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RP-HPLC Method Development and Validation for the Determination of Meloxicam in Bulk and Its Pharmaceutical Formulation

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ABSTRACT

A simple and reliable method called reversed-phase high-performance liquid chromatography (RP-HPLC) was developed and tested to measure the amount of the drug Meloxicam (MEL) bulk and in a pharmaceutical in bulk drug and Formulation. The samples separation was performed on a RP-C18 column (250 x 4.6 mm id, 5 micron particle size) with the mobile phase consisting of Acetonitrile: Phosphate Buffer (60:40) pH 3.6 with flow rate 1 ml/min at ambient temperature and the detection was done at a wavelength of 355 nm. The proposed HPLC method was statistically validated with respect to linearity, ranges, precision, accuracy, selectivity, LOD, LOQ and robustness. The retention time (RT) of Meloxicam was found to be 3.0 min. respectively. All the results were within the acceptable range. The measurements of Meloxicam were consistent for the amounts of 10 and 15 mg/ml. The reliability of the results, shown by the R^2 value, was 0.993.This new RP-HPLC method is simple, fast, sensitive, precise, accurate, and cost-effective for analyzing Meloxicam bulk and in a pharmaceutical in bulk drug and Formulation.

Keywords- Meloxicam, RP-HPLC, Validation.

INTRODUTION

Meloxicam is a type of non-steroidal anti-inflammatory drug (NSAID) that helps relieve pain and inflammation[1]. It's similar to another drug called piroxicam. Unlike many other NSAIDs, meloxicam works better at blocking a specific enzyme called COX-2, which is involved in causing pain and swelling, rather than the COX-1 enzyme, which helps protect the stomach lining.[2] Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) used for pain, swelling, and fever. It appears as a yellow crystalline powder with a molecular weight of about 351.39 g/mol. It melts at a temperature between 242 and 250°C, and its chemical formula is $C_{14}H_{13}N_3O_4S_2$.[3-4] High Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques. More than 85% of pharmaceuticals are analysed by HPLC. The separation is achieved by differential interaction of the analyte with the stationary and the mobile phases. Proper choice of the stationary phase and mobile phase is essential to achieve the desired separation.[5] The other variables which play important roles in method development are: the pH of mobile phase, types of buffer, flow rate, column temperature and detection wavelength etc. HPLC is the separation module which contains mainly the stationary and mobile phases having opposite polarity.[6] Method validation should be treated as a "final verification" of the method performance. As per ICH**,** method validation can be defined as (ICH) "establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce the desired result or product meeting its predetermined specifications and quality characteristics"[7] Method validation study includes parameters like system suitability, linearity, precision, accuracy, specificity, robustness, limit of detection, limit of quantification and stability of samples, reagents, instruments.[8]

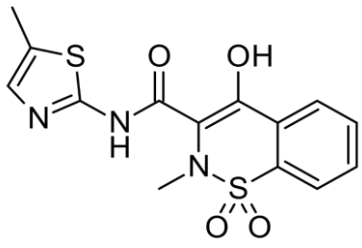

Figure 1. Chemical structure of meloxicam.

Chemical name: 4-Hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide.[9]

MATERIALS AND METHODS:

Meloxicam (Purity≥ 99% on anhydrous basis by HPLC) was gifted by Arch Pharmalabs Ltd. Thane. Analytical grade Orthophosphoric acid, Acetonitrile HPLC Grade, Methanol HPLC grade and Water HPLC grade were purchased from Merck Chemical Company (India).The Buffer was prepared by dissolving 17.418g (0.1M) of potassium dihydrogen phosphate in 980 mL of water, and pH was adjusted to 3.6 with Orthophosphoric acid and made upto1000 mL with water.

HPLC instrumentation and chromatographic conditions [10-12]

The chromatography was performed, with Youglin HPLC comprising UV Detector, UV-Visible Spectrophotometer Shimadzu Double beam -UV 1800, samples (20 µL) were injected by means of a Rheodyne injector fitted with a 20-µL loop. The samples separation was performed on a RP-C18 column (250 x 4.6 mm id, 5 micron particle size) with the mobile phase consisting of Acetonitrile: Phosphate Buffer (60:40) pH 3.6 with flow rate 1 ml/min at ambient temperature.[13]

FT-IR analysis

The FTIR spectra of a sample of pure MEL is shown in figure above. The FTIR spectrum of MEL showed a distinct peak at 3289 cm^{-1} (N–H stretching vibrations), 1619 cm^{-1} (C=N stretching vibrations), 1550 cm^{-1} (thizole ring), 1530 cm^{-1} (amide II band of the amide group), and 1152.6 cm^{-1} (S=O stretching vibrations). This FTIR spectra confirmed the drug.[14]

