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Determination of aliphatic amines in water by liquid chromatography using solid-phase extraction cartridges for preconcentration and derivatization

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Bond Elut C_{18} solid-phase extraction cartridges were used for preconcentration and pre-column derivatization with 3,5-dinitrobenzoyl chloride (DNB) of aliphatic amines in water. Conditions for analyte preconcentration and derivatization (including the volume of sample, concentration of reagent, time of reaction and pH) were investigated, using ethylamine, isopropylamine and dimethylamine as model compounds. On the basis of these studies, a rapid and sensitive method for the determination of aliphatic amines in water is presented. The analytes are retained and purified on the cartridges and then derivatized and desorbed by drawing in succession the DNB solution and acetonitrile. The collected extracts are subsequently chromatographed in a Hypersil ODS C_{18} column using acetonitrile–water for elution. The DNB derivatives are monitored at 230 nm. The method provides satisfactory reproducibility and linearity within the 0.050–1.0 mg l^{-1} concentration interval, the limits of detection being 2–5 μg l^{-1}. Analyte recoveries were in the 70–102% range, whereas the conversion yields compared with those observed for the solution derivatization were in the 79–107% range. The total analysis time (sample treatment plus chromatography) was about 15 min. The method was applied to the determination of ethylamine, isopropylamine and dimethylamine in tap and river waters.

Introduction

Short-chain aliphatic primary and secondary amines are widely distributed in the environment because they are used in several chemical and manufacturing industries. Aliphatic amines are also common components of biological systems as degradation products of organic materials such as amino acids and proteins. In addition to hygienic problems due to the stinging smell, these compounds may be hazardous to human health as they are sensitizers and irritants to the skin, eyes, mucous membranes and respiratory tract. In addition, they can react with certain nitrogen-containing compounds to form nitrosamines, which are potentially carcinogenic substances. Consequently, there is increasing interest in the determination of aliphatic amines in various aqueous matrices.

Chromatographic techniques are generally used for the determination of aliphatic amines in aqueous media and a number of methods using either gas chromatography (GC) or liquid chromatography (LC) are available. A common feature of such methods is the requirement for analyte preconcentration, as these compounds are usually present at trace levels. Analyte enrichment is usually accomplished by using extraction techniques. However, unlike many other compounds of environmental relevance, preconcentration of aliphatic amines by extraction methods is a difficult task, owing to their high polarity and water solubility. For this reason, chemical derivatization within the aqueous sample is often used to facilitate the extraction of the analytes into an organic solvent [liquid–liquid extraction (LLE)].[1–4] Derivatization is also a common step in the preconcentration of the analytes by solid-phase extraction (SPE).[5,6]

In LC methods, chemical derivatizations are also aimed at enhancing the sensitivity because aliphatic amines have no UV or fluorescence properties. Methods using derivatization usually provide improved sensitivity compared with those using electrochemical detection or indirect detection.[7,8] In this respect, several fluorogenic and UV reagents have been proposed, including *o*-phthalaldehyde (OPA), 9-fluorenylmethyl chloroformate, fluorescamine, 1-naphthyl isothiocyanate and phenyl isothiocyanate, among others.[2,5] In addition, (pre-column) derivatization may also be applied to improve the performance of the chromatographic step, as most aliphatic amines show poor chromatographic properties in typical LC systems (*e.g.*, under reversed-phase conditions). Different strategies and conditions combining derivatization and extraction, which allow the quantification of aliphatic amines at sub-ppm levels, have been proposed. However, in most of these methods, particularly in those using LLE, major drawbacks are the substantial sample handling involved and the long analysis times. This is because long reaction times are often necessary to achieve suitable reaction yields (even in methods using high reaction temperatures). In most of these procedures the reaction times are typically 20–60 min, but reaction times up to 180 min have also been reported.[9] Moreover, multiple LLE of the derivatized analytes is required to achieve acceptable recoveries and then large volumes of the organic solvents must be evaporated before the chromatographic step. In addition, in some instances the excess of reagent must be re-extracted or eliminated.[1,4] As a result, the analysis is very tedious and time consuming. The analysis can be simplified by using SFE for preconcentration of the derivatives previously formed within the aqueous samples,[5] but even in such methods some drawbacks persist (*e.g.*, long reaction times).

