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Preconcentration and dansylation of aliphatic amines using C_{18} solid-phase packings
Application to the screening analysis in environmental water samples

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Abstract

Precolumn preconcentration and derivatization on solid sorbents (Bond Elut C_{18} solid-phase extraction cartridges) of low-molecular-mass aliphatic amines in water samples have been performed using dansyl chloride (Dns-Cl) as derivatization reagent. Conditions for analyte preconcentration and derivatization such as volume sample, reagent concentration, time, pH and temperature reaction were optimised. On the basis of these studies a rapid and sensitive method for screening of aliphatic amines in waters is presented. Up to volumes of 5 ml, samples are drawn through the sorbent, the analytes retained are dansylated at basic pH, at 100 °C for 10 min or 85 °C for 15 min. The derivatized analytes are desorbed with 0.5 ml of acetonitrile. Twenty μl of the collected extracts are chromatographed in a Hypersyl ODS C_{18} column using an acetonitrile–imidazole (pH 7) gradient for elution. Seven amines and ammonium were separated within 9 min. The Dns derivatives were monitored at 333 nm with UV detection and at $\lambda_{excitation}$=350 nm and $\lambda_{emission}$=530 nm with fluorescence detection. The different signals are compared. Dynamic ranges from 10 to 250 μg/l and limits of detection at the microgram-per-litre level and relative standard deviations from 2 to 15% were obtained for all the amines. The total analysis time (sample treatment plus chromatography) was less than 25 min. The method was applied to determination and screening analysis of these analytes in real environmental water samples.

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1. Introduction

Low-molecular-mass aliphatic amines like methylamine, dimethylamine, ethylamine, diethylamine, *n*-propylamine, *n*-butylamine, are important intermediates in chemical and pharmaceutical industries. In addition to their industrial application, aliphatic amines may occur as biodegradation products of organic material like proteins and amino acids or other nitrogen-containing compounds. It is well known that aliphatic amines can react with nitrite, forming carcinogenic nitrosamines [1]. The moni-

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toring of alkylamines is of considerable interest as most of them are toxic, sensitizers and irritants to the skin, mucous membrane and respiratory tract. The European legislation fixes a content value of 0.5 and 1 mg l^{-1} for ammonium and nitrogen Kjeldahl for consumption waters, respectively, and between 15 and 85 mg l^{-1} for waste waters. Up to now, little information has been available on the occurrence of aliphatic amines in industrial waste waters and in surface waters.

Gas chromatography (GC) [2–6] and liquid chromatography (LC) [7–13] are the techniques generally used for the determination of these analytes. Table 1 summarises some of the procedures described in the literature for amine determination in water samples at low levels by using GC and LC. Due to the chemical properties of the aliphatic amines, and the low levels in the samples, most of the procedures described require sample enrichment techniques such as liquid–liquid extraction (LLE) or more recently solid-phase extraction (SPE) or solid-phase microextraction (SPME), often combined with pre- or post-column derivatization in HPLC procedures. Headspace SPME has been used in GC procedures [2,3].

Table 1
Conditions for derivatization of aliphatic amines

Reagent	Sample	Sample treatment	Derivatization			Detection	Recovery (%)	LOD	Refs.
			Time (min)	*T* (°C)	Medium				
GC procedures									
Pentafluobenzyladehyde	Waste water	Directly derivatized and followed by SPME and headspace	15–30	80	Water solution, pH 10	FID		0.4–26 ppb	[2]
	Waste water	SPME and headspace (30 min)	–	–	–	NPD or MS		3–56 ppb	[3]
	Water	Acidic samples	–	–	–	NPD		12–40 ppb	[4]
	Water	Acidic samples and headspace (80 °C and 15 min)	–	–	–	NPD		0.2–20 ppb (2° amines) 0.01–3 ppm (1° amines)	[5]
Benzenosulfonyl chloride	Waste and surface water	Directly derivatized and extracted with CH_2Cl_2	30	Room	Basic medium	MS		0.1 ppb	[6]
HLPC procedures									
N-Hydroxysuccinamidyl fluorescein-*O*-acetate (SIFA)	Standards	–	30	45	Borate buffer pH 8.5	Fl			[3]
3,5-Dinitrobenzoyl chloride (DBN)	Water	Derivatization on C_{18}-SPE cartridges	2	Room	Borate buffer pH 10	UV	79–107	2–5 ppb	[4]
Acridine-9-acetyl-*N*-hydroxysuccinimide	Waste water	Directly derivatized	10	50	Borate buffer pH 8–9	Fl	94–115	17–87 fmol	[5]
Dansyl chloride (DNS-Cl)	Water	On-line precolumn enrichment IRC-50 column	On-line	65	Borate buffer pH 11	CL	93–103	15–300 ppb	[6]
Phenylisothiocyanate	Domestic, surface and river water	Directly derivatized Clean-up of the derivatized solution (C_{18})	10+5	40	Carbonate buffer	UV		0.2–0.6 ppb	[7]
4-(5′,6′-Dimethoxybenzothizolyl) phenylisothiocyanate	Standards		30	80	NH_4OH	FL			[8]
Fluram	Water	Amberlite CG-120, collected in HCl, evaporated and redissolved in 1 ml borate buffer			pH 10	UV		0.02–1.2 ppb	[9]
9-Fluorenylmethylchloroformate (FMOC)	Water	Derivatization on C_{18}-SPE cartridges	2	Room	pH 10	FL	54–107	0.25–5 ppb	[20]
Dansyl chloride (DNS-Cl)	Waste water	Derivatization on C_{18}-SPE cartridges	15	85 °C	Carbonate buffer pH 9.5	UV-Fl	73–120	3–15 ppb	This work

FID, flame ionization detection; NPD, nitrogen–phosphorus detection; FL fluorescence detection; and CL, chemiluminescence detection.

