

## Estimation of Non micronized Piroxicam in SEDDS Formulation by HPLC Method

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### ABSTRACT

The different assay methods for the determination of Piroxicam in the pharmaceutical preparation have been reviewed. The determination of recoveries of Piroxicam has been described by HPLC method. The aim of the present study was to develop and validate analytical method for determination of non micronized Piroxicam in SEDDS formulation in hard gelatin capsules by High Performance liquid chromatography (HPLC) method. It is therefore necessary to study the behavior of non micronized Piroxicam when it is incorporated in SEDDS formulation. The HPLC method was developed using chromatopack, peerless basic C18, 250x4.6mm, 5 $\mu$ m analytical column. The mobile phase comprising of acetonitrile : potassium dihydrogen ortho phosphate buffer [pH-3.0] in the ratio (40:60) v/v. The flow rate was maintained at 1.0ml/min and elute was monitored by using U.V detector at 230nm. The retention time of Piroxicam was about 12 minutes. The method was validated for its specificity, accuracy, precision, and linearity, limit of detection(LOD), limit of quantification(LOQ), robustness and stability parameters. The linear regression analysis data for the calibration plots shows a good linear relationship over the concentration range of 5-150 mg/mL. The method showed good recoveries (98.0 – 99.8%) and has been applied to formulation without interference of excipients in the formulation. The result of method was reproducible and within official limits. The HPLC method has been proved more authentic as it can be used for the quantitative and entrapment efficient to determine non micronized Piroxicam in SEDDS formulation in hard gelatin capsules.

**Keywords:** Anti-Inflammatory drug, HPLC method, non Micronized piroxicam, SEDDS formulation

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### INTRODUCTION

There are many stages involved in drug development: for example, the technological stage, where the research focuses on getting a dosage form of the active substance. This involves different techniques being used to look for the most appropriate substance. However, some of these can damage the active substance, which can lose its effectiveness totally or partially. Therefore, once a dosage form is obtained it has to be analyzed with a proper analytical method. The great variety of analytical techniques available provides valuable information which makes it easier to interpret the behavior of a drug.

Some years ago, one or two analytical techniques were enough to study an active substance, however, nowadays more than two are required to study the same. Of all of these, the most popular is UV-vis spectrophotometry technique which is easier and simpler method of analysis. This technique is based on the absorbance capability of a substance at a specific wavelength. Interference with another substance that absorbs at the same wavelength limits the use of this technique. In recent years High Performance Liquid Chromatography (HPLC) has gained popularity since it is easy, quick and accurate. It is based on the detection of

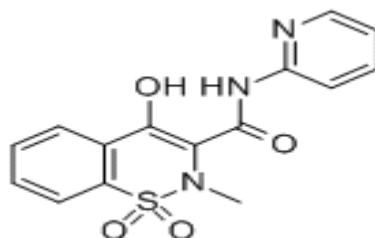
peaks that appear as a consequence of small changes. The number, location and shape of these peaks are used to identify a substance. This method is preferred over UV method as this will give accurate results which differentiates impurities and related substances which otherwise can interfere with the main active substance. Apart from this, the excipients used in the formulation also can be separated and prevent its interference with the active when HPLC method is used in the analysis. It is also important to develop stability indicating method for analysis of such final formulations.

Piroxicam(3E)-3-[hydroxy-(pyridine-2-ylamino) methylidene]-2-methyl-1,1 dioxo-benzo[e] thiazin-4-one (C<sub>15</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>S). Piroxicam is an Oxicam derivative belongs to Non-steroidal anti-inflammatory class of drugs (NSAIDs) [1-2]. Piroxicam is used to reduce the pain, inflammation, and stiffness caused by osteoarthritis and rheumatoid arthritis [3]. The anti-inflammatory activity of Piroxicam is due to the reversible

inhibition of Cyclooxygenase COX-1 resulting in disruption and production of prostaglandins [4]. Piroxicam also inhibits the migration of leucocytes in to sites of inflammation and prevents the formation of thromboxane A<sub>2</sub>, an aggregating agent, by the platelets. Piroxicam, 4-Hydroxy-2-methyl-N-(pyridin-2yl)-2H-1,2-benzothiazine-3-carboxamide 1, 1-dioxides is analgesic and anti-inflammatory agent [5-6]. Piroxicam is official in Indian Pharmacopoeia [7], British Pharmacopoeia [8], European Pharmacopoeia [9] and United States Pharmacopoeia [10].

