Determination of Amines in the Presence of Excess Ammonia by Ion Chromatography–Mass Spectrometry

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Abstract

A new method for the simultaneous determination of several amines in the presence of an excess of ammonia by ion chromatography–mass spectrometry detection was developed. Current methods using ion chromatography with suppressed conductivity detection are not selective enough to determine small amines at the required level of 10 µg/L or preferably lower in the presence of a large excess of ammonia (~1 mg/L) without resorting to time-consuming sample pre-treatment techniques. By using mass spectrometric detection, which is capable of resolving eluting compounds on their *m/z* values, an additional dimension of confirmation is added to the analysis. Detection based on the analytes *m/z* value overcomes problems such as co-elution or background interferences that complicate the quantification when using conductivity detection. The optimal conditions for mass spectrometry detection of amines in the presence of an excess of ammonia were investigated by a four-factor central composite design. The four factors investigated were scan time, cone voltage, probe temperature, and needle voltage. Evaluation of the obtained experimental data showed that detection limits were up to a factor of 100 lower when using mass spectrometry as the detection technique instead of the conventional suppressed conductivity detection. Detection limits of 1 µg/L and lower can be achieved for the six amines investigated in the presence of a large excess of ammonia (~1 mg/L).

Introduction

Over the past decade, interest in the analytical chemistry of volatile aliphatic amines has been mainly motivated by environmental concern (1–4). Amines are industrial chemicals with a wide range of applications. Anthropogenic sources include cattle feedlot operations, waste incineration, and sewage treatment. Amines are also emitted in car exhausts. Airborne amines are of special interest because of the role they play in various atmospheric processes. These amines, some of them toxic themselves, can undergo reactions to form a variety of products, among them the *N*-nitroso-amines that are potentially carcinogenic (5).

Sampling of amines from air is usually carried out by absorp-tion using impinger flasks containing (acidified) aqueous solutions (6,7) or sorption on solid sorbents (8,9). The inherent preconcentration of these sampling methods makes it possible to lower the detection limits of amines to the low parts per billion by volume (ppbv) concentration range in sampled air. Unfortunately, matrix components, such as ammonia, are also concentrated together with the amines.

Most analytical procedures reported for the analysis of amines feature separation methods. Gas chromatography (GC) is widely used in the analysis of amines owing to its simplicity, high resolving power, good sensitivity, short analysis time, and relatively low cost (2,8–15). In some cases, determination of amines can be carried out by direct injection of aqueous or salted solutions (7,16). Typical GC detectors used in amine determination include nitrogen–phosphorus detector (NPD), flame ionization detector (FID), and mass spectrometric detector (MSD). However, the determination of especially aliphatic amines is difficult due to the adsorption and decomposition of the solute on the column, leading to poor chromatographic behavior. Derivatization of amines is often employed to reduce solute polarity and to improve selectivity, sensitivity, and separation of the amines (2,11,17).

High-performance liquid chromatography (HPLC) is also often used in the determination of amines (5,18–21). Again, chromatographic performance is poor when using direct HPLC approaches. The vast majority of liquid chromatographic methods for amine determination utilize a two-step approach: separation of potential interferents in the sample and pre- or post-column formation of derivatives with better detectability. Note that aliphatic amines have low absorptivity in the UV range, thereby requiring derivatization if the common UV detector is to be used. Other detectors that have been applied include a coulometric detector, a fluorescence detector, a chemiluminescence detector, and a MSD.

Less common separation methods such as supercritical fluid chromatography (SFC) (22–24) and capillary electrophoresis (CE) (25) have also been used for the analysis of small amines. Good results could be obtained for alkaline extracts of atmospheric aerosol samples, especially for the combination of CE with laser induced fluorescence detection that enabled very low detection limits (~ 1 ng/L). However, also in these instances, derivatization was required before separation/detection.