Pan et al. [2] reported that compared to direct SPME, derivatization–SPME lowered limits of detection (LODs) by three orders of magnitude for analysis of amines in air and aqueous solutions. For HPLC procedures, enrichment by SPE has proved a more efficient choice than LLE. Despite the advantages of SPE, it has scarcely been used to preconcentrated low-molecular-mass amines from water samples. Usually these analytes are extracted with Amberlite CG-120 resin [13] or cation-exchange sorbents [14] while the derivatives can be extracted with C_{18} sorbent [11]. Different strategies and conditions combining derivatization and extraction, which allow the quantification at sub-ppm levels, have been proposed by Cobo and Silva [10]. Chemical derivatization in solution has long been accepted as an effective step [15], however as can be seen in Table 1, there are many drawbacks such as substantial sample handling, long reaction times, often high-temperatures, solvent consumption, etc. The use of SPE for extraction of the derivatives [11], previously formed on solution can solve some problems and with high concentration factors can provide similar or even lower detection limits than GC–MS with more affordable instrumentation [6]

However those problems directly related with the derivatization procedure still remain (sample handling, lack of automation, etc.). One possible solution to the problems of extra steps and interferences is to carry out analytical derivatizations on solid-phases [16].

In that direction, this research group has developed a simple methodology in which all steps of sample preparation, i.e., extraction, concentration, derivatization and transfer to the chromatographic system, are integrated in one device, and all steps can be performed on-line. The method is based on trapping the analytes on the sorbent. The analytes are then purified with a suitable solvent and derivatized by flushing the reagent through the cartridges. The analyte and the reagent are made to react for a given period of time. Then the reagent excess can be removed (if required) by flushing, the cartridges with an appropriate solvent. Finally, the derivatives are desorbed and collected for further processing [17] or transferred to a primary column in an on-line system [18,19].

We have recently demonstrated the utility of this methodology using the UV-reagent 3,5-dinitrobenzoyl chloride (DNB) and the aliphatic amines, ethylamine, isopropylamine and dimethylamine [8]. Also the support assisted derivatization of some aliphatic amines with 9-fluorenylmethyl chloroformate (FMOC) has been studied [20]. This methodology has been recently applied by Shangguan et al. [21] to the determination of amino acids and peptides with FMOC and silica sorbents.

This paper extends our methodology to the screening analysis of alkylamines in water samples by using dansyl chloride (Dns-Cl) as derivatization reagent. Derivatizations using dansyl chloride are generally carried out in aqueous acetone saturated with sodium carbonate with long reaction times at relatively high-temperatures and in many instances, extraction of the derivatized analytes to remove unreacted reagent is needed. However, it is interesting to study simplified dansylation procedures due to the sensitive detection limits that can be achieved. These detection limits can be improved because the dansylated derivatives provide chemiluminescence with oxalic acid bis(2,4,6-trichlorophenyl ester) ($TCPO/H_2O_2$). A rapid, straightforward method for preconcentration and derivatization followed by HPLC separation and detection of low-molecular-mass amines in environmental water at microgram-per-litre levels is proposed. Seven aliphatic amines and ammonium were systematically studied—for the first time in our knowledge—in order to obtain the best analytical conditions for retention and dansylation on Bond-Elut C_{18} cartridges. The HPLC separation conditions have been optimised. Dns derivatives were monitored at 333 nm with UV detection and at $\lambda_{excitation}$=350 nm and $\lambda_{emission}$=530 nm with fluorescence detection. Finally, the method was applied to the determination or screening of the amines studied in real water samples.

2. Experimental

2.1. Apparatus

The chromatographic system that was used consisted of a quaternary pump equipped with an automatic injector (1050 series) (Hewlett-Packard, Palo Alto, CA, USA) with a sample loop injector of

100 μl, and a high-pressure six-port valve (Rheodyne model 7000). The volume of sample injected was 20 μl. A fluorescence detector (Hewlett-Packard, 1050 Series; flow cell, 5 μl) was employed. The chromatographic signal was recorded at excitation wavelength of 350 nm, emission of 530 nm. For UV detection, a UV detector (Hewlett-Packard 1100 Series) was used, and the detection was carried out at 333 nm. The detectors were coupled in series and linked to a data system (Hewlett-Packard HPLC ChemStation) that was used for data acquisition and storage.

All the assays were carried out at room temperature.

2.2. Reagents

All the reagents were of analytical grade. Acetonitrile, methanol and acetone (Scharlau, Barcelona, Spain) were of HPLC grade. Methylamine (MA), ethylamine (EA), dimethylamine (DMA), *m*-propylamine (*n*-PRA), butylamine (BA), diethylamine (DEA), pentylamine (PeA), hexylamine (HA), 1,7-diamineheptane (internal standard) and dansyl chloride were obtained from Sigma (St. Louis, MO, USA). Sodium hydrogencarbonate (Probus, Badalona, Spain) and sodium hydroxide (Panreac, Barcelona, Spain) were also used. Imidazole (99%) was from Sigma, and the pH was adjusted to 7 with diluted chlorhydric acid.

