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PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1051-1060, Oct-Dec 2009

A validated RP-HPLC method for determination of Meloxicam in the Presence of its Impurities.

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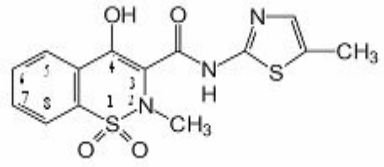
Abstract: A sensitive, specific, precise and cost effective RP-High-Performance Liquid Chromatographic method of analysis for meloxicam in presence of its impurities was developed. The method employed Hypersil Gold C18 (250 mm x 4.6 mm) column as stationary phase. The mobile phase consisted of 0.65% potassium dihydrogen orthophosphate (pH 6) and methanol in a ratio of 45: 55 *v/v*. This system was found to give good resolution of meloxicam and its impurities A, D, C (retention time 4.18, 5.32, 7.21, 9.13 min respectively). Method was validated as per ICH guidelines, in the concentration range of 5-25 µg/ml at 361nm.

Key words: RP-HPLC, Meloxicam, Meloxicam's impurities A, D, C.

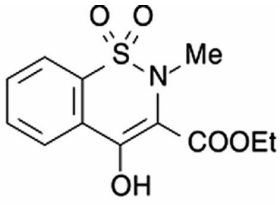
Introduction and Experimental:

Meloxicam (MEL) (4-hydroxy-2-methyl-N-(5-methyl-2-thiazoly)-2H-1,2-benzo-thiazine -3-carboxamide-1,1dioxide) ($C_{14}H_{13}N_3O_4S_2$) is a potent non-steroidal anti-inflammatory drug (NSAID) with a favorable COX-2 (cyclo oxygenase-2) selectivity [1], whose structure is shown as

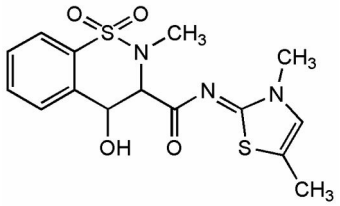


Meloxicam pharmacological activity is greatly affected by the substituents on parent oxicam moiety e.g. change in methyl to ethyl at 2 positions causes complete suppression of activity[2]. Any extraneous material present in the drug substance has to be considered an impurity even if it is totally inert or has superior pharmacological properties[3], there are five impurities reported for meloxicam in British pharmacopoeia[4] namely A, B, C, D and E, structure of A, C, D are

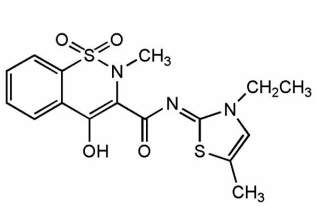
A.



C.



D.



There are many methods reported for the determination of meloxicam in individual and in combined dosage form, viz. spectrophotometric[5], electrophoretic[6], polarographic[7], RP-HPLC[8, 9, 10, 11, 12, 13]. The official method as per British Pharmacopoeia 2007 is a gradient RP-HPLC method. To the best of our knowledge, there is no isocratic method reported for the estimation of Meloxicam in presence of its impurities. Hence an attempt was made to develop and validate isocratic RP-HPLC method with optimum runtime and resolution for estimation of Meloxicam in presence of its reported impurities.

Experimental Data:

Meloxicam and its three impurities were kindly provided by Dr. Reddy Laboratories (Hyderabad, India). Acetonitrile (HPLC grade), Methanol (HPLC grade) and Water (HPLC grade) were purchased from Merck. (Mumbai, India).

Instrumentation

JASCO HPLC system (2000 series) comprising of JASCO PU – 2080 plus intelligent pump, JASCO MD-2010 plus multi wavelength detector and Rheodyne 7725i injector fitted with 20 µl capacity loop was used. Separations and quantitation was done on a Hypersil Gold C18 (250 mm x 4.6 mm) column.

Chromatographic conditions

The mobile phase was prepared by mixing methanol and solution of 0.65% potassium dihydrogen orthophosphate (pH 6) in a ratio of 55: 45 *v/v*. The mobile phase was filtered using 0.45 µm filter and degassed by ultrasonic vibrations prior to use. The flow rate was 1 ml min^{-1}. Column was maintained at 40^0 C.

Selection of detection wavelength

Meloxicam and all of its impurities show good absorbance at 361 nm so it was selected as the wavelength of detection (fig 1).

