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ANALYTICAL ESTIMATION OF TENOXICAM BY UV AND RP-HPLC METHODS

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ABSTRACT

Tenoxicam is an anti-inflammatory drug used to relive acute pain also it is used for the treatment of rheumatoid arthritis. But it is a BCS class II drug. So it has less solubility. To determine its solubility in in-vitro and *in-vivo* studies it is necessary to develop UV and RP-HPLC methods. The wavelength corresponding to maximum absorbance in methanol was found at 261nm using UV spectrophotometer. Beers law was obeyed in the concentration range of 0.25 µg/ml with regression coefficient 0.997. The slope of tenoxicam in RP-HPLC method was found to be 0.958 with regression coefficient 'r' of 0.998. The developed UV method was found to be precise as it shows less than 2 %RSD values for interday and intraday. The regression coefficient was found to be 0.997 and 0.998 for UV and RP-HPLC methods respectively which was close to unity indicates that at this concentration range tenoxicam was highly linear

INTRODUCTION

Tenoxicam (4-hydroxy-2-methyl-*N*-(pyridine-2-yl)-2*H*thieno- 1,2-thiazine-3-carboxamide-1,1-dioxide) (*1*) is a Non Steroidal Anti-Inflammatory Drug (NSAID), with analgesic and antipyretic properties.. It is indicated for treatment of rheumatoid arthritis and to control acute pain. Thetenoxicam has anti-inflammatory effects by the inhibition of the enzyme cyclooxygenase which results in the peripheral inhibition of prostaglandin synthesis. The inhibition of prostaglandins accounts for the peripheral analgesic effects of tenoxicam as prostaglandins sensitize pain receptors. Tenoxicam has anti-inflammatory activity due to the inhibition of the enzyme cyclo-oxygenase which inhibits the peripheral prostaglandin synthesis. The inhibition of prostaglandins results in peripheral analgesic effects as the prostaglandins sensitise pain receptors. Tenoxicamhas anti-pyretic activity due to the central action on hypothalamus which Causes peripheral dilation, increased blood flow and subsequent heat loss (University of leeds, 1988). The present research aims to develop simple, accurate and precise UV spectrometric method for determination of tenoxicam in bulk and pharmaceutical Formulations as per ICH Guidelines.

METHODOLOGY:

Instrument: Double beam UV-Visible spectrophotometer (Shimadzu)

Materials:

Tenoxicam was obtained as gift sample from Dr.Reddy's laboratory, Hyderabad, INDIA. Methanol, ethanol, acetone and chloroform used were of analytical grade and purchased from SD Fine Chemicals, India. All the other chemicals and reagents were of analytical grade.

Method:

Determination of solubility of tenoxicam:

To determine the appropriate solvent in which tenoxicam is stable and posses a relatively higher solubility, a set of aqueous

and non aqueous solvents were used and thus used for the development of UV spectrophotometric method of estimation for tenoxicam.

1. Aqueous solvents- Water, pH 7.4, pH 5.5, 0.1 N Hcl and 0.1 N NaOH.
2. Non aqueous solvents- Methanol, Ethanol, Acetone and Chloroform.

Scanning of absorption maxima λ_{max}oftenoxicam:

A simple, precise and reproducible method for estimation of tenoxicam was standardised usingtenoxicam solution which was prepared by dissolving 10 mg of tenoxicam in 5 ml of ethanol and was made up to 100 ml with phosphate buffer pH 5.5. This solution was placed in glass cuvettes and scanned from 400-200 nm using a UV-Visible spectrophotometer to determine the absorption maxima (λ_{max}). (PanwarMangal Singh. 2012).

