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Determination of dimethylamine and other low-molecular-mass amines using capillary electrophoresis with laser-induced fluorescence detection

Ewa Dabek-Zlotorzynska*, Wioleta Maruszak[1]

Analysis and Air Quality Division, Environmental Technology Centre, Environment Canada, 3439 River Road, Ottawa, Ontario, K1A 0H3, Canada

Abstract

The potential of capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection for the separation and determination of dimethylamine (DMA) and other low-molecular-mass amines involving precolumn derivatization with fluorescein isothiocyanate isomer I (FITC) was investigated. Different variables that affect derivatization (pH, FITC concentration, reaction time and temperature) and separation (buffer concentration, addition of various organic modifiers, applied voltage and length of capillary) were studied. The linearity, reproducibility and reliability of the method were evaluated. The estimated instrumental detection limit for a 2-s pressure injection of the FITC-DMA derivative was 50 pg/ml (10^{-9} *M*), using LIF detection with excitation and emission wavelengths of 488 nm and 520 nm, respectively. However, for practical reasons, a minimum of 5 ng/ml DMA should be subjected to the derivatization. The applicability of the described method to the extract of atmospheric aerosol samples was demonstrated.

Keywords: Dimethylamine; Amines

1. Introduction

Low-molecular-mass amines are an important class of environmental pollutants due to their odorous and toxic characteristic [1]. These compounds can be found in biological fluid [2], environmental samples [3] and industrial process streams [4], often at trace levels. They can be emitted into the environment from anthropogenic sources such as waste incineration, sewage treatment, automobile exhausts and various industries [3]. Amines are also emitted from animal wastes and biological activities. As lower secondary amines such as dimethylamine (DMA) and pyrrolidine, broadly distributed in environment, can form carcinogenic N-nitrosoamines [5,6] in the presence of nitrites or other nitrosation agents, their occurrence and determination have received a great deal of attention. For this reason, interest in the development of new and sensitive analytical methods to separate and to analyze these compounds continues unabated.

To date, gas chromatographic (GC) [3,7–9] and high-performance liquid chromatographic (HPLC) methods [10–25] are the most widely adopted techniques for the measurement of primary and secondary amines in various matrices. However, these methods have some inherent problems related to the difficulty in handling low-molecular-mass amines

*Corresponding author.

[1]On leave from Department of Chemistry, Warsaw University, Pasteura 1, 02-093 Warsaw, Poland.

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due to their high water solubility and volatility. Recently, capillary electrophoresis (CE) has emerged as a powerful analytical tool in the separation and determination of various species [26]. Besides the high efficiency and short analysis time that can be attained, CE offers great possibilities for microchemical analytical work since nanoliter samples can be readily injected and separated in a CE system. Additionally, CE is an extremely versatile separation method because selectivity can be changed essentially by addition of different modifiers to aqueous buffers or by changing buffer pH. When CE is coupled to a laser-induced fluorescence (LIF) detection of attomole amounts is achieved [27,28]. Currently, LIF is one of the most sensitive detection methods available for CE.

The aim of this study was to investigate the potential of CE coupled to LIF detection for the determination of DMA and other low-molecular-mass amines and to establish suitable conditions for such analyses. Because most amines show neither natural UV nor fluorescence properties the use of indirect UV detection or chemical derivatization is necessary for detecting derivatives of amines prior to CE separation. Chemical derivatization in solution has long been accepted as an effective modification technique in various separation methods such as GC and HPLC, improving the overall specificity, chromatographic performance and sensitivity for trace analysis [3,7–24]. As fluorescence detection and especially LIF is a very sensitive and selective detection mode, CE coupled to LIF detection was utilized. Various fluorescent derivatization reagents used in liquid chromatography [10–24] are readily applicable to CE [29–44].

In the present work, fluorescein isothiocyanate isomer I (FITC) was chosen as a reagent for derivatization of DMA and other amines. FITC has been widely used as a fluorescent derivatization agent in biochemical field [26]. FITC provides good sensitivity for primary and secondary amines [45]. The first use of this reagent in CE was reported by Cheng and Dovichi in 1988 [46] when subattomole analysis of some amino acids was demonstrated. FITC has been also shown to be a very useful precolumn derivatization reagent for CE with LIF detection of polyamines [35] and biogenic amines [36–41]. Most recently, Brumley and Kelliher [47] reported determination of some aliphatic amines in water by CE–LIF after derivatization with FITC. Our study includes an extensive evaluation of factors affecting optimal conditions for the derivatization reactions with DMA. The CE separation of seven amines is optimized and presented to illustrate the compatibility among the derivatization technique, CE separation and LIF detection. The developed method was used for the determination of dimethylamine in the extract of atmospheric aerosol samples.

