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Research Article

METHOD DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR ANALYSIS OF MELOXICAM USING DAD DETECTOR

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ABSTRACT

A simple, precise and accurate isocratic RP-HPLC method was developed and validated for determination of Meloxicam in bulk drug and tablets. Isocratic RP-HPLC separation was achieved on a LiChrospher RP-18 column (250 x 4.6 mm id, 5 micron particle size) using the mobile phase 'A' 0.1 M dipotassium hydrogen phosphate pH 4.0 with orthophosphoric acid and mobile phase 'B' Methanol . Mobile phase 'A': mobile phase 'B' premixed in the ratio of 65:35 v/v were used as mobile phase at a flow rate of 1.0 mL/min and the column oven temperature was 35 ˚C good. The retention time of Meloxicam (2H-1, 2-benzothiazine-3-carboxamide, 4-hydroxy-2-methy-N-(5-methyl-2-thiazolyl)-, 1, 1-dioxide) was about 4.31 min and its known impurity-B (5-methylthiazole-2ylamine) was about 2.26. The photodiode array detector was used to test the purity of the peaks, and the chromatograms were extracted at 254 nm. The method was validated for linearity, precision, accuracy, robustness, solution stability, and specificity. The method was linear in the concentration range of 150-450 µg/ml with a correlation coefficient of 0.999. The limit of detection (LOD) and limit of quantification (LOQ), respectively were 5 and 50 µg/mL for Meloxicam. The accuracy (recovery) was found to be in the range of 98.57%-101.69%. The drug was subjected to the stress conditions hydrolysis, oxidation, photolysis, and heat. Degradation products produced as a result of the stress conditions did not interfere with detection of Meloxicam; therefore, the proposed method can be considered stability-indicating.

***Key words**: Meloxicam, RP-HPLC separation, LiChrospher, ICH.*

INTRODUCTION

Derivative of oxicam are used to develop an array of pharmacological effects, including analgesic, fever reducer effects and rheumatoid arthritis. The formulations belonging to this group are relatively safe when compared with *acetaminophen* as they do not lead to stomach ulcers and bleeds when used in high doses [1]. At present, numbers of such formulations are globally marketed for clinical uses.

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Meloxicam (2H-1,2-benzothiazine-3-carboxamide,4-hydroxy-2-methy-N-(5-methyl-2-thiazolyl)-,1,1-dioxide) (Fig. 1) is a derivative of oxicam, which is abutting associated to piroxicam, and falls in the non steroidal anti-inflammatory (NSAID) enolic acid group. Meloxicam has analgesic and antipyretic activities due to selectively inhibit cyclooxygenase-2 over cyclooxygenase-1 [2-5]. The drug is also competent in the therapeutics of anti-rheumatic and pertinent conditions [4]. The average dose once in a day is 7.5mg and in severe pain conditions is 15mg.

The literature reveals that there are number of analytical techniques for determination and estimation of therapeutic and toxic blood

concentration by RP-HPLC [6-13], LC-MS [14], UV spectrophotometric methods [15, 16] and electrochemical methods [17-22] of Meloxicam individual or in the combinations formulations. Among all of the methods developed till now few of them is stability indicating methods. During the transportation, storage environmental factors play an important role in the stability of the formulation. Stress degradation study provides the information about the nature of the degradation product as well as intrinsic stability of the formulation [23-25]. Visitation of International conference on Harmonization (ICH) stability indicating methods emerge as clearly constitutional.

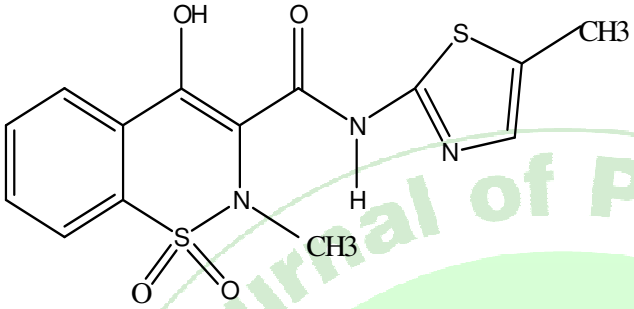
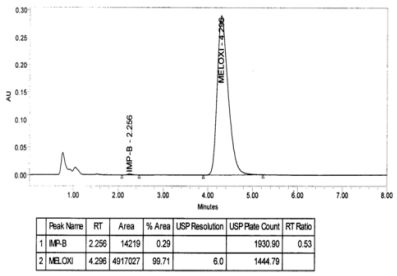