In recent years, we have developed a simple methodology for the determination of amines in which clean-up, enrichment and derivatization of the analytes are performed in SPE cartridges.[10,11] The method is based on trapping the analytes on the sorbent material. The analytes are then purified with a suitable solvent and derivatized by passing the derivatization reagent solution through the cartridges. After a given reaction time the excess of reagent is eliminated (if required) by flushing the cartridge with a suitable solvent and finally the analytes are desorbed and collected for chromatography. If large sample volumes are not needed, derivatization can also be performed in

a trapping column connected on-line with the analytical column and, therefore, the analysis can be fully automated.[12–14] The possibility of using the proposed derivatization technique has been illustrated for various compounds containing an amino group, including some aliphatic amines.[11] Regarding its application to real samples, the proposed methodology has been applied to the determination of amphetamines in pharmaceuticals and biofluids[10–14] and to the determination of some biogenic amines in biofluids.[15]

The aim of this work was to develop a simple and sensitive method for the determination of aliphatic amines in aqueous matrices using the solid support assisted derivatization method. The UV reagent 3,5-dinitrobenzoyl chloride (DNB) was used for derivatization. Although, to our knowledge, this reagent has never been used for aliphatic amines, it provided excellent conversion yields of amphetamines in very short reaction times and under mild conditions.[10] The reliability of the proposed procedure was investigated using the primary amines ethylamine and isopropylamine and the secondary amine dimethylamine as model compounds. In a previous study, conditions for the solution derivatization of the analytes were optimized. These conditions were then adapted to derivatization on C_{18} SPE cartridges. Finally, the method was applied to the determination of ethylamine, isopropylamine and dimethylamine in real water samples.

Experimental

Apparatus

The chromatographic system used consisted of a quaternary pump (1050 Series, Hewlett-Packard, Palo Alto, CA, USA) and an automatic sample injector (Hewlett-Packard 1050 Series) with a sample loop injector of 100 µl. The volume of sample injected was 20 µl. For detection, a UV detector (Hewlett-Packard 1100 Series) was used. The detector was linked to a data system (Hewlett-Packard HPLC Chem Station) for data acquisition and storage. Detection was carried out at 230 nm.

Reagents

All reagents were of analytical-reagent grade. Ethylamine and dimethylamine were obtained from Sigma (St. Louis, MO, USA), isopropylamine from T. J. Baker (Deventer, The Netherlands) and 3,5-dinitrobenzoyl chloride from Aldrich (Steinheim, Germany). Methanol and acetonitrile were of HPLC grade (Scharlau, Barcelona, Spain). Boric acid and sodium hydroxide were obtained from Panreac (Barcelona, Spain) and acetic acid from Probus (Badalona, Spain).

Preparation of solutions

Stock standard solutions of the analytes (1.0 g l^{-1}) were prepared in water. Working standard solutions of the analytes were prepared by dilution of the stock standard solutions with water. Water was de-ionized and filtered through 0.45 µm nylon membranes (Teknokroma, Barcelona, Spain). All solutions were stored in the dark at 2 °C.

Columns and mobile phases

For assays using solution derivatization, a LiChrospher 100 RP_{18} column, 125 × 4 mm id, 5 µm film thickness (Merck, Darmstadt, Germany) was used. The mobile phase was acetonitrile–water (40 + 60 v/v) at a flow rate of 1.0 ml min^{-1}. For assays using solid support assisted derivatization, the column was Hypersil RP_{18}, 250 × 4 mm id, 5 µm film thickness (Merck). The mobile-phase was also acetonitrile–water (40 + 60 v/v) but the flow rate was 0.75 ml min^{-1}. All solvents were filtered through 0.45 µm nylon membranes (Teknokroma) and de-gassed with helium before use.

