IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2018 Vol.:12, Issue:2

Trace Level Quantification of Diisopropyl Sulfate (PGI) in Abacavir Sulfate Drug Substance by Gas Chromatography with Mass Spectrometry (GC/MS)

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

K. Durga Raja[1], V.Saradhi Venkata Ramana[1], K.Raghu Babu [2], B. Kishore Babu[2], Vundavilli Jagadeesh Kumar [1*], K. S. R. Pavan Kumar [1], Hemant Kumar Sharma[1]

[1] Analytical Research Department, Aurobindo Pharma Limited Research Centre-II, Indrakaran (V), Kandi (M), Sangareddy Dist-502329, Telangana, India.

[2] Department of Engineering Chemistry, Andhra University, Vishakapatnam-530003, Andhra Pradesh, India.

Submission: 20 April 2018
Accepted: 27 April 2018
Published: 31 May 2018

Keywords: Abacavir Sulfate, Diisopropyl sulfate, GC/MS, Genotoxic or Mutagenic impurity

ABSTRACT

Simple and sensitive Gas chromatography with mass spectrometry (GC/MS) method was developed, optimized and validated for the determination of Mutagenic impurity i.e Diisopropyl sulfate (DPS) in Abacavir sulfate drug substance. The lower level of detection was achieved on Capillary GC column (DB-1, Fused silica capillary column; 30 m length; 0.32 mm internal diameter, coated with 100% dimethyl polysiloxane stationary phase of 1.0 µm film thickness with Electron Impact ionization (EI) in Selective Ion Monitoring (SIM) mode. The developed method was validated for specificity, linearity, accuracy, and precision. The detection limits of DPS obtained is 0.15µg/g. The method was found to be linear in the range between 0.5µg/g and 3.8 µg/g with correlation coefficient 0.9933. The average recovery range obtained for this impurity was 105.1%. The detail experimental approach is explained in this research paper.

HUMAN JOURNALS
www.ijppr.humanjournals.com

1.0 INTRODUCTION

Abacavir sulfate is chemically known as (1*S*,4*R*)-4-[2-amino-6-(cyclopropyl amino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol sulfate (2:1), it is a nucleoside reverse transcriptase inhibitor [1]. It is used for the treatment of acquired immunodeficiency syndrome (AIDS) caused by Human immunodeficiency virus type-1 (HIV-1) [2]. This nucleoside reverse-transcriptase inhibitor (NRTI) Abacavir sulfate drug is combined with Lamivudine, Tenofovir disoproxil fumarate, and Emtricitabine. These drugs are subjected to provide potent antiviral activity and are infrequently associated with mitochondrial toxic effects, lipoatrophy or neuropathy [3-6]. The molecular weight of Abacavir sulfate is 670.76 and the molecular formula is $(C_{14}H_{18}N_6O)_2.H_2SO_4$. It is marketed as a single dosage or formulated with other antiviral drugs like Lamivudine, Zidovudine etc. Numbers of FDA approved brands are available in the market, for example, Abacavir, Abacavir sulfate, and Lamivudine, Epzicom, Triumeq, and Trizivir etc. The chemical structure of Abacavir sulfate is shown in Figure 1.

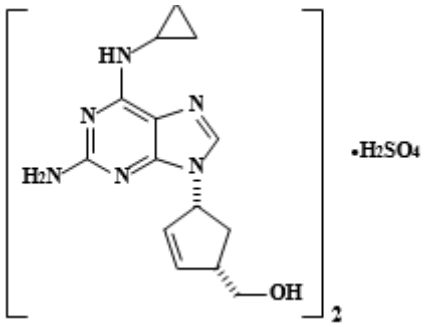


Fig. 1: Chemical structure of Abacavir sulfate

Compounds that are used in the synthesis of active pharmaceutical ingredients (API), or reaction byproducts that form during synthesis, have the potential to remain as impurities. Some of these compounds are potentially genotoxic impurities (PGI) and may raise concern about cancer and/or birth defects. To ensure that these undesired genotoxic impurities are reduced to an acceptable level. It is critical to monitor them with high accuracy throughout the completely technical manufacturing process and especially in the final product. In the last step synthesis of Abacavir sulfate, for the making of salt preparation, it may be a chance to form DPS, as isopropyl alcohol and sulfuric acid are used in this stage. Further, DPS is possibly carcinogenic to humans (group 2B) [7] and known mutagenic, which is under alkyl sulfates category [8-10] and having structural alert accordingly to QSARs Derek knowledge database. Further, in this step mono isopropyl sulfate impurity also remote chances to the