2.3. Columns and mobile-phases

Bond Elut C_{18} 200 mg (Varian, Harbor City, CA, USA) cartridges, were used to retain the analytes and later to perform an off-line derivatization.

A C_{18} LiChrospher (125×4 mm I.D., 5-μm film thickness) (Merck, Darmstadt, Germany) column was used as an analytical column for separation of the amine derivatives. An acetonitrile–imidazole solution (1 m*M*, pH 7.0) (50:50, v/v) mixture in gradient elution mode was used as eluent at flow-rate of 1 ml min^{-1}. Gradient acetonitrile–imidazole was used, (50:50) at zero time, (90:10) at 9 min, and (50:50) at 10 min. All the solvents were filtered with a 0.45-μm nylon membrane (Teknokroma, Barcelona, Spain) and degassed with helium before use.

2.4. Preparation of solutions

Standard solutions of the amine compounds were prepared by dissolving the pure compounds in water (1000 μg/ml). Working amine solutions were prepared by diluting the standard solutions in water. Dns-Cl solution (12.5 m*M*) was prepared by dissolving the pure compound in acetone. A mixture of Dns-Cl in acetone–carbonate solution (33.3 m*M*) pH 9.5 (2:3, v/v) was prepared daily. All solutions were stored in the dark at 4 °C. The dynamic ranges assayed were 0.3–5 mg l^{-1} and 0.01–0.25 mg l^{-1} by processing 1 and 5 ml, respectively.

2.5. Solution derivatization

The amines were derivatized according to the method described by Marcé et al. [22]. To 0.1 ml amine, 1 ml of 10 m*M* carbonate buffer (pH 9) and 0.9 ml of acetone containing 1.0 m*M* dansyl chloride were added successively, and the mixture was incubated at 70 °C for 10 min. An aliquot (20 μl) of the solution was injected into the HPLC system.

2.6. Extraction and derivatization into solid-phase supports

Solid-phase extraction cartridges were conditioned by drawing with 1.0 ml of methanol, followed by 1.0 ml of carbonate buffer (pH 12). Variable volumes of standard solution (1–25 ml) or 1–5-ml samples were then transferred to the cartridges. A total of 0.5 ml of 5 m*M* reagent [Dns-Cl in acetone–hydrogencarbonate buffer solution (pH 9.5, 20 m*M*), 2:3, v/v] was flushed through the cartridges, which were incubated at 85 °C for 15 min. After that, 1 ml of saturated carbonate solution carrying 0.1 *M* NaOH was flushed through the cartridges in order to eliminate residual reagent. The cartridges were dried under vacuum. The derivatives formed were desorbed from the cartridges with 0.5 ml of acetonitrile. The final amine concentrations were in the range 0.3–5 mg/ml for all the volumes processed. A 20-μl aliquot of the resulting mixture was finally injected into the chromatographic system.

2.7. Analysis of real water samples

The method was tested with water samples with

unknown ammonium and amines concentration. The environmental water samples were named: S1, irrigation ditch sample; S2, lake water sample; S3, tap water; S4, residual water from a factory. Water samples were collected, passed through a 0.45-μm filter and acidified to pH 2 with HCl.

Previously to the analysis, the samples were alkalised with NaOH to pH 10.5. The samples were spiked with the stock standard solutions of the individual analytes to give concentrations in the range of 0.1–2.5 mg l^{-1}. 1,7-Diaminoheptane was included in the sample as I.S. at a concentration level of ca. 1.51 mg l^{-1}.

Sample volumes of 5 ml or 0.2 (diluted to 1 ml) were placed into conditioned C_{18} cartridges. Then the analytes were derivatized in the solid-phase extraction cartridges as described above. The percent of analyte recovered after clean-up plus derivatization was calculated by comparing the peak area obtained for a particular assay with those obtained for standard solutions containing an equivalent amount of analyte.

3. Results and discussion

3.1. Solution derivatization

In order to have a reference for the derivatization reaction, amines were derivatized according to the conditions described by Marcé et al. [22], which are similar to that described by Cobo and Silva [10]. The mixture of amine, buffer and reagent was heated at 70 °C for 10 min. The response obtained for the different amines were assumed to produce 100% conversion yields of the analytes. Heating the reaction mixture destroyed the reagent excess and no reagent interference was obtained at the retention time of the analytes. The RSDs obtained under the described conditions are listed in Table 2.

3.2. Preconcentration and derivatization on C_{18}

Based on previous studies with different types of amines, Bond Elut C_{18} cartridges were selected for preconcentration and purification of the analytes [17]. The cartridges were conditioned with 1.0 ml of methanol followed by 1.0 ml of carbonate solution at pH 12 (10 m*M*). A 1-ml sample containing amine at 1 mg l^{-1} was passed through the cartridges (C_{18}, 200 mg). The different steps involved in this methodology were optimized. The retention efficiency was studied, and no amine elution was observed by passing the sample (1 ml) or by passing the reagent through the column. Although usually the Dns-Cl is prepared in acetone, in this case a mixture Dns-Cl dissolved in acetone–carbonate buffer 33.3 m*M*, pH 9.5 (2:3, v/v), was used in order to prevent amine elution. Based on previous studies the reagent concentration selected was 5 m*M* [17].

