Research Article

GC and GC-MS Detection of Alkyl Mesylates in Active Pharmaceutical Ingredients

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ABSTRACT

Methyl, ethyl, isopropyl, and hexyl mesylates were determined at trace levels in three active pharmaceutical ingredients [APIs: dolasetronmesylate (DOL), benztropinemesylate (BM), and dabigatranetexilatemesylate (DEM)] by a capillary gas chromatography method using flame ionization detection. A DB-WAX column (30 m × 0.53 mm × 1.0 µm) was used for method development and validation. Sample solutions were prepared in a non-polar solvent (*n*-hexane). The detection and quantitation limits obtained for the alkyl mesylates were 0.02 and 0.05 ppm, respectively. The method was validated following the procedures provided by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The linearity of the method was demonstrated, and it afforded correlation coefficients exceeding 0.99. The recoveries for DOL, BM, and DEM were in the ranges 100.8–107.1%, 97.1–100.3%, and 98.1–98.7%, respectively. Hence, this is a robust method for the determination of alkyl mesylates in these APIs. Also, this method has been verified by gas chromatograph equipped with mass spectrometry (GC-MS) for the detection of alkylmesylates.

Keywords: Alkyl mesylate; gas chromatography; flame ionization detector; method development; detection selectivity.

INTRODUCTION

Mesylates of short-chain alcohols (*n* = 1–6) are potent carcinogens. Recently, increased attention has been paid to the health risksassociated with even minute levels of mesylate esters such asmethyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), isopropyl methanesulfonate (IMS) and hexyl methane sulfonate (HMS)in drugs because of their potent mutagenic, carcinogenic, and teratogenic effects[1-13]. These mesylate esters can be derived from excess starting materials during pharmaceutical drug synthesis or are produced as by-products from the reaction between methane sulfonic acid (frequently used as a counter ion) and alcohols/acid chlorides (commonly used as reaction media in developed and industrialized processes). The detection of these compounds must be controlled below 1ppm as per the threshold of toxicological concern; note that the concentration was measured in parts per million[2-6]. Hence, it is imperative to develop suitable analytical methods that can meet the guideline requirements ofthe Food and Drug Administration (FDA) and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Mesylate esters cannot be analyzed directly by HPLC because they structurally lack chromophores. Since the target mesylate esters are fluids at room temperature with boiling points of 200°C or less, they can be separated and quantified by gas chromatography using the on-column injection technique. Ramjit*et al.*[7] reported a strategy that coupled capillary gas chromatography and mass spectrometry (MS) for the content analysis of MMS and EMS in drugs. Other studies adopted alternate methodologies like derivatizingmesylate esters into thiocyanate esters by means of headspace GC analysis and/orusing MS foridentification[5, 9-13]. These procedures were validated per ICH guidelines (ICHHT Guidelines, 2005). However, to the best of our knowledge no method has been reported that has a low detection limit and allows for the simultaneous quantification of MMS, EMS, IMS, and HMS in dolasetronmesylate (DOL), benztropinemesylate (BM) and dabigatran etexilate mesylate (DEM). We selected these drugs because they form salts in the final step, which involves the reaction between the alkyl sulfonic acid and short-chain alcohols (*n* = 1–6). Moreover, the presence of alkyl mesylate contaminants in these drugs has not been reported.

In this research work, we report a straightforward and sensitive strategy for the determination of MMS, EMS, IMS, and HMS in drugs based on capillary GC with flame ionization detection (FID). The method involves extraction with a non-polar solvent and splitless on-column injection for sample preparation and sample introduction, respectively. Quantification was accomplished via external standard calibration. The limits of detection and quantification (LOD and LOQ, respectively) of our method are 0.02 and 0.05 ppm with respect to 1000 mg/mLactive pharmaceutical ingredient (API), respectively. The study additionally incorporates method development and complete method validation. Also, verified by GC-MS for the detection of alkyl mesylates.

MATERIAL AND METHODS

Chemicals

Standards for MMS and EMS were procured from Sigma Aldrich, USA.IMS and HMS were obtained from TCI

Chemicals, Japan. HPLC-grade *n*-hexane was purchased from Spectrochem, India. Samples of DOL, BM, and DEM were procured from Techno Chemicals Ltd., India. The structures of MMS, EMS, IMS and HMS are shown in Figure 1.

