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A CAPILLARY GC-MS ANALYSIS OF RESIDUAL ETHANESULFONATES IN TENELIGLIPTIN

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ABSTRACT
The presence of residual sulfonate esters in drugs is potentially genotoxic impurities (PGIs), thus demanding a need for a specific method to quantify them in the drug as per regulatory authorities' guidelines. Accordingly, a GC-MS procedure was optimized and proved for the assessment of ethyl ethanesulfonate (EES) and isopropyl ethanesulfonate (IPES) in the Teneligliptin drug. The detection (LOD) and quantification (LOQ) limit values of EES and IPES were 3.75 and 11.25 ppm, respectively. A linear relationship was observed between 11.25 and 56 ppm. The mean recoveries of EES and IPES were 102.65 and 102.21%. The method is precise, accurate, specific, and linear; thus it can be employed to measure the EES and IPES in Teneligliptin and other gliptins.
Keywords: Ethyl Ethanesulfonate, Isopropyl Ethanesulfonate, Sulfonate Ester, Teneligliptin, GC-MS.
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INTRODUCTION

During the manufacturing process of pharmaceuticals, the remaining chemicals could leach the products[1-3], which are essentially be evaluated and eliminated or controlled as much as possible, since they do not possess therapeutic benefits but many of them are unfortunately harmful.[4] Nevertheless, they cannot be removed completely by employing practical manufacturing techniques.[5-6] Similarly, the resulting Teneligliptin may consist of ethane sulphonic acid, (a carcinogenic chemical) and PGIs such as EES and IPES while using ethanol, isopropanol, and ethanesulphonic acid during the synthesis.[7] Since the existing reports are not specific to EES and IPES[8], some other reports on various sulfonate esters using GC or LC9-18 are either complicated, expensive, or insensitive. Hence, there is a need for a specific and sensitive procedure for the assessment of EES and IPES in the Teneligliptin drug (Fig.-1). Accordingly, we sought to develop a simple and cost-effective method for detecting these two impurities by an easy-to-use derivatizing agent potassium iodide to convert EES and IPES to ethyl iodide and isopropyl iodide (Fig.-2).

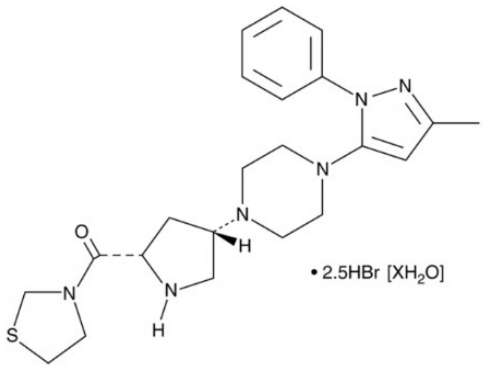

Fig.-1: Molecular Structure of Teneligliptin

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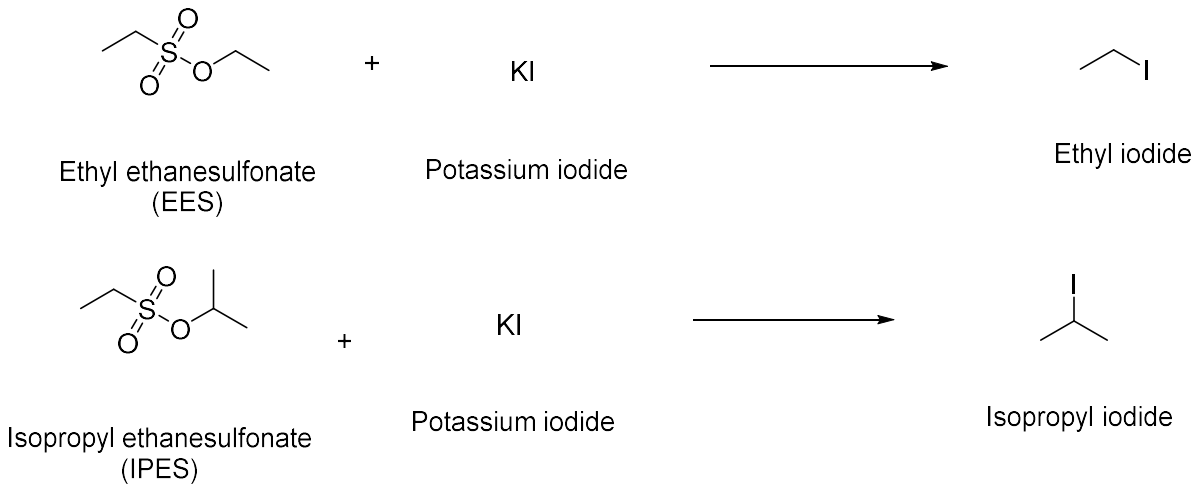


