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Determination of dimethylamine in groundwater by liquid chromatography and precolumn derivatization with 9-fluorenylmethylchloroformate☆

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Abstract

A reversed-phase liquid chromatographic method involving precolumn derivatization with 9-fluorenylmethylchloroformate (FMOC) has been developed for the determination of dimethylamine (DMA) in groundwater. FMOC reacts rapidly with DMA under mild conditions, and the derivative is separated from matrix components and FMOC degradation products on a C_{18} column using isocratic elution with a mobile phase of 0.03 *M* acetate buffer (pH 4.0)–acetonitrile (30:70, v/v). Mean recovery was 98.5 ± 4% and a detection limit of $4.5 \cdot 10^{-7}$ *M*, corresponding to an injected amount of 3.6 pmol, was estimated using fluorimetric detection with excitation and emission wavelengths of 265 and 310 nm, respectively. No matrix interferences were found even when highly coloured and/or cloudy samples were examined. Several mixtures containing eleven amines, diamines and amino acids can be resolved using this method.

Keywords: Water analysis; Derivatization, LC; Environmental analysis; Dimethylamine

1. Introduction

Dimethylamine (DMA) is present in untreated waste water discharges from aramide polymer manufacturing facilities where it is produced by the decomposition of N,N-dimethylacetamide in the solvent stripping step. DMA is the precursor of N-nitrosodimethylamine [1], a substance belonging to a major class of chemical carcinogens, and therefore, environmental protection authorities demand analytical monitoring of unconverted DMA in the aquatic environment close to waste treatment facilities.

The determination of low-molecular-mass aliphatic amines at trace levels is a difficult problem due to their high basicity and strong adsorption on solid surfaces. Gas chromatography is widely used for this purpose [2–4], and many derivatization methods have been developed to avoid adsorption effects; however, the GC methods have been mainly applied to the determination of aliphatic amines in air [5,6].

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Most primary and secondary amines exhibit poor chromatographic performance via direct high-performance liquid chromatography (HPLC) approaches [7,8], making quantitative trace analysis difficult. Chemical derivatization in solution has long been accepted as an effective modification technique in HPLC, improving the overall specificity, chromatographic performance and sensitivity for trace analysis.

Liquid chromatography with precolumn derivatization and fluorescence detection has been applied for the determination of traces of primary and secondary amines, using reagents such as *o*-phthalaldehyde (OPA) [9], fluorescamine [10], *m*-toluoyl chloride [11], dansyl chloride [12], and halogenonitrobenzofurazans [13–15]. OPA and fluorescamine are known to react only with primary amines to give the corresponding fluorophores. The use of *m*-toluoyl chloride is very tedious and not very sensitive or selective, and the reagent dansyl chloride requires long reaction times (1–15 h). Halogenonitrobenzofurazans are effective derivatization reagents, but they have mainly been applied to the determination of amines in air, polyamines and amino acids. Phenylisothiocyanate has been utilized for the determination of DMA in waste water samples but shows poor limits of detection as well as poor linearity [16]. Derivatization reagents like Luminarin I form a highly sensitive derivative due to their chemiluminescent properties but require very long reaction times for secondary amines (180 min) [17].

9-Fluorenylmethylchloroformate (FMOC) was introduced as a precolumn derivatization reagent for liquid chromatographic analysis of amino acids in 1983 [18]. FMOC forms derivatives with both primary and secondary amines and may be used to protect hydroxy groups [19]. The derivatives are fluorescent and absorb in the ultraviolet region, are formed in a reaction time of less than 1 min in a buffered aqueous solution at room temperature, and yield stable derivatives. Thus, a sample can be immediately stabilized by derivatization.

FMOC has not been previously applied to the chromatographic determination of DMA, and there are few references dealing with the use of this reagent for chromatographic separation of aliphatic amines, despite the fact that its application to amino acid separation has been well documented [20,21]. Bantok and Borcsok [22] described an FMOC derivatization method for polyamine separation in plant tissues with very low sensitivity. The method has also been applied to the determination of pirlimicine in urine [23]. Several derivatization columns have been developed where FMOC is covalently bonded to the inert polymer support, thus avoiding problems due to interferences by excess reagent [24]. Disadvantages of this technique are the complex polymer preparation and the peak widening.

In the present work, we have adapted the FMOC amino acid derivatization method for the detection of DMA by changing the derivatization procedure and the chromatographic conditions. The optimized method was applied to the determination of dimethylamine in subterranean waters close to factories which produce and purify effluents containing DMA.