Table 2
Comparison of recovery by performing solution derivatization and C_{18} SPE derivatization

Analyte	Solution derivatization RSD (%)	SPE C_{18} derivatization	
		Recovery (%)	RSD (%)
NH_4^+	25	(320±70)	22
Methylamine	25	(123±9)	7
Ethylamine	26	(135±14)	10
N-Propylamine	20	(146±16)	11
Butylamine	15	(130±16)	12
Diethylamine	17	(160±20)	13
Pentylamine	15	(133±11)	8
Hexylamine	14	(163±19)	12

Other parameters involved in the reaction such as pH of the medium, time of reaction and temperature were optimised. The reaction was carried out at basic pH, and the influence of this parameter was studied in the range 8.5–11.4 (Fig. 1). By performing the reaction at room temperature and a reaction time of 30 min, the conversion yields increased up to pH 9.5. Further pH increases did not significantly increase the signals.

In order to increase reaction efficiency, the temperature and time reaction were studied. The cartridges were heated in an oven at different conditions. Studies at 85 °C and time ranged from 0 to 60 min, and studies ranged from room temperature to 100 °C at 15 min were performed. As can be seen in Fig. 2 by increasing time and temperature the reaction efficiency increases. We selected as optimum condition 10 or 15 min at 100 or 85 °C, respectively. No degradation products were observed. The reaction products were desorbed from the cartridges with the minimum solvent volume (1 or 0.5 ml of acetonitrile) in order to achieve the maximum preconcentration factor. No differences in recoveries were obtained between using 0.5 or 1 ml

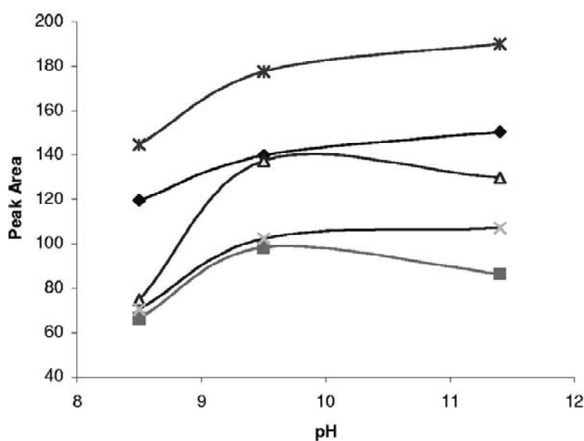

Fig. 1. Effect of the pH of the reaction on the analytical signal. Peak area versus derivatization pH. (♦) Methylamine, (□) ethylamine, (▲) *N*-propylamine, (×) butylamine, (*) pentylamine. Conditions: Dns-Cl 5 m*M*, borate buffer 20 m*M*, amine concentration 5 mg l^{-1}, room temperature and 30 min reaction time.

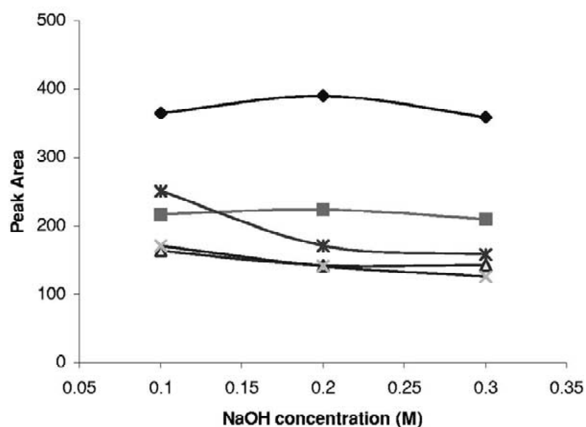

Fig. 3. Effect of the clean-up on the analyte signal. Peak area versus NaOH concentration (*M*) added to the clean-up solution. (♦) Methylamine, (□) ethylamine, (▲) *N*-propylamine, (×) butylamine, (*) pentylamine. Conditions: Dns-Cl 5 m*M*, borate buffer 20 m*M*, amine concentration 5 mg l^{-1}, pH 9.5, T=85 °C and 15 min reaction time.

solvent. However, if it is not necessary to perform sample concentration, 1 ml is the volume recommended.

The extracts were injected into a HPLC chromatographic system. The hexylamine determination by UV detection presented difficulties due to coelution together with the reagent excess. In order to solved this problem, a washing step after derivatization was included. Passing through the cartridges 1 ml of saturated carbonate solution, containing NaOH eliminated, the reagent excess. The NaOH concentration added was studied in the range 0.1–0.3 *M* (Fig. 3) and 0.1 *M* NaOH was selected as optimum in order to obtain the best signals. The peak area corresponding to the amine products decreased between