Solution derivatization

For the DNB method, 250 µl of the sample and 125 µl of 0.05 M borate buffer were placed in 2 ml glass vials, then 250 µl of the solution of DNB were added and the resulting mixture was left to react for a defined period of time. Finally, aliquots of 20 µl of the reaction solutions were injected into the chromatograph. The DNB solutions were prepared daily by dissolving the pure compound in acetonitrile. Borate buffer was prepared by dissolving boric acid in water. Then the pH was adjusted to appropriate values by adding 10% m/m NaOH. Each sample was derivatized in triplicate. Derivatizations were performed at ambient temperature.

Derivatization into solid-phase extraction cartridges

For analyte enrichment and derivatization, 1 ml Bond Elut C_{18} cartridges containing 100 mg of packing (Varian, Harbor City, CA, USA) were used. The cartridges were conditioned using 2 ml of methanol followed by 1 ml of 0.05 M borate buffer of pH 10.0. Next, variable volumes of the samples were drawn through the cartridges. The cartridges were then washed with 2 ml of 0.05 M borate buffer and dried with air. Derivatizations were then performed by drawing the DNB reagent or by drawing in succession an aliquot of the reagent and an aliquot of acetonitrile. Then the extracts were collected in 2 ml glass vials. Finally, 20 µl aliquots of the extracts were injected into the chromatograph. All operations were effected by flushing the cartridges with air using a 10 ml syringe. The DNB reagent and the buffer solutions were prepared as described below. Each sample was derivatized in triplicate. Derivatizations were performed at ambient temperature.

Analysis of real water samples

Water collected from the Turia river was filtered through 0.45 µm nylon membranes (Teknokroma) in order to remove any particulate matter, whereas tap water was processed directly. The samples were spiked with the stock standard solutions of the individual analytes to give concentrations of 0.05–1.0 mg l^{-1}. Aliquots of 5.0 ml of the samples were subjected to the optimized preconcentration–derivatization procedure. Each sample was derivatized in triplicate.

Results and discussion

In a first series of experiments, the conditions for the solution derivatization and subsequent chromatographic separation of the three amines under investigation were optimized. In this study, a LiChrospher RP 100 column, 125 × 4 mm id, 5 µm film thickness, was used. A mobile phase consisting of acetonitrile–water (60 + 40 v/v) at a flow rate of 1.0 ml min^{-1} was found to provide satisfactory separation of the DNB derivatives of the analytes with the minimum analysis times. Under such conditions, the retention times for the dimethylamine, ethylamine and isopropylamine DNB derivatives were 2.85, 3.69 and 5.27 min, respectively. The exccess of reagent eluted at a very short retention time (1.2 min) and therefore did not interfere with the compounds of interest.

Solution derivatization

According to previously published papers,[10,16] the main parameters affecting derivatizations with DNB (provided that the reactions are carried out in a medium containing acetonitrile) are the concentration of reagent, the time of reaction and the pH of the medium. Consequently, these parameters were optimized. In this study, standard solutions containing 10.0 mg l^{-1} of each of the analytes were assayed. First, different concentrations of DNB within the 5–20 mM concentration range were assayed. The reaction time was 2.0 min and the pH was adjusted to 9.5. It was observed that the peak areas of the analyte increased drastically as the concentration of reagent was increased from 5 to 10 mM. A further increase to 20 mM resulted in a moderate improvement. Concentrations higher than 20 mM could not be used, as an intense peak corresponding to the excess of reagent interfered with the measurement of the first-eluting ethylamine DNB derivative. Therefore, 20 mM DNB was selected for further work. With this concentration of reagent the reaction proceeded very rapidly and no significant differences in analyte responses were observed within the 0–10 min time interval. Moreover, the derivatives formed were found to be stable for at least 1 h. The effect of different borate buffers with pH ranging from 9.0 to 10.5 was also studied. The conversion yields increased up to pH 10.0 but further pH increases did not significantly increase the signals. Consequently, the general procedure for the solution derivatization was as follows: samples (250 µl) were mixed with 125 µl of 0.05 M borate buffer (pH 10.0) and with 125 µl of 20 mM DNB and the resulting mixture was immediately injected on to the LC column. Analyte responses obtained under such conditions were assumed to produce 100% conversion yields of the analytes.

