Headspace gas chromatography based methodology for the analysis of aromatic substituted quaternary ammonium salts

Niels van Boxtel, Kris Wolfs, Marta Guillén Palacín, Ann Van Schepdael, Erwin Adams*

KU Leuven – University of Leuven, Department of Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, Herestraat 49, O6N2, PB 923, 3000 Leuven, Belgium

A R T I C L E   I N F O
Article history:
Received 10 August 2016
Received in revised form 7 November 2016
Accepted 8 November 2016
Available online 9 November 2016

Keywords:
Headspace gas chromatography
Quaternary ammonium salts

A B S T R A C T
The analysis of quaternary ammonium salts (QAS) using GC is often performed by “in injector” pyrolysis to create volatile degradation products for quantification purposes. Besides the risk of severe system contamination, the application of this approach on aqueous samples is problematic. In this work, the sample is treated in a vial with 2,2-dimethoxypropane (DMP) under acidic catalysis. In addition to the removal of water and sample enrichment, the QAS are decomposed. As HS transfers only volatile compounds to the GC system, contamination is avoided. It was found that depending on the presence of benzyl, phenyl or methyl groups on the quaternary nitrogen; benzyl chloride, N,N-dimethylalaniline or chloromethane are formed respectively in the sealed vial. All these can be used as an analytical target. A calibration curve for benzyl chloride could be derived from the pure compound. Chloromethane was generated from pure benzylidimethylammonium chloride (BEDIDE), a pure QAS with benzyl and methyl groups, to construct a secondary calibration curve using a back analysis approach. It has been proven that by quantifying the formed analytical targets, the mass balance for the QAS under investigation was close to 100%. The presented procedure allows the quantification of any aromatic substituted QAS without the need for a matching reference, which is a major advantage over existing CE and LC methods. The proposed methodology was validated for mouth sprays containing benzethonium chloride (BZTCI) or benzoxonium chloride (BZOCI) and for denatonium benzoate (DB) in ethylene glycol (EG) based cooling liquids. Results showed that the approach provided excellent linearity ($R^2 > 0.999$) and limits of detection around 0.01 µg/vial for benzyl chloride. It was found that the reaction product of DMP and glycerol which was also present in the mouthspray and some cooling liquids, caused chromatographic interference with benzyl chloride. Treating those samples in the vial with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) after the enrichment step removes the interference and leaves a possible pathway for the simultaneous determination of glycerol in those samples.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Quaternary ammonium salts (QAS) are synthesised by reaction of tertiary amines with halogenated compounds and are mostly used as disinfectants, surfactants, corrosion inhibitors or pesticides. The analysis of QAS is usually performed using liquid chromatography (LC) [1–10], capillary electrophoresis (CE) [11–13] or gas chromatography (GC) [14,15]. Several of the LC methods use ion pairing agents which are known to cause slow equilibration between the mobile and stationary phase and which can never be washed completely from the column [16]. Moreover, sample preparation procedures often precede the LC process of QAS. This is certainly an issue when LC is coupled with mass spectrometry (MS) which is liable to matrix effects that cause ion suppression in the ion source. From CE, it is known that it does not offer the best repeatability/reproducibility [17]. In addition, LC and CE methods require for each QAS that is analysed a matching standard that can be used as reference, but which is not always easily available.

GC methods are based on pyrolysis GC in which a sample is introduced into a heated injector. This has the disadvantage that when not all the resulting pyrolysis products are volatile, parts of the GC system can become contaminated by the gradual build-up of residues that cannot be evaporated. The determination of QAS in aqueous samples poses an even larger challenge. Evaporation of large amounts of water in an injection liner is problematic due to the large resulting gas volume after evaporation leading to flooding...
of the injection liner. Moreover, the introduction of large amounts of water on a GC column leads to column damage unless special water resistant columns are used. Static headspace (sh5)-GC on the other hand allows clean sample introduction as only volatile sample constituents are introduced in the GC system. However, the use of sh5-GC in combination with aqueous samples is limited as the incubation temperature used should be kept below the boiling point of water. This limits the sensitivity for analytes with a high boiling point and large affinity for the aqueous matrix. Recently, a method was published in which an acetone acetal was used for the complete removal of water prior to GC analysis of a selection of typical high boiling polar residual solvents [18]. Acetone acetals such as 2,2-dimethoxypropane (DMP) react with water to form acetone and methanol under acidic catalysis. The fact that the resulting products are much more volatile than the analytes (which have a high boiling point), enables sample enrichment by simple evaporation using either a vacuum oven or a stream of nitrogen.

