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Reversed-phase liquid chromatographic behaviour of alkylamines with amperometric detection and its application to trace analysis

Masao Maruyama* and Takayuki Nagayoshi

Faculty of Science and Engineering, Chuo University, Kasuga, Bunkyo-ku, Tokyo 112 (Japan)

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ABSTRACT

The reversed-phase liquid chromatographic behaviour of tri- and dialkylamines with amperometric detection was studied. Peak retention and peak shape can be improved by the addition of ammonium ion (as competing base) to the mobile phase. The retention behaviour of alkylamines can be explained by both ion and solvophobic interactions. This method can be applied to the selective and sensitive determination of trace amounts of tri- and dialkylamines. In air samples the limit of determination of trimethylamine is a few μg/l and in water samples (direct injection) a few tenths of 1 mg/l.

INTRODUCTION

Amines have been separated by high-performance liquid chromatography with UV detection [1–3] and also by cation-exchange chromatography [4] and ion-pair chromatography [5]. Most aliphatic amines do not absorb in the ultraviolet region and it is difficult to monitor aliphatic amines directly by high-performance liquid chromatography (HPLC) with UV detection. Chemical derivatization techniques are often used to increase UV detectability. For example, aliphatic and aromatic amines were derivatized to N,N′-disubstituted ureas with phenyl isocyanate [1] and aliphatic amines to 2,4-dinitrophenyl derivatives with 2,4-dinitrofluorobenzene [6] and prior to HPLC.

The amperometric detector is one of the most sensitive and the most specific detectors available and responds to substances that are either oxidizable or reducible. Benzidine and its analogues [7] and aminophenyl isomers [8] have been determined by reversed-phase HPLC with amperometric detection.

In this paper, the reversed-phase HPLC behaviour of tri- and dialkylamines with amperometric detection and its application to trace analysis is described.

EXPERIMENTAL

Apparatus

Chromatograms were measured with a Yanagimoto (Kyoto, Japan) Model L-3200 HPLC system equipped with an amperometric detector. The amperometric detector contained a three-electrode cell, with a glassy carbon working electrode, platinum auxiliary electrode and Ag/AgCl reference electrode. Semi-differential voltammetric measurements were performed with a Yanagimoto Model VMA-010 cyclic voltammetric analyser. The column was 75 mm × 4.6 mm I.D. stainless steel, packed with Nucleosil C_{18} (Macherey-Nagel, Düren, Germany) of 3-μm particle size.

Chemicals

Alkylamines were purchased from Tokyo Kasei (Tokyo, Japan). Acetonitrile was of HPLC grade. All other chemicals and solvents were of analytical-

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reagent grade and used as received.

Stock solutions (1000 mg/l) of alkylamines were prepared in fresh glass-distilled water. The concentration of the stock solution was checked by acidimetry. Working standard solutions used for normalization and for the fortification of recovery samples were prepared by dilution of the stock solutions.

Chromatographic conditions

The mobile phase was acetonitrile–phosphate buffer (pH 7.0) (40:60, v/v) containing 0.05 *M* ammonium acetate. The flow-rate of mobile phase through the analytical column was 1.0 ml/min. All experiments were carried out at 25°C. The eluate was monitored with the amperometric detector. Measurements were made at as low a voltage as possible because it is difficult to use a high voltage routinely owing to increased background noise and a lack of stability. For monitoring trimethylamine, an applied voltage of 1000 mV *vs.* Ag/AgCl was selected.

RESULTS AND DISCUSSION

Cyclic voltammograms of alkylamines and applied voltages

Cyclic voltammograms of trialkylamines in acetonitrile–phosphate buffer (pH 7.0) (20:80, v/v) containing 0.1 *M* ammonium acetate were measured at potentials between 0 and 1500 mV *vs.* Ag/AgCl with a scan rate of 80 mV/s. A typical voltammogram of trialkylamines is shown in Fig. 1.

