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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF MELOXICAM IN PHARMACEUTICAL DOSAGE FORM

Mrunal Baburao Chavan*, Dr. S. C. Kale, Dr. Aijaz Sheikh and Dr. K. R. Biyani

Anuradha College of Pharmacy, Chikhli, Dist: Buldana 443201 Maharashtra.

***Corresponding Author: Mrunal Baburao Chavan**

Anuradha College of Pharmacy, Chikhli, Dist: Buldana 443201 Maharashtra.

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ABSTRACT

Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) used to treat rheumatoid arthritis, acute exacerbations of osteoarthritis, ankylosing spondylitis, and juvenile idiopathic arthritis. It is given in a single oral dose of 7.5mg, increased if necessary, and may be given by rectal suppository in doses similar to orally. Meloxicam has a greater inhibitory activity against the inducible isoform of cyclooxygenase (COX-2) than against the constitutive isoform (COX-1). COX-1 induces the synthesis of prostacyclin, which is responsible for vascular homeostasis, platelet aggregation, renal function, and gastric cytoprotection. Meloxicam's anti-inflammatory and analgesic properties are similar to non-selective NSAIDs, but it has both gastric mucosal and renal protective properties. The reported side effects of meloxicam are similar to those of NSAIDs, such as abdominal pain, anemia, and edema, with an increased risk of serious gastrointestinal adverse events, including ulceration and bleeding. The aim of the present study is to develop and validate the RP-HPLC method for estimating Meloxicam in Pharmaceutical Dosage Form, focusing on the application of suitable analytical techniques, optimization, and validation in accordance with ICH guidelines, while selecting the appropriate drug and developing an analytical methodology. In this study, we found that the pharmaceutical dose tablet formulations containing Meloxicam (MLC) may be accurately measured using the RP-HPLC method. The RP-HPLC technique is sensitive, accurate, precise, and repeatable; it also demonstrates high repeatability. Meloxicam (MLC) tablet dosage formulation analysis may also be conducted with success. These techniques do not experience any influence from additives, matrices, etc. To further understand these trials, additional research on other medication formulations is needed.

KEYWORD:- Meloxicam; RP-HPLC; Validation; ICH guidelines; Cyclooxygenase.

1. INTRODUCTION

Meloxicam, an oxicam derivative: 4-Hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2- benzothiazine-3-carboxamide 1,1-dioxide (Figure 1), is a nonsteroidal anti-inflammatory drug (NSAID). It is used in the management of rheumatoid arthritis, acute exacerbations of osteoarthritis, ankylosing spondylitis and juvenile idiopathic arthritis.

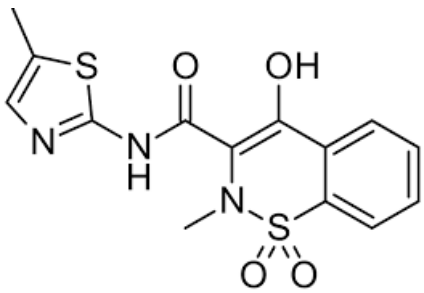

Figure 1: Meloxicam.

It is given in a single oral dose of 7.5mg, increased if necessary to a maximum of 15mg daily (7.5mg in the elderly).[1-3] It may also be given by rectal suppository in doses similar to those used orally. Meloxicam, an enol-carboxamide non-steroidal anti-inflammatory drug (NSAID) related to piroxicam, has long been used to treat acute pain and inflammation. In contrast to other NSAIDs, it has a greater inhibitory activity against the inducible isoform of cyclooxygenase (COX-2) than against the constitutive isoform (COX-1).[4-5] COX-1 induces the synthesis of prostacyclin, which is responsible for vascular homeostasis, platelet aggregation, renal function, and gastric cytoprotection. The expression of COX-2 isoform increases during inflammation. Consequently, although meloxicam's anti-inflammatory and analgesic properties are similar to non-selective NSAIDs, it has both gastric mucosal and renal protective properties. The reported side effects of meloxicam are similar to those of nonsteroidal anti-inflammatory drugs (NSAIDs), such as abdominal pain, anemia, and edema.[6-8] There is also an increased risk of

serious gastrointestinal (GI) adverse events, including ulceration and bleeding. This profile is prepared to discuss and explain physical characteristics, Proprietary and nonproprietary names of meloxicam. It also includes methods of preparation, thermal and spectral behavior, methods of analysis, pharmacokinetics, metabolism, excretion and pharmacology.[9-10]

The aim of the present study is to develop and validate the RP-HPLC method for estimating Meloxicam in Pharmaceutical Dosage Form, focusing on the application of suitable analytical techniques, optimization, and validation in accordance with ICH guidelines, while selecting the appropriate drug and developing an analytical methodology.