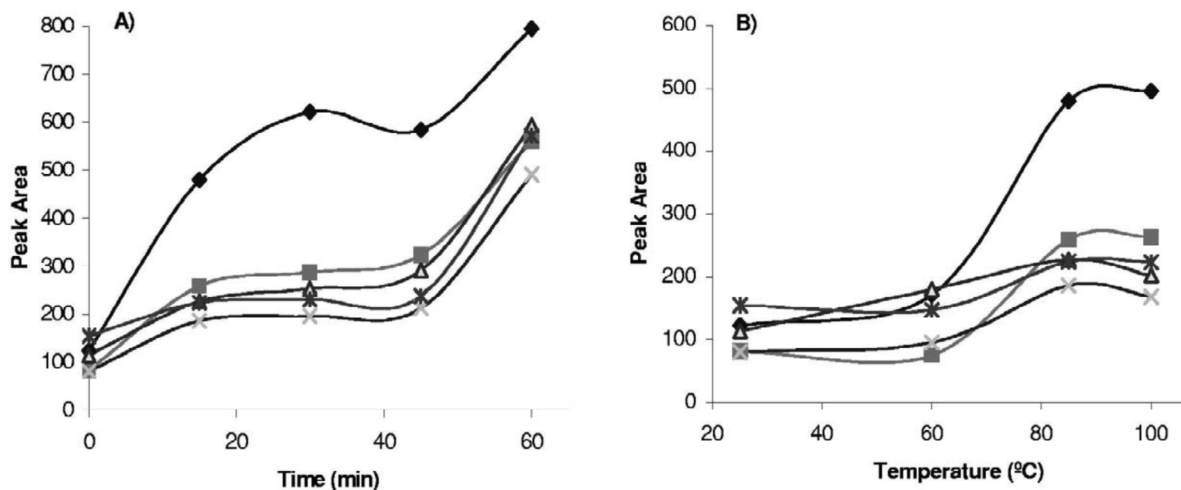

Fig. 2. Effect of the reaction time and temperature on the derivatization on C_{18} SPE. (A) Peak area versus time at 85 °C. (B) Peak area versus temperature (°C) at time=15 min. (♦) Methylamine, (□) ethylamine, (▲) *N*-propylamine, (×) butylamine, (*) pentylamine. Conditions: Dns-Cl 5 m*M*, borate buffer 20 m*M*, amine concentration 5 mg l^{-1}, pH 9.5, temperature and reaction time variable.

20 and 4% depending on the analyte. No reagent problems were observed by measuring fluorescence signals and it was not necessary to clean-up the cartridges in order to remove the reagent excess.

The efficiency of the proposed method was evaluated by calculating the percentages of the analytes (amine-derivates) in the collected extracts. These values were calculated by comparing the peak areas with those obtained with solution derivatization (see Table 2). These results suggest that derivatization on the SPE cartridges is more effective than the analogous solution derivatization, probably due to the reagent is concentrated into the solid material, thus resulting in a very high reagent to amine ratios, what can facilitate the reaction [23]. As can be seen in Table 2, the RSDs of the results improved by performing the derivatization on SPE cartridges.

Different volumes of standard solutions ranging from 1.0 to 25 ml were tested in order to increase the enrichment factor. The final concentrations of the samples were varied from 5.0 to 0.2 mg l^{-1} in such a way that the amount of analyte processed was the same in all the assays. The amines retained were derivatized and eluted following the procedure described above. Fig. 4 shows the recoveries obtained for the different volumes of sample assayed taking as reference 1 ml of sample volume. As can be deduced

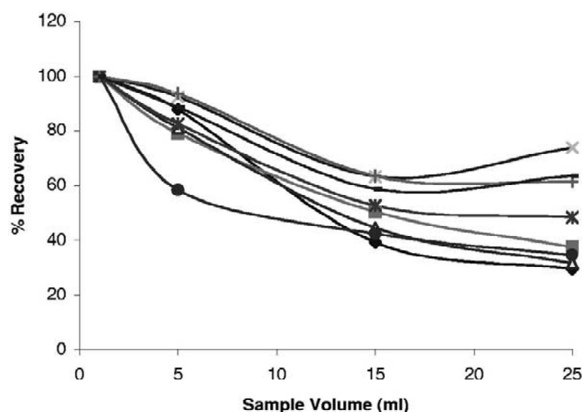

Fig. 4. Effect of the sample volume on analyte recoveries after preconcentration and derivatization on C_{18} SPE. Recovery versus sample volume for (♦) ammonium, (□) methylamine, (▲) ethylamine, (×) dimethylamine, (*) butylamine, (●) diethylamine, (+) pentylamine and (−) hexylamine. Conditions: Dns-Cl 5 m*M*, borate buffer 20 m*M*, amine concentration 5 mg l^{-1}, pH 9.5, *T*=85 °C and 15 min reaction time.

from this figure, at higher volumes losses by breakthrough were observed. Ammonium and amines like methylamine, dimethylamine, ethylamine and diethylamine have more losses than the others as sample volume increases due to its polarity. As a compromise, a sample volume of 5.0 ml was selected for further experiments. A dynamic ranged for the several amines in samples from 10 to 250 μg/l can be achieved. Amine solution was alkalised to pH 11 in order to improve amine retention, and recoveries between 85 and 115% (respectively to that obtained in neutral solutions) were obtained. However, ammonium recovery was lower, about 40%, probably due to its transformation to NH_3.

3.3. Chromatographic conditions

The chromatographic separation of the seven amines and ammonium under study were optimised. A LiChrospher RP18 100 column, 125×4 mm I.D., 5-μm film thickness, was used to separate the dansylamines. A mobile phase consisting of acetonitrile–imidazole, pH 7 (50:50, v/v), mixture in the gradient elution mode was used as eluent, at a flow-rate of 1 ml/min. The gradient was studied in order to have the best separation between the ammonium, amines and internal standard, and finally we selected: 50% of acetonitrile at time zero, 90% at time 9 min and 50% at time 10 min. Under such conditions, the retention times for ammonium, methylamine, ethylamine, dimethylamine, butylamine, pentylamine, hexylamine, and 1,7-diaminoheptane were 2.2, 3.2, 3.9, 4.8, 5.7, 6.2, 6.7, 7.8 and 8.2 min, respectively. The elution order was consistent with the polar character of these compounds, which decreases with increasing length of the aliphatic chain. Typical chromatograms obtained for a blank solutions and amines solution (10 mg l^{-1} of ammonium and 2.5 mg l^{-1} of each amine but for diethylamine 7.5 mg l^{-1}) processed under the optimal conditions are shown in Fig. 5.