Various analytical methods had reported in literature for estimation of Piroxicam individually and in combination with other drugs [11-12].

The main objective of the study is to develop and validate HPLC method so as to obtain an accurate, sensitive and precise for quantitative determination of non micronized Piroxicam in SEEDS formulation in hard gelatine capsule.



**Figure 1: Structure of Piroxicam**

## MATERIALS AND METHODS

### Instrumentation:

#### Materials:

Piroxicam was provided by Ramdev Chemical Pvt Ltd (Boisar Thana), Sodium Chloride, Potassium Di-hydrogen phosphate, Hydrochloric acid were used are AR grade of S.D.Fine. HPLC grade water was obtained from Milli Pore Merk water purification system.

Triethyl Citrate was purchased from Alfa Aesar a Johnson Matthey company, Acconon was obtained from Abitec and Tween -80 was purchase from Mohini Organics Pvt. Ltd (Mumbai).

#### Apparatus:

The analysis was performed by using the analytical balance GR-200 (AND), pH meter PHAN (Lab India), HPLC used was DG-U-

20A Shimadzu. Column used in HPLC is Chromatopacl, Peerless Basic C18,250x4.6mm,5µm, with a flow rate of 1.0 ml/min.

#### Chromatographic condition:

The chromatographic condition for optimized method is shown in (Table 1).

#### Mobile Phase:

The mobile phase consists of 600 volumes of buffer(dissolve about 6.81gm of Potassium dihydrogen Phosphate was dissolved in 1000mL water, adjust pH to 3.0 with orthophosphoric acid) which are filter through 0.45µm membrane filter and degassed in a sonicator for about 10 minutes and transferred to solvent reservoir.

The permutation and combination of the mobile phase were used and finally 10mM

potassium dihydrogen ortho phosphate buffer pH 3.0: Acetonitrile (50:50) v/v was selected as an appropriate mobile phase

which gave good resolution and acceptable system suitability parameters.

**Table 1: Optimized chromatographic conditions**

Parameter	Optimized condition
Chromatograph	DG-U-20A Shimadzu
Column used	Chromatopack, Peerless Basic C18, 250 x 4.6mm, 5mm
Wavelength	230nm
Flow rate	1.0mL/min
Injection volume	20µL
Mobile Phase	Acetonitrile and Buffer (40:60)
Column Temperature	40°C
Run Time	15 minutes
Diluent	Acetonitrile
Retention Time	12 min

## PROCEDURE

### Preparation of standard solution: : (11 ppm)

Weighed accurately about 22.0mg of Piroxicam standard in to a 100mL volumetric flask, dissolve in about 50mL diluent and dilute to volume with diluent. Further transfer 5.0mL of above solution in to a 100mL volumetric flask and dilute to volume with the dissolution media. Filter the solution through the 0.45µm filter.

### Calibration curve:

From the stock solution, measured volumes of working standards were prepared in the concentration range of 1- 5µg/mL. 20µL injections were made for each concentration in triplicate and were analyzed under optimized chromatographic conditions. A Calibration curve was plotted by using the response (peak area) versus concentration of drug. Regression equation was calculated.

### Procedure for analyzing SEDDS formulation:

Piroxicam SEDDS formulation was weighed equivalent to 10mg of Piroxicam and was transferred to a 100mL volumetric flask containing 25mL of mobile phase. The content of the flask was allowed to stand for 15minutes with intermittent sonication to ensure complete solubility of the drug and later it was made up to volume with mobile

phase. The test solution was filtered through 0.45µm membrane filter. The filtrate was finally diluted to 10X with mobile phase and appropriate dilutions were made to obtain concentration in the range of 1-5ug/mL. The steady base line was recorded by using the optimized chromatographic conditions. The assay was subjected for calculating regression equation. The procedure was repeated for 6 times and the percentage of drug in the formulation was calculated.

