RESEARCH ARTICLE

Method Development and Validation of Dimethyl Sulphate Content in Esomeprazole Magnesium Drug Substance by GC-MS

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Abstract

Dimethyl sulfate is universally used as an alkylating, sulfating and sulfonating agent in organic synthesis; hence it is one of the probable impurities during the synthesis of the esomeprazole magnesium. As per ICH M7, it is genotoxic and mutagenic, so it needs to be controlled as per the acceptable intake of dimethyl sulfate and daily sample dosage. This method validation can be achieved by the hyphenated technique of gas chromatography with mass spectroscopy, which is used to develop the dimethyl sulfate impurity in esomeprazole magnesium drug substance containing salt. The method is achieved with a DB-1 column with electron impact ionization source in sim mode ion under electronic pneumatic pressure control and deliberate oven ramp programming temperature was used. A dissolved, extracted and auto-injection sample was implemented for sample introduction in a splitless mode. Dichloromethane and 2N NaOH was used as a diluent. The calibration curve showed good linearity over the 1.69 to 8.40 ppm (limit: 5.56 ppm) and its correlation coefficient was >0.999. A limit of detection 0.50 ppm and limit of quantitation 1.69 ppm was achieved when the samples were prepared at 50 mg/mL. While recovery proved to be 106.8 to 113.5%, hence it signified the matrix effect.

Keywords: GC-MS, Esomeprazole magnesium, Carcinogenic/mutagenic, Validation.

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Introduction

Proton pump inhibitors (PPIs) are the most universally recommended class of medication for the action of heartburn and acid-related complaints. These are medications that work by decreasing the amount of stomach acid made by glands in the lining of the stomach.[1] It works by hindering the site of acid creation in the parietal cell of the stomach. Because there were millions of parietal cells constantly creating, the complete reticence of stomach acid creation is practically impossible. Completely, the drugs rectify esophagitis in 90–94% of patients. There are no important differences between the drugs' overall rectification and symptom progress rates. PPIs perhaps explain the wonderful safety of these medications. Nevertheless, side effects can arise. There are a number of PPIs available i.e., esomeprazole magnesium, lansoprazole potassium, rabeprazole sodium and pantoprazole sodium. All these drugs were structurally and chemically comparable, which were moderately limited comparisons of these drugs with each other.

Dimethyl sulphate (Figure 1) has a long history of use as war gas in World War, it is quickly absorbed by intake, exhalation and through contact of the skin. It is slowly broken down to methanol and sulfuric acid. Due to this lungs and brain showed a much more advanced degree of nucleic acid alkylation than the liver and kidneys. It is a diester of methanol and H_2SO_4, it is mostly used for a methylating agent[2] in the organic synthesis and is also used as a stabilizer, solvent, catalyst and sulfonation agent. DMS is a colorless, oily liquid with a faint onion-like odor at room temperature. The sundry applications of DMS is flavors, dyes, water treatment chemicals, surfactants, pesticides, rubber chemicals and pharmaceuticals. DMS is anticipated to be a human carcinogen; a man exposed more years develops primary cancer of the eye, kidney, heart, lung and central Nervous system. As per ICH M7 (R1) its acceptable intake (AI: 1.5 µg/day) and Esomeprazole Magnesium MDD-267mg hence spec limit is 5.6 ppm.

It is one of the probable impurities during the synthesis of drug, shown structural alert for the potential impurity[3-5] and it needs to control less than 5.6 ppm in the drug substance as per acceptable intake of Dimethyl sulphate and daily dosage of sample due to this defined threshold value, the analytical testing limits required for the detection and quantification of impurity is often in the µg/g. Therefore, gas chromatography (GC) hyphenated with MS is mostly to achieve the required specificity and sensitivity.

Very few reports are available in the literature on the determination of DMS in Esomeprazole magnesium drug and

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its impurities. Some of the methods for DMS determination by different techniques[6-15] on different drug substances. The literature survey on DMS Content did not reveal analytical methods for determining dimethyl sulphate impurity in any pharmaceutical drug containing a salt form.

To the best of our knowledge, no earlier reports have been discussed on trace level determination of as such Dimethyl Sulphate impurity content by mass spectroscopy for drug substances containing salt. A wide-ranging study was taken to develop a method for trace level determination of DMS with extraction procedure by GCMS and followed by validation.

Materials And Reagents

Dimethyl Sulfate impurity was purchased from Avra Synthesis Private Limited (Hyderabad, Telangana, India). Dichloromethane was obtained from Merck, (Vikhroli, Mumbai). Sodium hydroxide was purchased from Finar Chemicals.

Instrumentation

GC-MS analysis was performed using an Agilent 7890N GC system (Palo Alto, CA, U.S.A.) hyphenated with an Agilent 5975C inert XL EI/CI MSD with a triple axis Mass Spectrometer. An Agilent DB-1 (60 m x 0.32 mm id. x 5.0 µm) GC capillary column was used.

