



Quantification of methylamine in the headspace of ethanol of agricultural origin by selected ion flow tube mass spectrometry

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ABSTRACT

Selected ion flow tube mass spectrometry, SIFT-MS, has been used to investigate if absolute levels of trace compounds in the headspace of ethanol/water vapour mixture can be quantified. This case study was directed towards the analysis of methylamine in distilled ethanol of agricultural origin because of its relevance to quality control legislation in the distillery industry. This has required a detailed study of the ion chemistry occurring – initiated by H_3O^+ precursor ions – when ethanol/water vapour mixtures are introduced into the H_3O^+ /helium carrier gas swarm and has resulted in the construction of a full scheme of the complex ionic reactions that occur. It has been found that under the SIFT-MS flow reactor conditions (He pressure 130 Pa and temperature 299 K) the terminating ions of the several parallel and sequential reactions that occur are the proton bound ethanol clusters ions, $\text{C}_2\text{H}_5\text{OH}_2^+(\text{C}_2\text{H}_5\text{OH})_n$, with $n = 1, 2, 3$, proton bound trimer ($n = 2$) being the dominant species. These ethanol cluster ions can be used as precursor (reagent) ions for the chemical ionisation of the methylamine present in the ethanol/water vapour, which produces two characteristic product ions $\text{CH}_3\text{NH}_2\text{H}^+(\text{C}_2\text{H}_5\text{OH})_{1,2}$ that are used for the methylamine analysis. The ratio of the product ion count rate to the precursor ion count rate is used in an analogous way to the routinely used for SIFT-MS analyses to quantify the methylamine concentration. The results of calibration experiments show that using SIFT-MS it is possible to quantify methylamine in liquid ethanol/water mixtures at levels of 0.1 mg/L or greater.

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1. Introduction

Quantification of methylamine in distilled ethanol of biological origin and in spirit drinks is of interests and importance in the various phases of production and quality control in the distillery industry. The primary purpose of the present study was to test whether or not selected ion flow tube mass spectrometry (SIFT-MS) can be used to reliably quantify the concentration of methylamine in both drinks-grade refined ethanol produced by the distillation of fermented agricultural products and in spirit drinks. Methylamine, CH_3NH_2 , is the simplest primary biogenic aliphatic amine. Such amines, many of which are known to be hazardous to human health, are found, for example, in (waste) water [1], decaying foodstuffs such as fish [2] and in the fermentation broths used for ethanol production [3]. Methylamine and other toxic aliphatic amines are able to readily access the brain and spinal tissues and interfere

with neurological function [4]. Considerable amounts of these compounds can be produced during food fermentation processes or in food storage, especially if amino acid-decarboxylase positive microorganisms are present. Several different amines, including methylamine, have been identified in wine using high-performance liquid chromatography (HPLC) following the indirect process of derivatization [5,6]. Analyses of biogenic amines in foods, including methylamine and larger amines like histamine, putrescine, cadaverine and spermine, have received considerable attention recently and the methods used for their analyses are reviewed in [7].

Because of the potentially serious hazard to health, the production of ethanol by the distillation of fermented agricultural products is closely regulated. The levels of volatile bases containing nitrogen in the ethanol, including amines, must be known and these are usually expressed in grams of nitrogen per hectolitre of alcohol at 100 vol%. [8]. The raw material that is fermented to produce the ethanol must also be given. Because of the toxicity of methylamine, a legal limit is imposed on its concentration in spirit in some countries and regions. In the Czech Republic this limit is legislated as 1 mg/L of 100% ethanol [9]. Currently, the amount of nitrogen-containing bases is determined as the content of methylamine by

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titration with 0.005 mol/L solution of sulphuric acid using a colour change as an indicator or by gas chromatography [9].