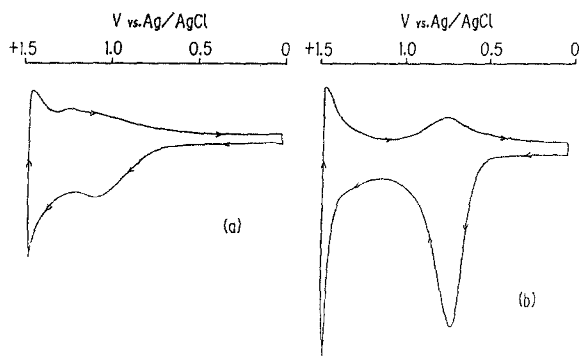

Fig. 1. Cyclic voltammograms of trialkylamines. Concentration, 1×10^{-3} *M*; medium, acetonitrile–phosphate buffer (pH 7.0) (40:60, v/v) + 0.1 *M* ammonium acetate; scan rate, 80 mV/s. (a) Trimethylamine; (b) tri-*n*-propylamine.

An oxidation peak due to one-electron oxidation to produce the cation radical was observed at a potential of about 700–1100 mV *vs.* Ag/AgCl. The peak potentials of trialkylamines are given in Table I. The optimum oxidation applied voltage was selected according to which trialkylamines were being measured. The cyclic voltammograms of dialkylamines were also measured under the same conditions as trialkylamines and the peak potentials are given in Table I.

Trialkylamines are more easily oxidized than dialkylamines and their peak potentials decrease with increasing size of the alkyl group owing to the stronger electron-donating power. The oxidation process of monoalkylamines is very different and more complex and they cannot be oxidized under the conditions mentioned above.

Chromatography of alkylamines

A typical chromatogram of trialkylamines measured under the conditions mentioned above is shown in Fig. 2. The compounds were clearly separated in order of increasing length of the alkyl group. In this chromatogram, the peak of tri-*n*-propylamine is broad with a low column efficiency, but a sharp peak can be obtained by increasing the concentration of acetonitrile in the mobile phase (Fig. 3). Fig. 3 shows a typical chromatogram of di- and tri-*n*-propylamine at an applied voltage of 1100 mV *vs.* Ag/AgCl.

Linearity and detection limit

The relationship between sample size and peak height was linear over the range 3–30 ng under the conditions mentioned above. The minimum detectable amount of trialkylamines was found to be a few nanograms based on a signal-to-noise ratio of $\geqslant 3$. This method is very sensitive and good repro-

TABLE I

PEAK POTENTIALS OF DI- AND TRIALKYLAMINES

Alkylamine	Peak potential (mV *vs.* Ag/AgCl)			
	Methyl	Ethyl	*n*-Propyl	*n*-Butyl
Di-	1280	1300	1230	1210
Tri-	1100	820	720	700

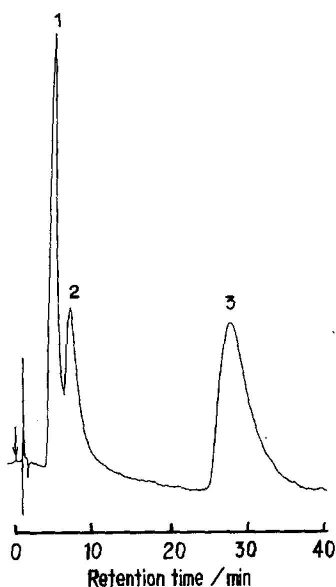

Fig. 2. Typical chromatogram of trialkylamines. Mobile phase, acetonitrile–phosphate buffer (pH 7.3) (20:80, v/v) + 50 m*M* ammonium acetate; flow-rate, 1 ml/min; applied voltage, 1000 mV *vs.* Ag/AgCl; temperature, 25°C. Peaks: 1 = trimethylamine (50 ng); 2 = triethylamine (50 ng); 3 = tri-*n*-propylamine (100 ng).

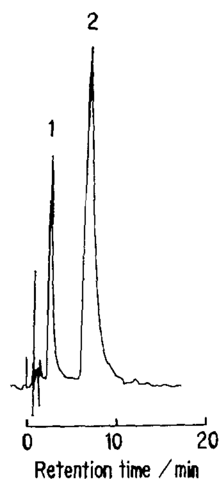

Fig. 3. Typical chromatogram of di- and tri-*n*-propylamine. Mobile phase, acetonitrile–phosphate buffer (pH 7.0) (40:60, v/v) + 50 m*M* ammonium acetate; flow-rate, 1 ml/min; applied voltage, 1100 mV *vs.* Ag/AgCl; temperature, 25°C. Peaks: 1 = di-*n*-propylamine (30 ng); 2 = tri-*n*-propylamine (100 ng).

ducibility was obtained for repeated injections [trimethylamine: sample size, 10 ng; relative standard deviation (n = 5) = 3.5%]. With dialkylamines, linearity was observed under almost the same conditions as for trialkylamines. The minimum detectable amount of dialkylamines was found to be *ca.* 10 ng.