Results And Discussions

Method Development and Optimization

The objective of this work is to be trace level determination of DMS in Esomeprazole Magnesium drug substance Method development initially started with the HPLC technique and then followed trials planned as follows:

High-Performance liquid chromatography (HPLC) Method

DMS is UV inactive; hence not possible to analyze as such determination by HPLC so the derivatization method was performed with 2-nitro phenol (Figure 2) in presence of base and temperature to formed p-nitroanisole (Figure 3) with sunfire C18 (150 × 4.6) mm, 3.5µm, mobile phase-A: 0.1% TFA (aq) and mobile phase-B: Acetonitrile and Water (65:35, v/v) was used as diluent at ƛ- 313 nm, DMS system suitability area good but sample interference was more at our concern RT so it impact recoveries hence further trails mentioned like below

Ion Chromatography(IC) Method

DMS have Characteristic nature of ionic hence planned by IC method to develop a method with Column: dionex ionpac AS17-C, ASRS-4m suppressor with 2.7 mm Na_2CO_3 +0.3 mm $NaHCO_3$ in 1-mL flow, 15 mA current, cell temp: 35°C, Column temp: 40°C but not getting sufficient area (Figure 4), tried with many modifications but did not get fruitful results hence development trails checked with another technique.

GC Method

Tried with derivatization methods but not getting noble results hence as such DMS content method developed in GC analysis, i.e. DB-1 (60 m x 0.53 mm id. x 3.0 µm) GC capillary column was used for Gaussian peak shape. DMS boiling point is 188°C so the oven temperature gradient started at 80°C held for 2 minutes, and it was ramped to 150°C at 10°C/minute and held for 5 minutes and then 240°C at 20°C/minutes and held for 10 minutes. DMS was soluble in dichloromethane, acetone and methanol among these all DMS intensities more in dichloromethane (DCM) solvent; hence method development started with DCM diluent and helium was used as carrier gas with a constant flow rate of 3.0 mL/min. The injector temperature was kept at 200°C in splitless mode. As such DMS content by GC analysis shown in (Figure 5), very less area observed, repeatability observed (n=6) and refine the line as well. A sensitive method is required to achieve LoD and LoQ at lower levels. So, when think about GCMS due to selective SIM ion we can reduce the matrix effects compared with conventional instruments like GC. Hence, these complications may be overcome with GC-MS instrument.

GC-MS Method

From GC development, the final GC method was adopted to GCMS with DB-1 (60 m x 0.32 mm id. x 1.8 µm) similar to the GC method with SIM mode even though sample interference was observed like the GC technique shown in (Figure 6).