### METHOD VALIDATION:

#### Linearity:

The method was linear in the concentration of 1 to 5µg ml for Piroxicam standard (Figure 2).

#### System precision:

The system precision was checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area response (Table-2) of five determinations should be measured and calculate the relative standard deviation.

#### Accuracy:

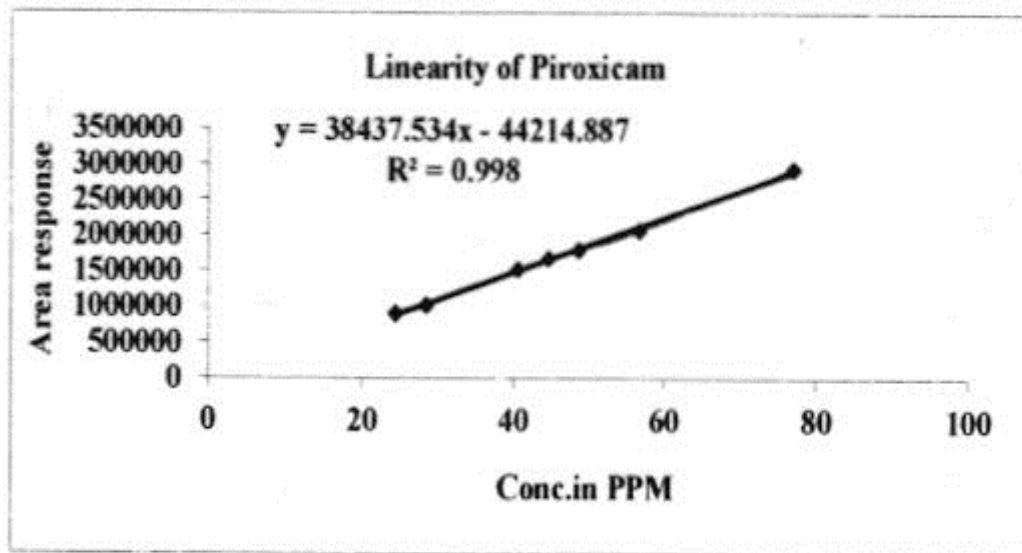
Accuracy for the assay of Piroxicam SEDDS was determined by applying the method in triplicate samples of mixture of placebo to which known amount of Piroxicam standard. The sample were filtered through

0.45µm membrane filter and injected into the chromatographic system.

conditions such as mobile phase ratio and pH of buffer (**Table 3**).

**Robustness:**

Robustness of the method was checked by making slight changes in chromatographic



**Figure 2: Linearity of Piroxicam**

**Table 2: System precision**

Sr. No	Piroxicam	
	Retention Time	Area Response
1	12.328	410844.017
2	12.335	410692.809
3	12.337	412970.306
4	12.343	410783.975
5	12.339	410784.876
Mean	12.336	411215.197
SD	0.00576	982.62392
RSD	0.00%	0.20%

**Table 3: Robustness**

Condition	% RSD
pH of the buffer (+ 0.2)	0.4507
pH of the buffer (- 0.2)	0.5321
Organic phase ratio (+ 2%)	0.4985
Organic phase ratio (- 2%)	0.7351

**Injection Profile:**

Inject blank (Dissolution Media) (1 injection) and Standard Preparation (5 injections) and check for system suitability.

**System Suitability:**

- The tailing factor for the Piroxicam from standard preparation should be NMT 2.0.
- The theoretical plates for the Piroxicam from standard preparation should be NLT 2000.

- RSD for the 6 replicate injections of the Piroxicam from standard preparation should be NMT 5.0.

The system suitability passes, inject sample preparation (1 injection) in to the chromatograph and the results (**Table 4**).

**Table 4: System suitability parameters**

Parameter	Piroxicam
Calibration rang ( $\mu\text{g/mL}$ )	5-150
Theoretical plates	NLT 2000
Tailing factor	NMT 2.0
% Recovery	98.0% - 99.8%

**Calculations and formula:**

Calculate the % release of Piroxicam by the below mentioned formula :

$$\% \text{ release of each individuals} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{1} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = Area response of Piroxicam in chromatogram obtained with sample preparation.

