RESEARCH PAPER

DEVELOPMENT AND VALIDATION OF GC-MS METHOD FOR THE QUANTIFICATION OF RESIDUAL DIMETHYL SULFATE, DIETHYL SULFATE AND DIISOPROPYL SULFATE CONTENTS IN RIMEGEPANT SULFATE DRUG SUBSTANCE

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ABSTRACT

New gas chromatography coupled with mass spectrometry (GC-MS) method was developed for the quantitation of three potential genotoxic impurities (PGI's) namely Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate in Rimegepant sulfate (RMP) drug substance in selective ion monitoring mode. Chromatographic separation of these impurities was achieved on capillary Rtx-35 GC column (Fused silica capillary column coated with 35% diphenyl and 65% dimethyl polysiloxane stationary phase), 30 m length; 0.32mm internal diameter and 1.0 µm film thickness. Helium gas used as carrier gas with Electron Impact ionization (EI) in Selective Ion Monitoring (SIM) mode by using liquid-liquid extraction sample preparation technique for these impurities. The mass fragment ions 95, 139 and 167 were selected for the quantification of Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate respectively. The Performance of the method validation was assessed by evaluating the specificity, linearity, sensitivity, precision and accuracy experiments. The established limit of detection (LOD) values for the genotoxic impurities was in the range of 0.4 ppm - 1.3 ppm and limit of quantification (LOQ) values were in the range of 1.3 ppm - 4.0 ppm. This developed method was found to be linear with correlation coefficient is greater than 0.990. The average recoveries for the accuracy were in the range of 104.4 - 105.6%. The Validation results demonstrated the good linearity, precision and accuracy of the method, which can be further adopted as an adequate quality control tool for quantitation of three potential genotoxic impurities (PGI's) at trace levels in Rimegepant sulfate drug substance.

Keywords: Rimegepant sulfate**,** Potential genotoxic impurities**,** GC-MS, Liquid-Liquid extraction and Method validation.

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INTRODUCTION

Rimegepant sulfate is a small and novel molecule inhibitor of the calcitonin gene-related peptide (CGRP) receptor that blocks the action of CGRP, a potent vasodilator believed to play a role in migraine headaches. Rimegepant sulfate, known under the brand name Nurtec ODT, is used to treat the acute treatment of migraine with or without aura in adults and the preventive treatment of episodic migraine in adults [1-2]. Migraine is one of the most common chronic neurologic diseases [3]. The illness is marked by recurrent headaches that range in intensity from mild to severe, as well as nausea, photophobia, phonophobia, cutaneous allodynia, and another phobia [4-5]. The headache attack lasts anywhere from 4 to 72 hours and happens once or twice per month on average. It is the third most prevalent and the second most incapacitating. most common neurological illnesses, which affect 12% of men and 33% of women for the rest of their lives [6,7]. The name migraine is taken from the Greek word "hemikrania," which was eventually changed to the Latin word "hemigranea." Such a word is translated as "migraine" in French. It frequently results in disability and job loss [8]. Rimegepant is an oral antagonist of the CGRP receptor developed by Biohaven Pharmaceuticals. It received FDA approval on February 27, 2020 for the acute treatment migraine headache, and was subsequently approved by the European Commission in April 2022 for both the treatment and prevention of migraines.

Rimegepant sulfate is chemically known as (5*S*,6*S*,9*R*)-5-amino-6-(2,3-difluorophenyl)-6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl-4-(2-oxo-2,3-dihydro-1H-imidazol[4,5-b]pyridine-1-yl)-1-piperidinecarboxylate hemisulfate sesquihydrate and has a chemical formula $C_{28}H_{28}F_2N_6O_3.0.5H_2SO_4.1.5\ H_2O$. Its molecular weight is 610.63 g/mol. The chemical structure of Rimegepant sulfate is shown in Fig. 1.

As shown in Fig. 2**,** in the final stage reaction of Rimegepant sulfate, Absolute alcohol, Purified water and Sulfuric acid were used for the salt form of Rimegepant. Methanol and Isopropyl alcohol solvents

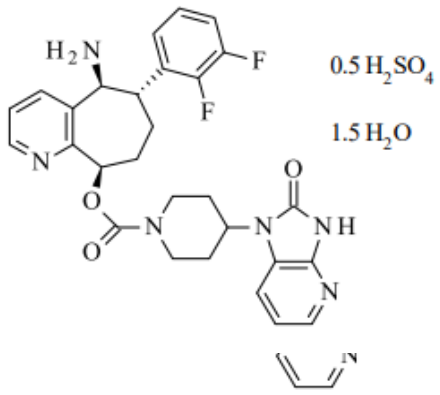
were also used for the preparation of various stages. Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate impurities were might be chance for form in manufacturing process. Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate potential impurities are having structural alert. International Agency for Research on Cancer (IARC) categorizes Dimethyl sulphate, Diethyl sulfate in Group 2A carcinogens and Diisopropyl sulfate in Group 2B carcinogen. These impurities are potent genotoxic chemicals which can directly alkylate DNA both in vitro and in vivo [9,10].

In the drug development, Control of impurities in active pharmaceutical ingredients (API) is an important aspect, ensuring product quality and minimizing safety risks. For impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects and different impurities may be observed throughout the development lifecycle of an active pharmaceutical ingredient (API).

According to Less-Than-Lifetime (LTL) exposures phenomena of ICH M7[10] for mutagenic/carcinogen impurities in the pharmaceutical industry, the risk assessment for carryover into the drug substance from the raw materials and intermediates to be appropriately controlled to fulfil the regulatory requirements. To achieve this, genotoxic assessment (identification of genotoxic impurity, control of impurity and generated data presentation) is must w.r.t. analytical approach and control of identified impurities based on TTC concept. Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate impurities are alkylating agents and show conventional structural alerts for genotoxic potentiality [11]. Hence genotoxicity is assumed, staged and further TTC concept is applied [12-15] as per EMEA [16] and FDA [17] guidelines. According to the maximum daily dose (75 mg per day) [18] the allowed limit is not more than 20 ppm for each impurity. According to TTC concept, we have developed a new GC-MS method for quantification of three genotoxic impurities in RMP drug substance. To attain the best quality of RMP drug, these three impurity levels should be monitored and controlled with appropriate analytical methods in RMP drug substance.