2. MATERIALS AND METHODS

2.1 Procurement of the drug

Meloxicam, a medication from Arch Pharma labs Ltd Thane, is available in a 10g package with a purity of 99.8 to be used as Reference drug while Muvera 15, Sun Pharma Lab. Ltd India which contains 15 mg dosage of Meloxicam to be used as test drug.

2.2 Method and Procedure

2.2.1 Identification and Characterization of drug

Meloxicam (MLC) were selected as model drug candidate for method development and validation. The drugs were kindly gifted from Pharmaceutical industry India. The procured drug was analyzed for different physical properties viz. color, odor, melting point, etc. The IR absorbance spectrum of MLC was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm^{-1} (11-15).

2.2.2 Selection of mobile phase

The mobile phases tested include methanol: water (90:10), methanol: water (80:20), acetonitrile: water (90:10), acetonitrile: phosphate buffer 10mm (90:10), acetonitrile: phosphate buffer 10mm (80:20), and acetonitrile: phosphate buffer (75:25) with pH 4.5.

2.2.3 Chromatographic conditions

The chromatographic conditions were established through trial and error, maintaining constant consistency throughout the method. The column was Inertsil 4.6 x 250 mm, with a particle size of 5 µm, stationary phases of C18 Inertsil, mobile phase of Acetonitrile: Phosphate Buffer (75:25), pH 4.5, and a sample size of 20 µL.

2.2.4 Validation of the method

Adjusting several UFLC settings (FDA, 1995, 1997, 2000, 1994, 1987; USP, 2000) confirmed the reliability of the UFLC approach (16). Calibration plot least-squares linear regression analysis verified the UFLC method's linearity (17), the limits of detection and quantification for the medicines mentioned were determined to be three and five epochs, respectively, above and below the baseline noise, The process adhered to the guidelines established by the United States Pharmacopoeia (USP, 2000), specificity (17), precision (18) accuracy (19), robustness (20) and ruggedness (21) were determined.

3. RESULTS AND DISCUSSION

3.1 Characterization of the drug

Meloxicam is a pastel yellow solid, practically insoluble in water, with higher solubility observed in strong acids and bases. It is very slightly soluble in methanol. Meloxicam has an apparent partition coefficient (log P)app = 0.1 in n-octanol/buffer pH 7.4. Meloxicam has pKa values of 1.1 and 4.2. Melting point of the meloxicam is found to be 152.95 ± 0.458 ºC.

3.2 Identification of the drug

3.2.1 UV Spectra analysis

The ultraviolet absorption spectrum of MLC was obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm, which was found to be 350 nm (Figure 2)

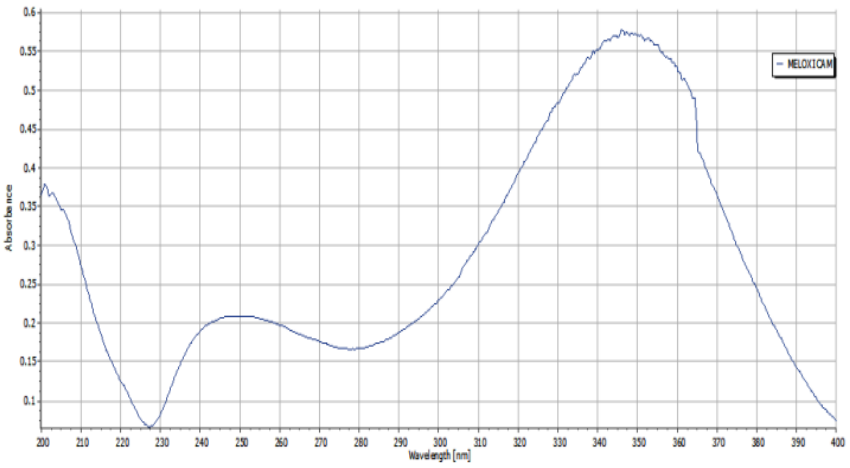

Figure 2: UV Spectra Analysis.