Figure 1; Structure of Meloxicam



	Peak Name	RT	Area	% Area	USP Resolution	USP Plate Count	RT Ratio
1	IMP-B	2.256	14219	0.29		1930.90	0.53
2	MELOXI	4.296	4917027	99.71	6.0	1444.79	

Figure 2; API chromatogram of Meloxicam

MATERIALS AND METHODS

Chemicals and reagents

Meloxicam (Purity≥ 99% on anhydrous basis by HPLC) was gifted by Ind Swift Labs Laboratories Ltd. (Mohali, India). Analytical grade Orthophosphoric acid, dipotassium Hydrogen Phosphate, Sodium hydroxide, Hydrogen peroxide and Hydrochloric acid were purchased from Merck Chemical Company (India) and Methanol was purchased from RANKEM (India). Distilled water prepared from Millipore was used throughout the study. Buffer was prepared by dissolving 17.418g (0.1M) of potassium dihydrogen phosphate in 980mL of water, and pH was adjusted to 4.0 with Orthophosphoric acid and made upto1000mL with water.

HPLC instrumentation and chromatographic conditions

The chromatography was performed, with WATERS (2695) HPLC equipment comprising quaternary pumps, U.V/Visible detector WATERS (2489), thermo controlled column oven, samples (20 µL) were injected by means of a Rheodyne injector fitted with a 20-µL loop. The operating system was controlled by use of Empower 2 software. The samples separation was performed on a LiChrospher RP-18 column (250 x 4.6 mm id, 5 micron particle size) with the mobile phase consisting of methanol and phosphate buffer (pH 4.0) with a ratio of 65: 35 (v/v) at 35˚C temperature. The flow rate was kept at 1.0 ml/min and the elute were observed at 254 nm.

Method Development

A number of trials on different column, buffer with different molar concentration, pH, flow rate, column temperature were investigated in the development of RP-HPLC method for suitable analysis of Meloxicam. These included phosphate buffer with different molar concentration from 0.01- 1.0 M with pH range from 3.0-7.2, C_{18} and C_8 columns, temperature from 25C˚ to 45C˚, flow rate 0.6 to 1.0mLmin^{-1}. And variation in the mobile phase composition was also investigated acetonitrile-water with pH 3.0-7.5 with NaOH and H_3PO_4 80:20 %v/v, methanol-water 50:50%v/v, methanol-water 85:15%v/v, methanol -phosphate buffer (pH 3.0–7.5), 65:35 %v/v, and acetonitrile-phosphate buffer (pH 3.0–7.5), 80:20 %v/v. The conformability of the mobile phase was choose on the basis of the receptiveness of the assay, suitability for stability studies, time required for the analysis and easy preparation.

System suitability

Verify that the system suitability parameters of the chromatographic system are adequate for the subjected analysis. The relative standard deviation of areas and retention time was calculated (Table I).

Table I System Suitability

Replicates	*Areas*	*Retention Time(min.)*
1	9648624	4.235
2	9661672	4.234
3	9664554	4.233
4	9678106	4.231
5	9692448	4.233
6	9712534	4.233
Average	***9676323***	***4.23***
Std .dev.	***23215.768***	***0.001***
%RSD	***0.24***	***0.031***

Construction of the calibration curve

A standard stock solution of 100 mg of Meloxicam was taken in a 100ml volumetric flask and dissolved in 10 ml 1.0 M NaOH, sonicated for 5 min. then 10ml of methanol was added again sonicated for 5 min. and finally volume was adjusted to 100 ml with mobile phase. Filter the solution through 0.45 µ filter. Then the solution is further diluted to prepare calibration samples in the concentration range of 150-450 µgmL^{-1} (50-150% of the target concentration i.e. 300 µg mL^{-1}).The column equilibration was attained by running mobile phase at 0.6 mL min^{-1} and baseline was monitored at 254 nm wavelength (Table II).