formation at trace levels. However, according to literature, the alkylating activity is dependent on the size of the alkyl group with the relative activity following the order methyl > ethyl > propyl > butyl. Beyond butyl, the activity is greatly minimized. For alkyl esters of dibasic (e.g., sulfate) and tribasic (e.g. phosphate) acids, the alkylating activity is completely eliminated if any of the alkyl groups are hydrolyzed (e.g., monoalkyl sulfate), in view of this information this impurity is non genotoxic in nature [11] and known safe levels have been established to human Hence, this impurity has not been considered for research work. This research paper describes a fast, reliable and validated GC-MS method that is capable of determining DPS in Abacavir sulfate drug substance.

Based on the current regulatory guidance's for genotoxic impurities, analytical methods should be developed to meet the required limit of 1.5mg/day daily intake of individual impurity [12]. This impurity limit is considered as 2.5 µg/g with respect to Abacavir sulfate maximum daily dose 600mg/day [13]. To the best of our knowledge, no GC, GC/MS or any other analytical technique methods are available in the literature for the trace level quantitative determination of this genotoxic impurity in Abacavir sulfate drug substance. Further, the method is validated to comply the requirements of ICH Validation guidelines [14]. The chemical structure of genotoxic impurity DPS is shown in Figure 2.

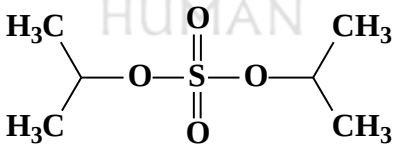


Fig. 2: Chemical structure of DPS

2.0 MATERIALS AND METHODS

Experimental

2.1 Chemicals, reagents, and samples

The investigated Abacavir sulfate drug substance was gifted from APL Research Centre laboratories (A division of Aurobindo Pharma Ltd., Hyderabad.), DPS obtained from TCI chemicals with 98.5% purity and water for chromatography lichrosolv purchased from MERCK, and Methylene chloride for analysis EMPART ACS.

2.2 Equipment

The gas chromatograph system with a mass spectrometer (GCMS), Agilent Technologies 7890B equipped with 5977A quadrupole mass selective detector (MSD) and GC sampler 80(Auto sampling unit). (Make: Agilent Technologies) was used. The data handling system, MASS HUNTER, version 0704 was used to monitor the output signals and for processing.

2.3 Chromatographic conditions

The analysis was carried out on Capillary GC column (DB-1, Fused silica capillary column; 30 m length; 0.32 mm internal diameter, coated with 100% dimethyl polysiloxane stationary phase of 1.0 µm film thickness) (Make: J & W Scientific, Santa Clara, CA, USA). Helium gas was used as carrier gas, column flow rate of 1.0ml/min with split 1:1 and maintaining column temperature as given below.

$$\text{Column oven temp.: } 100^{\circ}\text{C (3 min.)} \xrightarrow{20^{\circ}\text{C/min}} 240^{\circ}\text{C (5 min)}$$

The injector was maintained at 200^0C. The Ms. Source and Ms. Quad temperatures were kept at 230^0C and 150^0C respectively. MSD transfer line temperature was 250^0C and Dwell time (ms) 100. The data handling system Mass hunter was used to monitor the output signals and for processing. An aliquot of the sample or standard solution 2.0µL was injected. Ionization was carried out in the electron impact ionization mode (EI, 70 eV) and monitored in the selected ion monitoring (SIM) mode (Quantification ion m/z: 167& Qualifier ion m/z: 87) with low resolution.

2.4 Preparation of standard solution

2.4.1 Standard Stock solution

DPS standard solution is prepared by diluting DPS with Methylene chloride (approximately 6.4μg/mL) further it has been diluted with Methylene chloride for a stock standard solution, finally the standard solution consists approximately 0.25µg/mL. The prepared standard solution keeps in a cool place. Transfer 3 ml of standard stock solution (0.25µg/mL) into a clean and dry glass centrifuge tube add 15 ml of water and vortex the centrifuge tube for 1 min. Allow the two phases to separate. Collect the lower organic layer and transfer it into a 2 ml vial for injection.