3.2.2 FT-IR of Meloxicam

The IR absorbance spectrum of Meloxicam (MLC) was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1 (Table 1; Figure 3).

Table 1: FT-IR study of Meloxicam.

Sr. No	Functional group	Frequency Range cm-1
1	N-H stretching	3285.39
2	C-N stretching	1615.14
3	Thiazole ring	1547.79
4	amide II band of the amide group	1451.83
5	S=O stretching vibrations	1152.30

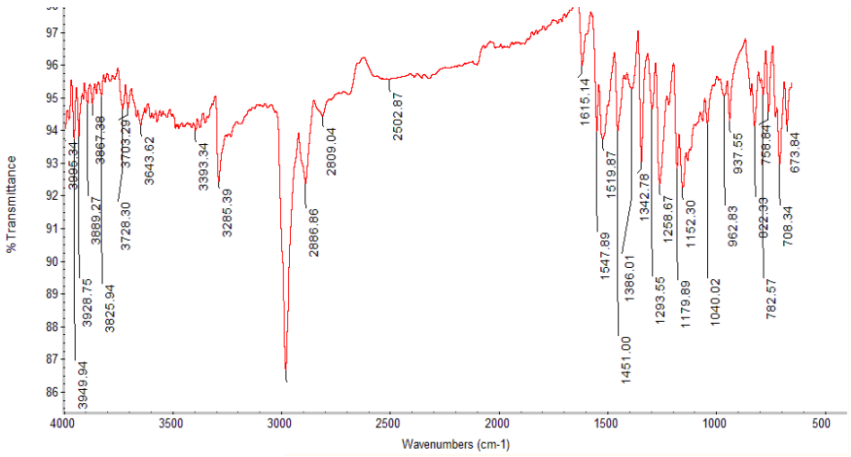

Figure 3: FT-IR study of Meloxicam.

3.3 Selection of the mobile phase

From various mobile phases tried, mobile phase containing Acetonitrile: Phosphate Buffer (75:25) pH 4.5 was selected, since it gives sharp reproducible retention time for MLC (Figure 4-7).

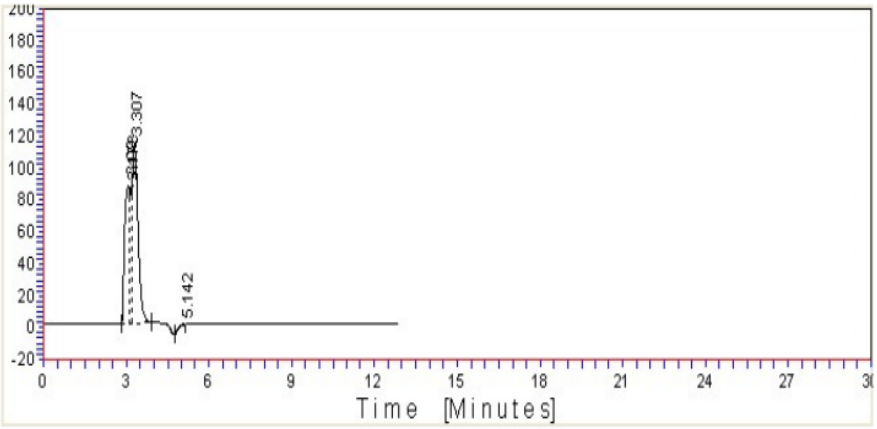

Figure 4: Trial Chromatogram obtained by using Acetonitrile: water (80:20) as mobile phase.

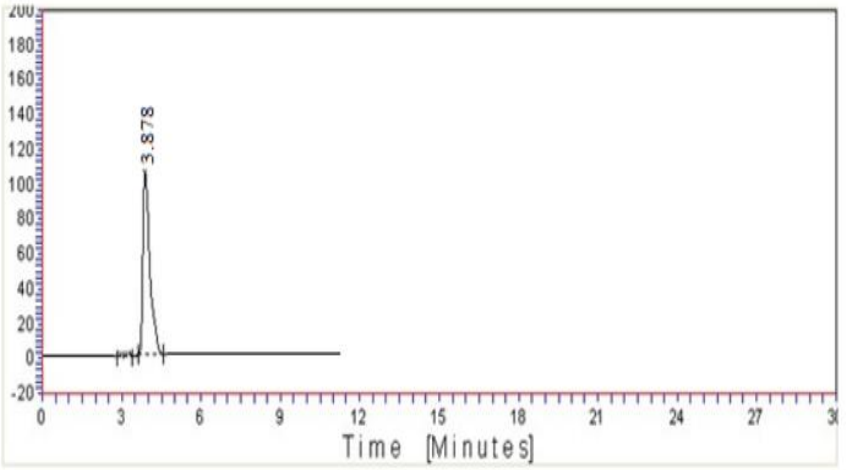

Figure 5: Trial Chromatogram obtained by using Acetonitrile: Phosphate Buffer 10mm (90:10) pH 5.5 as mobile phase.

