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Method Development and Dissolution Validation of Tenoxicam Using RP-HPLC as Per ICH Guideline

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ABSTRACT

Standard analytical procedure for newer drugs or formulation is essential to develop newer analytical methods and also Validate; which are accurate, precise, specific, linear, simple and rapid. The objectives of this paper work are as the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte (Tenoxicam) more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation for the determination of Tenoxicam dosage form for a routine quality control analysis and to validate the analytical method by HPLC for the determination of Dissolution of Tenoxicam and develop the analytical method in HPLC for studying the releasing behaviour and also study the characteristic of particular analyte in the available dosage form. The developed method was validated according to ICH guidelines. The data obtained from Linearity, Precision and Accuracy reveals that the method is linear, precise and accurate over the range of 10% to 150% of working concentration. Ruggedness of the method was evaluated under intermediate precision and results were found within acceptable limits. The robustness of the method was evaluated by altering the variables such as different paddle speed (98 rpm and 102 rpm), Different pH of Buffer (6.60 and 7.00) , Different pH of mobile phase (2.60 and 3.00) and Different flow rate (0.8 mL/min and 1.2 mL/min). The data obtained from individual condition and overall conditions including repeatability was found well within the limit. The Standard preparation and Test preparation is found stable up to 24 Hours at ambient temperature (25 ± 2°C). The system suitability parameters met the acceptance criteria, which were commenced during study of each individual validation characteristics.

Keywords: *Tenoxicam, RP-HPLC, dissolution, precision, accuracy, ruggedness.*

INTRODUCTION

The Code of Federal Regulations (CFR) 311.165c explicitly states that “accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented”. Of course as Scientists we would want to apply good science to demonstrate that the analytical method used had demonstrated accuracy, sensitivity, specificity and reproducibility. Finally the management methods had demonstrated uses to release its product are properly validated for its intended use so the product will be safe for human use. [1] Tenoxicam is an enolic acid derivative that inhibits high levels of COX-2 at the sites of inflammation and thus has anti inflammatory, analgesic, and antipyretic activity. This nonselective COX inhibitor is extensively used in the treatment of rheumatoid arthritis and osteoarthritis.[2,3] Several analytical methods are described in recent literature such as mass spectrometric[4], spectrofluorometric [5,6], potentiometric[7,8] , polarographic , infrared spectrophotometric[9] coulometric [10] spectrophotometric[11] derivative spectrophotometric[12]and high performance liquid chromatographic techniques [13,14,15]. The HPLC methods described in literature involve sample pre-treatment and troublesome buffers components in the mobile phase. The hyphenated LC-MS detection makes these methods available to only a few. On the other hand a majority of described spectrophotometric methods involves sample pre-treatment or use of derivation technique due to their application in the determination of the active compound in the presence of its degradation products.[16] The objectives of this paper work are as the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte (Tenoxicam) more specific, accurate and precise.

Dissolution medium: Mainly 6.8 phosphate buffer used as dissolution medium.

Procedure: 6.8 g of Potassium dihydrogen ortho phosphate in 1000 mL of water was mixed well; then pH 6.8 ± 0.05 with 1M sodium hydroxide was adjusted. Then the dissolution medium was deaerated by sonication.

Diluent: Dissolution medium used as diluent.

Mobile Phase Preparation:

Mobile Phase-A: 0.2 g of Sodium Lauryl Sulphate was accurately weighed and dissolved in 700 mL of Methanol and mixed well.

Mobile Phase-B: Then 6.8g of Potassium Dihydrogen Orthophosphate was accurately weighed and properly mixed with 1000ml of water.

Procedure: Mobile Phase-A and Mobile Phase-B was mixed well and adjusted the pH to 2.8 with dil. Ortho Phosphoric Acid. Then Filtered through 0.45µ-nylon membrane filter and also sonicated to degas.(The proportion was 70:100% v/v)

Blank Preparation: Dissolution medium used as blank preparation.

Methodology:

System Suitability:

Standard Solution Preparation: Accurately about 20.04 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Test preparation:

10 mL of aliquot was withdrawn after specified time interval and then same amount of dissolution medium was replenished and filtered through Whatman No. 1 filter paper. Then first 2 to 3 mL of the filtrate was discarded. Then filtrate was collected and injected in to HPLC.

Procedure for Dissolution: Six Capsules in individual jar containing dissolution medium was Introduced which was previously maintained at temperature 37°C ± 0.5°C and immediately instrument was operated as per the methodology. Area of the Standard preparation in five replicates was measured and Test preparation in single, by HPLC at the wavelength of 264 nm against blank preparation.

Validation Procedure:

Specificity:

Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interferences from blank and placebo.

Blank preparation: Dissolution medium used as blank preparation.

Placebo preparation:

Placebo equivalent to 20 mg of Tenoxicam was weighed and transfered to one of the jar of dissolution tester containing 1000 mL of dissolution medium previously maintained at temperature 37°C ± 0.5°C and was proceed further as per the test preparation .

Standard Solution Preparation:

Accurately about 20.0 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Test preparation:

10 mL of aliquot was withdrawn after specified time interval and then same amount of dissolution medium was replenished and filtered through Whatman No. 1 filter paper. Then first 2 to 3 mL of the filtrate was discarded. Then filtrate was collected and injected in to HPLC.

Procedure for Dissolution: Six Capsules in individual jar containing dissolution medium was introduced which was previously maintained at temperature 37°C ± 0.5°C and immediately instrument was operated as per the methodology. Area of Standard preparation in five replicates was measured and %RSD for Area from Standard preparation was calculated. Area of Placebo preparation and Test preparation was measured and checked for interference from placebo.

- **Linearity and Range:**

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the samples. Linearity at 7 levels, viz. 10%, 20%, 50%, 80%, 100%, 120% and 150% of working level concentration (200 ppm) was performed.

Range of analytical procedure is the interval between the upper & lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy & linearity.

The range is derived from the linearity studies.

Standard Solution Preparation:

Accurately about 20.0 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Standard Stock Solution:

Accurately about 20.0 mg of Tenoxicam working standard was weighed and transfered into 100 mL volumetric flask. Diluent was added and diluted up to the mark.

Procedure:

First level and last level in six replicates and remaining all other levels in triplicate was injected and %RSD, Correlation Coefficient, slope of regression line, y-intercept and residual sum of square was calculated.

Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The true value is that result which would be observed in the absence of error. Accuracy may often be expressed as percent recovery by assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method that is true for all practical purpose.Accuracy at 4 levels, viz. 10%, 50%, 100% and 150% of Test concentration was performed.

Standard Solution Preparation: Accurately about 20.0 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Level I - 10% of working concentration of Test preparation:

Placebo equivalent to 20 mg of Tenoxicam and 2.0 mg of Tenoxicam working standard in dissolution jar containing 1000 mL of dissolution medium was weighed and transfered which was previously maintained at temperature 37°C ± 0.5°C and continued dissolution process for 45 minutes. After specified time interval 10 mL of aliquot was withdrawl and filtered through Whatman No 1 filter paper. First 2 to 3 mL of the filtrate was discarded.At last filtrate/aliquot was collected and injected.

Level II - 50% of working concentration of Test preparation:

Placebo equivalent to 20 mg of Tenoxicam and 10.0 mg of Tenoxicam working standard in dissolution jar containing 1000 mL of dissolution medium was weighed and transfered which was previously maintained at temperature 37°C ± 0.5°C and continued dissolution process for 45 minutes. After specified time interval 10 mL of aliquot was withdrawl and filtered through Whatman No 1 filter paper. First 2 to 3 mL of the filtrate was discarded.At last filtrate/aliquot was collected and injected.

Level I - 100% of working concentration of Test preparation:

Placebo equivalent to 20 mg of Tenoxicam and 20 mg of Tenoxicam working standard in dissolution jar containing 1000 mL of dissolution medium was weighed and transfered which was previously maintained at temperature 37°C ± 0.5°C and continued dissolution process for 45 minutes. After specified time interval 10 mL of aliquot was withdrawl and filtered through Whatman No 1 filter paper. First 2 to 3 mL of the filtrate was discarded.At last filtrate/aliquot was collected and injected.

Level I - 150% of working concentration of Test preparation:

Placebo equivalent to 20 mg of Tenoxicam and 30 mg of Tenoxicam working standard in dissolution jar containing 1000 mL of dissolution medium was weighed and transfered which was previously maintained at temperature 37°C ± 0.5°C and continued dissolution process for 45 minutes. After specified time interval 10 mL of aliquot was withdrawl and filtered through Whatman No 1 filter paper. First 2 to 3 mL of the filtrate was discarded.At last filtrate/aliquot was collected and injected.

Procedure: Each level in triplicate was prepared and area was measured of each level in single and % recovery and RSD for % recovery was calculated.

Precision:

The precision of an analytical method is the closeness of agreement (degree of scatter) between series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed conditions.

System Precision:

A) System Precision of HPLC Apparatus:

Blank Preparation: Dissolution medium used as blank preparation.

Standard Solution Preparation:

Accurately about 20.0 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Procedure: Six Capsules in individual jar containing dissolution medium was Introduced which was previously maintained at temperature 37°C ± 0.5°C and immediately instrument was operated as per the methodology. Standard preparation in six replicates was injected and calculated %RSD for the area due to Tenoxicam.

B) System Precision of Dissolution Test Apparatus:

Blank Preparation: Dissolution medium used as blank preparation.

Standard Solution Preparation:

Accurately about 20 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Procedure: Six Capsules in individual jar containing dissolution medium was Introduced which was previously maintained at temperature 37°C ± 0.5°C and immediately instrument was operated as per the methodology was established and aliquot six times from any one jar was withdrawl. Area of test preparation in singlet was measured and %dissolution and RSD for % dissolution was calculated.

Method Precision (Repeatability):

Repeatability expresses the precision under the same operating conditions over a short interval of time.

Blank Preparation: Dissolution medium used as blank preparation.

Standard Solution Preparation:

Accurately about 20 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Test preparation:

10 mL of aliquot was withdrawn after specified time interval and then same amount of dissolution medium was replenished and filtered through Whatman No. 1 filter paper. Then first 2 to 3 mL of the filtrate was discarded. Then filtrate was collected and injected in to HPLC.

Procedure: Six Capsules in individual jar containing dissolution medium was Introduced which was previously maintained at temperature 37°C ± 0.5°C and immediately instrument was operated as per the methodology and Method precision was established and % dissolution was determined. Individual dissolution value, mean dissolution value and %RSD were calculated.

Intermediate Precision (Ruggedness):

Intermediate precision expresses within-laboratory variation on a different day, by a different analyst, using different instrument and using same lot of sample as specified under repeatability.

Blank Preparation: Dissolution medium used as blank preparation.

Standard Solution Preparation:

Accurately about 20 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Test preparation:

10 mL of aliquot was withdrawn after specified time interval and then same amount of dissolution medium was replenished and filtered through Whatman No. 1 filter paper. Then first 2 to 3 mL of the filtrate was discarded. Then filtrate was collected and injected in to HPLC.

Procedure: Six Capsules in individual jar containing dissolution medium was Introduced which was previously maintained at temperature 37°C ± 0.5°C and immediately instrument was operated as per the methodology and the procedure followed for method precision was repeated on a different day, by a different analyst and different dissolution apparatus. Also individual dissolution value, mean dissolution value and %RSD were calculated.

Robustness:

Blank Preparation: Dissolution medium used as blank preparation.

Standard Solution Preparation:

Accurately about 20 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Test preparation: Six Capsules in individual jar containing dissolution medium was introduced which was previously maintained at temperature 37°C ± 0.5°C and immediately instrument was operated as per the methodology**.**10 mL of aliquot was withdrawn after specified time interval and then same amount of dissolution medium was replenished and filtered through Whatman No. 1 filter paper. Then first 2 to 3 mL of the filtrate was discarded. Then filtrate was collected and injected in to HPLC.

Procedure:

Following condition changes were designed for confirming the robustness.

1) **Robustness Change in RPM:**

This above procedure was carried out by changing RPM of dissolution apparatus i.e. ± 2 rpm unit (98rpm and 102 rpm) and changes in results were observed.

2) **Robustness Change in pH of Buffer:**

This above procedure was carried out by changing pH of buffer i.e. ± 2 unit (6.6 and7.0) and changes in results were observed.

3) **Robustness Change in pH of Mobile Phase:**

This above procedure was carried out by changing pH of mobile phase i.e. ± 2 unit (2.6 and 3.0) and changes in results were observed.