While there are a few methods to determine the genotoxic compounds DMS and DES, the determination of DIPS has not been reported [19-21]. There are few references for Rimegepant sulfate (RMP) [22,23] literature survey revealed that currently there is no method for the quantification of

DMS, DES and DIPS in RMP drug substance till to date.Hence, it is aimed to develop and validate a sensitive and specific method for the trace level determination of the three genotoxic impurities in RMP drug substance by GC-EI-MS with selective ion monitoring (SIM) mode. The chemical structures of DMS, DES and DIPS are shown in Fig. 1.and its mass spectrums are shown inFig. 2 (a) to 2 (c)

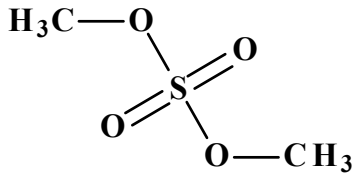


Rimegepant sulfate

Mol. Formula:$C_{28}H_{28}F_2N_6O_3$

$0.5H_2SO_4$. $1/2H_2O$

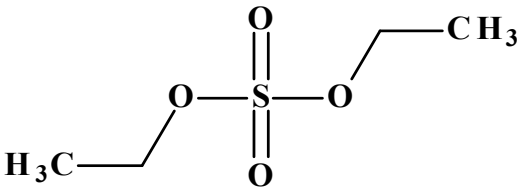
Mol. Wt:610.63



Dimethyl sulfate

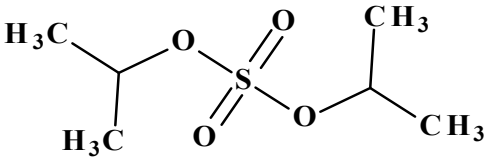
Mol. Formula:$C_2H_6O_4S$

Mol. Wt:126.13



Mol. Formula:$C_4H_{10}O_4S$

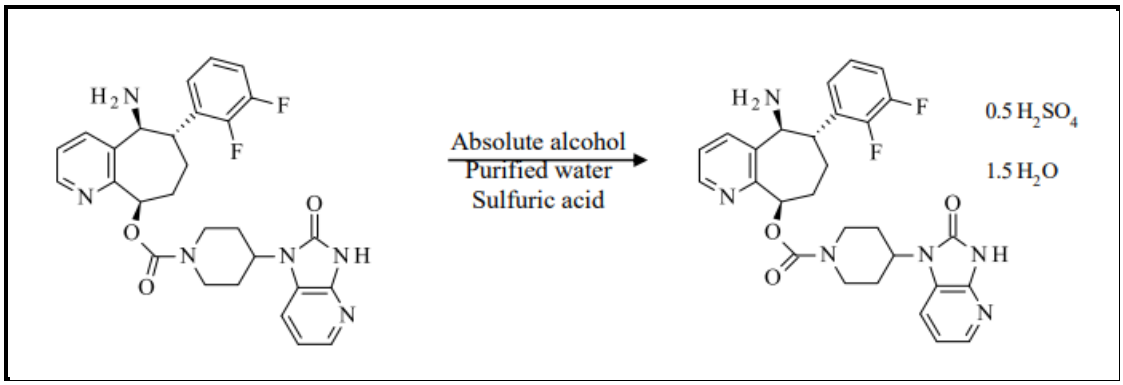
Mol. Wt:154.18



Mol. Formula:$C_6H_{14}O_4S$

Mol. Wt:182.24

Fig. 1:Chemical structure of Rimegepant sulfate, Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate impurities



Mol. Formula:

$C_{28}H_{28}F_2N_6O_3$

Molecular weight: 534.56

Mol. Formula:

$C_{28}H_{28}F_2N_6O_3$ $0.5H_2SO_4$ $1.5H_2O$

Molecular weight: 610.63

Fig. 2: Final stage reaction scheme of Rimegepant sulfate

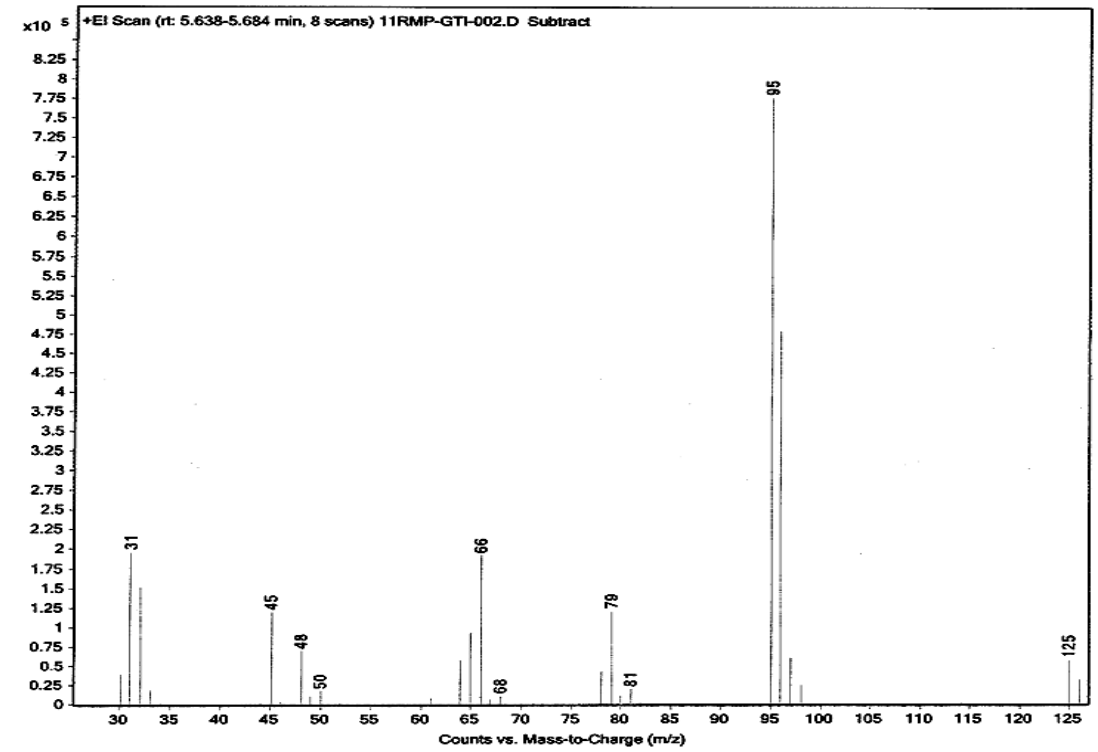


Fig. 2(a): Mass Spectrum of Dimethyl sulfate (m/z-126)