3.4. Analytical figures of merit

In order to evaluate the quantitative performance of the proposed method, standard samples containing analyte concentrations in the range 0.01–0.5 mg l^{-1} (sample volume 5 ml, elution volume 0.5 ml) or in

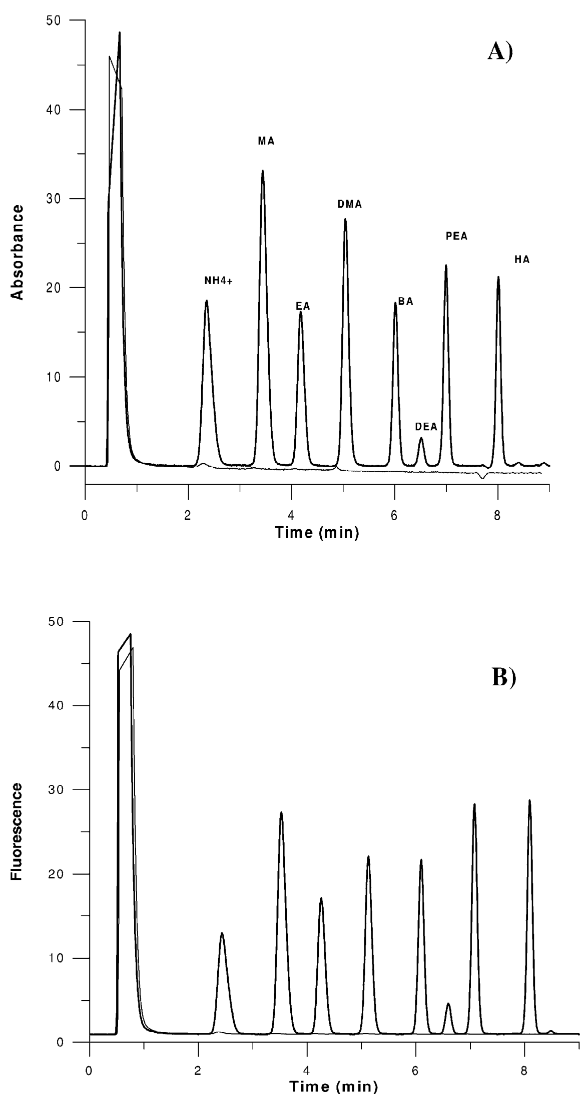

Fig. 5. Chromatograms obtained for a blank (water) (–) and for a standard solution (–) after the preconcentration and derivatization of the analytes by the proposed procedure. (A) UV detection (λ=333 nm), (B) fluorescence detection. For more details, see Section 2.

the range 0.1–5 mg l^{-1} (sample volume 1 ml, elution volume 1 ml) were assayed. For diethylamine the range was 0.03–1.5 mg l^{-1} (5 ml/0.5 ml) and 0.3–15 mg l^{-1} (1 ml/1 ml). The calibration graphs for both UV and fluorescence detection and other analytical features obtained for each amine are listed in Table 3. The sensitivities obtained depend on amine as can be seen in this table. The LODs were at the μg/l level for both fluorescence and spectrophotometric detection if 5 ml of sample is processed. Ammonium can be identified but it cannot be quantified for improving amine retention. Improved detection limits can be achieved by working with larger volumes of water. The intra-day precision for different sample volume (1, 5, 10 and 25 ml) can be seen in Table 4. Intraday results were slightly better than inter-day results because RSD values achieved for reproducibility were between 7 and 15%.

3.5. Analysis of real water samples

Spiking sample with known amounts of analytes (standard addition method) was used to validate the method. In order to improve the results, an internal standard (I.S.) 1,7-diaminoheptane was used.

Five ml of real water samples (spiked and not spiked) were passed trough the cartridges and processed according to the described procedure. For all samples tested, the slope obtained for the most polar analytes (ammonium, methylamine, ethylamine, and diethylamine) by using MOSA was lower than that obtained with standards. However for more non-polar analytes (butyl-, pentyl and hexylamine) the behaviour obtained was similar to that obtained with standard solutions. Thus, the low recoveries obtained, for the most polar analytes, testified to the presence of matrix effect. Fig. 6 shows the recoveries obtained for the different amines by comparing the analytical signal obtained for two real samples with the standard solution, at different concentrations, as can be seen the recoveries were not concentration dependent. In Table 5 are shown the recoveries obtained for different samples; as can be seen no differences in recoveries were obtained between the different samples processed, and a mean recovery for each analyte can be obtained ranging from 37±9 to 108±16%.