This work covers the use of full evaporation (FET)-HS-GC for the analysis of benzyl substituted QAS in aqueous samples after removal of water with DMP. First, a screening of typical QAS was performed to determine the resulting products and to evaluate the quantitative relationship of this approach. Finally, the method was applied to the analysis of denatonium benzoate (DB) in cooling liquids that contain both water and ethylene glycol (EG). The same approach was used to analyse benzenonium chloride (BZTCl) and benzoxyoxonium chloride (BZOCI) in mouth sprays.

2. Experimental

2.1. Reagents

DMP (98%), o-xylene (99%), N,N-bis(trimethylsilyl)trifluoroacetamide (95%) (BSTFA), sodium dodecyl sulphate (99.0%) (SDS) and N,N-dimethylaniline (99%) were purchased from Acros Organics (Geel, Belgium). Hydrochloric acid (37.5%) (HCl) was bought from VWR International S.A.S. (Fontenay-sous-Bois, France). DB (98%), BZTCl (99.0%), benzyldimethyldecylammonium chloride (BEDIDE) (97.0%), benzyl chloride (95%), sodium phosphate monobasic and trimethylphenoxy ammonium chloride (TMPACl) (98%) were from Sigma Aldrich (New Jersey, USA). Toluene (99.8%) was purchased from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany). Water was purified using a milliQ-system (Darmstadt, Germany). Phosphoric acid (85%) was obtained from Chemlab NV (Zedelgem, Belgium). Cooling liquids denatured with DB and consisting of about 50% v/v of EG and 50% v/v of water were purchased in a local shop while mouth sprays containing BZTCl and BZOCI respectively were obtained from a local pharmacy. The BZTCl mouth spray contained 435 μg/g BZTCl and was further composed of chlorhexidine digluconate, maltitol, ethanol, menthol, castor oil and water. The BZOCI content in the BZOCI spray was ~0.2%. This spray further consisted of sorbitol, ethanol, glycerol, peppermint oil, menthol, hydrochloric acid and water.

2.2. Chromatographic systems

2.2.1. GC analyses

HS-GC analyses with flame ionisation detection (FID) were carried out with an Agilent 6890 series GC equipped with a Perkin Elmer Turbomatrix 40 HS autosampler (balanced pressure system) (Waltham, MA, USA). GC–MS identifications and single ion monitoring (SIM) on m/z 91 and m/z 126 were performed with a Perkin Elmer Autosystem XL equipped with a Turbomatrix 40 HS autosampler and Perkin Elmer Turbomass mass spectrometer. The HS parameters used were as follows: equilibration temperature: 170 °C, equilibration time: 60 min, needle temperature: 180 °C, transfer line temperature: 190 °C, pressurization time: 1.0 min, injection time: 0.04 min, needle withdrawal time: 0.4 min, injection port temperature: 200 °C, carrier gas pressure: 130 kPa, split ratio 1:5, detector temperature FID: 250 °C. HS vials and PFTE-Sil caps were purchased from Perkin Elmer. All separations were carried out on an AT-1 column (30 m × 0.33 mm, df = 5.00 μm) from Grace Alltech (Deerfield, IL, USA). The GC oven program used for separations started at 40 °C. Immediately after injection, the column temperature was raised with a rate of 8 °C/min till 90 °C. Next, the column was further heated at 15 °C/min till 240 °C. This temperature was kept for 8 min. Finally, it was lowered to 40 °C again over a period of 15 min.

2.2.2. LC analyses

LC-UV analyses for the determination of DB in cooling liquids were performed using an ion pair method reported in literature [10]. A system, consisting of a Spectra system pump P1000 XR from Thermo Fisher Scientific (Waltham, MA, USA), an autosampler from Spark Holland with an injection loop of 20 μL (Emmen, the Netherlands) and a L-2400 UV detector set at 210 nm from Hitachi Elite LaChrom (San Jose, CA, USA) was utilized. The column used was a Waters (Milford, MA, USA) Symmetry C18 (100 Å, 5 μm, 150 mm × 3.9 mm I.D.) kept at 35 °C. The LC-system was monitored by Chromelon software (Dionex, Sunnyvale, CA, USA). The mobile phase consisted of acetonitrile and buffer solution pH 3 with 10 mM SDS (50:50, % v/v). The flow rate was 1.2 ml/min.