It would be very advantageous if the methylamine content was more quickly assayed than is currently possible using the techniques mentioned above. In this respect, our pilot experiments have suggested that SIFT-MS is a more rapid analytical method. SIFT-MS quantification is based on chemical ionisation using selected precursor (reagent) ions (H_3O^+ , NO^+ and O_2^+ are available) that are injected into a flow tube where they selectively react rapidly with trace gases present in air, breath or liquid headspace samples that are introduced into the helium carrier gas (but react only slowly with the major components of air and water vapour) resulting in characteristic product ions [10]. Thus, extensive studies of exhaled breath, urine and cell culture headspace and other systems have been carried that demonstrate well the value and potential of this analytical technique; the results of these studies have been reviewed in recent papers [10,11,12].

Previous work has revealed that the analysis of trace amounts of alcohols and amines in air or exhaled breath is best carried out using H_3O^+ precursor ions [13]. However, when the headspace of liquid ethanol or concentrated ethanol/water mixtures is applied directly to the input port of the SIFT-MS instrument, the flow rate of ethanol (headspace) vapour is such that the injected H_3O^+ precursor ions are totally consumed and protonated ethanol clusters dominate the product ions formed, as can be seen in the spectra shown in Section 3. Obviously, under such sample conditions the conventional mode of analysis using H_3O^+ ions to analyse trace amounts of compounds in the ethanol vapour cannot be achieved. However, all is not lost because the ethanol cluster ions can themselves be used as precursor ions for the analysis of trace compounds with which they react, amines being prime examples. But to achieve this it is essential to understand the complex ion chemistry occurring and to describe the detailed reaction scheme if accurate analyses are to be realised of the trace gases present in the ethanol/water vapour mixtures such as headspace of distilled ethanol and of spirit drinks. Such an approach has been previously attempted using proton transfer reaction mass spectrometry to obtain characteristic mass spectral fingerprints of the volatile compounds emitted by several different wine varieties [14].

However, neither the identification of the product ions nor the quantification of the trace compounds in the wine headspace was obtained.

Thus, the objective of the present study was first to understand the ion chemistry occurring in the SIFT-MS flow tube reactor in the presence of excess concentrations of ethanol vapour and from these gas phase analyses to quantify methylamine in liquid ethanol, validating these analyses using standard mixtures of methylamine in ethanol over a range of concentrations relevant to the limits imposed by current legislation.

2. Experimental

A Profile 3 SIFT-MS instrument (manufactured by Instrument Science Limited, Crewe, UK) was used for these studies. Its principle of operation and its special features have been outlined in previous papers [10,15]. Using this generation of SIFT-MS instruments, concentrations of trace gases can be determined down to the parts-per-billion, ppb, level in just a few seconds of data acquisition time, which allows the time profiles of some metabolites in single breath exhalations to be described. A special feature of these SIFT-MS instruments is that calibration is unnecessary to obtain absolute quantification [16]. This method has been described thoroughly in previous papers [10,16] so only some particular details are required here. The helium carrier gas was at a pressure of typically 1 Torr at the ambient temperature, which was 26 °C, and both pressure and temperature were recorded during every measurement. For headspace analyses, the liquid samples were contained in vials of volume 25 mL sealed with septa and the headspace volatiles were introduced into the helium carrier gas of the SIFT-MS instrument via a calibrated capillary, as illustrated in Fig. 1. A PEEK capillary was used with internal diameter of 0.25 mm providing a sample flow rate of 0.3 Torr L/s (24 sccm), which is routinely used for analyses of air samples containing water vapour at pressure equivalent to the saturation vapour pressure of water at 37 °C as pertains to exhaled breath or liquid headspace.