2. Experimental

2.1. Apparatus

Capillary electrophoresis separations were performed on a Beckman P/ACE 5510 CE system (Fullerton, CA, USA) with the anode on the injection side and the cathode on the detection side (normal polarity), since the negatively charged FITC-derivatized amines migrate toward the cathode under the influence of the electroosmotic flow. Unless otherwise stated, capillaries from Polymicro Technologies (Phoenix, AZ, USA) with 57 cm (50 cm to detector)×75 μm I.D., 375 μm O.D. were used. The temperature of the capillary in the P/ACE instrument was controlled at 20.0±0.1°C by means of a fluorocarbon liquid continuously circulated through the cartridge. The samples were injected by applying 3.45 kPa pressure for 2 s, and an approximate sample volume of 18 nl was calculated [48]. The separations were on-line monitored with a Beckman laser-induced fluorescence (LIF) detection system using a 4-mW argon ion laser with an excitation wavelength of 488 nm and emission wavelength filter of 520 nm. The electropherograms were acquired and stored on a personal computer using the Beckman P/ACE Station (Version 0.4).

2.2. Chemicals

The derivatizing agent fluorescein isothiocyanate isomer I (FITC), α-, β-, γ-cyclodextrins (α-CD, β-CD, γ-CD) and all amines used in this study were purchased from Aldrich (Milwaukee, WI, USA). 2,6-Di-O-methyl-β-CD (DM-β-CD), 2,3,6-tri-O-methyl-β-CD (TM-β-CD) and hydroxylpropyl-β-CD (HP-β-

CD) were purchased from Sigma (St. Louis, MO, USA). The chemicals used in the preparation of solution and all organic solvents were purchased from Fisher Scientific (Ottawa, ON, Canada). All solutions were prepared with deionized water.

2.3. Procedures

Standard solutions containing 1000 mg/l of each amine as hydrochlorides were prepared in water, stored at 4°C and used after dilution to required concentration. Unless stated, derivatization was performed using freshly prepared 1.1 m*M* FITC in acetone. All CE buffers were prepared daily from the 100 m*M* sodium tetraborate solution and ultrasonicated for 20 min before use.

Each day before starting analysis, the capillary was rinsed with 0.1 *M* NaOH and water for 5 min. Between each run the capillary was flushed with 0.1 *M* NaOH, water and the running buffer for 1 min.

2.4. Derivatization of amines

To the standard solution containing DMA or mixture of amines, 100 μl of 0.2 *M* sodium bicarbonate (pH 8.8) and 200 μl of 1.1 m*M* FITC acetone solution were added, and the total volume was made up to 1 ml with deionized water. Unless otherwise stated, the screwed capped reaction vessel was allowed to stand overnight in darkness and at room temperature (21°C). Before CE analysis, the derivatization mixtures were diluted five times with a running electrolyte.

The reagent blanks without amines were treated in the same manner. For the determination of the limits of detection, reaction mixture was diluted until it gave a signal-to-noise ratio of 3:1.

3. Results and discussion

According to the Edman degradation [49], FITC reacts with primary and secondary amines like phenyl isothiocyanate under alkaline conditions to form fluorescein thiocarbamyl derivatives. These derivatives exhibit strong fluorescence with an excitation wavelength which almost matches the 488 nm light provided by an argon laser that is used in at least one commercially available CE system with LIF detection.

3.1. Optimization of derivatization conditions

The conditions for the derivatization reaction were optimized using DMA. Although the derivatization conditions are based on the method developed for amino acids [50], the general aim of these experiments was to achieve the best possible compromise between high fluorescence intensity of DMA derivative and low side reaction products. For optimization of derivatization conditions for 100 μg/l of DMA, several parameters affecting the reaction were studied, including the chemical composition, concentration and pH of the buffer used, the amount of FITC, addition of organic solvents, reaction time and temperature. CE analysis of FITC-derivatized DMA was performed with a 20 m*M* borate buffer containing 10% acetone and with 25 kV voltage.