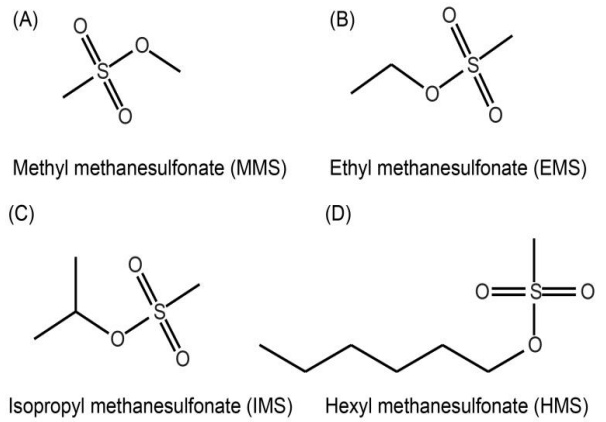

Figure 1: Structures of (A) methyl methanesulfonate (MMS), (B) ethyl methanesulfonate (EMS), (C) isopropyl methanesulfonate (IMS) and (D) hexyl methanesulfonate (HMS).

Equipment

Method development and method validation were carried out using a GC 7890N/MS 5973C system equipped with Auto Sampler 7683B (Agilent Technologies, Singapore). The output signals were acquired and integrated using Empower software version 3, on an Intel i3 workstation. The column used in this study was obtained from LCGC, India. Centrifugation and extraction of the alkyl sulfonates was performed using an instrument obtained from Eltek Centrifuge (Mumbai, India).

Preparation of solutions

Stock standard solutions were prepared by accurately weighing and transferring MMS, EMS, IMS, and HMS standards (~25 mg each) into a 100-mL volumetric flask, then diluting up to 100 mL with the sample solvent (*n*-hexane). Next, the stock standard solution (1 mL)was transferred by means of a pipette intoa 200-mL volumetric flask and filled up to the mark with *n*-hexane (200 mL). Each sample solution was prepared individually by accurately weighing and transferring each sample (i.e., DOL, BM, and DEM, ~1000 mg each) into a 20-mL centrifuge tube, and then adding *n*-hexane (2 mL). The sample solution was centrifuged at 5°C for 10 min at 5000 rpm to extract the alkyl sulfonates into the sample solvent. The supernatant was used as the sample solution for quantitative analysis of MMS, EMS, IMS, and HMS.

Chromatographic conditions

GC analysis was performed using aDB-WAX column in which the capillary (0.53 mm × 30 m, 1µm) was bonded with 100% polyethylene glycol. Nitrogen was used as the mobile phase gas for GC at a flow rate of 1.5 mL/min. For the FID, hydrogen was used as the fuel gas at a flow rate of 30 mL/min; zero air was used as the oxidizer gas ata flow rate of 300 mL/min; and nitrogen was used as the make-up gas for the remaining volume at a flow rate of 25 mL/min. Helium was used as the mobile phase gas at a flow rate of 1.5 mL/min for GC-MS. The oven temperature was first maintained at 80°C for 2 min, and the temperature was then increased to 200°C at a rate of 10°C/min and held at this level for 16 min. The injector and detector (FID) were maintained at 220 °C and 280 °C, respectively. Sample injection was carried out using an Agilent 7683B Series auto sampler. An inlet port liner with glass wool was obtained from Agilent. All injected solutions were chromatographed through the autosampler with the help of a split less liner, using an injection volume of 4µL.

RESULTS AND DISCUSSION

Method development and optimization

Achieving the desired LODs and LOQs using a commonly accessible instrument, i.e., a gas chromatograph with anFID, is the main challenge for the GC study of mesylate esters on a traditional PEG stationary phase. One way to obtain the desired sensitivity is to increase the sample weight injected into the GC system. Thus, increasing the sample load by using a wide-bore capillary column (internal diameter = 0.53 mm) with a high loading of bonded phase would be a good option. Utilizing a suitable initial column oven temperature in combination with a reasonable inlet temperature (200°C) may accommodate the huge increase in the injection volume without any significant deterioration of the plate count of the column.

The effects of concentration on the separation and quantification of MMS, EMS, IMS, and HMS were investigated by injecting 4 µL of the standard solutions and sample solutions. Poor precision was observed when smaller injection volumes were used, probably because of the formation of air bubbles or gaps during the injection. For example, when a 2µL injection volume was used to obtain the precision acceptance criterion (where %RSD (relative standard deviation) should be not more than 15.0%), unsatisfactory peak areas were obtained. Higher injection volumes affected the peak shape. Satisfactory peak areas with precise and accurate values were obtained with an injection volume of 4 µL. The initial column temperature was set at 80°C based on prior literaturereports[7, 13]; this temperature allowed baseline separation of the MMS, EMS, IMS, and HMS esters from interference peaks due to *n*-hexane (sample solvent).