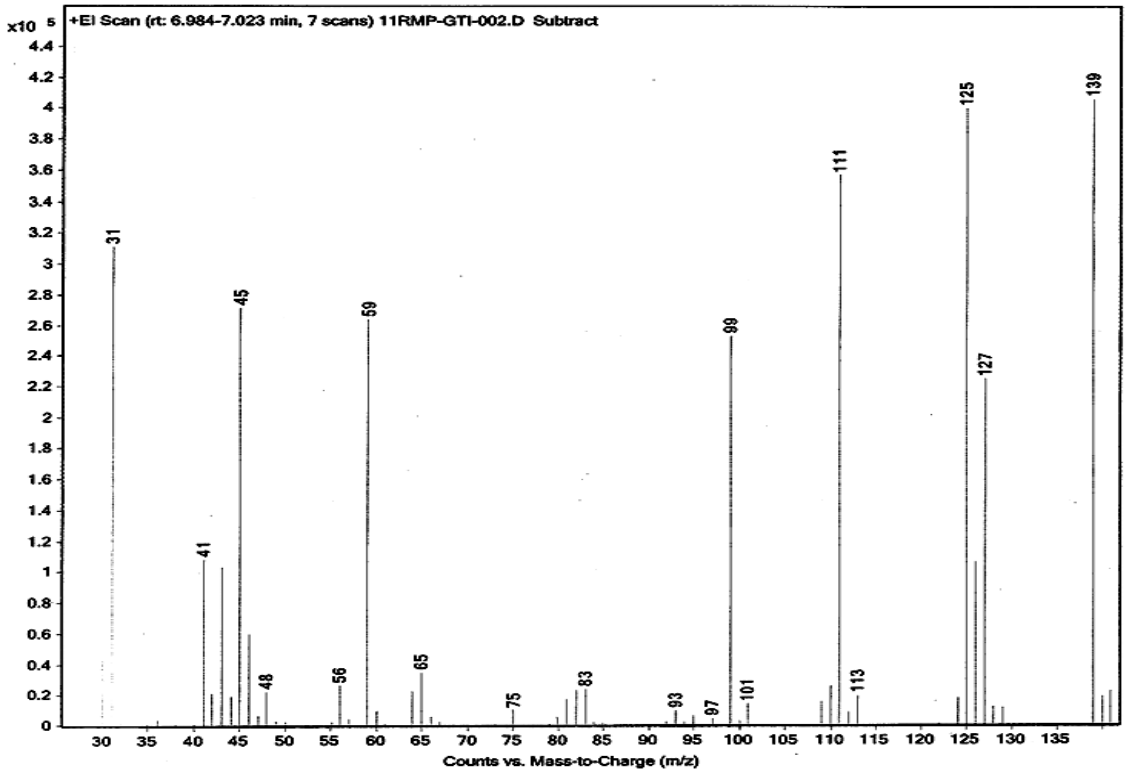


Fig. 2(b): Mass Spectrum of Diethyl sulfate (m/z-152, Spectrum shown m/z-139 [M-15])

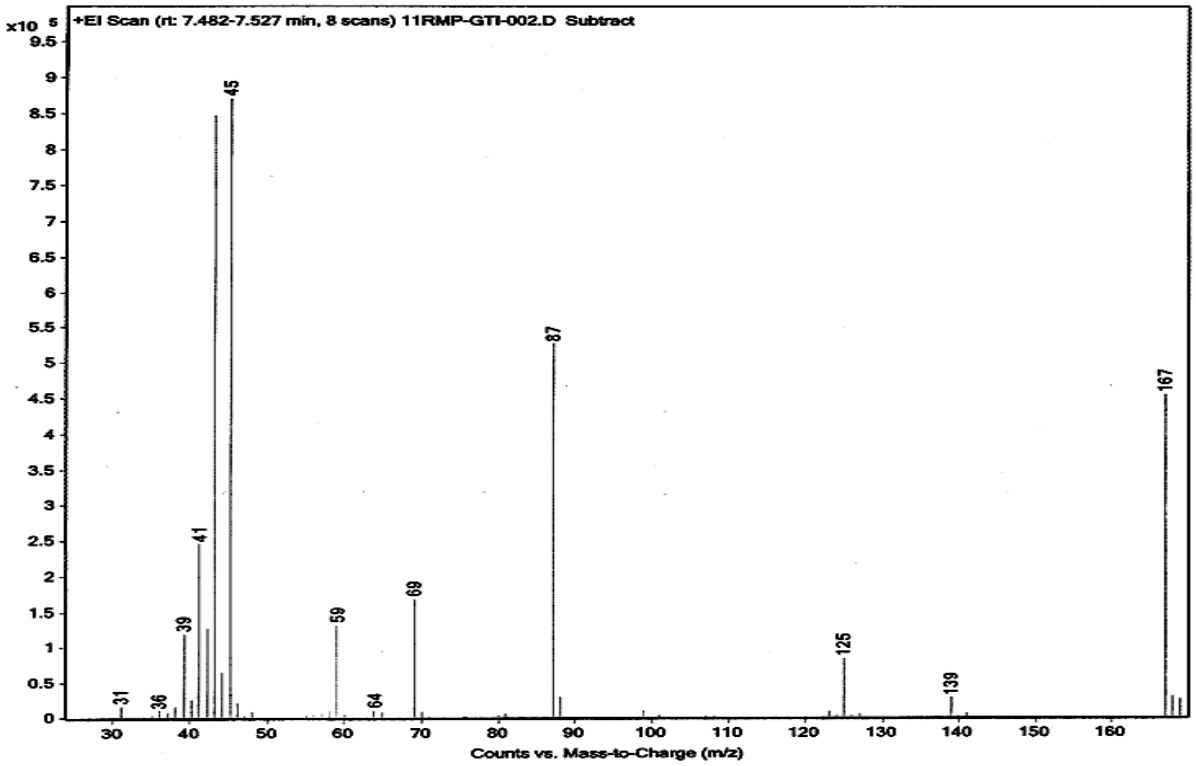


Fig. 2(c): Mass Spectrum of Diisopropyl sulfate (m/z-182, Spectrum shown m/z-167 [M-15]

MATERIAL AND METHODS

Chemicals and reagents

Dimethyl sulfate (DMS), Diethyl sulfate (DES) and Diisopropyl sulfate (DIPS) and pure samples of Rimegepant sulfate (RMP) were obtained from Chemical Research Division of APL Research Centre laboratories (A division of Aurobindo Pharma Ltd., Hyderabad, India). Formic acid (Grade: EMPARTA ACS) was procured from Merck, India. Water (Grade: HPLC) and Dichloromethane (Grade: GC) were procured from Rankem, India.

Standard solutions

Diluent: Dichloromethane

Standard stock solution: Weigh accurately about 31.5 mg of each Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate standard into a 25 mL of volumetric flask half-filled with diluent and make up to volume with diluent and mix well. Transfer 0.5 mL of this solution into a 25 mL volumetric flask and make up to the volume with diluent.

Standard solution: Transfer 1.0 mL of the above standard stock solution into a 25 mL volumetric flask and dilute to volume with diluent and mix well.

Standard solution vial: Transfer 1.0 mL of Formic acid into a clean glass centrifuge tube followed by add 0.5 mL of water and shaking the solution. To this, add 2.0 mL of

the above standard solution and shaking the solution about 1 min. Allow the two phases to separate. Collect the lower layer (Dichloromethane layer) and used it for analysis.

