# GC Quantification of Residual hexylmethane sulfonate in Dabigatran etexilate mesylate

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***Abstract:*** *A simple and a quantitative Gas chromatography (GC) method with oncolumn injection technique have been developed for the quantification of trace level hexyl methane sulfonate in Dabigatran etexilate mesylate. The chromatographic separation is achieved on DB-Wax, (30 m x 0.53 mm x 1.0 μm) capillary column and the instrument employed for the method development and validation was Agilent6890N GC equipped with flame ionization detector (FID). GC run time was 25 min employing programmed temperature with splitless mode oncolumn injection. In the developed method the limit of detection (LOD) of Hexyl methane sulfonate is 0.25 ppm and the limit of quantification (LOQ) of Hexyl methane sulfonate is 0.75 ppm. The method is validated for specificity, linearity, accuracy and precision. The developed method can be very well employed and can be used in the quality control laboratories to monitor and control the trace level of hexyl methane sulfonate in Active pharmaceutical ingredients (API)*

***Keywords:*** *Alkyl methyl sulfonates, Dabigatran etexilate mesylate, Gas chromatography (GC), Pharmaceutical analysis, Validation and quantification.*

## I. Introduction

Recently, the potential health hazards of trace amounts of mesylate esters, like hexyl methane sulfonate (HMS) in pharmaceuticals have attracted the attention of regulatory authorities. This mesylate ester is known to be potent mutagenic, carcinogenic and Teratogenic compounds [1-4]. Their presence in the pharmaceutical products may be result of left over starting material or formed as by products between methanesulfonic acid (often used as a counterion) and alcohols often used as solvents in manufacturing process. Although official guidelines have not been established, the concentration of these compounds are expected to be controlled at a level less than or equal $1 \mu gg^{-1}$[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 on column injection technique. Ramjit et al.[5] reported a method using capillary gas chromatography in combination with mass spectrometry (MS) for determination of methyl methane sulfonate and ethyl methane sulfonate in pharmaceuticals. A different approach was adopted by other researchers[6-8] using headspace GC after the mesylate esters was 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. However, to our knowledge, no method has been reported with lower detection and quantification of hexyl methane sulfonate by using capillary GC with flame ionization technique (FID) in Dabigatran etexilate mesylate (API).

This short communication describes a simple and sensitive method for determination of HMS, in pharmaceuticals using capillary GC with flame ionization technique (FID). The limit of detection was determined to be 0.25 ppm and limit of quantification was determined to be 0.75 ppm, with respect to 250 mg $mL^{-1}$ of API, respectively. The method utilizes an extraction with non-polar solvent and injects approach for sample preparation and introduction. The samples were injected in the splitless mode and quantification was achieved using a single point external standard calibration. The current research work deals with the determination of hexyl methane sulfonate in the drug substance. The work also includes the complete validation and method development.

## II. Experimental Data

### 2.1. Chemicals

Hexyl methane sulfonate (HMS) was purchased from Aldrich chemicals; LC grade n-Hexane was purchased from Rankem. Samples of Dabigatran etexilate mesylate (API) are received from Beijing Mesochem Technology Company Ltd., China. Structure of Hexyl methane sulfonate and Dabigatran etexilate mesylate are shown in Fig.1 and Fig.2.
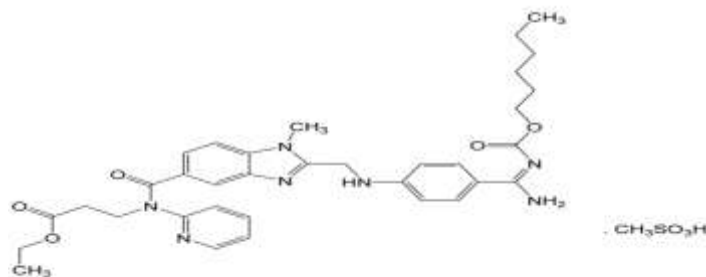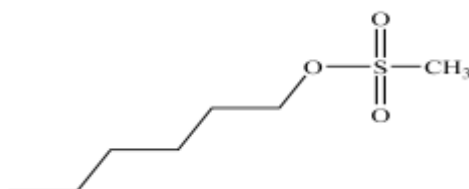
Fig.1 Dabigatran etexilate mesylate (API)



Fig. 2 Hexyl methane sulfonate (HMS)

### 2.2. Equipment

The GC system, used for method development and method validation was a GC 6890N equipped with Auto sampler 7683B manufactured by Agilent Technologies, Singapore. The output signal was monitored and processed using Empower software version 2 on a Pentium computer. Capillary GC columns used in this study were from J&W Scientific, Santa Clara, CA, USA.

### 2.3. Preparation of solutions

The stock solutions of hexyl methane sulfonate were prepared by dissolving *25* mg in 100 mL volumetric flask of the compound in the sample solvent (n-Hexane). The working standard solution (1.25 µg mL$^{-1}$) was prepared by further diluting0.5 mL of the stock solution into a 100 mL volumetric flask and diluting to volume with sample solvent.

