Research Article

A Novel Method Development and Validation for the Estimation of Meloxicam in Bulk and Dosage Forms by RP – UFLC

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ABSTRACT

Meloxicam is a non-steroidal anti-inflammatory drug and selective inhibitor of COX-2, it is used particularly for the management of rheumatoid arthritis. In this RP-HPLC method chromatographic separation was achieved on Isocratic elution of the mobile phase Acetonitrile: Ammonium acetate: methanol: glacial acetic acid (Proportion of mobile phase-45:40:10:5 %v/v/v/v) with the flow rate of 1 ml/min. Separation was performed on C18 column (150 mm × 4.6 mm inner diameter, 5 µm particle size). The flow rate was 1.0 ml/min and detector wavelength were kept at 363 nm for monitoring the separation. The method was developed and validated. The linearity range was found to be 10 - 50µg/ml. Regression coefficient was found to be 0.995. Precision study showed % RSD values are less than 2% in all selected concentrations. The recovery studies for Meloxicam were found to be in the range of 99.7 – 100.2%. System suitability parameters remain unchanged even if the composition of mobile phase and wavelengths were changed. The proposed method was very simple, precise, accurate and rapid for determination of Meloxicam from pure and its dosage forms.

Keywords: RP-UFLC, C18 column, Isocratic elution, %RSD.

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INTRODUCTION

Meloxicam is a benzothiazine that is piroxicam in which the pyridin-2-yl group is replaced by a 5-methyl-1,3-thiazol-2-yl group[5]. A non-steroidal anti-inflammatory drug and selective inhibitor of COX-2, it is used particularly for the management of rheumatoid arthritis. It has a role as a non-steroidal anti-inflammatory drug, an antirheumatic drug, a cyclooxygenase 2 inhibitor and an analgesic. It is a benzothiazine, a monocarboxylic acid amide and a member of 1,3-thiazoles[1]. Rare but important side effects include liver and kidney toxicity[8].

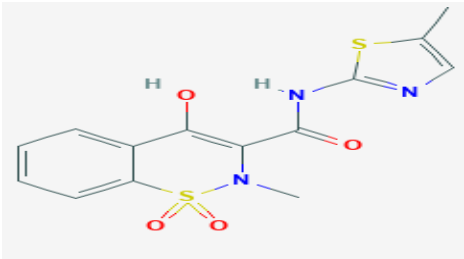

Fig No.1*:* Structure of meloxicam[2]

IUPAC name of Meloxicam is 4-hydroxy-2-methyl-*N*-(5-methyl-1,3-thiazol-2-yl)-1,1-dioxo-1λ,2-benzothiazine-3-carboxamide[2], and the molecular formula is $C_{14}H_{13}N_3O_4S_2$[2].

Meloxicam was patented in 1977 and approved for medical use in the United States in 2000. It was developed by Boehringer Ingelheim; however, it is also available as a generic medication. In 2018, it was the 34th most commonly prescribed medication in the United States, with more than 22 million prescriptions. An intravenous version of meloxicam (*Anjeso*) was approved for medical use in the United States in February 2020. Meloxicam is usually taken once a day, at a dosage of 7.5 mg[3].

To date, there are Various analytical techniques viz, UV spectrophotometry, fluorimetry, capillary electrophoresis[7], pulse polarography[9], electrochemical oxidation[10], electrochemical reduction and voltametry are reported for the analysis of MLX in pharmaceuticals[6] but there have been no studies like RP-UFLC method for detection of meloxicam in pharmaceutical formulations. To overcome this inadequacy, the aim and goal of the present paper was to develop and validate a new simpler methodology to quantify Meloxicam in tablet formulation using RP-UFLC method. In 2002, Dasandi et al developed methods for determining the concentration of meloxicam in plasma. In 2003, Baeyens et al developed methods also[3].

High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture[4]. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation

of the components as they flow out of the column[4]. HPLC has been used for manufacturing (*e.g.*, during the production process of pharmaceutical and biological products), legal (*e.g.*, detecting performance enhancement drugs in urine), research (*e.g.*, separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (*e.g.*, detecting vitamin D levels in blood serum) purposes[4].

MATERIALS AND METHODS

Chemicals and reagents

Meloxicam working standard powder was gifted by Cadila Pharmaceuticals Limited, secundrabad and was used without further purification. Meloxicam tablets containing 7.5 mg was purchased from local pharmacy, Bhimavaram. HPLC grade Methanol and Acetonitrile HPLC grade, Ammonium acetate (buffer) and Glacial acetic acid were purchased from laboratory of chemicals, Shri Vishnu college of pharmacy, Bhimavaram, Andhra Pradesh, India. All chemicals were of analytical grade unless stated otherwise and used as received. Purified HPLC grade water was obtained by reverse osmosis and filtration through a milli-Q system and was used to prepare all solutions.

