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TRACE-LEVEL QUANTIFICATION OF METHYL P-TOLUENESULFONATE, A GENOTOXIC SULFONATE ESTER IMPURITY, IN PREGABALIN API AND FINISHED DOSAGE FORMS BY A ROBUST RP-HPLC METHOD

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ABSTRACT

A highly sensitive HPLC approach has been developed in the direction of measure the quantity of genotoxic impurity of methyl p-toluenesulfonate in Pregabalin. The separation was carried out an Intersil column that is ODS-3V (250 × 4.6 mm, 5 µm) used as the stationary phase. This investigation was accomplished at a temperature of 25°C by a 20 µL injection volume and 1.0 mL/min flow rate. The Detection of methyl p-toluenesulfonate was performed through a UV detector set at 208 nm, and the entire run program for each injection was 40 minutes. A mobile phase containing water and acetonitrile (95:5, v/v) provided effective elution. The method was Validated according to ICH guidelines, and ensuring reliable, accurate results.The separation cores on obtaining was proceeding(LOQ) limit of quantitation for methyl p-toluenesulfonate was established at 0.31 ppm, indicating the method's strong sensitivity for trace-level impurity monitoring.

Keywords: Genotoxic Impurity, Methyl P-Toluenesulfonate, Forced Degradation, Pregabalin, RP-HPLC Method, Validation.

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INTRODUCTION

During the production of drug substances, various reactive reagents are commonly used, and traces of these chemicals may remain in the final product as impurities. Because such responsive species can exhibit toxic behaviour, including genotoxic, mutagenic, or carcinogenic effects, their levels must be tightly controlled, typically in relation to the highest daily dose.[1,2] These thresholds usually fall within the down microgram-per-millilitre range, making sensitive analytical methods essential. Techniques such as HPLC and GC are widely employed for detecting these low-level impurities.[3-7] Residual solvents like methanol or ethanol, which may persist from reaction or recrystallisation steps, can react to form alkyl tosylates, including methyl p-toluenesulfonate, the ester derivative of p-toluenesulfonic acid (P-TSA) **(Fig.-1).** These alkyl tosylates are recognised as potential genotoxic impurities (PGIs) because they are capable of alkylating DNA, leading to possible mutagenic, carcinogenic, or teratogenic outcomes.[8-10]

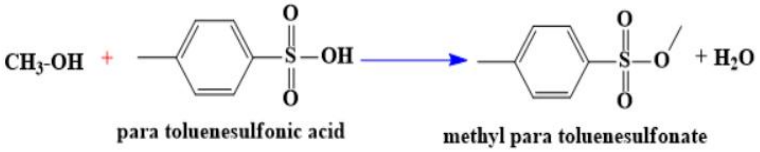

Fig.-1: Chemical Structure of Methyl p-toluenesulfonate (MPTS)

Regulatory agencies, including the (ICH) -International Conference on Harmonisation under guideline Q3A, and more recently the US FDA, have emphasised the need to control genotoxic and cancer-causing impurities in both drug constituents and finished products. These guidelines highlight the potential risks associated with trace-level reactive impurities and are importance of strict monitoring. This aligns with Sustainable Development Goal (SDG) 3-Good Health and Well-Being, which promotes access to safe, effective, and high-quality medicines. Pregabalin (Fig.-2) is a structural analogue of gamma-aminobutyric

acid (GABA), a key inhibitory neurotransmitter in the central nervous system. During its synthesis, p-toluenesulfonic acid is commonly employed as a process reagent.[11-13] Under certain conditions, this reagent can give rise to methyl p-toluenesulfonate, a known potential genotoxic impurity (PGI). As a result, small quantities of this impurity may stand in the final Pregabalin API- active pharmaceutical ingredient, making its accurate detection and control essential for product safety.[14-15]