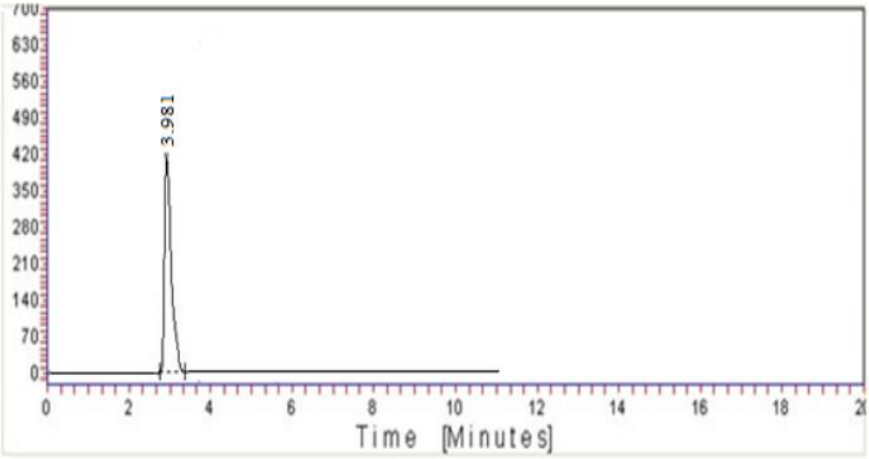

Figure 6: Trial Chromatogram obtained by using Acetonitrile: Phosphate Buffer 10mm (75:25) pH4.5 as mobile phase of MLC.

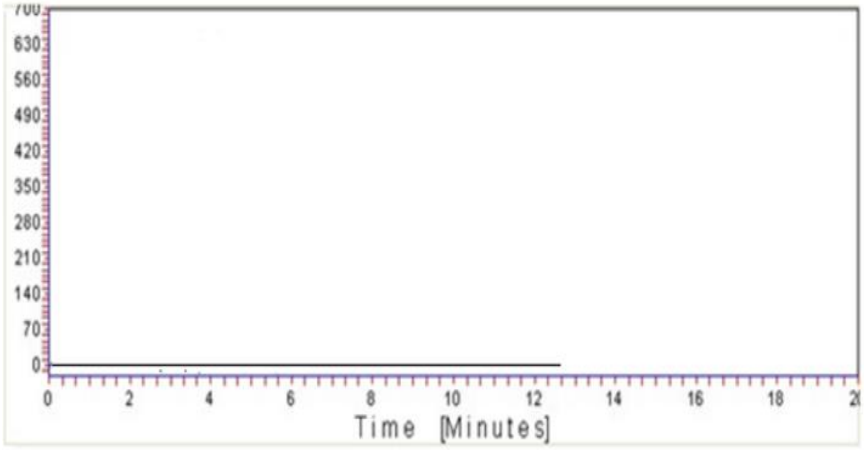

Figure 7: Blank Chromatogram obtained by using Acetonitrile: Phosphate Buffer 10mm (75:25) pH4.5 as mobile phase.

3.4 Application of proposed method for estimation of MLC in formulation

Equal volume (20µL) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content MLC was calculated by comparing a sample peak with that of standard (Figure 8).