Instrumentation

Different instruments used to carry out the present work such as, Digital balance, ELICO pH meter (Model LI - 120), Shimadzu UFLC system (LC – 10 ATvp solvent deliver modules), (SPD – 10 Avp UV - Visible detector), Sonicator – Sonica ultrasonic cleaner.

Chromatographic Conditions

Chromatographic separation was achieved on Isocratic elution of the mobile phase Acetonitrile: Ammonium acetate: methanol: glacial acetic acid (Proportion of mobile phase-45:40:10:5 %v/v/v/v) with the flow rate of 1 ml/min. Separation was performed on C18 column (150 mm × 4.6 mm inner diameter, 5 µm particle size). The flow rate was 1.0 ml/min and detector wavelength were kept at 363 nm for monitoring the separation. The column back pressure was maintained at 80 kg/cm. Integration of the detector output was performed using the Shimadzu Empower software to determine the peak area. The contents of the mobile phase were filtered through a 0.45-µm membrane filter and degassed by sonication before use. Injection volume was 20 micro litres and total run time was 5 min, and column temperature was maintained at ambient. The solution of Meloxicam was injected and the respective chromatogram was recorded. It was found that Meloxicam was eluted at 2.36 minutes and the peak splitting was observed. For this reason, different ratios of mobile phase with different solvents were tried to obtain good chromatogram with acceptable system suitability parameters.

Preparation of standard stock solution

The stock solution of Meloxicam was prepared by dissolving accurately weighed 10 mg in 100 mL of methanol to obtain a final concentration of 0.1 mg/ml. The prepared stock solution was stored at specified temperatures in amber glass scintillation vial. The diluted solutions were filtered through 0.45 µm membrane filter. From this stock solution Meloxicam calibration standards were freshly prepared prior to analysis prepared at concentrations of 30 µg/mL from a standard solution of 100 mg/mL by appropriate dilution with mobile phase.

Preparation of Sample Solution

Ten tablets of Meloxicam were weighed, crushed and mixed in a mortar and pestle to fine powder. 10mg of powder was taken and dropped into the volumetric flasks and 100 ml of mobile phase was added to the flask. The volumetric flasks were sonicated for 20 min to effect complete dissolution of the Meloxicam and the solution was then made up to the volume with mobile phase. Suitable aliquots of solution were filtered through a 0.45 µm nylon filter. Standard solutions were prepared by diluting stock solution with mobile phase to give a resultant solution of 30µg/ml.

Assay

A mass of not less than 10 tablets was prepared by grinding them to a fine, uniform particle size powder using a mortar and pestle. After calculating the average tablet weight, a composite equivalent to the 10 mg was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately, 100 ml of mobile phase was added, the solution was sonicated for 10 min, then diluted till 30µg/ml solution obtained serially with the same. The flask was equilibrated to room temperature, carefully filled to volume with the diluent, and mixed well. 20 µl of sample was taken and injected Isocratic HPLC system.

Method validation

The developed method was validated for assay of Meloxicam in accordance with ICH-Q2 (R1) guidelines[12].

Method Development and Optimization

The chromatographic conditions were optimized for the determination of Meloxicam within a short retention time (<3min). To accomplish these objectives, the chromatographic column was first chosen based on peak shapes and resolution. C18 column (150 mm × 4.6 mm inner diameter, 5µm particle size), maintained at ambient temperature (25 ^{0}C) was used for the separation and the method validated for the determination of Meloxicam in pharmaceutical dosage forms. The prepared samples were initially analyzed using a mobile phase consisting of Acetonitrile: Ammonium acetate (buffer): Methanol: Glacial acetic acid is taken as mobile phase in ratio of (45:40:10:5 v/v/v/v) at a flow rate of 1 ml per min and UV detection at 363 nm. The Retention time of Meloxicam was found to be 2.37 min. Optimized HPLC conditions for the estimation of Meloxicam were given in Table 1 and typical chromatogram of Meloxicam shown in Figure 2.