Method development

A detailed literature survey revealed that the mobile phase combination of 50mM potassium dihydrogen orthophosphate buffer (pH 5.5): ACN: MeOH in the ratio of 50:15:35 gave a good result for meloxicam with retention time 10 min. Trial was taken on the same mobile phase with HiQ Sil C18 (250 mm x 4.6 mm) column, the obtained retention time of drug (meloxicam) was 4.5 min but impurities of drug were not resolved from the peak of drug. Since our objective was to resolve the response of impurities from that of Meloxicam, we tried the combination of phosphate buffer: MeOH :: 80: 20 and 70: 30, but in both cases, Meloxicam did not elute up to 25 min even with column temperature of 35^0 C. We replaced the MeOH with ACN and now the ratio used was 70: 30 with temperature 35^0 C, here the retention time of meloxicam was 9.29 min, and the retention time of impurities were 9.66, 10.49, 10.8 for impurities A, C, D respectively. All the peaks were broad in shape and were not resolved from each other. The temperature was decreased to 30^0 C; a significant change in R_t (not in separation) was obtained with retention time of meloxicam and imp A at 11.76 and 11.72 min respectively. We changed the ratio to 75:25, a very broad peak was obtained after 22 min. Increase in temp to 35^0 C didn't change the shape here also broad peak of meloxicam was obtained after 18 min. With the same mobile phase in the ratio of 72: 28 the retention time obtained for meloxicam, imp A, C, D were 14.81, 12.49, 12, 27, 12.39 min respectively, but still peaks were broad but there was some resolution between Meloxicam and impurities.

Due to broad and unresolved peaks, various other mobile phase compositions were also tried, the observations for which are summarized in tabular form as below.

Mobile phase	Retention time (min.)				Remarks
	Meloxicam	Imp A	Imp D	Imp C	
Potassium dihydrogen orthophosphate 0.1% (pH 6): ACN: MeOH:: 50: 25: 25 at temperature 35^0 C (fig 2)	5.25	5.20	----	5.24	Unresolved and broad.
Potassium dihydrogen orthophosphate 0.1% (pH 6): ACN: MeOH:: 70: 10: 20 at 30^0 C	16.04	16.10	15.84	15.88	Unresolved and broad.
Potassium dihydrogen orthophosphate 0.1% (pH 6): ACN: MeOH:: 60: 20: 20 at 30^0 C (fig 3)	8.70	-----	----	7.52	Very broad peaks.
Water : MeOH :: 50: 50 at 35^0 C (fig 4)	2.21	2.41	2.38	2.40	Broad shape
Water : MeOH :: 70: 30 at 35^0 C (fig 5)	9.52	9.30	----	----	Broad shape

But peaks were still broad and unresolved so, finally after optimization the mobile phase selected was, methanol and solution of 0.65% potassium dihydrogen orthophosphate (pH 6) in a ratio of 55: 45 *v/v*, here retention time of meloxicam, imp A, D, C were 3.62, 5.37, 8.86, 10.41 min (Fig 6). The shape of drug peak was good with peak purity more than 950 (as obtained by PDA detector) but the shape of impurities peaks were separate but appeared as combination of more than two peaks, now the column was changed to HypersilGold (C18, potassium dihydrogen orthophosphate 0.65% (pH 6) in a ratio of 55: 45 *v/v*) here the retention time were 4.18, 5.32, 7.21, 9.13 min respectively (Fig 7).

Preparation of dilution

10 mg of drug was weighed and transferred to 25 ml volumetric flask containing about 15 ml methanol, ultrasonicated for 10 min and then the volume was made up to 25 ml with methanol. The solution was filtered using whatmann filter paper No.41. From the filtrate appropriate dilutions were made in mobile phase.

Method Validation

The method validation was done as per the ICH guidelines [14], and accordingly the parameters evaluated were:

1. Linearity and range.
2. Precision
3. Accuracy
4. Specificity
5. Limit of Detection
6. Limit of Quantification
7. Robustness

Linearity and Range

The 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 sample, was studied by analyzing five concentrations of the drug, and process was repeated for five times each. It was done over the range of 5-25 µg ml^{-1}.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by

1. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision, was studied by injecting three concentrations (10, 15, 20 µg ml^{-1}) of the drug, and process was repeated for three times each.
2. Intermediate: Intermediate precision expresses within-laboratories variations:
   I. Analysis on different days: was studied by injecting three concentrations (10, 15, 20 µg ml^{-1}) of the drug, and process was repeated for three times each, for three consecutive days.
   II. Analysis using different equipments: was studied by using JASCO HPLC system (2000 series) comprising of JASCO PU – 2080 plus intelligent pump, JASCO MD- 2070 plus UV/Vis detector and Rheodyne 7725i injector fitted with 50 µl capacity loop.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

1. Recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% and the percentage recovery was calculated.
2. Assay. Study was carried out using twenty tablets, each containing 75 mg meloxicam. Tablets were weighed and finely powdered. A quantity of powder equivalent to 10 mg was weighed and transferred to 25 ml volumetric flask containing about 15 ml methanol, ultrasonicated for 10 min and then the volume was made up to 25 ml with methanol. The solution was filtered using whatmann filter paper No.41. From the filtrate appropriate dilutions were made in mobile phase to obtain concentration of 20 µg ml^{-1}. The tablet sample solution was injected and chromatogram was obtained.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. here study was done using Impurities.

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Based on the Standard Deviation of the Response and the Slope, The detection limit (DL) may be expressed as:

$$DL = \frac{3.3\,\sigma}{S}$$

Where,

σ = the standard deviation of the response for the lowest conc. in the range.

S = the slope of the calibration curve.

Limit of Quantification (LOQ)
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Based on the Standard Deviation of the Response and the Slope, The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

Where,
σ = the standard deviation of the response for the lowest conc. in the range
S = the slope of the calibration curve.

Robustness
The robustness of an analytical procedure 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 and done by observing
- Influence variations of pH in a mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers);
- Temperature;
- Flow rate.

Results and Discussion:

Linearity and Range
The data obtained in the linearity experiments was subjected to linear-regression analysis. A linear relationship between peak areas and concentrations was obtained in the range of 5- 25 µg ml^{-1} with r^2 0.9962 (Table 1).

Precision
The developed method was found to be precise as the % RSD value for repeatability studies was less than 1%, where as the %RSD for interday precision was higher than that of repeatability study.

Specificity
Impurities were added to the stock solution and the mixture was subjected to chromatographic analysis and it was observed that impurity peaks were well resolved from peak of meloxicam (fig 7); system suitability parameters are shown in table 2. The method was considered to be specific since there was no interfering peak at the retention time of meloxicam and also the peak was well resolved from the peaks of all impurities. The peak purity profile of meloxicam by PDA detector also confirmed the specificity in which peak purity in front and tail have to be more than 900 and the obtained values was 942 and 968 respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ were found to be 219 ng ml^{-1} and 722 ng ml^{-1} respectively for meloxicam.

Accuracy
The results of recovery studies for accuracy determination are depicted in Table 1. Good recoveries (98.37-99.23%) and assay (101.79%) of the drug were obtained at each added concentration, indicating that the method was accurate.

Robustness
The results obtained by making small, deliberate change in some parameters were as follows:
- influence of pH: at pH 5.5 retention time of drug and its impurities A, D, C were increased to 5.41, 7.16, 9.92, 13.49 min respectively (fig 8). Where as no significant effect was obtained on retention time by increasing the pH more than 6 up to 6.3.
-Influence of mobile phase composition: 2 % change in mobile phase composition led to significant change the retention time (about 1 min).
- Different column: to perform this experiment C18 BDS (250 mm x 4.6 mm) column was used in which retention time were 4.42, 5.89, 7.74, 10.49 for meloxicam, Impurity A, D, C respectively (fig 9).
-Temperature: at 38^0 C retention time were 4.20, 5.36, 7.28, 9.30 min where as at 42^0 C 4.14, 5.18, 6.98, 8.76 min for meloxicam, impurity A, D and C respectively.

Discussion:

Impurities determination is an integral part of pharmaceutical analysis. Here a specific, accurate, precise and cost effective method for estimation of meloxicam in the presence of its impurities was developed which fulfill all parameters of validation (table 1) as per given in the ICH guidelines, but method developed was very much sensitive to deliberate variations in method parameters (pH, mobile phase composition, temperature). It caused considerable changes in the retention time, so it recommended that above mentioned parameters should be controlled well to reproduce the experiment.

Table 1: Validation Parameters

Parameters			**Obtained results**
Beer's law range			5-25 µg ml^{-1}
Regression equation (y^{*} = mx + C)			y = 45718x – 21682
r^{2} *			0.9962
Accuracy[a]	Recovery levels	80%	98.37%
		100%	98.15%
		120%	99.23%
	Assay		99.60% w/w
Precision[a] (%RSD)	Repeatability		less than 1.5%
	Interday		less than 2%
LOD			219 ng ml^{-1}
LOQ			722 ng ml^{-1}

Note:*- mark indicates average of 5 readings.
a- mark indicates average of 3 readings.

Table 2: System suitability parameters.