Analyte enrichment

Based on previous experiments with different types of amines, Bond Elut C_{18} cartridges were selected for preconcentration and purification of the analytes.[11] The cartridges were conditioned with 2.0 ml of methanol and reconstituted with 1.0 ml of 0.05 M borate buffer (pH 10.0). Different sample volumes ranging from 1.0 to10.0 ml were tested. The concentrations of the analytes in these samples were varied from 10.0 to 1.0 mg l^{-1} in such a way that the amount of the analyte processed was the same in all the assays. Therefore, the DNB to analyte concentration ratio was kept constant thorough the experiment. After sample loading, the analytes were purified by flushing with 2.0 ml of 0.05 M borate buffer (pH 10.0). Finally, the analytes were desorbed from the cartridges and subjected to the solution derivatization procedure described below.

It is interesting that the analytes should be desorbed from the cartridges in the minimum volume of solvent in order to achieve the maximum preconcentration factor. We tried different solvents for desorption: methanol, acetonitrile, 0.1 M acetic acid and different acetonitrile–water and acetonitrile–0.1 M acetic acid mixtures. The best results were obtained by flushing the cartridges with 0.1 M acetic acid. The minimum volume of acetic acid required to achieve quantitative desorption of the analytes was 2.0 ml. However, owing to the low pH of the collected extracts, derivatization under the previously described conditions resulted in very low analyte responses. For this reason, the derivatization procedure was modified as follows: aliquots of the collected extracts (250 µl) were neutralized with 125 µl of 0.1 M NaOH before adding the borate buffer (125 µl) and the reagent (125 µl). The percentages of analytes recovered were calculated by comparing the peak areas with those obtained for solutions containing an equivalent amount of amines and directly derivatized (taking into account the dilution produced by the addition of the NaOH solution).

Fig. 1 shows the recoveries obtained for the different volumes of sample assayed. As can be observed from this figure, dimethylamine was quantitatively retained in all instances. However, as the sample volume increased, losses by breakthrough were observed for the other amines, especially isopropylamine. As a compromise, a sample volume of 5.0 ml was selected for further work. The percentages of analyte recovered under the described conditions are listed in Table 1. It should be noted that larger sample volumes could most probably be processed by using C_{18} SPE cartridges with a higher loading capacity. However, the volumes of reagent and acetonitrile required to derivatize and desorb the analytes with suitable reproducibility would also be higher, thus resulting in a limited advantage.[10]

Derivatization in SPE

Chromatographic analysis of samples derivatized in SPE materials were performed in a Hypersil ODS C_{18} column (250 × 4.6 mm id) because in this way the analytes were totally resolved from peaks produced by the SPE material.

The usual treatment when using SPE cartridges for preconcentration and derivatization is to draw an aliquot of the reagent through the cartridges containing the trapped and purified analytes. Then the analytes and reagent are allowed to react for a defined period of time and the cartridges are flushed with a suitable solvent in order to eliminate the excess of reagent. The derivatives formed are finally desorbed and injected into the chromatograph. In the present instance and according to the results in the previous sections, the general procedure can be simplified. Since the reaction proceeded very rapidly and unreacted DNB did not interfere in the chromatographic separation, derivatization and desorption were effected simply by flushing the cartridges with a volume of the DNB

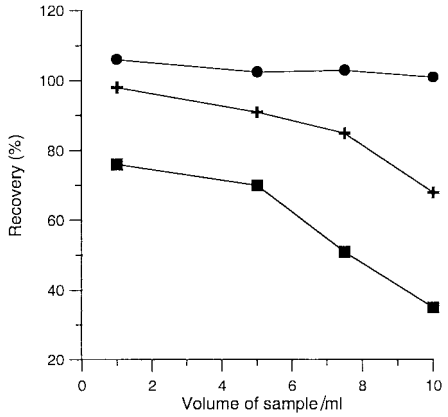

Fig. 1 Effect of the volume of sample on analytes recoveries after preconcentration into the SPE cartridges: (●) dimethylamine; (+) ethylamine; and (■) isopropylamine. For other experimental details, see text.