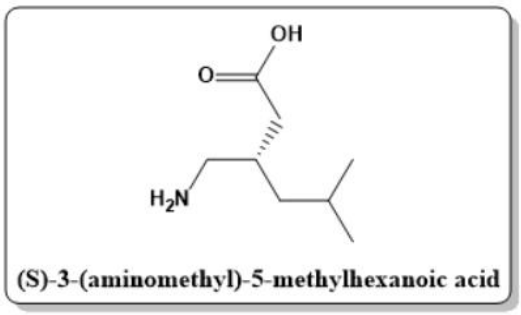

Fig.-2: Chemical Structure of Pregabalin

A review of the available literature indicates that no analytical method has been previously described for quantifying methyl p-toluenesulfonate in Pregabalin. Therefore, this study focus ondeveloping a speedy, specific, and robust HPLC technique capable of detecting this impurity at trace levels, in accordance with ICH guidelines.[16]

EXPERIMENTAL

Materials and Methods

Methyl *p*-toluenesulfonate (MPTS), acetonitrile, and HPLC-grade water were sourced from confirmed suppliers, and Pregabalin API was obtained from Innosyn Life Sciences. Commercial Pregabalin capsules were purchased locally.

Instrumentation

Chromatographic analysis was performed on a Waters e2695 HPLC system using DAD, supported by a Bandelin ultrasonic bath, a Thermo Orion pH meter, and a Mettler Toledo analytical balance.

Preparation of Methyl *p*-toluenesulfonate Stock Solution

Accurately weighed 1.514 mg of MPTS was assigned to a 100-mL volumetric flask, dissolved in about 50 mL of diluent with sonication, and made up to volume.

Preparation of Methyl *p*-toluenesulfonate Standard Solution (1.514 µg/mL)

A 1.0-mL aliquot of the stock solution was diluted to 10 mL with diluent, giving a concentration corresponding to 1.5 ppm relative to a 10 mg/mL sample.

Preparation of Sample Solution: About 100.28 mg of Pregabalin was dissolved in 5 mL of diluent and diluted to volume in a 10-mL flask.

Preparation of Sample Spiked Solution

Approximately 100.33 mg of Pregabalin was dissolved similarly, followed by the addition of 1.0 mL of the MPTS stock solution, and the mixture was diluted to 10 mL.

Method Development

UV scanning of Pregabalin (200–400 nm) indicated a maximum absorbance at 208 nm, and the chromatographic parameters were subsequently optimized to obtain clear separation and satisfactory peak characteristics for MPTS. Figure-7 presents the chromatogram of the spiked Pregabalin sample, while Table-1 summarises the system suitability results.

Detection Method

The Separation was achieved by using a Inertsil (250 × 4.6 mm, 5 µm) ODS-3V column, using a mobile phase is water and acetonitrile (95:5, v/v), filtered through a 0.45-µm PVDF membrane. The flow rate

was look after at 1.0 mL/min, column temperature at 25°C, detection at 208 nm, injection volume at 20 µL, and total runtime at 40 minutes.

RESULTS AND DISCUSSION

Specificity was evaluated by analyzing the blank, placebo, standard, sample, and spiked sample solutions under the improved chromatographic conditions. The corresponding results are presented in Table-1 and Fig.-3 to 7. The study validated that no interfering peaks were observed at the retention time of impurity. Observed at the retention time of methyl *p*-toluenesulfonate, demonstrating that the method is selective for detecting this impurity in Pregabalin.

