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Short communication

Trace analysis of residual methyl methanesulfonate, ethyl methanesulfonate and isopropyl methanesulfonate in pharmaceuticals by capillary gas chromatography with flame ionization detection

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Abstract

A capillary gas chromatographic method using flame ionization detection was developed and validated for the trace analysis (ppm level) of methyl methanesulfonate, ethyl methanesulfonate, and isopropyl methanesulfonate in pharmaceutical drug substance. The method utilizes a megabore capillary column with bonded and crosslinked polyethylene glycol stationary phase. A dissolve-and-injection approach was adopted for sample introduction in a splitless mode. The investigated sample solvents include acetonitrile, ethyl acetate, methylene chloride, 1,2-dichloromethane, and toluene. Aqueous mixtures of acetonitrile and water can also be used as sample solvent. A limit of detection of about 1 μg/g (1 ppm) and limit of quantitation of 5 μg/g (5 ppm) were achieved for the mesylate esters in drug substance samples. The method optimization and validation are also discussed in this paper.

Keywords: Pharmaceutical analysis; Alkyl methanesulfonates; Methanesulfonates

1. Introduction

Recently, the potential health hazards of trace amounts of mesylate esters, including methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), and isopropyl methanesulfonate (IPMS), in pharmaceuticals have attracted the attention of regulatory authorities. These mesylate esters are known to be potent mutagenic, carcinogenic and teratogenic compounds [1–4]. Their presence in the pharmaceutical products may be the result of leftover starting materials, or formed as by-products between methanesulfonic acid (often used as a counterion) and alcohols (often used as solvents in manufacturing process) [5]. Although official guidelines have not been established, the concentration of these compounds are expected to be controlled at a level less than or equal to 1 μg/g [6–8]. Therefore, it is of great importance to develop analytical methods that are sensitive enough and meet all the regulatory requirements.

The pure mesylate esters are liquids at ambient temperature with a boiling point around 200 °C. Therefore, it is feasible to separate and quantify these compounds by gas chromatography. Ramjit et al. [5] reported a method using capillary gas chromatography in combination with mass spectrometry (MS) for the determination of MMS and EMS in pharmaceuticals. 0.5 μg of MMS or 1.3 μg of EMS per gram of active pharmaceutical ingredient (API) was quantified using MS operated in the single-ion-monitoring mode. A different approach was adopted by other researchers using headspace GC after the mesylate esters were converted into thiocyanate esters through derivatization. MS detection also was used for the headspace analysis [9]. The analysis of the mesylate esters using HPLC is not straightforward because of the specific chemical and physical properties of these compounds.

This short communication describes a simple and sensitive method for the determination of MMS, EMS, and IPMS

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in pharmaceuticals using capillary GC with flame ionization detection (FID). The limit of detection and limit of quantitation were determined to be about 1 μg and 5 μg per gram of API, respectively. The method utilizes a dissolve-and-inject approach for sample preparation and introduction. The samples were injected in the splitless mode and quantitation was achieved using a single point external standard calibration. Factors affecting method development and validation will be discussed.

2. Experimental

2.1. Instrumentation

An Agilent 6890 GC (Agilent, Palo Alto, CA, USA) equipped with an auto sampler was used in the experiment. Data acquisition and processing were conducted using the Waters Empower software.

2.2. Chemicals

MMS, EMS, toluene, and 1,2-dichloroethane were purchased from Aldrich Chemical (Milwaukee, WI, USA). IPMS was purchased from Acros Organics (Acros Organics, Geel, Belgium). HPLC grade acetonitrile, ethyl acetate, and dichloromethylene were purchased from EM Science (an affiliate of Merck KGaA, Darmstdt, Germany). HPLC grade equivalent water was obtained from an in-house Millipore Milli-Q-Gradient ultrapure water system (Millipore, Billerica, MA, USA). This study also involves two proprietary Johnson & Johnson Pharmaceutical Research & Development compounds.

2.3. Preparation of solutions

The stock solutions of mesylate esters were prepared by dissolving 2.8 mg/mL each of the compounds in sample solvent, which include acetonitrile, 80% acetonitrile in water, ethyl acetate, dichloromethylene, 1,2-dichloroethane, and toluene. The diluted stock solution was prepared by pipetting 5.0 mL of the stock solution into a 100-mL volumetric flask and diluting to volume with sample solvent. The working standard solution (4 μg/mL each) was prepared by further diluting 7.0 mL of the diluted stock solution into 250 mL. The 0.04 μg/mL solution of the mesylate esters was prepared by diluting the working standard solution 100-fold. The sample solution was prepared by accurately weighing about 40 mg of the drug substance into a 2-mL GC vial and adding 1.0 mL of sample solvent.