4) **Robustness Change in flow of Mobile Phase:**

This above procedure was carried out by changing flow of mobile phase i.e. ± 2 unit (0.8ml/min and 1.2ml/min) and changes in results were observed.

The procedure followed for method precision was repeated by using above changes in method one by one. Also individual dissolution value, mean dissolution value and %RSD were calculated.

Solution Stability:

Standard Solution Preparation:

Accurately about 20 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Test preparation: Six Capsules in individual jar containing dissolution medium was introduced which was previously maintained at temperature 37°C ± 0.5°C and immediately instrument was operated as per the methodology and 10 mL of aliquot was withdrawn after specified time interval and then same amount of dissolution medium was replenished and filtered through Whatman No. 1 filter paper. Then first 2 to 3 mL of the filtrate was discarded. Then filtrate was collected and injected in to HPLC.

Procedure:

The dissolution as per methodology was carried out; Test preparation and Standard preparation as per the methodology was prepared and Area of Standard preparation and Test preparation at initial was measured and kept them on bench top at room temperature. Area of Standard preparation and Test preparation at different time interval viz, after 12 Hours and 24 Hours were measured. Also Standard preparation freshly each time was prepared and the Area of Standard preparation was measured. % of Tenoxicam dissolved at every time interval from Test preparation was calculated. Also % difference of Tenoxicam in initial Standard preparation as well as 12hrs and 24hrs w.r.t freshly prepared Standard preparation was calculated.

- **System Suitability:**

Standard Solution Preparation:

Accurately about 20 mg of Tenoxicam working standard was taken into 100 mL volumetric flask. Diluent was added and dissolved properly and diluted up to the mark with diluent. Then 5.0 mL of above solution was added to 50 mL and volume made with diluent (20 ppm).

Procedure:

Areas of Standard preparation in five replicates were measured and %RSD of Area of Tenoxicam was calculated due to Standard preparation.

Results and Discussion for Tenoxicam by RP-HPLC Method

- Method Development of Tenoxicam:

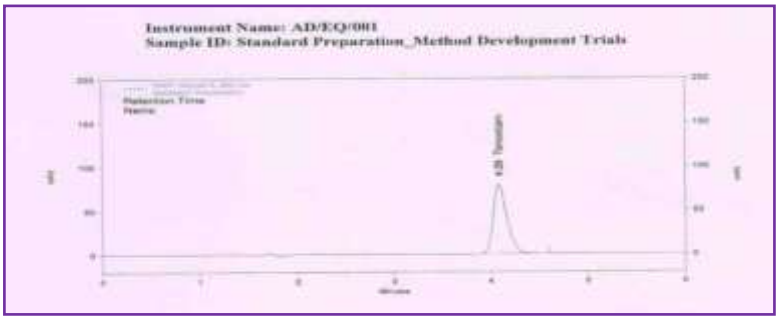

Fig-1: Chromatogram for Method development

- **Validation Observation and Results:**
- **Specificity:**

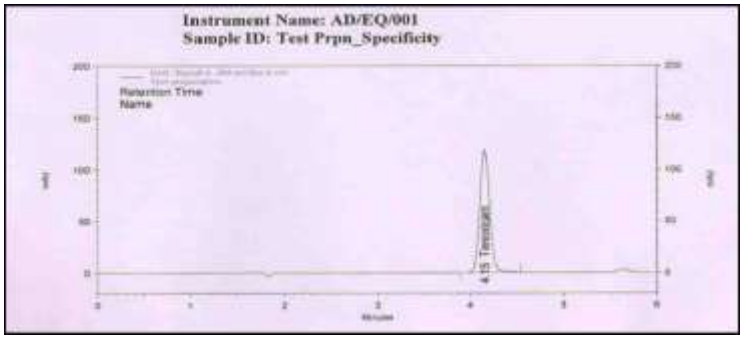

Fig-2: Chromatogram of Test Preparation for Specificity

Linearity and Range:

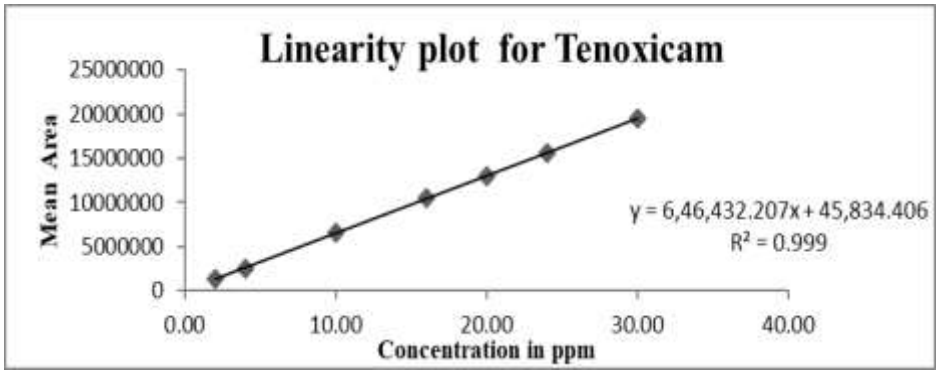

Fig-3: Linearity Plot for Tenoxicam

System Precision:

Table-1: Standard for System Precision of Dissolution by RP-HPLC Method:

Injection No.	Area	Theoretical Plates	Asymmetry
1	1779506	8156	1.04
2	1779632	8180	1.03
3	1781441	8126	1.07
4	1779314	8191	1.04
5	1778089	8164	1.07
Mean	1779596	8163	1.05
Std. Dev.	1200.42	-	-
% RSD	0.07	-	-