The first experiments involved the introduction of the headspace vapour from mixtures of ethanol in water at varying dilutions up to 97% ethanol. Distilled ethanol of biological origin, strength 97%,

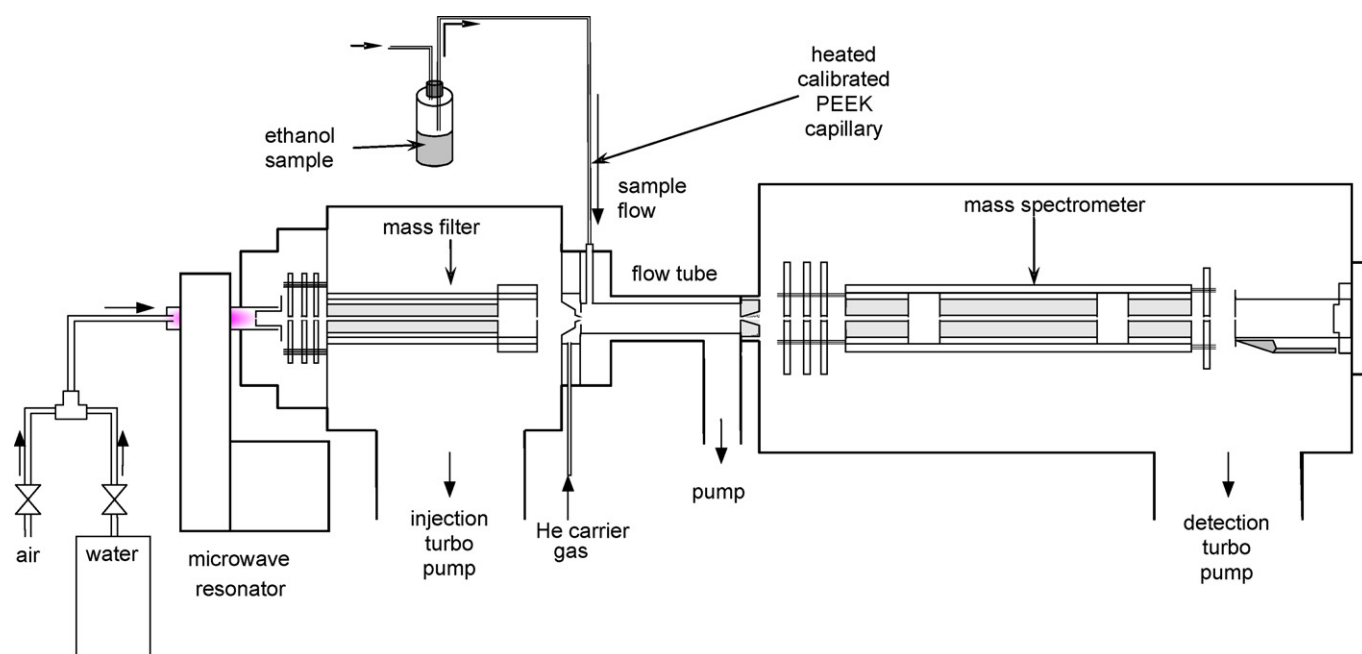


Fig. 1. Schematic diagram of SIFT-MS configured for headspace analyses of ethanol/water vapour/trace compound samples.

was used to prepare these mixtures (supplied as a quality control sample from a production plant at the distillery BIOFERM – liho-var Kolín, a.s.). Methylamine has been added to these mixtures at a concentration of 10 mg/L to allow its clear identification on the mass spectra, recorded in the full scan mode. Characteristic ions of methylamine were indeed seen that originate from the reactions of ethanol cluster ions with the trace methylamine. Hence, a kinetic database library entry was constructed that allows this amine to be quantified in the headspace above the ethanol. These experiments were followed up by introducing known amounts of methylamine into pure ethanol liquid (producing concentrations of 0.1–0.4 mg/L) at two different temperatures and duly analysing the liquid headspace by monitoring the signals of ions included in this library entry that are signatures of methylamine and which can ultimately be used to determine the concentration of this compound in the liquid ethanol. Finally, calibration plots of headspace concentration as a function of the liquid concentrations were constructed and interpreted in the terms of the Henry's law coefficients.