2. Experimental

2.1. Apparatus

The chromatographic system consisted of a Philips (Cambridge, UK) PU 4100 pump and a Rheodyne M-7125 (Berkeley, CA, USA) sample injection valve equipped with a 20-μl sample loop. Alternatively, a Waters (Milford, MA, USA) M-510 pump was used.

UV spectrophotometric detection was carried out with a Waters 486 UV spectrophotometric detector. The wavelength was set at 264 nm. Fluorimetric detection was performed with a Perkin-Elmer LS-3 spectrofluorimeter (Perkin-Elmer, Norwalk, CT, USA) fitted with a flow cell. Data acquisition and processing were carried out by a Philips PU 485 digital integrator.

A pH meter Crison Micro pH 2000 (Crison Barcelona, Spain) was used for eluent preparation and sample treatment.

Derivatization reagents and analytes were mixed in a Heidolph Reax 2000 vortex mixer.

2.2. Reagents

9-Fluorenylmethylchloroformate (FMOC) was purchased from Fluka (Buchs, Switzerland) and was prepared by dissolving the appropriate amount of FMOC in anhydrous acetonitrile. Hexane (Aldrich, Steinheim, Germany) was used as the extraction solvent. Acetonitrile was purchased from Romil Chemicals (Loughborough, UK). All were of HPLC grade. All other chemicals were of analytical reagent grade.

Boric acid was purchased from Panreac (Barcelona, Spain) and anhydrous sodium acetate and glacial acetic acid from Merck (Darmstadt, Germany).

Standard solutions of dimethylamine (DMA), other amines and amino acids examined were prepared with the following reagents without further purification. Dimethylamine hydrochloride, diethylamine hydrochloride, benzylamine hydrochloride, cadaverine dihydrochloride, putrescine dihydrochloride, methylamine hydrochloride, *n*-butylamine, proline, anhydrous cysteine hydrochloride and glycine were all purchased from Sigma (St. Louis, MO, USA). Aniline and ammonia were from Merck and proline from Fluka.

Samples were filtered through a Millipore Millex-SLGV025BS 0.22-μm filter.

2.3. Derivatization procedure

Groundwater samples were homogenized and filtered through a 0.22-μm membrane filter. Derivatization was carried out by mixing 400 μl of the filtered sample or DMA standard solution, 500 μl of 15 m*M* FMOC dissolved in anhydrous acetonitrile and 100 μl of 1 *M* borate buffer (pH 8.0). The solution was vortexed for 120 s in a 3-ml glass vial fitted with a Teflon-head screw cap. The reaction mixture was then neutralized by adding 5 μl of glacial acetic acid, and the excess of FMOC was extracted by adding 2 ml of hexane and vortex mixing for 30 s. The organic phase was discarded, and 20 μl of aqueous phase were injected into the chromatographic system.

2.4 Chromatographic conditions

Chromatographic separation was carried out on a Spherisorb ODS-2 column (25 cm × 4 mm I.D.) (Teknokroma, Barcelona, Spain), filled with 10 μm C_{18} bonded phase particles. The optimum composition of the mobile phase for separating FMOC–DMA derivative from FMOC degradation products and other sample materials was found to be a mixture of 0.03 *M* acetate buffer pH 4.0 and acetonitrile in the proportion of 30:70 (v/v).

Isocratic elution was used at a flow-rate of 1.0 ml/min and ambient temperature. Fluorimetric detection was performed at an excitation wavelength of 265 nm, monitoring the emission at 310 nm. Quantitation was achieved by the external-standard method using calibration graphs constructed by plotting the FMOC–DMA elution peak height versus the concentrations of DMA in a series of standard solutions processed as described above.

3. Results and discussion

3.1. Derivatization procedure

The chemical characteristics of DMA differ from those of amino acids due to its low molecular mass, high basicity and high volatility, which produces loss of analyte in diluted alkaline solutions. Thus it was necessary to modify the usual order of reagent addition to avoid the loss of DMA that was observed when the sample solutions were made alkaline before the addition of the derivatizing reagent.

No significant changes in the derivative yield or the reaction rate were observed when the reaction mixture was buffered at various pHs between 8 and 12. However, the stability of the derivative was slightly higher at pH 8.0. At this pH the elution peak of the derivative remains

constant for more than 70 min after the derivatization reaction has been stopped.