Table 1 Efficiencies of the preconcentration, preconcentration plus derivatization and derivatization steps. The concentration of each analyte in the samples was 1.0 mg l^{-1} (n = 3)

Compound	Recovery after preconcentration ± standard deviation (%)	Recovery after preconcentration and derivatization ± standard deviation (%)	Estimated conversion yield ± standard deviation[a](%)
Dimethylamine	102 ± 5	81 ± 5	79 ± 6
Ethylamine	91 ± 3	77 ± 6	85 ± 7
Isopropylamine	70 ± 4	75 ± 2	107 ± 7

[a] Calculated as the ratio (percentage after preconcentration and derivatization/percentage after preconcentration) × 100.

solution (prepared in acetonitrile). We observed that, for the SPE cartridges used in the present study, the minimum volume that provided reproducible results was 0.5 ml. In order to ensure that the derivatives were totally desorbed from the cartridges, derivatization and desorption were performed by flushing in succession 0.25 ml of the reagent and 0.25 ml of acetonitrile.

The efficiency of the proposed method was evaluated by calculating the percentages of the analytes in the collected extracts. These values were calculated by comparing the peak areas with those obtained for samples containing an equivalent concentration of amine and derivatized in solution.

As expected, the time elapsed between the addition of the reagent and the injection of the derivatives into the chromatograph had a negligible effect on the percentages. The concentration of the DNB reagent was also optimized. Different solutions of DNB with concentrations ranging from 10 to 25 mM were tested. As for the solution derivatization method, 20 mM DNB was found to provided the highest values and no improvement was observed for higher concentrations. It should be noted that washing the cartridges with borate buffer of pH 10.0 before adding the reagent ensured that the amines were in a suitable form for reaction. Therefore, the presence of buffer in the collected extracts was not necessary. No significant improvements in the percentages were observed when the pH of the buffer was adjusted to 10.5. Therefore, 0.05 M borate of pH 10.0 was also used for the derivatization into SPE cartridges.

Under the described conditions, the percentages of the analytes in the collected extracts (that is, after extraction and derivatization) were in the 75–81% range, as shown in Table 1. In this table are also listed the estimated reaction yields, which were calculated taking into account the percentages of the analytes after the preconcentration and purification step. The values obtained were in the 79–107% range, which means that the proposed procedure can be considered very effective. The estimated derivatization yield for isopropylamine was significantly higher than those observed for the other amines. These results suggest that derivatization in the SPE cartridge is more effective than the analogous solution derivatization. Similar behaviour was previously found for other amino compounds. This behaviour was explained by the fact that during derivatization, the reagent is concentrated into the solid material, thus resulting in very high reagent to amine concentration ratios, which seems to facilitate the reaction.[11,17] Typical chromatograms obtained for a blank (water) and a solution containing the analytes at a concentration of 0.75 mg l^{-1} under the proposed conditions are shown in Fig. 2.

Linearity, reproducibility and sensitivity studies

In order to evaluate the quantitative performance of the proposed method, standard samples containing the analytes in the 0.050–1.0 mg l^{-1} concentration range were assayed. According to the literature, this concentration interval is suitable for monitoring aliphatic amines in tap water and surface water.[2,5] The linearity was tested by analysing samples at six different concentrations within the studied interval. The results obtained are given in Table 2. Although significant intercepts were found for dimethylamine and isopropylamine, the residuals did not show any non-linear trend. Therefore, the calibration graphs can be considered linear over the concentration range studied. The accuracy and reproducibility were examined at different levels of concentration. As observed in Table 2, the method provided intra- and inter-day relative standard deviations (RSDs) ranging from 3 to 9% and from 6 to 12%, respectively. These values can be considered suitable at