Fig.-2: Derivatization Reaction

EXPERIMENTAL

Materials

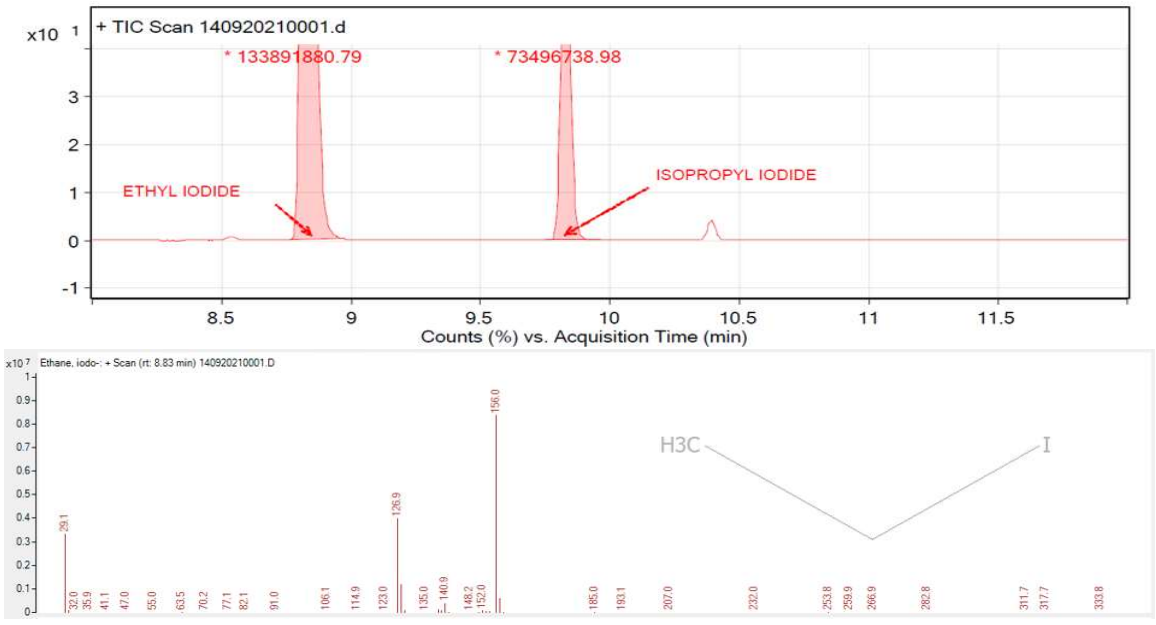
Ethyl and isopropyl ethanesulfonates and dichloromethane were procured from Sigma-Aldrich, India.

Instrumentation

The analysis was conducted using a GC system consists single quadrupole MS (Agilent 5977B GC/MSD, USA). Impurities were separated by DB-624 column (60 m length * 0.25 mm * 1.4 µm film thickness).

Optimized GC-MS Conditions

For optimization, a range of columns (HP-5, DB-1, DB-1701, and DB-624) with various thicknesses were employed. Columns with HP-5 (60 * 0.32 * 1.0) and DB-1 (60 * 0.25 * 0.25) expressed acceptable retention periods at lower temperatures. However, the stationary phases showed bleeding and the baselines shifted over time, the DB-624 (60 * 0.25 * 1.4) exhibited the best optimization conditions. The other optimized conditions are: initial temperature 50 °C, held 4 minutes, rose to 240 °C at 20 °C/min, held 10 minutes; split ratio 10:1; He was used as the carrier gas (flow rate: 1.0 mL/min). GC-MS interface, quad, and ion source temperatures were 200, 150, 200, and 250 °C; ionizing energy: 70 eV. The process of SIM ion selection was conducted by scanning EES and IPES from 29 - 150 Da using scan mode by selecting the stable ions at *m/z* 127, 156, and 170 (Fig.-3).