Our technique for MMS, EMS, IMS, and HMS ester analyses utilized an extraction and injection approach. Several factors were considered when selecting the sample solvent, including purity, extraction ability and chemical compatibility with the compounds of interest. The purity of the sample solvent plays a critical role in the detection of low concentrations (ppm) of mesylate esters. We found that HPLC-grade solvents generally allow for interference - free analysis. 1,3-Dimethyl-2-imidazol

idinone (DMI), dimethyl sulfoxide (DMSO), and *N, N*-dimethylformamide (DMF), which are used in residual solvent analysis, have high boiling points, andare therefore incompatible with the high-boiling mesylate esters. Hence, it was preferable touse anon-polar solvent such as *n*-hexane. Earlier effort compares theresults obtained in this study with those of several previous studies[7, 13]. The data reveal that the present techniqueis extremely responsive for the determination of MMS, EMS, IMS, and HMS in the APIs. The limitation of the method is that only a 10µLGC syringe, rather than a 5µL syringe, would be suitable for an injection volume of 4µL. The sample solution was centrifuged at 5°C to avoid the loss of sample solvent (*n*-hexane), which began to evaporate when the centrifugation procedure was carried out at ambient temperature.

Method validation

Validation of our method was conducted as perICH guidelines[4]. The validation parameters were specificity, accuracy, precision, sensitivity, linearity, robustness, ruggedness and solution stability.

Limit of quantification

In the pharmaceutical field, the LOQ is defined as the lowest amount of the compound of interest that can be quantitatively estimated with appropriate precision and accuracy. The LOQ was estimated to be less than or equal to 0.05 ppm for MMS, EMS, IMS, and HMS based on the accuracy and precision data. The retention times of each peak in the GC and GC-MS chromatograms, system suitability and precision are given in Table1. The obtained data show that the method is highly sensitive for the determination of MMS, EMS, IMS and HMS in the drugs DOL, BM, and DEM.

Linearity and precision

The linearity of the technique was determined by injecting a sequence of eight standard solutions to cover the concentration range from the 0.02 to 5 ppm for MMS, EMS, IMS, and HMS. The precision results for the LOQ are given in Table 1. Each correlation coefficient was greater than 0.99, as determined from a plot of peak area against concentration in Table 1. The experimental results also indicate the excellent precision of this method, even without the use of an internal standard. Repeat injections were performed for the MMS, EMS, IMS and HMS standard solutions. For six injections of the standard solution, the RSD was less than 2.5%. Overall, these data show that the method is linear and precise, as well as suitable for the analysis of MMS, EMS, IMS and HMS in the drugs DOL, BM, and DEM.

Accuracy and solution stability

The accuracy of the method was determined by analyzing drug samples to which known concentrations of MMS, EMS, IMS, and HMS had been added. The concentrations tested were 0.05, 2.5 and 5 ppm. The recoveries were in the range between 90 – 105%. The accumulation of the drug in the injection liner, which can negatively affect the recovery, is avoided because this method is based on an extraction and injection approach. Therefore, it is necessary to replace the injection liner after every sequence of injections. The method was also validated in terms of solution stability at room temperature for 24 h. A standard solution containing MMS, EMS, IMS, and HMS at concentrations of 2.5 ppm was injected at regular intervals over the course of 7 days at ambient temperature. Recovery was in the range of 95–105%, confirming the stability of the solution. The GC and GC-MS chromatograms for MMS, EMS, IMS and HMS LOQ solutions are shown in Figure 2–7. The achieved accuracy and solution stability data show that the method is accurate and the solution is stable for the analysis of MMS, EMS, IMS, and HMS in the drugs DOL, BM, and DEM.