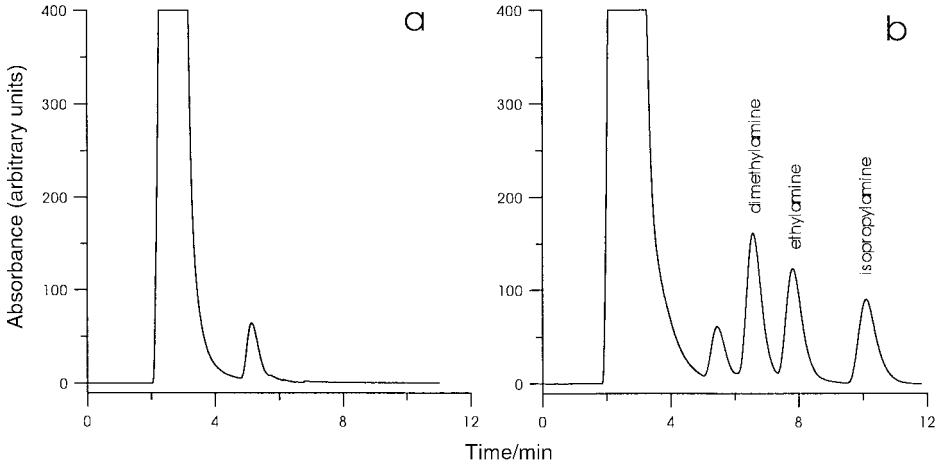

Fig. 2 Chromatograms obtained for (a) a blank (water) and (b) a standard sample containing 0.75 mg l^{-1} of each of the analytes, after preconcentration and derivatization of the analytes by the proposed procedure. For other experimental details, see text.

Table 2 Analytical data for determination of aliphatic amines

Compound	Linearity (n = 18) $y = ax + b$	Reproducibility, RSD (%) Intra-day (n = 3) 0.05 mg l^{-1}	0.50 mg l^{-1}	1.0 mg l^{-1}	Inter-day (n = 6) 0.05 mg l^{-1}	0.50 mg l^{-1}	1.0 mg l^{-1}	Limit of detection/ µg l^{-1}
Dimethylamine	$a \pm s_a$: 6698 ± 241 $b \pm s_b$: 576 ± 87 r^2 = 0.991	9	7	7	12	9	8	2
Ethylamine	$a \pm s_a$: 5823 ± 141 $b \pm s_b$: 126 ± 75 r^2 = 0.990	6	5	5	11	9	9	5
Isopropylamine	$a \pm s_a$:4070 ± 119 $b \pm s_b$: 285 ± 67 r^2 = 0.990	5	3	7	6	7	9	5

the tested concentration levels. The accuracy was also satisfactory (see Table 3).

The limits of detection (LODs) were estimated by analysing solutions of decreasing concentration of each analyte. Before analysing each sample, water was processed in order to detect possible contaminants and/or memory effects. The LODs were established as the concentration required to generate a signal-to-noise ratio of 3. The values obtained were 2 μg l^{-1} for ethylamine and 5 μg l^{-1} for isopropylamine and dimethylamine. Additional work would be required to confirm these values in order to make them acceptable for regulatory agencies.

Although less sensitive than methods using some fluorogenic reagents,[18] the proposed procedure provide similar LODs to other LC methods using UV reagents in combination with LLE/solvent evaporation methods;[2,3] the LODs are also comparable to those reported for assays involving derivatization followed by SPE.[5] The values are also of about the same order of magnitude as those given by GC methods using special techniques for the injection of large sample volumes.[19,20]

Application to real water samples

The described method was applied to the analysis of tap and river waters. Samples were spiked to produce three different concentrations, 0.05, 0.25 and 0.75 mg l^{-1}. Unspiked samples were also processed. Examples of the chromatograms obtained are shown in Fig. 3. As can be observed, the chromatograms obtained for the spiked samples are very similar to those obtained for standard solutions and no interferences were observed from other compounds potentially present in the samples (*e.g.*, ammonia). On the other hand, none of the amines investigated were detected in the samples tested.