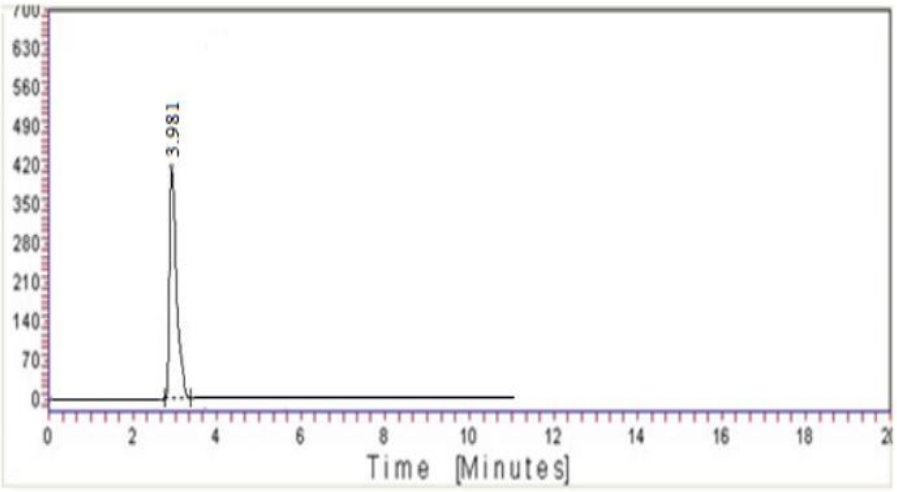

Figure 8: Chromatogram obtained by formulation of MLC.

3.5 Validation of the method

Accuracy was ascertained on the basis of recovery studies performed by standard addition method (Table 2). Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method (Table 3). Specificity was measured as ability of the proposed method to obtain well separated peak for MLC without any interference from component of matrix. Mean retention time for – MLC – 3.981 The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix. Linearity and range: According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions was recorded. MLC marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug (Table 4). The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate. The system suitability results should lie within the limit. Hence the method was robust (Table 5). Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision accuracy (Table 6). After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by following procedure described under experimental and results. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation (Table 7).

Table 2: Results and statistical data for Recovery study of MLC.

Sr. No	wt. of formulation	Amount of Drug Added in (µg/ml)	Peak Area of stand.	Peak Area of sample	% Recovery
	MLC	MLC	MLC	MLC	MLC
1	126	1	438956.1	437200.3	99.6
2		1		439395.1	100.1
3		1		439834.0	100.2
4		2		438517.1	99.9
5		2		443784.6	101.1
6		2		435883.4	99.3
7		3		441589.8	100.6
8		3		442467.7	100.8
9		3		442906.7	100.9

Table 3: Results and Statistical data of precision study.

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	MLC	MLC	MLC	MLC	MLC
1	10	126	438956.1	440273.0	100.3
2		125.9		440711.9	100.4
3		126		441150.9	100.5

Table 4: Observations of Linearity and Range study for MLC.

Sr. No.	%Label claim	Peak area MLC
1	80	351164.9
2	90	395060.5
3	100	438956.1
4	110	488851.7
5	120	526847.3

Table 5: Result of Robustness study of MLC.

Sr. No.	Condition	Parameter	Peak Area	RT
01	Change of wavelength	348 nm	438956.1	3.984
02		350nm	438956.1	3.981
03		352 nm	438956.1	3.980
04	Change in Temperature	30 °C	438854.2	3.983
05		25 °C	438956.1	3.981
06		20 °C	438456.2	3.979
07	Change in Flow rate	0.8 ml/min	438898.3	3.985
08		1ml/min	438956.1	3.981
09		1.2 ml/min	438987.6	3.978
10	Change in Mobile Phase	70:30	438901.5	3.986
11		75:25	438956.1	3.981
12		80:20	438974.2	3.979

Table 6: Limit of detection (LOD) and Limit of quantitation (LOQ).

Sr. No.	Drug Name	LOD µg/ml	LOQ µg/ml
1	MLC	0.81	1.97

Table 7: Summary of laboratory mixture and marketed formulation analysis by RP-HPLC Method

Sr. no.	Sample	Statistical data	MLC	
			% Estimation	%Recovery
1.	Standard Laboratory mixture	Mean	100.13	-
		S.D.	0.208	-
		C.V.	0.002	-
2.	Muvera 15	Mean	100.47	100.28
		S.D.	1.012	0.616
		C.V.	0.010	0.006

4. CONCLUSIONS

In this study, we found that the pharmaceutical dose tablet formulations containing Meloxicam (MLC) may be accurately measured using the RP-HPLC method. The RP-HPLC technique is sensitive, accurate, precise, and repeatable; it also demonstrates high repeatability. Meloxicam (MLC) tablet dosage formulation analysis may also be conducted with success. These techniques do not experience any influence from additives, matrices, etc. To further understand these trials, additional research on other medication formulations is needed.

5. Conflict of interest

None.

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