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"DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF TENOXICAM IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM."

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ABSTRACT:

The present study describes a simple, accurate, precise and cost-effective reverse phase High Performance Liquid Chromatographic method for estimation of tenoxicam in their pharmaceutical dosage form. The separation was carried on Kromasil, C18, 250 mm X 4.6 mm, 5 µm. Detection was done using UV detector at isocratic point 368 nm. The developed method employed mobile Acetonitrile: Buffer (60: 40 % v/v), with flow rate 1.0 ml/min. High linearity of the developed method was confirmed over concentration range 1-8 µg/ml for tenoxicam with the correlation coefficient of 0.999. The Percentage RSD for precision of the method was found to be less than 2%. The percentage recoveries for tenoxicam was found to be in range 98.00-102.00 w/v. Peaks was obtained at retention time 2.9 min for for tenoxicam. By using all the above parameters, a simple, accurate, precise and cost-effective method were developed, optimize and validate.

KEYWORDS: RP-HPLC, Method optimization , development, tenoxicam .

INTRODUCTION:

Analytical chemistry is the analysis of material samples to gain an understanding of their chemical composition and structure. During last few decades, analytical chemistry has witnessed extensive development in terms of sophistication, quantitation and Instrumentation. Consequently, newer analytical techniques (such as hyphenated techniques FTIR, GCMS, LCMS, HPLC, HPTLC etc.) and their areas of

application have increased considerably because of the stringent requirements for testing and monitoring of the drugs for approval; the demand on quality, validation data and performance of analytical methods have gained an importance.

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter both qualitatively (what is present) and quantitatively (how much is present). [1-8]

4-hydroxy-2methyl-n-(pyridinyl-2-yl)-2h-thieno [2, 3-e] is the chemical name for tenoxicam. Non-steroidal anti-inflammatory medication -1, 2thiazine-3-carboxamide 1, 1- dioxide It is used to treat rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis by reducing inflammation, oedema, stiffness, and pain. In BP, it is official**.**

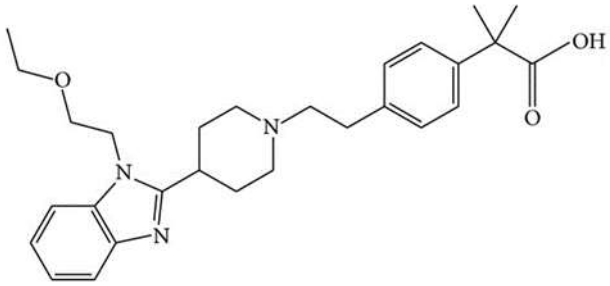

Fig 1: Structure of Tenoxicam

Tenoxicam is a nonsteroidal anti-inflammatory medication sold under the trade names Mobiflex and others (NSAID). It's used to treat rheumatoid arthritis, osteoarthritis, ankylosing spondylitis (a kind of arthritis that affects the spine), tendinitis (tendon inflammation), bursitis (inflammation of a fluid-filled sac around joints and around the bones), and periarthritis of the shoulders or hips (inflammation of tissues surrounding these joints**).**

Material and Method

Table 1: List of apparatus/ instruments used.

Sr. No.	Instrument	Make	Series/Model
1	Balance	Aczet	CY224
2	PH-Meter	Labman	LMPH-10
3	HPLC	Agilent	1260 Infinity II
4	UV-spectrophotometer	Jasco	550
5	Ultrasonicator	Bio Technics India	12L300H

Table 2: List of chemicals used

Sr. No.	Chemicals/ Reagents/ Solvents	Supplier	Grade
1	Methanol	Merck	HPLC grade
2	Acetonitrile	Merck	HPLC grade
3	Water	Siddhi Lab	HPLC grade
4	Ortho-phosphoric Acid (OPA)	Merck	HPLC grade
5	Ethanol	Merck	Analytical
6	DMF	Merck	Analytical
7	DMSO	Merck	Analytical
8	HCl	Merck	Analytical