- **Method Precision:**

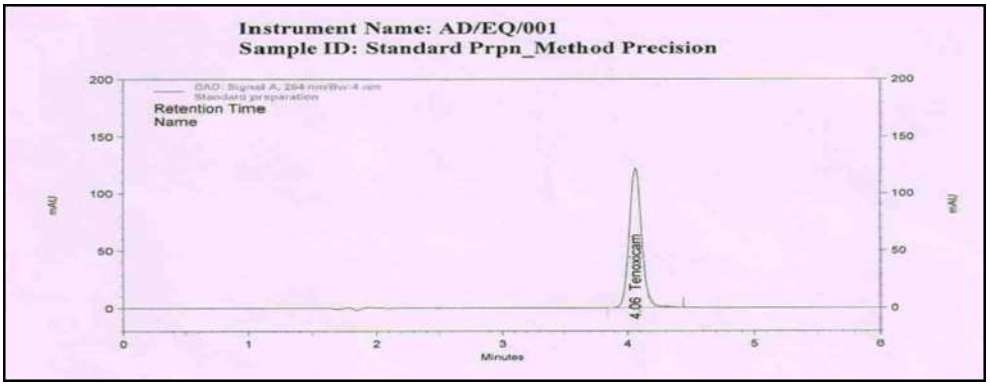

Fig-:4 Chromatogram of Standard Preparation for Method Precision

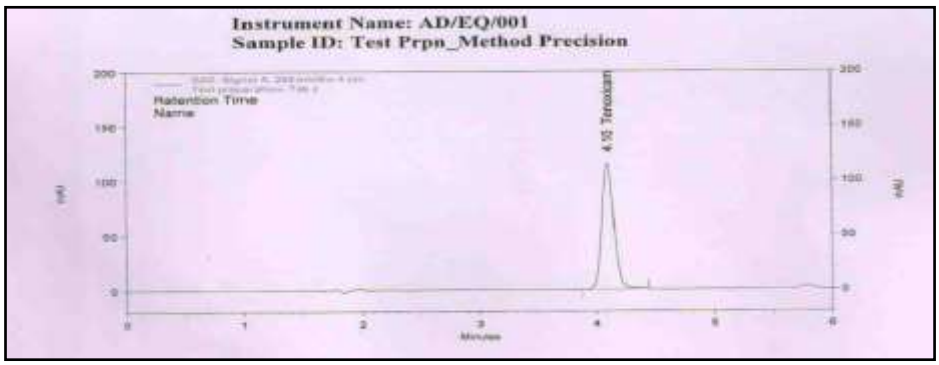

Fig-5: Chromatogram of Test Preparation for Method Precision

- **Intermediate Precision:**

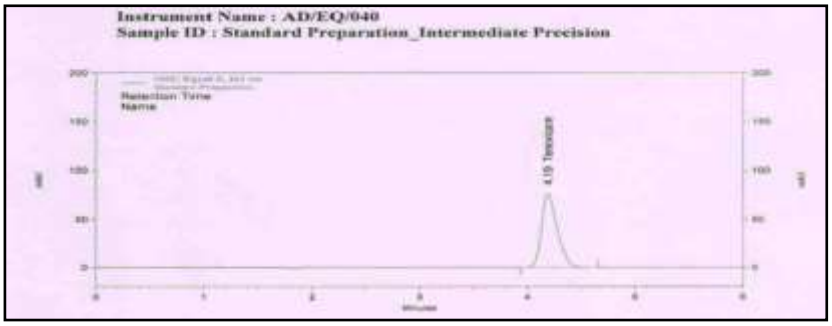

Fig-:6 Chromatogram of Standard Preparation for Intermediate Precision

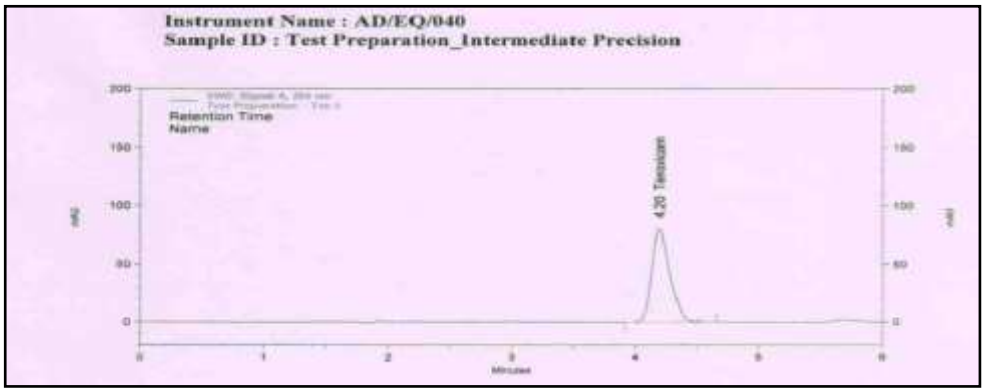

Fig-:7 Chromatogram of Test Preparation for Intermediate Precision

Robustness:

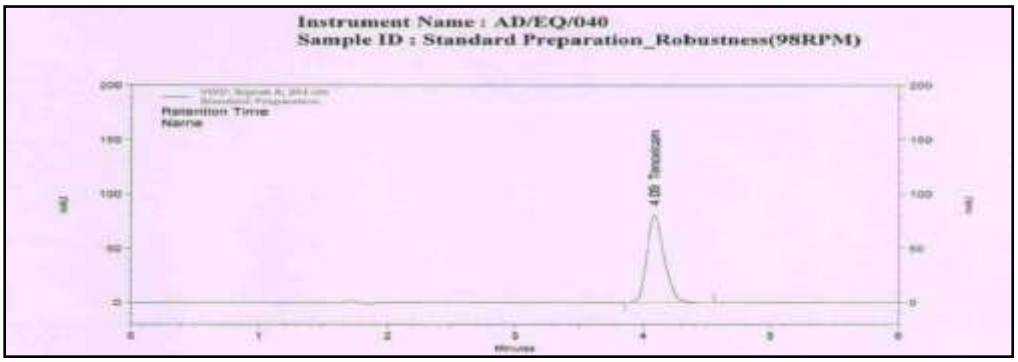

Fig-: 8Chromatogram of Standard Preparation for Change in RPM-98

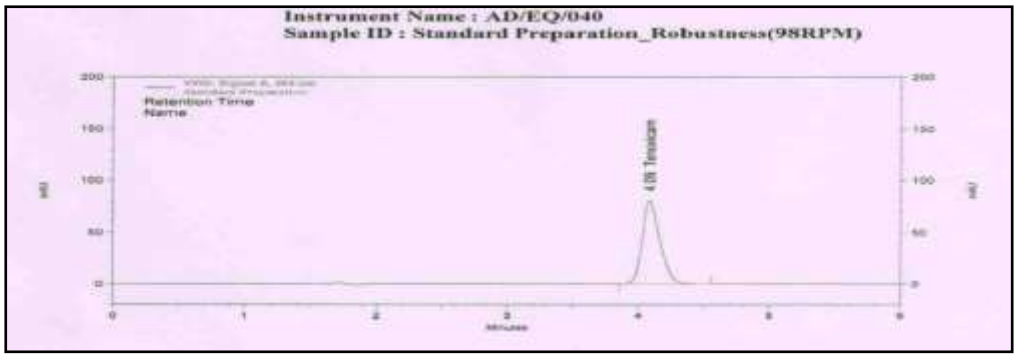

Fig-:

9Chromatogram of Test Preparation for Change in RPM-98

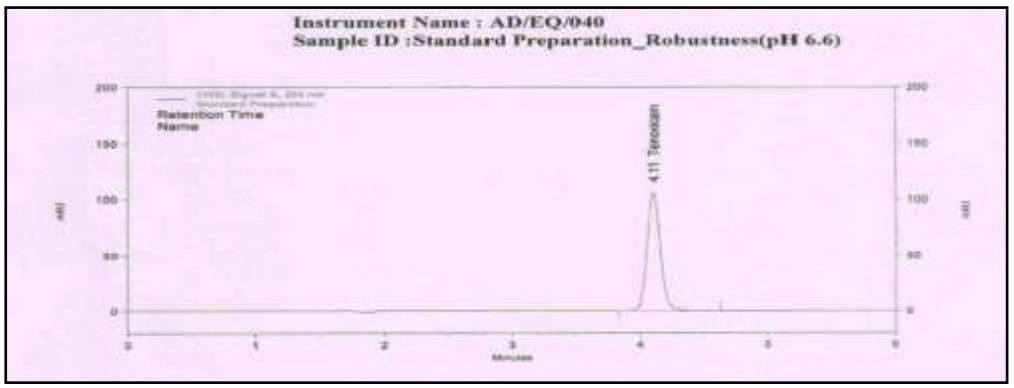

Fig-:10 Chromatogram of Standard Preparation for Change in Buffer pH 6.6

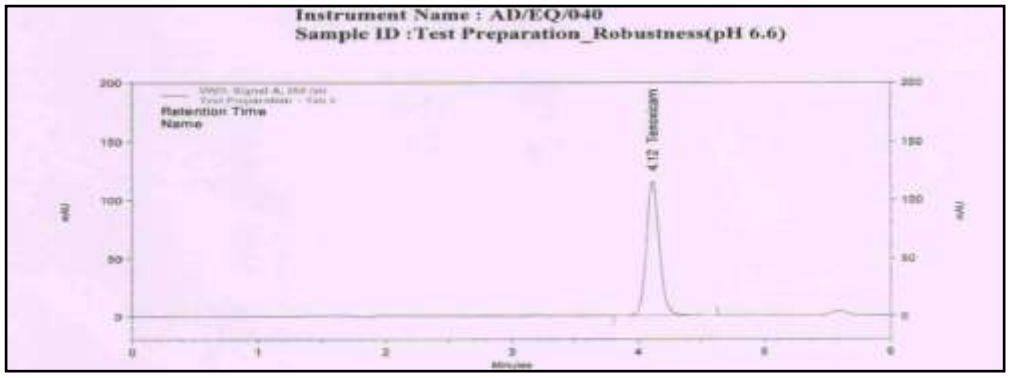

Fig-:11 Chromatogram of Test Preparation for Change in Buffer pH 6.6

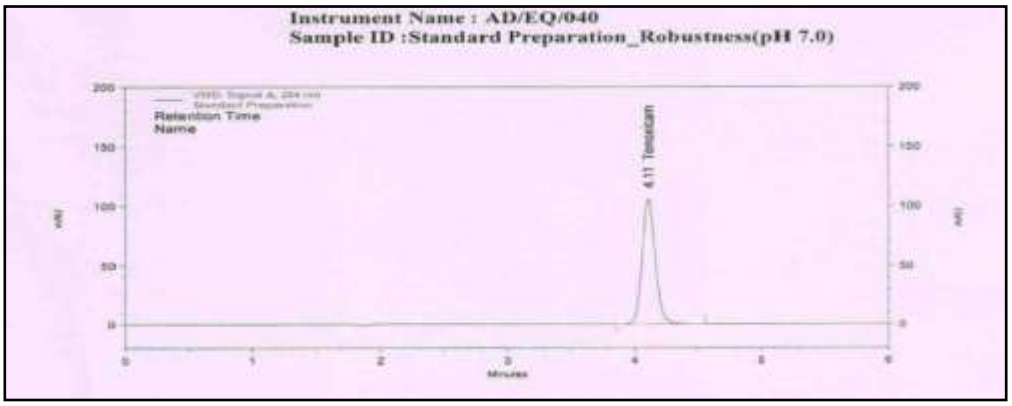

Fig-: 12Chromatogram of Standard Preparation for Change in Buffer pH 7.0

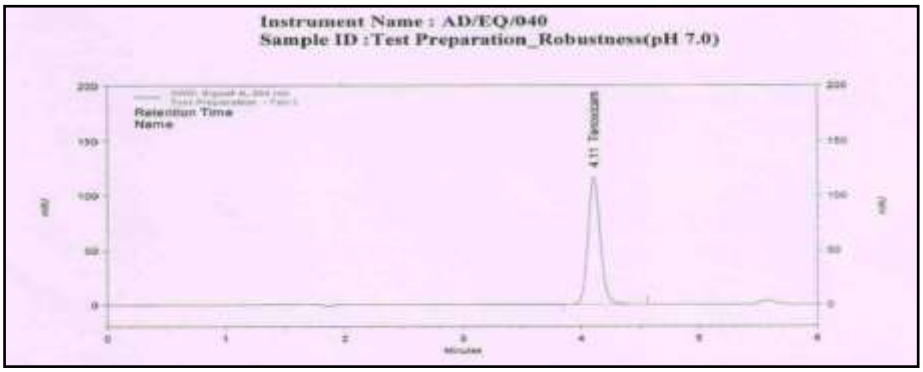

Fig-:13 Chromatogram of Test Preparation for Change in Buffer pH 7.0

- **Change In flow 0.8ml/min:**

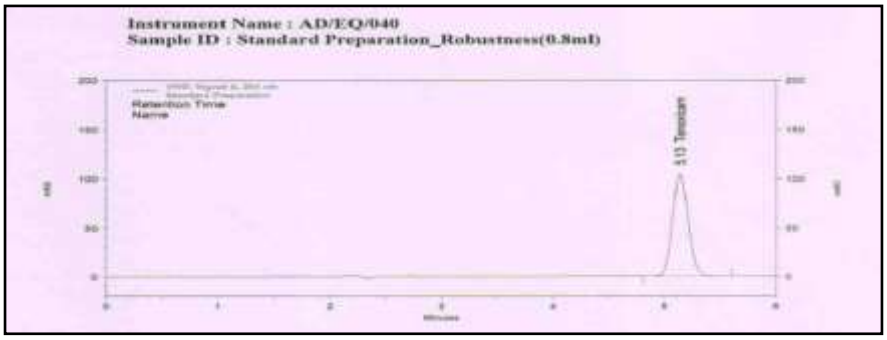

Fig-14: Chromatogram of Standard Preparation for Change in flow 0.8ml/min

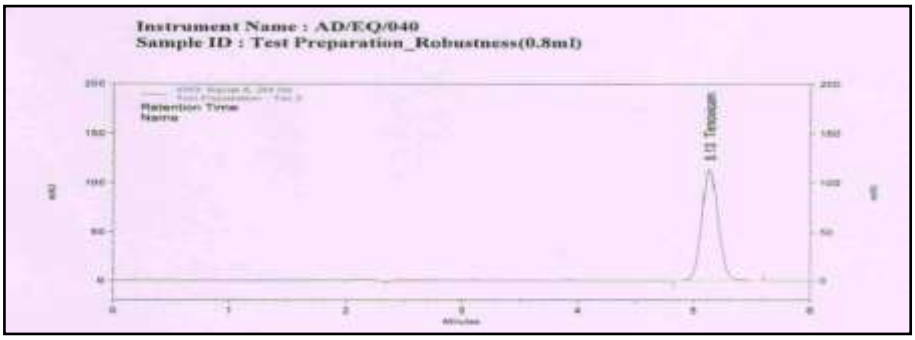