3. Results

3.1. Ion chemistry in water vapour and ethanol vapour mixtures

The major product ions that result when the headspace of ethanol/water mixture containing a trace of methylamine is sampled into the carrier gas are revealed by full scan (FS) SIFT-MS spectra over the mass-to-charge ratio, m/z , range from 10 to 200 as exemplified in Fig. 2 for three ethanol/water dilutions. As can be seen, the H_3O^+ precursor ions react with water and ethanol molecules producing both hydrated hydronium ions $H_3O^+(H_2O)_{1,2,3}$, protonated ethanol and its hydrates $C_2H_5OH_2^+(H_2O)_{0,1,2}$ and ultimately at high ethanol concentrations the ion at m/z 139, which is the protonated ethanol ion to which three ethanol molecules are clustered, $C_2H_5OH_2^+(C_2H_5OH)_3$. This ion is so dominant at the high concentration of ethanol that it can, in principle, be used as a precursor ion for SIFT-MS analyses of very basic molecules with large proton affinities such as amines [10]. A point to note is that small signals of O_2^+ ions at m/z 32 are ever present on SIFT-MS spectra when using H_3O^+ precursor ions. These are typically at a signal level of 1% or less of the H_3O^+ signal and thus play little part in SIFT-MS analyses except at sub-ppb levels [10]. However, it is worthy of note for the present study that these ions are at the same m/z value of 32 as protonated methylamine, $CH_3NH_2H^+$ (see Section 3.2).

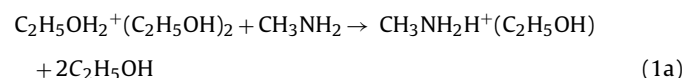
The progression of this ion chemistry is best followed using the multiple ion monitoring (MIM) mode of SIFT-MS operation in which the signal levels of the H_3O^+ precursor ion and the product ions, as revealed by the FS spectra shown in Fig. 2, are monitored as the headspace of the ethanol/water mixtures at increasing concentrations is introduced into the helium carrier gas. The sample data in Fig. 3 clearly show that the $H_3O^+(H_2O)_{0,1,2,3}$ and the $C_2H_5OH_2^+(C_2H_5OH)_{0,1,2}$ ions are removed as the ethanol concentration in the liquid solution is increased above 10% to be replaced by the $C_2H_5OH_2^+(C_2H_5OH)_3$ ion at m/z 139 together with a smaller signal of the higher order ethanol cluster ion $C_2H_5OH_2^+(C_2H_5OH)_4$ at m/z 185 that finally reaches about 15% of the total ion signal at the highest ethanol fraction in the liquid. Also present are minor signals (<1%) of the proton bound dimer $C_2H_5OH_2^+ \cdot C_2H_5OH$ at m/z 93 and its monohydrate at m/z 111.

The generalised scheme of the complex ion chemistry occurring is shown in Fig. 4. In these studies H_3O^+ is the precursor ion that initiates the ion chemistry. At high concentrations of water the H_3O^+ ions are rapidly hydrated to $H_3O^+(H_2O)_{1,2,3}$ ions. Reactions of organic molecules M (e.g., ethanol) with H_3O^+ ions are dominated by exothermic proton transfer reactions to pro-

duce MH^+ ions (e.g., $C_2H_5OH_2^+$), which can also react with the water molecules present to form hydrated protonated ethanol ions. The latter can also be produced in the reactions of the hydrated H_3O^+ ions with M. Then sequential reactions that involve ligand switching of H_2O molecules by C_2H_5OH molecules form protonated ethanol cluster ions. In Fig. 4 the formation of $H_3O^+(H_2O)_{1,2,3}$ ions and the $C_2H_5OH_2^+(C_2H_5OH)_{1,2,3}$ ions is shown in the vertical and horizontal directions, respectively and production of the mixed clusters is possible by alternative routes, as indicated. All ions from the $H_3O^+(H_2O)_{1,2,3}$ group are efficiently removed by their reactions with ethanol molecules. Note that $H_3O^+(H_2O)_3$ does not react directly with ethanol [13] and thus the pathway for its removal begins by thermal dissociation in the helium carrier gas to $H_3O^+(H_2O)_2$ which then rapidly reacts with ethanol molecules. The double arrows indicate the possibility of reverse reaction at the temperature of the helium carrier gas of about 26 °C. Thus, the concentrations of each pair of ions connected by double arrows can approach ion–molecule kinetic equilibrium. Hence, as is evident in Fig. 3, the terminating ion is largely the protonated ethanol trimer at m/z 139 at all but the largest concentrations of ethanol in the liquid mixture when the contribution of protonated ethanol tetramer at m/z 185 becomes significant.