Blank solution vial: Transfer 1.0 mL of Formic acid into a clean glass centrifuge tube followed by add 0.5 mL of water and shaking the solution. To this, add 2.0 mL of Dichloromethane and shaking the solution about 1 min. Allow the two phases to separate. Collect the lower layer (Dichloromethane layer) and used it for analysis.

Test solution vial: Accurately weigh and transfer about 200 mg of test sample into a clean glass centrifuge tube, add 1.0 mL of Formic acid and dissolve. To this, add 1.0ml of water and shake the solution about 1 min and add 2.0 mL of Dichloromethane and shake the solution for about 1 min. Allow the two phases to separate. Collect the lower layer (Dichloromethane layer) and use for analysis.

Spiked solution vial: Accurately weigh and transfer about 200 mg of test sample into a clean glass centrifuge tube, add 1.0 mL of Formic acid and dissolve. To this, add 1.0ml of water and shake the solution about 1 min and add 2.0 mL of Standard solution and shake the solution for about 1 min. Allow the two phases to separate. Collect the lower layer (Dichloromethane layer) and use for analysis.

GC-MS Conditions: The analysis was carried out on the Agilent GCMS-5977A and GCMS-5977B gas chromatograph equipped with 7890B GC System auto sampler and data handling system having Mass Hunter solution software. The instrument was run in EI mode. Rtx-35, (30m × 0.32 mm I.D, 1.0 µm film thickness, Agilent Technologies, USA) column consists of 35% diphenyl and 65% dimethyl polysiloxane as a stationary phase. Chromatographic method conditions were used as shown in Tables-1 to Table-3.

Table 1: Gas chromatograph conditions

Instrument	Agilent 7890B		
Column	Rtx-35, 30 m × 0.32 mm I.D. × 1.0 µm Film thickness		
Carrier gas	Helium		
Injector temperature (°C)	220°C		
Injection type	ALS mode (Auto liquid sampler)		
Column oven program	Heating rate (°C/min)	Initial temperature (°C)	Hold time(min)
	---	60	2
	20	200	11

Flow rate (mL/min)	2.0
Injection volume (µL)	2.0
Split ratio	10:1
Run time (min)	20

Table 2: Gas chromatography mass spectrometer conditions

Instrument	Agilent GCMS-5977A and 5977B Single Quad MS
MS Transfer line temperature (°C)	250
MS Source temperature (°C)	230
MS Quad temperature (°C)	150
Function type	SIM (selective ion monitoring)
Gain factor	5

Table 3: SIM Time segments

Solvent delay time	Group Name	Mass (m/z)	Dwell time (ms)	Resolution
5.0 min	Dimethyl sulfate	95*, 96**	50, 50	Low
	Diethyl sulfate	139*, 125**	50, 50	
	Diisopropyl sulfate	167*, 125**	50, 50	
*Quantifier ion and **Qualifier ion.				
Timed MS Detector: The MS must be ‘‘Detector Off’’ after 10.0 min				

RESULTS AND DISCUSSION

Method development: The objective of the present work is, to establish a simple GC-MS-SIM method for the determination of DMS, DES and DIPS contents in RMP drug substance. Initially, method development experiments were executed by using GC equipped with flame ionization detector (FID) based on the tendency of volatility and polarity of the analytes. These analytes having high boiling points. Due to this reason, these analytes were not coming in statistic headspace mode. We have made few trials by changing different diluents, columns and chromatographic conditions in GC with FID in direct injection mode. Three impurities having very poor response in GC. Hence, not getting the desired levels peaks (20ppm) in GC. Due to the lower response of these impurities by GC-FID technique, we have chosen a gas chromatography electron ionization mass spectrometry (GC-MS-EI) technique in SIM mode for good separation and desired sensitivity.

Further, development trials were initiated in

direct liquid injection technique using the DB-624 GC column (stationary phase: 6% cyanopropyl and 94% dimethyl polysiloxane, Make: Agilent). Initially, Methanol used as a diluent. Standard, sample and spiked sample solutions were prepared and injected into the GC-MS. Unfortunately, in spiked sample injection, DMS, DES and DIPS peaks were very less areas observed as compared to standard solution. In the evaluated spiked sample solution, all the analytes might be binded to the sample matrix because of the Rimegepant sulfate is in salt form. In this trial, Background interference was also encountered. In general, drug substances are in free or slat form depending upon various factors and salt form often improve the solubility and stability. Rimegepant sulfate is in salt form as it contains specifically a hemisulphate sesquihydrate salt. Salts are generally soluble in water. During optimization procedure we have tried with few of diluents i.e., chloroform, diethyl ether and ethyl acetate and different columns. Finally, Methylene chloride was used as extraction solvent and using the Rtx-35 GC column (stationary phase, 35% diphenyl and 65% dimethyl polysiloxane, Make: Restek).

During the further development, sample solution was prepared with water, i.e, 100 mg of Rimegepant sulfate dissolved in 2ml of water and extracted with 3 mL of Methylene chloride. Because water and methylene chloride both are polar and immiscible liquids, Methylene chloride separates from water as an organic layer (lower layer). DMS, DES and DIPS standard solution was prepared in methylene chloride diluent at desired concentration (20 ppm each impurity) level. Standard solution, Sample solution and Spiked sample solutions were prepared as per above mentioned water and methylene chloride extraction procedure and these solutions were analysed. In the obtained chromatograms, high-quality baseline and there are no unknown peaks observed in sample chromatogram. Unfortunately, in spiked sample DMS, DES and DIPS peaks are very less as compared to standard solution, so recovery not meet the acceptance criteria.

To improve the peak area response of DMS, DES and DIPS as well as to minimize the sample matrix effect during development activity, initially 100 mg of Rimegepant sulfate sample was dissolved in 1.0 mL of Formic acid and added 1.0 mL of water. Rimegepant sulfate solubility is more in formic acid as compared to water due to acid's ability to promote ionization and dissolution of the Rimegepant sulfate molecule. After few trails of modification of exctration procedure, finally below

mentioned exctration procedure has given satisfactory results.

Standard vial preparation: Transfer 1.0 mL of Formic acid into a clean glass centrifuge tube followed by add 0.5 mL of water and shaking the solution. To this, add 2.0 mL of the above standard solution and shaking the solution about 1 min. Allow the two phases to separate. Collect the lower layer (Dichloromethane layer) and used it for analysis.

Blank vial preparation: Transfer 1.0 mL of Formic acid into a clean glass centrifuge tube followed by add 0.5 mL of water and shaking the solution. To this, add 2.0 mL of Dichloromethane and shaking the solution about 1 min. Allow the two phases to separate. Collect the lower layer (Dichloromethane layer) and used it for analysis.