2.4. Operating conditions

The GC separation was conducted on an Agilent DB-WAX column with a dimension of 30 m × 0.53 mm and a film thickness of 1 μm. Helium was used as carrier gas at a constant flow of 5 mL/min. The GC oven temperature program utilized an initial temperature of 80 °C (unless otherwise specified) and an initial holding time of 1 min, then increased at 16 °C/min to 200 °C. The final temperature was held for 1 min.

A flame ionization detection (FID) system was used. The H_2, air, makeup flows were kept at 40, 350 and 20 mL/min, respectively. The detector temperature was set at 220 °C.

The samples were injected with the Agilent 6890 series auto sampler. The inlet temperature was kept at 120 °C. A straight glass injection liner with glass wool was obtained from Restek, (Restek, Bellefont, PA, USA). The samples were injected in a splitless mode with a 5-μL injection volume unless otherwise specified.

3. Results and discussion

3.1. Method development and optimization

GC analysis of mesylate esters on the traditional polyethylene glycol stationary phase was previously reported [5]. The challenge was to achieve the desired detection and quantitation limit using the most commonly available instrument, i.e. a gas chromatograph with a FID system. To obtain the desired sensitivity, one approach is to increase sample amount injected into the GC system. The adoption of a megabore capillary GC column (0.53 mm I.D.) with a high capacity bonded stationary phase seems to be the obvious choice. Relatively high flow rate of carrier gas (5 mL/min) and suitable initial column temperature in combination with a moderate inlet temperature (120 °C) may allow a relatively large injection volume without significant deterioration in column efficiency.

The effect of injection volume on separation and quantitation of the mesylate esters was investigated by injecting between 1 and 20 μL of the working standard solution containing 4 μg/mL each of the esters. The results show that the peak widths (about 0.028 min) of the mesylate esters are independent of injection volume within the tested range. Further studies were not done to determine the maximum injection volume that the chromatographic system could handle because interfering peaks from the sample solvent started being detected when the injection volume was greater than 10 μL in our experiments. An injection volume of 5 μL was chosen for this method.

The effect of initial column temperature on the separation of the mesylate esters was investigated. Fig. 1 shows the chromatograms of the working standard prepared in ethyl acetate. The initial column temperature was varied from 40 to 100 °C. An aliquot of 5 μL of the sample was injected in the splitless mode. The results show that the peak shape and peak width were not affected by the initial column temperature. An initial column temperature of 80 °C was chosen, which allowed baseline separation of the three mesylate esters from each other and from interfering peaks in the sample solvent.

