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Development and validation of an automated static headspace gas chromatography–mass spectrometry (SHS-GC–MS) method for monitoring the formation of ethyl methane sulfonate from ethanol and methane sulfonic acid

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ABSTRACT

An automated sample preparation and analysis procedure was developed to monitor the formation of ethyl methane sulfonate from reaction mixtures containing ethanol and methane sulfonic acid. The system is based on a liquid handling robot combined with a static headspace module. The formed ethyl methane sulfonate is analysed after derivatisation with pentafluorothiophenol using static headspace-gas chromatography–mass spectrometry (SHS-GC–MS).

Using the automated reaction–derivatisation–headspace GC–MS system, the formation of ethyl methane sulfonate can be monitored in different reaction mixtures under different reaction conditions, including temperature, water content and pH. Excellent linearity, repeatability and robustness were obtained, allowing the system to be used in kinetic studies.

1. Introduction

Sulfonic acids are widely used for salt formation during the synthesis and production of active pharmaceutical ingredients (APIs) [1]. In the presence of low molecular weight alcohols, such as methanol, ethanol or isopropanol, sulfonic acids can lead to the formation of corresponding sulfonates. These esters are considered as potential alkylating agents that may exert genotoxic effects in bacterial and mammalian cell systems [2], and therefore their potential presence as trace level impurities in active pharmaceutical ingredients (APIs) is a concern which needs to be appropriately managed and controlled as directed in recent regulatory guidances and communications [3,4]. In order to better understand the mechanisms and kinetics governing the formation of these sulfonate esters, a series of experimental studies has been initiated by a group of innovative multi-national pharmaceutical companies operating within the framework of the Product Quality Research Institute (PQRI). In a first stage, the formation of ethyl methane sulfonate (EMS) from methane sulfonic acid (MSA) and ethanol was studied. Therefore an analytical procedure was needed to monitor EMS in ethanol/MSA reaction mixtures and the developed method should allow the evaluation of different reaction conditions, including presence of water or bases, different pH, reaction temperature and reaction times.

For the determination of alkyl esters of sulfonic acids in APIs different methods have been developed and used, as described in a recent review by Elder et al [5]. Direct analysis of alkyl esters of methanesulfonates by gas chromatography (GC) was used by Ramijt et al. [6] and Li [7], respectively in combination with mass spectrometric (MS) and flame ionization (FID) detection. Although ppm (μg/g) sensitivity was obtained, direct injection of sulfonates

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in API matrix can lead to inlet contamination and/or solute degradation [7,8]. In addition, we also observed occasional formation of sulfonate esters in heated inlet systems (through sample pyrolysis and flash reaction with solvents). To avoid the introduction of non-volatile and reactive material in the GC inlet, extraction methods such as (micro-) liquid-liquid extraction, solid phase micro-extraction (SPME) and solid phase extraction (SPE) [9] were tested for selective extraction and enrichment of sulfonate esters. Extraction methods such as SPME are however restricted to aqueous API solutions (or aqueous reaction media).

As an alternative to gas chromatography, liquid chromatography (HPLC) methods have also been developed for the analysis of alkyl and aryl sulfonate esters [8]. Although thermal decomposition of the API is less likely to occur, reaction between alcohols (in the mobile phase or solvent) and trace levels of acids (present as impurities in the API or intermediate) could potentially lead to formation of sulfonate esters and consequently to false positive results. Moreover, the stability of sulfonate esters in aqueous solutions and mobile phases can be questioned.

To overcome problems with solute stability prior to and during analysis, esters of methane sulfonic acid were also determined by GC after derivatisation with sodium thiocyanate by Lee et al. [10]. The corresponding alkylthiocyanates and alkylisothiocyanates were analysed by static headspace (SHS) coupled to GC–MS. High sensitivity and acceptable repeatability were achieved. The major drawback of this method was the (slow) hydrolysis of alkyl mesylate esters in the aqueous reaction mixture. Recently, another derivatisation method was described by Alzaga et al. [11] allowing determination of methyl, ethyl and isopropyl esters of sulfonic acids in API's at sub-ppm level. The method was based on in-situ derivatisation using pentafluorothiophenol (PFTP), followed by static headspace and GC–MS analysis. This method could be applied to aqueous and non-aqueous (dimethyl sulphoxide) API solutions. For accurate quantification, corresponding internal standards were synthesized using deuterated alcohols. By derivatisation of the sulfonate esters with PFTP, the formation reaction is stopped and the static headspace sampling avoids contamination of the analytical system. Excellent sensitivity, linearity, repeatability and solute stability (of the derivatised solutes) were obtained, and therefore this method was used as a basis for the current study. However in contrast to the work of Alzaga et al. [11], this work did not focus on the determination of trace levels of EMS in API, but upon the formation of EMS from concentrated reaction mixtures. High precision and reproducibility over a wide linear dynamic range of the analytical method are thus required. The derivatisation-headspace GC–MS method was fully automated using a robotic system and applied to the analysis of methane sulfonic acid/ethanol reaction mixtures. The automated method and its validation in terms of linearity, repeatability and robustness are described in this paper. In addition, some examples of the monitoring of ethyl methane sulfonate formation in different reaction mixtures and different conditions are shown.