Test vial preparation: Accurately weigh and transfer about 200 mg of test sample into a clean glass centrifuge tube, add 1.0 mL of Formic acid and dissolve. To this, add 0.5 mL of water and shake the solution about 1 min and add 2.0 mL of Dichloromethane and shake the solution for about 1 min. Allow the two phases to separate. Collect the lower layer (Dichloromethane layer) and use for analysis.

Spiked vial preparation: Accurately weigh and transfer about 200 mg of test sample into a clean glass centrifuge tube, add 1.0 mL of Formic acid and dissolve. To this, add 0.5 mL of water and shake the solution about 1 min and add 2.0 mL of Standard solution and shake the solution for about 1 min. Allow the two phases to separate. Collect the lower layer (Dichloromethane layer) and use for analysis. These prepared vial solutions analysed in GC-MS by using Rtx-35, 30m long with 0.32mm i.d.,1.0µm particle diameter column consists of 35% diphenyl and 65%-dimethylpolysiloxane as stationary phase and passing helium as carrier gas. The temperature of column oven is used initially 60°C is maintained for 2 min and then increased to 200°C at a rate of 20°C/min followed by holding at 200°C for 11 min. In this methodology, satisfactory results were obtained. The developed method was used for validation study to evaluate its performance characteristics.

Method validation study parameters: Proposed the quantification of DMS, DES and DIPS contents in Rimegepant sulfate drug substance has been validated as per ICH guidelines [24] to prove its competence. The validation parameters studied were specificity, sensitivity, limit of

detection (LOD), limit of quantification (LOQ), linearity, accuracy and precision (Repeatability and method precision) and robustness and system suitability.

System suitability: Analyzed the Standard solution containing 20ppm of each impurity six times as per the GC-MS method. Summarizes the percentage relative standard deviation for the standard solution.

Specificity: The specificity of the developed GC-MS-EI method was indicated by showing the m/z peaks in the method as 95 for DMS, 139 for DES and 167 for DIPS. Specificity is the ability of the method to measure the analyte response in presence of all impurities in Rimegepant sulfate drug substance. To evaluate the specificity experiment, all individual analyte solutions were prepared and injected individually to confirm retention time and solutions of Rimegepant sulfate drug substance (Control sample), Rimegepant sulfate drug substance spiked with analytes at specification level (Spiked sample) and Rimegepant sulfate drug substance spiked with analytes and all other known residual solvents at specification level (All spiked sample) were injected to confirm any interference of other solvent peaks with analyte peaks. From the chromatogram of individual injections of analytes, control sample, spiked sample, all spiked sample, it is observed that there is no interference (co-elution) from the sample matrix indicated that the method is selective and specific for the determination of DMS, DES and DIPS contents in RMP drug substance. A typical representative overlaid GC-MS chromatogram were shown in Fig.3 (a) to 3 (e) and summary of specificity results are shown in Table-4 and Table-5.

Table4: Summary of the retention time (RT) and Mass (m/z) values for all the components

Name of the solvent	Retention time (min)	m/z value
Methanol	1.1	32
Ethanol	1.3	46
Isopropyl alcohol	1.3	59 [M-1]
t-Butanol	1.4	59 [M-15]
n-Hexane	1.6	86
Ethyl acetate	2.1	88
Methyl tertiary butyl ether	2.1	73 [M-15]

Tetrahydrofuran	2.3	72
1,4-Dioxane	3.4	88
N,N-Diisopropylethylamine	3.6	129
Dimethyl sulfate	5.7	125 [M-1]
Diethyl sulfate	7.0	139 [M-15]
Diisopropyl sulfate	7.5	167

Table5: Specificity experiment results

Impurity	Quantifier ion	Standard		Control sample		Spiked sample		% Recovery
		RT (min)	Area	RT	Area	RT	Area	
Dimethyl sulfate	95 [M-31]	5.66	46608	5.66	ND	5.67	50478	108.3
Diethyl sulfate	139 [M-15]	7.00	21707	7.00	ND	7.02	22251	102.5
Diisopropyl sulfate	167 [M-15]	7.51	32363	7.51	ND	7.52	33421	103.3