3.2. Ion chemistry in water vapour and ethanol vapour mixtures containing trace amounts of methylamine

The question now is whether or not the terminating ions $C_2H_5OH_2^+(C_2H_5OH)_{1,2,3}$ can be used to quantify trace amounts of methylamine in ethanol solution in a way analogous to the use of $H_3O^+(H_2O)_{1,2,3}$ ions in traditional SIFT-MS analyses. The SIFT-MS spectra in Fig. 2 reveal that when methylamine is present at trace concentrations in the ethanol liquid (and in the vapour), ions appear in the spectra at m/z values of 78 and 124 that are certainly not present in the spectra obtained using the pure ethanol/water mixture. Thus, these two ion species are due to the presence of methylamine in the ethanol. They are identified as $CH_3NH_2H^+(C_2H_5OH)$ and $CH_3NH_2H^+(C_2H_5OH)_2$. They are produced, predominantly, in the reaction of the $C_2H_5OH_2^+(C_2H_5OH)_2$ precursor ion at m/z 139 with methylamine, CH_3NH_2 :



These are examples of the well-understood ligand switching reactions [8,13]. Reaction of $C_2H_5OH_2^+(C_2H_5OH)_{1,2,3}$ cluster ions with CH_3NH_2 presumably lead to the same products as do reactions (1a) and (1b), because no other product ions are evident in the mass spectra. Thus, the ions at m/z 78 and 124 can be used to detect methylamine in the headspace of the ethanol/water vapour/methylamine mixtures.

At low concentrations of ethanol (below 2% by volume in water) the H_3O^+ precursor is the dominant ion on the mass spectra and thus proton transfer to CH_3NH_2 occurs leading to formation of $CH_3NH_2H^+$ at m/z 32. Thus, in Fig. 2a (0.1% ethanol in water) the ratio of signal intensities at m/z 32 to m/z 19 is elevated above the photoionisation background of 1% to about 3%. At increasing ethanol concentrations, the absolute intensity at m/z 32 ($CH_3NH_2H^+$) decreases because the H_3O^+ precursors ion signal is reducing and becomes insignificant above some 2% ethanol concentration. The residual signal seen at m/z 32 at ethanol concentrations above 2% is mostly due to photoionisation of O_2 in the flow tube by ultraviolet radiation penetrating from the microwave discharge ion source and is not related to the presence of CH_3NH_2 .