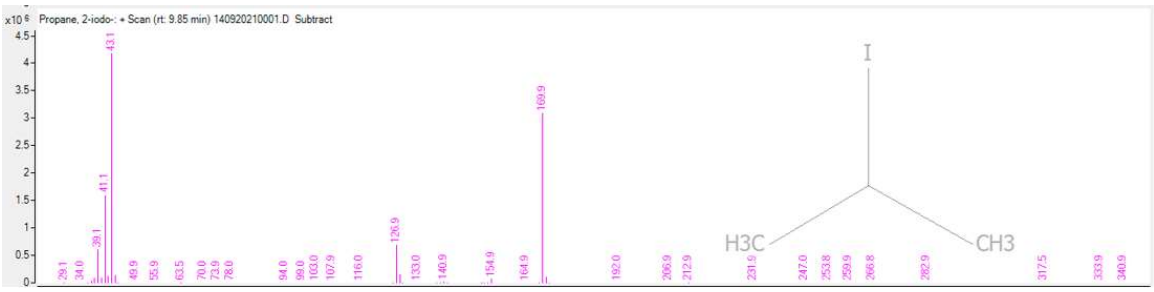


Fig.-3: GC-MS Pattern of EES and IPES Standards

Diluent-1: Dimethyl sulfoxide (DMSO)
Diluent-2: 40 g of potassium iodide in 100 mL Milli-Q water.

Blank Solution
The blank solution was prepared with 0.5 mL of each diluent 1 and 2 in a headspace vial and crimped the vial immediately (Fig.-4).

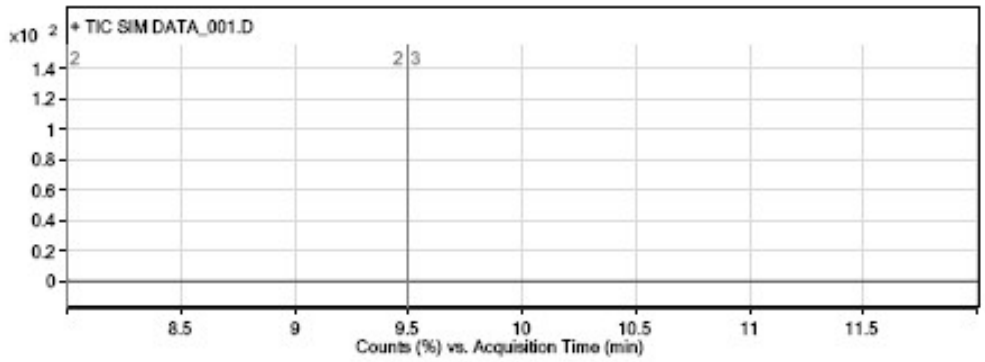


Fig.-4: Blank Chromatogram

Standard Stock Solutions
Stock Solution 1
About 75 mg of each standard (EES and IPES) were transferred to 50 mL standard flasks and diluted with diluent-1.

Stock Solution 2
1.0 mL Stock solution 1 was transferred into a 50 mL standard flask, diluted the contents to the mark with diluent-1. Further 1.0 mL of the above solution was pipetted out into a 20 mL standard flask and diluted the contents using diluent-1.

Standard Solution
In the headspace vial, 0.5 mL of diluent-1 and 0.5 mL of diluent-2 were transferred and crimped. The standard chromatogram is reproduced for easy perception (Fig.-5).

