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Sensitive determination of aliphatic amines in water by high-performance liquid chromatography with chemiluminescence detection

S. Meseguer Lloret, C. Molins Legua, J. Verdú Andrés, P. Campíns Falcó*

Departament de Química Analítica, Facultad de Química, Universitat de Valencia, C/Dr. Moliner 50, E46100 Burjassot, Valencia, Spain

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Abstract

A sensitive method has been developed for liquid chromatographic determination of short aliphatic amines in water samples. Analytes are preconcentrated and dansylated on solid sorbents (C_{18} solid-phase extraction cartridges). The dansyl derivatives are chromatographed and post-column mixed with peroxyoxalate (TCPO) and H_2O_2 in order to perform chemiluminescence detection. Optimal results have been obtained using a sample volume of 5 ml. The method has been applied to the quantification or screening of several aliphatic amines: methylamine, ethylamine, butylamine, diethylamine, pentylamine and hexylamine. The screening procedure has been developed including also polyamines (putrescine, cadaverine, spermidine and spermine). The results obtained by using chemiluminescence (CL) detection have been compared with other detection systems (fluorescence and UV). The sensitivity can increase from 3 to 75 times respect UV detection and from 2 to 10 times respect fluorescence detection depending on the amine. The detection limits achieved were between 0.15 and 0.9 μg/l.
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1. Introduction

Short-chain aliphatic primary amines are widely distributed in the environment due to their use in several chemical and manufacturing industries. These aliphatic amines are also common components of biological systems as degradation products of organic material such amino acids and proteins. Besides hygienic problems due to the stinging smell, these compounds may be hazardous to human health as they are sensitises and irritants to skin, eyes, mocous membranes or respiratory tract. In addition, they can react with certain nitrogen-containing compounds to form nitrosamines, which are potentially carcinogenic substances [1].

Aliphatic amines are compounds that occur at small quantities in some kind of waters, so analysis methods should be sensitive. Selectivity and sensitivity usually have been achieved by combination of analyte derivatization, a separation technique (liquid chromatography (LC), gas chromatography (GC) or capillary electrophoresis (CE)) and a highly sensitive detection method [chemiluminescence (CL), electrochemical or mass spectrometric methods]. CL detection techniques have proved to be valuable tools for sensitive measurement at ultra trace levels, with cheap and simple optical system, providing low background noise, and wide dynamic range [2].

The chemiluminescence from synthetic compounds, including lophine derivative, luminol type compounds, adamantyldioxetane acridinium esters, peroxyoxalate esters and ruthenium complexes, have been widely used. Although luminol type is one of the most used chemiluminogenic compound [3], only a few compounds such as *N*-(4-aminobutyl)-*N*-ethylisolumino (ABEI), *N*,*N*′-disuccinimidyl carbonate (DSC) [4,5], 4-isothiocyanatophthalhydrazine (ILITC) [6], 6-isothiocyanatobenzo[*g*]phathalazine-1,4(2H,3H)-dione (IPO) [7], and 4-(6,7-dihydro-5,8-dioxothiazolo[4,5-*g*]phthalazin-2-yl)benzoic acid *N*-hydroxysuccinimide ester (TPB-suc) [8] have been reported as CL reagents for the determination of amino compounds.

Tris(bipyridyl)ruthenium(III) [$Ru(bipy)_3$] has been reported for the determination of a variety of nitrogen-containing analytes without requiring derivatization of the analyte [4,9]. The [$Ru(bipy)_3$] CL method is highly selective to ternary amines, but was not relatively sensitive compared with other CL determination techniques. Usually this reagent is electrogenerated, giving electrogenerated chemiluminescence (ECL) [10]. Despite the large theoretical potential of ECL only certain reactions have found widespread

* Corresponding author. Tel.: +34-96-3544436; fax: +34-96-3544436.
E-mail address: pilar.campins@uv.es (P. Campíns Falcó).

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applicability in analytical science, because significant limitations such as the presence of interfering species, the complex optimisation and the lack of the reproducibility make ECL less attractive than conventional CL [11].

In the presence of a fluorescer, oxalated derivatives are the most efficient non-biological CL emiters. Bis-(2,4,6-trichlorophenyl) oxalate (TCPO), bis(2,4-dinitrophenyl) oxalate (DNPO) and bis[2-(3,6,9-trioxadecyloxycarbonyl)-4-nitrophenyl] oxalate are the most commonly used. Efficiency and flexibility are, therefore, the main advantages of these systems, which required the presence of a fluorophore, which is usually obtained from the analyte after a derivatization reaction. The labelling reagent for this reaction has been also investigated. Reagents such as dansyl chloride (Dns-Cl) [12–14] for biogenic amines and amphetamines, naphtalene-2,3-dialdehyde (NDA) or anthracene-2,3-dialdehyde (ADA) [15] for primary amines and luminarin [16] for primary and secondary amines, combined with post-column mixing with TCPO and H_2O_2, have been used.