Table 3: HPLC System

HPLC System HPLC Binary Gradient System	
Model No.	1260 Infinity II
Make	Agilent
Pump	DEAX02386
Detector	DEACX16446
Column	Kromasil C18 column (250 mm X 4.6 mm i.d. 5µm).
Software	Openlab EZ Chrome

Table 4: Analytical Balance

Analytical Balance: Azcet High Precision Balance	
Model	CY 224C
Maximum	220 gm
Minimum	0.001gm
pH Meter: Digital pH meter	
Make	LabMan

Preparation of standard stock solution :

Weighed accurately 10 mg of Tenoxicam and transferred to 50 mL volumetric flask. Added 30-35 mL of 0.1 N NaOH solution, sonicated to dissolve it completely, made the volume up to the mark with 0.1 N NaOH solution. Further Diluted 0.5 mL to 20 mL with mobile phase. (5 PPM of Tenoxicam).

API Sample Preparation: Weighed accurately 10 mg of Tenoxicam and transferred to 50 mL volumetric flask. Added 30-35 mL of 0.1 N NaOH solution, sonicated to dissolve it completely, made the volume up to the mark with 0.1 N NaOH solution. Further Diluted 0.5 mL to 20 mL with mobile phase. (5 PPM of Tenoxicam)

Selection of analytical wavelength from the spectrophotometric method: Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis. The standard solution was scanned between 200-400 nm. The wavelength of maximum absorption was determined for drug tenoxicam and it was 368 nm.

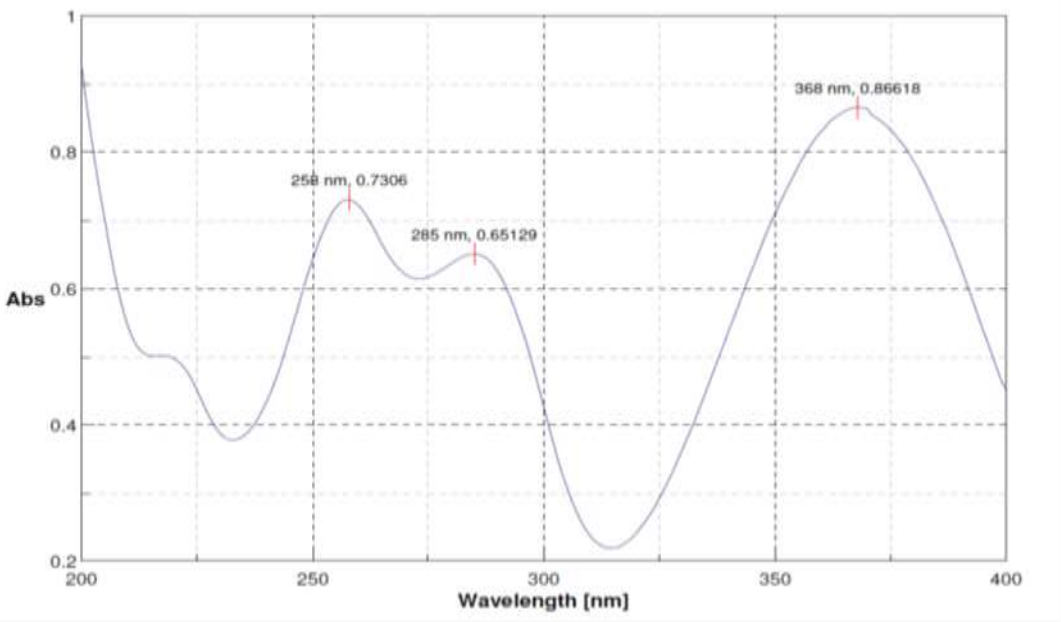

Fig. 2: UV spectrum of Tenoxicam

Table 5: Optimized Chromatographic Conditions:

Parameter	**Description**
Mode	Isocratic
Column Name	Kromasil C18, (250 mm X 4.6 mm i.d.) 5µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	368 nm
Column Oven temp	35ºC
Mobile Phase	Methanol: Water (70: 30)
Flow Rate	1.0 ml/min

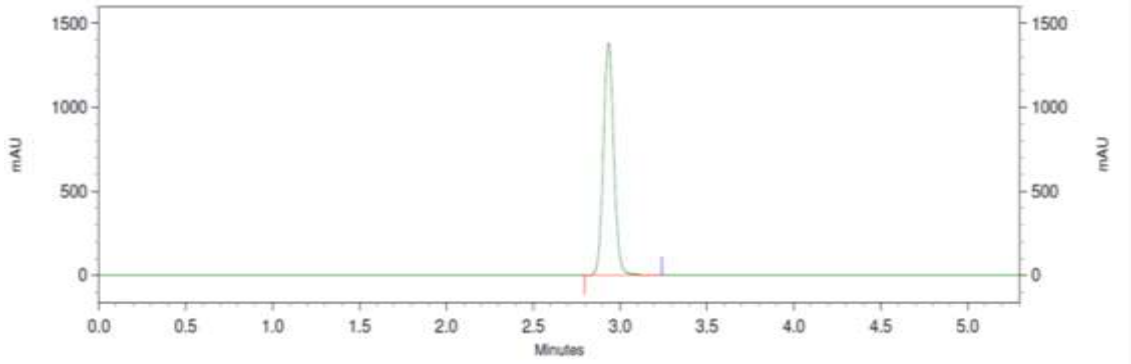

Fig 3: Chromatogram for optimized RP-HPLC condition (Tenoxicam)

RESULT AND DISCUSSION :

Analysis of test samples (Assay)

a) Assay Calculation:

$$\% \text{ Assay} = \frac{\text{sample area}}{\text{std mean area}} \times \frac{\text{std wt.}}{20} \times \frac{1}{50} \times \frac{50}{\text{sample wt.}} \times \frac{20}{1} \times 100$$

$$\frac{16170241}{16100420} \times \frac{10.2}{100} \times \frac{10}{20} \times \frac{100}{10.3} \times \frac{20}{10} \times 100$$

$$1.004336595 \times 0.102 \times 0.5 \times 9.70873786 \times 2 \times 100$$

% Assay =99.46%

Validation of RP-HPLC Method:

The developed method for estimation of Tenoxicam was validated as per ICH guidelines for following parameters.

1) **FILTRATION STUDY:** Filtration study of an analytical procedure checks the interference of extraneous components from the filter, deposition on filter bed and compatibility of the filter with the sample. This study will be conducted with a sample of **tablet.**

Filter study performed by using Centrifuged sample (Unfiltered), Sample passed through 0.45 µ PVDF filter and 0.45 µ Nylon filter, by discarding 5 mL of solution. **(Performed on Tablet sample)**

Table. No. 6 Analytical data of Filter Test for Tenoxicam

Sample	Area	% Absolute difference
Unfiltred	5189743	NA
0.45 µ PVDF filter	5176283	0.26
0.45 µ Nylon filter	5155971	0.65

2) **STABILITY OF ANALYTICAL SOLUTION**

Stability study was conducted for standard solution and test solution. Test solution stability was performed using test sample. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analyzed after 12 hours and 24 hours.

Table. No. 7 Analytical data of Tenoxicam for solution Stability

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	5176294	NA	**Initial**	5162809	NA
12 Hours	5153371	0.44	**12 Hours**	5139148	0.46
24 Hours	5131056	0.87	**24 Hours**	5120608	0.82

3) **SPECIFICITY:** Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

Blank, standard solution and test sample prepared and injected to check peak purity. Blank does not showed any interference at R.T. of Tenoxicam. Peak purity for both standard as well as sample were within limits. Sample solution exhibit same R.T. as that of standard solution. Hence developed chromatographic method passed the criteria for specificity.