2.3. Preparation of solutions and samples

2.3.1. GC analyses

2.3.1.1. Screening samples of different quaternary ammonium salts.

An overview of the QAS included in this study is given in Table 1. Solutions of pure QAS available as their chloride salt (BZTCl, BEDIDE and TMPACl) and DB having benzoate as counter ion with concentrations of 15 mg/mL were prepared in methanol. For BZTCl, BEDIDE and TMPACl, 100 μL of each solution was transferred to a HS vial. As DB is not a chloride salt, the reaction did not proceed and so the procedure was adapted: 100 μL of methanolic DB solution and 10 μL of HCl were introduced in a HS vial. After evaporation to dryness using a vacuum oven at 30 °C (≤0.1 kPa, 90 min), vials were sealed with a PFTE-Sil cap and analysed using the described HS–GC method to identify the major volatile decomposition (or pyrolysis) products.

BZOCI was not available as pure salt. Therefore, 10 μL of mouth spray containing BZOCI was treated with 1 mL of DMP (to which 10 μL of HCl was added) for water scavenging. This procedure was carried out directly in the HS vial which was dried under vacuum afterwards. Next, the vial was sealed with a PFTE-Sil cap.

2.3.1.2. Internal standard solution.

An amount of 200 mg of toluene was dissolved in 10 mL of o-xylene and diluted 10 times by transferring 5.0 mL of the solution to a volumetric flask of 50 mL. o-Xylene was used to bring the solution up to volume.

2.3.1.3. Calibration solutions for the determination of benzyl chloride.

An amount of 200 mg of benzyl chloride was dissolved in 10 mL of o-xylene and diluted 10 times by transferring 5.0 mL to a volumetric flask of 50 mL and bringing to volume with o-xylene. A 6-point calibration series was prepared in the range from 100 to 600 mg/L. Before bringing to volume with o-xylene, each time 4.0 mL of internal standard solution was added. Calibration was performed by analysing 10 μL of each solution.

2.3.1.4. Calibration solutions for the determination of chloromethane.

A solution of BEDIDE in methanol was prepared by dissolving 20 mg
in 25 mL of methanol. A 6-point calibration curve in the range from 1 to 10 mg/L was prepared using appropriate dilutions in methanol. A volume of 200 µL of each calibration solution was transferred into a HS vial and methanol was removed by vacuum evaporation. Then, 10 µL of o-xylene was added to the vial which was sealed with a PTFE-Sil cap.

2.3.1.5. Sample preparation for the HS analysis of denatonium benzoate in cooling liquids. Due to the high amount of EG (50%), samples were diluted 20-times with water from which an aliquot of 1.0 mL was transferred to a HS vial. After adding 10.0 mL of DMP and 50 µL of HCl, the mixture was left for 5 min to react. After the water scavenging process, samples were placed in a vacuum oven at 30 °C for 2 h to evaporate all solvents and HCl. After the drying step, another 1.0 mL of DMP and 10 µL of HCl were added to react with residues of EG. Once again, samples were evaporated before adding 10 µL of o-xylene to the sample vial. Finally, vials were sealed with a PTFE-Sil cap and analysed with the described HS method. In case of FID detection, 100 µL of BSTFA was transferred into the sample vial for silanisation and evaporated to dryness before addition of 10 µL o-xylene and closure of the vial.

2.3.1.6. Sample preparation for the HS analysis of benzethonium chloride in a mouth spray. A mouth spray containing BZTCl was diluted 100 times in water. A volume of 1.0 mL of the resulting solution was brought into a HS vial and 10.0 mL of DMP and 50 µL of HCl were added. After reacting for 5 min, samples were evaporated to dryness in a vacuum oven at 30 °C. Before sealing the vials with a PTFE-Sil cap, 10 µL of o-xylene was added.