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We are interested in the determination of small amines for two reasons: the determination of total base in clean room air and the determination of volatile amines in exhaled breath. In clean rooms, airborne molecular contamination (AMC) is monitored on a routine basis. One of the AMC categories, total volatile base (26) is generally considered to consist of ammonia and the volatile amines. Specifications are often set at 10 ppbv and lower in clean room air. Amines in exhaled breath are considered biomarkers for renal and liver disorders (27–31); these amines are present at concentration levels of 10 ppbv or lower. Although from very different application fields, these analyses both require that the amines can be determined in the presence of a large excess of ammonia.

In this paper, we report on the determination of amines using ion chromatography–mass spectrometry (IC–MS) (32). IC was chosen because this technique allows separation of the amines without the need for tedious and time-consuming derivatization reactions (33–38). However, the presence of a large ammonia excess greatly complicates the chromatographic separation. By using MS, the detector can be made highly selective by tuning to the m/z ratios of the selected amines, thereby allowing good quantification without the need for a complete baseline separation.

Experimental

Reagents and standards

High-purity methanesulfonic acid (MSA) was prepared from an EluGen II MSA Cartridge (Dionex, Sunnyvale, CA) with a Dionex EG50 eluent generator and was used as the eluent in the IC analysis. Standard solutions of methylamine (MMA), dimethylamine (DMA), trimethylamine (TMA), ethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA), and ammonia were prepared by serial dilution of certified stock standards (1000 µg/mL) of MMA, DMA, TMA, MEA, DEA, TEA (Alltech Associates, Inc., Deerfield, IL), and ammonium (NH_4Cl in H_2O), CertiPUR grade (Merck, Darmstadt, Germany) with ultrapure water.

Instrumentation

The analytical system consisted of a Dionex ICS3000 coupled to a MSQ Plus single quadrupole spectrometer (ThermoFisher Scientific, Waltham, MA). A standard electrospray interface was used in a positive ionization mode. Samples were injected on an analytical column using a Dionex AS50 autosampler. Separation was performed on a medium hydrophobic Dionex Ionpac CS18 analytical column (250 mm length × 2 mm i.d., 6 µm 55% cross-linked ethylvinyl benzene-divinylbenze grafted with carboxylated functional groups) with a Dionex Ionpac CG_{18} guard column (50 mm length × 2 mm i.d.) using a 4 mM isocratic methanesulfonic acid eluent at a flow rate of 0.25 mL/min. The eluent was generated by a Dionex EG50 eluent generator combined with a continuously regenerated cation trap column (CR-CTC) in order to minimize the base line shift caused by contaminants in the eluent. A 2-mm cation self regenerated suppressor (CSRS Ultra II) in external water mode was placed in-line with the analytical column. The reduction of high background signals enabled the use of suppressed conductivity detection with

an ICS3000 conductivity detector (CD) in combination with MS detection. System control and data collection was carried out using Dionex Chromeleon 6.80 software. Optimal conditions (e.g., IC columns, eluent, flow rate) for the IC separation with suppressed conductivity detection were determined using the Virtual Column feature of the Dionex Chromeleon software system.