FMOC was used in acetonitrile solution instead of the usual acetone–acetonitrile mixture because a significant loss of derivatized adducts in the hexane extraction step was detected when acetone was present in the reaction mixture. Acetonitrile decreases the organic content of the reaction mixture and also the solubility of the most hydrophobic derivatized compounds. Also, the use of acetonitrile has an additional advantage because the final composition of the injected mixture approaches that of the mobile phase used.

The excess of the derivatizing reagent was extracted with hexane instead of pentane, which is normally used as the extraction solvent in amino acid derivatization. Hexane is more manageable due to its lower volatility and produces quick and clear phase separation.

3.2. Chromatographic separation

The chromatographic behaviour of the FMOC–DMA adduct on the C_{18} column was examined using several mixtures of acetonitrile and acetate buffer as mobile phases with different buffer concentrations and pHs. The UV spectrophotometric detector at 264 nm was used for this study.

Fig. 1 shows chromatograms of a derivatized DMA standard solution and the corresponding reagent blanks. The FMOC–DMA adduct is eluted at 5.65 min, close to a peak eluting at 4.97 min. It was observed that this peak disappears if no acetic acid was added to the reaction mixture. After neutralization of the reaction mixture with glacial acetic acid this peak increases slowly, indicating that it is produced by acetylation of a component of the reaction solution. The only compound resulting from FMOC hydrolysis which is able to produce an acetyl derivative is 9-fluorenylmethanol. Therefore the substance eluted at 4.97 min could be 9-fluorenylmethylacetate (FMA), although further identification by mass spectroscopy may be necessary to confirm this assumption.

Different acids were unsuccessfully tested to eliminate the peak close to that of the FMOC–DMA derivative. Hydrochloric acid produces a strong distortion in the FMOC–DMA peak, whereas benzoic acid does not allow a quantitative recovery of DMA. Thus neutralization with acetic acid was retained and a careful optimization of the mobile phase composition was carried out to improve the resolution between the FMA and the FMOC–DMA adduct peaks.

The effect of the aqueous–organic solvent ratio on the capacity factor of FMOC–DMA and the resolution between FMOC–DMA and FMA peaks was studied using a set of 0.01 *M* pH 4.5 acetate buffer–acetonitrile mixtures with aqueous buffer percent ranging from 20% to 45%. The results, plotted in Fig. 2, show that buffer amounts lower than 20% produce a derivative elution very close to the dead time of the column ($t_0 = 1.60$ min), while for values higher than 50%, too long retention times were obtained. On the other hand, an increase in the buffer amount over 30% produces a rapid deterioration in resolution between the FMOC–DMA adduct peak and the preceding FMA peak.

The use of gradient elution did not improve the resolution because, as shown in Fig. 2 for buffer percentages lower than 30%, resolution remains nearly constant. For that reason an isocratic regime was used, and a proportion of acetate buffer to acetonitrile of 30:70 (v/v) was selected for further studies.

The effect of buffer pH on the retention of FMOC–DMA adduct and FMA is small since both compounds are not ionizable in the pH range suited to bonded-phase liquid chromatography. However, a small loss in resolution was observed at pH higher than 4.0. Thus, this value was finally selected.

Buffer solution concentration does significantly affect the resolution between the FMA and FMOC–DMA peaks. For a 0.03 *M* acetate buffer at pH 4.0, a resolution of 1.9 was reached which may be improved at higher buffer concentrations. As that value was sufficient to achieve an accurate estimation of DMA and to avoid salt precipitation in the system due to the high acetonitrile content in the mobile phase, a final eluent composition of 0.03 *M* pH 4.0 acetate

