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GC-MS METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF GENOTOXIC TOSYLATES IN TENELIGLIPTIN

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ABSTRACT

In this study, a specific and robust Gas Chromatography-Mass Spectrometry (GC-MS) method was meticulously developed, optimized, and validated for the precise determination of the genotoxic tosylate impurities in Teneligliptin. The method was optimized on an Agilent 5977B GC/MSD system equipped with a capillary column (HP-5MS, 30 m × 0.32 mm x 1.0 µm) using helium as the carrier gas. Detection of the impurities was achieved using selective ion monitoring mode at specific mass-to-charge ratios. The method excelled in validation for various parameters, including specificity, sensitivity, precision, linearity, accuracy, selectivity, and robustness. It demonstrated linear behavior in the concentration range of 0.54, 0.55, and 0.53 to 3.0 ppm with high correlation coefficients. The detection and quantitation limits of genotoxic tosylates for Teneligliptin were also determined.

Keywords: Toluene sulfonates, Tosylates, Teneligliptin, GC-MS, Validation.

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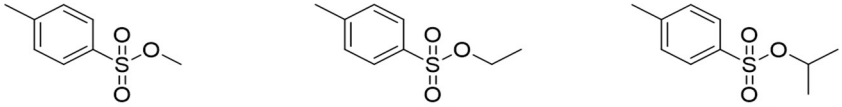
INTRODUCTION

The use of Teneligliptin has proven effective in the treatment of type 2 diabetes mellitus, as supported by various studies.[1-6] Teneligliptin operates by elevating the levels of naturally occurring substances known as incretins, which play a crucial role in regulating blood sugar by enhancing insulin release, particularly post-meal. The synthesis of drug substances typically involves multiple manufacturing steps. When elucidating the rationale for impurities, it is essential to consider the inclusion of raw materials, impurities arising from the manufacturing process, intermediates, and degradation products.[7-10] Throughout the manufacturing process of Teneligliptin drug substances, residual solvents and catalysts may be present, but the complete elimination of these contaminants from drug substances may not be achievable.[11] Toluenesulphonic acid, a known carcinogenic chemical, was utilized in the manufacturing process of the Teneligliptin API. In this preparation, process solvents (methanol, ethanol, and isopropanol) are employed, and there is a likelihood of these solvents forming respective tosylates, namely, methyl toluenesulfonate (MTS), ethyl toluenesulfonate (ETS), and isopropyl toluenesulfonate (ITS). The structure, molecular formula, and molecular weight are given in Fig.-1. It's important to note that MTS, ETS, and ITS are recognized as potential genotoxic impurities (PGIs). Considering the recommended daily maximum dose of 40 mg for Teneligliptin, specific benzene derivatives such as MTS, ETS, and ITS must be limited to less than 37.5 µg/g in the drug. Therefore, essentially, we need to have a suitable analytical method with a sensitive, precise, and accurate procedure for the quantitation of PGIs (MTS, ETS, and ITS) in drug substance samples.[12-14] For a better understanding of the earlier reports and to understand the importance of the proposed method, the most relevant methods' details are discussed in Table-1.



Structure of MTS
MF: $C_8H_{10}O_3S$
MW: 186.23 g/mol

Structure of ETS
MF: $C_9H_{12}O_3S$
MW: 200.26 g/mol

Structure of ITS
MF: $C_{10}H_{14}O_3S$
MW: 214.28 g/mol

Fig.-1: Molecular Formula and Molecular Structure of MTS, ETS, and ITS

Table-1: Comparison of Methods

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Method	Outcome
RP-HPLC[15-17]	Most of the reported methods control only the alkyl mesylates, not tosylates.
MS (HPLC-MS)[18]	Mostly, the reported procedures control only the alkyl mesylates.
HILIC-ESI-MS[19]	The method controls only the alkyl mesylates.
HPLC-MS/MS[20-22]	This method focused on one or two classes of sulfonate esters. However, efficiently quantified the alkyl mesylates but not effectively with the besylates and tosylates.
GC-MS[23-28]	The reported methods control only alkyl mesylates.
Proposed method	The limit of detection (LOD) and limit of quantitation (LOQ) values obtained are (0.18, 0.18, and 0.17 ppm) and (0.54, 0.55, and 0.53 ppm), respectively, for MTS, ETS, and ITS. It's a low-level control by the GC-MS method for Teneligliptin drug substance compared to the above methods on different sulfonates.