Table 1 - System suitability, precision and linearity

%RSD of area response for 2.5 ppm				
Injection	MMS	EMS	IMS	HMS
%RSD	2.4	1.0	1.2	0.8
Criterion	Not more than 15%			
Retention time of peaks				
Equipment	MMS	EMS	IMS	HMS
GC (FID)	14.1 min	14.4 min	13.7 min	22.7 min
GC-MS (TIC)	10.6 min	10.9 min	10.2 min	18.4 min
%RSD of retention time				
Injection	MMS	EMS	IMS	HMS
% RSD	0.01	0.01	0.01	0.01
Criterion	Not more than 0.5%			
Tailing factor				
Injection	MMS	EMS	IMS	HMS
1	1.0	1.1	0.9	1.0
Criterion	Not more than 2.0			
Resolution				
Injection	MMS	EMS	IMS	HMS
1	2.5	3.5	NA	22.5
Criterion	Not less than 1.5			
%RSD of area response for LOQ				
Injection	MMS	EMS	IMS	HMS
%RSD	4.1	5.4	4.6	1.3
Criterion	Not more than 15.0%			
Summary of Linearity data				
Standard (ppm)	MMS peak area	EMS peak area	IMS peak area	HMS peak area
0.02	300	970	680	750
0.50	640	2013	1382	1506

1.00	1156	3772	3073	3052
2.00	2475	7268	5432	5552
2.50	3022	8751	6945	7256
3.00	3514	11002	8389	9523
4.00	4908	15105	11594	12568
5.00	6131	18161	13829	15265
Slope	1192.3	3559.5	2730.0	3026.2
Intercept	82.52	362.49	266.23	117.44
Correlation (r^2)	0.9985	0.9983	0.9985	0.9970

EMS, ethyl methane sulfonate; HMS, hexyl methane sulfonate; IMS, isopropyl methanesulfonate; MMS, methyl methanesulfonate

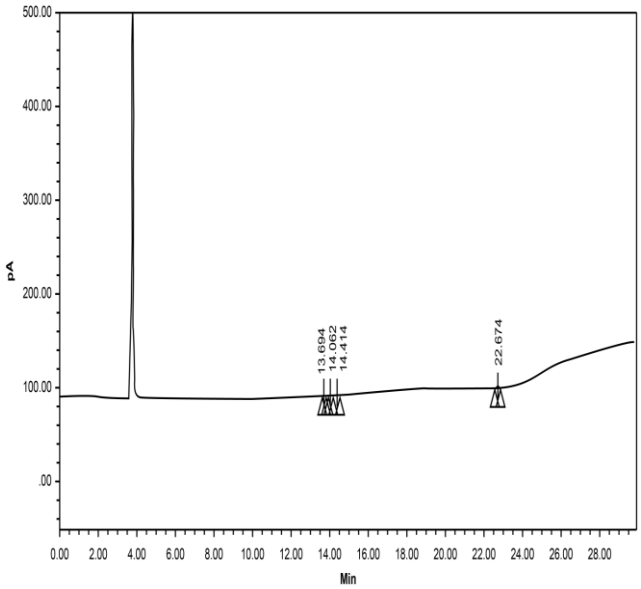

Figure 2: Gas chromatogram of methyl methane sulfonate (MMS), ethyl methane sulfonate (EMS), isopropyl methane sulfonate (IMS) and hexyl methanesulfonate (HMS) in a LOQ solution.

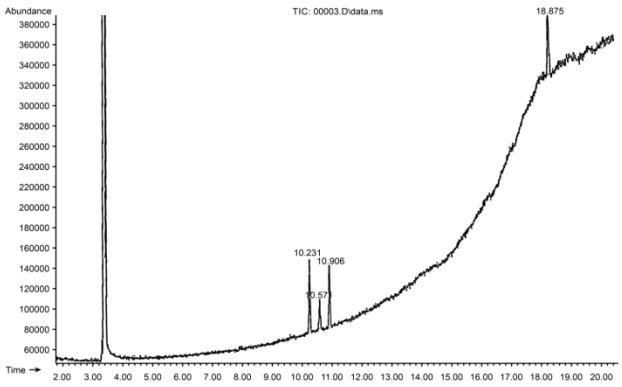

Figure 3: Total ion chromatogram of methyl methane sulfonate (MMS), ethyl methane sulfonate (EMS), isopropyl methane sulfonate (IMS) and hexyl methane sulfonate (HMS) in a LOQ solution.