In peroxyoxalate chemiluminescence, the initial excited state product does not return to the ground state by photon ejection, it can undergo energy transfer with a suitable fluorophore which in turn my then exhibit its characteristic fluorescence emission. A model for mechanism of peroxyoxalate chemiluminiscence as applied to detection in LC was proposed in [17].

We have recently reported an off-line procedure using solid-support assisted derivatization for the determination of alkylamines in different water samples by using dansyl chloride [18]. The present paper studied the post-column chemiluminescence generation with oxalic acid bis(2,4,6,-trichlorophenyl ester) (TCPO/H_2O_2) of the dansylated amines in order to increase the sensitivity of the procedure for low molecular mass amines in environmental water for achieving low ng/ml levels. Six aliphatic amines (methylamine, ethylamine, butylamine, pentylamine, hexylamine and diethylamine) were systematically studied. Other aliphatic amines such as biogenic amines (cadaverine, putrescine, spermidine and spermine) have also been included in the screening study.

Finally, the method has been applied to the determination or screening of amines in real water samples. The results obtained have been compared with those previously obtained by using UV and fluorescence detection and dansyl chloride reagent [18,19] or other reagents such as 3,5-dinitrobenzoyl chloride (DNB) [20], 9-fluorenylmethylchloroformate (FMOC) [21] and *o*-phthalaldehyde/*N*-acetylcysteine (OPA/NAC) [22].

2. Experimental

2.1. Apparatus

The chromatographic system used consisted of a quaternary pump (Hewlett–Packard Series 1050) equipped with a high-pressure six-port valve (Rheodyne model 7000). The volume of the sample loop was 20 μl.

The post-column instrument (PCX 5100), from Pickering Labs. (Mountain View, CA, USA), consisted of two isocratic pumps. The chemiluminescence detector was a Jasco CL-1525 (Ishika-cho, Hachioji-shi, Tokio, Japan) which was coupled in series and linked to a data system Borwin-chromatography software and the interface Hercule lite (JMBS, France) used for data acquisition and storage. The model CL-1525 detector is equipped with Jasco's uniquely designed coiled PTFE tube flow cell placed inmediatelly next to a highly sensitive end-on photomultiplier. All the assays were carried out at room temperature.

2.2. Reagents

All the reagents were of analytical grade. Acetonitrile (J.T. Baker, Deventer, The Netherlands), methanol and acetone (Scharlau, Barcelona, Spain) were of HPLC grade. Methylamine (MA), ethylamine (EA), butylamine (BA), diethylamine (DEA), pentylamine (PeA), hexylamine (HA), putrescine (Put), cadaverine (Cad), spermine (Spm), spermidine (Spd) and dansyl chloride were obtained from Sigma (St. Louis, MO, USA). TCPO, bis(2,4,6-trichlorophenyl) oxalate, tetrahydrofuran and imidazole (99%) from Fluka (Steinheim, Switzerland) and H_2O_2 (30%), sodium carbonate and sodium hydroxide from Merck (Darmstadt, Germany) were also used.

2.3. Columns and mobile phases

Bond Elut Varian C_{18} 100 mg, (Harbor City, CA, USA), were used to retain the analytes and to perform the off-line derivatization.

A LichroCart RP 18 (125 × 4 mM i.d., 5 μm particle diameter) (Merck) column was used for separation of the amine derivatives. An acetonitrile-imidazole solution (1 mM, pH = 7.0) (50:50, v/v) mixture in the gradient elution mode was used as eluent at flow rate of 1.5 ml/min.

Two different mobile phase gradients were used depending on the analytes assayed. For aliphatic amines, gradient elution mode acetonitrile-imidazole was used, being 50:50 at zero time, 80:20 at 9 min, and 50:50 at 10 min (*Gradient 1*). When polyamines were included in the mixture, the gradient elution mode acetonitrile-imidazole was: 50:50 isocratic from 0 to 13 min, gradient elution 90:10 at 17 min, isocratic 90:10 until 19 min, and 50:50 at 20 min (*Gradient 2*).