EXPERIMENTAL

Reagents and Materials
Ethyl acetate, MTS, ETS, and ITS were purchased from Spectrochem Pvt Ltd. (India).

Instrumentation
Agilent GC 7890B with mass detector.

Preparation of Solutions
Preparation of Blank Solution
Ethyl acetate was used as a diluent.

Preparation of Standard Stock Solutions
About 10 mL of diluent was added to a 100 mL volumetric flask. Then, 50 mg of MTS, ETS, and ITS were added to the flask and made up to volume with diluent. Further, 1.0 mL of this solution was diluted to 50 mL in a 50 mL standard.

Preparation of Standard Solutions
1.0 mL of the above diluted standard stock solution was further diluted in a 50 mL volumetric flask. The standard solution's chromatogram is shown in Fig.-2.

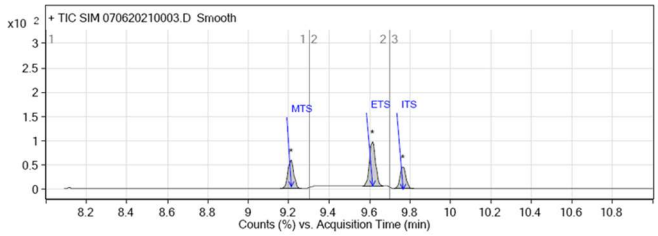

Fig.-2: Standard Chromatogram

Preparation of Test Solutions
About 500 mg of the Teneligliptin sample in a 5 mL standard flask was made up to the volume. The content was shaken well, filtered, and the solution was used for injection. (The final concentration: 100 mg/mL).

Method Development and Optimization
During the method development,[29-31] different chromatographic parameters were taken for optimization to obtain an acceptable peak shape with acceptable recoveries to satisfy the GC system suitability. These parameters include flow rate (1.0–1.5 mL/min, constant flow), initial column temperature (80–150 °C), and injector temperature (+10 °C to the column temperature). Also, a range of capillary GC columns (HP-5, DB-1, DB-1701, and DB-624 with different film thicknesses) was screened for the method optimization. Among them, the DB-624 (60 m length × 0.25 mm width × 1.4 µm film thickness) and DB-1 (60 m × 0.25 mm × 0.25 µm) columns gave reasonable retention times at lower temperatures. However, these stationary phases showed more bleed and had a baseline shift over the temperature range used. Satisfactory levels of selectivity, sensitivity, resolution, and chromatographic separation with a stable baseline were achieved only on DB-1701 (30 m × 0.25 mm × 1 µm) with helium as carrier gas. In the selective ion monitoring

(SIM) process, the MTS, ETS, and ITS were scanned from 29 to 150 Da range, and selected the major and more stable ions of MTS (*m/z* 155 and 186), ETS (*m/z* 91 and 200), and ITS (*m/z* 172 and 214). The total ion chromatogram (TIC) and mass spectrum are shown in Fig.-3 and 4, respectively. The optimized chromatographic details are briefed in Table-2.

