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Journal of Chromatography A, 1023 (2004) 27–31
JOURNAL OF CHROMATOGRAPHY A
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Liquid chromatographic determination of trimethylamine in water

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Received 24 April 2003; received in revised form 22 September 2003; accepted 3 October 2003

Abstract

A method for the selective determination of trimethylamine (TMA) in aqueous matrices by liquid chromatography is reported. The proposed procedure is based on the derivatization of the analyte with 9-fluorenylmethyl chloroformate (FMOC) in a precolumn (Hypersil C_{18}, 30 μm, 20 mm × 2.1 mm i.d.) connected on-line to the analytical column (LiChrosphere 100 RP_{18}, 5 μm, 125 mm × 4 mm i.d.). Gradient elution was performed with a mixture of acetonitrile–water–0.05 M borate buffer (pH 9.0). The method has been applied to the direct determination of TMA in water within the 0.25–10.0 μg/ml concentration interval, and can also be adapted to the determination of TMA over the range 0.05–1.0 μg/ml by incorporating a preconcentration stage with C_{18} solid-phase extraction (SPE) cartridges. Good linearity, reproducibility and accuracy was achieved within the tested concentration intervals. The limits of detection at 262 nm were 50 and 5 ng/ml for the direct method and for the method involving preconcentration, respectively. The proposed conditions allowed the selective determination of TMA in the presence of other primary and secondary short-chain aliphatic amines. The utility of the described procedure has been tested by determining TMA in different water samples.

Keywords: Water analysis; Trimethylamine

1. Introduction

The analysis of short-chain aliphatic amines in water samples is often problematic owing to their high polarity and water solubility, and also to the low concentrations present in real samples. Liquid chromatography (LC) is well suited for the analysis of these amines in aqueous matrices (water, biofluids). However, since short-chain aliphatic amines are rather insensitive towards common LC detectors, a chemical derivatization is generally required. Derivatization can also be used for more convenient sample preparation and chromatographic separation.

Several UV and fluorogenic reagents have been proposed for the derivatization of short-chain aliphatic amines before LC, including 9-fluorenylmethyl chloroformate (FMOC) [1,2], *o*-phtalaldialdehyde [3] or dansylchloride [3,4], and new reagents are constantly being developed [5–7]. However, those reagents are reactive only to primary and secondary amines. In contrast, only a few procedures have been developed for the derivatization of tertiary amino groups, and they rarely include tertiary aliphatic amines. In this sense, the most popular approach is probably the tertiary amine-catalysed condensation of acetic anhydride with citric acid or with malonic acid [8,9]. However, this reaction is subjected to interference by water and thus, it can not be carried out in aqueous phases. Other proposals involve high temperatures and/or very long times of reaction [10]. Therefore, the development of methods for the derivatization of tertiary aliphatic amines for LC continues to be of great interest.

In a previous paper, we demonstrated that FMOC can be used to derivatize the tertiary amphetamine *N*-methylpseudoephedrine under mild conditions [11]. The same reagent has been successfully used for the determination of traces of primary and secondary short-chain aliphatic amines following their derivatization into solid supports [12]. The aim of the present study was to explore the utility of FMOC in the determination of tertiary short-chain aliphatic amines. Derivatizations have been carried out into a C_{18}-packed precolumn connected on-line to the analytical column. Trimethylamine (TMA) has been used as a model of compound, as its determination in aqueous matrices is of increasing interest. Recent methods for this compound are generally based on gas chromatography (GC) [13–17].

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0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.10.003

The limits of detection (LODs) reported in such methods are typically in the 4–50 ng/ml range. Assays based on potentiometry have also been reported [18,19] giving LODs in the 30–170 ng/ml interval. However, to our knowledge, no LC methods with precolumn derivatization have been reported.

2. Experimental

2.1. Reagents and solutions

All the reagents were of analytical grade. Trimethylamine (TMA), methylamine, ethylamine, propylamine, *n*-butilamine, *n*-pentilamine, dimethylmine and diethylamine were obtained from Sigma (St. Louis, MO, USA), and 9-fluorenylmethyl chloroformate was purchased from Aldrich (Steinheim, Germany). Methanol and acetonitrile were of HPLC grade (Scharlau, Barcelona, Spain). Sodium hydroxide, 85%-phosphoric acid and boric acid were obtained from Panreac (Barcelona, Spain).