Table 1: Showing Optimized HPLC conditions for the estimation of Meloxicam

S. No	Parameter	Description/Value
1.	Stationary Phase	C18 column (150 mm × 4.6 mm inner diameter, 5 µm particle size)
2	Mobile Phase	Acetonitrile: Ammonium acetate(buffer): Methanol: Glacial acetic acid is taken as mobile phase in ratio of (45:40:10:5 v/v/v/v)
3	Flow rate	1 ml/min
4	Detection Wavelength	363 nm
5	Detector	SPD – 10 Avp UV - Visible detector
6	Elution	Isocratic
7	Injection volume	20µl
8	Column Temperature	25°C
9	Run time	5 min
10	Diluent	Mobile Phase

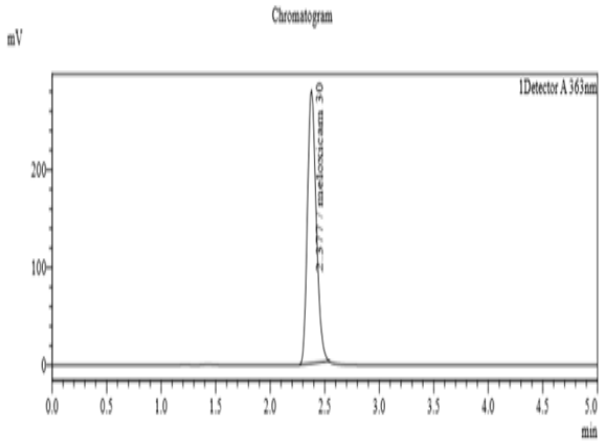

Figure 2: Showing typical chromatogram of Meloxicam

RESULT AND DISCUSSION

Validation

The method was validated with respect to parameters including Specificity, System suitability, linearity, precision and accuracy, Robustness, Ruggedness.

System suitability

System suitability and chromatographic parameters were validated such as number of theoretical plates; asymmetry factor and tailing factor were calculated. The acceptance limit was ±2% for the percent coefficient of variation (% CV) of the peak area and the retention time of Meloxicam. The % RSD of the meloxicam was 0.048 and tailing factor was 1.236.

Table 2: Showing System suitability parameters of Meloxicam

Drug	Injection	Area
Meloxicam	Injection1	1578891
	Injection2	1579910
	Injection3	1578191
	Injection4	1578888
	Injection5	1577989
	Average	1578773.8
	Standard Deviation	753.87
	% RSD	0.048
	Theoretical plates	3259
	Tailing factor	1.236

Specificity

Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. Studies are designed to evaluate the degree of interference, if any, which can be attributed to other analytes, impurities, degradation products, reagent "blanks" and excipients.

Acceptance Criteria: Placebo chromatogram should not show any peak at the retention time of Meloxicam.

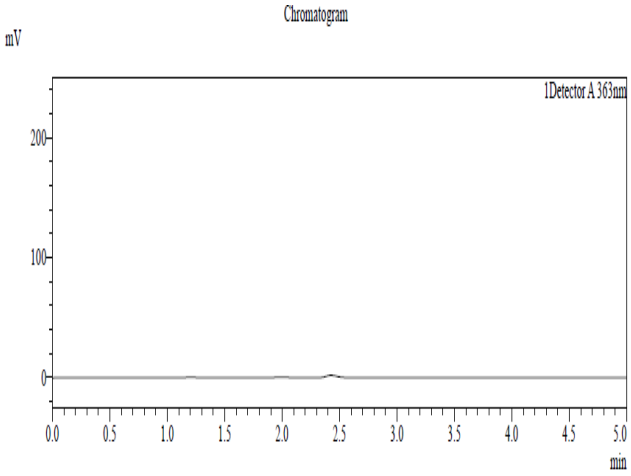

Figure 3: Showing Blank Chromatogram of Meloxicam

Placebo interference

A study of placebo (placebo batch no: 200003291) interference from excipients was conducted. Equivalent weight of placebo taken as per the test method and placebo interference was conducted in duplicate.

Accuracy

The accuracy of the proposed analytical method was determined by recovery experiments. The recovery studies were carried out at three different concentration levels in triplicate (50, 100, and 150%). The analyzed samples yielded high recovery values from the developed method. The % recovery results of the method are given in Table 3.

Table 3: Accuracy (Recovery) of Meloxicam

Sample ID	Concentration (mg/mL)		Avg %Recovery	Mean %RSD
	Drug	Spiked		
S_1 : 50 %	30	20	99.9	0.11
S_2: 100%	30	30	99.7	0.32
S_3 : 150 %	30	40	99.9	0.24

Linearity

The linearity of the calibration curve for Meloxicam was calculated and constructed by plotting the mean peak area versus concentration. The correlation coefficient of regression r^2 = 0.995 over a concentration range (10 to50 µg/ml), the representative linear regression equation for Meloxicam Y = 50505x+90334 as shown in Figure 3, and the corresponding results given in Table 4.