Fig-15: Chromatogram of Test Preparation for Change in flow 0.8ml/min

- **Change in Flow 1.2ml/min:**

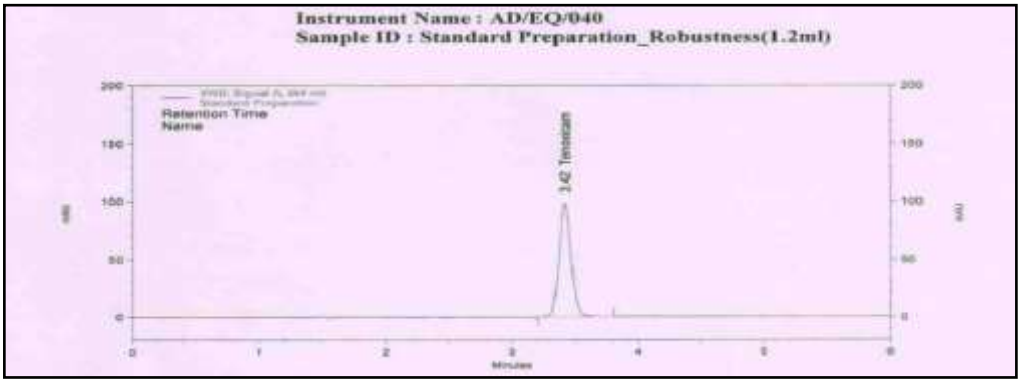

Fig-:16 Chromatogram of Standard Preparation for Change in flow 1.2ml/min

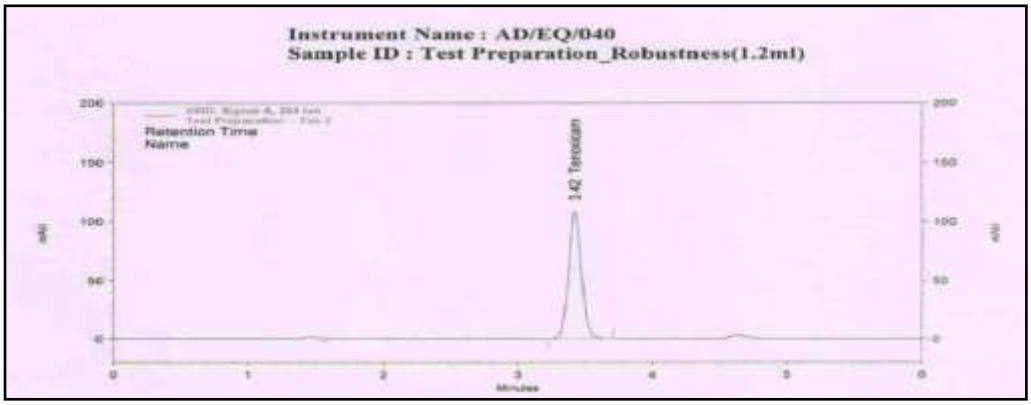

Fig-:17 Chromatogram of Test Preparation for Change in flow 1.2ml/min

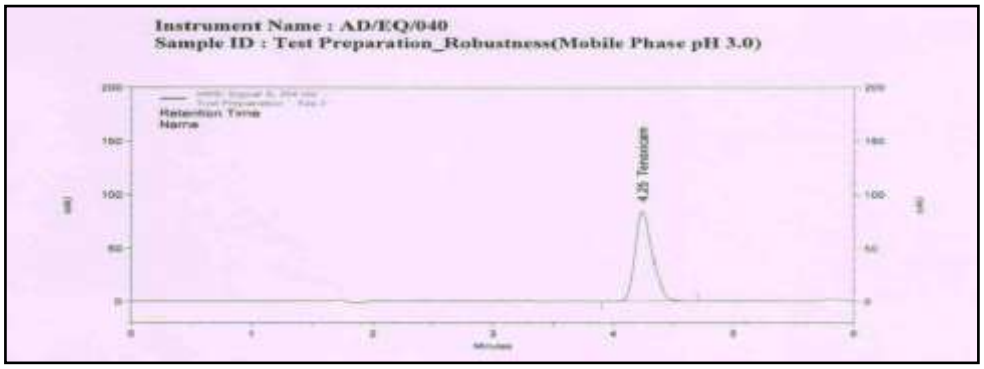

Fig-18: Chromatogram of Test Preparation for Change in pH of mobile phase 3.0

- **Solution Stability**:
- Solution stability initial:

Table-32: Solution stability initial of Tenoxicam by RP-HPLC:

	Area of Tenoxicam in	
Time in Hours	**Standard preparation**	**Test preparation**
Initial	12961217	14246949

Table-33: Solution stability initial showing % dissolution for standard by RP-HPLC:

Time in Hours	**% Tenoxicam in Standard preparation**	**Difference**
Initial	95.4	NA

Table-34: Solution stability initial showing % dissolution for Test by RP-HPLC:

Time in Hours	**% Tenoxicam in Test preparation**	**Difference**
Initial	100.5	NA

- **Solution Stability 12 Hrs:**

Table-35: Standard Preparation of Solution stability 12hrs by RP-HPLC:

Injection No.	**Area**	**Theoretical Plates**	**Asymmetry**
1	**12979921**	**7228**	**1.12**
2	**12972867**	**7251**	**1.11**
3	**12965707**	**7199**	**1.10**
4	**12964631**	**7217**	**1.12**

5	**12971507**	**7195**	**1.12**
Mean	**12970927**	**7218**	**1.11**
Std. Dev.	**6161.98**	-	-
% RSD	**0.05**	-	-

Table-36: Solution stability 12hrs by RP-HPLC:

	Area of Tenoxicam in	
Time in Hours	**Standard preparation**	**Test preparation**
After 12 Hrs	12959205	14243390

Table-37: Solution stability 12hrs showing % dissolution for Standard by RP-HPLC:

Time in Hours	**% Tenoxicam in Standard preparation**	**Difference**
After 12 Hrs	95.8	0.4

Table-38: Solution stability 12hrs showing % dissolution for Test by RP-HPLC:

Time in Hours	**% Tenoxicam in Test preparation**	**Difference**
After 12 Hrs	100.9	0.4

- **Solution Stability 24Hrs:**

Table-39: Standard Preparation of Solution Stability 24hrs by RP-HPLC:

Injection No.	**Area**	**Theoretical Plates**	**Asymmetry**
1	12995727	4261	1.24
2	12994065	4252	1.24
3	12996679	4247	1.27
4	12991424	4243	1.27
5	12997276	4226	1.26
Mean	**12995034**	**4246**	**1.26**
Std. Dev.	**2354.99**	-	-
% RSD	**0.02**	-	-

Table-40: Solution stability 24hrs by RP-HPLC:

	Area of Tenoxicam in	
Time in Hours	**Standard preparation**	**Test preparation**
After 24 Hrs	12964313	14251229

Table-41: Solution stability 24hrs showing % dissolution for Standard by RP-HPLC:

Time in Hours	**% Tenoxicam in Standard preparation**	**Difference**
After 24 Hrs	94.6	0.8

Table-42: Solution stability 24hrs showing % dissolution for Test by RP-HPLC:

Time in Hours	**% Tenoxicam in Test preparation**	**Difference**
After 24 Hrs	99.7	0.8

- **Validation Summary:**

Validation Parameter	**Acceptance criteria**	**Results**
Specificity	There should not be any peak from blank and placebo at the retention time of main peak	No interference was found at the retention time of main peak.
	Retention time of main peak from Test preparation should be similar to that of Standard preparation.	Retention time of main peak from Test preparation is similar to that of Standard preparation.
	The main peak from test preparation should exhibit the maxima at the same wavelength as that from standard preparation.	The main peak from test preparation exhibits the maxima at the same wavelength as that from standard preparation.

Specificity	Peak purity for the main peak in standard preparation and Test preparation should not be less than 0.99.	Peak purity for the main peak in Standard preparation and Test preparation is 1.000.
Linearity and Range		
RSD at Level I 10%	RSD = Not more than 2.0%	0.66%
RSD at Level II 20%		0.02%
RSD at Level III 50%		0.06%
RSD at Level III 80%		0.07%
RSD at Level V 100%		0.04%
RSD at Level VI 120%		0.01%
RSD at Level VII 150%		0.01%
Coefficient of correlation	Not less than 0.999	0.9999
Slope of regression line	Report the value	646432.207
y-intercept	Report the value	45834.40559
Residual sum of square	Report the value	12340626926
Range	Report the value	2.00 – 30.03 ppm

Accuracy		
10% level	1) Recovery at each level, mean recovery and overall mean recovery should be between 95.0% and 105.0 %. 2) RSD for each level and overall RSD should not be more than 5.0%.	Minimum-103.4% Maximum-104.5% Mean-103.9% RSD-0.53%
50% level		Minimum-100.7% Maximum-100.5% Mean-100.6% RSD-0.12%
100% level		Minimum-99.9% Maximum-100.1% Mean-100.0% RSD-0.10%
150% level		Minimum-99.8% Maximum-99.9% Mean-99.9% RSD-0.06%
Overall mean recovery		Recovery = 101.1% RSD =1.73%

Validation Parameter	Acceptance Criteria	Results
System Precision		
System Precision of HPLC	**RSD for Area NMT 2.0%**	**RSD = 0.03%**
System Precision of Dissolution	**RSD for the % dissolution should not be more than 2.0 %.**	**RSD = 1.29%**
Method Precision		
Method Precision	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-95.7% Maximum-103.1% Mean-98.9%
	RSD for % dissolution of six capsules should be less than 5.0 %.	RSD-2.54%
	95% Confidence Interval	**2.63**
Intermediate Precision		
Intermediate Precision	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-100.6% Maximum-101.7% Mean-101.2%
	RSD for % dissolution of six results should be less than 5.0 %.	RSD-0.46%
	RSD for % dissolution of twelve results should not be more than 5.0%	**RSD-2.10%**

Validation Parameter	Acceptance Criteria	Results
Robustness- Speed - 98 rpm	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-98.9% Maximum-102.6% Mean-100.2%
	RSD for % dissolution of six results should be less than 2.0 %.	RSD-1.86%
	RSD for % dissolution of nine results should be less than 5.0%	RSD-2.23%
Robustness- Speed - 102 rpm	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-99.5% Maximum-103.0% Mean-101.6%
	RSD for % dissolution of six results should be less than 2.0 %.	RSD-1.62%
	RSD for % dissolution of nine results should be less than 5.0%	RSD-2.48%
Robustness-Change in pH of Buffer 6.60	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-101.8% Maximum-102.6% Mean-102.2%
	RSD for % dissolution of six results should be less than 2.0 %.	RSD-0.33%
	RSD for results should be <5.0%	RSD-2.44%

Validation Parameter	Acceptance Criteria	Results
Robustness-Change in pH of Buffer 7.00	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-102.3% Maximum-102.4% Mean-102.3%