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

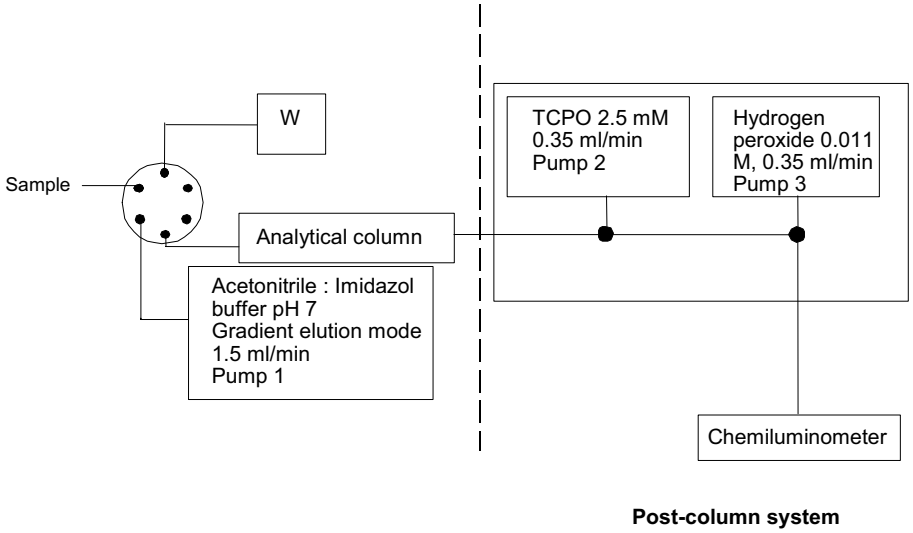

Fig. 1. Assembly used for separation of dansylated amines and on-line post-column chemiluminescence detection.

standard solutions in water. Dns-Cl 12.5 mM solution was prepared by dissolving the pure compound in acetone. A 2:3 (v/v) mixture of Dns-Cl in acetone and carbonate solution (33.3 mM) pH 9.5, was daily prepared. All solutions were stored in the dark at 4 °C.

2.5. 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 10 mM (pH 12). Variable volumes of standard solution (1–25 ml) or 1–5 ml sample were then transferred to the cartridges. 0.5 ml of 5 mM reagent (Dns-Cl in acetone-hydrogencarbonate buffer solution (pH 9.5, 33.3 mM), 2:3 v/v) was flushed through the cartridges, which were incubated at 110 °C in an oven for 10 min. The derivatives formed were desorbed from the cartridges with 0.5 or 1 ml of acetonitrile. A 20 μl aliquot of the resulting mixture was finally injected into the chromatographic system.

2.6. Post-column generation of chemiluminescence

H_2O_2 (11 mM, prepared in acetonitrile) and TCPO [2.5 mM, prepared in acetonitrile-tetrahydrofuran (75:25)] were used as post-column reagents.

The post-column system was running continuously during all runs at a flow rate of 0.35 ml/min, with TCPO (pump 2) and H_2O_2 (pump 3) (see the assembly on Fig. 1). The dansyl derivatives, previously separated in the analytical column, were transferred to the post-column system, where the intermediate generated from the TCPO and H_2O_2 served to excite the Dns-amine derivatives so that they could undergo chemiluminescence emission. The distance between the reagents confluence and the detector was a tube of 5 cm (i.d. 0.508 mm). This was the minimum possible distance in the assembly.

2.7. Analysis of real water samples

The method was tested in real water samples with unknown amine concentration. The environmental water samples were named as S1: tap water, S2: irrigation ditch water, S3: lake water, S4: residual water from a factory and S5: marine water. Water samples were collected, filtered through 0.45 μm and acidified to pH 2 with HCl. Previously to the analysis, the samples were alkalised with NaOH to pH 10.5.

Sample volumes of 0.02 (for methylamine determination and screening analysis) or 0.1 ml (for pentylamine quantification), both diluted up to 1 ml for S4, were placed into conditioned C_{18} cartridges. For S1–S3, and S5, sample volumes of 5 ml were processed in order to analyse any amine present in the sample. The samples were also spiked with known concentrations of amines standard solutions as it is shown in Table 1. Then the analytes were derivatized in the solid−phase extraction cartridges as described above and the elution volume was 1 ml, followed by HPLC separation and post-column chemiluminescence generation.

3. Results and discussion

3.1. Preconcentration and pre- and post-column conditions

The procedure used for pre-column derivatization have been selected on the bases of previous studies [18]. According to our previous work with different types of amines, Bond Elut C_{18} cartridges were selected for preconcentration and derivatization of the analytes [19]. Sorbents with different characteristics were assayed: SCX, C_{18}, C_2, C_8,

Table 1
Spike of the samples

Sample	Spiked concentration (μg/l)						Found spiked concentration (μg/l)					
	MA	EA	BA	PeA	HA	DEA	MA	EA	BA	PeA	HA	DEA
S1	20	32			40	64	15	31			50	88
			80		80	128			106		97	136
S2	20	32	40	40			19	26	39	51		
S3			40	40					47	51		
	40	64	80	80		128	52	72	80	88		133
S4	100	160	200	200	200	320	90	120	178	194	186	360
	100		200	200	200		112		222	189	202	
				200	200					218	211	
S5	20	32					17	39				
	40	65			80		31	68			110	

S1: tap water sample; S2: irrigation ditch water sample; S3: lake water sample; S4: residual water from a factory and S5: marine water sample) and found amine concentration (μg/l) for spiked concentrations by employing the corresponding calibration graphs. MA: methylamine; EA: ethylamine; BA: butylamine; PeA: pentylamine; HA: hexylamine; and DEA: diethylamine.