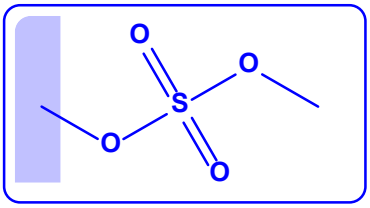

Figure 1: Chemical Structure of Dimethyl Sulphate

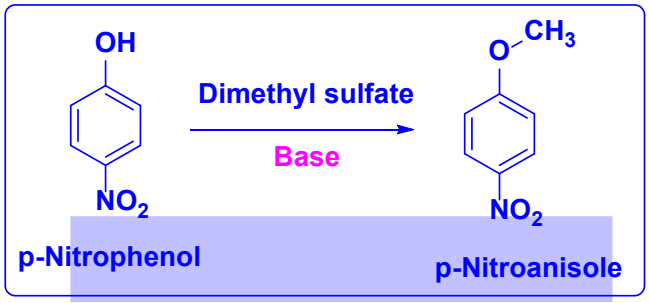

Figure 2: DMS derivatization with 2-Nitro phenol

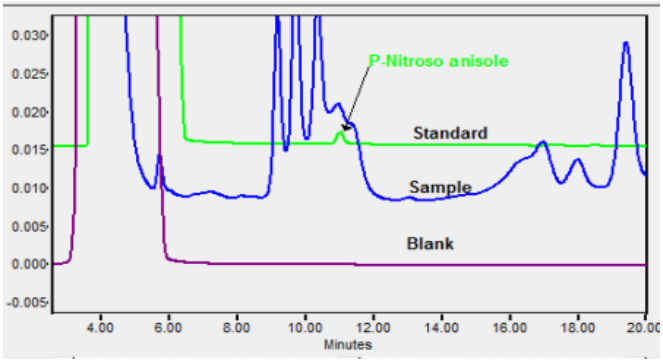

Figure 3: Derivatization chromatogram in HPLC

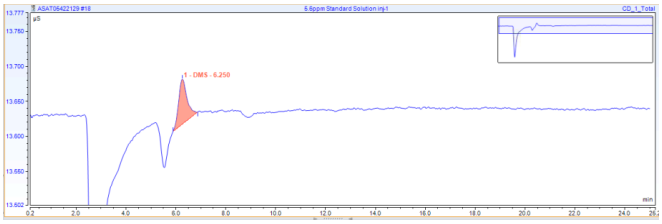

Figure 4: IC Chromatogram

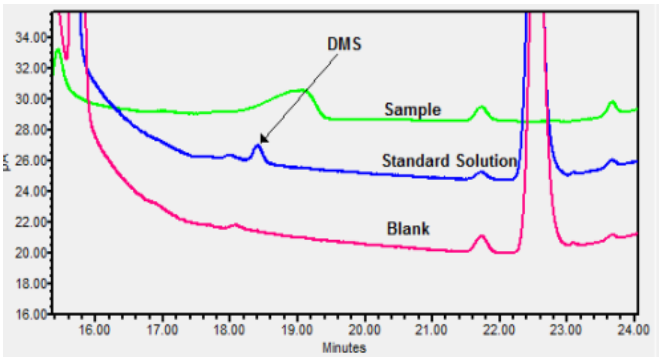

Figure 5: As such DMS content in GC Chromatogram

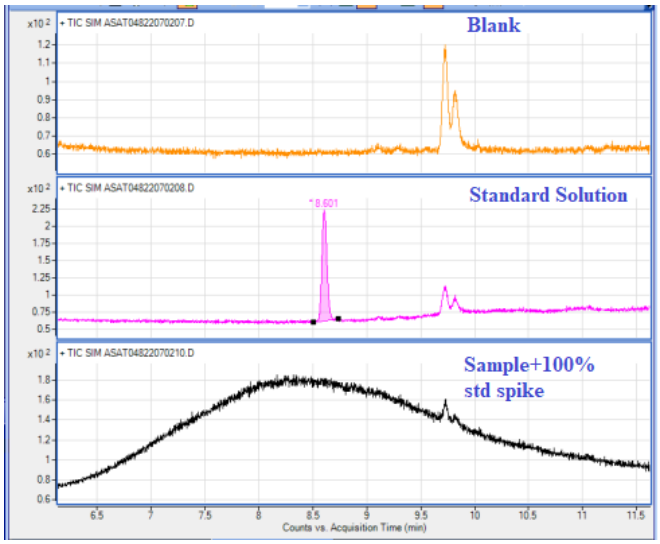

Figure 6: Sample interference in GCMS Chromatogram

Sample interference can be removed by extraction technique; sample was in salt form hence more soluble in water but DMS reacts with water. It degrades so 1N NaOH is used as aqueous media so diluent is 1:1 ratio of DCM and 1N NaOH shown in (Figure 7). There is no impact on the standard solution due to DMS more soluble in organic media than aqueous media, in this method results, sample interference was less and in 100% spiking sample, enough recovery was not observed.

To overcome the whole sample interference and for better recoveries, NaOH normality was increased to 2N from 1N. So, 1:1 ratio of DCM and 2N NaOH used for sample preparation, in this ratio sample was completely soluble in aqueous media; hence no interference was observed at RT of DMS in test sample and achieved good recoveries in the range from LoQ to 150%.