2.4.2 Sample solution

Accurately weigh and transfer about 300 mg sample into a clean and dry centrifuge tube, add 15 ml of water to dissolve the sample. Then add 3 ml of Methylene chloride and vortex the centrifuge tube for 1 min. Allow the two phases to separate. Collect the lower organic layer and transfer it to a 2 ml vial for injection.

2.4.3 Blank solution

Transfer 15 ml of water into a clean and dry glass centrifuge tube, add 3 ml of Methylene chloride and vortex the centrifuge tube for 1 min. Allow the two phases to separate. Collect the lower organic layer and transfer it into a 2 ml vial for injection.

2.4.4 Method development and optimization

For this research work, initially, trails were performed on GC FID detector for the trace level determination. However, due to low response of DPS peak in FID detector, GC/MS technique has been chosen, as DPS peak is not detected at specification levels. However, the response is observed at higher levels only. It is evident that mass spectroscopy detectors including electron impact(EI) or Chemical ionization(CI) operating in the SIM mode offer the more sensitive and selective detection(compound specific) in most of the GC methods. The GC/MS analysis for DPS is performed on an Agilent (Model No: 7890B / 5977A) by using a DB-1 capillary column with a dimension of 30m×0.32mm ID×1.0µm film thickness. Due to its non-polar stationary phase (100% dimethyl polysiloxane), this column has been chosen, since it is better to retain DPS with good peak shape and resolves with other peaks. Further hard ionization technique (EI) mode is selected; because of selective ion, monitoring (SIM) is employed. Trails were performed with low boiler solvents like methanol, methylene chloride, methyl tertbutyl ether, cyclohexane, and acetone to get the maximum response for DPS. However, abacavir sulfate drug substance has not dissolved in any of these solvents. Hence, to overcome this solubility issue Liquid-Liquid extraction technique has been chosen. Moreover, in this extraction technique sample matrix interference is less and no need to clean inlet port often. In extraction technique, a mixture of water and Methylene chloride solvents has been chosen since drug substance is soluble in a mixture of water and Methylene chloride and has better polarity. DPS standard solution is prepared by diluting DPS with Methylene chloride (approximately 6.4μg/mL) is injected through the auto-injector into GC/MS(in

SCAN mode). After taking acquisition target analyte DPS was extracted by Software [(mass-to-charge ratio(m/z)]. The DPS GC/MS spectrum was shown in Figure 3.