Sl. no.	Parameters	Meloxicam	Imp A	Imp D	Imp C
1	Resolution with previous peaks	4.12	2.34	3.96	3.51
2	Asymmetry	1.16	1.54	1.34	1.21
3	Plate no.	891	2708	2755	4440
4	HETP	35.61	108.3	110.2	177.6

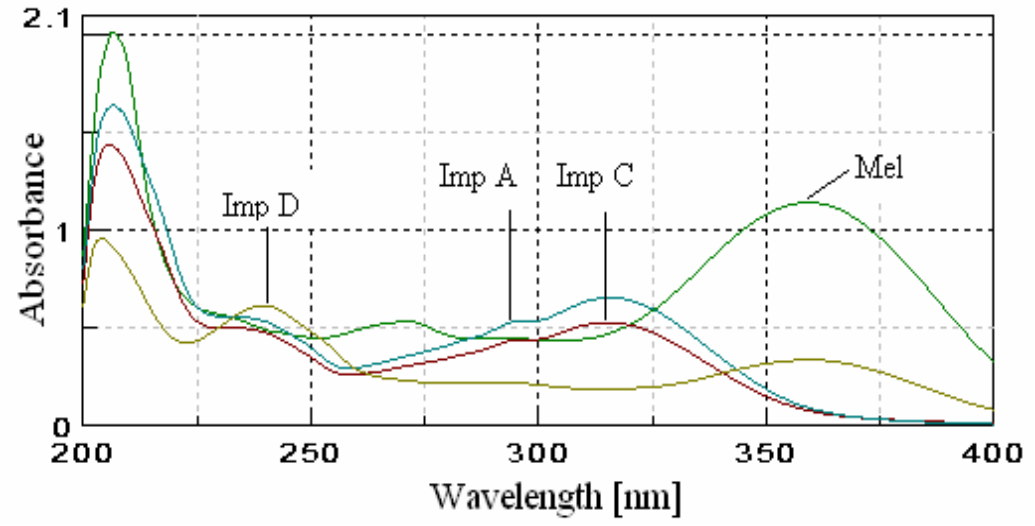


Fig 1: UV absorbance spectra of meloxicam and its impurities A, C, D.

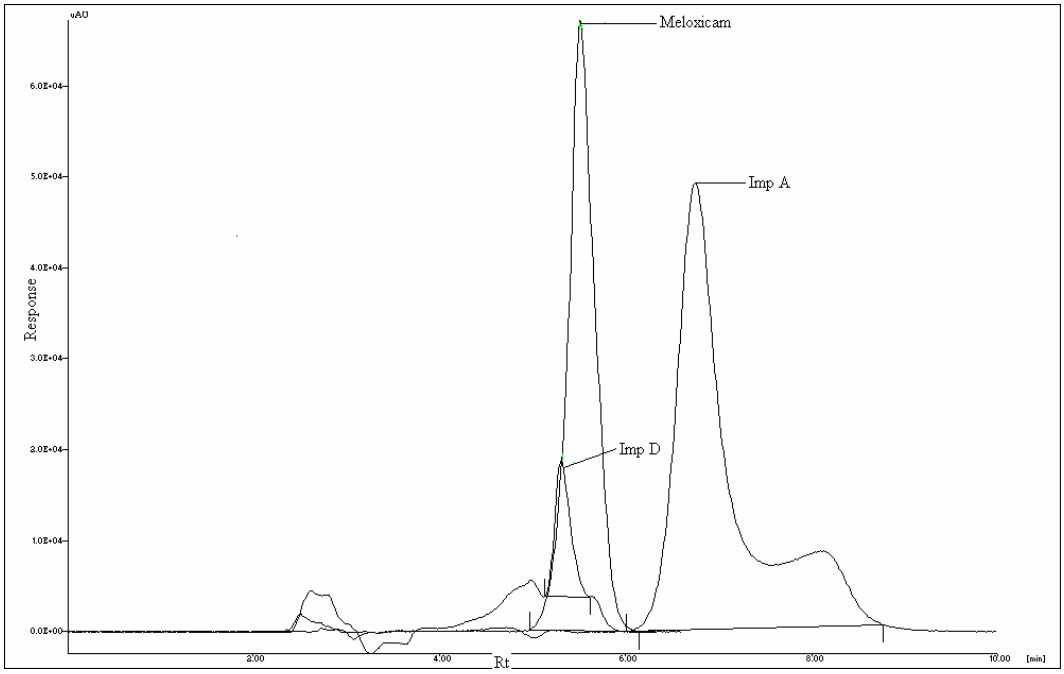


Fig 2: chromatogram of meloxicam and its impurities A,D having retention time 5.25,5.20,5.24 min respectively with HiQ Sil C18 (250 mm x 4.6 mm) column.