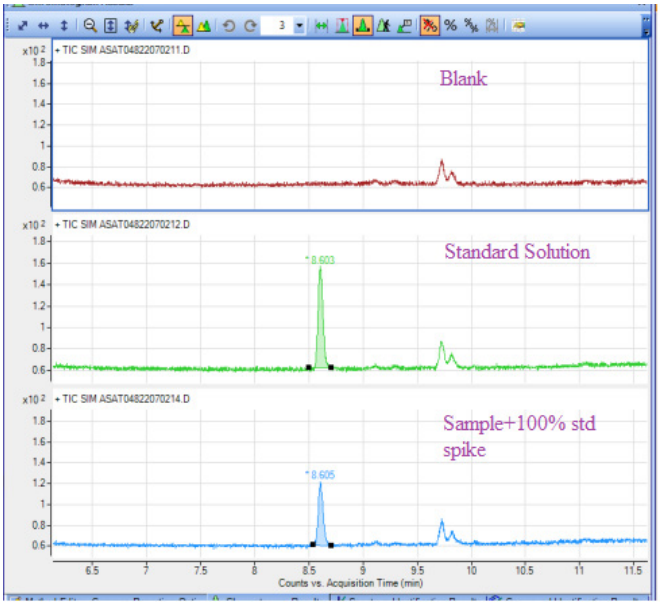

Figure 7: Sample interference reduced in GCMS Chromatogram with 1N NaOH

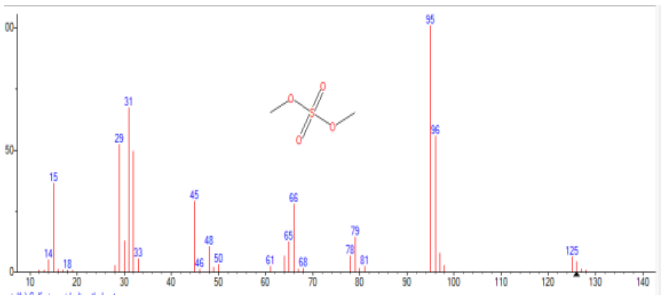

Figure 8: Dimethyl Sulphate impurity EI-Mass spectrum

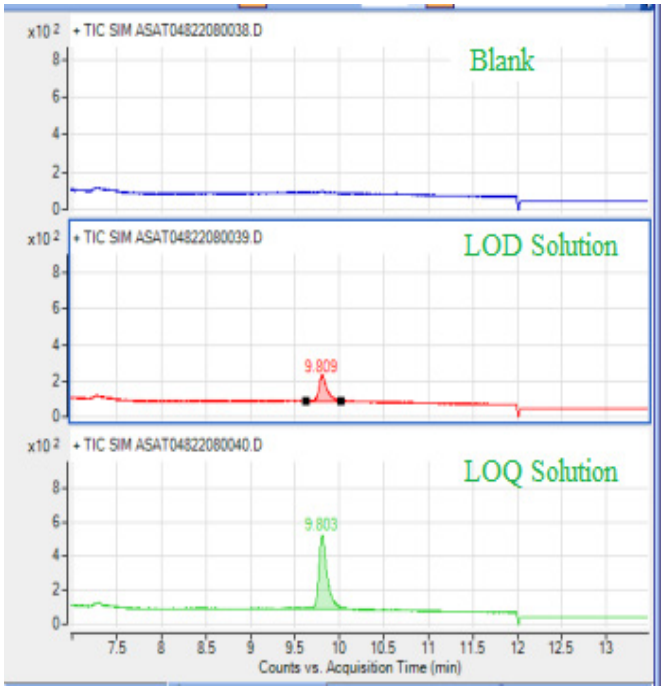

Figure 9: LOD and LOQ establishment for DMS

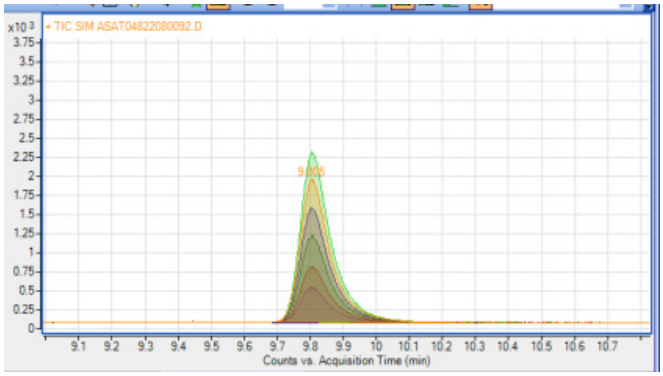

Figure 10: Linearity Chromatograms Overlay

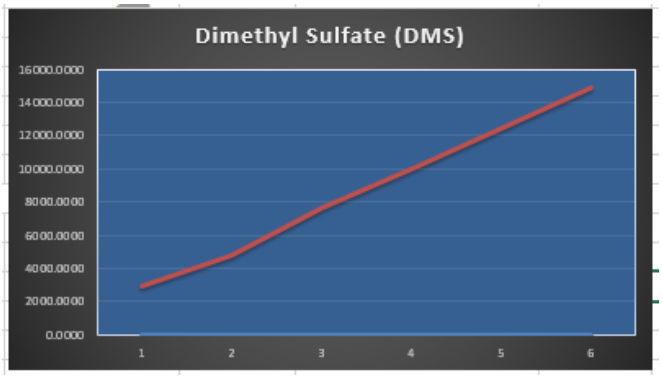

Figure 11: Linearity graph

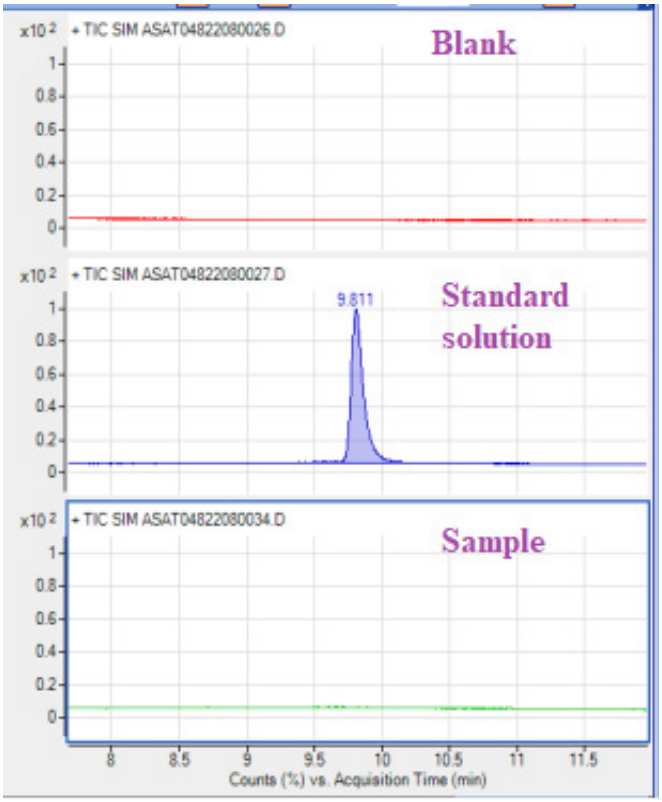

Figure 12: Selectivity Chromatogram

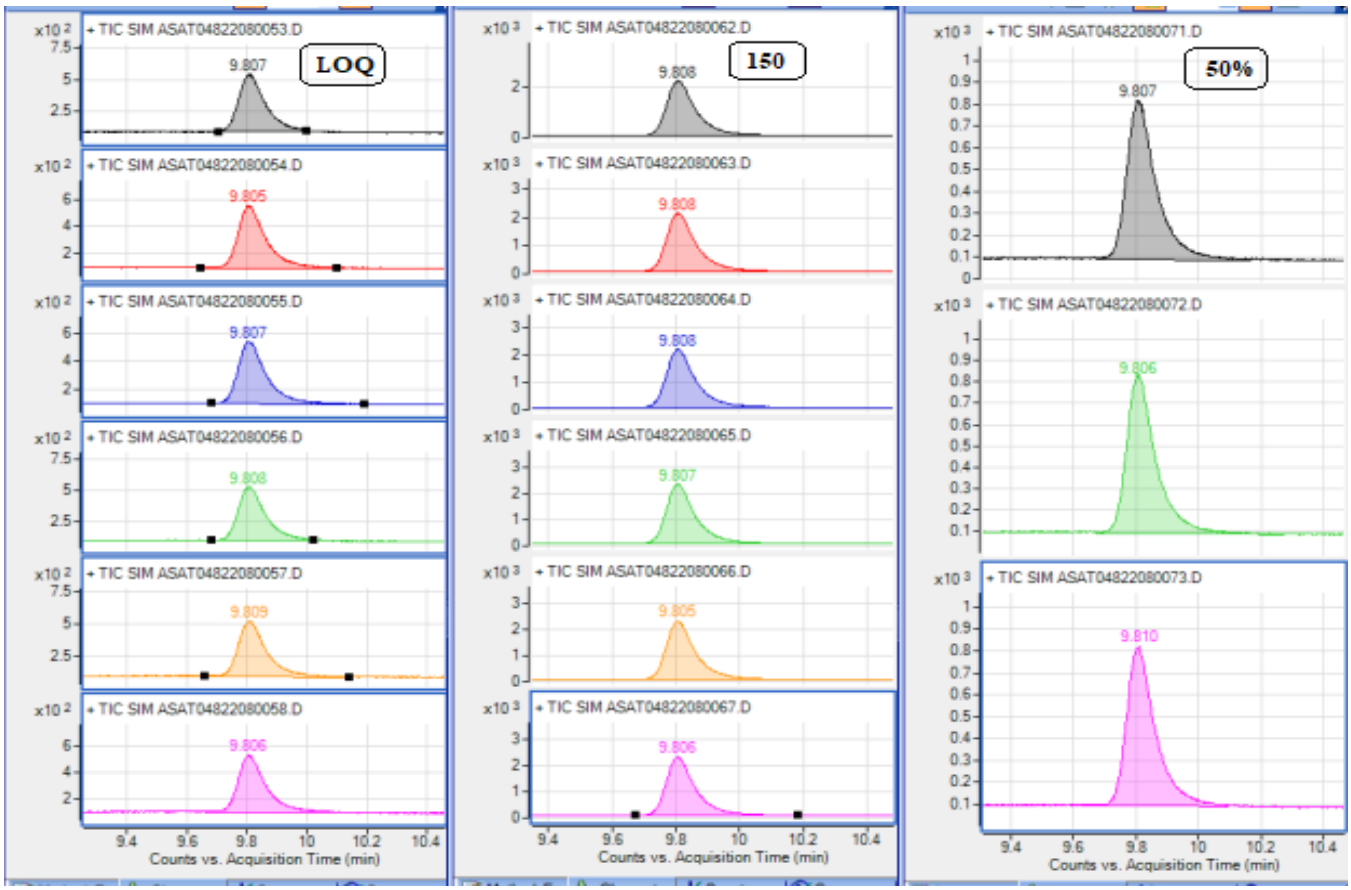

Figure 13: Accuracy at LOQ, 150% and 50%

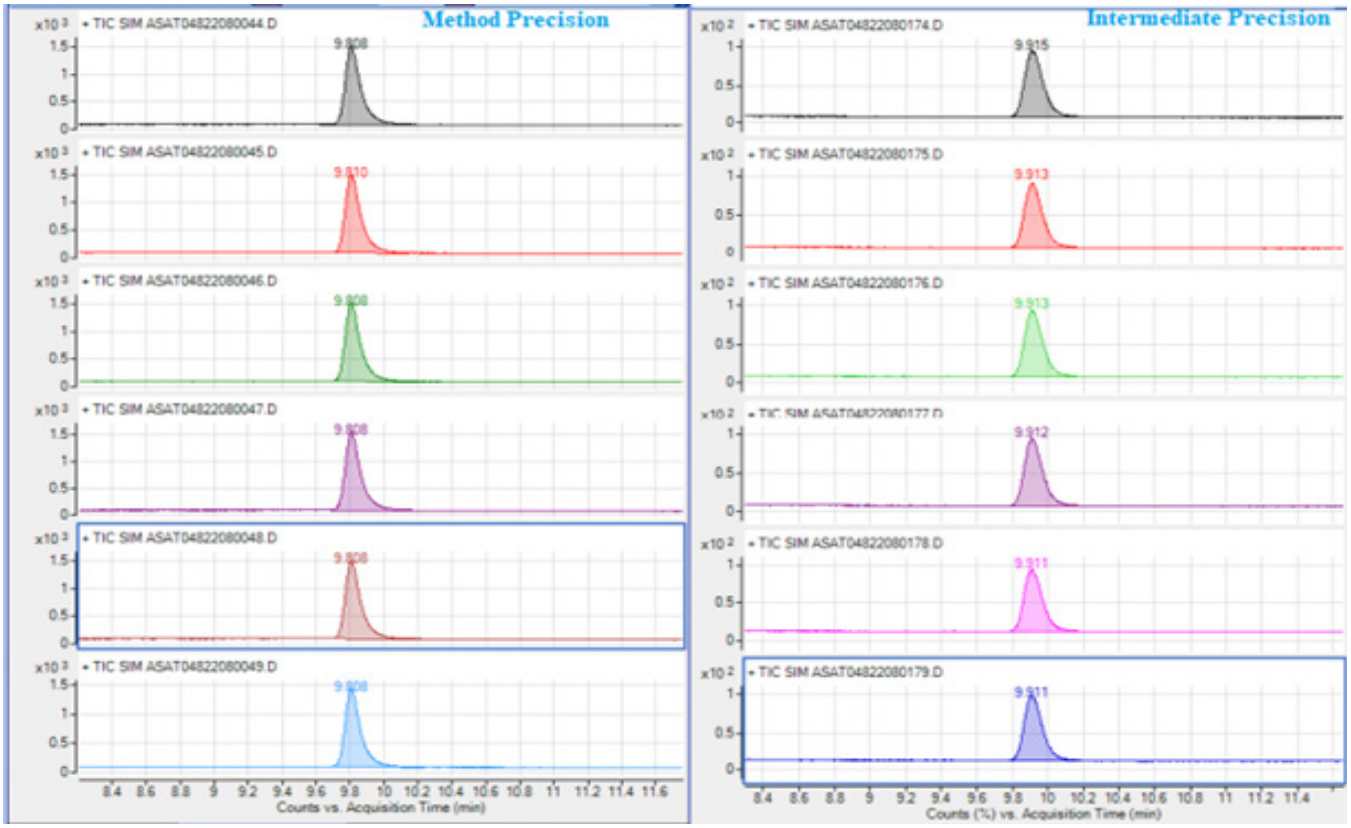

Figure 14: Method precision and Intermediate precision

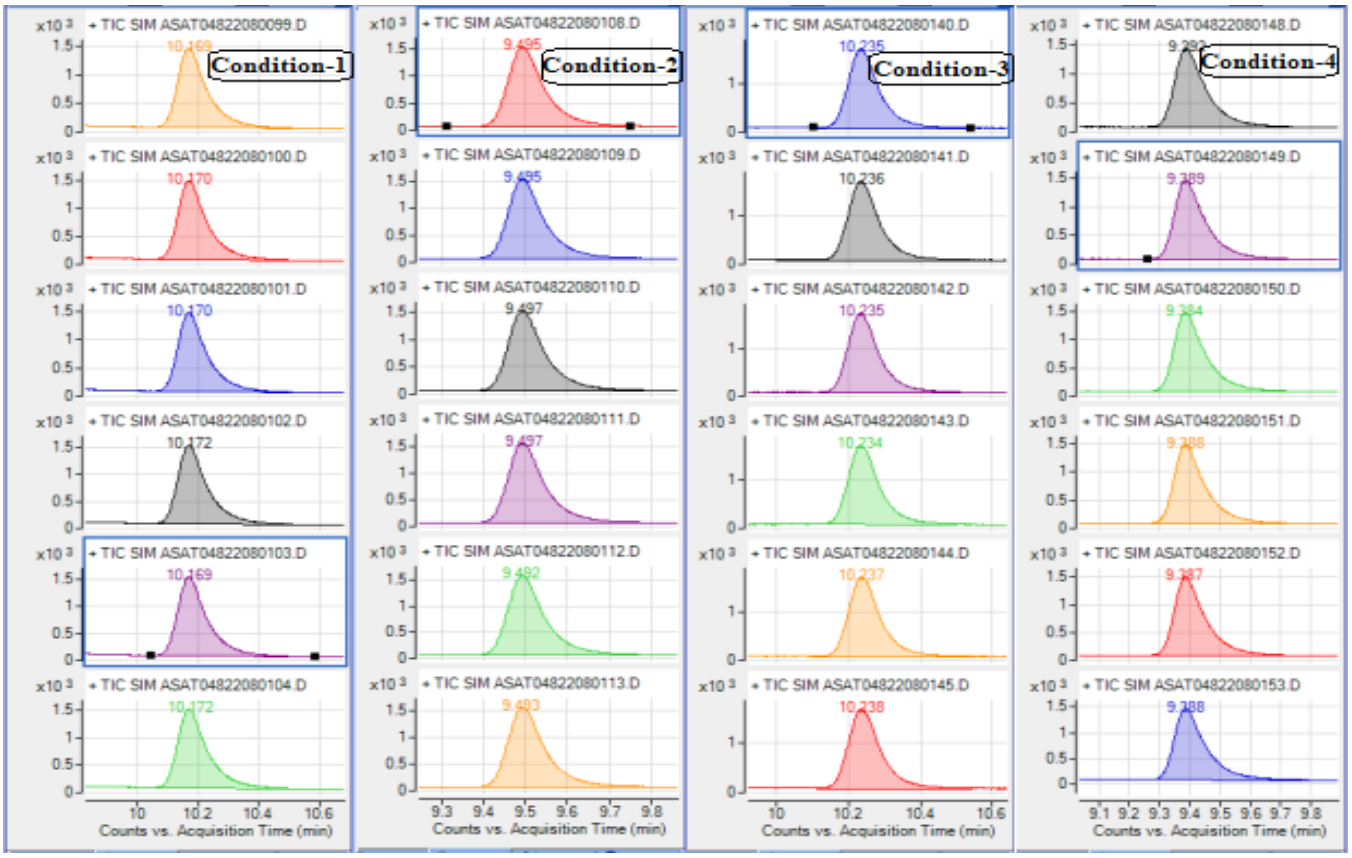

Figure 15: Robustness chromatographic data

Method Validation

Preparation of Standard Solutions