2. Experimental

2.1. Chemicals

Methane sulfonic acid (MSA), methane sulfonyl chloride (MSC), ethyl methane sulfonate (EMS), pentafluorothiophenol (PFTP), dimethyl sulfoxide (DMSO), 2,6-lutidine, di-isopropyl ethyl amine (Hunig's base) and ethanol (absolute, EtOH) were obtained from Sigma-Aldrich (Beerse, Belgium). Pentafluoroanisole (PFA), sodium sulfate (anhydrous) and sodium hydroxide were from Acros Organics (Thermo Fisher, Geel, Belgium) and d_6-ethanol (d_6-EtOH) was from Biosolve (Valkenswaard, NL)

2.2. Internal standard preparation

1 g methane sulfonyl chloride was mixed with 1 mL d_6-ethanol in a reaction tube, closed with a Teflon lined screw cap. The reaction mixture was heated for 72 h at 70 °C. After cooling, 2.5 mL water was added followed by 2.5 mL diethylether (CAUTION: volatile acidic vapours). The formed d_5-ethyl methane sulfonate (d_5-EMS) was extracted in the ether phase. This phase was separated, dried over sodium sulfate, concentrated under nitrogen and diluted in 10 mL acetonitrile (CAUTION: genotoxic material). The solution was stored at 4 °C. The exact concentration of the internal standard in this solution was checked by GC–MS using liquid injection and using EMS as external standard. The analytical conditions were similar to the conditions used for headspace analysis (see below). The use of methane sulfonyl chloride resulted in a much higher reaction yield and higher concentration of the deuterated internal standard than the previously described method using methane sulphonic acid [11].

2.3. Solutions

The following solutions were prepared:

- Reaction mixture: MSA was diluted at a typical concentration of 100 mg/mL (around 1.04 M) in ethanol. Bases or water can also be added to this reaction mixture. This reaction mixture is premixed and 1 mL aliquots are transferred to several 2 mL vials.
- Derivatization solution: mixture of pentafluorothiophenol (6.4 mg/mL) and sodium hydroxide (20 mg/mL) in water.
- Internal standard solution: mixture of 50 ng/μL pentafluoroanisole (IS 1) and 100 ng/μL d5-EMS (synthesized, IS 2) in acetonitrile.
- Dilution solvent in SHS vials: DMSO/H_2O (1:1).
- External standard solution for validation: EMS was diluted at different concentrations between 5 and 500 μg/mL in ethanol, acetonitrile or in reaction mixture (see above) for linearity and reproducibility tests.

2.4. GC–MS analysis

GC–MS analyses were performed on a Agilent 6890GC-5973MSD system (Agilent Technologies, Wilmington, DE, USA), equipped with a Gerstel dual rail MPS2 sampler (Gerstel GmbH, Mülheim, Germany). A schematic diagram of the sampler is shown in Fig. 1. The available vial trays were filled as follows:

- Tray C: 98 position temperature controlled tray for 2 mL reaction vials. The vials contain 1 mL reaction mixture (MSA in ethanol).
- Tray D: 32 position tray for 20 mL vials. The vials contain 2 mL DMSO/water (1:1 mixture).
- Tray E: 2 trays with each 5 mL and 10 mL vials containing IS solution, derivatisation reagent solution and wash solvents.

The typical sample preparation sequence is as follows:

- Transfer 20 μL reaction mixture from heated tray (Fig. 1, C) at time $t = x$ to 20 mL headspace vial (with 2 mL DMSO/water) in tray D.
- Add 20 μL IS solution (from E to D).
- Add 100 μL derivatisation solution (from E to D).
- Perform headspace analysis (using headspace syringe B and agitator/heater F).

Between the liquid sample handling steps, syringe washing is performed using the wash solvents in the E trays.