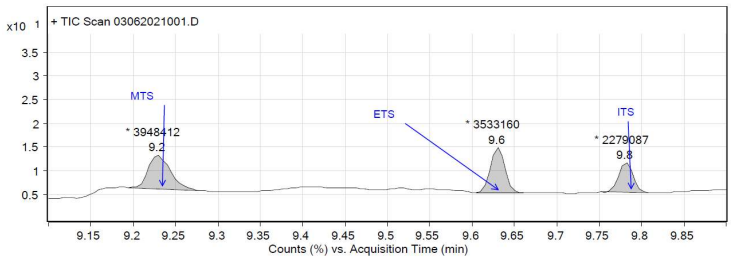

Fig.-3: TIC Scan of MTS, ETS, and ITS

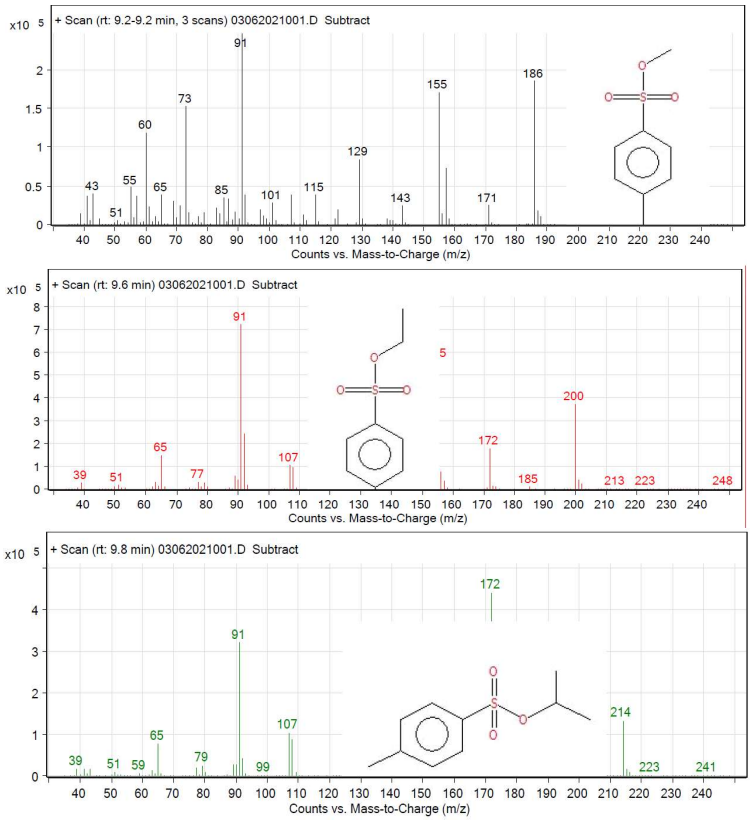

Fig.-4: Mass Spectra of MTS, ETS, and ITS

Table-2: Optimized Chromatographic Conditions for Tosylates

Parameter	Optimized condition
Column	HP-5MS, 30 m × 0.32 mm x 1.0 µm
Carrier gas	Helium
Diluent	Ethyl acetate
Flow rate	1.5 mL/min
Injector temperature	240 °C
Split ratio	5:1
Temperature Ramping	Initial temp.: 80 °C, halt: 2 min; ramping: 20 °C/min up to 260 °C, halt: 4 min
Injection volume	2.0 µL
Run time	15 min
Source temperature	230 °C
Auxiliary temperature	245 °C

Solvent delay	8.0 min
Detector off	11.0 min
Quad temperature	150 °C
SIM	MTS (*m/z* 155 and 186); ETS (*m/z* 91 and 200); ITS (*m/z* 172 and 214)

RESULTS AND DISCUSSION

System Suitability

The blank and six standard solutions were prepared and injected for system suitability. The relative standard deviation (RSD) is less than 10% as shown in Table-3.

Table-3: System Suitability

Preparation	MTS	ETS	ITS
STD-1	16755.56	25684.38	13104.33
STD-2	17321.00	26234.00	13205.00
STD-3	17383.00	26546.00	13113.00
STD-4	16844.00	25731.00	12613.00
STD-5	16948.00	26001.00	12761.00
STD-6	16964.00	25682.00	12667.00
Average	17035.927	25979.73	12910.56
SD	256.93	353.13	259.02
% RSD	1.5	1.4	2.0

Specificity

Based on the results, no interference was observed during the retention of analyte components. As prescribed, the ICH-listed solvents were prepared while the other solvents were at a 0.1 % level. Interestingly, none of the process solvents exhibited mass fragments of the selected ion, thus indicating the absence of interference on MTS, ETS, and IPS analytes.

Detection and Quantitation Limits

A progression of a low-level concentration of the impurities of MTS, ETS, and ITS solutions was prepared and injected into the GC-MS and recorded. From the obtained peak area results, the slope, intercept, and % RSD were calculated, and the LOD and LOQ were predicted. Six LOQ injections were performed into GC-MS, and the results, whereas three LOD injections were performed, and the obtained results are shown in Tables-4, 5, and 6. The LOD and LOQ were determined as 0.18, 0.18, and 0.17 ppm and 0.54, 0.55, and 0.53 ppm, respectively, for MTS, ETS, and ITS. The LOQ TIC is shown in Fig.-5.