Construction of calibration curve for estimation of tenoxicam:

Preparation of primary stock solution:

Accurately weighed 100 mg of the tenoxicam was transferred to a volumetric flask and dissolved in 5 ml of ethanol and then volume was made up to 100 ml with phosphate buffer pH 5.5 to give a solution of strength 1 mg/ml.(Kalian Hazra, Ravi Kumar. 2015)

Preparation of secondary stock solution: 10 ml of the above primary stock solution was pippeted out and transferred to a volumetric flask and the volume was made up to 100 ml with phosphate buffer pH 5.5. The concentration of the solution obtained is 100 µg/ml.

Preparation of working samples:

Various concentrations of tenoxicam i.e., 1, 5,10,15,20 and 25 µg/ml were prepared by diluting varying volumes of secondary stock solution. Absorbance was measured at 261 nm and the values obtained were plotted against the concentration to get a standard calibration curve.

Reproducibility:

This UV method was also studied for reproducibility by analyzing three individually weighed samples of tenoxicam. Then working samples of tenoxicam were prepared and estimated as described above. To explicit reproducibility relative standard deviation (RSD) was calculated. (Mandrescu M. 2013).

Analytical Method Validation Parameters

Intra-day variability:

To obtain intra-day variability the tenoxicam working solutions prepared were stored for 8 hrs at room temperature and absorbance was measured at 261 nm using UV visible spectrophotometer.All the measurements were performed in triplicate.(Vishal VasantHolkar. 2015)

Inter-day variability

To obtain inter-day variability the tenoxicam working solutions prepared which was stored at room temperature were analysed on another day by measuring the absorbance at 261 nm using UV visible spectrophotometer**.**All the measurements were performed in triplicate.

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed analytical method. Repeatablity was determined by preparing six replicates of 5 µg/ml concentration of the sample and the absorbance was measured. All the measurements were performed in triplicate. (Kiran Sharma. 2012.)

Development of RP-HPLC method for estimation of tenoxicam in rat plasma:

Instrument and chromatographic conditions:

High Pressure Liquid Chromatography instrument (Shimadzu, Sphinchrome software series, Japan) equipped with Shimadzu LC-10 a pumps and UV-Visible SPD-10 AVP detector was used for estimation of tenoxicam. The samples were chromatographed on a reverse phase Hibar C_{18} (4.6 i.d. × 150 mm, 5 µm) column. Different mobile phase were tested in order to find the sensitive conditions for the sharp peaks characteristic of the selected internal standard such as aceclofenac and tenoxicam. Acetonitrile: water (61:39 v/v) was selected as mobile phase with pH adjusted to 2.5 with formic acid, at a flow rate of 1.0 mL/min and at a a column temperature of 25°C ± 1. The injector having the capacity of 0-25 µL, Rheodyne Hamilton type was used to inject 25 µL of samples.

Sample that are filtered through 0.45µm Millipore filter paper using sample filtration syringe system were used to inject in the system and UV detection was observed at 375 nm.(Ganga Gavhad. 2015)

Preparation of stock solution of tenoxicam:

10 mg of tenoxicam was weighed accurately and dissolved in 100 ml of mobile phase.

Preparation of internal standard solution

1 mg of aceclofenac was dissolved in 10 ml of mobile phase. From this solution, it was diluted to obtain 0.15 µg/ml of aceclofenac solution.

Extraction procedure:

Blood sample, 0.5 ml volume was collected in ependroff tube from retro orbit sinus of male wistar albino rats weighing around 225 gm to 250 gm. 20% sodium citrate solution (anticoagulant) was added and the sample was subjected to centrifugation at 15000 rpm for 15 mins(M. AsadullahMadni. 2016).

Clear plasma of 100 µl, 25µl of aceclofenac (IS, 0.15 µg/ml in mobile phase) solution and mobile phase was added to make the volume up to 1ml. The tubes were tightly capped, vortexed for 10 min and centrifuged for 20 min at 3000 rpm. Then the supernatant clear sample was filtered through 0.45 µm Millipore filter paper and then 25 µl of sample was injected into the system and the chromatogram of IS was obtained (JL Mason . 2005).