The dissolving solvent used for the preparation of the standards was dichloromethane. Accurately weighed 10.0 mg of DMS impurity standard in 10 mL volumetric flask (stock-1) dissolve up to the mark with diluent and 55 µL of stock-1 into 20 mL volumetric flask (stock-2) containing some diluent and made up to mark with diluent and then final standard prepared with 1mL of stock-2 into 10 mL volumetric flask and made up to mark with diluent and cyclomixed, on the basis of the test sample 50 mg/mL of preparation, the latter DMS corresponds to 5.6 ppm of SPEC LIMIT.

Table 1: System Suitability

Standard Solution	*Impurity area of DMS*
Injection-1	8419.45
Injection-2	8436.1
Injection-3	8645.92
Injection-4	8815.95
Injection-5	8701.72
Injection-6	8798.69
Average	8636
Standard Deviation	173.3
% RSD	2.0

Table 2: Linearity

Linearity	*Conc of DMS in ppm (w.r.t. Test)*	*DMS Imp Area*
Level-1 (LOQ)	1.6638	2947.16
Level-2 (50%)	2.7730	4805.88
Level-3 (75%)	4.1595	7604.02
Level-4 (100%)	5.5460	9954.65
Level-5 (125%)	6.9324	12407.22
Level-6 (150%)	8.3189	14865.61
Correlation Coefficient		1.000

Table 3: Precision at Range

Preparation	*LOQ Precision*	*50% Precision*	*Method Precision*	*150% Precision*
Preparation-1	1.7574	3.0434	5.8644	8.8246
Preparation-2	1.9133	3.0991	5.849	8.4885
Preparation-3	1.8518	2.93	5.8495	8.7424
Preparation-4	1.7366		6.0049	9.1196
Preparation-5	1.8168		5.8421	8.975
Preparation-6	1.8364		5.5252	8.9636
Average	1.8187	3.024	5.8225	8.8523
Std Deviation	0.065	0.086	0.158	0.221
% of RSD	3.6	2.8	2.7	2.5

Blank preparation

Transfer 5mL of diluent into 10 mL centrifuge tube and added 5 mL 2N NaOH Solution, cyclomixed and inject the bottom layer (organic layer).

sample Preparation

Weigh and transfer about 250 mg of test sample into 10 mL of centrifuge tube dissolve in 5 mL of diluent and add 5 mL of 2N NaOH Solution and cyclomix, separate the layers and filter the bottom layer (Organic layer) through 0.45 µ nylon syringe filer and inject.

Instrumentation and Method Conditions

In GC-MS analysis DB-1 (60 m x 0.32 mm id. x 5.0 µm) GC capillary column was used, the oven temperature gradient

Table 4: Accuracy results

Level	*Obtained Conc*	*Amount added*	*% of recovery*	*Avg*	*Standard deviation*	*% of RSD*
LOQ-1	1.7978	1.6636	108.06			
LOQ-2	1.9573	1.6544	118.30	113.5	5.1	4.5
LOQ-3	1.8944	1.6596	114.14			
50% Level-1	3.1134	2.7653	112.58			
50% Level-2	3.1704	2.7667	114.59	111.8	3.3	3.0
50% Level-3	2.9974	2.7719	108.13			
100% Level-1	5.9993	5.5742	107.62			
100% Level-2	5.9836	5.5453	107.90	107.98	0.19	0.2
100% Level-3	5.9840	5.5411	107.99			
150% Level-1	9.0276	8.3183	108.52			
150% Level-2	8.6838	8.2951	104.68	106.8	1.96	1.8
150% Level-3	8.9435	8.3383	107.25			

Table 5: Cumulative % RSD from Method Precision and Intermediate Precision (Ruggedness)