Stock standard solutions of TMA and the other aliphatic amines (1.0 g/l) were prepared in water. Working solutions of the analyte were prepared by dilution of the stock solutions with water, and then, the pH was adjusted to 10.0 by adding 0.5 M sodium hydroxide. Water was deionized and filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain). All solutions were stored in the dark at 2 °C.

2.2. Apparatus and chromatographic conditions

The chromatographic system consisted of a quaternary pump (Hewlett-Packard 1050 Series, Palo Alto, CA, USA), a 100 μl sample loop injector, and a UV detector (Hewlett-Packard 1046 Series). The detector was linked to a data system (Hewlett-Packard HPLC Chem Station) for data acquisition and storage. The signal was monitored at 262 nm.

A LiChrospher 100 RP_{18}, 5 μm, 125 mm × 4 mm i.d. (Merck, Darmstadt, Germany) column was the analytical column. The mobile-phase was a mixture of acetonitrile–water–0.05 M borate buffer (pH 9.0) in gradient elution. The mobile phase flow rate was 1.0 ml/min. The 0.05 M borate buffer was prepared by dissolving boric acid in water; then the pH was adjusted to the appropriate value by adding 0.5 M sodium hydroxide.

All solvents were filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain) and degassed with helium before use.

2.3. Derivatization procedure

On-line solid support assisted derivatization was accomplished into a precolumn 20 mm × 2.1 mm i.d., dry-packed with a Hypersil ODS-C_{18}, 30 μm, stationary phase (Merck). The precolumn was connected to the analytical column by means of a high pressure six-port switching valve (Hewlett-Packard). Before each analysis the precolumn and the analytical column were equilibrated with a mobile-phase of acetonitrile–water. At the beginning of each assay the gradient elution program was started, and the switching valve was rotated. In such a way, the percentage of borate buffer in the precolumn was progressively increased, the eluent being sent to waste. At 2.5 min, 25 μl of the samples were injected into the precolumn. After a delay time of 0.5 min, an aliquot of 50 μl of 1 mM FMOC was injected. The trapped analyte and the reagent were left to react for 0.5 min. Finally, the switching valve was turned to the original position, so the TMA–FMOC derivative was transferred to the analytical column for chromatography. The FMOC solutions were prepared daily by dissolving the pure compound in acetonitrile.

Each sample was derivatized in triplicate and all assays were carried out at ambient temperature.

2.4. Preconcentration into C_{18} SPE cartridges

For analyte enrichment, 1 ml Bond Elut C_{18} cartridges containing 100 mg of packing (Varian, Harbor City, CA, USA) were used. The cartridges were conditioned with 1 ml of methanol followed by 1 ml of 0.05 M borate buffer of pH 9.0. Samples (5.0 ml) were drawn through the cartridges, and then the cartridges were washed with 1 ml of 0.05 M borate buffer and dried with air. Next, the retained TMA was desorbed from the cartridges with 0.5 ml of 0.1 M phosphoric acid. The extracts were basified by adding 100 μl of 0.5 M sodium hydroxide. Finally, 25 μl of the resulting mixture were injected into the chromatographic system for derivatization and chromatography.

Each sample was derivatized in triplicate and all assays were carried out at ambient temperature.