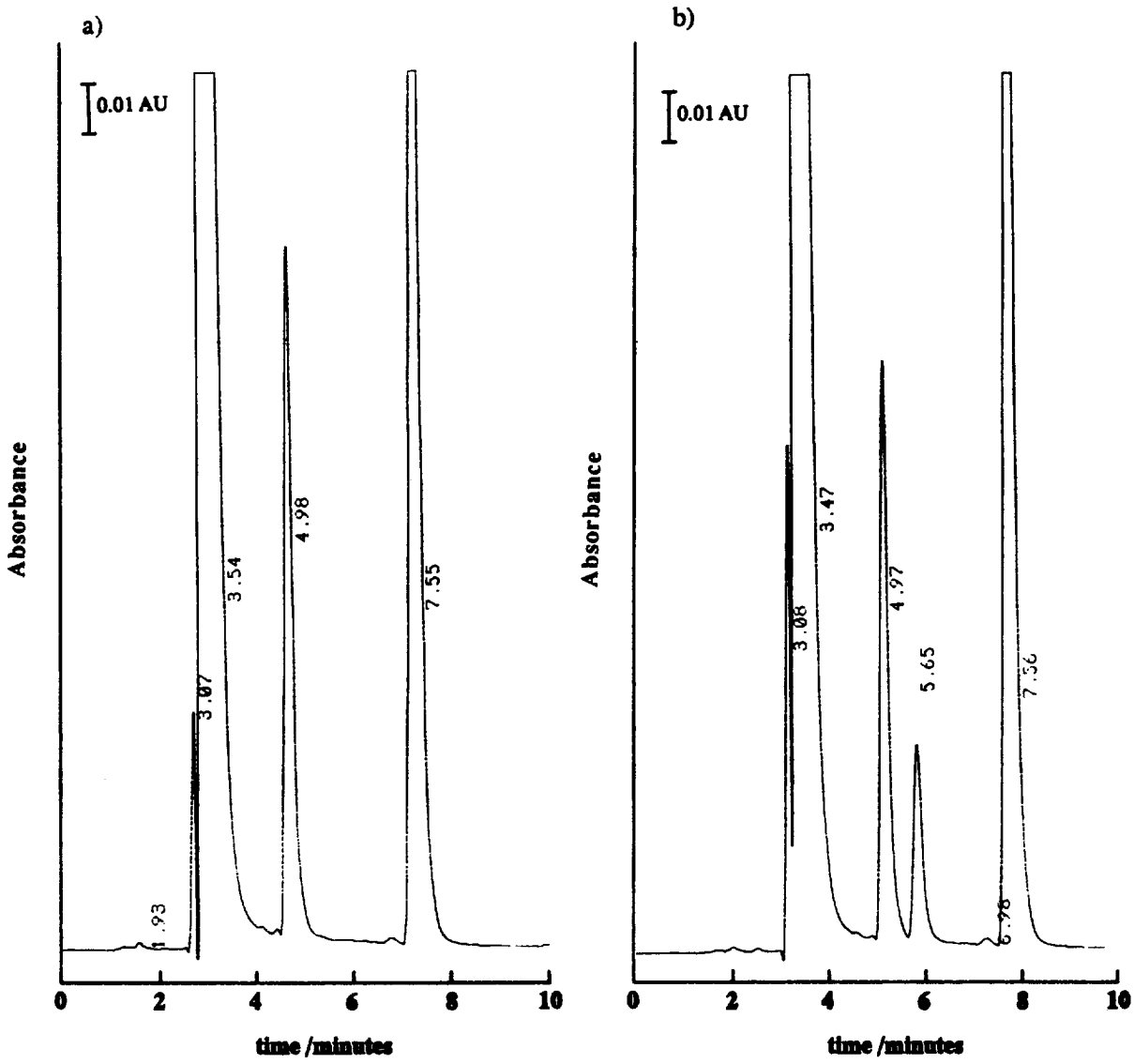

Fig. 1. Chromatograms corresponding to (a) reagent blank and (b) $5 \cdot 10^{-5}$ *M* standard DMA.

buffer and acetonitrile 30:70 (v/v) was selected. Using these separation conditions and a flow-rate of 1.0 ml/min, the retention time of FMOC–DMA derivative at 20°C was 5.70 min ($k' = 2.33$). Other peaks in the chromatogram were assigned to degradation products of the excess of FMOC: 9-fluorenylmethylmethanol at 3.54 min, 9-fluorenylmethylacetate at 4.98 min and dibenzofulvene at 7.55 min.

