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DEVELOPMENT AND VALIDATION OF ASSAY METHOD FOR MELOXICAM TABLETS BY RP-HPLC

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ABSTRACT

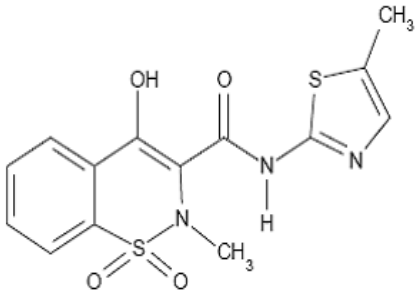
A simple, rapid, and accurate reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the determination of meloxicam .The separation was carried out using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 60: 40. The pH of the mobile phase was adjusted to 7.0 with triethylamine. The column used was X Terra C18 (150 × 4.6 mm, 5 µm) with flow rate of 0.8 mL/min using UV detection at 344nm. The total run time was 6 min and the retention time of meloxicam was 2.4 min. The described method was linear for the assay of meloxicam over a concentration range of 10 µg/mL respectively. Results of the analysis have been validated statistically and by recovery studies. The Limit of quantification and Limit of detection were found to be 0.135 µg/mL and 0.05µg/mL respectively. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise, and accurate, which is useful for the routine determination of meloxicam bulk drug and its pharmaceutical dosage form.

Keywords: Meloxicam, HPLC.

INTRODUCTION

Meloxicam is non-steroidal anti inflammatory drug (NSAID), registered as an anti-inflammatory and analgesic agent for management of pain arising from different conditions such as rheumatoid arthritis and osteoarthritis in human as well as animal. The therapeutic index of meloxicam is higher when compared with other NSAIDs like piroxicam, diclofenac, and indomethacin.

MELOXICAM STRUCTURE



MATERIAL AND METHODS

Analytically pure Meloxicam was provided by Lara Laboratory, Hyderabad as gift samples. HPLC grade acetonitrile was purchased from Merck & Co. Glass wares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Triple distilled water is used for all purpose. The commercial combined dosage form was purchased from local pharmacy.

Instrumentation

The method was performed by using HPLC system (Waters with Empower2 Software) containing C_{18} (150 x 4.6mm, 5µ) column with UV- PDA detection.

CHROMATOGRAPHIC CONDITIONS

The mobile phase consisted of phosphate buffer : Acetonitrile (adjusted to pH 7.01 using Triethylamine) in the ratio of 60:40 v/v. The contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 10 min. The mobile phase was pumped from

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the solvent reservoir to the column at a flow rate of 0.8 ml/min and the injection volume was 20µL. The column temperature was maintained at ambient temperature. The eluents was monitored at 344 nm.

PREPARATION OF THE MELOXICAM STANDARD & SAMPLE SOLUTIONS

Accurately 50.07 mg of Meloxicam working reference standard was weighed and transferred into a 50 mL clean dry volumetric flask. 10 mL of diluent was added and sonicated for 10 min for complete dissolution of the drug. Finally the volume was made up to the mark with the diluent.

Standard solution

3 mL of standard stock solution was pipetted into a 100 mL volumetric flask and diluted up to the mark with diluent. Filtered through 0.45µ Millipore nylon filter.

Sample Stock Solution

20 tablets were weighed and the average weight was determined. Tablets were crushed into fine powder. Accurately weighed and transferred 1001.1 gm of powder equivalent to 50 mg of meloxicam into 50 mL volumetric flask, 20 mL of diluent was added and sonicated for 20 minutes with intermittent shaking. Volume was made up to the mark with the diluent. Mixed well and centrifuged at 5000 RPM for 8 minutes.

Sample Solution

3 mL of supernatant sample stock solution was pipetted into a 100 mL volumetric flask and diluted up to the mark with diluent. Filtered through 0.45µ Millipore Nylon filter.

Instrumentation

The method was performed by using HPLC system (Waters with Empower2 Software) containing C_{18} (150 x 4.6mm, 5µ) column with UV- PDA detection.

METHOD VALIDATION PARAMETERS

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility as per ICH guidelines. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision was expressed with respect to the repeatability, intra and inter-day variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

EXPERIMENTAL PROCEEDINGS

Experimental Approach towards Method DevelopmentThe proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility as per ICH guidelines. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision was expressed with respect to the repeatability, intra and inter-day variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

ACCURACY

Theaccuracy of the method was determined by measuring drug recoveries by the standard spiking method. The accuracy of the method was assessed by adding know amount of standard solution (50%, 100% and 150% of the sample concentration) to the pre-analysed sample solution of 100% concentration. The mean recovery obtained by standard spike ranged from 99.93-99.28% for amlodipine and 100.81% - 100.05 for Meloxicam with %RSD less than 1. High recovery values indicate assay procedure is highly accurate.