Effect of the concentration of acetonitrile in the mobile phase on the retention of trialkylamines

The retention times of trialkylamines altered with variation of the concentration of acetonitrile in the mobile phase. Some results are given in Table II.

For trimethylamine, the retention time increased with increase in the concentration of acetonitrile, for tri-*n*-propyl- and tri-*n*-butylamine it decreased and for triethylamine it scarcely changed. These results indicate that the elution order of trialkylamines can be reversed by increasing the concentration of acetonitrile in the mobile phase. This elution behaviour is not the same as that in ordinary reversed-phase liquid chromatography.

Retention mechanism of tri- and dialkylamines

Many workers have observed cation-exchange characteristics during the reversed-phase chromatography of cations on silica-based column [9–15], but the retention mechanism of reversed-phase ion-pair liquid chromatography is not yet clearly established.

In the present instance, the retention mechanism can be explained as follows. There are two processes involved: ion interaction (silanophilic) and solvophobic interactions. For more hydrophobic solutes, such as tripropyl- and tributylamines, solvophobic interactions predominate, whereas for the less hydrophobic trimethylamine silanophibic interactions dominate.

Ammonium acetate added as a supporting electrolyte plays an important role as a competing base. Increasing the ammonium ion concentration in the eluent can improve the peak retention and peak shape (Fig. 4). This change can be explained as follows: because of its relatively high concentration, ammonium ion is preferentially adsorbed by the silanol groups, thus minimizing the adsorption of amine. However, concentrations of ammonium ion in the eluent above 50 m*M* do not improve the peak retention and peak shape further.

TABLE II

RETENTION TIMES OF TRIALKYLAMINES (min)

R_3N	Acetonitrile: buffer solution				
	7:3 (pH 7.76)[a]	6:4 (pH 7.74)[a]	5:5 (pH 7.74)[a]	3:7 (pH 7.50)[a]	2:8 (pH 7.42)[a]
Methyl	5.3	4.4	4.2	2.4	2.2
Ethyl	3.5	3.3	3.6	2.8	3.2
n-Propyl	3.2	4.0	5.2	8.8	13.0
n-Butyl	3.8	6.1	10.8	–	–

[a] Apparent pH.

Application to trace analysis

Small amounts of trimethylamine in air and water were determined by this HPLC method as a demonstration of its application to trace analysis.

Determination of small amounts of trimethylamine in air. A Sep-Pak C_{18} cartridge was used for the trace determination of trimethylamine in air. A Sep-Pak C_{18} cartridge was washed with 5 ml of acetonitrile, then dried by passing a stream of dry nitrogen at 80–100 ml/min for 30 min. The collection efficiency of trimethylamine was investigated by using two Sep-Pak C_{18} cartridges in series. All the trimethylamine was recovered on the first cartridge.

The sampling and analytical procedure was as follows. A 2–100-l air sample was passed through the Sep-Pak C_{18} cartridge with a stream of dry nitrogen at 0.5–1.5 l/min. The trimethylamine adsorbed on the cartridge was extracted with a few millilitres of acetonitrile–water (30:70, v/v) containing 50 mM ammonium acetate. The eluate was diluted to 10 ml with acetonitrile. A 10–50-μl aliquot of the solution was injected into the liquid chromatograph and the eluate was monitored with the amperometric detector at an applied voltage of 1000 mV *vs.* Ag/AgCl. The minimum detectable amount of trimethylamine was found to be 1 ng.