The fluorescence intensity and amount of side reaction peaks of three buffer systems (carbonate, borate and phosphate) at different pH values containing 10% (v/v) acetone were compared. Table 1 shows relative fluorescence peak intensity of DMA-FTC derivatized in various buffer solutions. As expected [50], the reaction rate increases with increasing pH value. The best intensity was obtained with buffers at pH 10. However, an increase of side reaction peaks is also observed. The degree of derivatization is also influenced by the type of buffer. Best results were obtained with the carbonate buffer compared to the borate and phosphate buffers

Table 1
Relative fluorescence peak intensities (%) of DMA-FITC derivatized in various buffer solutions after different reaction times

Buffer	pH	Relative intensity[a] (%)	
		5 h	24 h
Phosphate	8.6	22	45
Carbonate	8.8	87	100
Borate	8.9	61	66
Carbonate	9.7	139	127
Phosphate	10.0	121	127

[a] Normalized to response for DMA-FITC derivative in carbonate buffer (pH 8.8) and after 24 h. Conditions: 100 μg/ml DMA, 20 m*M* buffer, 20% (v/v) acetone, 0.22 m*M* FITC in reaction solutions.

at similar pH. The reaction rate was not significantly affected by changing the concentration of the carbonate buffer in the range 5–50 m*M* in the reaction solution, therefore the carbonate buffer at concentration of 20 m*M* and pH 9 was used in further studies.

The signal intensity also depends on the FITC concentration in the reaction solution. A saturation type curve was obtained showing a plateau above 0.1 m*M* (a 100-molar excess). A 100-molar excess of FITC seems to be the optimum for DMA derivatization in standard solutions, but in further experiments 0.22 m*M* of FITC in the reaction mixture was used.

Because organic solvents can enhance or quench the fluorescence [51], the influence of acetone, acetonitrile, methanol, tetrahydrofuran (THF) and dimethylformamide (DMF) which were necessary as solvents for FITC was investigated. Nearly similar intensity of FITC-derivatized DMA was obtained with acetone and acetonitrile. Methanol decreases the fluorescence intensity to about 10% compared to acetone. When THF or DMF were used no response for DMA derivative was observed. The signal intensity was almost similar when the reaction solution contained acetone at a concentration range of 15% to 40% (v/v). Lower intensity was observed in 50% (v/v) acetone. Thus, 20% acetone solution was selected for further experiments.

As reported previously [45], the derivatization reaction of primary and secondary amines using FITC is relatively slow. Fig. 1 shows the derivatization efficiency for DMA increases with time and after 8 h a plateau is reached. Since an 8-h derivatization procedure is not convenient, we suggest an overnight (16 h) derivatization procedure for the secondary amines.

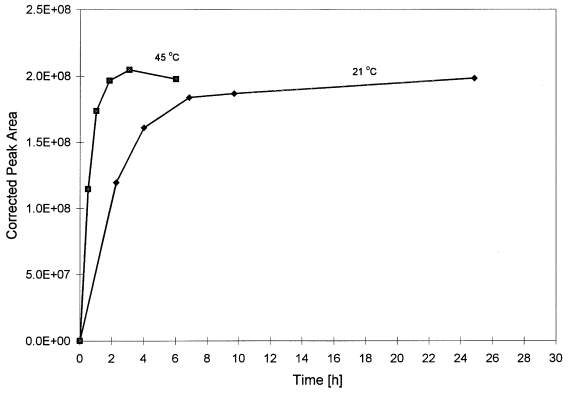

Fig. 1. Effect of reaction time and temperature on the formation of DMA derivative. Conditions as in Section 2.4.

Reaction temperature is a critical parameter for the derivatization reaction. The effect was examined in acetone. As expected [43], the rate of reaction of DMA with FITC increased with temperature. Fig. 1 shows the derivatization yield as a function of incubation time at 45°C. As can be seen, the derivatization of DMA practically completed after 2 h. However, since raising the reaction temperature also enhanced side reactions, heating was not found useful.

3.1.1. Stability studies

The stability of the DMA derivative at room temperature and at 4°C in the dark was studied over a period of 7 days. No significant change in the corrected peak area for DMA derivative was found (R.S.D.$<$5%), indicating favorable stability of the derivative.

On the basis of these results, the optimum derivatization conditions were established as formulated in Section 2.4.