Experimental procedure

No prior work was published on IC–MS detection of the six amines we were interested in. Therefore, no optimal conditions were available from literature, and the optimal conditions for MS detection were investigated by a four-factor central composite design. The four factors investigated were needle voltage, cone voltage, probe temperature, and scan time. The response measured was the signal-to-noise (S/N) ratio for each analyte. Variation ranges for the four main parameters scan time (1–5 s), cone voltage (40–130 V), probe temperature (300–500°C), and needle voltage (3.0–5.0 kV) were derived from the user manual of the MSQ Plus. All experiments were carried out using a standard mixture containing 10 µg/L MMA, DMA, TMA, MEA, DEA, and TEA in a 1 mg/L matrix of ammonia and an injection volume of 50 µL. After separation on the Ionpac CS_{18} analytical column, mass spectra were collected in a full-mass scan mode (18 to 160 m/z) during a run-time of 15 min using the Chromeleon software. Experimental design and evaluation of the obtained data was performed using MINITAB™ statistical software (Minitab Ltd., Coventry, U.K.). The response optimizer feature of the software was used to find the optimum settings for the MS factors.
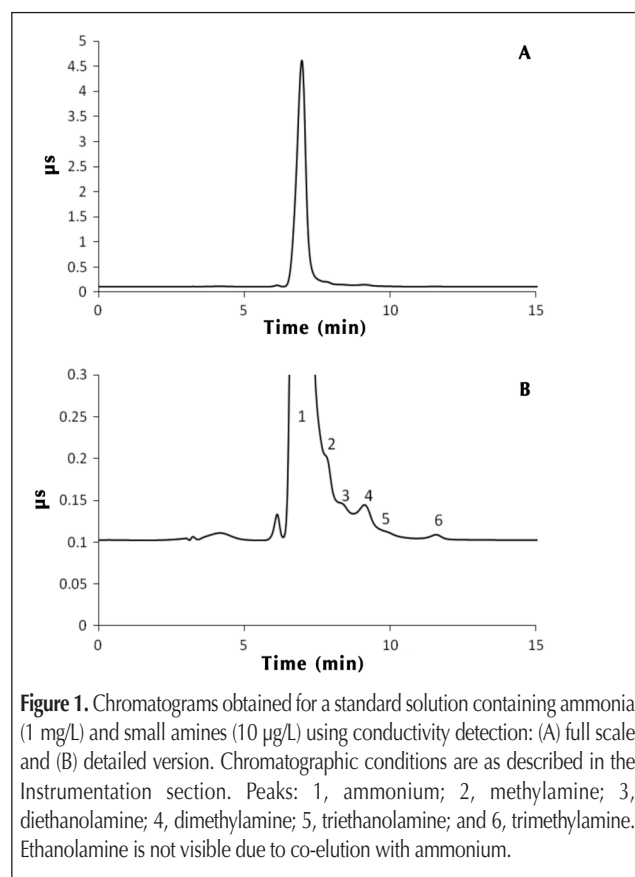

Figure 1. Chromatograms obtained for a standard solution containing ammonia (1 mg/L) and small amines (10 µg/L) using conductivity detection: (A) full scale and (B) detailed version. Chromatographic conditions are as described in the Instrumentation section. Peaks: 1, ammonium; 2, methylamine; 3, diethanolamine; 4, dimethylamine; 5, triethanolamine; and 6, trimethylamine. Ethanolamine is not visible due to co-elution with ammonium.

Results

IC with suppressed conductivity detection

A typical chromatogram is shown in Figure 1 for a standard mixture of six small amines in the presence of an excess of ammonia by suppressed conductivity detection, using the chromatographic conditions as listed in the instrumentation section. The standard mixture contains some typical volatile amines that can be found in exhaled breath or clean room air. When using the optimal chromatographic conditions (as obtained from the Virtual Column feature of the Dionex Chromeleon software system) in combination with suppressed conductivity detection, MEA cannot be detected due to co-elution with ammonium. Due to the large excess of ammonium, separation of ammonium and MEA with retention times of 6.9 and 7.5 min, respectively, could not be established. Four other amines (MMA, DEA, DMA, and TEA) with retention times of 7.8, 8.3, 9.1, and 9.8 min, respec-

tively, are quite difficult to quantify due to incomplete separation from the large ammonium peak. TMA, with a retention time of 11.5 min, is the only amine which is completely separated from the ammonium peak. The detection limits (concentration corresponding to 3 times the standard deviation of the blank level) for MMA, DMA, TMA, MEA, DEA, and TEA as obtained when using suppressed conductivity are reported in Table I. In general, quantification of the selected amines by suppressed conductivity detection leads to detection limits of 10–50 μg/L. The applications we are interested in required detection limits for all six amines of 10 μg/L and preferably lower in the presence of a large excess of ammonia (~1 mg/L). There is only little room for improvement when using conductivity detection without resorting to time-consuming pre-concentration and derivatization reactions. Simply lowering the eluent concentration towards 2 mM MSA will increase the run-time significantly from 15 to 20 min and still results in co-elution of ammonium and MEA. To enable quantification by means of conductivity detection derivatization reactions such as for example ammonia removal from water by precipitation with $MgNH_4PO_4 \cdot 6H_2O$ or reaction with a zeolite (37,38) are required. The selectivity of these two ammonia removal techniques is yet unknown and very critical for our applications, any precipitation of the selected amines should be avoided. Further investigation related to the selectivity towards ammonia is required. Subsequent to the ammonia removal, pre-concentration of the amines can be performed on a Dionex trace cation concentrator low pressure column (TCC-LP1).