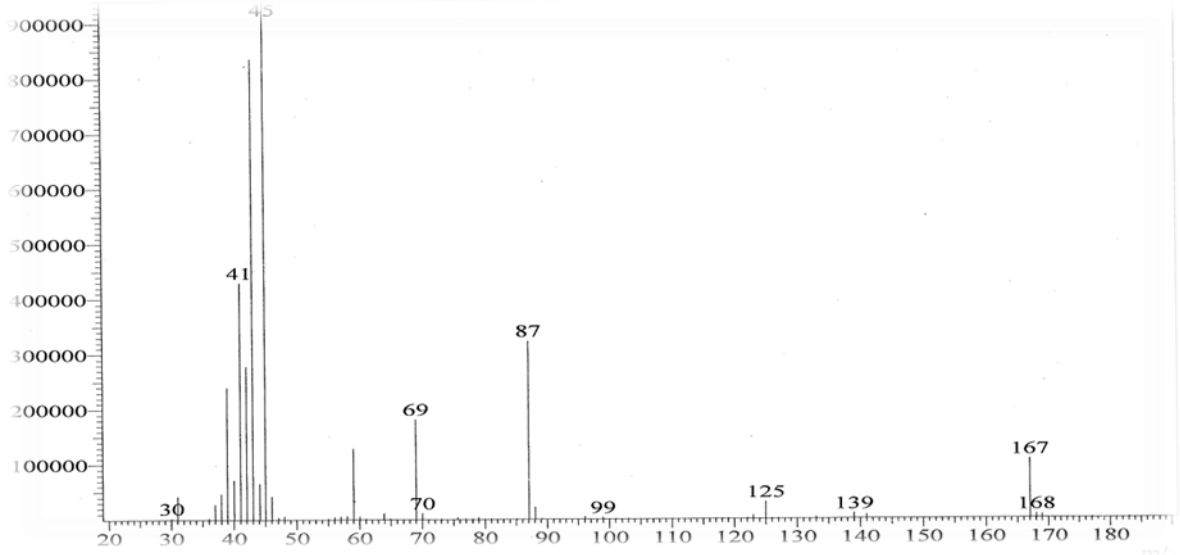


Fig. 3: GC mass spectrum of DPS

The Quantification and Qualifier ions were selected m/z-167 and m/z-87 respectively. Finally, the standard solution consists approximately 0.25µg/mL was prepared and transferred 3 ml into a clean and dry glass centrifuge tube added 15 ml of water and vortex the centrifuge tube for 1 min to separate for two phases in liquid-liquid extraction. Collected the lower organic layer, transfer it into a 2 ml vial, and then allowed it for acquisition. In the same manner, sample and recovery have been attained at desired specification level. Column temperatures & split modes were modified to get better DPS peak shape & optimized based on boiling point of DPS and its response. Finally, the optimized method was validated as per International Conference on Harmonization (ICH) guidelines [14].