AS = Average area response of Piroxicam in chromatogram obtained with standard preparation.

WS = Weight of Piroxicam standard taken in mg

DS = Dilution of Standard preparation

DT = Dilution medium volume

P= Potency of Piroxicam Standard in % w/w on as is basis

LC= Label claim of Piroxicam in mg/ Capsule.

**Data interpretation:**

From the above results, it can concluded that retention time and area response are consistent as evidenced by relative standard deviation. Hence it can be concluded that the system precision parameter meets the requirement of method development.

**RESULTS AND DISCUSSION**

The self emulsifying drug delivery system (SEDDS) formulation of non micronized Piroxicam is a novel and versatile approach for overcoming the formulation difficulties

of drugs with poor aqueous solubility. The present developed method is novel for the determination of non micronized Piroxicam in SEDDS formulations. The method was found to be specific as excipients in the formulation did not interfere in the estimation of piroxicam in SEDDS formulation.

Piroxicam standard having concentration 50mg/ml was scanned in UV- region between 200-400 nm.  $\lambda$  max of Piroxicam was found to be at 230 nm. The Piroxicam peak in the sample was identified by comparing with the Piroxicam standard and the retention time was found to be around 12 minutes.. The estimation Piroxicam was carried out by RP-HPLC using Mobile phase having a composition volumes of phosphate buffer, 40 volumes of Acetonitrile and 60 volumes of buffer (40 : 60 v/v). The ratio pH was found to be 3.0. Then finally filtered using 0.45 $\mu$  nylon membrane filter and degassed in s onicator for 10 minutes. The column used was C18 Inertsil ODS 3V (150 mm x 4.6 mm x 5  $\mu$  particle size). Flow rate of Mobile phase was 0.8 mL/min, System suitability parameters such as the tailing factor for the Piroxicam from standard preparation should be NMT 2.0, the theoretical plates for the Piroxicam from standard preparation should be NLT 2000, RSD for the 6 replicate injections of the

Piroxicam from standard preparation should be NMT 5.0.

The results of analysis showed that the amount of drug was in good agreement with label claim of developed SEDDS formulation. It was observed that there were no marked changes in chromatogram, The results indicated that the developed formulation was stable up to 12-14 hours which was sufficient for completing the analytical procedures. The developed method was specific and reproducible for the quantitative determination of Piroxicam in SEDDS formulation with a good resolution and high sensitivity.

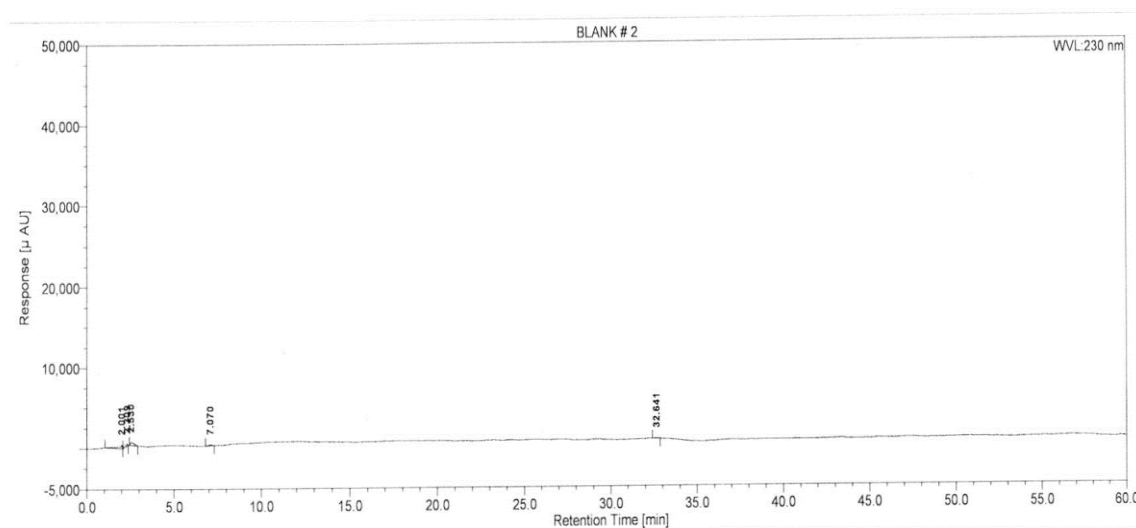
The Accuracy limit is the % recovery should be in the range of 98.0% to 99.8%. The developed method shows that the accuracy is well within the limit, which

shows that the method is capable of showing good accuracy.