CN and Bond Elut Certify (C_8 and SCX mixed phase). The success of the methodology depends mainly on the amine retention and not on the derivatization reagent used [19]. Once the analytes are retained, a small volume of reagent and buffer solution are mixed and loaded into the cartridge. In this case a mixture Dns-Cl dissolved in acetonecarbonate buffer (see Section 2) was used in order to prevent amine elution [18]. Other parameters involved in the reaction such as pH of the medium, time of reaction and temperature were optimized. The influence of pH, time and temperature reaction were studied in the ranges 8.5–11.4, 20–100 °C and 0–60 min, respectively and the best conditions were: pH 9.5, 100 °C and 10 min. The reaction products formed are eluted with organic solvent such as CH_3CN or mixtures CH_3CN water, MeOH-buffer etc. Small volumes of solvent is sufficient to elute the reagent products formed, 0.5 or 1 ml of CH_3CN. The formed dansylated amines were injected on the chromatographic system and were post-column mixed with TCPO and H_2O_2.

Post-column parameters were optimized. According to [23,24], the parameters affecting the response of a TCPO chemiluminescence detection are: temperature, pH, water content, solvent, catalyst, TCPO concentration, H_2O_2 concentration, reservoir materials, volume of mixing tube, cell volume of detector and flow rates of the different streams. Based on previous studies [13] and the literature [22,23] appropriate parameters were established previously: room temperature, acetonitrile was used as solvent, imidazole as catalyst, the flow rates were less than 0.6 ml/min, the length of the mixing tube was the minimum for the assembly, the cell volume was fixed by the instrument, the concentration of TCPO must be higher than 0.1 mM and the ratio TCPO-H_2O_2 close to 5. Two of the most critical parameters were optimized: the imidazol concentration (0.1 mM and 5 M) and pH (5–7). The imidazole concentration gave a maximum in the CL signal-to-noise ratio at about 1 mM, and the pH at 7, the flow being 1.5 ml/min.

The reaction product between TCPO and H_2O_2 is, 2,4,6-trichlorophenol which can produce a quenching effect on CL. In order to avoid this product, both reagents were prepared separately and mixed during the reaction procedure (see Fig. 1) It was observed that the sensitivity increased by a factor of 5 when the TCPO reagent was included in the system before the H_2O_2 reagent (see Fig. 1) and a mixture acetonitrile: tetrahydrofurane was used as TCPO solvent. TCPO concentration was varied between 0.5 and 5 mM, the flow being 0.35 ml/min. However, no big differences were observed between 2.5 and 5.0 mM concentrations and 2.5 mM was chosen. High H_2O_2 concentration reduced the chemiluminescence intensity and increased the baseline (background), working in the range 11–5 mM the analytical signal was stable.

The final recommended procedure for dansylation and chemiluminescence generation for alkylamines is summarised in Table 2.

Table 2
Optimal conditions for amines determination

Stage	Experimental parameters
	1 ml methanol
Pre-column	1 ml carbonate buffer pH 12, 10 mM
Preconcentration	1 ml/5 ml sample
Derivatization on C_{18} cartridges	0.5 ml Dns-Cl reagent
	T^a 110 °C, 10 min
	Elution 1 ml MeCN
Chromatographic separation	Sample injection: 20 μl
of dansyl derivatives	Gradient: 0 min 50:50, 9 min (80:20) (MeCN-imidazole buffer), flow rate 1.5 ml/min
Post-column	
Chemiluminescence reaction of the dansyl derivatives	TCPO 2.5 mM, 0.35 ml/min
previously separated	H_2O_2 11 mM, 0.35 ml/min

Table 3
Recoveries for the different aliphatic amines in the mixture respect the individual amine solution

Amine	Recovery (%, $n = 3$) $(S_{mixture}/S_{individual}) \times 100$
Methylamine	108 ± 14
Ethylamine	100 ± 11
Butylamine	114 ± 3
Pentylamine	88 ± 3
Hexylamine	78 ± 5
Diethylamine	109 ± 16

Concentrations of 0.1 mg/l for MA, EA, BA, PeA and HA, and 0.2 mg/l for DEA.

The efficiency of the derivatization process was established for each amine individually and for all of them together. As can be seen in Table 3 the results were similar, and no significant differences were observed when the reaction took place in presence of all the amines tested.

Different volumes of standard solutions ranging from 1.0 to 25 ml were tested in order to increase the enrichment factor. The concentrations were varied from 200 to 4 μg/l, 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 in Section 2. Fig. 2 shows the recoveries obtained for the different volumes of sample assayed taking as reference 1 ml of sample volume. As can be deduced from this figure, severe losses by breakthrough were observed when 25 ml of sample were taken. Sample volumes of 5 or 10 ml could be used in order to improve detection limits of the amines determination.

3.2. Chromatographic conditions

Two chromatographic conditions were used depending on the analytes studied. The chromatographic separation of the six monoamines MA, EA, BA, DEA, PeA and HA was done according to the conditions previously established [18]. Typical chromatograms obtained for a blank and an amine mixture are shown in Fig. 3A.