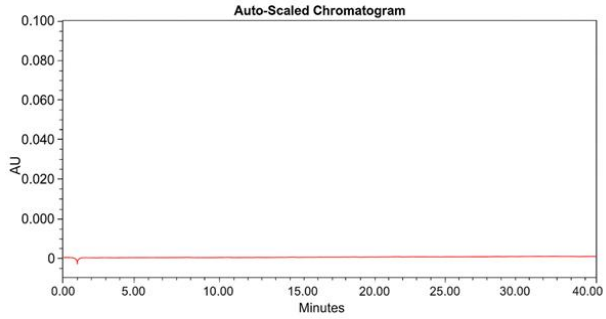

Fig.-3: Baseline Response of Blank Chromatogram

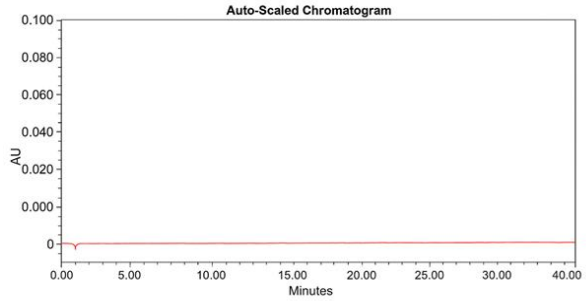

Fig.-4: Chromatogram of Baseline Response of Placebo

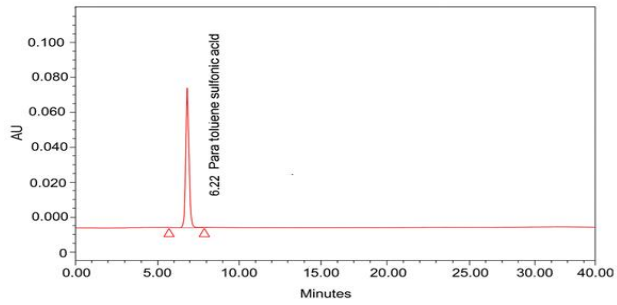

Fig.-5: Chromatogram of Paratoulene Sulfonic Acid Sample

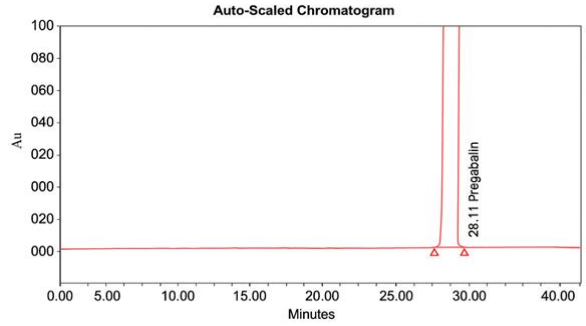

Fig.-6: Chromatogram of Pregabalin Sample

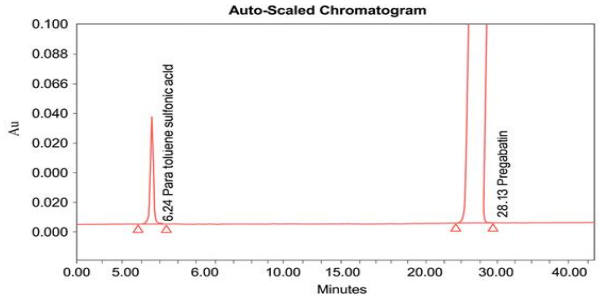

Fig.-7: Spiked Sample Chromatogram

Table-1: Specificity Results

Tests	Name	R.T(min)	Response in Blank	Response in Placebo
T1	Blank matrix	ND	—	—
T2	Placebo preparation	ND	—	—
T3	Reference Standard	6.22	No interference	No interference
T4	Sample under study	ND	—	—
T5	Sample fortified with analyte	6.24	No interference	No interference

System Suitability (System precision)

A standard solution prepared according to the test procedure was injected six times into the HPLC system, and the impurity peak areas were recorded for each injection. As shown in Table-2, the %RSD of the methyl p-toluenesulfonate peak area was 1.70%, which is well below the acceptance limit of 10%. This confirms that the system performance is precise and suitable for analysis.

Table-2: Precision Results

Entry	Injection ID	Recorded Peak areas
E1	Run–1	31452
E2	Run–2	31458
E3	Run–3	30568
E4	Run–4	31985
E5	Run–5	30784
E6	Run–6	30895
Avg.		31190
Std. Dev.		531.2697
%RSD		1.70

Method Precision

Method precision is evaluated by preparing six control samples and six samples spiked with the impurity at the specification level, followed by analysis as described in the test method. All samples were prepared in accordance with the validated procedure, and the results from this precision study are provided in Table- 3.