Using a combination of two sample pre-treatment techniques is not only time-consuming, but it might also introduce unnecessary errors in the analysis results. By using MS detection, which is capable of resolving eluting compounds on their m/z values, an additional dimension of confirmation is added to the analysis. This selectivity of MS overcomes co-elution or background interferences that complicate the quantification when using conductivity detection.

IC with mass spectrometry detection

The optimal conditions for MS detection were investigated by a four-factor experimental design. The effect of the four main factors on the S/N ratio was evaluated using response surface plots, as shown in Figure 2. The optimal conditions for MS detection of all six amines in the presence of an excess of ammonia in one single run can be derived from these experimental data. During
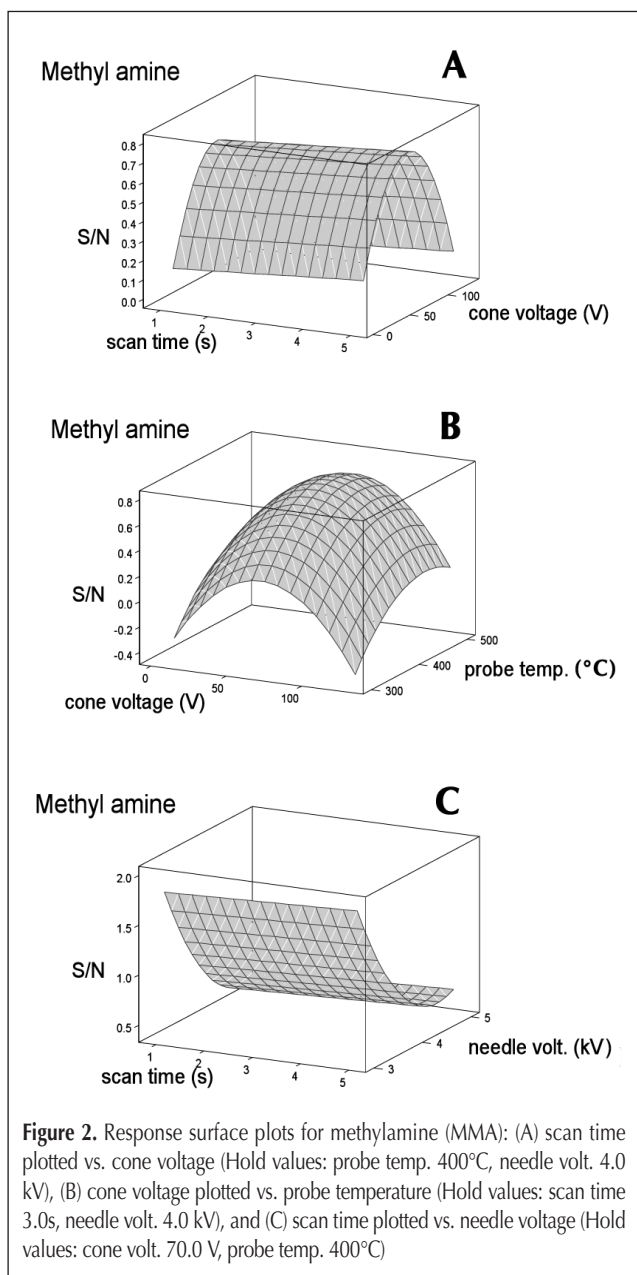

Figure 2. Response surface plots for methylamine (MMA): (A) scan time plotted vs. cone voltage (Hold values: probe temp. 400°C, needle volt. 4.0 kV), (B) cone voltage plotted vs. probe temperature (Hold values: scan time 3.0s, needle volt. 4.0 kV), and (C) scan time plotted vs. needle voltage (Hold values: cone volt. 70.0 V, probe temp. 400°C)