3.2. Choice of separation conditions

Optimization of the separation conditions was achieved through testing the migration behavior of derivatized mixture of seven amines. Test substances were methylamine (MEA), dimethylamine (DMA), diethylamine (DEA), dipropylamine (DPA), piperidine (PIP), pyrrolidine (PYR) and morpholine (MOR). The effect of various parameters such as concentration of buffer, addition of various organic modifiers, applied voltage and length of capillary were optimized to achieve best separation, the highest sensitivity and the shortest analysis time.

Initially, experiments performed using a 20 m*M* borate buffer at pH 9.0 and with an applied voltage of 25 kV were successful in resolving DPA, DMA and MEA. The resolution of the other FTIC-derivatized amines was unsatisfactory. Thus, complete separation of all seven amines was not achieved with a buffer containing no organic modifier. The use of different organic solvents such as methanol, acetoni-

trile and acetone at the concentrations ranging from 5% to 30% was then tested. The best results were obtained on addition of 30% (v/v) acetone. However, the migration time was longer than 30 min and PIP, DEA and PYR were not baseline resolved.

For a further improvement of the resolution, addition of a range of various cyclodextrins (α-CD, β-CD, γ-CD, DM-β-CD, TM-β-CD and HP-β-CD) of different cavity size and rim substitution was investigated. Many papers have been published demonstrating the effect of cyclodextrins on the improvement of CE separation of various analytes [52]. Figs. 2 and 3 show the electrophoretic mobility of the studied derivatized amines using a 20 m*M* borate buffer containing 20% acetone and in the presence of the cyclodextrins at concentrations ranging from 5 m*M* to 15 m*M*. The various CDs showed different migration patterns of the various analytes. Generally, the electrophoretic mobility of the CD–analyte complexes is lower (shorter migration times) than that of the uncomplexed analytes, and the stronger this complexation was, the faster the analytes migrated toward the detector. The migration times of derivatized amines were shortened as γ-CD was added to the buffer. Addition of α-CD strongly affected the electrophoretic mobility of the reagent peak but did not influence the migration pattern or times of the tested derivatized amines. Among the unmodified CDs, only β-CD improved the separation efficiency with baseline resolution of PIP and DEA. Similar effects on the separation resolution were obtained with DM-β-CD and TM-β-CD. However, DM-β-CD yielded much better peak shape than β-CD or TM-β-CD. With HP-β-CD, worse resolution was observed among the various analytes and the migration times of derivatized amines were shortened.

Based on these results, the best separation conditions were found to be 20 m*M* borate buffer pH containing 20% acetone and 5 m*M* DM-β-CD. As can be seen in Fig. 4, the separation of seven derivatized amines is obtained within 10 min using a 47-cm capillary (40 cm to detector) with theoretical plate numbers of about 130 000. However, DEA and PYR were not baseline resolved. In addition to organic modifier, the concentration of the electrophoretic buffer is also an important separation parameter. Use of higher concentrations of borate led to slightly better resolution, but the increase of the migration time and electrophoretic current are also observed. The effect of the applied voltage was also investigated resulting in selecting a running voltage of 25 kV.

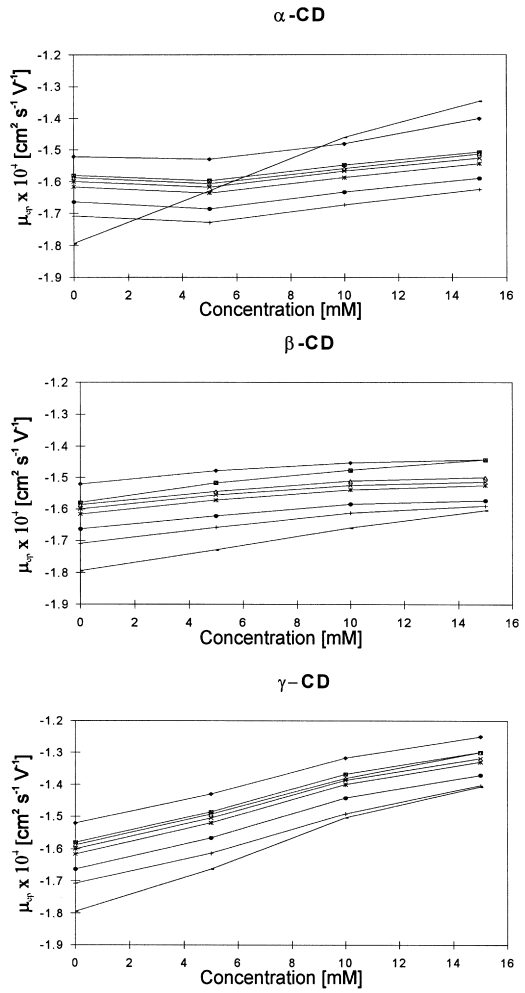

Fig. 2. Electrophoretic mobility of the tested amine derivatives as a function of α-CD, β-CD and γ-CD concentration. Running buffer, 20 m*M* borate, 20% acetone containing various concentration of CDs. Lines (◇) DPA; (⊠). PIP; (Δ) DEA; (x) PYR; (*) MOR; (●) DMA; (+) MEA; (−) FITC. Other conditions as in Section 2.4.