Table-3: Method Precision Results

S. No.	No. of Preparations	% w/w impurity in Control samples	% Recovery of impurity spiked samples
1	Preparation 1	ND	103.8
2	Preparation 2	ND	100.1
3	Preparation 3	ND	101.5
4	Preparation 4	ND	99.5
5	Preparation 5	ND	100.7
6	Preparation 6	ND	102.3
Average		NA	101.3
SD		NA	1.5702
%RSD		NA	1.55

Limit of Quantization (LOQ) and Limit of Detection (LOD): Limit of Quantisation (LOQ) and Limit of Detection (LOD):

A solution prepared at the LOQ (0.3028 µg/mL) and LOD (0.099 µg/mL) levels of methyl p-toluenesulfonate was injected six consecutive times to evaluate sensitivity and precision at these concentrations. The results obtained from the LOQ precision assessment are summarised in Table-4.

Table-4: LOQ precision results

S.No.	Methyl p-toluenesulfonate
1	6245
2	6198
3	6085
4	6174
5	6223
6	6347
Avg.	6212
Std. Dev.	86.2601
%RSD	1.39

Linearity

Linearity for the methyl p-toluenesulfonate impurity was evaluated by preparing a series of solutions ranging from the LOQ level up to 150% of the target concentration. The detector response showed a strong linear relationship across this range, with a correlation coefficient exceeding 0.99. The detailed linearity data are presented in Table-5.

Table-5: Linearity Studies for Methyl p-toluenesulfonate

S.No.	Linearity Level	Concentration (ppm)	Area response
1	LOQ	0.303	6295
2	50	0.757	15845
3	75	1.136	23098
4	100	1.514	31254
5	125	1.893	39176
6	150	2.271	46587
Correlation coefficient (r^2)			0.9998
Slope			20521.2739
Intercept			115.8525
% Y-intercept			0.37

Accuracy

The accuracy of the method was assessed by preparing recovery samples at three concentration levels—LOQ, 100%, and 150% of the target concentration. For each level, samples were prepared in triplicate. The recovery results, summarised in Table-6, confirm that the method provides accurate measurements across the evaluated range.

Table-6: Recovery Studies for Methyl p-toluenesulfonate

% Level	Added (µg)	Founded (µg)	% Recovery	Mean % Recovery	% RSD
LOQ Level-1	0.7581	0.7499	98.92	99.7	0.75
LOQ Level-2	0.7581	0.7612	100.41		
LOQ Level-3	0.7581	0.7566	99.80		
100% Level-1	1.5145	1.5047	99.35	99.1	0.23
100% Level-2	1.5145	1.4985	98.94		
100% Level-3	1.5145	1.4992	98.99		
150% Level-1	2.2751	2.2014	96.76	97.1	0.39
150% Level-2	2.2751	2.2185	97.51		
150% Level-3	2.2751	2.2097	97.13		

An investigation was performed to confirm whether the standard and spiked sample solutions remained stable during the analysis. Under different storage conditions, including room-temperature bench-top conditions and refrigeration at 2–8°C for each condition, the initially prepared standard, sample, and spiked solutions were compared against freshly prepared standard solutions to assess any changes in response. Based on these comparisons, all three types of solutions remained stable for up to 48 hours under both ambient and refrigerated storage.

CONCLUSION

A robust RP-HPLC method was developed and validated for the minute-level determination of the genotoxic impurity methyl p-toluenesulfonate in Pregabalin, following the recommendations of the ICH guidelines. The method demonstrated reliable performance in terms of specificity, precision, linearity, sensitivity (LOD and LOQ), accuracy, and solution stability. Overall, the validated method is appropriate for its expected application and can be effectively used for the standard quality-control testing of Pregabalin before its release to the market.

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CONFLICT OF INTERESTS

The authors confirm that they have no competing interests related to this work.

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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