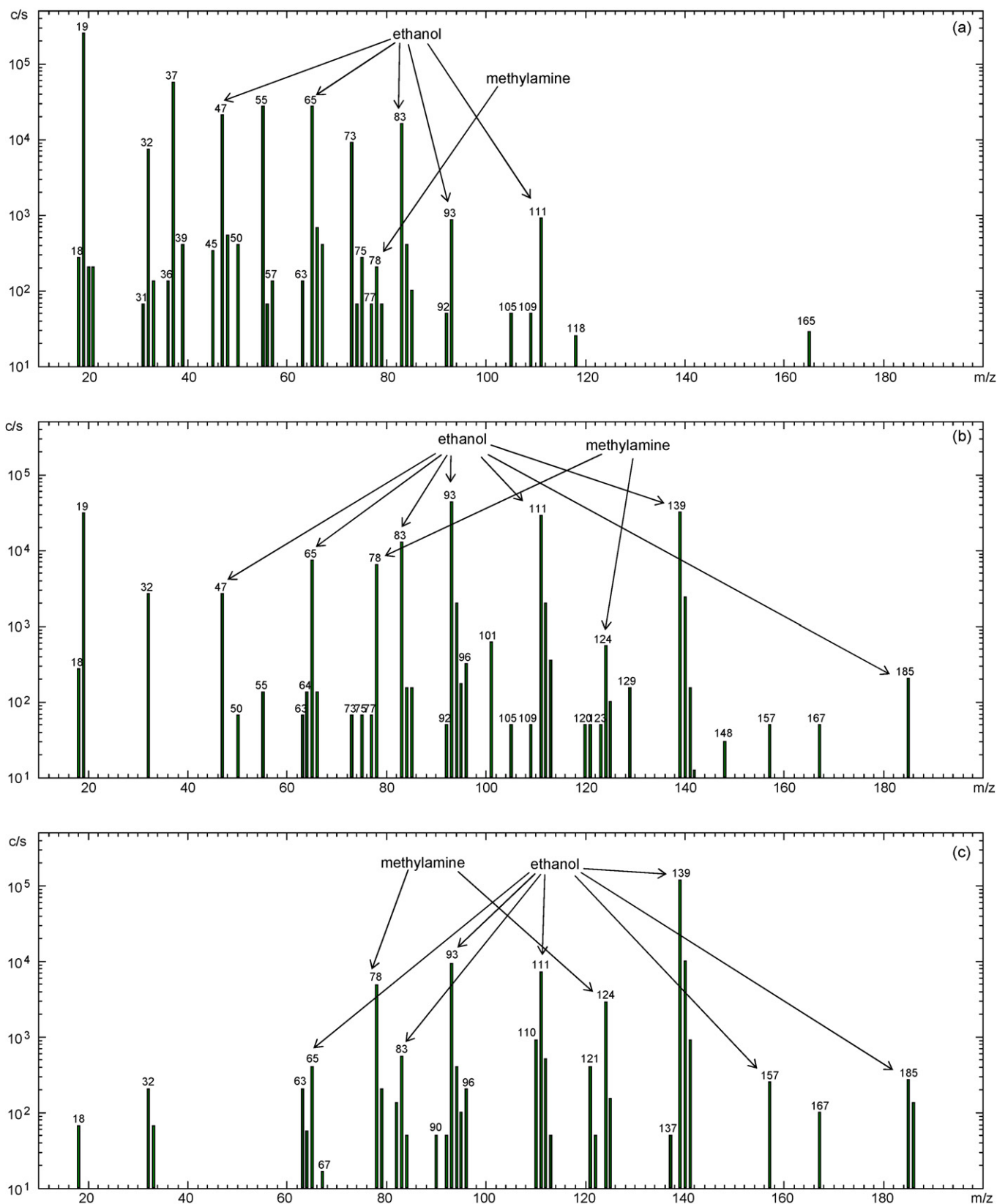


Fig. 2. Full scan (FS) SIFT-MS spectra showing ion count rate per second, c/s, on a semi logarithmic scale as a function of mass-to-charge ratio, m/z , obtained when the headspace above mixtures of ethanol in water is introduced into the SIFT-MS helium carrier gas. Three different concentrations of ethanol in water have been used (a) 0.1%, (b) 1% and (c) 97%. All mixtures contained methylamine at a concentration of 10 mg/L. See the text for interpretation of these spectra.

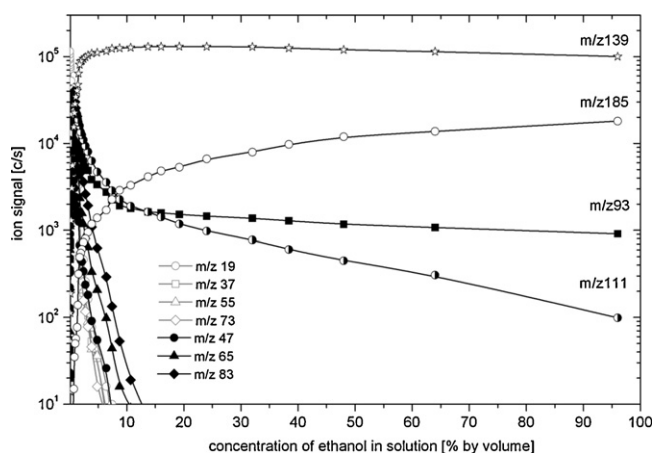


Fig. 3. Count rates, c/s, of the precursor and product ions as a function of the concentration of ethanol in ethanol/water mixture expressed as % by volume of ethanol. These experimental data were obtained at a solution temperature 20 °C and a fixed headspace sample flow rate of 0.3 Torr L/s (24 sccm). Note the dominance of m/z 139 at the high ethanol concentrations.

