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A new method of development and validation of Methanesulfonic acid ester (Methyl, Ethyl) impurity content in dabigatran etexilate mesylate by GC MS

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Abstract

We attempted to establish a method for estimating methane sulfonic acid methyl or ethyl ester in the dabigatran etexilate Mesylate in bulk and pharmaceutical dosage form. Dabigatran is an anticoagulant used in deep vein thrombosis and lung thrombosis. Methane sulfonic acid and ethyl sulfonic acid are DNA ethylating agents and genotoxic. These compounds can be estimated using the GC MS method using Helium as a carrier gas, and the compounds are estimated to be at 79 and 400 masses. The 60% methanol is used as diluent and blank. The method is validated as per the procedures of ICH, and all the validation parameters are within the acceptable limit of the impurities guidelines, as stated in the Q4 section. The different batches were analyzed using the same technique, and no impurities were found; hence, the batches were passed.

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INTRODUCTION

Dabigatran is an ant-clotting agent used to prevent stroke and harmful blood clots in deep veins of the legs and lungs. If a person is suffering from atria fibrillation, that means irregular heartbeat [1]. Hence, Dabigatran is used to treat blood clots in the deep veins of the legs and deep vein thrombosis or in the lungs. It is a pulmonary embolism and reduces the risk of these clots. Dabigatran etexilate is an oral prodrug and hydrolyzed to convert active Dabigatran, and the drug was approved by the FDA in 2010 [2]. The significant brand available is Pradaxa by Boehringer Ingelheim Pharmaceutical Limited.

Ethyl methanesulfonic acid has been used to induce mutations in mouse embryonic fibroblasts, yeast, and human lymphocytes. Ethyl methanesulfonate is a DNA ethylating agent, mutagenic to plants and animals, and carcinogenic in mammals [3]. It has been used as a model alkylating agent in studies of DNA repair processes. EMS induces base substitutions of guanine-cytosine (G/C) to adenine-thymine (A/T).

EMS also generates point mutations and single nucleotide polymorphisms in genomes [4]. EMS is a potential chemical mutagen used to induce mutations in rice, wheat, and Arabidopsis thaliana. Methyl Methanesulfonic acid (MMS) methyl Mesylate is an alkylating agent and carcinogen. It is also a suspected reproductive toxicant and also a skin/ sense organ toxicant. It is used in cancer treatment [5].

MATERIALS AND METHODS

MATERIALS

Dabigatran Pure drug, Ethyl Methanesulfonic acid and Methyl Methanesulfonic acid, Helium, Nitrogen, Methanol, Water HPLC grade.

METHODS

Preparation of Standard and Sample solutions

Methanesulfonic acid methyl ester and ethyl ester standard solution

Transfer 10 mg of each methane sulfonic acid methyl and ethyl ester into a 10 ml of the volumetric flask, fill the flask with diluent, and Transfer 100 µl of methane sulfonic acid methyl and ethyl ester stock solution into a 10 ml of volumetric flask and makeup to the mark with the same diluents [6]. The above solution contains about five ppm of methyl and ethyl ester concerning 50 mg/ml of sample concentration [7].

Preparation of sample solution: Transfer 250 mg of sample into a 5 ml volumetric flask and fill the flask with diluent. The final concentration of the sample and standard were prepared at five ppm [8].

Optimization:

The GC MS Instrument is Made Agilent and made of MS 5975C triple-axis detector with 7890A, column DB-5ms, and a column length of 30 mts with a Diameter of 0.25 mm SCOT column is used; the silicon solid support, the temperature of the Injection port is maintained at 140^{o}c used the Helium as carrier gas and Mobile phase comprise of H_2 and O_2 with a ration of 40 and 400 ml/min. The detector temperature is 250oC, the flow rate maintains 0.8 ml/min, and the flow rate is maintained at 0.8 ml/min. The Mass Spectroscopy uses a Quadruple mass analyzer and Ion/Dwell in groups 79.0, 400 ml, and 80.0, 400 ml [9][10][11][12].

Precision

The precision is measured by injecting six replicate injections of similar concentrations of EMS and MMS into a GC MS.