Calibration curve:

Standard solutions containing 4, 8, 16, 20 and 24 µg/ml of tenoxicam were prepared in mobile phase. An aliquot of drug free plasma 100 µl was accurately measured into a stoppered centrifuge vials followed by the addition of 100 µl of tenoxicam working solution and 0.15 µg/ml solution of aceclofenac (I.S).(Vijay R Salunkhe, Snehal J Patil. 2014)

RESULTS AND DISCUSSION:

Solubility: With aqueous solvents no absorbance was produced due to poor solubility. With non aqueous solvent absorbance was produced. But when the drug was first dissolved with non- aqueous solvent like ethanol and made up the volume with aqueous solvent absorbance was produced**.** Hence water with ethanol was used as solvent for preparing stock solutions.

Absorbance of tenoxicam for different concentrations is given in **Table 1.** Calibration graph of tenoxicam is given in **Fig.2**. From the calibration graph slope value was found to be 0.026 and regression was found to be 0.997 which was close to unity indicates positive correlation between concentration and absorbance of tenoxicam.

Scanning of absorption maxima λ_{max}oftenoxicam

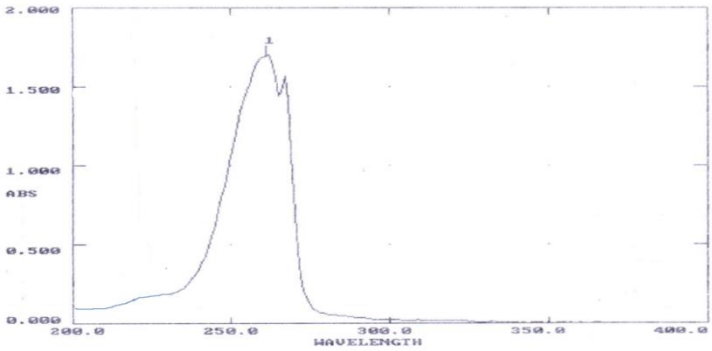

Fig. 1: λ_{max} of tenoxicam in phosphate buffer 5.5

The λ_{max} of tenoxicam was found to be 261 nm.

The absorption maxima of tenoxicam are given in **Fig.1.**

Table 1: Concentration Vs absorbance values for estimation of tenoxicam (n=3

Concentration (µg/ml)	Absorbance ±S.D	RSD
0	0	0
1	0.043±0.052	0.61
5	0.132±0.067	1.25
10	0.269±0.053	0.41
15	0.398±0.059	0.58
20	0.510±0.064	0.28
25	0.674±0.070	1.26

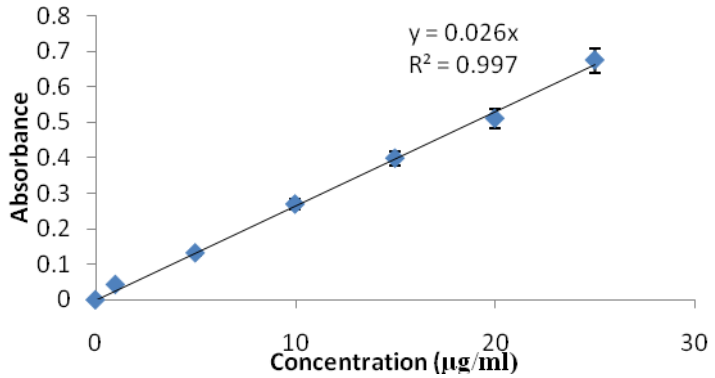

Fig. 2 : Calibration curve for estimation of tenoxicam using UV method

Absorbance of tenoxicam for different concentrations is given in **Table 1.** Calibration graph of tenoxicam is given in **Fig.2**. From the calibration graph slope value was found to be 0.026 and regression was found to be 0.997 which was close to unity indicates positive correlation between concentration and absorbance of tenoxicam.

Reproducibility:

Reproducibility werereported in **Table 1**as RSD**.**From the data it was revealed that the relative standard deviation is less than 2 which indicates good reproducibility.