In samples with high amine content, lower sample volume was required and no matrix effect was found. According to this, 0.2 ml sample diluted to 1 ml was processed in residual water samples, and as can be seen in Fig. 7, analytes such as ammonium, methyl-, dimethyl- and pentylamine, were screened. This sample was fortified with a mixture of these amines at different concentration levels and in all cases good

Table 3
Analytical figures of merit of amine-dansyl derivatives detected by UV or fluorescence

Analyte	$(a \pm s_a)$	$(b \pm s_b)$	$s_{y/x}$	n	r^2	LOD (μg/l)	D
MA	(7±3)	(470±10)	6.3	15	0.991	2	FL
EA	(1±2)	(465±9)	4	9	0.997	2	FL
DMA	(10±2)	(255±7)	3	10	0.996	3	FL
BA	(−1±1)	(346±7)	3	15	0.995	3	FL
DEA	(8±1)	(74±2)	2	7	0.990	4	FL
PeA	(−4±2)	(425±10)	6	15	0.990	2	FL
HA	(−3±2)	(388±10)	6	14	0.992	2	FL
MA	(18±4)	(567±19)	8	15	0.992	3	UV
EA	(2±3)	(495±18)	8	14	0.990	6	UV
DMA	(64±4)	(442±17)	10	8	0.990	6	UV
BA	(−1.6±2.5)	(331±14)	7	14	0.990	9	UV
DEA	(5±2)	(66±3)	3.5	11	0.990	15	UV
PeA	(−2.9±4)	(306±18)	7	11	0.990	8	UV
HA	(10±1)	(177±4)	2	12	0.997	15	UV
MA*	(−1±6)	(85±3)	10	6	0.996	48	UV
*EA**	(−11±4)	(58±2)	7	8	0.997	93	UV
*DMA**	(−2±2)	(47.5±0.8)	3	10	0.9992	75	UV
*BA**	(−18±4)	(36±1)	5	8	0.995	90	UV
*DEA**	(11±5)	(7±1)	4	4	0.990	340	UV
*PeA**	(−11±4)	(37±2)	6	9	0.991	90	UV
*HA**	(−20±4)	(35±1.5)	5	6	0.994	70	UV

Conditions: sample volume processed 5 ml (or 1 ml)* and elution volume 0.5 ml (or 1 ml)*. D, detection.

recoveries were obtained (nearly to 100% for all the amines) indicated the absence of matrix effect. *t*-Test for comparison of slopes gave that the slopes obtained by applying MOSA are similar to those obtained for calibration curve with standards for $\alpha=0.01$.

Table 6 lists the found concentration and relative error (%) of the spiked waters, by applying the MOSA or the calibration graph with standards, taking into account the percent recovery. Good results were obtained in both cases.

Table 4
Repeatability data (RSD %)

Analyte	Repeatability RSD(%)			
	1 ml	5 ml	10 ml	25 ml
MA	8	4	4	4
EA	4	4	9	14
n-PRA	4	–	8	15
BA	4	8	6	2
DEA(*)	–	14	15	8
PEA	4	–	6	3
HA	–	4	3	–

Sample volume processed: 1 ml (5 mg l^{-1}), 5 ml (1 mg l^{-1}), 10 ml (0.5 mg l^{-1}) and 25 ml (0.2 mg l^{-1})
*Analyte: dimethylamine, 15, 1.5 and 0.6 mg l^{-1}, respectively.

4. Conclusions

Derivatization in C_{18} is more effective that the analogous solution derivatization. The optimum derivatization conditions have been optimised (pH reaction 9.5; temperature and time reaction 85 or 100 °C and 15 or 10 min, respectively, and sample volume 5 or 1 ml). The proposed procedure is very simple, rapid and required a less time-consuming operation than that typically involved in dansylation derivatization procedures. The total analytical (sample treatment and chromatography) is less than 25 min.

Up to 5 ml of sample volume can be processed with low losses of the most polar analytes. The recoveries are independent of the sample origin and dependent on the analyte. The matrix effect can be corrected by applying MOSA or by using the calibration graphs with standards taking into account the (%) recoveries.

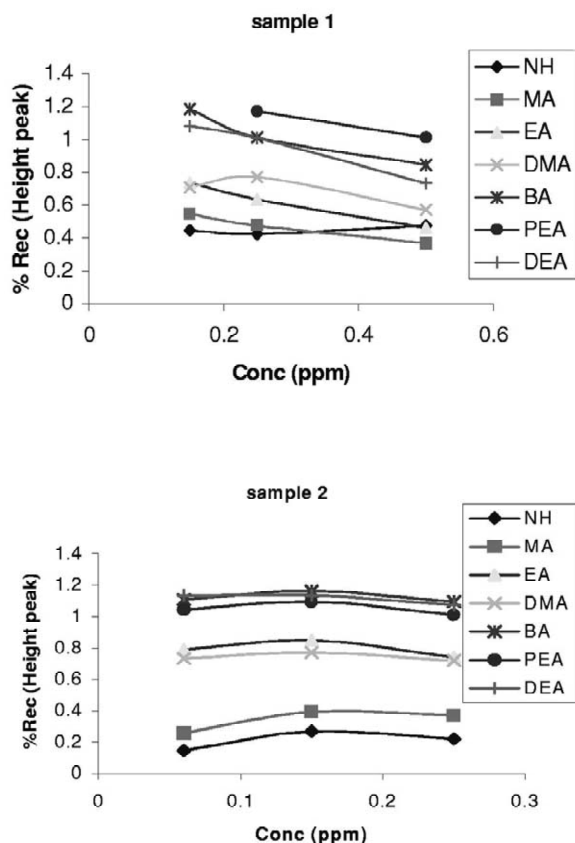

Fig. 6. Recoveries (REC) obtained for different water samples fortified with amines at different concentration levels. Conditions: Dns-Cl 5 m*M*, borate buffer 20 m*M*, pH 9.5, $T=85$ °C and 15 min reaction time, sample volume 5 ml, elution volume 1 ml.