Precision LOD

LOD level methane sulfonic acid methyl and ethyl ester standard solutions preparations made by dissolving 10 µl of methane sulfonic methyl ester and ethyl ester stock solution transferred into a 10 ml volumetric flask containing 5 ml of diluent, dilute to volume with diluent and mixed well [Table 1].

Precision of LOQ level

LOQ solution is prepared by transferring 33 µl of methane sulfonic acid methyl ester and ethyl ester stock solutions into a 10 ml volumetric flask containing 5 ml of diluent and diluting the flask up to the volume with the same solvent and mixing for a few minutes. This solution is injected six times to perform the LOQ level precision [Table 2].

Accuracy at LOQ level

Continue from the precision at the LOQ level, and the last three injections from the precision at the LOQ level were taken for accuracy at LOQ level calculations. Individual stock solutions of methane sulfonic acid methyl and ethyl ester were used.

Accuracy at LOQ level

Continue from the precision at the LOQ level. The last three injections at the LOQ level were taken for accuracy at LOQ level calculations, and individual stock solutions were used: methane sulfonic acid methyl and ethyl ester.

Sample + 50% standard solution

Weight accurately 250 mg of sample to be examined into a 5 ml volumetric flask containing 2 ml of 50% standard solution and diluted to volume with diluent and mixed well.

Sample + 100% LOQ level standard solution

Weigh accurately 250 mg of sample to be examined into a 5 ml volumetric flask containing 2 ml of LOQ standard solution and diluted to volume with diluent and mixed well.

Sample + 150% standard solution

Weigh accurately 250 mg of sample to be examined into a 5 ml volumetric flask containing 2

Table 1 Precision and Accuracy

	MSA Methyl Ester			MMS Ethyl Ester		
	Mean	SD	RSD	Mean	SD	RSD
Precision	2089	4.3	2.1	1586	59	3.7
LOD s/n	2.3	-	-	3.1	-	-
LOQ s/n	9.6	-	-	9.8	-	-
Precision at LOD (0.5 ppm)	815	28	3.4	669	26	3.9
Precision at LOQ (1.7 ppm)	663	18	2.7	482	18	3.7
Accuracy at LOQ 50% - Recovery	99	-	-	98	-	-
Accuracy at LOQ 100% - Recovery	103	-	-	101	-	-
Accuracy at LOQ 150% - Recovery	99	-	-	99	-	-
Average Recovery	99	-	-	99	-	-
Range	663	18	2.7	482	18	3.7

Table 2 Linearity

Concentration (%) of methane sulfonic acid methyl ester.	Area	Concentration (%) of methane sulfonic acid ethyl ester.	Area
33	687	33	468
50	1044	50	844
80	1645	80	1290
90	1831	90	1442
100	2093	100	1625
110	2226	110	1858
120	2409	120	1977
150	3069	150	2489
Slope	20.42	Slope	16.45
Y-intercept	29	Y-intercept	-63
Correlation coefficient	0.9994	Correlation coefficient	0.9987

ml of 150% standard solution and diluted to volume with diluent and mixed well.

Linearity

The various concentrations were prepared from 50% to 150% and injected into the optimized conditions of GC. The correlation coefficient and other conditions were measured [Table 2].