Precision:

The results were reported as%RSD. The precision result showed a good reproducibility (Table 1) with percent relativestandard deviation less than 2. The results of intraday and interday precision studies areshown in **Table 2**

Table 2: Intra-day and Inter-day absorbance of tenoxicam

Concentration (µg/ml)	Intra-day Absorbance ±S.D	Inter-day Absorbance ±S.D	RSD (%)
1	0	0	0
2	0.015±0.0031	0.018±0.0038	1.5
3	0.024±0.0018	0.025±0.0019	0.8
4	0.034±0.0017	0.037±0.0011	1.4
5	0.047±0.0035	0.045±0.0028	1.3
6	0.065±0.0029	0.061±0.0021	0.6
7	0.081±0.0042	0.082±0.0022	1.2
8	0.089±0.0019	0.09±0.0012	0.9
9	0.092±0.0025	0.094±0.0027	0.8
10	0.108±0.0026	0.11±0.0015	0.7

Table 3 Concentration Vs peak area ratio of tenoxicam and aceclofenac using RP-HPLC method

Concentration (µg/ml)	Peak area ratio	Co-efficient of variation
0	0	0
4	1.216	1.26
8	7.864	3.25
16	14.625	1.53
20	19.54	2.89
24	23.156	3.12

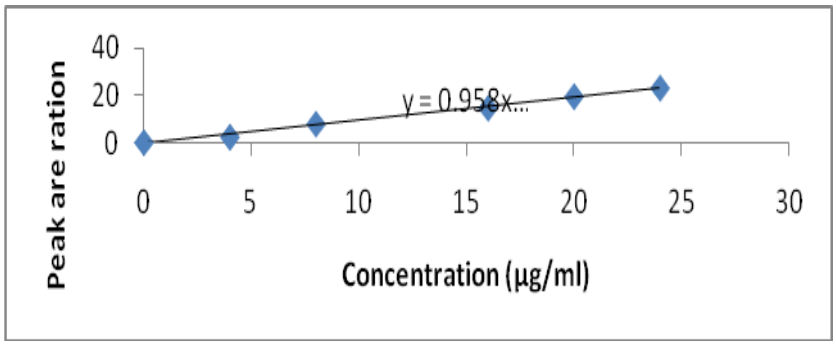

Fig. 3: Calibration curve of tenoxicam using RP-HPLC method

The proposed method provides a simple, accurate, economical and convenient method for the analysis of tenoxicam using UV spectrophotometry.The wavelength corresponding tomaximum absorbance in methanol was found at 261nm. Beers law was obeyed in theconcentration range of 0.25µg/ml with regression coefficient of 0.997. The developed UVmethod was found to beprecise as it shows less than 2%RSD values for interday and intraday. The method was also found to be rugged and robust as the % RSD values were found to be less than 2.

RP-HPLC method for estimation of tenoxicam

The quantification of chromatogram was performed using peak area ratios of the drug to internal standard and values are shown in **Table 3**. A standard graph was plotted between the plasma concentrations of tenoxicam and the peak area ratio of tenoxicam to aceclofenac (I.S) is shown in **Fig 3.**