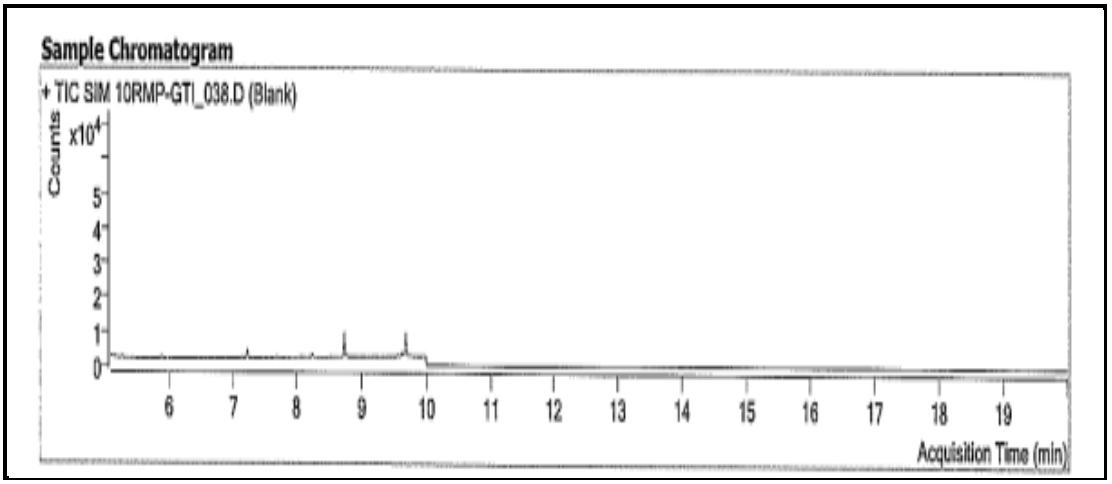


Figure 4(a): Typical GC-MS chromatogram of Blank solution

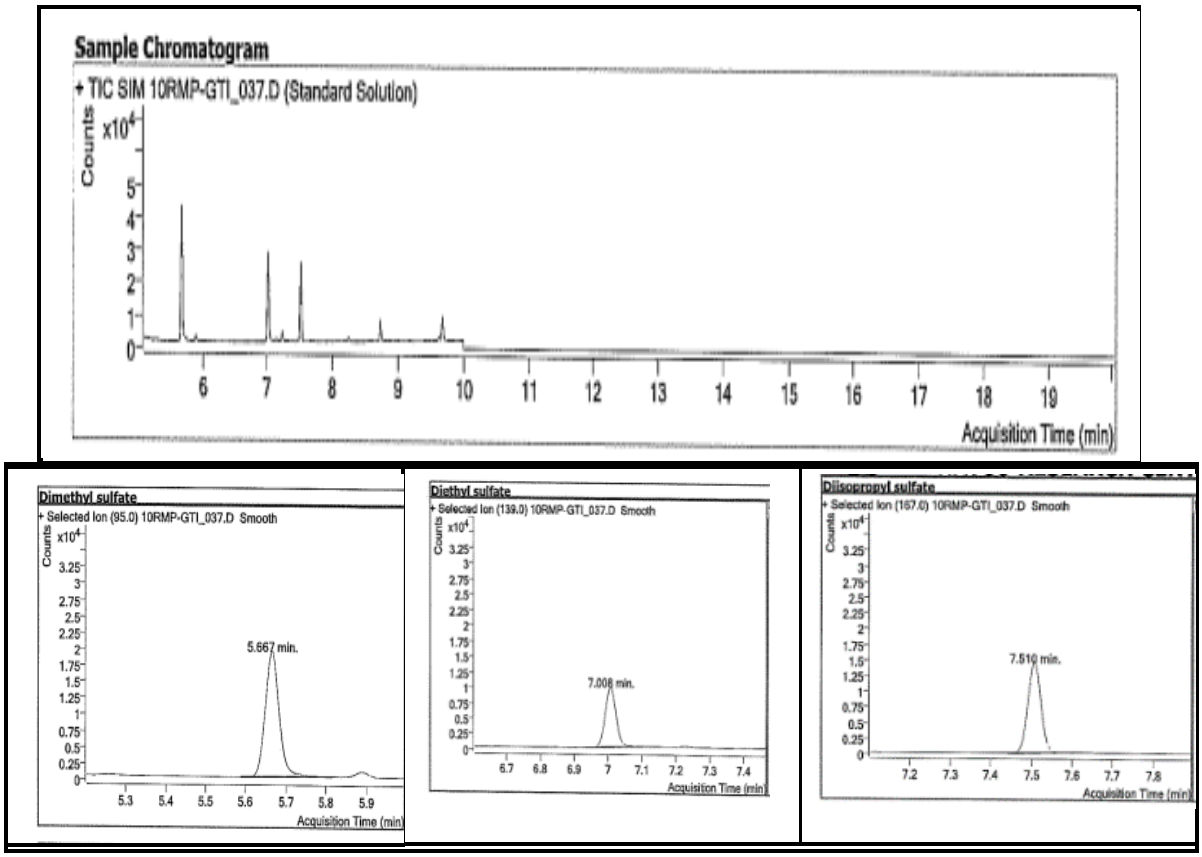


Figure 4(b): Typical GC-MS chromatogram of Standard solution

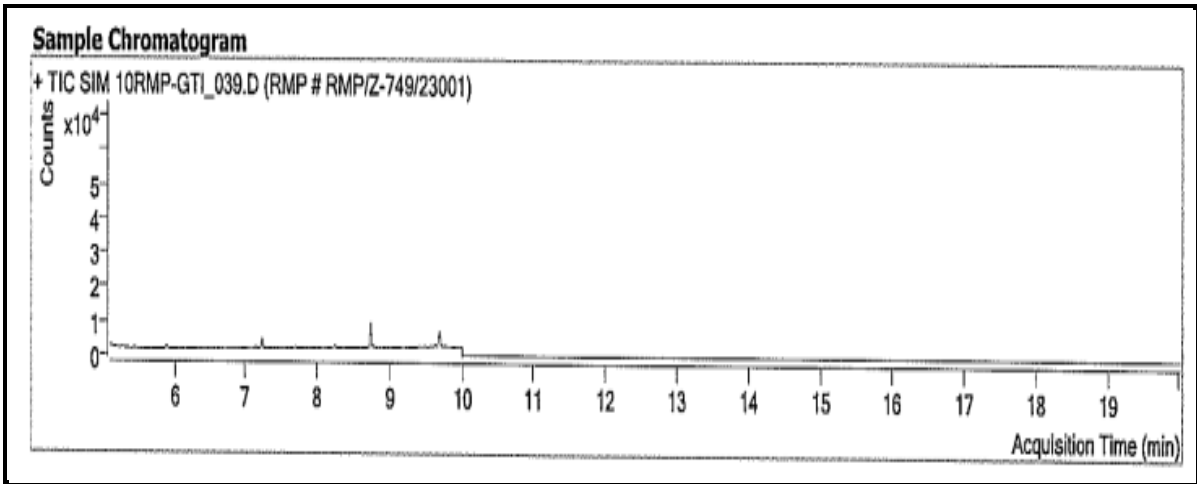


Figure 4(c): Typical GC-MS chromatogram of Rimegepant sulfate drug substance (as such sample)

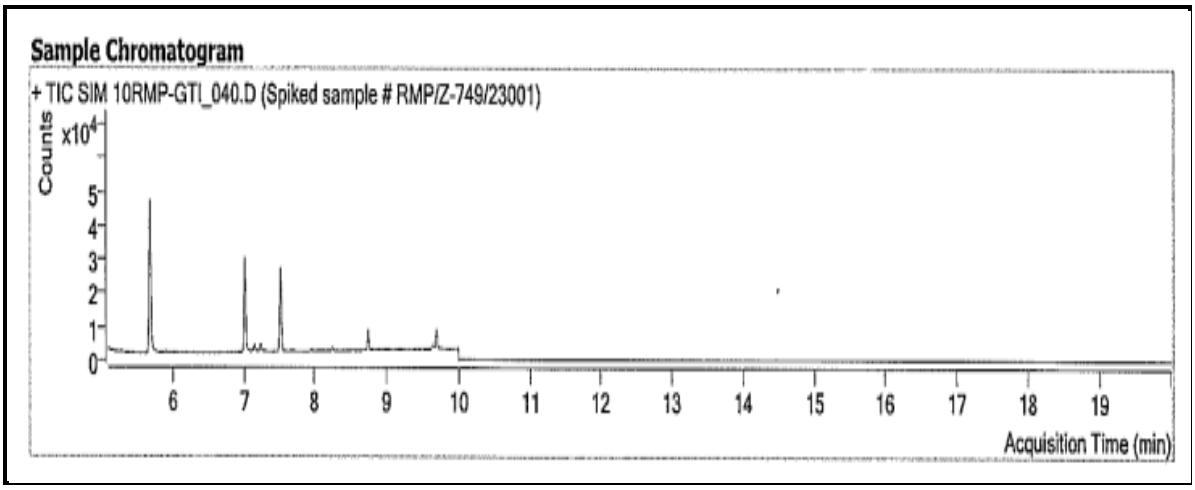
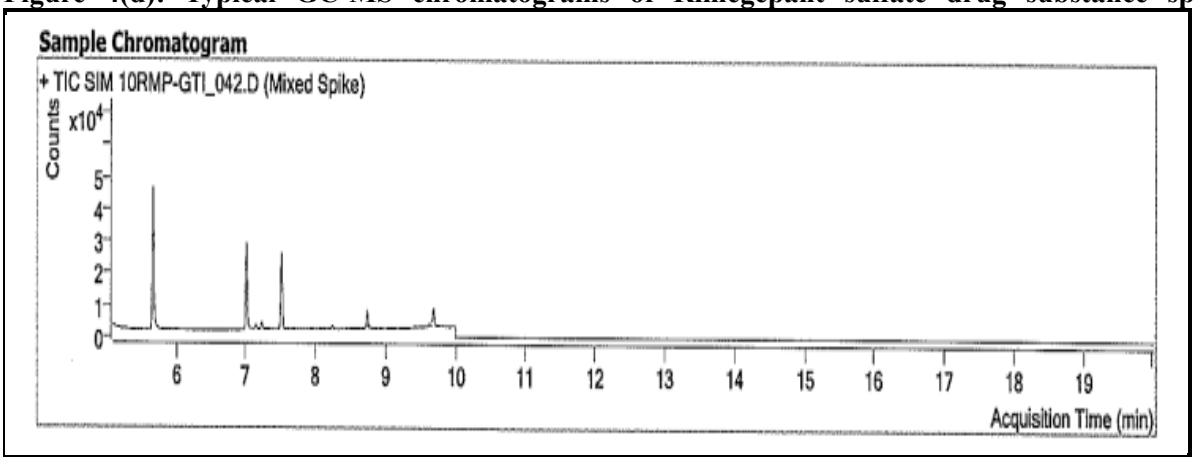