Table-4: LOD Results

Injection No.	s/n		
	MTS	ETS	ITS
1	335	36	269
2	337	37	530
3	650	552	264
Mean	441	208	354

Table-5: LOQ Precision

Preparation	MTS	ETS	ITS
STD-1	4378	7084.00	3308
STD-2	4285	6964.00	3243
STD-3	4331	7077.00	3265
STD-4	4408	7185.00	3353
STD-5	4225	6932.00	3230
STD-6	4329	7003.00	3228
Average	4326	7041	3271
S.D.	65.3361	92.8815	49.8855
% RSD	1.5	1.3	1.5

Table-6: LOD and LOQ Values

Standard	LOQ		LOD	
	Concentration (ppm)	s/n	Concentration (ppm)	s/n
MTS	0.54	1231	0.18	398.6
ETS	0.55	158.5	0.18	52.6
ITS	0.53	967.5	0.17	319.2

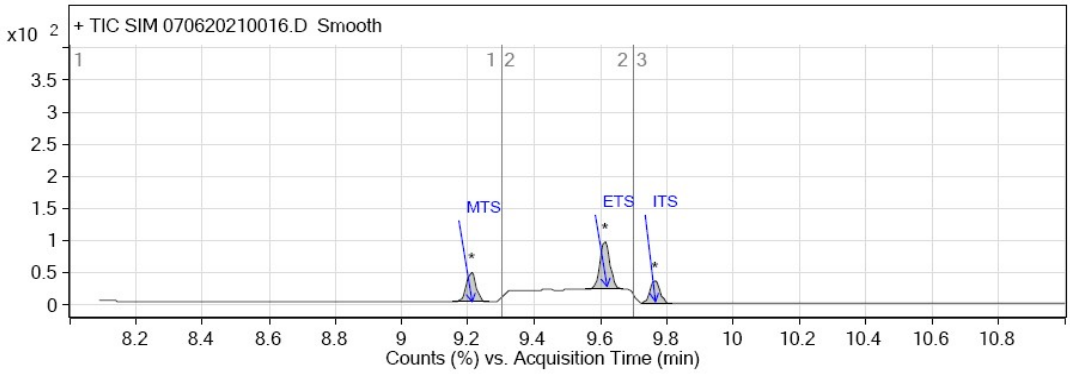

Fig.-5: LOQ Chromatogram

Linearity and Range

A series of solutions, LOQ, 50, 75, 100, 125, and 150% were made from the stock and injected into the GC-MS system. The calculated slope value, intercept, and correlation coefficient, and the obtained values of peak area and corrected concentrations are shown in Table-7. The concentration levels were recorded to identify the linear range of results, leading to the establishment of a defined range.

Table-7: Linearity Results

Linearity (%)	MTS		ETS		ITS	
	Conc.(ppm)	Mean Area	Conc.(ppm)	Mean Area	Conc.(ppm)	Mean Area
Linearity-1 (QL)	0.54	4277.0	0.55	6967.5	0.53	3229.0
Linearity-2 (50)	1.00	8111.0	1.00	12999.0	1.00	6134.5
Linearity-3 (75)	1.50	12618.0	1.50	20062.0	1.50	9458.0
Linearity-4 (100)	2.00	16788.0	2.00	26979.5	2.00	13109.0
Linearity-5 (125)	2.50	20943.0	2.50	33630.0	2.50	16357.0
Linearity-6 (150)	3.00	24940.0	3.00	39263.5	3.00	19068.0
Slope	8437.6		13355.1		6539.0	
% Intercept	-1.2		-0.6		-1.9	
Cor. coefficient	0.9998		0.9994		0.9985	
Reg. coefficient	0.9997		0.9988		0.9993	

Method Precision

To showcase the repeatability of sample injections, six spiked sample solutions were accurately made by spiking the standard at a 100% concentration level. The %RSD for each component has been calculated and represented in Table-8, and the spiked sample TIC is shown in Fig.-6.

Batch Analysis and Accuracy

The sample was prepared and injected to observe the results. The sample was spiked at LOQ, 50, 100, and 150% levels of the standard concentration. All of them were spiked thrice, and the % recovery was calculated. The obtained results were between 95 and 110% and the results are represented in Table-9.

Robustness

The optimized developed GC-MS conditions were further verified for system suitability by altering a few chromatographic conditions as follows, and the obtained responses are recorded in Table-10.Change in initial column oven temperature of 80 °C: (a) Oven temp diverted to 72 °C, (b) Oven temp diverted to 88 °C. Change in injector temperature of 240 °C: (a) Injector temp diverted to 224 °C, (b) Injector temp

diverted to 264 °C. Change in flow from 1.5 mL/min: (a) Flow varied to +10% of initial flow (1.35 mL/min), (b) Flow varied to –10% of initial flow (1.65 mL/min).