2.3.1.7. Sample preparation for the HS analysis of benzoxyonium chloride in a mouth spray. A mouth spray containing BZOCl was diluted 250 times in water. A volume of 1.0 mL of the resulting solution was brought into a HS vial and 10.0 mL of DMP and 50 µL of HCl were added. After reacting for 5 min, samples were evaporated to dryness in a vacuum oven at 30 °C. Before sealing the vials with a PTFE-Sil cap, 10 µL of o-xylene was added.

2.3.2. LC analyses
2.3.2.1. Preparation of the mobile phase. A 0.1 M buffer solution of NaH₂PO₄ and phosphoric acid was prepared by adjusting the pH to 3.0. Next, 100 mL of the buffer was diluted 10 times in a volumetric flask of 1000 mL. Before bringing to volume, 2.88 g of SDS was added. Finally, 500 mL of the resulting buffer solution was mixed with 500 mL of acetonitrile.

2.3.2.2. Sodium dodecyl sulphate solution. An amount of 560 mg of SDS was dissolved in water using a volumetric flask of 100 mL.

2.3.2.3. Denatonium benzoate standard solution. An amount of 40 mg of DB was weighed and dissolved in 50.0 mL of water using a volumetric flask. This solution was diluted 10 times by transferring 10.0 mL to a volumetric flask of 100 mL. Next, 5.0 mL of this solution was transferred to a 50 mL volumetric flask; 35 mL of ethanol was added and brought up to volume with water. Finally, 5.0 mL of the resulting reference solution was diluted to 10.0 mL with SDS solution.

2.3.2.4. Sample preparation for the LC analysis of denatonium benzoate in cooling liquids. A volume of 5.0 mL of sample was transferred into a volumetric flask of 10 mL and brought up to volume using the SDS solution.

2.4. Procedures
2.4.1. Screening study of degradation compounds
For all compounds under investigation, vials were prepared as described in 2.3.1.1. and analysed using the parameters listed in 2.2.1. The data were collected using MS in full scan mode for identification and FID in parallel.

The effect of the equilibration time on the decomposition yield was investigated as example for BZTCl and BEDIDE. The vials prepared as described in 2.3.1.1. were analysed using the parameters listed in 2.2.1, but with different equilibration times varying from 35 to 60 min. The resulting peak areas for benzyl chloride and chloromethane were measured.
2.4.2. Calibration for benzyl chloride

Three correction curves were constructed. One was FID based, while the two others were SIM based (m/z 91 and m/z 126). Each six point calibration curve was constructed using the vials prepared in duplicate according to 2.3.1.3. The peak areas for benzyl chloride were corrected with the obtained peak areas for toluene which was used as internal standard.

2.4.3. Calibration for chloromethane

Decomposition of QAS like BEDIDE results in the formation of either benzyl chloride or chloromethane (see also Table 1). The benzyl chloride content of the vials prepared as described in 2.3.1.4. was determined using the analytical parameters from 2.2.1 with FID detection and the calibration curve obtained from 2.4.2. It was assumed that from the number of mmols of BEDIDE and the number of mmols of benzyl chloride, the number of mmols of chloromethane can be calculated. As the original amount of QAS with methyl groups like BEDIDE in the vials is known and the amount of formed benzyl chloride is measured, the formed amount of chloromethane in a vial can be calculated using Formulae (1) and (2):

\[
\text{mmoles QAS left} = \left( \frac{m_{\text{QAS}}}{M_{\text{QAS}}} \right) - \left( \frac{m_{\text{BCl}}}{M_{\text{BCl}}} \right)
\]

In which:
- mQAS = total mass in mg of QAS in HS vial
- MQAS = molecular mass of QAS
- mBCl = mass (mg) of released benzyl chloride
- MBcl = molecular mass of benzyl chloride.

Using Formula (1) the amount of QAS that can produce chloromethane is calculated enabling the determination of the amount of chloromethane in a vial. Since one mole of QAS (even with more than one methyl group) produces one mole of chloromethane:

\[
m_{\text{CM}} = \text{mmoles QAS left} \times M_{\text{CM}}
\]

In which:
- MCm = mass (mg) of chloromethane
- MCM = molecular mass of chloromethane.

From these calculations and the peak areas measured for chloromethane, a calibration curve was constructed.