3.3. Quantification of methylamine in ethanol solution

SIFT-MS quantification using the $C_2H_5OH_2^+(C_2H_5OH)_{1,2,3}$ precursor ions can be carried out in an analogous way to the use of the conventional $H_3O^+(H_2O)_{1,2,3}$ precursor ions that exploits the general method for calculating absolute trace gas concentrations from the precursor and product ion signal levels [16]. With reference to the SIFT-MS master quantification equation (equation (15) in [16]) all that is required is to enter the signal levels of the $C_2H_5OH_2^+(C_2H_5OH)_{1,2,3}$ ions (m/z 93, 111, 139 and 185), which have been corrected for differential diffusion and mass discrimination, as the precursor ion signals, and the signal levels of the $CH_3NH_2H^+(C_2H_5OH)_{1,2}$ ions (m/z 78 and 124), which have also been corrected for differential diffusion and mass discrimination, as the product ion signals. The collisional rate coefficient for the exothermic switching reactions (1a) and (1b) is calculated to be $1.6 \times 10^{-9} \text{ cm}^3/\text{s}$ by the Su and Chesnawich parameterisation [17], using the known polarisability ($4 \times 10^{-24} \text{ cm}^3$) and permanent dipole moment (1.31 D) of the CH_3NH_2 molecule. The resulting kinetic library entry in the format of the SIFT-MS software (Trans Spectra Limited, version 3.1.238 or later versions) is given in Table 1. Note that m/z 19 must be formally included amongst the precursors ions in order to instruct the software to use this entry when the H_3O^+ ions are selected by the injection mass filter.

Table 1
SIFT-MS kinetics library entry for calculation of methylamine concentration using the proton bound ethanol clusters as the precursor ions.

Methylamine.EtOH(H_3O^+)	
5 Precursors	
93	1.7e-9 1.0
111	1.6e-9 1.0
139	1.6e-9 1.0
185	1.6e-9 1.0
19	2.3e-9 1.0
2 Products	
78	1.0
124	1.0

In order to test the above formulated SIFT-MS kinetic library entry, validation experiments were carried out in which pure ethanol was spiked with known amounts of methylamine at levels below the limits set by health and safety legislation. Thus, 10, 20, 30 and 40 μL of a solution of methylamine in water at a concentration of 3 g/L was added to 30 mL of 97% distilled ethanol/water mixture creating solutions containing 0, 0.1, 0.2, 0.3 and 0.4 mg/L of methylamine. The headspace of these solutions was introduced into the SIFT-MS instrument at a known flow rate via the heated PEEK capillary (see Fig. 1) and the concentration of methylamine was determined as described above. Headspace concentrations of methylamine were obtained at two solution temperatures of 20 and 50 °C and the data are given in the plots in Fig. 5. The linearity of these plots demonstrates that gas phase/liquid phase equilibrium is established and the slopes of the regression lines relate the vapour phase concentration of methylamine to its solution concentration. The linearity of the calibration plots is good with $R^2 > 0.97$ and the limit of detection of methylamine in the ethanol/water solution is better than 0.1 mg/L. Note that repeated analyses of the pure quality control sample did not reveal any methylamine above the limit of detection; these results are represented as zero values on both plots in Fig. 5. The slopes of these plots provide the Henry's law coefficients for the volatility of methylamine from ethanol as 0.076 atm/M at 20 °C and 0.31 atm/M at 50 °C, which serve as the calibration required to determine the concentration of methylamine in ethanol solution. The increasing volatility of methylamine with temperature is related to its enthalpy of evaporation and like the Henry's law coefficients themselves represent instrument independent parameters.