Table I. LOD* of Amines Using Conductivity and MS Detection

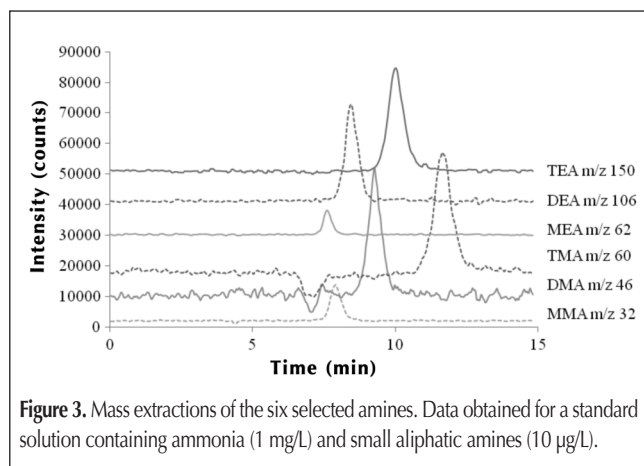| Analyte	Conductivity LOD (μg/L)	Mass spectrometry LOD (μg/L)
Methylamine	8.2	0.49
Dimethylamine	4.1	0.75
Trimethylamine	9.3	1.25
Ethanolamine	> 50	0.23
Diethanolamine	11.0	0.22
Triethanolamine	21.9	0.41

* LOD = limit of detection.

Figure 3. Mass extractions of the six selected amines. Data obtained for a standard solution containing ammonia (1 mg/L) and small aliphatic amines (10 μg/L).

this development work, optimal S/N ratios were obtained using the following conditions: a scan time of 3 s, a cone voltage of 61 V, a probe temperature of 482°C, and a needle voltage of 3.0 kV. Evaluation of the experimental data showed that the optimal condition for the needle voltage is equal to or perhaps even lower than the 3.0 kV, which was used as the minimal value in the four-factor central composite design. By using MS for the determination of MMA, DMA, TMA, MEA, DEA, and TEA in the presence of an excess of ammonia under the optimal conditions (according to our experimental data), the detector can be made selective for all six amines during one single sample run. This selectivity of MS overcomes co-elution or background interferences. All mass spectrometry data was collected in a full-mass scan mode for a standard solution containing 1 mg/L ammonia and 10 μg/L of MMA, DMA, TMA MEA, DEA, and TEA. Mass extractions were made for all amines separately and are presented in Figure 3. The mass extractions for DMA and TMA show a negative drop in the baseline at approximately 7 min. This is most likely due to signal suppression associated with the large excess of ammonia. Detection limits for MMA, DMA, TMA, MEA, DEA, and TEA in the presence of an excess of ammonia are up to a factor of 100 lower when using MS detection instead of suppressed conductivity detection. The detection limits for the six amines are reported in Table I. During this development work, all mass spectra were collected in a full-scan mode (18 to 160 *m/z*). When using SIM channels, possibly even lower detection limits can be achieved.

Discussion and Conclusion

When using the optimal chromatographic conditions in combination with suppressed conductivity detection, it is impossible to quantify the six amines we are interested in at the required level of 10 μg/L in the presence of a large excess of ammonia (~1 mg/L) without resorting to time-consuming pre-concentration and derivatization reactions.

The developed method for the simultaneous determination of MMA, DMA, TMA, MEA, DEA, and TEA in the presence of a large excess of ammonia by IC–MS lowers the detection limits up to a factor of 100. Detection limits of 1 μg/L and lower were achieved. Quantification of all six amines in the presence of a large excess of ammonia is perfectly possible for the samples used in this

experimental set-up at the required concentration level of 10 μg/L and even lower.

By using MS detection, which is capable of resolving eluting compounds on their *m/z* values, an additional dimension of confirmation is added to the analysis. This selectivity of MS overcomes co-elution or background interferences. It enables the quantification of all six amines in one single sample run without the need of time-consuming sample pre-treatment techniques and thus introducing unnecessary errors in the analysis results.

Acknowledgments

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