Table II Linearity (n=3)

S. No.	*Parameters*	*Meloxicam*
1	***Linearity range (µg/ml)***	150-450
2	***R^2***	0.9992
3	***Slope***	32152.29
4	***Intercept***	88256.469
5	***Y=mX+c***	32152.29x+88256.469

Detection (LOD) and Quantification (LOQ) Limits

To determine the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantification as an exact value and the quantification 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. The formulae LOD = 3.3 × standard deviation and LOQ = 10 × standard deviation (Table III a, b).

Table III a LOD @ 5µg/ml

S. No	*LOD Height*	*LOD Area*
Average	149.16	2863
Standard deviation	5.12	21.15
%Relative standa deviation	3.43%	0.74%

Table III b LOQ @ 50µg/ml

S. No	*LOQ Height*	*LOQ Area*
Average	386.66	19825.33
Standard deviation	6.65	111.14
%Relative standa deviation	1.72%	0.56%

Recovery

Previously analyzed samples of Meloxicam (300µg/ml) were spiked with 50, 100, and 150% extra Meloxicam standard and the mixtures were analyzed. The experiment was performed in triplicate. Recovery (%) was calculated for each concentration (Table VI).

Table VI Accuracy (n=3)

S. No	*Replicates*	*Proposed conc. be spiked (% target conc.)*	*Amount add (ppm)*	*Amount recovered (ppm)*	*Recovery (%)*
1	1	50	156.90	158.11	100.77
	2	50	157.40	158.40	100.64
	3	50	159.90	162.60	101.69
2	1	100	300.20	296.34	98.71
	2	100	299.10	295.92	98.94
	3	100	300.50	296.19	98.57
3	1	150	450.70	449.29	99.69
	2	150	450.10	447.69	99.46
	3	150	451.21	451.11	99.98
Percentage recovery: 98.57%-101.69%					

Precision

The precision study was carried out in accordance with ICH recommendations by estimating the response of the Meloxicam at concentrations 150µg/ml in triplicate. The results are reported in terms of RSD (Table V).

Table V Precision

S. No	*Replicates*	*Assay (%w/w)*
1	1	99.56
2	2	99.95
3	3	100.21
4	4	99.93
5	5	100.18
6	6	100.20
	Average	***100.01***
	SD	***0.25***
	%RSD	***0.25***

Stress degradation study

In acidic condition:

From the standard stock solution 3mL was pipette out and transferred in to 10mL volumetric flask and was allowed to hydrolyze with 10mL 1N HCl.Then the volumetric flask was kept at 60˚C for one hour on thermostatic water bath. After which the volume was made up to the mark with diluents.

In alkali condition:

From the standard stock solution 3mL was pipette out and transferred in to 10mL volumetric flask and was allowed to hydrolyze with 10mL 0.1 N NaOH. Then the volumetric flask was kept at 60˚C for one hour on thermostatic water bath. After which the volume was made up to the mark with diluents.

In water condition:

From the standard stock solution 3mL was pipette out and transferred in to 10mL volumetric flask

and was allowed to hydrolyze with 10mL water. Then the volumetric flask was kept at 60˚C for one hour on thermostatic water bath. After which the volume was made up to the mark with diluents.

In hydrogen peroxide condition:

From the standard stock solution 3mL was pipette out and transferred in to 10mL volumetric flask and was allowed to oxidize with 10mL of 30% hydrogen peroxide. Then the volumetric flask was kept at 60˚C for one hour on thermostatic water bath. After which the volume was made up to the mark with diluents.

For all Blank preparation was also treated in same way except Meloxicam (Table VI).

Table VI Stress degradation Study

S. No	*Drug*	*Status*	*Purity Angle*	*Purity Threshold*	*Peak Purity*
1	Meloxicam	Real time sample	0.027	0.693	Passes
2	Meloxicam	Heated with 10ml of 0.1N HCl	0.026	0.979	Passes
3	Meloxicam	Heated with 10ml of 0.1N NaO	0.028	0.466	Passes
4	Meloxicam	Treated with 10 ml of H_2O_2	0.044	30.054	Passes
5	Meloxicam	Heated with 10ml of water 70°C	0.027	2.047	Passes

Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of Meloxicam. Robustness was determined by changing the mobile phase, flow rate, concentration mobile phase and pH (Table VII).