3.3. Analytical characterization

In order to evaluate the characteristics of this CE–LIF system, the linearity, reproducibility and

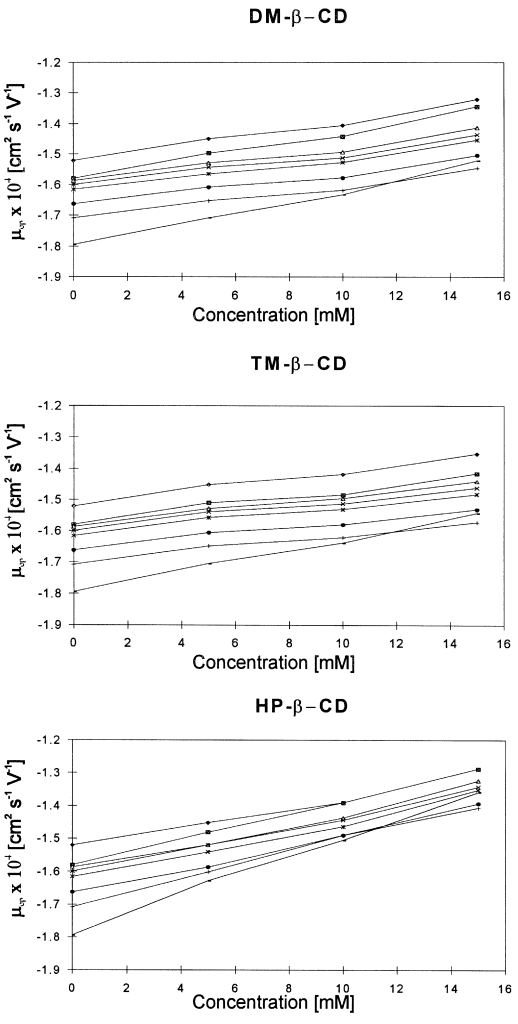

Fig. 3. Electrophoretic mobility of the tested amine derivatives as a function of DM-β-CD, TM-β-CD and HP-β-CD concentration. Conditions and lines are the same as in Fig. 2.

limit of detection for the six amines were determined. The quantitative applicability of the method for the determination of DMA and other low-molecular mass amines was evaluated at seven different amounts of analytes taken for derivatization over the range 5–1000 μg/l using 20 m*M* borate buffer with 20% acetone and 5 m*M* DM-β-CD. The calibration graphs were established with the corrected peak area. The linear least-squares standard calibration graphs were linear with correlation coefficient $r^2>0.998$ in a 2-s pressure injection for all the amines examined (Table 2).

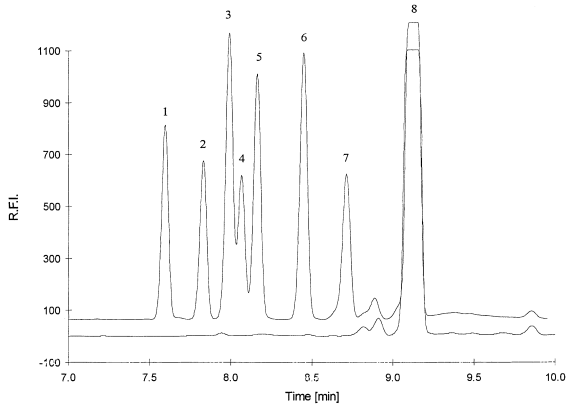

Fig. 4. LIF electropherograms of 5-fold diluted standard (upper chart) and reagent blank (lower chart). Conditions: buffer; 20 m*M* borate–20% acetone–5 m*M* DM-β-CD; capillary, 47 cm×75 μm (40 cm effective length), injection, 2 s, pressure; applied voltage, 25 kV (~50 μA). Peaks: 1=DPA; 2=PIP; 3=DEA; 4=PYR; 5=MOR; 6=DMA; 7=MEA; 8= FITC. Diluted concentration of PYR and DMA (100 μg/l), other peaks (200 μg/l).