LINEARITY

Calibration curve constructed by taking concentration on X-axis and area response on Y-axis, displayed good linearity over the concentration range. The polynomial regression for the calibration plot showed linear relationship with coefficient of correlation. Linearity range and correlation coefficient obtained for Meloxicam was found to be 1-7 µg/mL, 0.999 and 8-56 µg/mL, 0.998 respectively.

PRECISION

System precisions, method precision were performed separately for assay. % RSD of system precision, method precision was found for Meloxicam. Low % RSD of all precisions indicates method is precise.

ROBUSTNESS

The robustness was carried out to demonstrate that the optimized method was unaffected by varying the flow rates and wave length. The results show that changes in flow rates (0.65 or 0.85 mL/min) and wave length changes (236 nm or 238 nm) did not show effect on the asymmetry and efficiency.

RUGGEDNESS

The ruggedness was performed separately by different analysts. % RSD of ruggedness for analyst-1, analyst-2 were found for Meloxicam.

LOD and LOQ

LOD and LOQ was calculated from the formula 3.3 x (σ/S) and 10 x (σ/S), respectively where, σ is

standard deviation of intercept and S is the mean of slope. The LOD and LOQ can also be determined by S/N.

The limit of detection of meloxicam was found to be 0.02µg/ml. The limit of Quantification of meloxicam was found to be 0.135 µg/ml.

ASSAY

System suitability solution was injected before performing analysis. System suitability results obtained USP plate count-2522.1, USP tailing-1.6 and % RSD three replicate standards- 0.04 indicated suitability of chromatographic system for assay analysis.

Amount and % Label Claim of meloxicam present in tablet were found to be 7.48 mg and 99.8%, respectively.

RESULT AND DISCUSSION

Table 1. Results for System Suitability

S.No	Name	Retention time (min)	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1.	Meloxicam	2.455	1822870	201515	2552.1	1.6

Table 2. Accuracy

Accuracy	Area	% Recovery	Mean Recovery	Overall Mean Recovery
50%	2733633	99.7%	Mean=99.53%	Mean = 99.58%
50%	2726428	99.4%	S.D = 0.152	S.D = 0.07
50%	2730536	99.5%	%RSD = 0.15	%RSD = 0.68
100%	3644844	99.7%	Mean = 99.66%	
100%	3642998	99.6%	S.D = 0.057	
100%	3645468	99.7%	%RSD = 0.05	
150%	4556055	99.7%	Mean = 99.56%	
150%	4539986	99.3%	S.D = 0.23	
150%	4556135	99.7%	%RSD = 0.23	

Table 3.Calibration curve of Meloxicam

S.No	Level	Concentration	Retention time (min)	Peak Area
1	I	10 µg/mL	2.453	634405
2	II	20 µg/mL	2.452	1294705
3	III	30 µg/mL	2.448	1856592
4	IV	40 µg/mL	2.443	2479121
5	V	50 µg/mL	2.448	2962653
6	VI	60 µg/mL	2.452	3365663
7	VII	75 µg/mL	2.457	4825300
Slope				59179
Intercept				21703
Correlation Coefficient				0.999

Table 4. Precision

Injection	Retention time	Peak area
Injection-1	2.468	1873693
Injection-2	2.466	1879960
Injection-3	2.459	1878730
Injection-4	2.459	1887804
Injection-5	2.458	1889616
Injection-6	2.466	1879961
Mean	2.462	1881961
Standard Deviation	0.00046	6624.7
%RSD	0.018	0.35

Table 5. Robustness

S.No	Flow Rate (in mL/min)	System Suitability Results		RT (in Min)
		USP Plate Count	USP Tailing	
1	0.6	2656.9	1.7	2.79
2	0.8(actual)	2541.6	1.6	2.45
3	1.0	2614.9	1.5	2.17