3.0 RESULTS AND DISCUSSION

3.1 Method validation

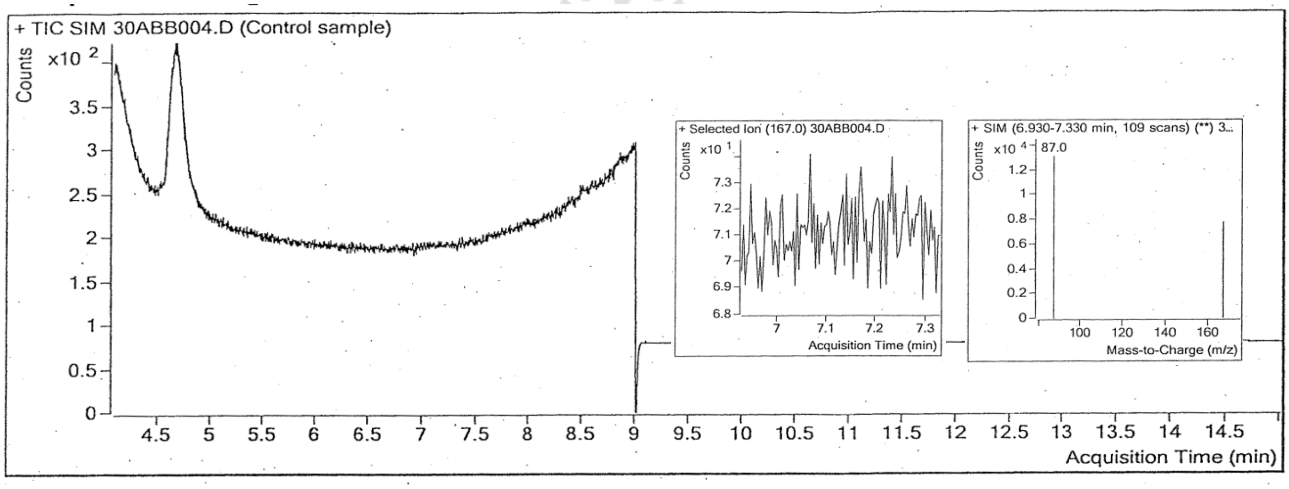
3.1.1 Specificity

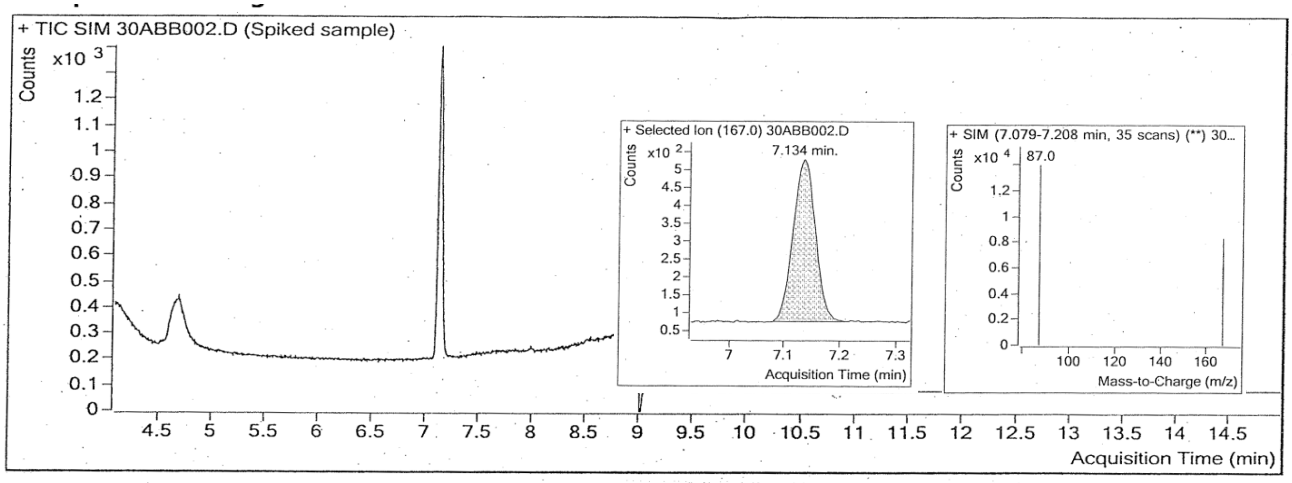
As per ICH guidelines, specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the developed GCMS method was verified in presence of residual solvents like Ethanol, Ethyl acetate, Isopropyl alcohol and cyclopropylamine, which were used in the Abacavir sulfate

process. Abacavir sulfate sample solution (control sample), Abacavir sulfate drug substance spiked with DPS at specification level (Spiked Sample) and Abacavir sulfate drug substance spiked with DPS and all other known residual solvents at specification level (All Spiked Sample) were injected into GCMS to confirm any co-elution of DPS and with any other known residual solvents. Typical GCMS spectrograms of a control sample, spiked sample, and all spiked sample are shown in Figure.3. Specificity results are shown in Table.1 and these experimental results indicating that DPS peak is homogeneous from all other known residual solvents.

Table 1: Specificity experiments results

Sample	DPS Response (counts/area)	DPS content (µg/g)
Control sample	Not detected	Not detected
Spiked sample	1266	2.5
All spiked sample	1251	2.5





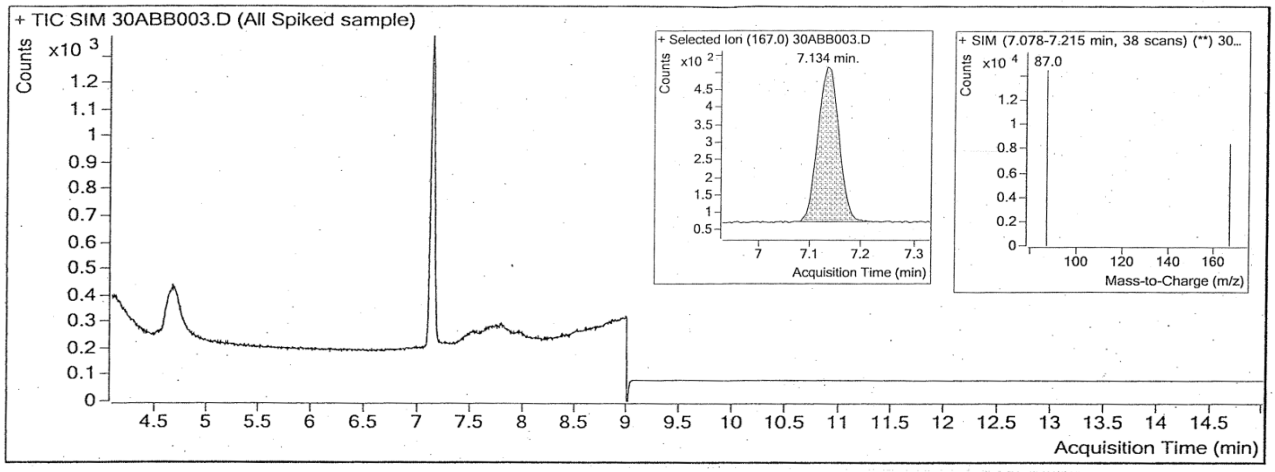


Fig. 4: Typical GCMS spectrograms of the control sample, spiked sample, and all spiked sample