3.3. Detection

Although UV spectrophotometric detection at 264 nm allows DMA estimation down to the $5 \cdot 10^{-6}$ *M* level, fluorescence detection can improve DMA detectability. As the fluorescence characteristics of the FMOC–DMA derivative have not been reported previously, excitation and emission spectra of the FMOC–DMA de-

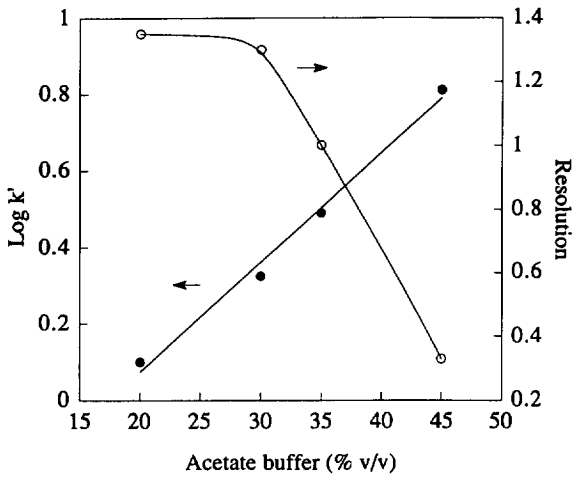

Fig. 2. Effect of eluent composition on the FMOC–DMA derivative capacity factor (●) and FMOC–DMA resolution peak (○). See text for chromatographic conditions.

rivative were obtained by repeated injections of the mixture resulting from the derivatization of a standard solution of $5 \cdot 10^{-5}$ *M* DMA. The emission spectrum was obtained by fixing an excitation wavelength of 264 nm and detecting the repeated injections at different emission wavelengths ranging from 280 to 370 nm. An analogous experiment was done to obtain the excitation spectrum. Collected data are displayed in Fig. 3. Maximum values of excitation and emission spectra of the FMOC–DMA derivative correspond to 265 and 310 nm, respectively.

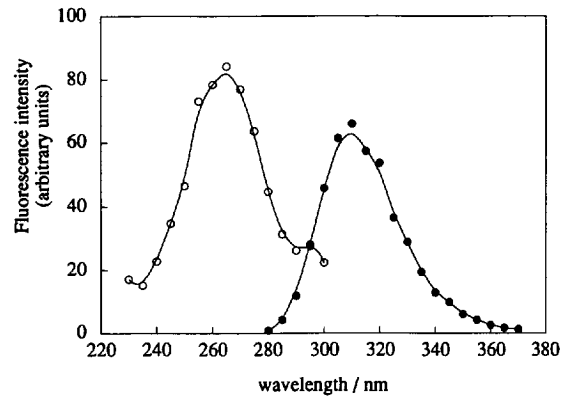

Fig. 3. FMOC–DMA excitation (○) and emission (●) spectra (265 nm maximum excitation and 310 nm maximum emission wavelength). Chromatographic conditions: 0.03 *M* pH 4 acetate buffer–acetonitrile (30:70, v/v); isocratic elution; flow-rate, 1 ml/min.

3.4. Calibration

A calibration graph was constructed using the optimum separation and detection conditions for a set of standard solutions of DMA with concentrations ranging from $1.0 \cdot 10^{-6}$ to $1.0 \cdot 10^{-3}$ *M*. Aliquots of 20 μl of the reaction mixture were injected.

Quantitation was carried out with the external-standard method by plotting the detector signal (peak height) versus the molar concentration of DMA in the standard. Least squares analysis for a set of 13 standard DMA solutions gives a slope of $(2.21 \pm 0.01) \cdot 10^{5}$ mol^{-1} l and an intercept of 0.78 ± 0.67, with a linear correlation coefficient of 0.9998.

The limit of detection was evaluated as the concentration of DMA giving a signal equal to five times the peak-to-peak noise of the baseline and was found to be $4.5 \cdot 10^{-7}$ *M*, which corresponds to an amount of FMOC–DMA derivative injected equivalent to 0.16 ng of DMA.

3.5. Interferences

A set of amino acids and amines of industrial and environmental importance were selected and derivatized with FMOC as described above. All of the chosen compounds, listed in Table 1, react

Table 1
Interfering compounds and capacity factors

Compound	*k'*
Glycine	0.25
Proline	0.52
Ammonia	0.97
Methylamine	1.23
Acetic acid	2.12
Dimethylamine	2.56
Benzylamine	2.87
n-Butylamine	3.22
Aniline	3.41
Diethylamine	4.28
Putrescine	5.65
Cadaverine	6.87

with FMOC. In Fig. 4 chromatograms of a standard solution containing $6 \cdot 10^{-5}$ *M* DMA and a mixture of eleven compounds at the same concentration level, including amines, amino acids and ammonia, are shown.