By running seven replicates of the standard (100 μg/l), each FITC-derivatized amine showed high reproducibility in terms of the corrected peak area or migration times. The R.S.D values of the corrected peak areas were between 2.5% and 5%. Relatively stable migration times (R.S.D. less than 0.5%) could be obtained when the capillary was rinsed with sodium hydroxide after each run. The day-to-day reproducibility (R.S.D.<4%) is affected by any changes of the capillary surface that affect the electroosmotic flow. Therefore, it is better to rely on relative migration times, whose reproducibility was found to be much better.

Further instrumental sensitivity (dilution after derivatization) and derivatization sensitivity (dilution before derivatization) were evaluated as described by Lallije and Sandra [43]. A similar calibration response was found for FITC-derivatized DMA when the derivatization step was performed at low analyte concentrations ($y=1\,493\,788x-223\,057$, $r^2=0.998$) and that obtained by the dilution of the relatively high concentration (500 μg/l) used for derivatization ($y=1\,529\,225x-247\,120$, $r^2=0.999$; y=corrected peak area, x=concentration). These data suggest that the efficiency of the derivatization reaction under the present conditions is similar in very low and high concentration of DMA. However, handling and

Table 2
Linearity, sensitivity and detection limit of the proposed method

Amine	Correlation coefficient	Sensitivity[a] CPA/conc.	Detection limit	
			(pg/ml)	(*M*)
DPA	0.9981	618 199	90	$9\cdot10^{-10}$
PIP	0.9987	513 715	110	$1\cdot10^{-9}$
DEA	0.9985	849 949	65	$9\cdot10^{-10}$
MOR	0.9999	767 368	80	$9\cdot10^{-10}$
DMA	0.9999	1 495 919	50	$1\cdot10^{-9}$
MEA	0.9892	432 968	150	$5\cdot10^{-9}$

[a] Slope of calibration curves obtained in the 5–1000 ng/ml range. CPA=corrected peak area.

derivatization for practical reasons require a minimum of ~100 times larger concentration than the estimated detection limit. As can be seen in Table 2, detection limits (based on signal-to-noise ratio equal to 3) for the six amines range from 50 pg/ml (10^{-9} *M*) for DMA up to 150 pg/ml for MEA (10^{-9} *M*). The proposed CE method provides equivalent or better detectability than those obtained by HPLC or GC (Table 3). Increasing the injection time can further lower the detection limits of the developed procedure.

3.4. Application

Ion chromatography (IC) is extensively used in this laboratory for the analysis of inorganic and organic anions and cations in aqueous extracts of atmospheric aerosols collected on thin PTFE filters [53]. Some unknown peaks are sometimes present in these extracts analyzed by IC. The proposed method was utilized to identify and determine of amines in such samples in order to confirm the IC results. As can be seen in Fig. 5, DMA and DEA are present in the atmospheric aerosol extract in addition to other inorganic cations.

4. Conclusions

This paper has demonstrated the suitability of CE–LIF for determination of the FITC-derivatized low-molecular-mass amines. The derivatized amines are stable, highly fluorescent and can be detected in an extremely low concentration. Optimum separation for seven investigated amines was obtained using 20 m*M* borate–20% acetone–5 m*M* DM-β-CD. The detection limits are about 1 n*M* and thus are equiva-

Table 3
Comparison of the detection limits reported for dimethylamine

Separation method	Sample pretreatment	Detection	Detection limit (*M*)	Reference
GC	2,4-BSC[a]-derivatized	Mass spectrometry	$2\cdot10^{-8}$	[7]
	2,4-DNFB[b]-derivatized	Mass spectrometry	$1\cdot10^{-8}$	[7]
HPLC	FMOC[c]-derivatized	Fluorescence	$2\cdot10^{-8}$	[14]
	FMOC-derivatized	Fluorescence	$4\cdot10^{-7}$	[15]
	2-NPO[d]	Chemiluminescence	$5\cdot10^{-7}$	[16]
	ITDT[c]	Fluorescence	$5\cdot10^{-8}$	[23]
CE	FITC-derivatized	LIF	$1\cdot10^{-9}$	This work

[a] Benzenesulfonyl chloride.
[b] 2,4-Dinitrofluorrobenzene.
[c] 9-Fluorenylmethyl chlorformate.
[d] Bis(2-nitrophenyl)oxalate.
[e] 5-Isothiocyanato-1,3-dioxo-2-*p*-tolyl-2,3-dihydro-1*H*-benz[*de*]isoquinoline.