Table 6. LOD and LOQ

S. no	Drug Name	LOD	LOQ
1	Meloxicam	0.02µg/mL	0.135µg/mL

Table 7. Parameters

S. No.	Parameter	Results			
1.	System suitability	The tailing factor for meloxicam peak is 1.6			
2.	Limit of Detection LOD	Limit of detection (LOD) 0.02µg/mL			
3.	Limit of Quantitation LOQ	Limit of quantification for meloxicam 0.135µg/mL			
4.	System precision	%RSD for peak areas of meloxicam 0.35			
5.	Method precision	% RSD for peak areas of meloxicam 0.25			
6.	Intermediate precision	% RSD for peak areas of meloxicam is 0.18			
7.	Linearity	The correlation coefficient value 0.999			
8.	Accuracy	Mean % recovery 99.06.			
9.	Robustness	Effect of flow rate variation			
			RT	Tailing factor	Plate count
		0.6 mL	2.79	1.7	2656
		0.8 mL	2.45	1.6	2541
		1 mL	2.17	1.5	2614
		Effect of mobile phase composition Variation			
		10% less(org)	2.58	1.4	1855
		actual	2.45	1.6	2514
		10% more(org)	2.25	1.6	2224

Fig 1. System Suitability Parameters

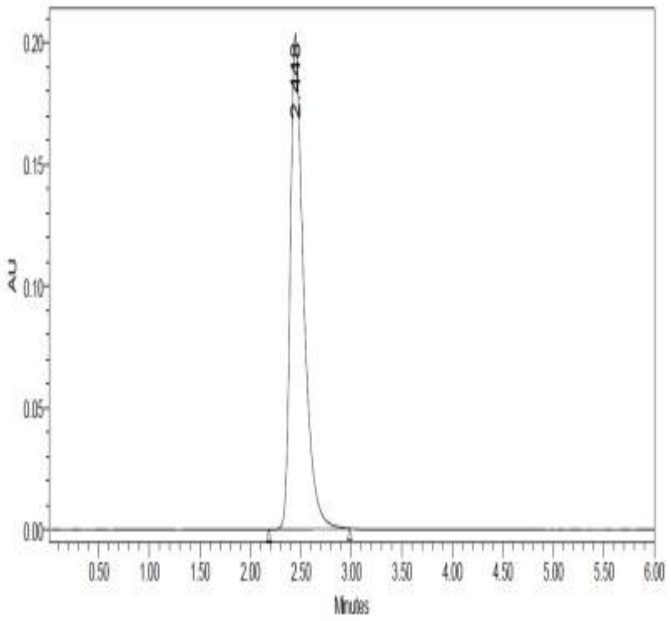
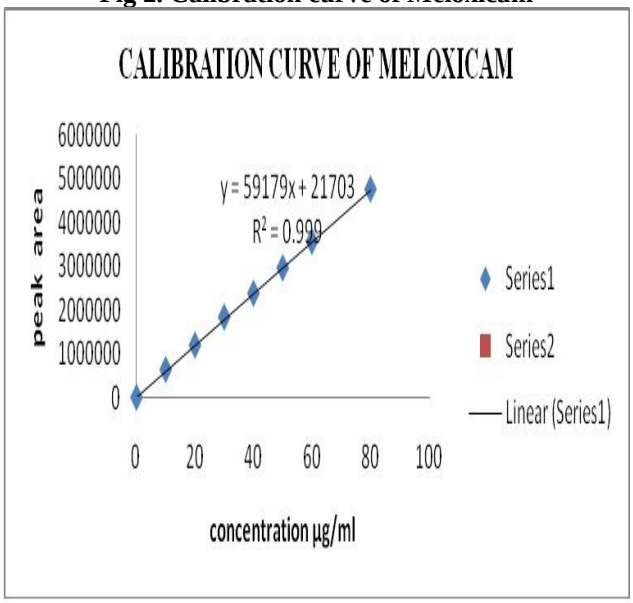


Fig 2. Calibration curve of Meloxicam



CONCLUSION

The objective of the present study was to develop a HPLC method for the estimation of meloxicam in tablet dosage form. The need for development of the analytical method was identified due to the drawbacks of the existing methods such that the present method developed was much simple, precise, accurate and economic when compared to the earlier works. chromatographic separation was performed on XTerra C18 (150x 4.6 mm,) 5µm at a wavelength of 344 nm using a isocratic program for 6 min, by using mobile phase of acetonitrile and potassium dihydrogen orthophosphate buffer pH 7 (60:40 v/v) in 3000mL of purified water (HPLC grade).

The method was validated by evaluating linearity, accuracy, precision, limit of quantitation, limit of detection. The results conclude that the method was suitable for its intended use for the estimation of meloxicam in formulation.

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CONFLICT OF INTEREST

No interest

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