. 2. Total ion chromatogram of a spiked water solution (10 μg/l) extracted for 30 min with direct immersion of a carbowax–divinylbenzene 65 μm fiber. (1) *o*-Toluidine; (2) *p*-chloroaniline; (3) 2,4- and 2,5-dichloroaniline; (4) 3,5-dichloroaniline and (5) 3,4-dichloroaniline.

ane–divinylbenzene gave the best sensitivity, the analytes were not fully desorbed from the fiber into the GC injector and a memory effect was observed. A 65-μm carbowax–divinylbenzene phase was finally chosen for development of the analytical method, since it showed good sensitivity and no memory effect.

Further preliminary tests on these aromatic amines showed that SPME with direct immersion of the fiber into the aqueous sample gives a much better performance than a head space procedure, even though these compounds are quite volatile (Fig. 4). The extraction was carried out for 30 min on 5 ml of 10 μg/l standard water solutions in 10 ml vials at room

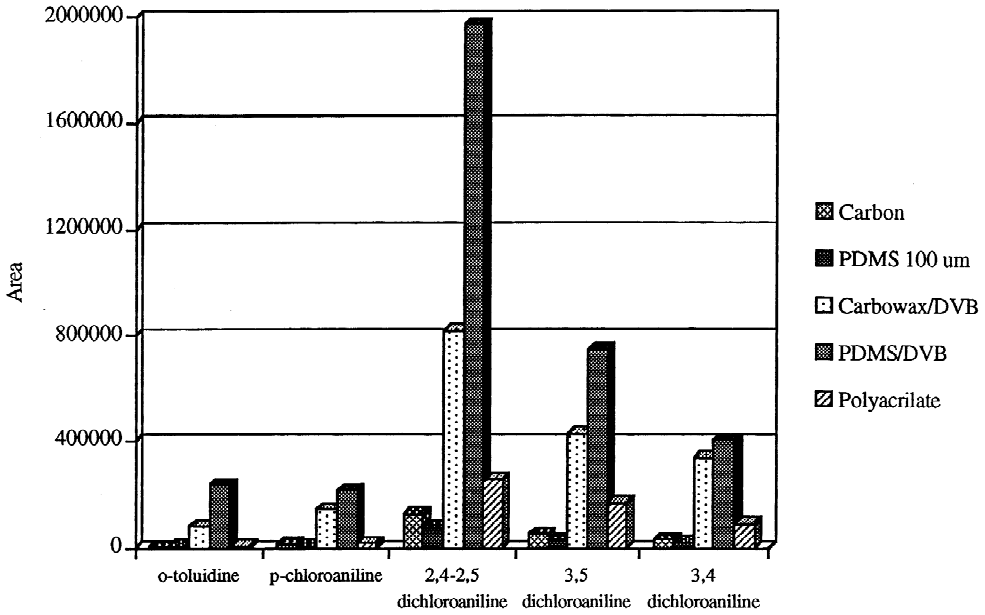

Fig. 3. Comparison of the performances of different SPME coating fibers.

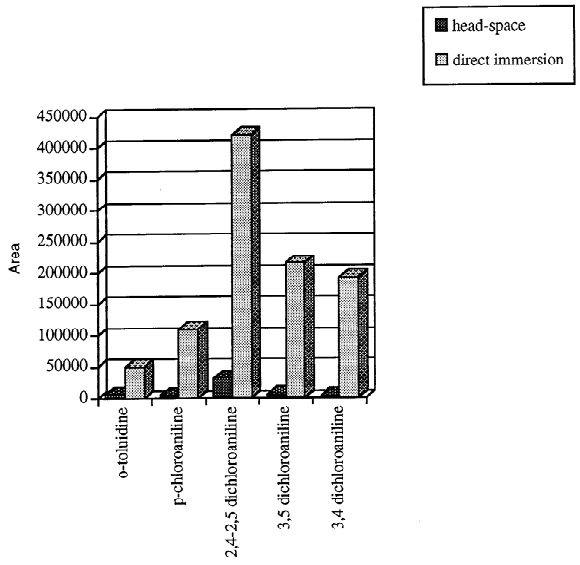

Fig. 4. Comparison of head-space and direct immersion SPME procedures. Aqueous standard solutions (5 ml; 10 μg/l) were extracted in the two exposure techniques for 30 min at room temperature under magnetic stirring.

temperature with magnetic stirring. For subsequent analyses, therefore, the fiber was dipped directly into the aqueous sample.