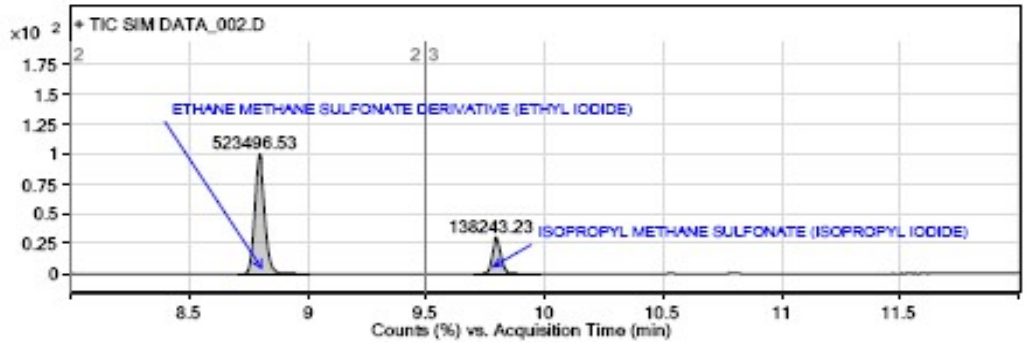


Fig.-5: Standard Chromatogram

Sample preparation
40 mg Sample was transferred in the 20 mL headspace vial and 0.5 mL of each diluent 1 and 2 were added and crimped immediately (Fig.-6).

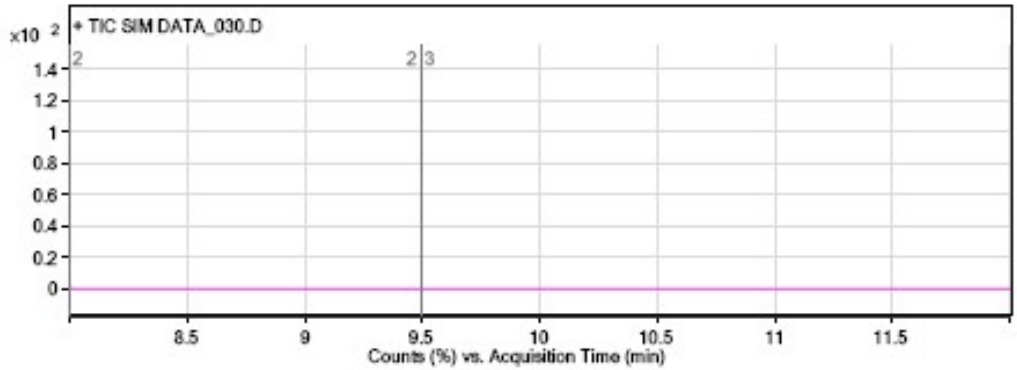


Fig.-6: Sample Chromatogram

RESULTS AND DISCUSSION

Method Validation
Based on the ICH and USFDA guidance for analytical procedures, the current procedure was validated.[19-20]

System Suitability
Various concentrations of EES and IPES solutions were introduced into the GC-MS and the outcome of all the study parameters are shown in Table-1. In this study, the % RSD of EES and IPES peak results from the standard solutions were found to be lesser than 15% of the acceptance criterion.

Table-1: System Suitability Data

	EES Response	IPES Response
Std-1	523496.53	188243.23
Std-2	562143.50	186350.31
Std-3	560295.87	188324.84
Std-4	559273.86	185971.42
Std-5	579843.78	182241.88
Std-6	591965.92	183062.31
Average	562836.58	185699.00
S.D.	23259.542	2560.093
% RSD	4.13	1.38

Specificity
Teneligliptin was subjected to a specificity study through the injection of solvents used during the method development. The results of the study indicate the absence of interference between the retention times of analyte components.

Limit of Detection and Quantification
The calibration curve method was used to determine the LOD and LOQ values and reproduced in Table-2. For EES and IPES, the LOD and LOQ values were 3.75 and 11.25 ppm, respectively. Based on the RSD peak areas found from LOQ preparations, it was 2.60 (EES) and 3.45% (IPES).

Table-2: LOQ level precision

	EES Response	IPES Response
Concentration	11.25 ppm	11.25 ppm
Std-1	160202.00	48337.61
Std-2	170471.38	52223.60
Std-3	168474.17	51898.94
Std-4	170394.84	52856.88
Std-5	171885.57	52893.02

Std-6	171575.45	53035.86
Average	168833.90	51874.32
S.D.	4395.128	1787.919
% RSD	2.60	3.45

Linearity

A stable baseline was conditioned for the system and column. Observations were recorded as per protocol. Linearity was observed from LOQ to 150% with correlation coefficients of 0.9969 (EES) and 0.9977 (IPES). The results are provided in Table-3.