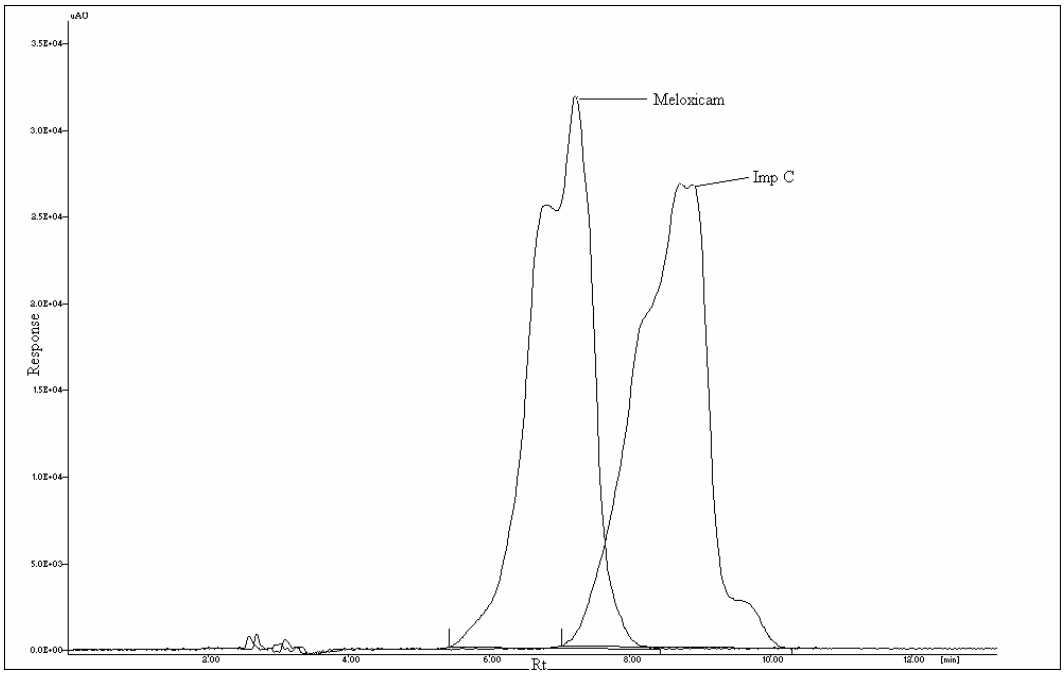


Fig 3: chromatogram of meloxicam and its impurity C having retention time 8.70, 7.52 min respectively with HiQ Sil C18 (250 mm x 4.6 mm) column.