Different aspects were investigated to evaluate the applicability of this technique to the pollutants in question.

3.1. Extraction time profiles

The first step in development of the SPME method was to determine when equilibrium of the analytes was reached between the aqueous and the polymeric phases. Standard water solutions (10 μg/l) were prepared and extracted under magnetic stirring, varying the exposure time of the fiber to the sample from 5 to 90 min. A fresh solution was used for each time interval. The area counts were monitored in relation to time (Fig. 5). Even after 90 min, equilibrium was not achieved; in fact, the difference between two successive times exceeds the variability of the method, which was around 5% (see Section 3.3). For routine analysis, it is not necessary to reach equilibrium if constant extracting conditions are maintained, therefore, in further studies, we dipped the fiber into the solution for 30 min.

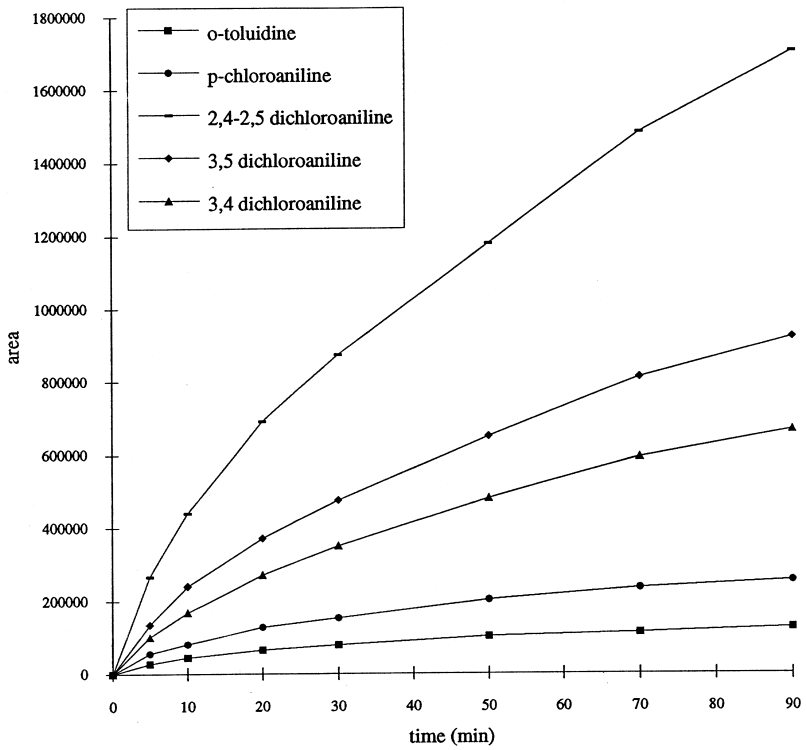

Fig. 5. Equilibration time profiles under magnetic stirring.

3.2. Linearity

Calibration curves were calculated in the concentration range of 0.05–5 μg/l. All procedures were carried out in triplicate to evaluate the inter-day reproducibility. Linearity was good for all of the aromatic amines, as shown by the determination coefficients, r^2, in Table 2 ($0.9991 < r^2 < 0.9996$). This means that, within this range, these pollutants can be easily quantified.

3.3. Precision

The reproducibility of the method was determined by eight extractions of 10 μg/l spiked water samples under identical operating conditions (30 min ad-

Table 2
Main method parameters for the aromatic amines investigated

Compound	Coefficient of determination (r^2)[a]	Limit of detection (μg/l)[b]	Relative standard deviation (%) ($n=8$)
o-Toluidine	0.99908	0.025	5.6
p-Chloroaniline	0.99936	0.010	5.1
2,4-2,5 Dichloroaniline	0.99948	0.002	4.5
3,5-Dichloroaniline	0.99962	0.005	5.0
3,4-Dichloroaniline	0.99918	0.007	5.5

[a]Average of three experiments performed on different days.
[b]Lowest detectable concentration with a signal-to-noise ratio of approximately three.

sorption with tolerance of only a few seconds). Fresh solutions were prepared for each extraction. The relative standard deviation [R.S.D. (%)] of the area counts is around 5% for all compounds, showing the good precision of this method (Table 2); in SPME literature, other analytes presented a reproducibility that was either similar or sometimes worse [21,31]. Our SPME analysis of chloroanilines for direct immersion of the fiber into water samples showed a better precision than that obtained on head-space analysis of the same pollutants in a soil matrix [8].