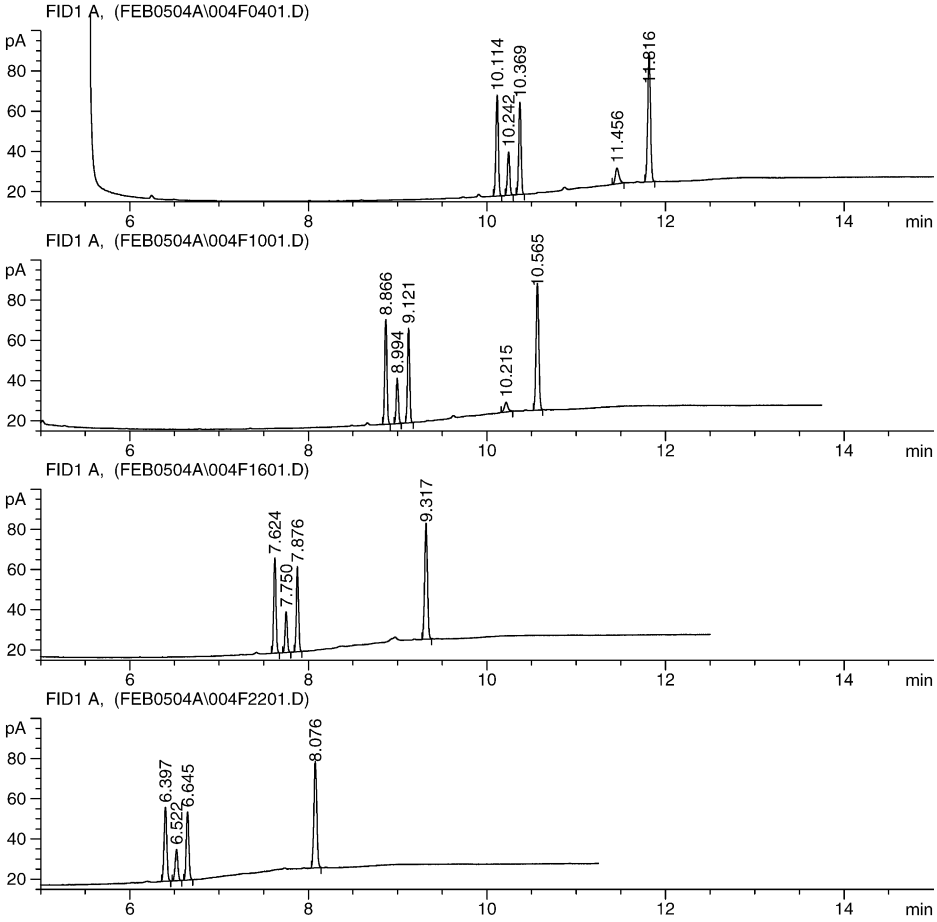

Fig. 1. Chromatograms showing the effect of initial column temperature on separation of IPMS, MMS, and EMS (4 μg/mL each). Initial temperature (from top to bottom): 40, 60, 80, and 100 °C. The first three peaks are IPMS, MMS, and EMS in the order of retention times.

This method utilizes a dissolve-and-inject approach for the residual mesylate esters analysis. Several factors were considered in selection of a sample solvent, including the purity, its ability to dissolve the analyte, and its chemical compatibility with the compounds of interest. To detect the mesylate esters at 1 μg/g level, the purity of sample solvent is critical. It has been observed in our laboratory that the HPLC grade solvents are generally suitable. Relatively volatile solvents such as acetonitrile, ethyl acetate, methylene chloride, 1,2-dichloroethane, and toluene were possible candidates. Fig. 2 shows the chromatograms of a few common sample solvents that can be used for the trace analysis of the mesylate esters. In each case 5 μL of the solvent was injected. The tested sample concentration of drug substances was in the range of 40–100 mg/mL. Because the mesylate esters have relatively high boiling point, solvents often used in residual solvent analysis, including 1,3-dimethyl-2-imidazolidinone (DMI) and *N*,*N*′-dimethylformamide (DMF) are less suitable. The use of aqueous mixtures as sample solvents is acceptable. For example, a mixture of acetonitrile–water (80:20, v/v) was successfully used for one of the in-house compounds for the residue analysis. The mesylate esters showed reasonable stability in the aqueous solution. This is important because many pharmaceuticals are in salt forms, which sometimes show limited solubility in pure organic solvents.

3.2. Method validation

The validation work was conducted according to the ICH (International Conference on Harmonization) guidelines [10–13]. The validated method parameters include specificity, accuracy, precision, sensitivity, linearity, and solution stability.

The detection limit (LOD) of the method for the mesylate esters was estimated from a chromatogram of a solution containing about 0.04 μg/mL each of the esters (Fig. 3). From the chromatogram, a signal-to-noise ratio of 16, 25 and 29, was obtained for MMS, EMS, and IPMS, respectively. A second instrument (same instrument manufacturer) was used to repeat the experiment and similar results were obtained. A drug substance sample at 40 mg/mL was spiked with 0.04 μg/mL each of the mesylate esters and the corresponding chromatogram is shown in Fig. 4. All three peaks have a signal-to-noise of > 3, indicating that this method is capable of detecting 0.04 μg/mL level of the esters in the drug substance, which is equivalent to about 1 μg each of the