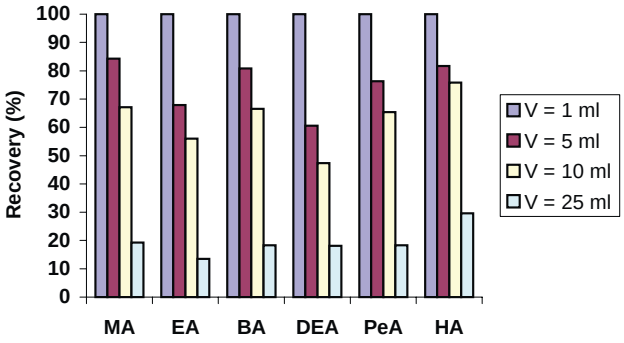

Fig. 2. Recoveries obtained for the different amines, when different sample volumes were processed. Measured concentration: 0.150 mg/l MA, 0.24 mg/l EA, 0.3 mg/l BA, PeA, HA, and 0.48 mg/l DEA.

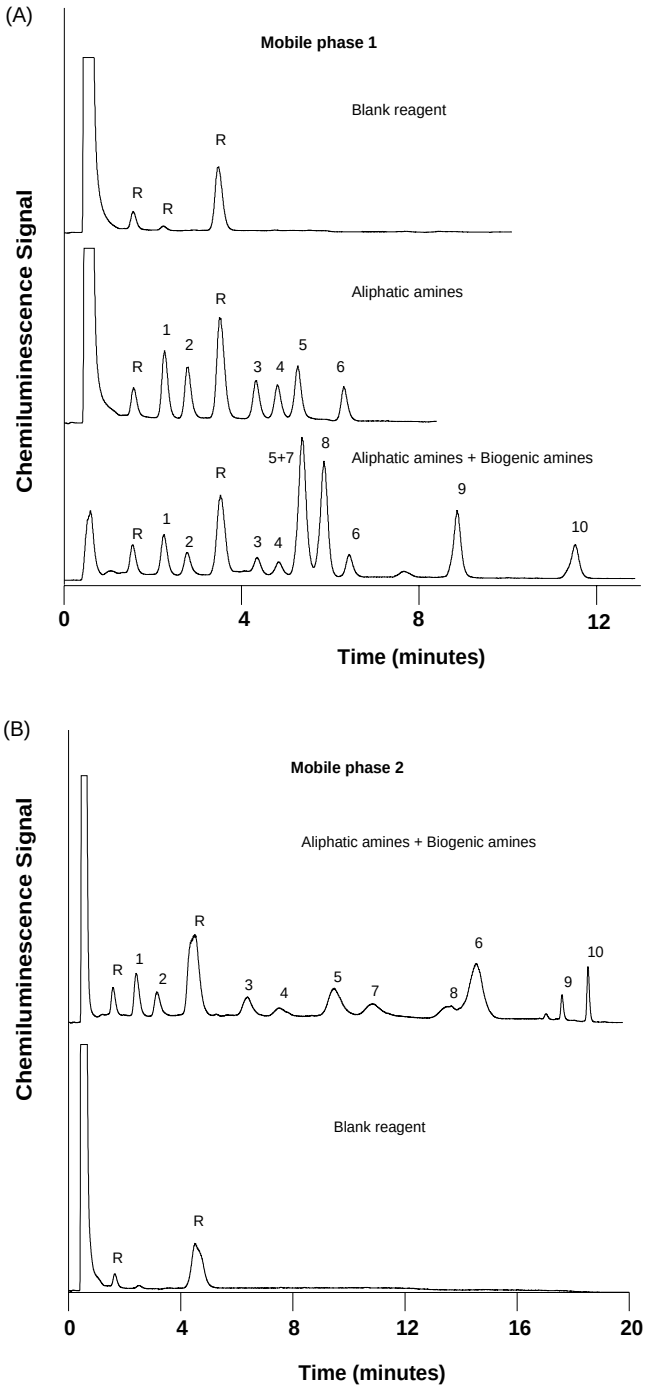

Fig. 3. Chromatograms corresponding to: (A) blank reagent, standard solutions of aliphatic amines and aliphatic amines plus biogenic amines, by using mobile phase 1 and (B) blank reagent and standard solutions of aliphatic amines plus biogenic amines, by using mobile phase 2. (R) Reagent; (1) MA (measured concentration 0.150 mg/l); (2) EA (0.240 mg/l); (3) BA (0.3 mg/l); (4) DeA (0.48 mg/l); (5) PeA (0.3 mg/l); (6) HeA (0.3 mg/l); (7) Put (0.3 mg/l); (8) Cad (0.3 mg/l); (9) Spd (0.3 mg/l); (10) Spm (1.12 mg/l).