Figure 4(d): Typical GC-MS chromatograms of Rimegepant sulfate drug substance spi



ked with

DMS, DES and DIPS (spiked sample)

Figure 4(e): Typical GC-MS chromatograms of Rimegepant sulfate drug substance spiked with DMS, 3-DES and DIPS including all residual solvents (all spiked sample)

Limit of detection and Limit of quantification

The sensitivity of an analytical method was demonstrated in terms of the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ concentrations were estimated in this method, Specification level standard solution was injected in to GC-MS and S/N ratio for all analytes were recorded. Based on these values, the LOD and LOQ values of DMS, DES and DIPS were predicted. At LOQ level S/N ratio was $\geq$ 10 and LOD level S/N ratio was $\geq$ 3 for all analytes. Each predicted concentration was verified for precision by preparing the solutions containing DMS, DES and DIPS about its detection limit and quantification limit concentrations. The LOD and LOQ solutions were injected six replicates into GC-MS. The % RSD values for the precision of DMS, DES and DIPS were

ranged from 0.8 to 4.9 and 1.6 to 2.3 for LOD and LOQ respectively. The details of the précised LOD and LOQ values are shown in Table-6. The overlaid GC-MS chromatograms of LOD solution and LOQ solution are shown in Fig.4 (a) and 4 (b).

Table6: LOD and LOQ Precision

Name of the impurity	Average peak area		S/N Ratio		% RSD		Concentration (ppm)	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Dimethyl sulfate	1487	4332	4.0	12.2	0.8	2.1	0.42	1.27
Diethyl sulfate	1276	3679	4.3	11.7	4.9	2.3	1.32	3.99
Diisopropyl sulfate	1248	3712	3.2	10.4	1.8	1.6	0.96	2.92

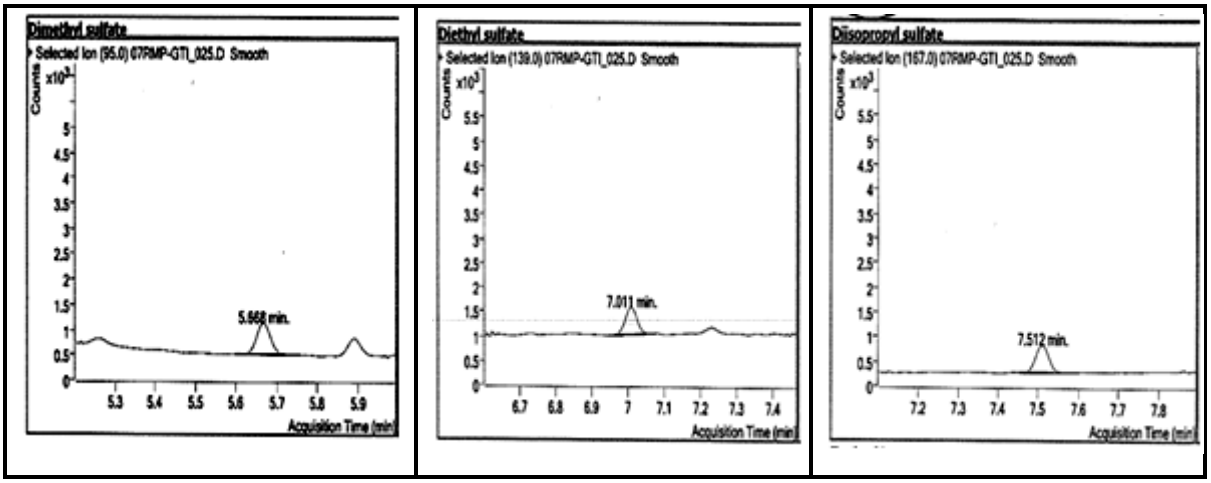


Fig.5 (a):Typical GC-MS chromatogram of LOD solution

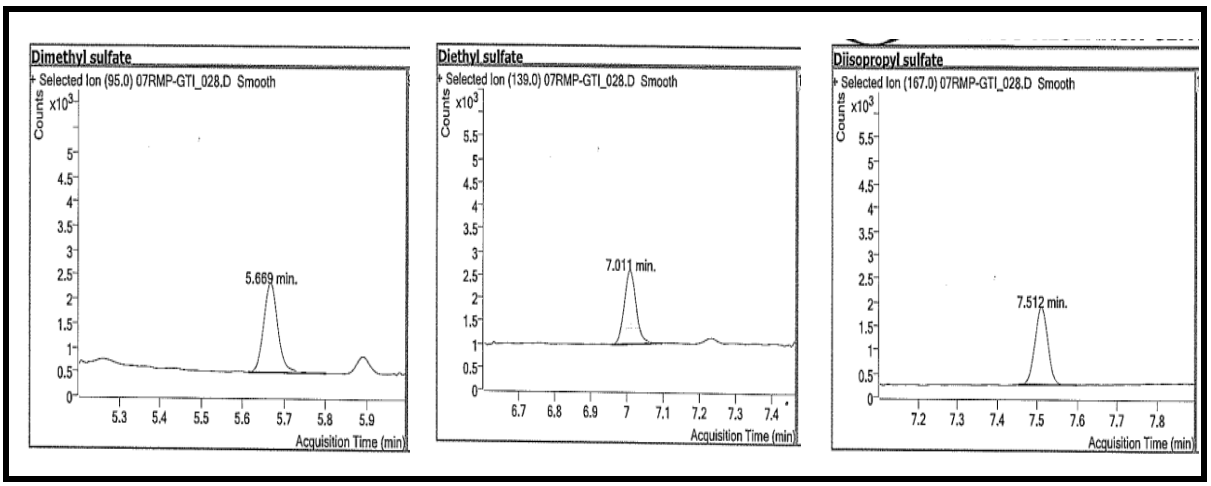


Fig.5 (b): Typical GC-MS chromatogram of LOQ solution

Linearity and Range

The linearity was evaluated by measuring the response of DMS, DES and DIPS seven different concentrations (LOQ, 30%, 50%, 75%, 100%, 125% and 150%) were prepared across the range concentrations were studied in the range of LOQ to 150% of the specification level. The derived correlation coefficients were in the range of 0.9974–0.9981 indicating the best fitness of the linearity curves of the developed method. The calculated statistical results are shown in Table-7.