Table VII Robustness

S. No	*Parameters*	*Change*	*Area %RSD*	*Resolution between Imp. and Meloxicam*
1	Flow rate	0.6	0.11	7.2
		1.0	0.43	6.2
2	pH	6.8	0.200	6.3
		7.2	0.27	6.0
3	Mobile pha composition	Mobile phase A: Mobile phase B (61:39)	0.363	6.33
		Mobile phase A: Mobile phase B (65:35)	0.46	7.2
4	Column temperature	35°C	0.19	6.8
		45°C	0.21	5.5

Stability

The stability of the sample solution (300µg/ml) was analysis by repeating samples during the course of experimentation on the same day and also after storage of the drug solution (Table VIII).

Table VIII Solution stability at 300 µg/ml

S. No.	*Time of injection*	*Area*	*Cumulative % RSD*
1	Initial	9152012	-
2	4 Hr	9113618	0.3
3	8 Hr	9145518	0.22
4	12 Hr	9151869	0.2
5	16 Hr	9147843	0.18
6	20 Hr	9065253	0.38
7	24 Hr	8862794	1.16
8	28 Hr	8836527	1.47

RESULTS AND DISCUSSION

HPLC method development and optimization

Several systematic trials were performed to optimize the chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of Meloxicam formulations. The UV absorption spectrum of Meloxicam formulation was observed maximum at wavelength 254 nm and the baseline was also observed smooth without noise. For the initial method development various buffers were prepared with different molar concentration, different pH and were used with different ratios of acetonitrile, but the elution of the Meloxicam was eluted very early and was merged with impurity-B and the peak shape of the Meloxicam was having fronting and the resolution between the impurity-B and Meloxicam was less than 1.0. Phosphate buffer with different molar concentration 0.1 to 1.0 M with different pH along with the different ratios of methanol was used. Finally the optimized mobile phase composition for the Meloxicam was 0.1 M of phosphate buffer having pH 4.0 with OPA ratio of 65:35 v/v with methanol, flow rate was 1.0mLmin^{-1} and the column oven temperature was 35 ˚C. Good peak shape, symmetry factor and resolutions between impurity-B and Meloxicam were observed.

Validations parameters

The method was validated in terms of linearity, precision and measurement of peak area as well as repeatability of sample application, accuracy and specificity. A representative calibration curve of Meloxicam was obtained by plotting the mean peak area of Meloxicam against the concentration over the range of 50-150% of target concentration of Meloxicam (300µg/ml). The test results obtained are indicative of good correlation between area and concentration of Meloxicam. The coefficient of correlation is found to be 0.9996 for Meloxicam, which lies well within the acceptance criteria. Therefore the method is linear over the entire selected range. The relative standard deviation of area is 1.04 % and retention time is 0.2% for Meloxicam peak, peak tailing factor of Meloxicam peak is 1.3 and column efficiency of Meloxicam peak is 4695.4, which lies well within the acceptance criteria. This indicates that the method is suitable for Meloxicam. The LOD and LOQ S/N ratio and relative standard deviation of area is 0.74% and 0.56%, which lays within the acceptance criteria. Purity angle and purity threshold of Meloxicam capsule was found to be 0.027 and 0.693 respectively at real time, purity angel and purity threshold of Meloxicam capsule was found to be 0.026 and 0.979; 0.028 and 0.466; 0.044 and 30.054; 0.027 and 2.047 respectively when treated with HCl, NaOH, H_2O_2 and Water. Purity angle should be less than purity threshold hence the peak of Meloxicam is pure and the analytical method is specific for Meloxicam. It was observed that the excipients present in formulation did not interfere with the peak of Meloxicam. The result obtained from accuracy studies showed that recovery is 98.57%-101.69% for Meloxicam which is well within the range 98%-102%. The method therefore may be defined accurate in the range considered. Based on test result of linearity, accuracy and precision, the range of method is established as 50%-150% of target concentration of Meloxicam. Deliberated changes in Mobile phase composition, temperature, flow rate of mobile phase and pH of buffer, system suitability parameters of these

changes lies well within the limit. So method should be robust. There is slight variation in areas of test solution of Meloxicam capsules with time. After 28 hours the cumulative %RSD value is 1.47% for Meloxicam, which is well within the acceptance criteria therefore it can be established that the product in solution form should be stable for 28 hours.

The results indicate that the proposed HPLC method was found to be simple, specific, rapid, precise and accurate for estimation of Meloxicam in its formulations.

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