Table No. 8: Results of specificity

Description	**Observation**
Blank	No interference at R.T. of Tenoxicam in blank
Placebo	No interference at R.T. of Tenoxicam in placebo
Standard solution	Peak purity was 0.998 for Tenoxicam
Test sample	Peak purity was 0.997 for Tenoxicam

4) **Linearity and Range**

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.

Table no: 09 Result and statistical data of linearity of Tenoxicam

Level	Conc. (µg/mL)	Area	Mean	% RSD
10%	0.51	497591	500520	0.665
		499831		
		504138		
50%	2.55	2556247	2544472	0.436
		2534186		
		2542983		
100%	5.10	5136856	5142300	0.311
		5129759		
		5160284		
125%	6.32	6472641	6450033	0.304
		6439410		
		6438049		
150%	7.65	7598364	7625468	0.353
		7652148		
		7625891		

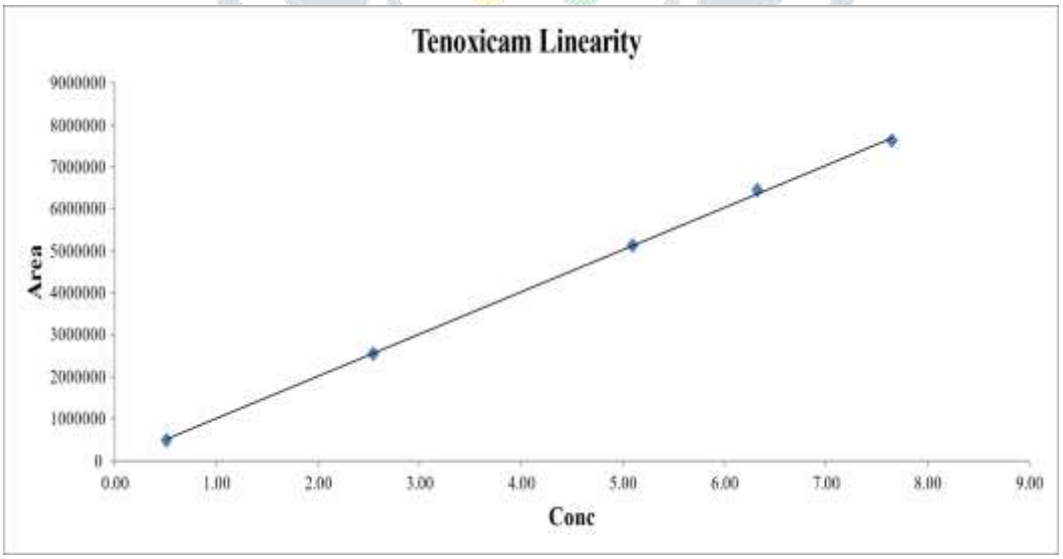

Fig.8.41: Linearity graph of Tenoxicam

Table No. 10 Data for calibration curve of Tenoxicam

Parameters	Result
Detection Wavelength	368 nm
Beer's law limit	0.5- 7.6 µg/mL
Slope	1007086.588
Intercept	-5612.308822
Correlation coefficient (R^2)	0.9998

5) ACCURACY (RECOVERY):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added. Recovery of analytical procedure was found well within acceptance criteria at all 3 levels. % Recovery not get hampered by changed in analyte concentration

Table no. 11 Result and statistical data of accuracy for Tenoxicam

Level (50 %)	Area	Recovered conc	Added conc	% Recovery
50	2573164	2.54	2.55	99.61
	2511081	2.48	2.50	99.20
	2543716	2.52	2.55	98.82
100	5149207	5.09	5.05	100.79
	5161071	5.10	5.10	100.00
	5167159	5.11	5.15	99.22
150	7566284	7.48	7.55	99.07
	7581091	7.50	7.50	100.00
	7579148	7.49	7.55	99.21

Chromatograms of Accuracy for drug:

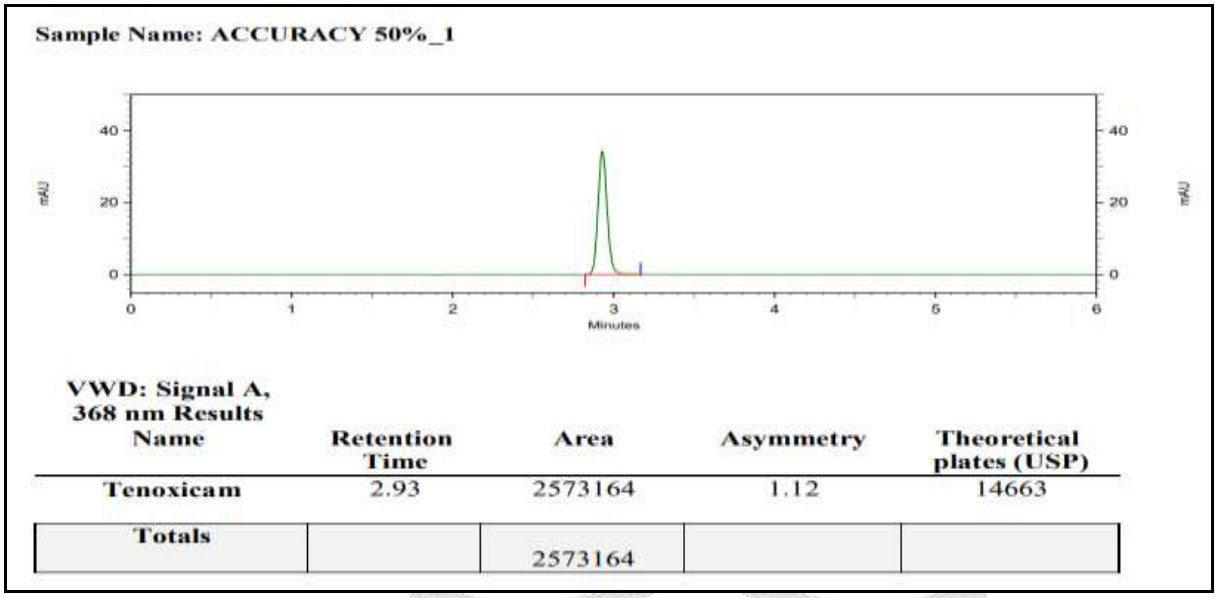

Fig.8.20: Chromatograms of Accuracy for drugs at level of 50% -

6) PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on API sample. % RSD was found well within acceptance limit and hence method is precise.

A) Intraday Precision:

Table no.12 Analytical data Intraday Precision of Tenoxicam

Sample	Area	% Assay
Sample 1	5103814	100.93
Sample 2	5125843	99.38
Sample 3	5129713	100.44
Sample 4	5105831	98.99
Sample 5	5176984	99.40
Sample 6	5146023	100.76
Mean		**99.98**
STD DEV		**0.824492**
% RSD		**0.825**

B) Interday Precision:

Table no. 13 Analytical data Interday Precision of Tenoxicam

Sample	Area	% Assay
Sample 1	5106813	100.56
Sample 2	5113982	99.72
Sample 3	5109143	100.61
Sample 4	5176317	99.95
Sample 5	5176256	100.93
Sample 6	5106521	99.57
Mean		**100.22**
STD DEV		**0.550842**
% RSD		**0.550**
Precision plus intermediate precision	**Mean**	**100.103**
	STD DEV	**0.68016**
	% RSD	**0.679**

Acceptance Criteria:

The % RSD for the six samples NMT 2.0.

For Both: % RSD for 12 sample (Precision and Intermediate Precision samples) NMT 2.0 %

Conclusion: Precision: The %RSD of method precision is 0.53 & 0.495 Therefore, the HPLC method for the determination of tenoxicam is precise.