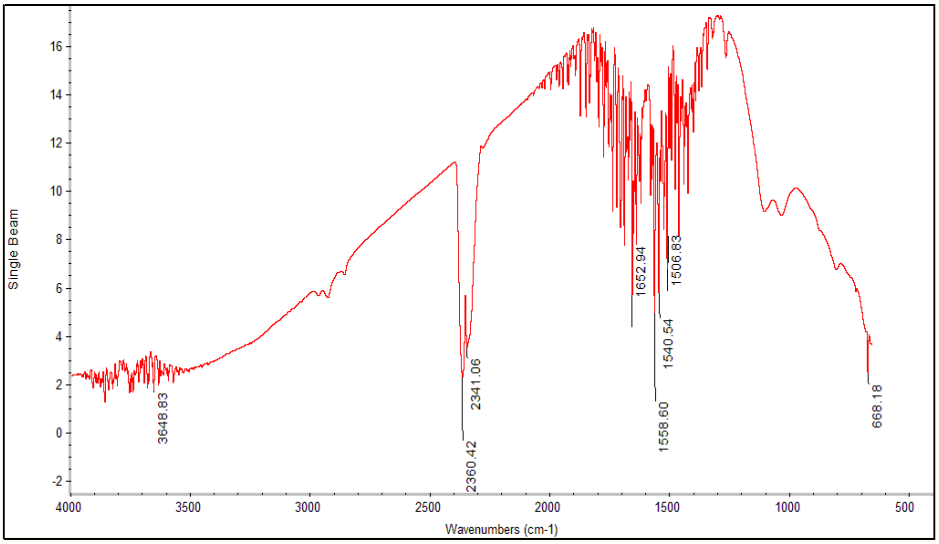

Figure 2 : FTIR Spectra of MEL

RESULTS AND DISCUSSION

Preparation of standard stock and sample solution

Preparation of standard stock solution 10 mg of Meloxicam were weighed accurately in a 100 ml volumetric flask respectively. 80 ml of the mobile phase was added, sonicated to dissolve and diluted to volume with the mobile phase. Further, 1 ml of this solution was diluted to 10 ml, respectively, with the mobile phase. The resultant mixture was subjected to HPLC analysis in developed chromatographic conditions.[15-17]

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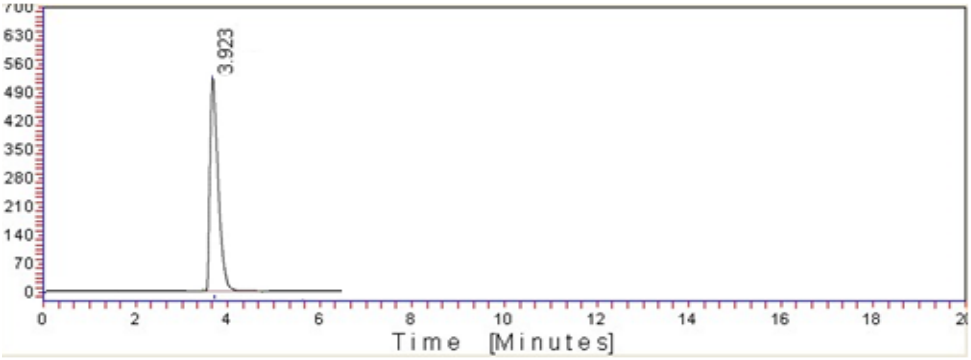

Figure 3: Chromatogram obtained by using Acetonitrile: Buffer (60:40) pH 3.6 as mobile phase.

UV Spectroscopy Analysis

The ultraviolet absorption spectrum of MEL was obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm. The wavelength maxima (λmax) The wavelength of 355 nm was selected as suitable detection wavelength because of clear flat baseline.[18]

Preparation of calibration curve:

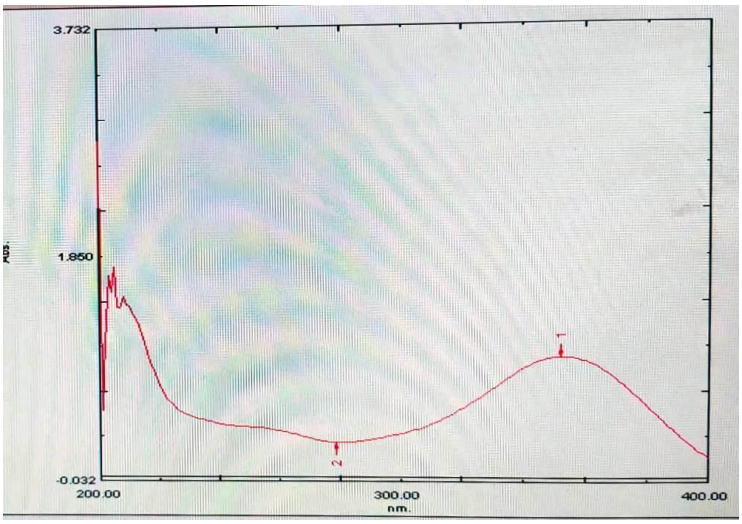

Figure 4 : - UV Spectra of MEL

Table .1: Standard calibration curve for MEL

Sr. No.	Conc. (µg/ml)	Peak area
1	2	34895.63
2	4	69791.26
3	6	104686.9
4	8	129582.5
5	10	174478.2
6	12	209373.8
7	14	244269.4
8	16	279165
9	18	314060.7
10	20	348956.3