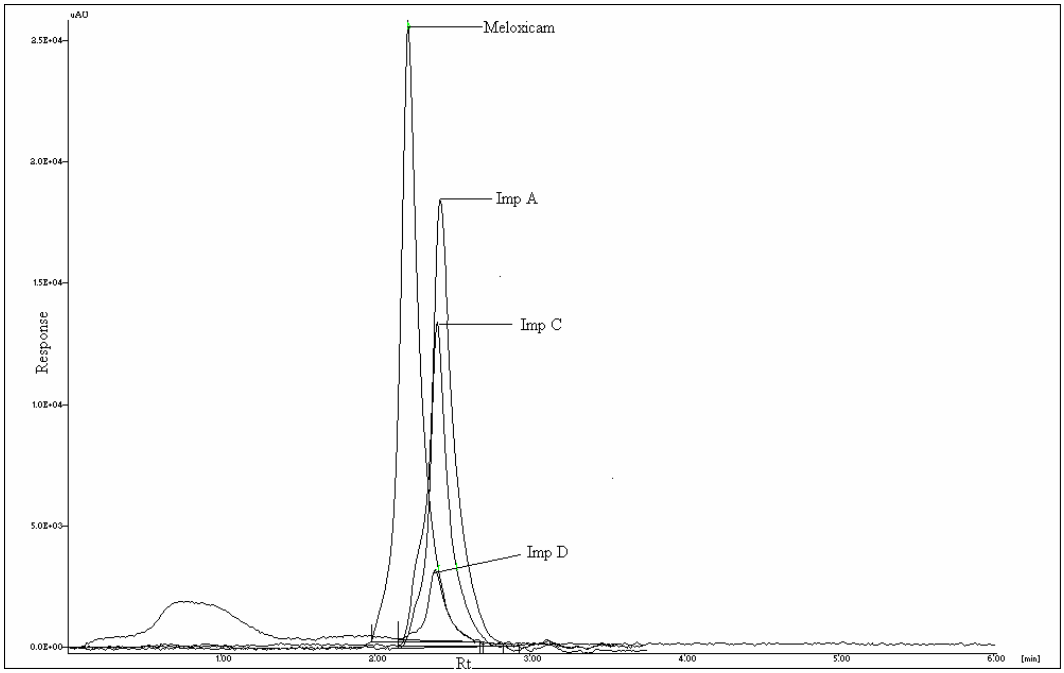


Fig 4: chromatogram of meloxicam and its impurities A,D,C having retention time 2.21, 2.41, 2.38, 2.40 min respectively with HiQ Sil C18 (250 mm x 4.6 mm) column.

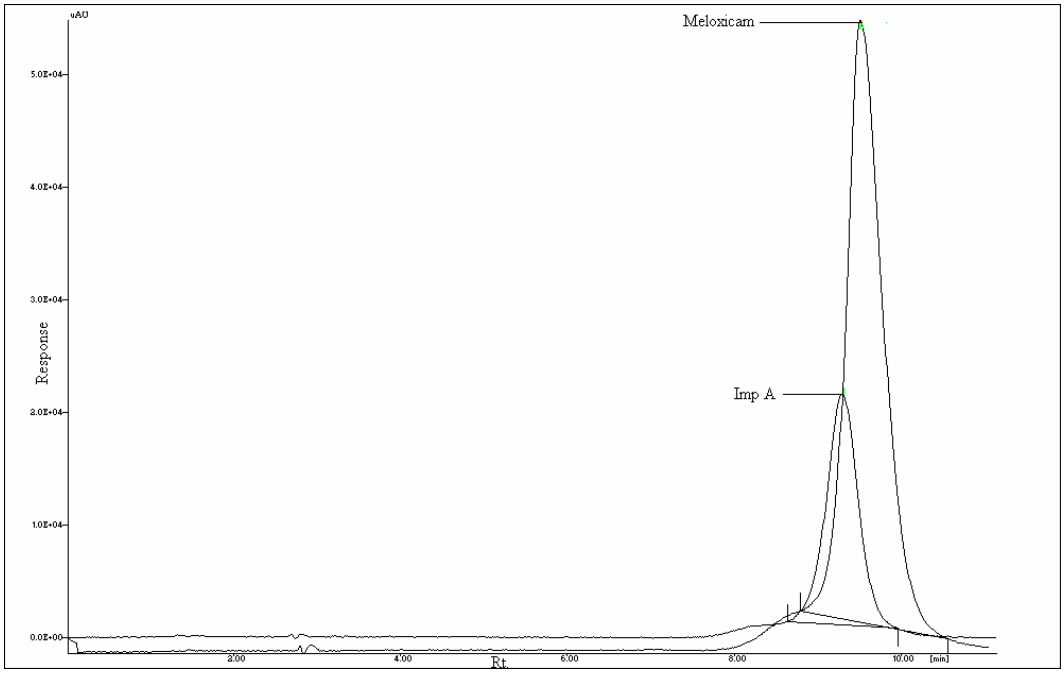


Fig 5: chromatogram of meloxicam and its impurity A having retention time 9.52, 9.30 min respectively with HiQ Sil C18 (250 mm x 4.6 mm) column.

