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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, O&N2, PB 923, 3000 Leuven, Belgium

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ABSTRACT

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*-dimethylaniline 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 benzyldimethyldecylammonium 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 (BZTCl) or benzoxonium chloride (BZOCl) and for denatonium benzoate (DB) in ethylene glycol (EG) based cooling liquids. Results showed that the approach provided excellent linearity ($R^2 \geq 0.999$) 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.

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 pretreatment 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

* Corresponding author.
E-mail address: Erwin.Adams@pharm.kuleuven.be (E. Adams).

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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 (sHS)-GC on the other hand allows clean sample introduction as only volatile sample constituents are introduced in the GC system. However, the use of sHS-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 benzethonium chloride (BZTCl) and benzoxonium chloride (BZOCl) in mouth sprays.

2. Experimental

2.1. Reagents

DMP (98+%), o-xylene (99%), *N,O*-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 (99%), sodium phosphate monobasic and trimethylphenyl 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 BZOCl 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 BZOCl content in the BZOCl 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 PTFE-Sil caps were purchased from Perkin Elmer. All separations were carried out on an AT-1 column (30 m × 0.53 mm, d_f = 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 C_{18} (100 Å, 5 μm, 150 mm × 3.9 mm I.D.) kept at 35 °C. The LC-system was monitored by Chromeleon 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 PTFE-Sil cap and analysed using the described HS-GC method to identify the major volatile decomposition (or pyrolysis) products.

BZOCl was not available as pure salt. Therefore, 10 μL of mouth spray containing BZOCl 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 PTFE-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