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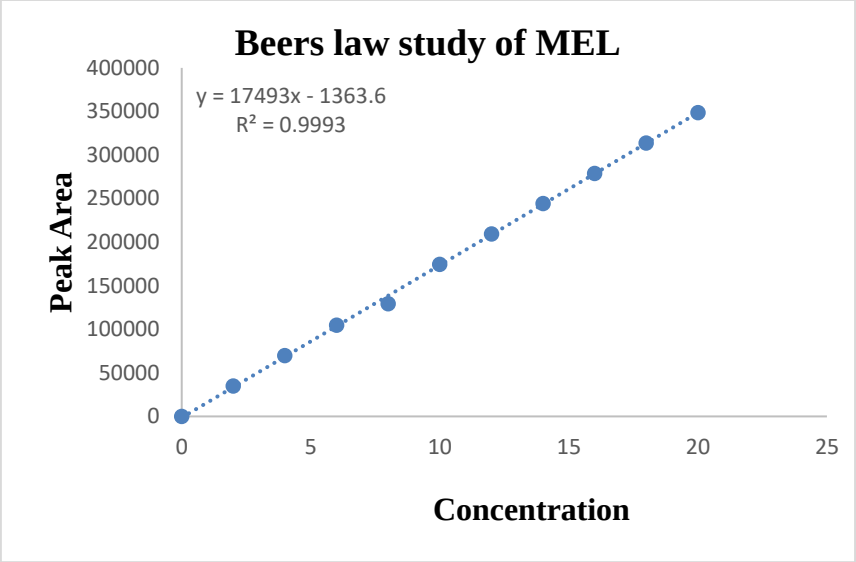

Figure 5: Standard calibration curve for MEL

System suitability test: System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.[19]

Table 2 : Result of System Suitability Study

Sr. No	Retention time (Rt) (Min)	Peak Area	Capacity Factor (K')	Tailing Factor (T)	No. of Theoretical plates (N)
1	3.923	27385.5	6.38	1.12	6992.0
2	3.888	27402.8	6.38	1.12	6992.0
3	3.923	27385.5	6.36	1.11	6931.6
4	3.967	27399.7	6.36	1.11	6931.6
5	3.912	27460.1	6.38	1.12	6992.0
Statistics					
Mean	3.922	27405.66	6.372	1.12	6967.84
S.D.	0.028	31.8671147	0.0109	0.008	33.08
C.V.	0.217	0.00116279	0.171	0.714	0.474

Estimation of MEL in laboratory sample:[20]

The amount of each drug estimated in laboratory mixture was calculated using following formula –

$$\% \text{ Estimation} = \frac{A_t}{A_s} x \frac{D_s}{D_t} x \frac{W_s}{W_t} x\, 100$$

Where-

At = Area count for sample solution.

As = Area count for standard solution.

Ds = Dilution factor for standard.

Dt = Dilution factor for sample.

Ws = Weight of standard (mg)

Wt = Weight of sample (mg)

Table 3 : Results and statistical data for estimation of MEL in lab Sample

Sr No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Drug Estimation
	MEL	MEL	MEL	MEL	MEL
1	15	15	27385.5	27330.73	99.8
2		15		27303.34	99.7
3		15		27275.96	99.6
				Mean	99.70
				±S.D.	0.100
				C.V.	0.001

Validation parameters:

a) Accuracy: It was ascertained on the basis of recovery studies performed by standard addition method.

Table 4 : Results and statistical data for Recovery study of MEL

Brand name : Muvera 15 **Avg wt -** 190 mg

Sr. No.	Wt. of tablet powder taken (mg)	Amount of Drug Added in	Wt of Std Drug	Peak Area of stand	Peak Area of sample	% Recovery
1	190	2	15	27385.5	12678.81	100.8
2	190	4			12691.38	100.9
3	190	6			12716.54	101.1
					Mean	100.93
					S.D.	0.153
					C.V.	0.002

b) Precision: 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.[21]

Table 5 : 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
	MEL	MEL	MEL	MEL	MEL
1	10	190	27385.5	27440.27	100.2
2		189.9		27358.11	99.9
3		190		27467.66	100.3
				Mean	100.13
				±S.D.	0.208
				C.V.	0.002

c) Ruggedness:

The studies of ruggedness were carried out under two different conditions-

1) Days

2) Analyst.