Table-3: Linearity

	EES		IPES	
	Concentration (ppm)	Average Area	Concentration (ppm)	Average Area
Linearity-1 (QL)	11.25	165336.69	11.25	50280.61
Linearity-2 (50%)	18.75	310170.89	18.75	98339.96
Linearity-3 (75%)	28.13	449669.8	28.13	144105.02
Linearity-4 (100%)	37.5	582956.63	37.5	189981.97
Linearity-5 (125%)	46.88	732950.78	46.88	240819.85
Linearity-6 (150%)	56.25	825337.85	56.25	273845.95
Correlation coefficient	0.9969		0.9977	
Regression coefficient	0.9939		0.9954	
Slope	14720.113		4982.99	

Precision and Accuracy

Using the six replicate preparations of a standard solution containing 37.5 ppm of EES and IPES, the method precision was evaluated. Based on six replicates, the %RSD of EES and IPES were 2.56 and 3.31, respectively, and the RSD is much lower than the acceptance criteria of 15% as shown in Table-4. Hence, the method is precise. A recovery test was performed by spiking EES and IPES samples with 50, 100, and 150% of their calculated QL levels. The results are reproduced in Table-5. The recoveries of EES and IPES were observed between 80 and 120% for all precision levels. According to the accuracy value, the average recovery was well within the acceptable limits.

Table-4: Method Precision of EES and IPES

Preparation	EES Response	IPES Response
Quantity	37.5 ppm	3.75 ppm
Preparation-1	39.4	39.54
Preparation-2	39.38	39.49
Preparation-3	38.33	38.11
Preparation-4	41.41	42.17
Preparation-5	39.4	39.82
Preparation-6	39.96	40.17
Average	39.65	39.88
S.D.	1.01	1.32
% RSD	2.56	3.31

Table-5: Accuracy

Quantity	Recovery (%)	
	EES	IPES
QL level	100.59	96.53
50%	109.51	108.26
100%	105.73	106.36
150%	101.63	103.73
Mean	102.65	102.21

Robustness

Robustness was studied even by the effect of minor changes in temperature and flow rate of EES and IPES at 37.5 ppm. In Table-6, the summary of comparative standard deviations of the EES and IPES peak areas is provided for better perception. In the solution using each of the modified GC conditions, the RSD values were within 5% is an indication of this method's robustness.

Table-6: Robustness

EES							
	As per the method's idle condition	Oven temperature (50°C)		Injector temperature (200 °C)		Flow rate (1.0 mL/min)	
		45	55	180	220	0.9	1.1
%RSD	4.13	3.28	3.97	2.21	2.44	1.22	1.33
IPES							
	As per the method's idle condition	Oven temperature (50°C)		Injector temperature (200 °C)		Flow rate (1.0 mL/min)	
		45	55	180	220	0.9	1.1
%RSD	1.38	1.09	1.54	3.21	3.33	0.98	1.41

Mass Spectral Analysis

In GC-MS experiments, 8.8 and 9.8 minutes rt were observed for EES and IPES. For the EES derivative (ethyl iodide), a parent ion peak is observed at *m/z* 156, representing the molecular formula C_2H_5I. Major fragments also have peaks associated with mass values of 156 and 127. The parent peak of the molecular formula C_3H_7I can be seen in the mass spectrum of the IPES derivative (isopropyl iodide) is represented by *m/z* 170. Other major fragment peaks reflect masses of 170 and 127.

Method Recommendation

As an outcome of the optimized results, the following data (Table-7) is recommended for determining EES and IPES content by GC-MS.

Table-7: Recommended parameters of EES and IPES

Substance	LOD (ppm)	LOQ (ppm)	Retention time (minutes)
EES	3.75	11.25	8.8
IPES	3.75	11.25	9.8

CONCLUSION

A sensitive GC-MS method was reported to estimate EES and IPES in Teneligliptin through a simple derivatization method. From the validated parameters it is clear that the developed procedure is precise, reliable, linear, robust, and accurate. Hence, we recommend the developed method to determine the quantity of EES and IPES in Teneligliptin and a range of other gliptins as well.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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