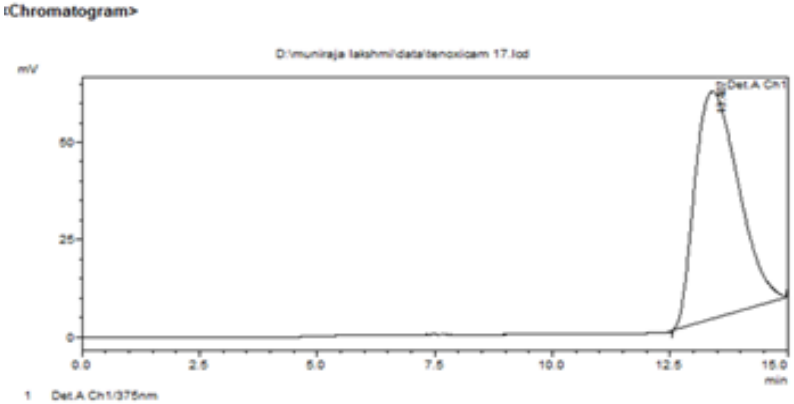

Fig. 4: HPLC chromatogram of tenoxicam (16 µg/ml) in mobile phase

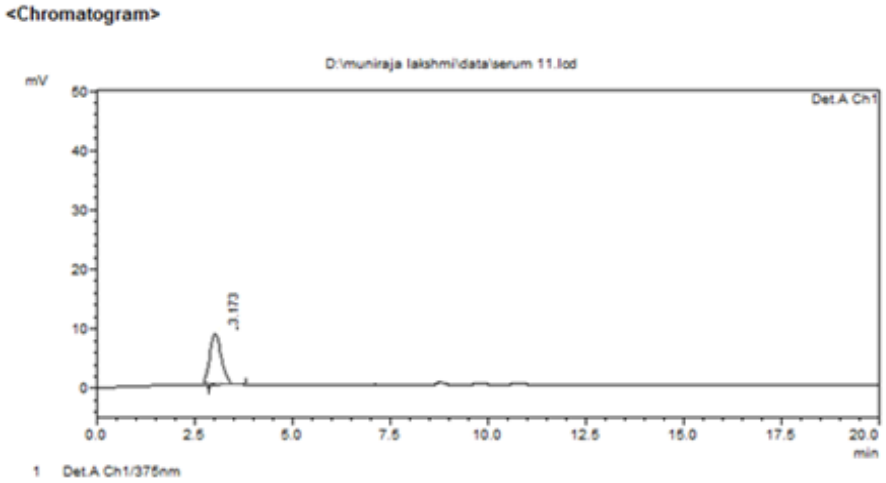

Fig. 5: HPLC chromatogram of rat plasma

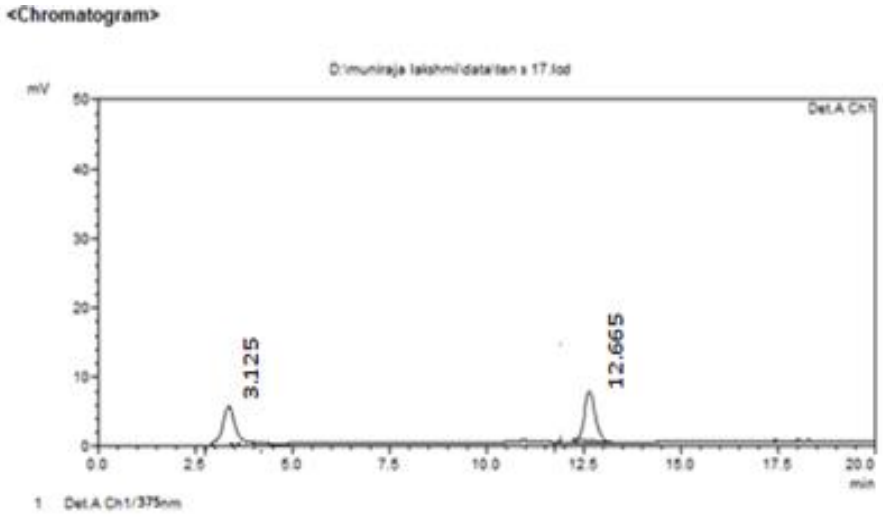

Fig. 6: HPLC chromatogram of tenoxicam in rat plasma

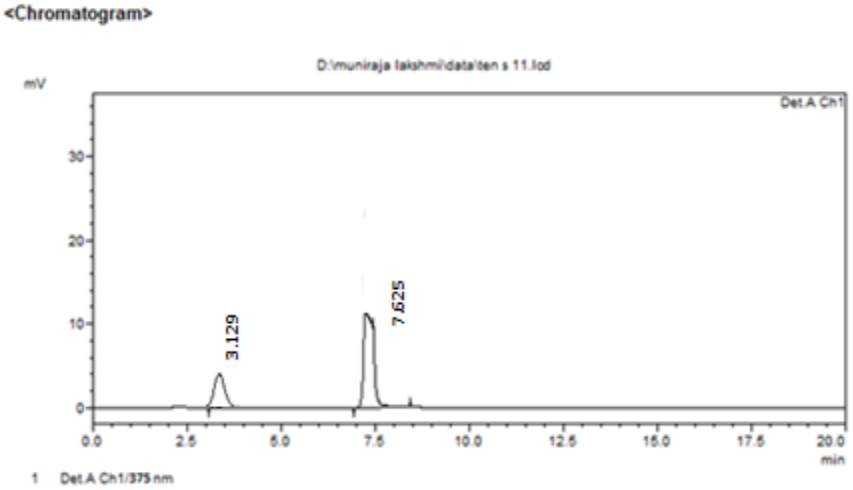

Fig. 7: HPLC chromatogram of aceclofenac in rat plasma

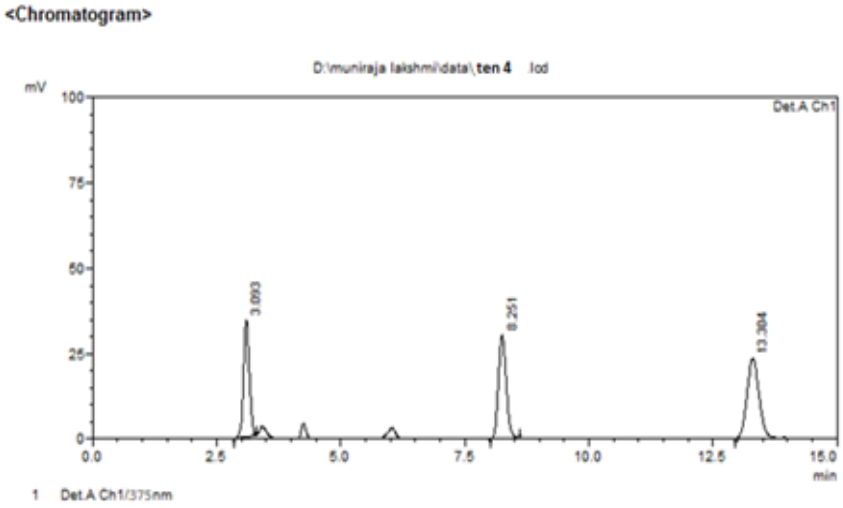

Fig. 8: HPLC chromatogram of tenoxicam (4 µg/ml) and IS in rat plasma

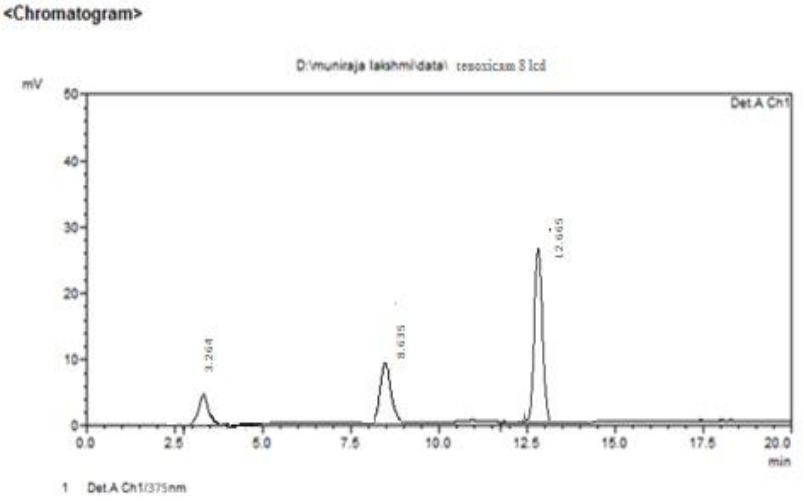

Fig. 9: HPLC chromatogram of tenoxicam (8 µg/ml) and IS in rat plasma

Peak area ratio of tenoxicam and aceclofenac at different concentrations in rat plasma was observed and given in **Table 3**and calibration graph is given in **Fig. 3.** The HPLC chromatogram of tenoxicam in mobile phase, HPLC chromatogram of rat plasma, HPLC chromatogram of tenoxicam in rat plasma, HPLC chromatogram of IS in rat plasma, HPLC chromatogram of tenoxicam (4 µg/ml) and IS in rat plasma and HPLC chromatogram of tenoxicam (8 µg/ml) and IS in rat plasma are shown in **Fig. 4, 5, 6, 7, 8 and 9** respectively. The slope of tenoxicam in HPLC method was found to be 0.958 with regression coefficient 'r' of 0.998 which was close to unity indicates that at this concentration range tenoxicam was highly linear. This method is simple, sensitive and successfully applied in pharmacokinetic studies.