Table 4: Showing Linearity data of Meloxicam

S. No	Linearity Level	Concentration mg/ml	Area
1	I	10	657216
2	II	20	1049974
3	III	30	1578891
4	IV	40	2067748
5	V	50	2673574
Correlation Coefficient			0.995

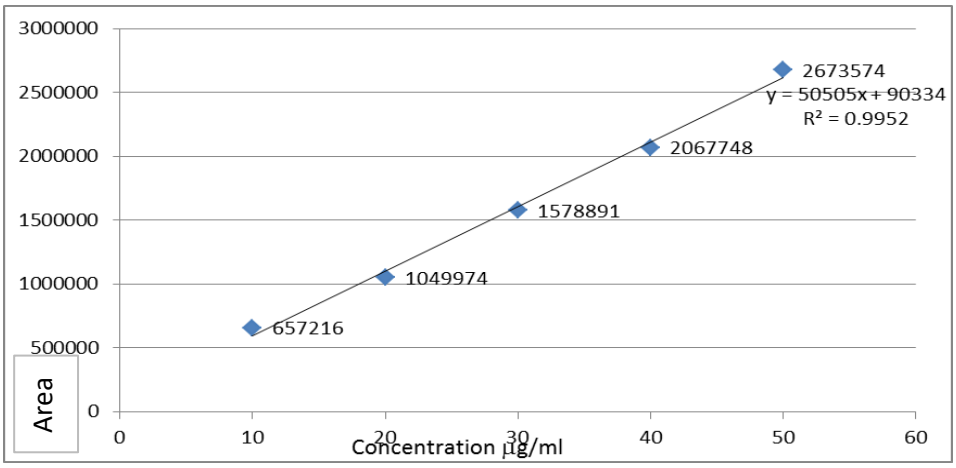

Figure 3: Showing Linearity of Meloxicam

Precision (Reproducibility)

In order to demonstrate the reproducibility of the method for the assay of a tablet pharmaceutical preparation, six replications were injected in to the system. The resultant % RSD for peak area was found to be 0.022. The results were shown in Table 5.

Robustness

Preliminary experiments revealed that amongst the many operating parameters involved. The buffer pH is the most influential parameter on the repeatability of the method, when suitable precautions have been taken with regard to instrumental aspects of injection and capillary conditioning. The method was employed with changing the mobile phase composition and flow rate of the mobile phase. The results remained unaffected by small variations in these parameters. The % RSD was found to be 0.08%.

Table 5: Showing Precision of Meloxicam

Drug	Injection	Area
Meloxicam	Injection1	1578990
	Injection2	1578910
	Injection3	1578191
	Injection4	1578987
	Injection5	1578991
	Injection6	1578813
	%RSD	0.022

Table 6: Robustness of Meloxicam

S. No	Flow Rate (ml/min)	Area	%RSD	System Suitability Results	
				Plate Count	Tailing
1	Less flow 0.9	1592030	0.031	3321	1.21
		1591121			
		1591887			
2	Actual flow 1	1593356	0.005	3401	1.203
		1593415			
		1593512			
3	More flow 1.1	1595321	0.059	3456	1.2
		1596213			
		1597213			

Table 7: Robustness of Meloxicam

S. No	Mobile phase composition	Area	%RSD	System Suitability Results	
				Plate Count	Tailing
1	Less Org	1592131	0.036	3321	1.21
		1591020			
		1591797			
2	Normal	1593351	0.008	3401	1.203
		1593515			
		1593612			
3	More Org	1595121	0.007	3456	1.2
		1595113			
		1594913			

Ruggedness

The method was employed on instrument and with three different operators. In these experiments, three standard solutions of Meloxicam were assessed on each of three occasions and the results showed no significant statistical differences between operators or between instruments. The % RSD value was found to be 0.271. The results for Ruggedness were presented in the Table 8.

Table 8*:* Ruggedness values of Meloxicam

Drug	Analyst	Instrument	Column	Assay(avg)	%RSD
Meloxicam	1	Shimadzu UFLC	C_{18} column (150 mm ´ 4.6 mm i.d. 5m)	99.80%	0.251
	2			100.80%	0.271
	3			99.72%	0.249

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Meloxicam from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claim and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Meloxicam in pure form and its dosage form and also can be used for dissolution or similar studies.

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