3.1.2 Limit of detection and Limit of quantification/ Linearity

The limit of detection (LOD) and limit of quantification LOQ) values of DPS was determined using visual evaluation. The predicted concentrations of LOD and LOQ of DPS was verified for precision by preparing the solutions containing at about predicted concentrations and injected each six times into GCMS and calculating the %RSD of peak areas. The series of solutions were prepared using DPS at concentration levels from LOQ to 150% of specification level (2.5µg/g) and each solution was injected and calculating the statistical values like slope, intercept, STEYX and correlation coefficient from linearity plot drawn for concentration versus area. The statistical experimental values are shown in Table 2 and Linearity plot has been presented in Figure 5.

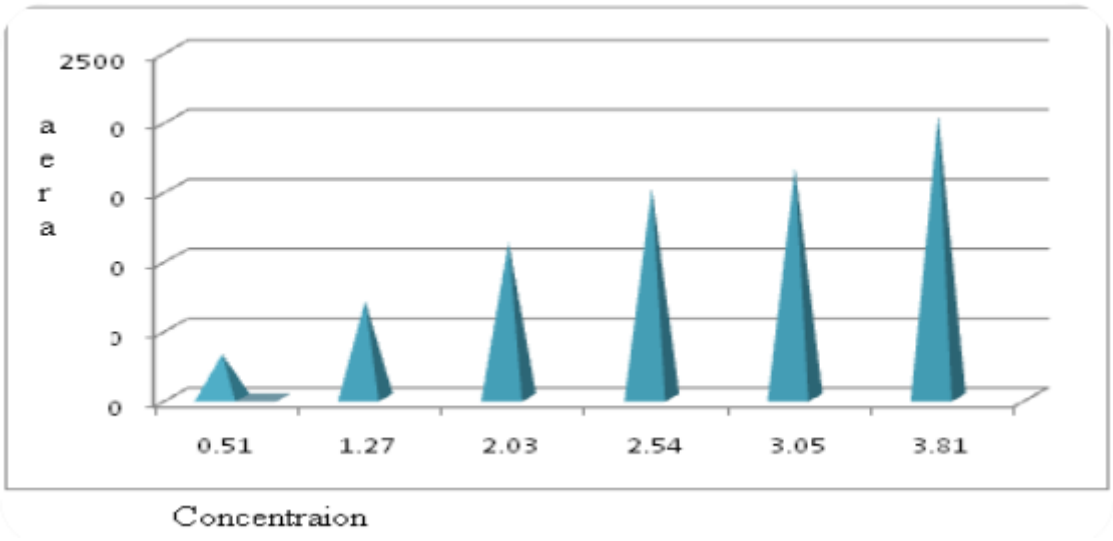


Fig. 5: Linearity plot between concentration and area

Table 2: LOD/LOQ and Linearity experiments results

Statistical parameters	Results
Correlation coefficient	0.9933
Concentration range (µg/g)	0.51 – 3.81
Calibration points	6
Intercept	51.789
Slope(S)	534.1612
Limit of detection(µg/g)	0.15
Limit of quantification(µg/g)	0.51
Precision for Limit Of Detection (%R.S.D)	2.1
Precision for Limit Of Quantification (%R.S.D)	0.9

3.1.3 Precision

The system precision of the method was checked by injecting standard solution for six replicates and method precision was checked by preparing the six individual sample solutions by spiking the DPS at specification level (2.5µg/g) to the drug substance and injected into GCMS. The results of system precision experiment and method precision experiment are shown in Table 3.

Table 3: Precision experiments results

System Precision	**Injection**	**DPS Area**	**Mean**	**SD**	**%RSD**	**95% Confidence interval (±)**
	1	1405	1407	31	2.2	3.3
	2	1357				
	3	1443				
	4	1387				
	5	1422				
	6	1429				
Method Precision	**Sample**	**DPS (µg/g)**	**Mean**	**SD**	**%RSD**	**95% Confidence interval (±)**
	1	2.66	2.81	0.09	3.2	0.09
	2	2.76				
	3	2.86				
	4	2.78				
	5	2.91				
	6	2.86				

3.1.4 Accuracy

To prove the recovery for developed GCMS method, standard addition experiments were conducted in triplicate preparations (Abacavir sulfate drug substance sample solutions were prepared in by spiking with DPS) at LOQ, 50%, 100% and 150% of specification level and recoveries of DPS was determined. The obtained recovery values lie between 102.0 and 109.1 shows method is accurate. The accuracy experiment results are reported in Table 4.