	RSD for % dissolution of six results should be less than 2.0 %.	RSD-0.04%
	RSD for % dissolution of nine results should be less than 5.0%	RSD-2.47%
Robustness-Change in pH of Mobile Phase 2.60	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-101.5% Maximum-104.0% Mean-102.4%
	RSD for % dissolution of six results should be less than 2.0 %.	RSD-0.91%
	RSD for % dissolution of nine results should be less than 5.0%	RSD-2.56%
Robustness-Change in pH of Mobile Phase 3.00	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-99.1% Maximum-102.0% Mean-100.5%
	RSD for % dissolution of six results should be less than 2.0 %.	RSD-0.98%
	RSD for % dissolution of nine results should be less than 5.0%	RSD-2.02%

Validation Parameter	Acceptance Criteria	Results
Robustness-Change in flow rate of mobile phase 0.8 mL/min	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-99.8% Maximum-102.6% Mean-100.8%
	RSD for % dissolution of six results should be less than 2.0 %.	RSD-1.01%
	RSD for % dissolution of nine results should be less than 5.0%	RSD-2.10%
Robustness-Change in flow of mobile phase 1.2 mL/min	% Dissolution : NLT 70% (Q) in 45 minutes	Minimum-99.9% Maximum-102.8% Mean-101.1%
	RSD for % dissolution of six results should be less than 2.0 %.	RSD-1.05%
	RSD for % dissolution of nine results should be less than 5.0%	RSD-2.18%
Solution Stability		
Solution Stability of standard preparation		
After 12 Hours	**Difference with initial Area - Not more than 2.0%**	**Difference = 0.4%**
After 24 Hours		**Difference = 0.8 %**
Solution Stability of Test preparation		
After 12 Hours	**Difference with initial Area - Not more than 2.0%**	**Difference = 0.4 %**
After 24 Hours		**Difference = 0.8 %**

Validation Parameter	Acceptance Criteria	Results
Filter Paper Interference		
Standard preparation (Unfiltered)	Variation of Area should not be more than 2.0 %.	----
Standard preparation (Filtered through Whatman No.1)		% Variation = 1.7
Standard preparation (Filtered through Whatman No.41)		% Variation = 1.6
Standard preparation (Filtered through Whatman No.42)		% Variation = 1.7
Standard preparation (Filtered through 0.45 µ syringe filer)		% Variation = 1.8
System Suitability		

% RSD	NMT 2.0%	Minimum = 0.01% Maximum = 0.22%
Theoretical Plates	Not less than 2000	Minimum = 3768 Maximum = 8176
Asymmetry	Not more than 2.0	Minimum = 1.05 Maximum = 1.30

Sr. No.	**Parameter**	**% RSD for Area**	**Theoretical Plates**	**Asymmetry**
1	Specificity	0.09	8136	1.06
2	Linearity and Range	0.04	4051	1.28
3	Accuracy (Recovery)	0.01	3960	1.30
4	System Precision (HPLC)	0.03	8176	1.05
5	System Precision (Dissolution apparatus)	0.07	8163	1.05
6	Method Precision	0.12	8170	1.07
7	Intermediate Precision	0.04	3768	1.29
8	Robustness-Speed change-98 rpm	0.03	4156	1.29
9	Robustness-Speed change-102 rpm	0.02	4103	1.28
10	Robustness-Change in pH of Buffer 6.60	0.04	7382	1.11
11	Robustness-Change in pH of Buffer 7.00	0.22	7331	1.11
12	Robustness-Change in pH of Mobile Phase 2.60	0.03	5000	1.15
13	Robustness-Change in pH of Mobile Phase 3.00	0.03	4025	1.26
14	Robustness-Change in Flow Rate 0.8 mL/min	0.02	6939	1.12
15	Robustness-Change in Flow Rate 1.2 mL/min	0.03	6239	1.09
16	Solution Stability – Initial	0.02	7407	1.11
17	Solution Stability – After 12 Hours	0.05	7218	1.11
18	Solution Stability – After 24 Hours	0.02	4246	1.26

- **System Suitability:**

Sr. No.	**Parameter**	**% RSD for Area**	**Theoretical Plates**	**Asymmetry**
19	Filter Paper Interference	0.07	8027	1.08
Minimum		**0.01**	**3768**	**1.05**
Maximum		**0.22**	**8176**	**1.30**
Mean		**0.05**	**6131**	**1.16**
Limit		**Not more than 2.0%**	**Not less than 2000**	**Not more than 2.0**

- **Discussion:**

The observations and result obtained for each parameter including Specificity, Linearity, Accuracy (Recovery), Method Precision (Repeatability), Intermediate precision (Ruggedness), Robustness, Solution stability and System suitability lies well within the acceptance criteria.

Specificity of the method was demonstrated by analyzing Blank preparation, Placebo preparation, Standard preparation, Test preparation and Blank preparation, Placebo preparation, did not show any interference.

The data obtained from Linearity, Precision and Accuracy reveals that the method is linear, precise and accurate over the range of 10% to 150% of working concentration.

Ruggedness of the method was evaluated under intermediate precision and results were found within acceptable limits.

The robustness of the method was evaluated by altering the variables such as different paddle speed (98 rpm and 102 rpm), Different pH of Buffer (6.60 and 7.00) , Different pH of mobile phase (2.60 and 3.00) and Different flow rate (0.8 mL/min and 1.2 mL/min). The data obtained from individual condition and overall conditions including repeatability was found well within the limit.

The Standard preparation and Test preparation is found stable up to 24 Hours at ambient temperature (25 ± 2°C). The system suitability parameters met the acceptance criteria, which were commenced during study of each individual validation characteristics.

CONCLUSION

Since the results are within acceptance criteria for all parameters, therefore, the method is considered as validated and suitable for routine and stability analysis.The developed RP-HPLC method was found to be simple, precise, accurate and rapid for determination Tenoxicam in their bulk forms. The mobile phase is simple to prepare and economical. This method can be easily and conveniently adopted for routine analysis of Tenoxicam.From data it was concluded that the method is linear, precise and accurate over 10% to 150% working concentration range.This method was successfully applied for the identification, quantitative analysis, homogeneity tests and stability tests of all compounds in a solid dosage form.Hence, the method can be easily and conveniently adopted for routine estimation of Tenoxicam in solid dosage form. As the process is precise and accurate, drug also stable for long hours; this process can be applied for forced degradation study and multimedia profiling.

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