3. Results and discussion

3.1. Derivatization and elution conditions

According to previous works, an acetonitrile–water mixture was selected as the mobile-phase [12]. However, since derivatizations with FMOC require a basic medium the pH within the precolumn was increased by increasing the percentage of borate buffer (pH 9.0) in the mobile phase before sample injection. Different experiments were carried out under a variety of elution conditions in order to check whether TMA reacted with FMOC to produce an adequate signal for monitoring this compound. In this study, the concentration of FMOC was 25 mM, and the delay time between sample injection and injection of the reagent (t_1) was 0.5. The time between the injection of the FMOC and the transfer of the TMA derivative to the analytical column (time of reaction,

Table 1
Time schedule and conditions used in the determination of TMA

Cumulative time (min)	Valve position	Action	Elution conditions[a]	
–	1	Conditioning of the precolumn and analytical column	60:40 acetonitrile–water	
0	2	Start of the gradient elution	at 0 min	60:40 (v/v) acetonitrile-water
			at 1.5 min	60:40 (v/v) acetonitrile-0.05 M borate buffer (pH 9.0)
2.5	2	Sample injection (25 μl)		
2.5–3.0	2	analyte purification		
3.0	2	Reagent injection (50 μl, 1 mM)		
3–3.5	2	Reaction and elimination of unreacted FMOC	at 3.5 min	60:40 (v/v) acetonitrile–water
3.5	1	Transfer of the product of reaction		
3.5–15	1	Chromatography and detection	at 10 min	70:30 (v/v) acetonitrile–water
			at 15 min	100% acetonitrile
15	1	End		

[a] mobile phase flow rate, 1.0 ml/m.

t_2) was also fixed at 0.5 min. The concentration of TMA in the samples was 10.0 μg/ml.

In most of the tested conditions, it was observed that the reaction between TMA and FMOC resulted in an intense and well-defined chromatographic peak, whereas peaks corresponding to the excess of reagent and/or subproducts eluted at retention times lower than that of the TMA–FMOC derivative. Best resolution of the peak of interest in the minimum time of analysis was obtained under the elution conditions listed in Table 1.

The effect of other experimental variables on the derivatization yields of TMA was examined. The concentration of FMOC was evaluated in the 1–25 mM interval. Both t_1 and t_2 were 0.5 min. Although external standards of TMA–FMOC were not available (and therefore, the absolute analyte conversion yield could not be established), the responses obtained for the derivative of TMA were approximately constant within the tested concentration interval. This indicates that maximum TMA conversion was reached even with 1 mM FMOC. Moreover, best baseline was obtained with 1 mM FMOC, and therefore, this was the concentration used in further work. It should be noted that for this concentration of FMOC and for TMA 10 μg/ml (the maximum concentration assayed in the present work), the reagent to analyte concentration ratio was about 6. Unlike solution derivatization, this may be enough in the solid support assisted derivatization approach to reach maximum analyte conversions [20]. Lower concentrations of reagent were not used in order to ensure an adequate FMOC-to-amine concentration ratio within the precolumn, particularly in samples with other amines potentially present.

The effect of the time of reaction was evaluated by changing t_2. Times in the 0.5–1.5 min range were assayed. It was observed that the signal of the analyte remained constant or even decreased with increasing t_2 due to breakthrough. Consequently, a reaction time of 0.5 min was selected as the best option for derivatization of TMA.

3.2. Selectivity

In order to test the selectivity of the proposed method, different short-chain primary and secondary amines were assayed under the conditions listed in Table 1. The amines tested were methylamine, ethylamine, propylamine, *n*-butylamine, *n*-pentylamine, dimethylamine and diethylamine. Indeed, all these amines reacted with FMOC, but their derivatives showed retention times different to that of the TMA–FMOC. Nevertheless, in order to prevent an excessive consumption of FMOC in the presence of other potentially present amines, a purification step was incorporated in the analytical procedure. Purification was effected by flushing the precolumn with the mobile phase for a defined period of time (t_1) before injecting the FMOC reagent. Different values for t_1 within the 0.5–1.5 min interval were assayed.

All the amines tested were flushed from the precolumn already after 0.5 min, with the only exception of diethylamine. Increasing this time caused a loss of TMA. A flushing time of 0.5 min was therefore preferred.

On the basis of the above results, the conditions finally selected for the derivatization and chromatography of TMA are those listed in Table 1. Fig. 1 shows the chromatograms obtained for a blank (water) and a sample containing TMA under such conditions.