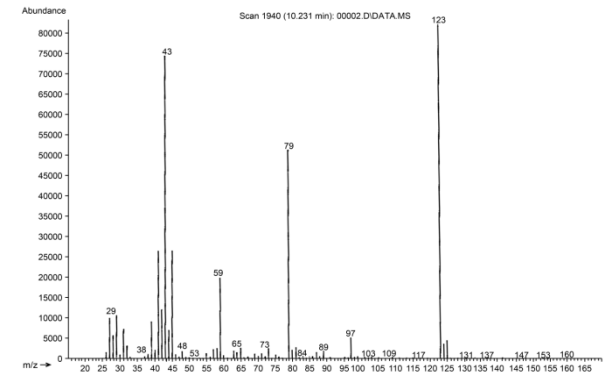

Figure 4: Mass spectrum of isopropyl methane sulfonate (IMS) in a LOQ solution.

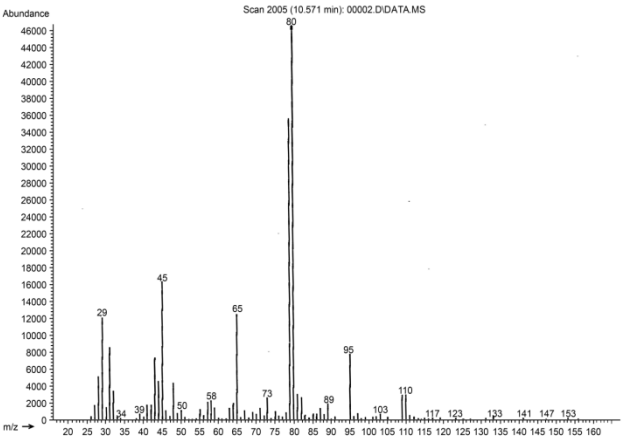

Figure 5: Mass spectrum of methyl methanesulfonate (MMS) in aLOQ solution.

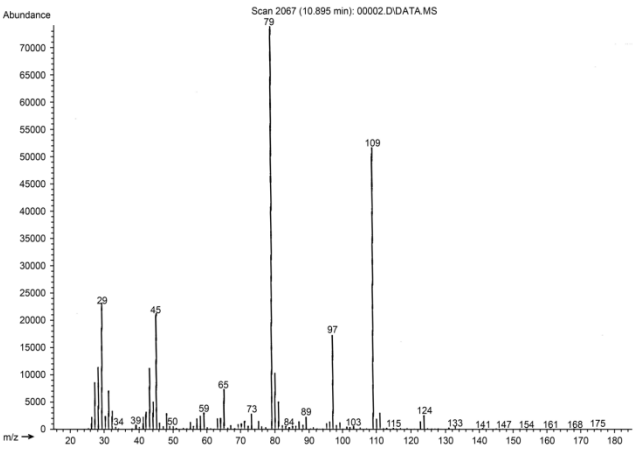

Figure 6: Mass spectrum of ethyl methane sulfonate (EMS) in a LOQ solution.

CONCLUSION

A highly responsive GC / GC-MS method for the trace analysis of MMS, EMS, IMS, and HMS in pharmaceutical drugs has been developed and validated according toICH guidelines. The FID used in this method is readily available in most testing laboratories in the pharmaceutical industry and is relatively simple to use. The LOD and LOQ of our method are 0.02 and 0.05ppm, respectively, for MMS, EMS, IMS and HMS in the drugs DOL, BEM, and DEM. The results show that this method can be used for

the routine analysis of the aforementioned impurities in APIs.

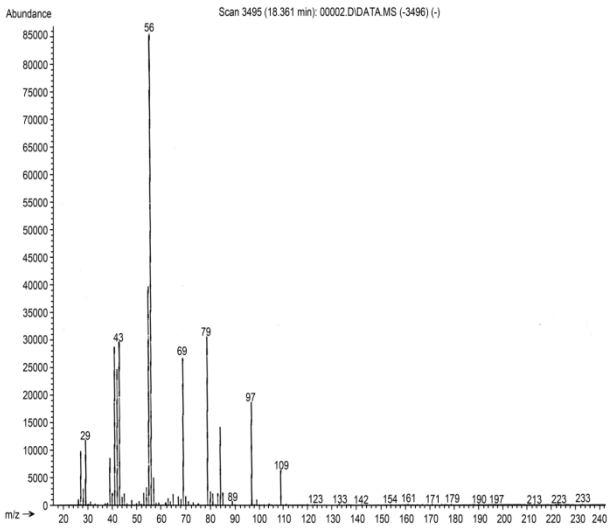

Figure 7: Mass spectrum of hexyl methanesulfonate (HMS) in a LOQ solution.

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