Quantitative estimation of DMA was unaffected by the presence of the above-mentioned compounds in the sample. Ammonia derivative was eluted at the same time as 9-fluorenylmethanol, whereas polyamines like putrescine and cadaverine, which are produced by microbiological protein degradation and may also be present in groundwater, are strongly retained due to their ability to bond two molecules of derivatizing agent. There is also a possibility of finding in these types of untreated waters amino acids like glycine, cysteine and proline, and other aliphatic amines, such as diethylamine, methylamine and *n*-butylamine, since such amines have similar industrial uses as DMA. All of them were completely separated from the DMA peak and can also be quantified independently.

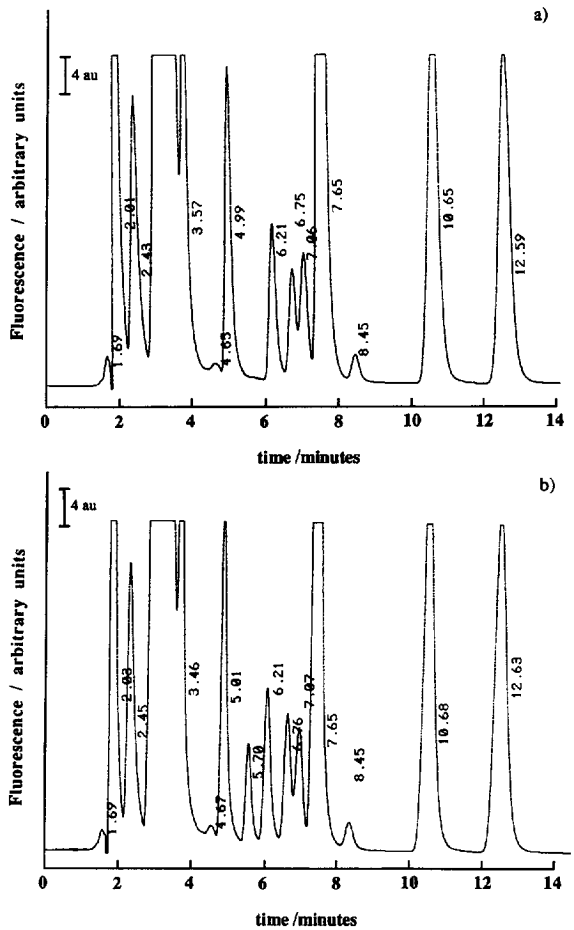

Fig. 4. Samples of (a) interferences pointed out in Table 1 except DMA and (b) containing DMA. Concentration of compounds was $6 \cdot 10^{-5}$ *M*. Chromatographic conditions as in Fig. 3.

3.6. Analytical application

The developed method was applied to the determination of DMA in subterranean water taken from three different pools close to the microbiological degradation pit next to an aramide polymer synthesis plant. Once samples were taken, they were immediately acidified with hydrochloric acid to avoid volatilization of DMA in a neutral medium.

DMA was not found in groundwater at concentrations higher than the limit of detection of our method. These results are justified by the fact that the purification pit was recently built and is structurally sound. Chromatograms of a groundwater sample and an aliquot of the same sample spiked with DMA to reach a concentration of $6 \cdot 10^{-6}$ *M* are presented in Fig. 5. No matrix interferences were observed through the complete set of fifteen examined samples taken over a period of a year and at three different sampling points.

Analytical accuracy was evaluated through recovery studies carried out by adding different amounts of DMA to filtered samples. After filtering, a 400-μl aliquot was used for the derivatization reaction. No matrix effects were observed when comparing the slopes of a standard addition line and a calibration graph constructed using pure DMA standard under the same separation and detection conditions. Therefore, the external-standard calibration method was used for the recovery studies. Results presented in Table 2 show that recoveries varied between 91% and 103% ($n = 9$) with a mean value of 98.5% and a relative standard deviation of 4%.