Table 4: Accuracy experimental results

Level	Amount added (µg/g)	Amount found (µg/g)	% Recovery	Mean	SD	%RSD
LOQ-1	0.52	0.53	101.9	105.1	2.96	2.8
LOQ-2	0.52	0.56	107.7			
LOQ-3	0.52	0.55	105.8			
50% level-1	1.29	1.28	99.2	102.0	3.13	3.1
50% level-2	1.30	1.37	105.4			
50% level-3	1.30	1.32	101.5			
100% level-1	2.59	2.66	102.7	106.4	3.86	3.6
100% level-2	2.60	2.76	106.2			
100% level-3	2.59	2.86	110.4			
150% level-1	3.89	4.52	116.2	109.1	6.32	5.8
150% level-2	3.89	4.05	104.1			
150% level-3	3.88	4.15	107.0			

4.0 CONCLUSION

A reliable and sensitive validated GCMS method for the determination of Diisopropyl sulfate in Abacavir sulfate drug substance is presented. Based on validation data, it is concluded that method is Specific, Sensitive, Linear, Precise, Accurate and Suitable. Hence, GCMS method can be employed in the routine analysis.

5.0 ACKNOWLEDGEMENTS

The authors gratefully acknowledge the management of APL Research Centre-II (A Division of Aurobindo Pharma Ltd.), for allowing us to carry out the present work. The authors are also thankful to the colleagues of Analytical Research Department and Chemical Research Department for their cooperation.

6.0 REFERENCES

[1] De Clercq E. New anti-HIV agents and targets. Med Res Rev. 2002;22(6):531–565.
[2] Pomerantz RJ, Horn DL. Twenty years of therapy for HIV-1 infection. Nat Med. 2003; 9(7): 867-873.
[3] Paul ES, Camlin T, Ann CC, Margaret AF et al. Abacavir-Lamivudine versus Tenofovir-Emtricitabine for initial HIV-1 Therapy. N Engl J Med. 2009;361:2230-2240.

[4] DeJesus E, Herrera G, Teofilo E et al. Abacavir versus zidovudine combined with lamivudine and efavirenz, for the treatment of antiretroviral-naive HIV-infected adults. Clin Infect Dis. 2004;39(7):1038-1046.
[5] Gallant JE, Staszewski S, Pozniak AL et al. Efficacy and safety of Tenofovir DF vs Stavudine in combination therapy in antiretroviral-naive patients, a 3-year randomized trial. JAMA. 2004; 292(2):191-201.
[6] Gallant JE, DeJesus E, Arribas JR et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med. 2006;354(3):251-260.
[7] Diisopropyl sulfate, 1992 IARC monograph
[8] Alexander A, Lisa B, Joel B, Dave B, et al. Principles and procedures for implementation of ICH M7 recommended (Q) SAR analyses. Regulatory Toxicology and Pharmacology. 2016; 77:13-24.
[9] Ashby J, Tennant, RW. Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. Mutat Res. 1988;204(1):17-115.
[10] Hoffmann GR. Genetic effects of dimethyl sulfate, diethyl sulfate, and related compounds. Mutat Res. 1980;75(1):63-129.
[11] David Y. L and Yin Tak. W Reducing Carcinogenicity and Mutagenicity through Mechanism Based Molecular Design of Chemicals, Part 9: Designing safer chemicals
Hand Book of Green Chemistry, 2010, Online ISBN: 9783527628698, DOI: 10.1002/9783527628698
[12] International Conference on Harmonization of technical requirements for registration of Pharmaceuticals for human use, M7, Assessment and control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to limit potential carcinogenic risk, 2014.
[13] EPZICOM, US FDA drug label, VIIV Healthcare.
[14] International Conference on Harmonization of technical requirements for registration of Pharmaceuticals for human use, Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005.