CONCLUSION

The developed UV and RP-HPLC method for estimation of tenoxicamcan be concluded to be simple, accurate, reliable and economical. By using the UV method, beers law was obeyed in the concentration range of 0.25µg/ml with regression coefficient 0.997 and the slope of tenoxicam in HPLC method was found to be 0.958 with regression coefficient 'r' of 0.998 which was close to unity indicates that at this concentration range tenoxicam was highly

linear The developed UV and RP-HPLC method was found to beprecise as it shows less than 2%RSD values for interday and intraday. The proposed method is specific without and interference of excipients and hence can be used for the routine analysis of tenoxicam in bulk and in pharmaceutical formulation.

REFERENCES:

1. Ganga Gavhad.2015. Development and validation of RP-HPLC method for the simultaneous determination of cinnamaldehyde and curcumin in pharmaceutical formulation of lozenge, International Journal of Pharmaceutical and Chemical Sciences, 4 (3) : 311-316

2. JL Mason. 2005. Simple method for the analysis of tenoxicam in human plasma using high-performance liquid chromatography, Journal of Chromatography Biomedical Sciences and Applications,66(2),410-415.

3. Kalian Hazra, Ravi Kumar. 2015. UV-Visible spectrophotometric estimation ofcurcumin in nanoformulation, International journal of pharmacognosy, 2(3):127-130.