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i) Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 6.[22]

Table 6 : Results and statistical data of Interday Study

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	MEL	MEL	MEL	MEL	MEL
1	10	190	27385.5	27303.34	99.7
2		189.9		27275.96	99.6
3		190		27412.89	100.1
				Mean	100.13
				±S.D.	0.208
				C.V.	0.002

ii) Intraday: It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The percent label claim was calculated using formula No. 28. Result and statistical data are shown in Table No. 7.

Table 7: Result and statistical data for Intraday.

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	MEL	MEL	MEL	MEL	MEL
1	10	190	27385.5	27467.66	100.3
2		189.9		27495.04	100.4
3		190		27522.43	100.5
				Mean	100.40
				±S.D.	0.100
				C.V.	0.001

iii) Different analyst : The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

d) Specificity: Specificity was measured as ability of the proposed method to obtain well separated peak for MEL without any interference from component of matrix. Mean retention time for –MEL– 3.923.

f) Robustness: 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.[22-23]

Table 8: Result and statistical data of Different analyst study

Sr. No	% Label claim	
	ANALYST I	ANALYST II
	MEL	MEL
1	100.3	100.3
2	100.4	99.77
3	100.5	99.61
4	101	99.8
5	101.1	100.18
Mean	100.66	99.932

± S.D	0.364691651	0.293376891
C.V	0.003623005	0.002935765

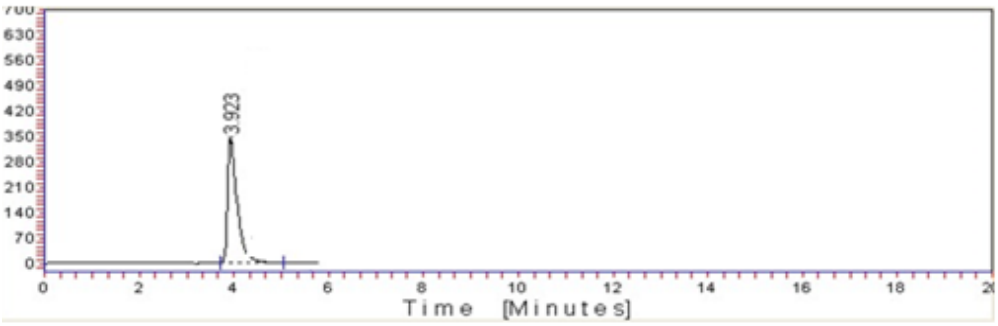

Figure 6: Chromatogram obtained by formulation of MEL

f) Limit of Detection (LOD) and Limit of Quantitation (LOQ): As per ICH guideline both LOD & LOQ were performed on the basis of standard deviation of the response and slope and expressed by following formulae respectively.

$$\text{LOD} = \frac{3.3\sigma}{S}, \qquad \text{LOQ} = \frac{10\sigma}{S}.$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

Table 9 : Result of Robustness study of MEL

Sr. No.	Condition	Parameter	Peak Area	RT
01	Change of wavelength	353 nm	27355.1	3.923
02		355 nm	27385.8	3.921
03		357 nm	27388.2	3.923
04	Change in Temperature	30 ºC	27370.3	3.923
05		25 ºC	27385.5	3.923
06		20 ºC	27305.2	3.923
07	Change in Flow rate	0.8 ml/min	27385.5	4.011
08		1ml/min	27385.5	3.923
09		1.2 ml/min	27385.5	3.901
10	Change in Mobile Phase	50:50	27301.8	3.899
11		60:40	27385.5	3.923
12		70:30	27467.1	4.011

Table 10 : LOD & LOQ of MEL

Sr. No.	Drug Name	LOD µg/ml	LOQ µg/ml
1	MEL	0.51	1.45

CONCLUSION:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. HPLC is highly valued in analysis because it's easy to use and very specific and sensitive, making it great for studying complex samples. It's often used to measure drugs in formulations and to analyze their metabolites in biological

fluids, allowing for the estimation of multiple components without needing to separate them first, even in tiny amounts. In this study, we used HPLC to measure Meloxicam (MEL) in tablets. We focused on important factors for developing the analytical method using an Inertsil column and a UV detector. We prepared standard and sample solutions of MEL in different mobile phases. We found that increasing the amount of methanol sharpened the peaks, while more water made them broader. The best mobile phase was Acetonitrile and Phosphate Buffer (60:40) at pH 3.6, with a detection wavelength of 355 nm. This setup provided good resolution and the right retention time, with a tailing factor of less than 2.

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