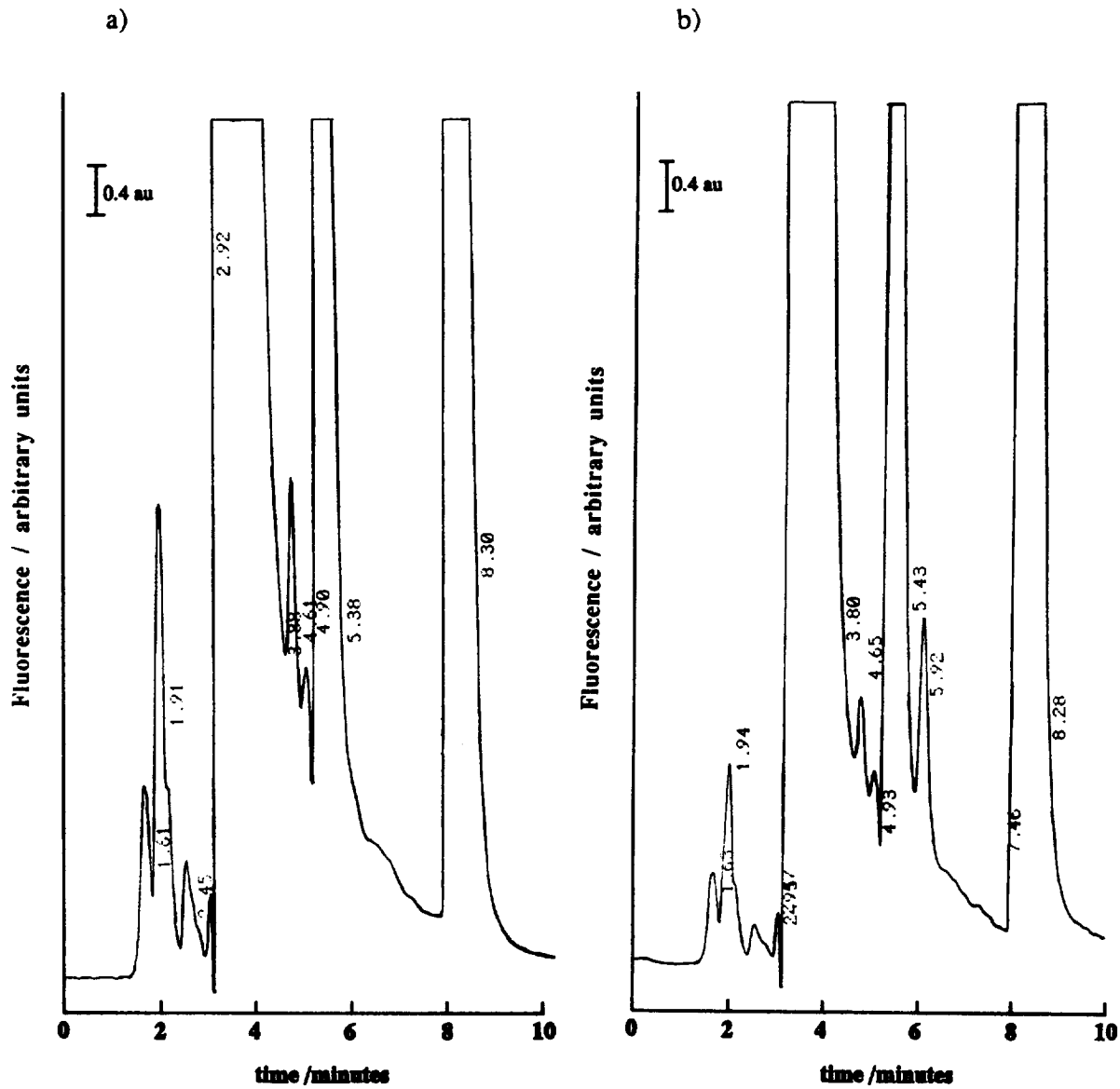

Fig. 5. Chromatograms of (a) a groundwater sample and (b) the same sample spiked with DMA standard ($6 \cdot 10^{-6}$ *M*). Chromatographic conditions as in Fig. 3.

Reproducibility (within-day precision) was verified in the analysis of five aliquots of the same groundwater sample spiked with $6 \cdot 10^{-6}$ *M* of DMA, and the relative standard deviation was found to be 5.7% ($n = 5$).

4. Conclusion

9-FMOC is a derivatizing agent useful for reversed-phase liquid chromatographic determination of DMA in groundwater samples at low

Table 2
Recovery studies on ground water samples

Groundwater sample	DMA standard additions		
	[DMA] added (μg/l)	[DMA] measured (μg/l)	Recovery (%)
1	360	350	97.2
2	360	370	102.7
3	360	360	100.0
4	360	370	102.7
5	360	350	97.2
6	360	340	94.4
7	360	350	97.2
8	360	330	91.2
9	360	370	102.7

(μg/l) concentration levels. The reagent allows quick and selective amine quantitation with excellent recovery and linearity. The method is free from matrix effects and allows simultaneous detection of a number of other amines, polyamines and amino acids.

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