In presence of other amines, such as biogenic amines like putrescine, cadaverine, spemine and spermidine, the compounds putrescine and PeA coeluted (see Fig. 3A), so the chromatographic conditions were modified in order to

Table 4
Some analytical figures of merit of dansyl-amine derivatives detected by chemiluminescence

Amine	V (ml)	$b \pm S_b$ (l/mg)	$a \pm S_a$ (V)	$S_{y/x}$	r^2	n	LI (μg/l)
MA	1	1900 ± 120	25 ± 14	17	0.9887	5	17–200
EA	1	1010 ± 30	−3 ± 5	6	0.9987	4	3–320
BA	1	570 ± 40	−8 ± 9	12	0.9904	4	13–400
PeA	1	820 ± 50	−8 ± 12	15	0.9920	4	7–400
HA	1	630 ± 20	2 ± 6	7	0.9969	4	7–400
DEA	1	239 ± 17	7 ± 6	8	0.9856	5	13–640
MA	5	10500 ± 500	16 ± 14	15	0.9973	3	4.3–40
EA	5	4300 ± 500	−17 ± 18	20	0.9629	5	0.7–64
BA	5	1790 ± 160	−5 ± 8	9	0.9838	4	3–80
PeA	5	3000 ± 300	−9 ± 14	16	0.9732	5	2–80
HA	5	2800 ± 200	−9 ± 10	11	0.9847	5	1.7–80
DEA	5	860 ± 50	3 ± 4	5	0.9919	4	4–128
MA	10	22300 ± 1500	52 ± 10	15	0.9915	4	3–10
EA	10	10100 ± 500	3 ± 6	9	0.9944	4	0.5–16
BA	10	5600 ± 600	4 ± 8	12	0.9787	4	2–20
PeA	10	9000 ± 500	1 ± 7	10	0.9935	4	1–20
HA	10	10300 ± 300	5 ± 4	6	0.9984	4	1–20
DEA	10	2870 ± 150	2 ± 3	5	0.9943	4	3–32

b: Slope of the calibration graph; a: ordinate; n: number of standards; and LI: linear interval.

obtain better separation of the amines (see Section 2). Typical chromatograms obtained for a blank solution and for a solution containing the ten amines processed under the optimal conditions are shown in Fig. 3B. As can be seen the mobile phase 2 provided good separation for the 10 amines.

3.3. Analytical figures of merit

In order to evaluate the quantitative performance of the proposed method, standard sample volumes of 1, 5 and 10 ml were assayed. The calibration graphs peak height (V) versus standard concentration (mg/l) for chemiluminescence detection and other analytical features obtained for each amine by using mobile phase 1 are listed in Table 4. Calibration curves were established with a single value at each concentration level. Good linearity was obtained for the six amines assayed. The detection limits (LODs, established as the concentration required to generate a signal-to-noise ratio of 3) achieved are given in Table 5. Standard sample volumes between 1 and 25 ml were assayed. The elution volume was 1 ml for all volumes except for 5 ml standard sample volume, being 1 and 0.5 ml of acetonitrile. The best detection limits were obtained processing 5 ml of standard sample and eluting with 0.5 or 10 ml of standard sample and an elution volume of 1 ml. The study of inter-day precision for different standard sample volumes (1, 5, and 10 ml) can be seen in Table 6. The method provided good precision, independently of the standard sample volume. Intra-day precision is also given in Table 6 for a standard sample volume of 1 ml, and was slightly better than inter-day results.

Although using volumes of 5 ml of sample the precision was poor for diethylamine, others like pentylamine improved this parameter from 20 to 0.9% (see Table 6), so 5 ml was selected as optimum sample volume in order to achieve good detection limits.

3.4. Analysis of real water samples

The proposed method was applied to the analysis of several real water samples (see Section 2). Spiked samples with

Table 5
Limits of detection (μg/l) obtained for chemiluminescence detection at different sample volumes and different elution volumes

Detection reagent	CL (Dns-Cl/this work)					UV (Dns-Cl/[18])	FL (Dns-Cl/[18])	UV (DNB/[20])	FL (FMOC/[21])	FL (OPA/NAC/[22])
Sample volume (ml)	1	5	5	10	25	5	5	5	5	10
Elution volume (ml)	1	1	0.5	1	1	0.5	0.5	0.5	0.5	2
MA	5	1.3	0.9	0.8	2	3	2	–	0.5	5
EA	0.8	0.2	0.15	0.15	0.2	6	2	5	0.25	6
BA	4	0.9	0.5	0.6	0.8	9	3	–	1	10
PeA	2	0.6	0.3	0.3	0.5	8	2	–	5	23
HA	2	0.5	0.3	0.3	0.3	15	2	–	2.5	–
DEA	4	1.3	0.8	0.8	0.9	15	4	–	–	–

Comparison with those obtained with other detection systems and reagents: CL: chemiluminescence; UV: ultraviolet, FL: fluorescence and Dns: dansyl chloride, DNB: 3,5-dinitrobenzoyl chloride, FMOC: 9-fluorenylmethylchloroformate, OPA/NAC: *o*-phthalaldehyde/*N*-acetylcysteine.

Table 6
Reproducibility data (R.S.D.)