Table7: Linearity

Name of the impurity	Conc. range (ppm)	Slope	Intercept	% y-Intercept	Correlation coefficient	Residual sum of squares
Dimethyl sulfate	1.27 - 29.66	2266.1190	41.2218	0.10	0.9974	0.9947
Diethyl sulfate	3.99 - 30.05	1048.1047	-667.7991	-3.29	0.9979	0.9958
Diisopropyl sulfate	2.92 - 29.47	1601.8926	-973.2495	-3.16	0.9981	0.9962

Accuracy

Accuracy experiment was performed by spiking the known amounts of DMS, DES and DIPS at LOQ level, 50%, 100% and 150% levels (with respect to 20 ppm limit) into Rimegepant sulfate drug substance. In the accuracy experiment, Rimegepant sulfate sample solutions (control sample) were prepared without spiking any impurity in triplicate and injected into GC-MS. Further, Rimegepant sulfate sample solutions (spiked sample) were prepared in triplicate by spiking with the all the impurities (DMS, DES and DIPS) at LOQ level, 50% level (10 ppm), 100% level (20 ppm) and 150% level (30 ppm) and injected into GC-MS. Control samples, Spiked samples were analysed and the percentage recoveries were calculated. The average % recovery values of four levels (LOQ, 50%, 100% and 150% levels) for twelve determinations for 105.0 (DMS), 104.4 (DES) and 105.6 (DIPS). The complete validated accuracy results are shown in Table-8.

Table8: Accuracy

	Dimethyl sulfate				Diethyl sulfate				Diisopropyl sulfate			
Accuracy parameter	LOQ level	50% level	100% level	150% level	LOQ level	50% level	100% level	150% level	LOQ level	50% level	100% level	150% level
Amount added (ppm)	1.26	9.85	19.63	29.44	3.98	10.00	19.92	29.87	2.91	9.79	19.50	29.25
Amount found (ppm)	1.43	10.22	18.89	31.38	4.38	10.53	19.34	31.39	3.20	10.50	19.23	31.10
% Recovery	113.2	103.8	96.2	106.6	110.0	105.3	97.1	105.1	110.0	107.3	98.6	106.3
%RSD	1.5	1.7	1.9	8.3	0.5	0.8	2.0	5.2	1.1	1.4	2.2	5.0
Overall % recovery	105.0				104.4				105.6			

Precision

The system precision has been demonstrated by injecting six replicate injections of standard solution into the GC-MS system and the relative standard deviation results obtained for the system precision experiment were 1.3 (DMS), 1.6 (DES), 1.3 (DIPS) respectively. Repeatability (Method Precision) experiment was performed by prepared six sample solutions were using single batch of Rimegepant sulfate drug substance spiked with DMS, DPS and DIPS about known concentration (20 ppm) level and injected into GC-MS. The relative standard deviation for the content results of the Method precision experiment 2.1 (DMS), 2.1 (DES) and 2.1 (DIPS). The intermediate precision was the inter-day variation (ruggedness) defined as the degree of reproducibility obtained by following the same procedure as mentioned for Method precision experiment. Ruggedness of the method was evaluated by preparing six individual sample preparations (same sample which was used in Method precision experiment) by spiking DMS, DES and DIPS to Rimegepant sulfate drug substance and injected into different column, different instrument and different analyst on different days. The achieved precision (System precision, Method precision and Intermediate precision) experiment results are shown inTable 9 and Table 10.

Table9: System Precision

Sample ID	Dimethyl sulfate	Diethyl sulfate	Diisopropyl sulfate
Injection-1	37863	19052	29211
Injection--2	38645	19385	29527
Injection-3	37677	18529	28575

Injection-4	37515	18871	28990
Injection-5	37173	18708	28630
Injection-6	37598	18808	28721
Mean	37745	18892	28942
S.D.	496	297	374
% RSD	1.3	1.6	1.3

Table10.Method Precision (MP) and Intermediate Precision (IP)

Sample ID	Dimethyl sulfate		Diethyl sulfate		Diisopropyl sulfate	
	MP (ppm)	IP (ppm)	MP (ppm)	IP (ppm)	MP (ppm)	IP (ppm)
Sample-1	18.93	19.50	19.28	20.04	19.06	19.54
Sample-2	18.53	19.23	18.98	19.70	18.92	19.48
Sample-3	19.21	19.77	19.77	20.13	19.72	19.61
Sample-4	18.98	19.79	19.34	20.17	19.23	19.85
Sample-5	19.18	19.80	19.28	19.97	18.98	19.51
Sample-6	19.77	20.79	20.08	20.43	19.86	19.65
Mean	19.10	19.81	19.46	20.07	19.30	19.61
S.D.	0.41	0.53	0.40	0.24	0.40	0.13
%RSD	2.1	2.7	2.1	1.2	2.1	0.7
Overall statistical data						
Mean	19.46		19.76		19.45	
S.D.	0.58		0.45		0.33	
% RSD	3.0		2.3		1.7	

Solution stability

Standard solutions, controlled sample and sample spiked with Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate impurities at 20 ppm was prepared and kept at room temperature as well as in the refrigerator at 2-8 ºC. The results indicates that Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate in standard solution, the sample solution and spiked solution were stable upto 24 h at 2-8ºC as well as at room temperature. The results were statistically identical with the initial value without measurable loss and more over % difference between the standard and spiked sample areas of each impurity was below the 10.

CONCLUSION

A GC-MS method has been developed, optimized and validated for the determination of Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate in Rimegepant sulfate (RMP) drug substance. The results from the validation experiments proved that the optimized method was specific, sensitive, linear, precise, accurate, robust and rugged for the determination of three potential genotoxic impurities (PGI's)

namely Dimethyl sulfate, Diethyl sulfate and Diisopropyl sulfate in Rimegepant sulfate (RMP) drug substance and can be used in the routine

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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All authors contributed equally to the conception, analysis, writing, and revision of the manuscript and approved the final version.

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