3.3. Analytical performance data

The reliability of the described method was evaluated by processing standard samples containing the analytes in 0.25–10.0 μg/ml concentration range. The linearity was tested by analysing samples at four different concentrations within the studied interval. The proposed procedure provided adequate linearity ($y = (942 \pm 42)x + (642 \pm 234)$, $R^2 = 0.98$). The method also provided suitable reproducibility, with intra-day and inter-day coefficients of variation of 9% ($n = 3$) and 11% ($n = 6$), respectively (at a concentration of 5.0 μg/ml).

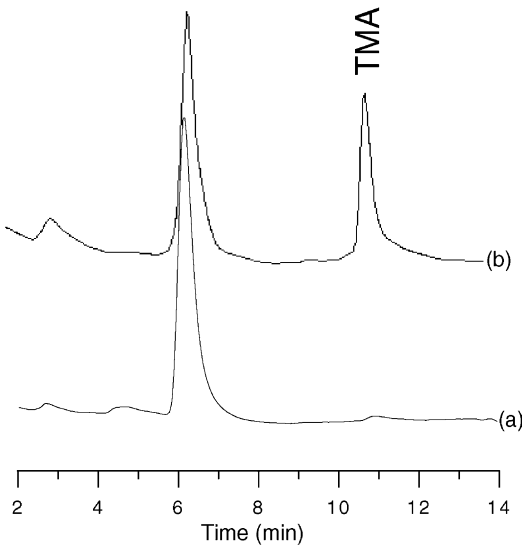

Fig. 1. Chromatograms obtained for (a) a blank (water) and for (b) a standard solution containing 2.5 μg/ml of TMA. For other experimental details, see text.

The limit of detection (LOD, established as the concentration required to generate a signal-to-noise ratio of 3), was estimated by analysing solutions of decreasing concentration of TMA. Before analysing each sample, water was processed. In such a way, it was confirmed that there were no contaminants and/or memory effects. The value obtained was 50 ng/ml.

In principle, the described conditions may be suitable for most applications concerning the determination of TMA in industrial and waste water, or in biological samples [6,19]. However, methods capable of determining concentrations of TMA below 1.0 μg/ml may be required, for example in the analysis of environmental water samples [16,17]. Although analyte enrichment is possible through the injection of large sample volumes into precolumns, such as that used in the present study for derivatization, we obtained unsuccessful results for TMA. The reason is that, owing to the polarity of TMA and the small dimensions of the precolumn, losses of the analyte by breakthrough occurred even for sample volumes as low as 0.5 ml.

As an alternative, enrichment of the analyte was effected off-line on SPE cartridges according to the procedure previously described for primary and secondary amines [12]. The sample volume was 5.0 ml, and the concentration of TMA ranged from 0.05 to 1.0 μg/ml. The recovery of TMA obtained within the tested concentration interval was (96 ± 11)% ($n = 12$), thus resulting in an enrichment factor of about 8. The linearity was suitable ($y = (8729 \pm 182)x + (776 \pm 291)$, $R^2 = 0.97$), and the intra-day and inter-day coefficients of variation were 11% ($n = 3$) and 13% ($n = 6$), respectively (for 0.05 μg/ml TMA). The LOD was 5 ng/ml. It is interesting to note that this value of about one order of magnitude higher than the LODs typically encountered for primary and secondary aliphatic amines [12]. This suggests that the reaction yield for this tertiary amine is significantly lower than the conversion yields obtained for primary and secondary aliphatic amines. Similar results were observed in the reaction between FMOC and primary, secondary and tertiary amphetamines [11].

The accuracy of the two proposed procedures was evaluated by processing standard solutions containing TMA at different concentrations within the tested concentration interval. As observed in Table 2, suitable accuracy was achieved, with relative errors ranging from +10% to −8%. In order to test possible interference by diethylamine (the only short-chain aliphatic amine that could be retained in the precolumn), samples containing 0.1 μg/ml of TMA and diethyalmine were also assayed. The diethylamine to TMA concentration ratios in these samples were 1 and 50. Finally, a sample containing a mixture of several aliphatic amines besides TMA was processed. No significant interference was observed in any of the samples assayed (see Table 2).

Table 2
Accuracy for the determination of TMA ($n = 3$).