Analyte	Intra-day ($n = 3$ R.S.D., %)	Inter-day ($n = 3$ R.S.D., %)		
	1 (ml)	1 (ml)	5 (ml)	10 (ml)
Methylamine	6	0.8	11	7
Ethylamine	10	4	10	17
Butylamine	3	6	16	13
Pentylamine	5	20	0.9	9
Hexylamine	11	1.3	3	8
Diethylamine	16	10	29	9

Sample volumes processed 1, 5 and 10 ml. Measured concentration from all sample volumes 0.150 mg/l MA, 0.24 mg/l EA, 0.3 mg/l BA, PeA, HA and 0.48 mg/l DEA. Intra- and inter-day standard deviations provided by this method by using different sample volumes

known amounts of analytes were used to validate the method accuracy. The found spiked concentrations calculated employing the corresponding standard calibration graph for each amine are shown in Table 1. For samples S1 (tap water), S2 (irrigation water), S3 (lake water) and S4 (residual water), the found spiked values for all amines and concentrations were close to the real ones, with mean recoveries (±standard deviation) of 113% (±22, $n = 7$), 100% (±19, $n = 4$), 114% (±11, $n = 7$) and 99% (±11, $n = 12$) for S1, S2, S3 and S4, respectively. These values can be considered acceptable taking into account the concentration levels assayed and indicated the absence of matrix effect. However, for sample S5-sea water amines containing one or two C gave recoveries near 100 (97 ± 22, $n = 4$) for the spikes, while amines with more number of C atoms produced higher recoveries between 150 and 200%. Then a matrix effect was observed for those amines in sea water, and the amine concentration must be calculated by using standard additions method (MOSA) instead of the standard calibration equation if those amines would be present in the samples.

Methylamine was found and quantified by using the standard calibration graph in lake water (8.8 ± 0.3 μg/l, $n = 3$), irrigation water (7 ± 3 μg/l, $n = 3$) and industrial water (6000 ± 700 μg/l, $n = 3$), in which also pentylamine (3100 ± 200 μg/l) was observed. In tap water, diethylamine was found and quantified by using the standard calibration graph (13 ± 3 μg/l, $n = 3$) or standard addition method (15 μg/l, $n = 5$) with similar results. None of the analytes were detected in the sea water. Industrial and sea waters were also analysed by using the LC procedure described in [18] which uses pre-column solid-supported dansylation and fluorescence detection. The procedure did not detect any amine for the sea water as the proposed method and for the industrial water the results were: (6040 ± 900 μg/l, $n = 3$) and (2960 ± 160 μg/l) for methylamine and pentylamine, respectively. Both values can be considered statistically equal (at a confidence level of 95%) to those obtained by the proposed method.

Fig. 4 shows the chromatogram obtained for the screening analysis of the industrial waste water. In this case, mobile phase 2 was applied, in order to separate and distinguish the

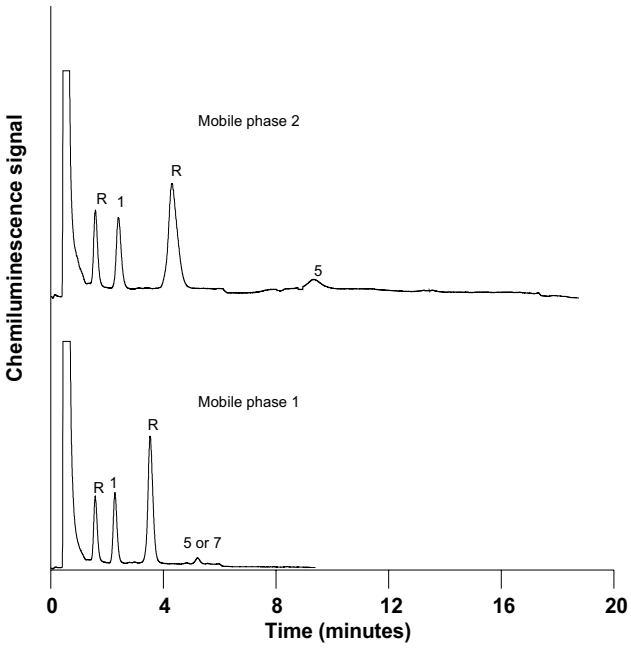

Fig. 4. Chromatograms corresponding to an industrial waste water (processed sample volume: 0.02 ml diluted up to 1 ml), by using mobile phase 1, and mobile phase 2. (R) reagent; (1) MA; (5) PeA; (7) Put.

presence of putrescine and/or pentylamine which coeluted with mobile phase 1 and 0.02 ml of water sample dilute to 1 ml were processed. The results obtained confirmed the presence of pentylamine and methylamine. The limits of detection obtained for the screening procedure, established as the concentration required to generate a signal-to-noise ratio of 3, were: 66, 53, 15, 200, 11, 7.5, 27, 7.6, 11 and 60 μg/l for methylamine, ethylamine, butylamine, diethylamine, pentylamine, putrescine, cadaverine, hexylamine, spermidine and spermine, respectively.