4. Kiran Sharma. 2012. Development and validation of UV spectrophotometric method for the estimation of curcumin in bulk drug and pharmaceutical dosage forms, International journal of drug development & research, 4 (2):375-380.

5. Asadullah Madni. 2016. Determination of tenoxicam in the plasma by reverse phase hplc method using single step extraction technique: a reliable and cost effective approach, Acta Poloniae Pharmaceutica- Drug Research, Vol. 73 (5): 1129-1134.

6. Mandrescu M. 2013.Ultraviolet spectrophotometric determination of tenoxicam using iodine solution as reagent,Journal of Applied Chemistry, 113(2):598-603.

7. PanwarMangal Singh. 2012. Visible Spectrophotometric Estimation of Tenoxicam from Tablets, International Journal of Pharmaceutical & Biological Archives,3(4):993-995.

8. University of leeds, 1988. International experience with tenoxicam: a review, Scandinavian Journal of Rheumatology, 73:23-27.

9. Vijay R Salunkhe, Snehal J Patil. 2014. UV spectrophotometric and hplc method development of quercetin and curcumin in polyherbalchurna and its validation, International Journal of Pharmaceutical and Phytopharmacological Research, 4(1):8-12

10. Vishal Vasant Holkar. 2015. Development and validation of uv spectrophotometric method for the estimation of curcumin in bulk and pharmaceutical formulation, World Journal of Pharmacy and Pharmaceutical Sciences, 4 (5):1456-1463.