2.4.4. Method validation

2.4.4.1. Linearity. The generalized linearity depends on the combined response for benzyl chloride and chloromethane. For both compounds a six point calibration curve was created and assessed.

2.4.4.2. Precision. Repeatability was expressed as RSD% value using at least 4 measurements.

2.4.4.3. Recovery. Recovery in this procedure relies on the complete conversion of the analyte into the target compounds and their proper determination. Three solutions of DB in 50% v/v EG with known concentrations (60, 80 and 100 µg/g) were analysed. The GC results of the commercial samples were compared with those obtained with an LC method from literature [10]. For GC analysis, commercial samples were spiked with DB as a supplementary validation.

For the BZTCI mouth spray, three solutions of BZTCI with known concentrations in water (10, 15 and 20 µg/g) were analysed. For the quantification of chloromethane, a baseline correction was performed. GC results obtained for the commercial mouth spray were compared with the label claim. For the BZOCl mouth spray, GC results were also compared with the labelled value.

2.4.4.4. Sensitivity. The limit of quantification (LOQ) was determined based on a signal-to-noise ratio (S/N) of 10.

2.4.5. Analysis of commercial samples

2.4.5.1. HS-GC analysis of cooling liquid samples. Two typical automotive cooling liquids were analysed with the validated protocol. Detection was performed using either MS operated in SIM mode to detect both toluene (m/z 91) and benzyl chloride (m/z 91 and m/z 126) or FID after BSTFA silanisation to avoid chromatographic interference.

2.4.5.2. HS-GC analysis of benzethonium chloride in a mouth spray. HS-GC analysis of BZTCI was performed at 170 °C with an equilibration time of 60 min. Benzyl chloride and chloromethane amounts were measured using FID and the amount of BZTCI was calculated according to Formulae (1) and (2).

2.4.5.3. HS-GC analysis of benzonium chloride in a mouth spray. HS-GC analysis of BZOCl was performed at 170 °C with an equilibration time of 60 min. Quantification of this QAS was done by calibration with benzyl chloride.

2.4.5.4. LC-UV analysis of denatonium benzoate in commercial cooling liquids. Cooling liquids were diluted ten times with water and analysed with the described protocol using DB for calibration.

3. Results & discussion

3.1. Screening of various quaternary ammonium salts

In literature, debenzylation of the quaternary nitrogen in benzyl substituted QAS is proposed as the favoured thermal decomposition pathway [19]. In the case of DB, this yields lidocaine and benzyl benzoate, both having boiling points over 300 °C making them unusable for a HS approach. Converting the DB into a chloride salt should produce benzyl chloride with a boiling point of 179 °C instead. The data obtained from the procedure described in 2.4.1. were compared with a NIST mass spectral library. The identified degradation products of the QAS studied (see Table 1) were benzyl chloride as major product (Fig. 1), chloromethane, N,N-dimethylaniline and N,N-dimethyldecylamine (Fig. 2). Looking at the structures of the QAS and formed decomposition products, a set of simple rules to predict the result can be proposed depending on the substitution of the quaternary nitrogen atom:

- if a benzyl group is present, benzyl chloride is formed.
- if a benzyl group and methyl groups are present, 1 molecule of benzyl chloride and 1 molecule of chloromethane are formed.
- if the aromatic substituent is a phenyl group then the corresponding aniline analogue is formed.

As a result, benzyl substituted QAS without methyl groups on the quaternary nitrogen like DB and BZOCl can be quantified using benzyl chloride only, while for those bearing also a methyl group, quantification should be based on the combination of the produced amounts of all the chlorinated species being benzyl chloride and chloromethane. For phenyl and methyl substituted QAS like TMPACl, N,N-dimethylaniline can be used as calibrant. N,N-dimethyldecylamine which is found in case of BEDIDE, is the tertiary amine formed after removing the benzyl group. As BEDIDE is one of the components of benzalkonium chloride which contains several benzyldimethylalkanes (ranging from C₈ to C₁₈), it...
3.2. Equilibration time

In HS analysis the equilibration time is an important factor and is chosen in such a way that a stable gas phase is formed without decomposition. In this case however, a stable gas phase with complete decomposition is the goal. From Fig. 3 it can be concluded that the decomposition is complete after 60 min as both benzyl chloride and chloromethane peak areas are stable and repeatable.