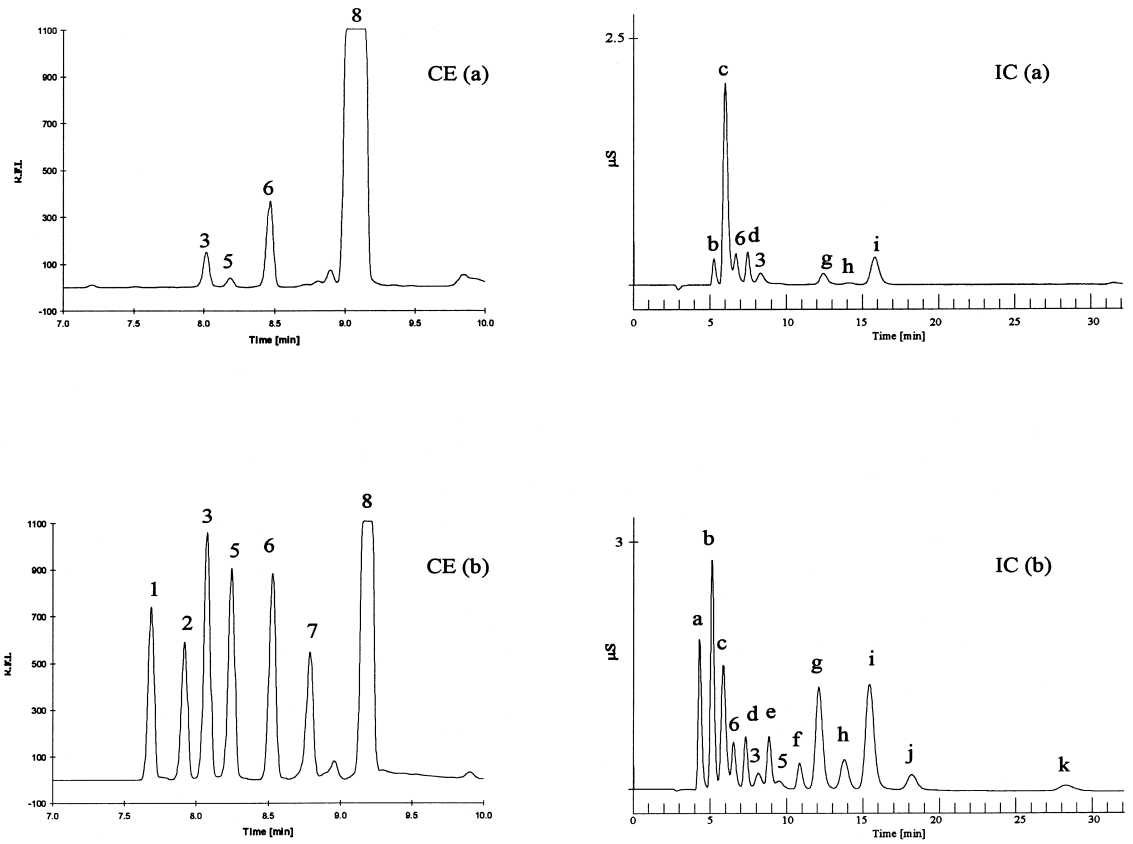

Fig. 5. CE and IC comparative analyses of the aqueous extract of atmospheric aerosol (a=extract, b=standard). CE: Experimental conditions and peaks as in Fig. 4. IC: Columns; Dionex IonPac CG12A (50 mm×4 mm I.D.) and CS12A (250 mm×4 mm I.D.); eluent, 18 m*M* methanesulphonic acid; flow-rate, 1.0 ml/min; detection, suppressed conductivity, CSRS AutoSuppression, injection volume, 50 μl. Peaks: a=lithium, b=sodium, c=ammonium, d=potassium, e=rubidium; f=cesium, g=magnesium, h=manganese, i=calcium, j=strontium, k=barium. Other peaks as in Fig. 4. Concentration of amines in IC standard solution: 500 μg/l.

lent to or better than the range of the detection limits obtained by GC or HPLC. The proposed CE–LIF method seems to be an attractive choice for the determination or confirmation of DMA and other low-molecular-mass amines in atmospheric aerosols and other environmental samples.

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