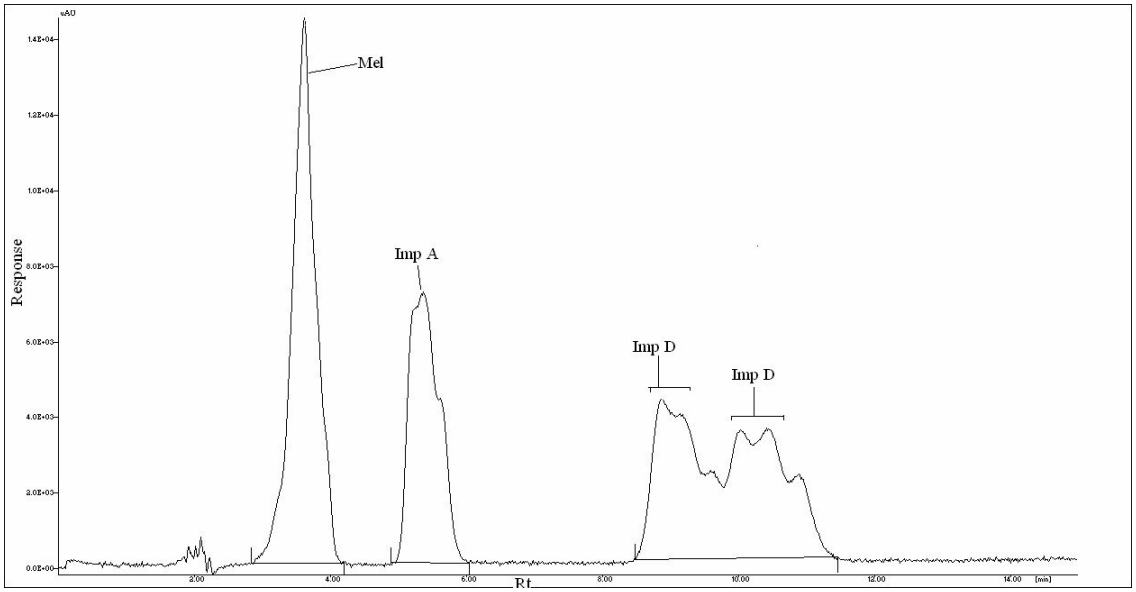


Fig 6: Representative chromatogram of meloxicam and its impurities A, D, C at retention time of 3.62, 5.37, 8.86, 10.41 min. respectively with HiQ Sil C18 (250 mm x 4.6 mm) column.

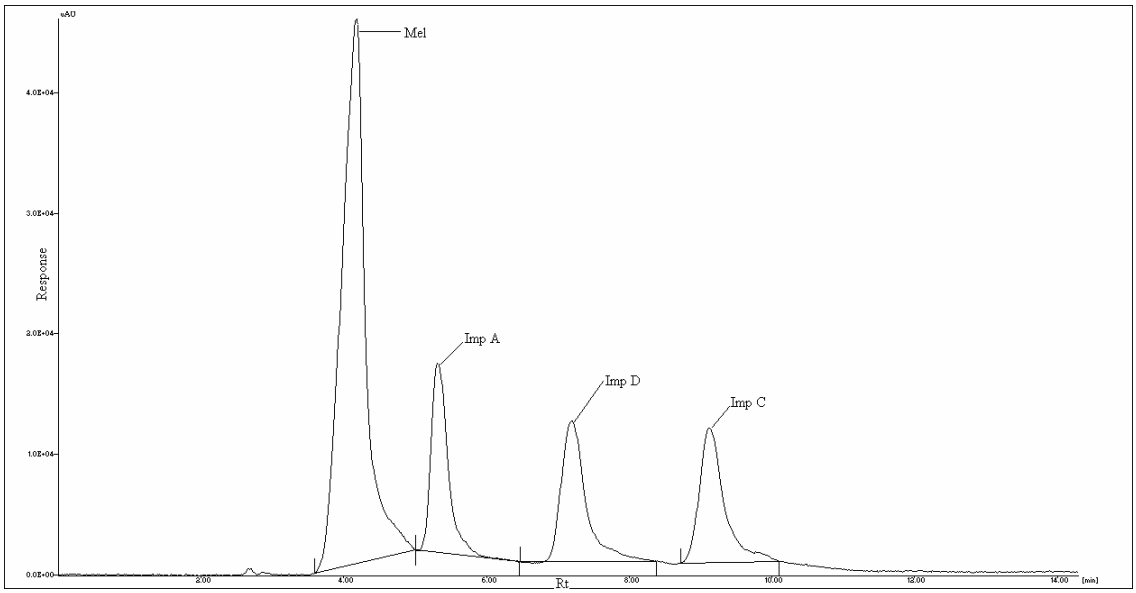


Fig 7: Representative chromatogram of meloxicam and its impurities A, D, C with retention time of 4.18, 5.32, 7.21, 9.13 min. respectively using HypersilGold C18 (250 mm x 4.6 mm) column.