3.3. Method validation HS-GC method

3.3.1. Linearity

A calibration curve of benzyl chloride was constructed in the range of 1–6 μg/vial by repetitive injections. This range was selected in function of the analysed samples and calibration solutions were prepared as described in 2.3.1.3. The obtained R² value was higher than 0.999, indicating that a good linear relationship exists between analyte response and the amount of analyte. The use of an internal standard for calibration was necessary to correct for variations on the sample volume as the analysed sample volume was only 10 μL. o-Xylene was added to the sample vials before sealing to avoid possible viscosity effects in the gas phase, causing quantification errors [20].

The obtained calibration curve for chloromethane following the procedure described in 2.4.3. showed good linearity as well (R² = 0.999) and was used to calculate the mass balance for QAS producing both benzyl chloride and chloromethane.

3.3.2. Recovery and precision

Results for the three DB recovery experiments were as follows: 102.2% (RSD: 0.70%, n = 6) for 60 μg/g DB, 101.3% (RSD: 2.5%, n = 6) for 80 μg/g DB and 99.1% (RSD 2.0%, n = 6) for 100 μg/g DB. These values were close to 100% and RSD values were acceptable, demonstrating that the method is suitable for the analysis of DB in cooling liquids. For comparison, the LC method (see 2.2.2) yielded recovery values between 98.0% and 100.0% and RSD values below 1.0%.

Recovery values for BZTCI (as sum of benzyl chloride and chloromethane) in aqueous samples amounted to 99.7% (RSD: 6.9%, n = 4) for 10 μg/g BZTCI, 100.5% (RSD: 4.3%, n = 4) for 15 μg/g BZTCI and 102.6% (RSD: 5.3%, n = 4) for 20 μg/g BZTCI. These results show that a mass balance of 100% can be obtained without needing a matching reference. The amounts of benzyl chloride and chloromethane were used to calculate the corresponding amount of BZTCI from which they both originated. These results imply that any QAS producing both benzyl chloride and chloromethane can be quantified using another QAS with the same decomposition products.

3.3.3. Sensitivity

The LOQ for DB was found to be 0.1 μg/vial. When a sample volume of 1.0 mL is brought into the HS vial, this corresponds to a concentration of 0.1 mg/L. For comparison, with the LC method described in 2.2.2, a concentration of 0.025 mg/mL yielded a S/N of 10.

3.4. Analysis of denatonium benzoate in commercial cooling liquid samples

Two cooling liquid samples consisting mainly of water and EG (with some glycerol and anti-corrosive agents added) were analysed with the method described in 2.3.1.5. HCl was added as catalyst in the DMP reaction, but also to ensure complete conversion of DB into its chloride form. After evaporation of the solvents,
another aliquot of 1.0 mL DMP was added to ensure the complete conversion of EG to 2,2-dimethyl-1,3-dioxolane [21]. Analysis of the samples resulted in very complex chromatograms (Fig. 4). The benzyl chloride peak is situated around 12.3 min on the tail of a much bigger peak which was identified using the NIST library as 2,2-dimethyl-1,3-dioxolane-4-methanol. This compound is the reaction product of DMP and glycerol. The latter is often added to cooling liquids. Further evaluation of the MS-data revealed that another compound co-eluted with benzyl chloride.

Based on the MS spectrum of the co-eluting compound with a molecular mass of 145 Da and its comparison with the NIST library, it was proposed to be 1-(2,2-dimethyl[1,3]dioxan-4-yl)ethanol.

As the most abundant characteristic m/z values of benzyl chloride (m/z 91 and m/z 126) are not present in the mass spectrum of this interfering compound, it was decided to use MS operated in SIM mode using m/z 91 and m/z 126 for the quantification of DB in cooling liquids. As both compounds have an alcohol function, another approach is to apply BSTFA and so circumvent the interference and to enable FID detection. BSTFA will react with the alcohol function to form a trimethylsilyl derivative, thereby changing the properties of the interfering molecules. In Fig. 5, a BSTFA treated sample is compared with an untreated sample. Following the procedure above, the peak interfering with benzyl chloride is completely removed and also 2,2-dimethyl-1,3-dioxolane-4-methanol is sufficiently removed so that a more reliable quantification of benzyl chloride can be performed.