The sample solution was prepared by accurately weighing about *500* mg of sample into a 20 mL volumetric flask and adding 2 mL of sample solvent. Shake well for 5 minutes to extract hexyl methane sulfonate into sample solvent. Use the upper layer as sample solution for analysis to quantify HMS present in the sample.

### 2.4. Chromatographic conditions

The gas chromatographic column employed was 100% polyethylene glycol (PEG) stationary phase coated capillary column (0.53 mm x 30 m, 1µm). Helium was used as carrier gas at 1.5 mL min$^{-1}$ flow rate, hydrogen at 35mL min$^{-1}$and air at 350 mL min$^{-1}$. GC oven temperature was maintained initially at 80 °C for 2 min, then raised to 200 °C at a rate of 10 °C min$^{-1}$and held for 16 min. Injector was maintained at 200 °C and the detector at 250 °C. Flame ionization detector (FID) was used for detection. The samples were injected with the Agilent 7683B series auto sampler. A straight glass injection liner with glass wool was obtained from Agilent. The samples were injected in a splitless mode with a 4-µL injection volume.

## III.      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 quantification 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 concentration injected into the GC system. The adoption of megabore capillary GC column (0.53 mm I.D.) with a high capacity bonded stationary phase seems to be the obvious choice. Suitable initial column temperature in combination with a moderate inlet temperature (200°C) may allow a relatively large injection volume without significant deterioration in column efficiency.

The effect of concentration on separation and quantification of HMS was investigated by injecting 4 µL of the working standard solution and sample solution. Further studies were not done to determine the maximum injection. An injection volume of 4 µL was chosen for this method. No further studies were done to determine

the initial column temperature. An initial column temperature of 80°C was chosen based on available literature, which allowed baseline separation of the hexyl mesylate ester from interfering peaks in the sample solvent.

This method utilizes an extract and injects approach for the residual hexyl mesylate ester analysis. Several factors were considered in selection of a sample solvent, including the purity, ability to extract and its chemical compatibility with the compounds of interest. To detect the mesylate ester at very low ppm, the purity of sample solvent is critical. It has been observed in our laboratory that the HPLC grade solvents are generally suitable and free from interference. Because the mesylate esters have relatively high boiling point, solvents often used in residual solvent analysis including 1, 3-dimethyl imidazolidinone (DMI), Dimethyl sulphoxide (DMSO) and N, N Dimethyl formamide (DMF) are less suitable. The use of non-polar solvent (n-Hexane) is found to be suitable.

### 3.2. Method validation

The validation work was conducted according to the ICH(International Conference on Harmonization) guidelines[10-12]. The validated method parameters include specificity, accuracy, precision, sensitivity, linearity, robustness, ruggedness and solution stability. In the pharmaceutical industry, the quantification limit (LOQ) was defined as the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The LOQ was determined to be less than or equal to 0.75ppm for HMS based on the precision and accuracy data discussed below. HMS Spiked at LOQ level in sample chromatogram shown in Fig. 3.
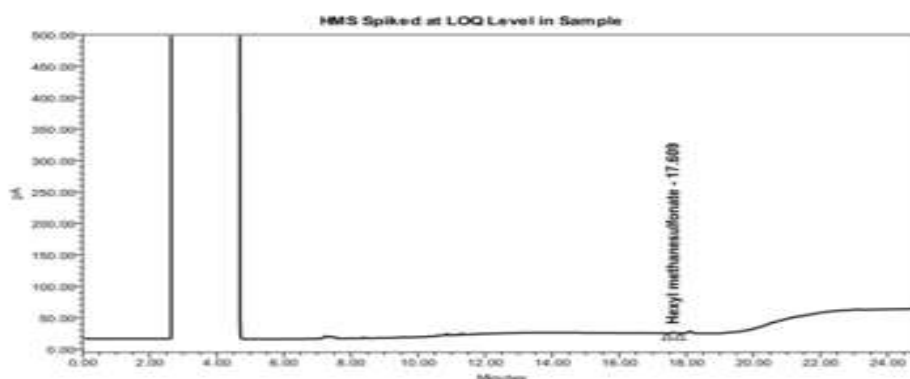


Fig. 3 HMS Spiked at LOQ level in sample

Linearity of the method was determined by preparing and analyzing a series of 7 standard solutions to cover the concentration range of LOQ to 10 ppm for HMS. Regression analysis of the peak area versus concentration data yields an $R^2$> 0.99 for each of the three calibration curve (Table 1)

**Table 1.** Summary of linearity results

| Levels checked | 7 levels; from LOQ to 10 ppm (with respect to target level concentration) | |
|---|---|---|
| | Peak Area observed(in pA) | |
| Level (% with respect to target level concentration) | Level ($\mu g\ mL^{-1}$ with respect to target level concentration) | Hexyl methane sulfonate |
| 15 | 0.7935 | 5.977 |
| 40 | 2.1160 | 14.865 |
| 80 | 4.2320 | 30.755 |
| 100 | 5.2900 | 39.396 |
| 120 | 6.3480 | 46.829 |
| 160 | 8.4640 | 62.374 |
| 200 | 10.5800 | 77.775 |
| Trend line equation | | Y=7.3879x-0.2092 |
| Regression coefficient | | 0.9998 |