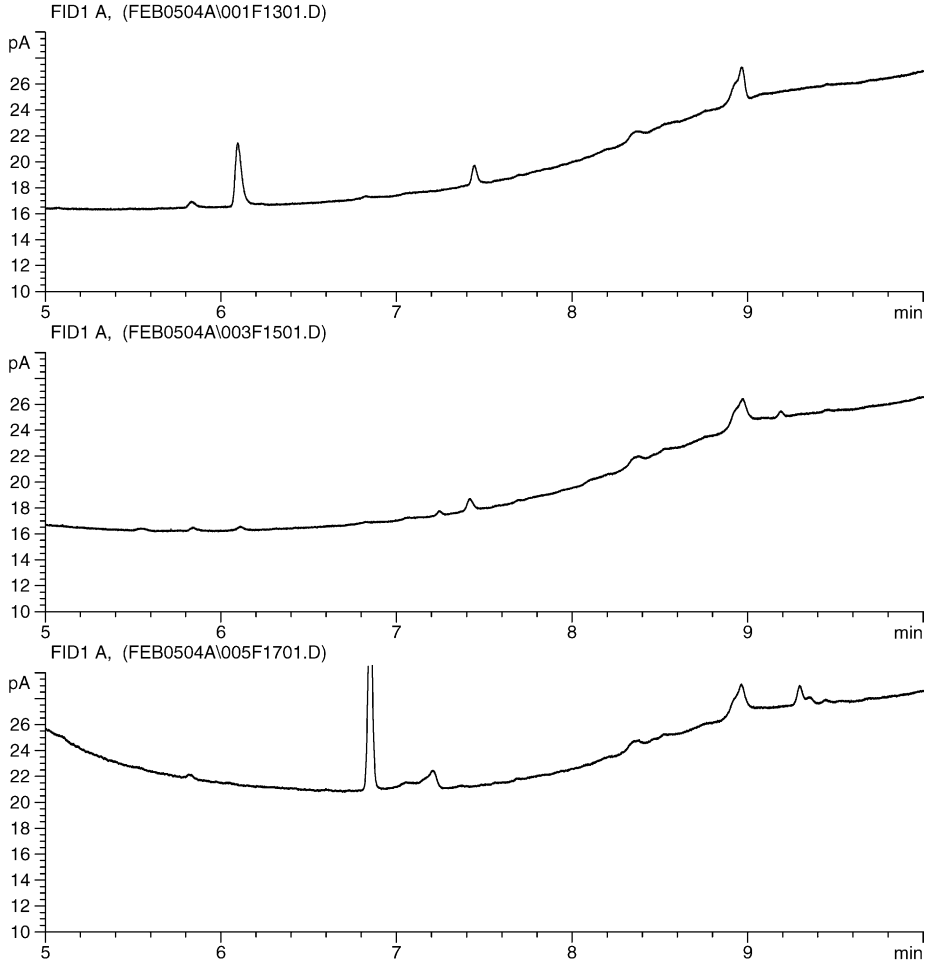

Fig. 2. Chromatograms of sample solvents. From top to bottom: ethyl acetate, methylene chloride, and toluene (initial column temperature 80 °C).

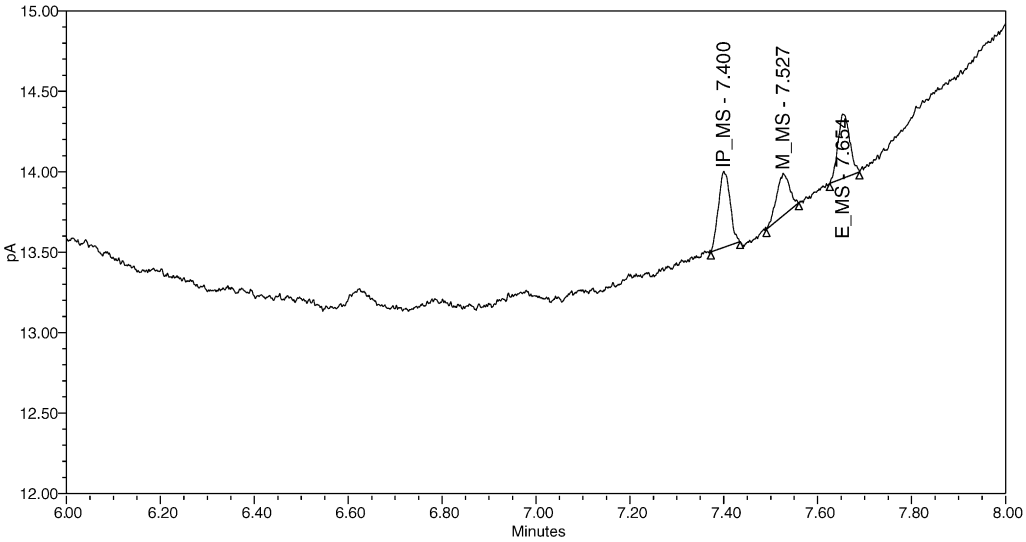

Fig. 3. Chromatogram of a standard solution containing 0.04 μg/mL each of IPMS, MMS and EMS.