3.4. Limits of detection (LODs)

Detection limits differ substantially for the various compounds analysed. LODs were calculated by comparing the signal-to-noise ratio (S/N) of the lowest detectable concentration to a S/N of three. Results are presented in Table 2 and they are lower than the detection limits of other current methods [6,10–12]. For example, the United States Environmental Protection Agency (EPA) method 1625 [29] for the detection of semivolatile toxic organic pollutants by GC–MS achieves LODs for these compounds of around 10 μg/l, like EPA method 8270 [30], while the analytical method described here has much lower detection limits.

3.5. Effect of salt

The addition of salt, usually sodium chloride (NaCl), as about a 60% saturated solution, often improves the sensitivity of this extraction method, increasing the ion strength of the aqueous phase and salting the analytes out of solution into the fiber coating [19,31]. We made a comparison using the same operating conditions and adding 2 g of NaCl to 10 ml of a spiked water solution (60% saturated solution). All of the analyses were performed in triplicate.

Fig. 6 shows how the peak response depends on the salt content. The addition of salt does not improve the sensitivity much, despite the significant difference (Student's test; $\alpha < 0.05$). The addition of salt normally increases the amount of analyte adsorbed by the fiber, especially for polar compounds [22,28,31].

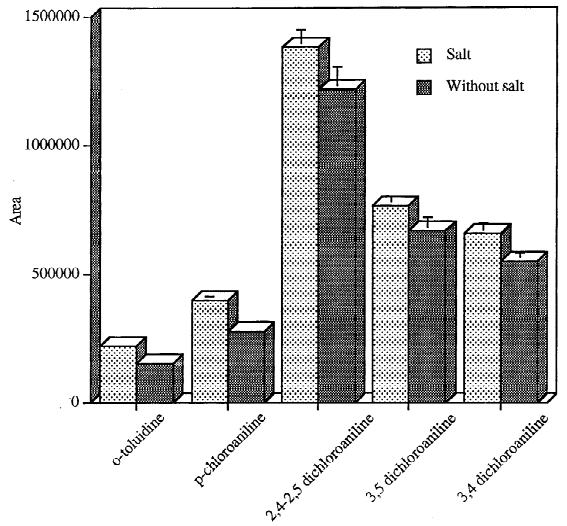

Fig. 6. Effect of salt at pH 7.6. Aqueous spiked samples were salted by adding NaCl (60% saturated solutions). The experiments were performed in triplicate.

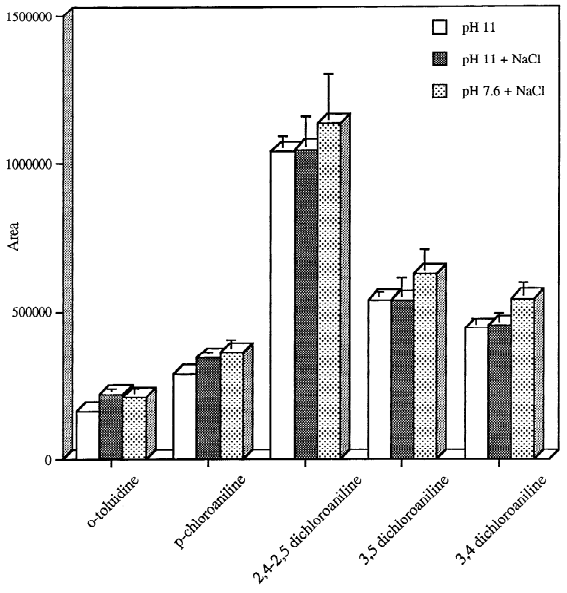

Fig. 7. Effect of pH with and without NaCl. Salted aqueous solutions were 60% saturated by NaCl. The adjustment of the pH was achieved by adding NaOH. The experiments were performed in triplicate.