Chromatogram of Interday Precision:

% RSD for 12 sample (Precision and Intermediate Precision samples) NMT 2.0 %

7) ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Effect of variation in the flow rate of the mobile phase

1. Change in flow Rate

Table no. 14 Data for change in flow rate Tenoxicam

Sr. No.	System Suitability parameter	Tenoxicam		Limits
		As such+10%	As such-10%	
1	Peak area response	5081423	5008149	
2	Theoretical plates	14865	14214	NLT 2000
3	Asymmetry	1.11	1.13	NMT 2.0
4	% Assay	100.74	100.78	

2. Change in wavelength:

Table No. 15 Analytical Data for change in wavelength for Robustness of Tenoxicam

Sr. No.	System Suitability parameter	Tenoxicam		Limits
		As such+3 nm	As such-3 nm	
1	Peak area response	4976124	4928749	
2	Theoretical plates	14638	14745	NLT 2000
3	Asymmetry	1.12	1.12	NMT 2.0
4	% Assay	100.82	99.72	

3. Change in Column Oven Temperature (COT)

Table No. 16 Analytical Data for change in COT for Robustness of Tenoxicam

Sr. No.	System Suitability parameter	Tenoxicam		Limits
		As such+2^0C	As such-2^0C	
1	Peak area response	5238147	4862764	
2	Theoretical plates	14873	13276	NLT 2000
3	Asymmetry	1.12	1.14	NMT 2.0
4	% Assay	100.80	100.46	

Table no. 17 change in parameter

Change in Parameter	Standard area	Theoretical plates	% Assay
Wavelength by +3 NM	4976124	14638	100.82
Wavelength by -3 NM	4928749	14745	99.72
Flow rate by +10%	5081423	14865	100.74
Flow rate by -10%	5008149	14214	100.78
Column oven temp by +2ºC	5238147	14873	100.80
Column oven temp by -2ºC	4862764	13276	100.46

8) Limit of Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope. The limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

***Where,* SD= Standard deviation of the Y intercept**

***S* = Slope**

Tenoxicam- LOD = 3.3 x 57057.222/ 682788.749 = 0.28 µg/ml

The LOD of Tenoxicam was found to be 0.28 µg/ml

9) Limit of Quantitation

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope.

The quantitation limit (LOQ) may be expressed as

LOQ = 10 (SD)/S

***Where,* SD = standard deviation of Y intercept**

S = Slope

Tenoxicam- LOQ = 10 x 57057.222/ 682788.749 = 0.84 µg/ml

The LOQ of Tenoxicam was found to be 0.84 µg/ml

Table no. 17: Result data of LOD and LOQ

Name	**LOD**	**LOQ**
Tenoxicam	0.28	0.84

10) System suitability test:

Sample solution of Tenoxicam were injected three times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from three replicate injection.

System suitability is a Pharmacopeial requirement and is used to verify, whether the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from Five replicate injection of standard drug solution and the results are recorded.

Table no. 18: Results for system suitability test of Tenoxicam

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_1	5163842	1.11	14529
2	Standard_2	5139751	1.12	14367
3	Standard_3	5139706	1.12	14298
4	Standard_4	5173149	1.11	14327
5	Standard_5	5173054	1.11	14554
Mean		**5157900**	**1.11**	**14415**
STD Dev		**17013.89812**		
% RSD		**0.33**		

Conclusion:

A literature survey revealed that several methods have been reported for the determination of Tenoxicam in bulk drug or pharmaceutical dosage forms separately, but no one method was developed for estimation of tenoxicam by using RP-HPLC method. Hence, in the present study, a new, sensitive, and suitable reversed-phase high-performance liquid chromatography method was developed and validated for the determination of tenoxicam in bulk drug and pharmaceutical dosage form.

The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation, and improved selectivity as well as sensitivity. The regression coefficient (r^2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in the tablet dosage form. The % percent RSD was also less than 2.0 % showing a high degree of precision of the proposed method.

Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in the pharmaceutical industry for the bulk drug of Tenoxicam and also in the pharmaceutical dosage form.

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