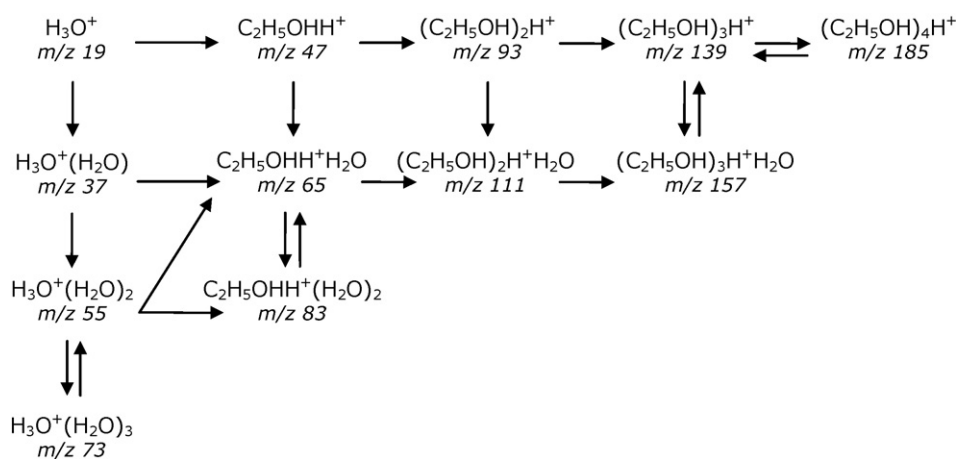


Fig. 4. The full scheme of the ion chemistry reaction system of H_3O^+ with ethanol and water vapour. For the meaning of the double arrows and further explanation of this scheme, see the text.

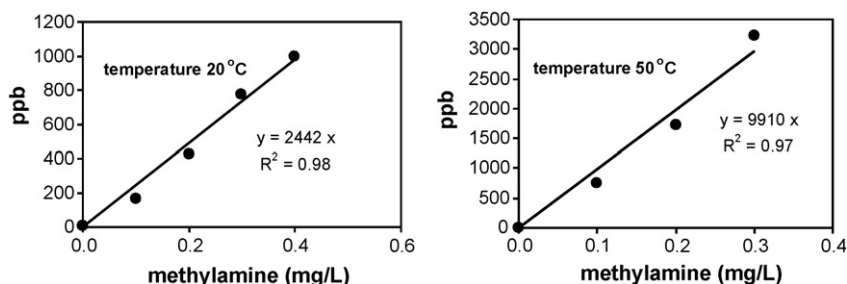


Fig. 5. The variation of the headspace concentration of methylamine, as measured by SIFT-MS in parts per billion, ppb, with its concentration in 97% ethanol liquid samples prepared as described in the text. The kinetic library entry given in Table 1 is used for the headspace methylamine analyses.

Concluding remarks

SIFT-MS has been used for the first time to quantify absolute levels of a trace compound in gas/vapour samples in the presence of excessive amount of ethanol vapour. This paper presents the results of a case study of the quantification of methylamine in distilled ethanol of agricultural origin. The results of calibration experiments demonstrate that it is possible to quantify methylamine in ethanol at concentrations of 0.1 mg/L or greater. Such analyses are much quicker than liquid phase analyses and as such can be of great value to the distillation industry and for the real time monitoring of fermentation and distillation systems. The concepts and methodology used for this analysis can be now used with confidence to analyse other polar or basic compounds present in ethanol or alcoholic beverages with ethanol concentrations between 2% and 97%, as long as switching reactions like reaction 1(a) and 1 (b)(1) are exothermic. This will have to be experimentally confirmed for specific cases; however, experience with this type of ion chemistry [8,13] indicates that polar molecules with proton affinities larger than that of ethanol will be accessible to analysis by SIFT-MS after appropriate chemical ionisation schemes have been established.

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