The analysis results for cooling liquid sample 1 were: with GC–MS in SIM 91 mode 40.5 μg/g (RSD: 3.7%, n = 4), in SIM 126 mode 37.9 μg/g (RSD: 6.9%, n = 4), with GC-FID after BSTFA treatment 40.7 μg/g (RSD: 2.0%, n = 4) and with LC-UV 35.6 μg/g (RSD: 0.1%, n = 3). For cooling liquid sample 2, these values were 93.2 μg/g (RSD: 3.3%, n = 4), 85.9 μg/g (RSD: 7.0%, n = 4), 77.4 μg/g (RSD: 3.5%, n = 4) and 82.5 μg/g (RSD: 0.6%, n = 3) respectively. The run time of a chromatogram with the LC method was about 20 min while this was about 25 min for the GC method.
Fig. 3. Obtained peak areas of (a) benzyl chloride and (b) chloromethane from BZTCI and BEDIDE vs. different equilibration times.

Fig. 4. TIC chromatogram of a cooling liquid sample after analysis with the developed HS method. BCl = benzyl chloride, int. = interference.
In case of the BSTFA treatment, samples were spiked with DB as well. The recovery of the spiked amount of DB was found to be 99.6% (RSD = 3.7%, n = 4) and 100.1% (RSD = 3.1%, n = 4) for sample 1 and 2, respectively. Both samples were also analysed using an LC-method and the obtained results for both samples are comparable indicating that the GC-FID method is suitable for the quantification of DB in cooling liquids. In comparison to GC–MS, GC-FID is in general far more robust as the ion source used for MS is prone to suffer from signal drift [21]. This signal drift can only be corrected by using expensive isotopes of the analytes of interest.

3.5. Analysis of benzethonium chloride in a mouth spray

Analysis and quantification of the sample was performed by the procedure described in 2.4.5.2. The concentration of BZTCI in the mouth spray was found to be 438 μg/g (RSD = 3.7%, n = 6). This analysis result corresponds to 100.7% of the labelled value.

3.6. Analysis of benzoxonium chloride in a mouth spray

As with the commercial cooling liquids, the same interfering peak was also seen here with GC–MS. Therefore, 100 μL of BSTFA was added to the sample vial after solvent evaporation to silanise the interfering compound with the alcohol function. Samples were evaporated to dryness again. The concentration of BZOCl was found to be 2010 μg/g (RSD = 1.6%, n = 6), which is about the same as the label claim (0.2%) of the mouth spray. This is an indication that the method has an adequate recovery towards BZOCl.

4. Conclusions

From the obtained results it can be concluded that the use of HS-GC is an excellent tool to quantify several aromatic substituted QAS by calibration with the most abundant volatiles that are released after heating of a sample in a HS vial. In contrast to pyrolysis methods in which samples are introduced directly in a hot injection liner, this approach offers a clean way of sample introduction. After screening of several QAS, it was revealed that all benzyl substituted QAS released benzyl chloride as the major volatile product when chloride was the anion of the salt. Even when a particular QAS produces multiple volatiles, reliable quantification is still possible. It has been demonstrated that for QAS producing benzyl chloride and chloromethane, the mass balance is close to 100%. This means that it is possible to quantify all QAS that produce benzyl chloride and chloromethane without the need of having a matching reference standard. This is an important advantage towards LC or CE methodologies in which matching reference standards are necessary for quantification. In combination with an in-vial water scavenging procedure, a powerful GC method for the analysis of QAS in aqueous samples is established. The method was validated for the analysis of DB in cooling liquids during which adequate recovery values (99.1–102.2%) were obtained with RSD values not exceeding 2.5%. Obtained R² values were always equal to or larger than 0.999. Finally, the methodology was applied to commercial cooling liquid samples and the analysis of BZTCI and BZOCl in mouth sprays. For the cooling liquid samples and the BZOCl mouth spray, an interference that appeared to be an alcohol-like molecule, made accurate analysis impossible at first instance. Silanisation of the sample after DMP treatment removed this interference and enabled analysis of the samples. An advantage is also that all reactions can be performed in one HS vial.

References