#### CONCLUSION

SEDDS formulation prepared shows release intended for the treatment of rheumatoid arthritis. There are no methods developed for quantitative determination of Piroxicam in SEDDS formulation. Precision and accuracy for Piroxicam were comparable with other HPLC method previous described in the literature. The standard deviation and %RSD calculation for the proposed method are low, indicating high degree of precision of the method. Hence, it can be concluded that the proposed chromatographic method was accurate, precise and selective can be employed successfully for the determination of Piroxicam in bulk and SEDDS formulation.

Product Name :-	PIROXICAM CAPSULES	Batch No. :-	
Sample Name :-	BLANK	A.R.No. :-	
Sample No. :-	2	Test :-	RELATED SUBSTANCES
Sample Type :-	unknown	Stage :-	
Program File :-	PIR_CAP_REL_1	Pack Details :-	1
Method File :-	PIR_CAP_REL_1	Vial Number :-	51
Date Acquired :-	24.11.2012 16:20:38	Injection Volume :-	20.0 µl
Qnt Last Update :-	18.12.2012 12:42:10	Wavelength :-	230 nm
Instrument ID :-	1080-HPLC-0008	Run Time :-	60.00 mins
Column No. :-			
Sequence Name :-	PIR_REL_1_1080-MS-1200527_2411		
Sequence Path :-	STAR\PIROXICAM CAPSULES\SEQUENCES\2012\PIR_REL_1_1080-MS-1200527_2411.SEQ		
Parameters :-	1		