Table 3
Relative standard deviation (%) of extractions performed in triplicate with and without salt at different pH values

Relative standard deviation (%)	*o*-Toluidine	*p*-Chloroaniline	2,4–2,5-Dichloroaniline	3,5-Dichloroaniline	3,4-Dichloroaniline
pH 7.6 with salt	13.9	11.2	14.9	13.2	10.0
pH 7.6 without salt	3.3	2.0	3.9	4.8	6.0
pH 11 with salt	8.8	5.1	10.5	14.6	8.7
pH 11 without salt	3.6	0.9	4.9	5.1	7.7

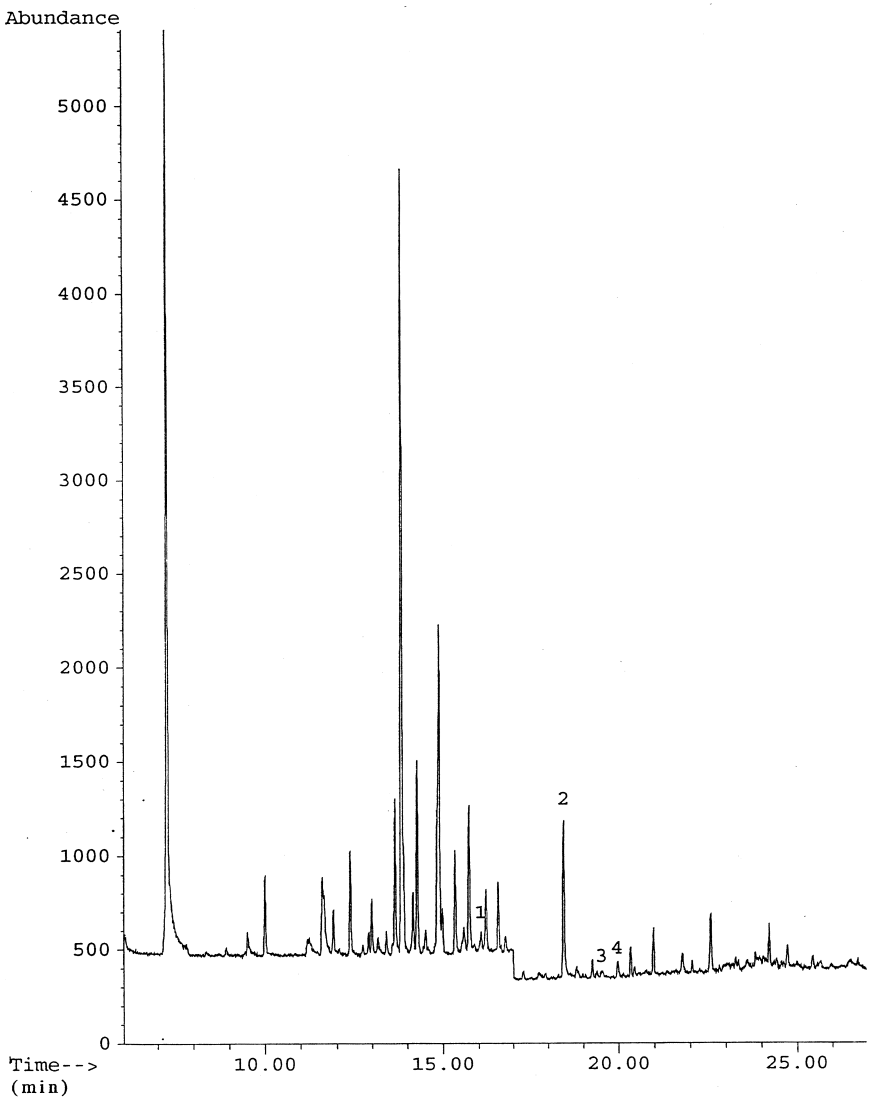

Fig. 8. Total ion chromatogram of a groundwater sample collected near an industrial area north of Milan, Italy, for the quantitative analysis of aromatic amines with SPME–GC–MS. (1) *p*-Chloroaniline; (2) 2,4- and 2,5-dichloroaniline; (3) 3,5-dichloroaniline and (4) 3,4-dichloroaniline.