Injection ID	*Method precision*	*Intermediate precision*
Precision-1	5.9993	4.6987
Precision-2	5.9836	4.4017
Precision-3	5.9840	4.5401
Precision-4	6.1431	4.5581
Precision-5	5.9764	4.3086
Precision-6	5.6523	4.5798
Average	5.9565	4.5145
Mean (For n=12)	5.2355	
STDEV	0.7666	
% of RSD	14.6	

Table 6: Robustness results

	Condition-1	*Condition-2*	*Condition-3*	*Condition-4*
Injection-1	9612.71	9018.22	9978.28	10156.44
Injection-2	9761.43	9197.10	10302.84	10234.65
Injection-3	9646.48	9169.93	10322.65	10254.47
Injection-4	10007.18	9426.48	10030.96	10286.43
Injection-5	10067.95	9515.82	10230.33	10366.95
Injection-6	10182.60	9447.75	10052.04	10200.61
Average	9880	9296	10153	10250
Std dev	237.9	195.6	150.2	72.7
% of RSD	2.4	2.1	1.5	0.7

started at 100°C held for 2 minutes and it was ramped to 160°C at 10°C/minute and held for 4 minutes and then 250°C at 20°C/minutes and held for 7 minutes. An ultra-inert liner containing glass wool was used. Helium was used as carrier gas with a constant flow rate of 2.8 mL/min. The injector temperature was kept at 200°C in splitless mode. The mass detector was operated in electron impact mode (70 eV). The source and quadrupole temperatures were set to 230 and 150°C, respectively. Injection volume 2 µL. The MSD transfer line temperature was set at 260°C. Detection was achieved using a single ion monitoring (SIM) mode with a dwell time of 100 ms. The data was collected between 7 to 12.0 minutes only to moderate the source contamination from diluent and sample matrix. For DMS impurity, the molecular ion at m/z 95 was monitored (Figure 8). Data was acquired and processed using Agilent Masshunter software. Method Validation. According to our in-house validation guidelines for limit test methods, the following validation parameters were evaluated.

Validation Parameters

The method was validated according to the mentioned parameters. System suitability shown in (Table 1) proved system was in good condition followed by LoD (0.50 ppm), LoQ (1.66 ppm) establishment shown in (Figure 9), LoD states the lowest amount of the standard can be detected but not essentially quantified as an exact value and LoD signal to noise observed more than 3, LoQ states LoQ solution concentration should be less than 50% of specification limit and the lowest amount of the analyte in a sample that can be quantified with suitable accuracy and precision as an exact value and signal to noise ratio observed more than 10.

Linearity is generating test results are directly proportional to analyte concentration within given range hence it concludes dimethyl sulphate was soluble in all levels of dilutions. The calibration curves showed good linearity over the concentration range (LoQ to 150%) of 1.63 to 8.15 ppm. The correlation coefficient was >0.999 (Figure 10 and 11, table 2).

Selectivity is the ability of an analytical method to differentiate the analyte in the presence of other components in a sample. This was demonstrated by analysis of blanks, system suitability and test sample (Figure 12). Established the solution stability from standard solution, test solution and spiked test sample solution at 100% level over a period of 15 hours at room temperature.

Precision was evaluated by injection of six replicates of sample solutions that were prepared by spiking drug substance test samples at LoQ, Spec limit and 150% (Table 3). Recovery was evaluated by spiking samples with Dimethyl Sulphate at LoQ, 50%, Spec limit and 150% of Spec limit and comparing the analyte peak area against a pure standard of the same concentration. The analyte could be fully recovered (113.5% at LoQ level, 111.8% at 50% level 107.9 % at Spec limit and 106.8% at 150%) and shown in Table 3 to 5 and (Figure 13 and 14), no additional matrix effect was observed. In Esomeprazole Magnesium test samples, Dimethyl Sulphate impurity was not detected.

Method precision and intermediate precision are analytical methods well-defined as proximity in the repetitive measurements. It is established by using six different preparations of test sample spiked at specification level along with duplicate preparations of the test sample. An analytical method's ruggedness/Intermediate precision (Figure 14) also performed with different analysts, day and column. The cumulative (Method Precision and Intermediate Precision) %RSD of the dimethyl sulphate impurity content (Table 5) was within the limit (should not be more than 25.0).

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage, it was performed various parameters like column flow rate and oven, as such column flow 2.8 mL/min so 10% variation means 2.5, 3.1 mL/min and oven temperature 100°C so performed ± 5°C means 95 and 105°C even these various parameters also system suitability was within acceptable limits and shown in (Table 6 and Figure 15).

The above experimental data on the various method validation parameters proves that this method designed to determine dimethyl sulphate impurity content by GCMS is precise, accurate, linear, selective, rugged and robust. Solution stable up to 15 hours and robust and range from LoQ to 150%.

Conclusions

A GC-MS method for the determination of dimethyl sulphate was developed. Mass spectrometry ensured that the method was sufficiently sensitive to control the impurity at a trace level. The method was validated and fulfilled as per ICH guidelines for analytical method validation criteria for trace level.

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