Synthetic air samples fortified with trimethylamine were prepared in a 2-l gas sampling bottle. The recoveries of trimethylamine are given in Table III; in each instance, 75–90% recoveries were achieved. The limit of determination is a few μg/l. This method can easily be used for the selective and

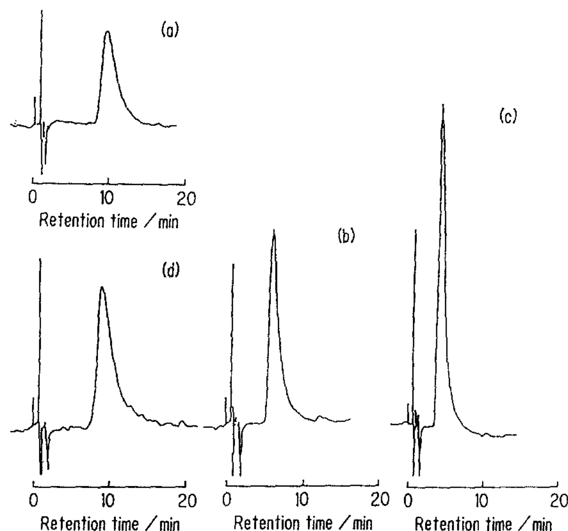

Fig. 4. Effect of the concentration of ammonium ion in the eluent on the chromatogram of trimethylamine. Mobile phase: (a) acetonitrile–phosphate buffer (pH 7.0) (40:60, v/v); (b) (a) + 30 mM ammonium acetate; (c) (a) + 50 mM ammonium acetate; (d) (a) + 50 mM sodium acetate. Amount of trimethylamine, 30 ng; flow-rate, 1 ml/min; applied voltage, 1000 mV *vs.* Ag/AgCl; temperature, 25°C.

TABLE III

RECOVERIES OF TRIMETHYLAMINE IN AIR

Trimethylamine (μg)		Recovery (%)
Taken	Found	
5	3.8	76
10	8.5	85
20	17.6	88
40	35.1	86

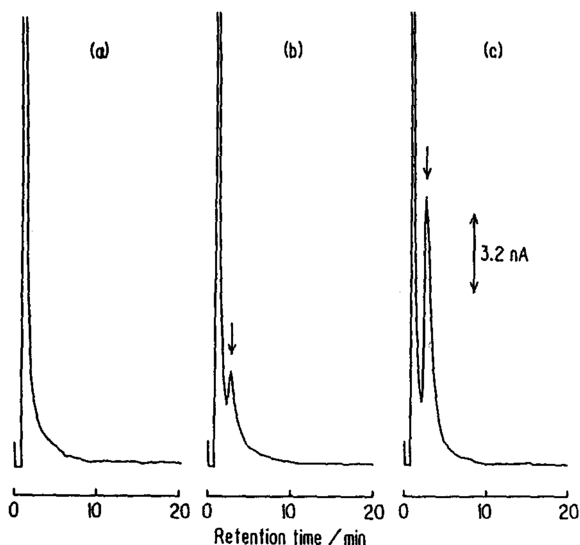

Fig. 5. Typical chromatogram of trimethylamine in water. Concentration of trimethylamine: (a) blank (river water); (b) 0.5 mg/l; (c) 2.0 mg/l. For chromatographic conditions, see the text.

sensitive determination of trace amounts of trimethylamine in air.

Determination of small amounts of trimethylamine in water. A 10-μl sample of water fortified with trimethylamine was injected directly into the chromatograph through a membrane filter (0.45 μm). The eluate was monitored with the amperometric detector at an applied voltage of 1000 mV *vs.* Ag/AgCl. Fig. 5 shows typical chromatograms of trimethylamine in water (Tama River, Japan) at fortification levels of 0.5 and 2.0 mg/l.

The limit of determination is 0.2 mg/l. For ultratrace analysis, trimethylamine is transferred from the aqueous to the vapour phase by bubbling a stream of nitrogen through the water sample (the pH of the water sample has to be taken into consideration) and the vapour obtained is swept through a Sep-Pak C_{18} cartridge to collect the trimethylamine.

CONCLUSIONS

The retention behaviour of tri- and dimethylamines here is different to that in ordinary reversed-phase liquid chromatography. Ammonium acetate added as a supporting electrolyte plays an important role as a competing base. This behaviour can be explained by both ion and solvophobic interactions.

This method can be used for the simple determination of small amounts of trimethylamine in environmental samples.

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