3.6. Effect of pH

The amount of aromatic amines adsorbed by the fiber should be enhanced by a high pH, since these conditions ensure that amines are in their neutral form. The simultaneous presence of salt and basic operating conditions should improve the sensitivity of the SPME extracting method [19,21].

The evaluation of the pK_a values of these aromatic amines, with a rule-based system, HazardExpert 2.0 (produced by Compudrug), showed that, at pH 7.6, their neutral form is highly favoured. This behaviour was confirmed by operating under basic conditions (pH 11) and observing no increase in the sensitivity (Fig. 7).

Fig. 7 presents a comparison, performed in triplicate, of a neutral salted solution (pH 7.6), previously shown to give a higher response than the sample without salt, and a basic solution (pH 11) with and without salt. As expected, the adjustment of the pH did not enhance the sensitivity. At pH 11, the addition of salt did not lead to a significant increase in the amount of analyte adsorbed by the SPME fiber, particularly for dichloroaniline isomers. This might be explained by assuming that using salt in basic conditions may prolong the extraction time because diffusion and adsorption are slower than in pure water [21]. *o*-Toluidine and *p*-chloroaniline presented the highest enhancement factors with added salt under basic and neutral conditions. Only the increases of *o*-toluidine and *p*-chloroaniline were significant (Student's test; $\alpha = 0.05$).

Another interesting point is the decrease in the precision of the method, expressed by the coefficient of variation of three values, for the analysis with salt in comparison with the unsalted samples, at any pH value (Table 3). To our knowledge, no evidence of this worsening of precision due of the addition of salt is described in literature on SPME.

3.7. Environmental samples

To apply this analytical method on a real water sample, we analysed aromatic amines in groundwaters collected near a contaminated area north of Milan, Italy. We analysed various well samples of raw water, before treatment with active carbon for potabilization. No dilutions or salt additions were carried out on environmental samples. The pH of the well samples that were analysed was around 7.6. Fig. 8 presents the chromatogram of the extracted ions from the dirtiest well, showing *p*-chloroaniline, 3,5 dichloroaniline and 3,4-dichloroaniline in very low concentrations (up to 0.05 μg/l), and 0.23 μg/l of 2,4- and 2,5-dichloroaniline, as presented in the bar graph in Fig. 9. Values below 0.1 μg/l conform to the limits set by Italian regulations for pesticides in general; in fact, in Italy, no specific legal limits exist for aromatic amines.

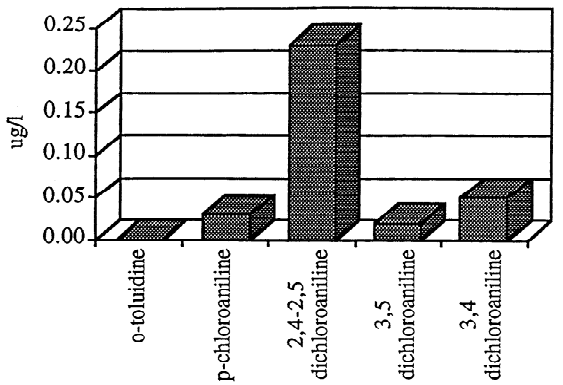

Fig. 9. Content of aromatic amines in groundwaters collected near an industrial area north of Milan, Italy.

4. Conclusions

The analytical method optimised in this study, based on SPME combined with GC–MS, for the detection of some dangerous environmental pollutants, such as the aromatic amines, proved to be simple, rapid, precise and sensitive.

It detects these polar compounds even in trace amounts, without derivatization, in water samples. The analytical method is applicable to real environmental samples with matrix effects.

In view of the interest in this class of pollutants and the satisfying results obtained with this method, it is our aim to extend the list of aromatic amines investigated to include other compounds with important toxicity, which are widely found in the environment.

Acknowledgements

Financial support for this work was provided by the Commission of the European Communities (Project ENV4-CT95-0020). E.F. is the recipient of a fellowship from Fondazione Lombardia per l'Ambiente, Milan, Italy. L.M. is the recipient of a fellowship from Banca Commerciale Italiana. Thanks to Prof. G. Gini, Politecnico of Milan (Italy), for the use of HazardExpert 2.0. We are grateful to USSL 67 (Garbagnate, Milan, Italy) for providing groundwater samples and to Supelco for technical support.

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