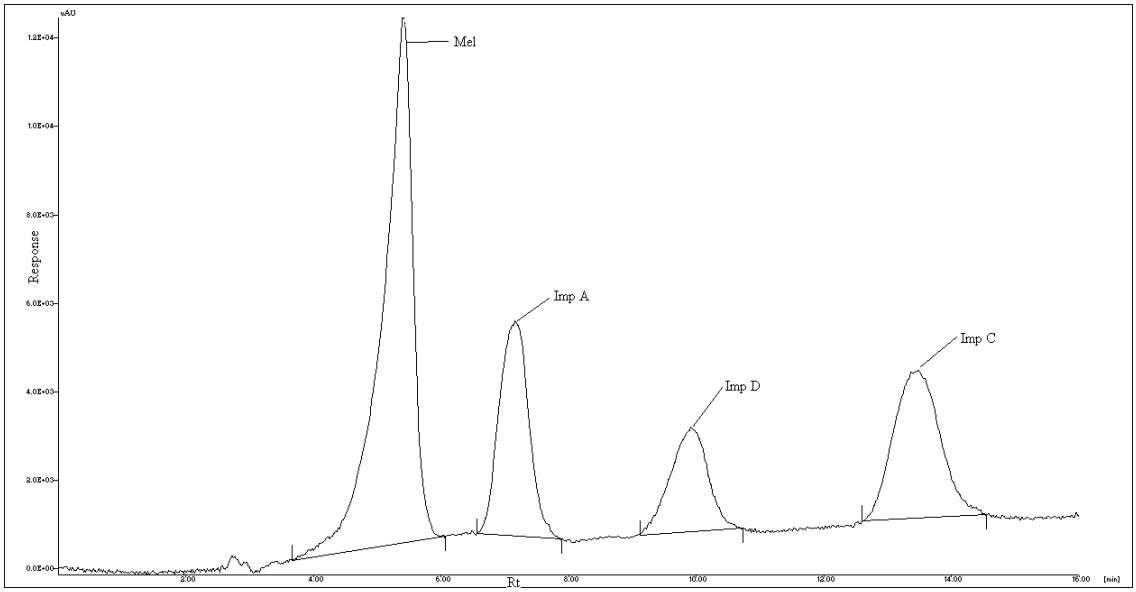


Fig 8: Chromatogram of meloxicam and its impurities A, D, C at pH 5.5 having retention time 5.41, 7.16, 9.92, 13.49 min respectively.

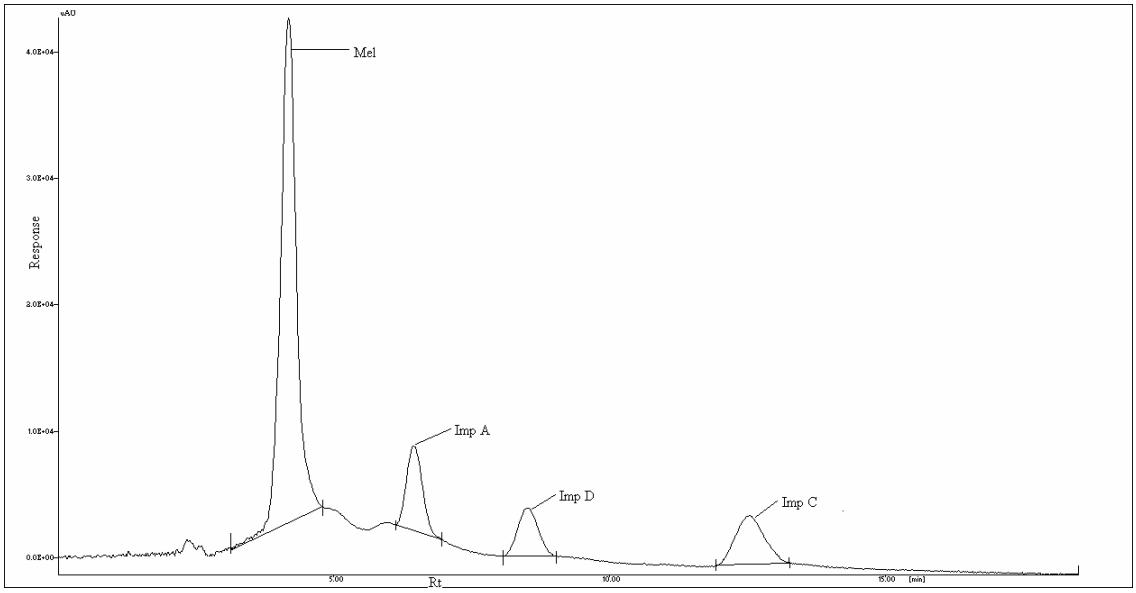


Fig 9: Chromatogram of meloxicam and its impurities A, D, C with C18 BDS (250 mm x 4.6 mm) column having retention time 4.42, 5.89, 7.74,10.49 min respectively.

Acknowledgement:
The authors wish to express their gratitude to Dr. Reddy Laboratories, Hyderabad (India), for providing gift sample of meloxicam and its impurities. The authors are also thankful to Principal, AISSMS College of Pharmacy for providing necessary facilities to carry out the research work.

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