The experiment result also shows that this method has excellent precision without using an internal standard. Multiple injections were made for the standard solution containing 1.25 $\mu gmL^{-1}$ of the hexyl methane sulfonate. For the six injections of the standard solution, the R.S.D was in the range of 1.7%.Accuracy of the method was determined by analyzing a drug substance samples spiked with known concentration of the hexyl mesylate ester. The spiked levels were at 0.7, 5 and 10 $\mu gmL^{-1}$.The recovery was in the range of 100.8-107.1%,

97.1-100.3% and 98.1-98.7% respectively. Because this method uses the extraction and inject approach, accumulation of drug substance in the injection liner is avoided which may have negative effect on the recovery. Therefore the injection liner may not be replaced after every sequence of injections.

Method also validated for solution stability at room temperature. Standard solution of HMS, at concentration of $1.25\mu gmL^{-1}$ was injected at regular intervals upto 7 days at room temperature. The recovery was in the range of 95-105% which conforms solution is stable. HMS Spiked at 100% level and Individual standard solution mass spectra were confirmed by GC/MS and mass spectrum shown in Fig. 4.
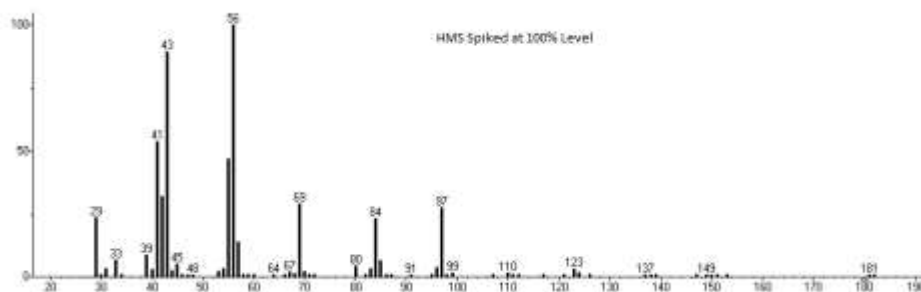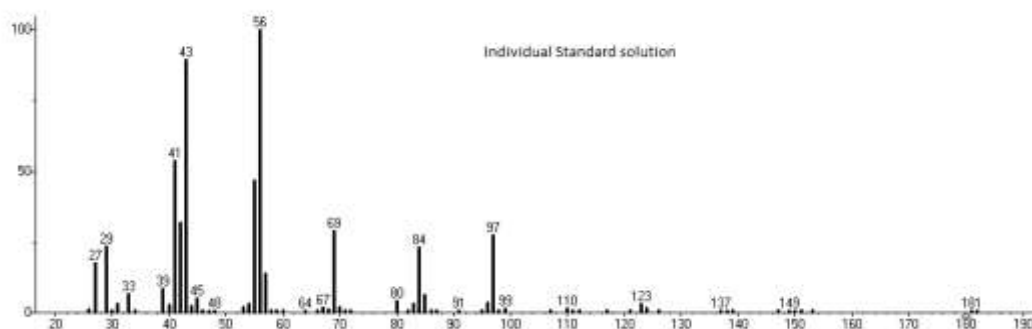


Fig.4 HMS Spiked at 100% level Mass spectrum and Individual standard solution



## IV.      Conclusion

A simple and sensitive GC method has been developed and validated for the trace analysis of hexyl mesylate ester in pharmaceuticals which is also confirmed by GC/MS. 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 testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect 0.25 ppm and quantify 0.75ppm level of hexyl mesylate ester in pharmaceutical drug substances.

## References
[1]    U. H. Ehling, R.B. Cumming, and H. V. Malling, Mutation Research, 5, 1968, 417-428
[2]    G. L. Petzold and J. A. Swenberg, Cancer Research, 38, 1978, 1589
[3]    R. D. Synder and J. D. Regan, Mutation Research, 91, 1981, 307
[4]    G. A. Sega, A. E. Sluder, L. S. McCoy, J. G. Ownens and E. E. Generoso, 159, 1986, 55
[5]    H. G. Ramjit, M. M. Singh and A. B. Codington, Journal of Mass spectroscopy, 31, 1996, 867
[6]    G. Colli, D. Mauleon and J. Vessman, Pharmeuropa, 12, 2000, 401
[7]    P. Leigh and M. Bowker, Pharmeuropa, 12, 2000, 734
[8]    C.R. Lee, F. Guivarch, C. N. Vandau, D. Tessier and A. M. Krstulovic, International journal of pharmaceutics, 195, 2000, 159
[9]    C.R. Lee, F. Guivarch, C. N. Vandau, D. Tessier and A. M. Krstulovic, Analyst, 128, 2003, 857
[10]   ICH Q2 (R1), Validation of analytical procedures: Text and Methodology, Fed. Reg (19 May 1997) 62:27463
[11]   ICH Q3A (R2), Impurities in new drug substances, Fed. Reg (11 February 2003) 68:6924
[12]   ICH Q3B (R2), Impurities in new drug products, Fed. Reg (19 May 1997) 62:27454