Sample	Added concentration (μg/ml)	Determined concentration (μg/ml)	Relative error (%)
Standard[a]	5.0	5.03	+0.6
Standard	0.1	0.11	+10
Standard	0.2	0.195	−3
Standard	0.5	0.46	−8
Standard	0.1 + 0.1 μg/ml of diethylamine	0.11	+10
Standard	0.1 + 5.0 μg/ml of diethylamine	0.11	+10
Standard	1.0 + methylamine + ethylamine + propylamine + butylamine + pentylamine + diethylamine + dimethylamine (1.0 μg/ml each)	0.84	−16
Tap water	0.1	0.11	+10
Tap water	0.5	0.52	+4
Sea water	0.5	0.55	+10
Ground water	0.5	0.45	−10
Waste water[a]	10.0	9.9	−1

[a] Obtained by the direct method.

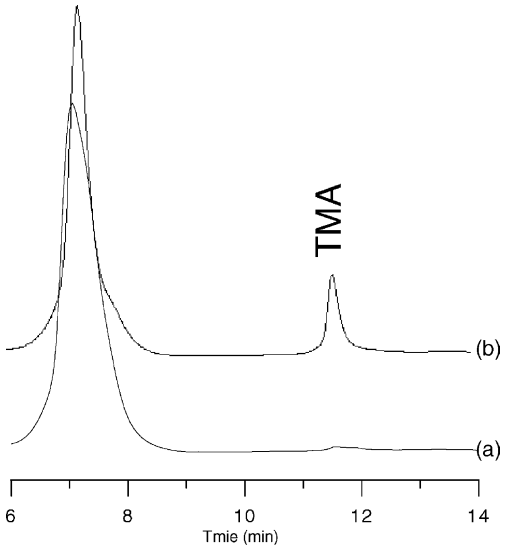

Fig. 2. Chromatograms obtained for (a) sea water, and for (b) sea water fortified with 1.0 μg/ml of TMA, after SPE. For other experimental details, see text.

3.4. Application to real water samples

The reliability of the described method was tested by analysing tap water, ground water, sea water and waste water. Ground water, sea water and waste water were previously filtrated with 0.45 μm nylon membranes in order to remove any particulate matter. Waste water was analyzed by the direct method, whereas the other samples were then subjected previously to SPE. None of the samples analyzed contained TMA. As an illustrative example, in Fig. 2 are shown the chromatograms obtained for sea water and for sea water spiked with TMA.

Samples fortified with TMA were processed, and the concentration of TMA was established from the calibration curves obtained from the standard solutions. As observed in Table 2, the results obtained were comparable to those obtained for standard solutions. Therefore, the quantitative performance of the method can be considered suitable for these kind of samples.

4. Conclusions

The results of this study demonstrate the utility of FMOC for the sensitive analysis of TMA in water samples using the derivatization into C_{18} supports. Although FMOC has been proposed as a reagent for primary and secondary amines, the solid support assisted derivatization method permits the formation of TMA in very short times of reaction and under very mild conditions (pH 9.0 at ambient temperature).

The described method is very simple, as derivatizations are performed in an on-line mode, and allows the determination of TMA within the range 0.25–10.0 μg/ml in about 15 min. If required, the method can be applied to the determination of TMA at lower concentrations (0.05–1.0 μg/ml) by preconcentrating the analyte into C_{18} SPE cartridges. The LODs are comparable to those reported by methods for TMA using GC. Additional advantages over previous methods involving derivatization are the fact that the reagent is commercially available, and that the reaction can be carried out into aqueous media. Moreover, the presence of other primary and/or secondary short-chain aliphatic amines did not interfere, with diethylamine as the only exception. Very large concentrations of this amine would interfere with the determination of TMA due to the consumption of FMOC.

Acknowledgements

The authors are grateful to the Ministerio de Ciencia y Tecnología of Spain for financial support received for the realization of Project PPQ2000-1641. C. Cháfer expresses her gratitude to the Ministerio de Educación, Cultura y Deporte of Spain for pre-doctoral grant.

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