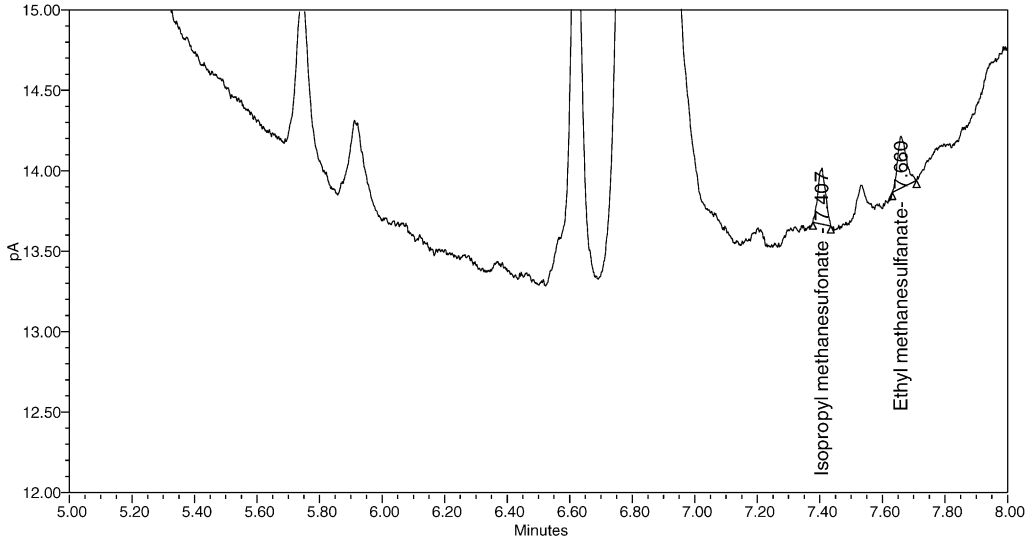

Fig. 4. Chromatogram of a drug substance sample spiked with 0.04 μg/mL each of IPMS, MMS and EMS.

mesylate esters per gram of API (1 ppm). In the pharmaceutical industry, the quantitation limit (LOQ) was defined as the lowest amount of anlalyte in a sample that can be quantitatively determined with suitable precision and accuracy. The LOQ was determined to be less than or equal to 5 μg/g (5 ppm) for MMS, EMS or IPMS, based on the precision and accuracy data discussed below.

Linearity of the method was determined by preparing and analyzing a series of 5 standard solutions to cover the concentration range of 0.04–4 μg/mL. Regression analysis of the peak area versus concentration data yield an $R^2 > 0.99999$ for each of the three calibration curves.

The experimental results also show that this method has excellent precision without using an internal standard. Multiple injections were made for the standard solutions containing 0.2 and 4 μg/mL each of the esters. For six injections of the 4-μg/mL solution, the R.S.D. of the peak area was in the range of 0.44–0.85%. For six injections of the 0.2-μg/mL solution, the R.S.D. was in the range of 1.7–3.8%.

Accuracy of the method was determined by analyzing drug substance samples spiked with known amount of the mesylate esters. The spiked levels were at 0.2, 0.4 and 1.6 μg/mL. The recovery was in the range of 78–123%, 82–105%, and 80–104%, respectively. Because this method uses the dissolve-and-inject approach, for every sample injection, about 200 μg of the drug substance is introduced in the injection port. The accumulation of drug substance may have negative effect on the recovery. Therefore the injection liner should be replaced after every sequence of 15–20 injections.

4. Conclusion

A simple and sensitive GC method has been developed and validated for the trace analysis of mesylate esters in pharmaceuticals. The validation has been conducted according to ICH guidelines. Compared with the previously reported methodologies, this method utilizes a FID detector, which is readily available in most of the quality control testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect 1 μg/g and quantify 5 μg/g level of the mesylate esters in pharmaceutical products. However, more sensitive methods such as GC-MS may have to be used for quantitation of these compounds at levels <5 μg/g.

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