The concentrations calculated for the spiked samples by using the calibration equations obtained for the standard samples are summarized in Table 3. As can be seen, the results are comparable to those observed for standard solutions containing the same concentrations of the analytes. This means that the performance of the proposed preconcentration–derivatization procedure was similar for all types of samples tested.

Conclusions

The results of this work show that the use of SPE cartridges for enrichment and derivatization is a valid alternative for the determination of aliphatic amines in water samples. The proposed procedure is very simple and rapid, as the reaction with DNB proceeds very rapidly and time-consuming operations typically involved in previously described LC methods (*e.g.*, elimination of unreacted reagent or solvent evaporation) are not necessary. The time required for analysis (sample pretreatment plus chromatography) is about 15 min.

Conventional SPE cartridges packed with C_{18} sorbent provided suitable recoveries of the analytes for a sample volume of 5.0 ml. Sample volumes > 5.0 ml resulted in breakthrough of some of the analytes, which is a limitation of the method. Nevertheless, the proposed procedure can be applied with satisfactory accuracy and reproducibility to the determination of aliphatic amines at low- to sub-ppm concentrations. No significant differences were observed in the quantification of the analytes between the different types of samples tested.

Table 3 Accuracy for the determination of aliphatic amines (n = 3)

Compound	Concentration added/mg l^{-1}	Concentration determined ± standard deviation/mg l^{-1}		
		Standard samples	Tap water	River water
Ethylamine	0.05	0.047 ± 0.003	0.058 ± 0.005	0.059 ± 0.005
	0.25	0.25 ± 0.02	0.23 ± 0.01	0.22 ± 0.02
	0.75	0.77 ± 0.05	0.78 ± 0.03	0.73 ± 0.05
Dimethylamine	0.05	0.054 ± 0.004	0.059 ± 0.004	0.050 ± 0.02
	0.25	0.25 ± 0.01	0.25 ± 0.02	0.24 ± 0.02
	0.75	0.78 ± 0.04	0.80 ± 0.07	0.75 ± 0.07
Isopropylamine	0.05	0.052 ± 0.002	0.052 ± 0.003	0.055 ± 0.005
	0.25	0.25 ± 0.02	0.26 ± 0.01	0.27 ± 0.02
	0.75	0.74 ± 0.05	0.73 ± 0.06	0.79 ± 0.05

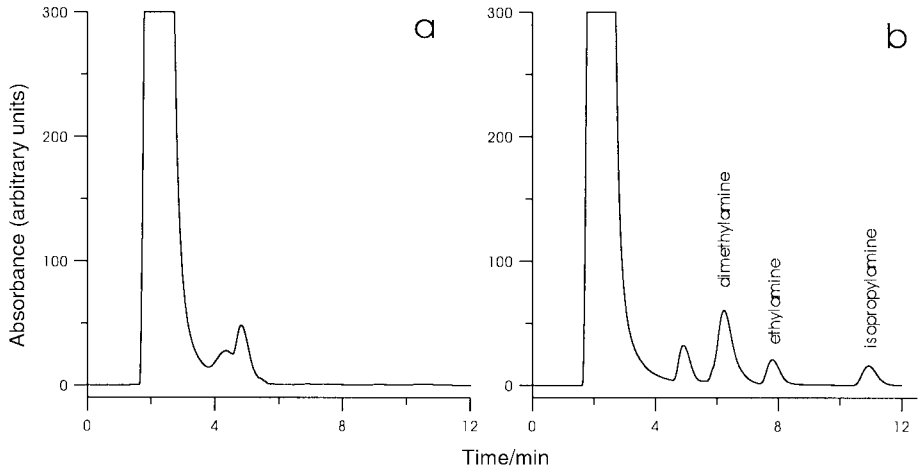

Fig. 3 Chromatograms obtained for (a) blank river water and (b) river water spiked with 0.25 mg l^{-1} of each of the analytes, after preconcentration and derivatization of the analytes by the proposed procedure. For other experimental details, see text.

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