**Figure 3: Chromatogram representing the blank**

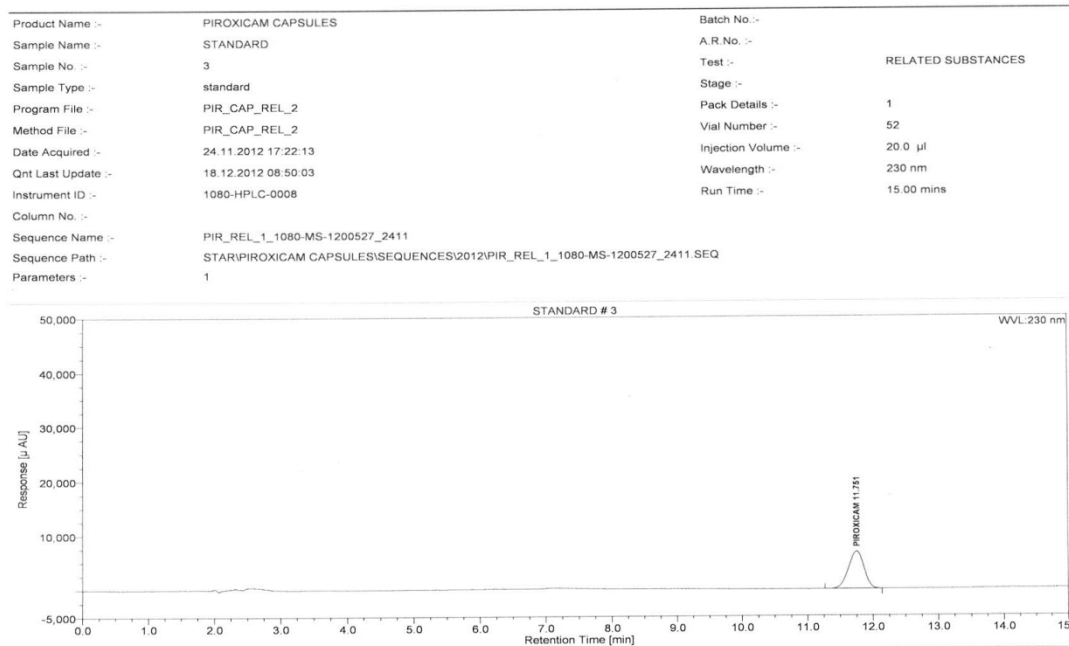


Figure 4: Chromatograph of Piroxicam standard

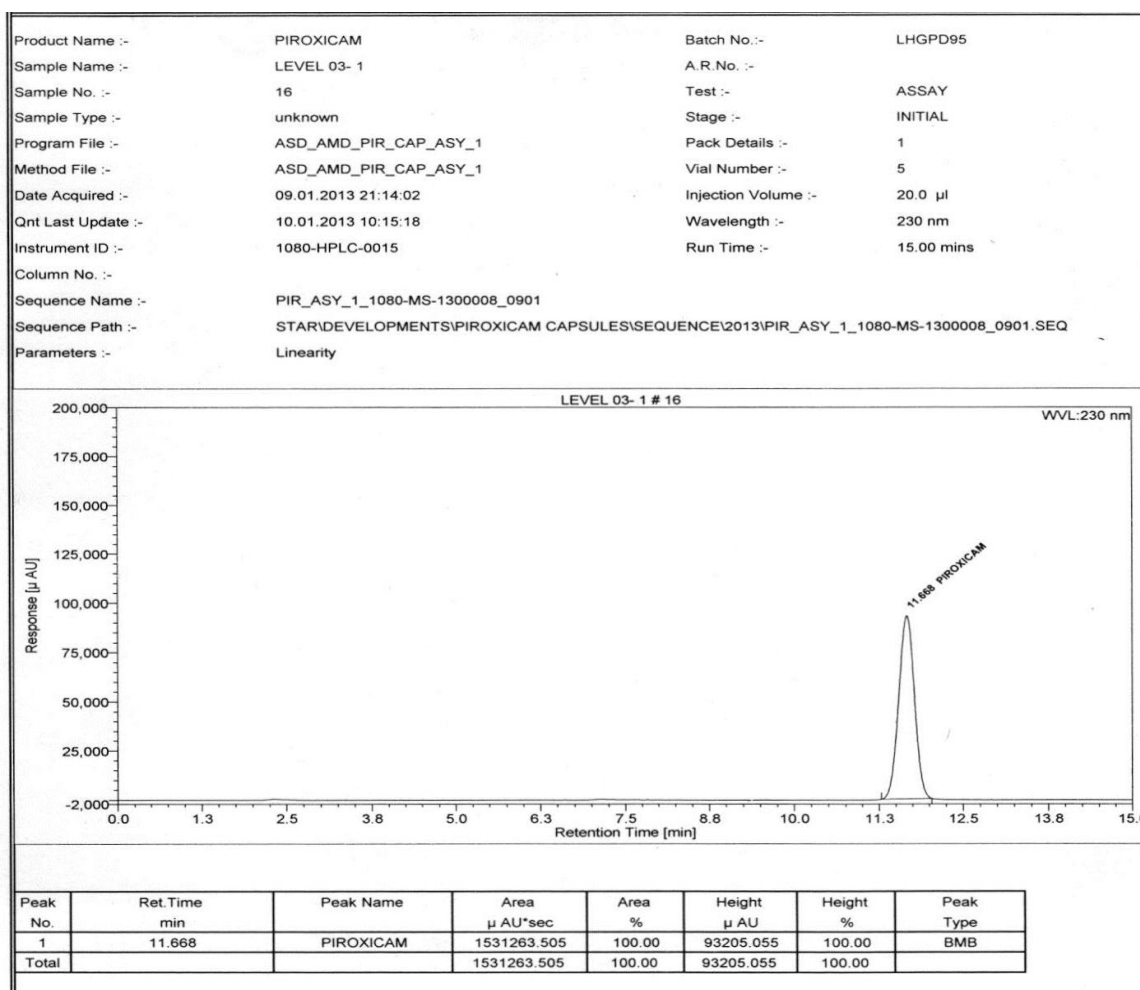


Figure 5: Typical Chromatograph of non micronized Piroxicam in SEEDS formulation

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