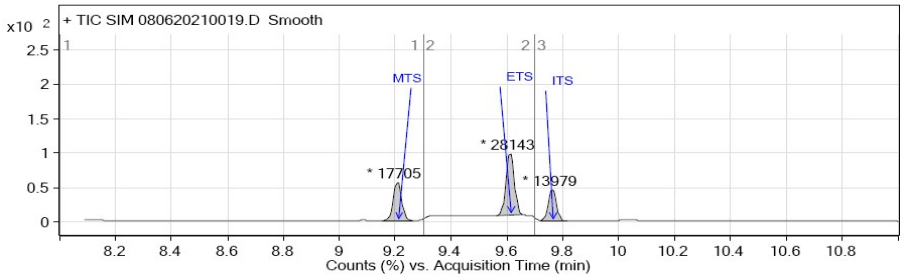

Fig.-6: Spiked Sample Chromatogram

Table-8: Method Precision Results at 2.0 ppm

Preparation	MTS	ETS	ITS
1	16788	26979	13109
2	17643	27132	13112
3	16813	27054	13098
4	16699	26698	12978
5	16854	28555	12932
6	17021	26752	13077
Average	16969.67	27195.00	13051.00
SD	346.47	687.51	76.76
% RSD	2.04	2.53	0.59

Table-9: Recovery Results in Percentage

Level	MTS	ETS	ITS
QL	100.9	101.7	108.8
50%	100.9	107.8	110.0
100%	100.5	100.5	106.2
150%	95.0	98.5	99.8
Mean	99.33	102.13	106.20

Table-10: Robustness Results

As per the method's ideal condition			Oven Temp. (ºC)		Injector Temp. (ºC)		Flow Rate (mL/min)	
			80		240		1.5	
			72	88	224	264	1.35	1.65
ETS	RSD	1.5	1.7	4.1	5.1	4.9	2.9	3.1
MTS	RSD	1.4	1.8	2.1	2.3	2.1	3.1	2.9
ITS	RSD	2.0	1.8	1.4	2.1	2.3	2.1	3.1

The discussion section of the experimental study on the development and optimization of a GC-MS method for quantifying MTS, ETS, and ITS impurities in Teneligliptin hydrobromide hydrate provides a comprehensive analysis of the method's effectiveness and significance. Here's a brief discussion of the key points.

Significance of Teneligliptin in Diabetes Management

The discussion begins by emphasizing the importance of Teneligliptin in managing elevated blood sugar levels, particularly in combination with diet and exercise. It highlights Teneligliptin's efficacy as a DPP-4 inhibitor, essential for managing postprandial blood glucose levels.

Challenges in Impurity Analysis

Despite the therapeutic benefits of Teneligliptin, its synthesis involves various manufacturing steps, leading to the inclusion of impurities like MTS, ETS, and ITS. These impurities, recognized as potential genotoxic impurities, necessitate strict monitoring to comply with regulatory standards.

Analytical Method Comparison

The discussion contrasts the newly developed GC-MS method with previous analytical approaches, highlighting limitations in controlling specific impurities or adequately addressing tosylates. It underscores the need for more precise analytical methods to ensure compliance with regulatory guidelines.

The Superiority of the Proposed Method

The proposed GC-MS method is lauded for its superior sensitivity, precision, and accuracy in quantifying MTS, ETS, and ITS impurities. It fills the gap in existing methods by providing a more effective means of impurity quantification, crucial for regulatory compliance.

Overall, the discussion section reinforces the importance of the developed GC-MS method in addressing the analytical challenges associated with impurity quantification in Teneligliptin hydrobromide hydrate, thereby contributing to the quality control and safety of the drug product.

CONCLUSION

A sensitive GC-MS technique was developed, optimized, and validated for the quantification of three potential MTS, ETS, and ITS impurities in Teneligliptin. The method's effectiveness was assessed by executing experiments with specificity, linearity, precision, and accuracy. The findings demonstrated that the technique is effective in determining even trace amounts of MTS, ETS, and ITS in Teneligliptin.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. 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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