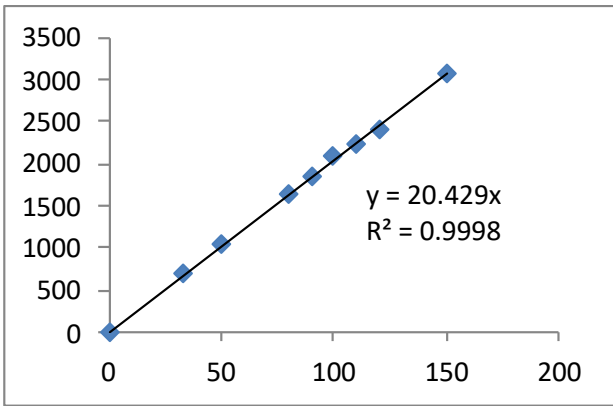

Figure 1

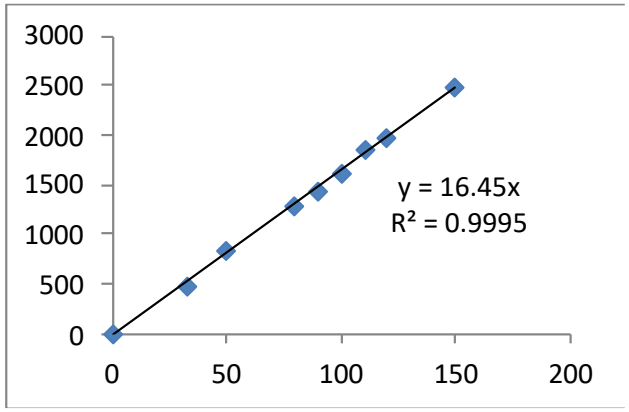

Figure 2

RESULTS AND DISCUSSION

Stability for sample and standard solutions

The chromatographic conditions and system settings are maintained per the requirements [Table 3. 4, 5]. The standard solution of about five ppm and the sample solution of approximately five ppm are prepared, and the stability will be

verified for both the sample and standard solutions.

SUMMARY AND CONCLUSION

Prepare a standard solution of 5 ppm each and approximately similar sample concentration using the same mobile phase as 60% methanol. The method is validated for its precision, LOD and LOQ, accuracy, robustness, and ruggedness were performed.

The linearity of the experiment is done by injecting 33% to 150% of the original concentration level. The slope correlation coefficients were plotted and found to be within the acceptable limit. The accuracy of the experiment is checked to ensure the exactness of the procedure. This is done by injecting 50%, 100%, and 150% levels of standard solution added to the blank or sample solution. In all the cases, the percentage of recovery was between 98 – 102% for both methane sulfonic acid methyl ester and methane sulfonic acid ethyl ester. The results proved that the experiment accurately determines specified impurities in the Dabigatran in API and formulations.

Table 3 Standard solution stability

S.No	Parameter	Injection	Acquired date & time	MSAME	MASEE
1	Solution stability	Start (0 Hrs)	20/11/2023 18:52	1845	1006
2		End (~8 Hrs)	21/11/2023 03:14	1796	911

Table 4 Sample solution stability

S.No	Parameter	Injection	Acquired date & time	MSAME	MASEE
1	Sample Solution stability	Start (0 Hrs)	20/11/2023 20:52	0	0
2		End (~14 Hrs)	21/11/2023 10:45	0	0

Table 5 Spiking solution stability

S.No	Parameter	Injection	Acquired date & time	MSAME	MASEE
1	Spiking Solution stability	Start (0 Hrs)	20/11/2023 20:35	1855	1022
2		End (~7 Hrs)	21/11/2023 03:31	1728	982

The experiment's precision is achieved by injecting the six preparations of the standard with the optimized chromatography conditions. The average peak area, standard deviation, and relative standard deviation were calculated. The results were found to be 2.1 and 3.7respectively for MMS and MES. The % RSD for methane sulfonic acid methyl ester and ethyl ester should not be more than 15.0 based on the above results, revealing that the system meets the required system suitability criteria.

The detection limit is carried out using the signal-to-noise ratio method, and the LOD concentrations of MMS and MES are 0.5 and 0.5 ppm, respectively. The LOQ concentrations of MMS and MES are 1.7 and 1.7 ppm, respectively. We followed precision to the LOQ level by injecting 1.7 ppm of MMS and MES into the chromatography system six times. The average area, SD, and RSD are compared with acceptable criteria. The same experiment was also carried out for different batches to find the LOQ level of MMS and MES, and there were no detection levels in the batches.

The range of the MMS and MES can be done by injecting six replicate injections of LOQ level and 150% level of the standard into the specified experimental conditions and verifying their reliability with the acceptable criteria. Finally, a stability study is conducted to see the 0 hours to up to 14 hours of the solution preparation if no degradation is detected. A different batch analysis was performed. From the data, we can conclude that the methane sulfonic acid methyl and ethyl ester were not detected in all batches of Dabigatran etexilate Mesylate.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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