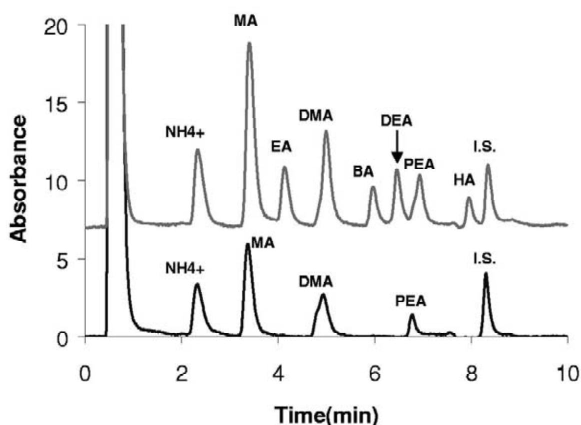

Fig. 7. Chromatograms corresponding to residual water sample (S4) (a), spiked with 1.25 mg l^{-1} of each analyte (b), by following the proposed procedure. Sample volume processed 0.2 ml diluted to 1 ml. For more details, see Section 2.

In samples containing amines at ppm levels, waste water, 0.2 ml sample diluted to 1 ml, was processed. In this case, any matrix effect was observed, and amine recoveries were around 100% in almost every amine studied.

The proposed procedure can be applied with satisfactory accuracy and reproducibility to the determination of seven aliphatic amines at low and sub-ppm concentration levels and ammonium identification. No significant differences were observed in the quantification of the analytes between the different sample types tested. The procedure has been used for screening of ammonium and amines in a waste water sample.

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Table 5
Recovery obtained for different real water sample by applying the proposed procedure

Analyte	Sample 1		Sample 2		Sample 3		Mean recovery (%)	
	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)
Ammonium	45±3	6	25±3	14	38±6	17	37±9	25
Methylamine	46±9	19	38±2	5	65±9	14	51±14	27
Ethylamine	69±7	11	79±5	7	88±19	22	80±14	17
Dimethylamine	74±5	6	74±3	4	83±13	16	78±9	11
Butylamine	101±17	17	112±4	3	103±20	19	106±13	12
Diethylamine	109±11	10	105±4	4	109±14	13	107±8	8
Pentylamine	94±18	20	112±4	3	119±14	11	108±16	15

(*) Sample 4 (residual water sample) − 0.2 ml diluted to 1 ml. For more details see Section 2.

Table 6
Concentrations found in the real samples by applying standard addition method MOSA or by applying calibration graphs with standards taking into account the mean recovery (%)

Analyte	Added conc.	Sample 1		Sample 2		Sample 3		Sample 4 (*)	
		MOSA	Calib	MOSA	Calib	MOSA	Calib	MOSA	Calib
Methylamine	0.15	0.15 (0%)	0.11(26%)	0.15 (0%)	0.13 (13%)	0.15 (0%)	0.17 (13%)	1.14 (0.9%)	1.13 (2%)
	0.25	0.25 (0%)	0.21 (16%)	0.25 (0%)	0.24 (4%)	0.25 (0%)	0.27 (8%)	2.61 (4%)	2.56 (2.4%)
Ethylamine	0.15	0.15 (0%)	0.11 (26%)	–	–	0.15 (0%)	0.15 (0%)	1.34 (16%)	0.98 (15%)
	0.25	0.25 (0%)	0.21(16%)	0.25 (0%)	0.24 (4%)	0.25 (0%)	0.23 (9%)	2.49 (0.4%)	2.58 (3.2%)
Dimethylamine	0.15	0.14 (7%)	0.18 (20%)	0.16 (7%)	0.14 (7%)	0.15 (0%)	0.16 (7%)	1.13 (2%)	1.18 (2.6%)
	0.25	0.28 (12%)	0.25 (0%)	0.25 (0%)	0.3 (20%)	0.25 (0%)	0.28 (12%)	2.56 (2.4%)	2.40 (4%)
Butylamine	0.15	0.13 (13%)	0.15 (0%)	0.15 (0%)	0.14 (7%)	0.15 (0%)	0.15 (0%)	1.22 (6%)	1.08 (6%)
	0.25	0.28 (12%)	0.25 (0%)	0.25 (0%)	0.27 (8%)	0.25 (0%)	0.22 (12%)	2.52 (0.8%)	2.56 (2.4%)
Pentylamine	0.15	0.14 (7%)	0.15 (0%)	0.15 (0%)	0.16 (7%)	0.14 (7%)	0.15 (0%)	1.08 (6%)	1.21 (5%)
	0.25	0.27 (8%)	0.27 (8%)	0.25 (0%)	0.26 (6%)	0.25 (0%)	0.24 (4%)	2.57 (2.8%)	2.50 (0%)
Diethylamine	0.75	0.83 (10%)	0.77 (3%)	0.75 (0%)	0.74 (1%)	0.77 (3%)	–	3.75 (0%)	–
	0.45	0.44 (2%)	0.41 (8%)	0.46 (2%)	0.42 (7%)	0.40 (12%)	–	7.50 (0%)	7.51 (0.1%)

(*) Sample 4: added concentration 1.5 and 2.5 mg l^{-1}, dimethylamine 3.75 and 7.50 mg l^{-1}.

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