3.5. Comparison with other detection systems

The chemiluminescence detection allows to reach the lowest detection limits as can be seen in Table 5. For each procedure the best sample volume and elution volume were considered. These parameters can increase from 75 to 3 times respect UV and from 10 to 2 times respect Fluorescence detection by using the dansylated derivatives depending on the amine. Other fluorescence reagents as FMOC and OPA/NAC provided also LODs higher than those obtained by CL detection and amine dansylated derivatives as can be seen in Table 5.

The repeatability and reproducibility of fluorescence and chemiluminescence detections both with dansylated derivatives are comparable, R.S.D.s between 3 and 15% (calculated from three replicates of 5 ml of a mixture containing the amines at 1 mg/l level except dimethylamine at 15 mg/l) and Table 6 values, respectively.

By using fluorescence detection and dansylation, a sample clean-up step is needed in the solid-support assisted

derivatization in order to clean the blank reagent. This step produces partial elution of some amines, specially the most polar ones. This behaviour was also observed in all real water samples studied in [18], and the concentration in the sample had to be calculated by using the MOSA method. However, by using chemiluminescence detection, the clean-up step was not included in the procedure, and higher recoveries were obtained for the most polar amines. No matrix effect was obtained for all kind of samples assayed. Only for the sea water and for amines with more than two C atoms the recoveries were different to those obtained with amine standards.

4. Conclusions

This paper studied the chemiluminescence detection of amines in order to improve their detection limits. The procedure used solid-support assisted dansylation as pre-column treatment [18]. The dansylated derivatives were chromatographed and mixed with TCPO/H_2O_2 in order to generate chemiluminescence.

The quantification of aliphatic amines at low ppb levels is possible with satisfactory accuracy and reproducibility. These concentration levels are adequated for monitoring aliphatic amines in real water samples.

No significant differences were observed in the quantification of the analytes between the different sample types tested.

The method also served for screening purposes in presence of other aliphatic amines, such as the polyamines spermine, spermidine, putrescine or cadaverine. An screening procedure has been optimised in order to distinguish ten amines.

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References

[1] P. Simon, C. Lemacon, Anal. Chem. 59 (1987) 480.
[2] C. Dodeigne, L. Thunus, R. Lejeune, Talanta 51 (2000) 415.
[3] M. Yamaguchi, H.H. Nohta, J. Chromatogr. A 950 (2002) 1.
[4] T. Kawasaki, M. Maeda, A. Tsuji, J. Chromatogr. 328 (1985) 121.
[5] K. Nakashima, M. Maeda, A. Tsuji, J. Chromatogr. 530 (1990) 154.
[6] S.R. Spurlin, M.M. Cooper, Anal. Lett. 19 (1986) 2277.
[7] J. Ishida, N. Horike, M. Yamaguchi, Anal. Chim. Acta 302 (1995) 61.
[8] H. Yoshida, R. Nakao, T. Matsuo, H. Nohta, M. Yamaguchi, J. Chromatogr. A 907 (2001) 39.
[9] J.B. Noffsinger, N.D. Danielson, Anal. Chem. 59 (1987) 865.
[10] X. Wang, D.R. Bobbitt, Talanta 53 (2000) 337.
[11] A.W. Knight, Trends Anal. Chem. 18 (1999) 47.
[12] M. Cobos, M. Silva, J. Chromatogr. A 848 (1999) 105.
[13] C. Molins Legua, P. Campins Falcó, A. Sevillano Cabeza, Anal. Chim. Acta 378 (1999) 83.
[14] C. Molins Legua, P. Campins Falcó, A. Sevillano Cabeza, Analyst 123 (12) (1998) 2871.
[15] P.J.M. Kwakman, H. Koelewijn, I. Kool, U.A.Th. Brinkman, G.J. De Jong, J. Chromatogr. 511 (1990) 155.
[16] M. Tod, J.Y. Legendre, J. Chalom, H. Kouwtli, M. Poulou, R. Farinotti, G. Mahuzier, J. Chromatogr. 594 (1992) 386.
[17] G. Orosz, R.S. Givens, R.L. Schowen, Crit. Rev. Anal. Chem. 26 (1996) 1.
[18] S. Meseguer Lloret, C. Molins Legua, P. Campíns Falcó, J. Chromatogr. A 978 (2002) 59.
[19] C. Molins Legua, P. Campíns Falcó, S. Meseguer Lloret, Chromatographia 58 (2003) 15.
[20] J. Verdú Andrés, P. Campins Falcó, R. Herráez Hernández, Analyst 126 (2001) 1683.
[21] J. Verdú Andrés, P. Campins Falcó, R. Herráez Hernández, Chromatographia 55 (2002) 129.
[22] Y. Moliner Martinez, C. Molins Legua, P. Campins Falcó, Talanta 62 (2004) 373.
[23] R. Weinberger, J. Chromatogr. 314 (1984) 155.
[24] N. Hanoaka, J. Chromatogr. 503 (1990) 155.