Original Article

Novel RP-HPLC Method Development and Validation of Meloxicam Suppository

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ABSTRACT

Objective: A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Meloxicam drug (MLX) in pharmaceutical mixture. **Methods:** Effective chromatographic separation achieved using a phenomenex luna C_{18} (4.6 mm, 250 mm, 5 μm) column with isocratic elution by the mobile phase composed of 0.02 M Potassium dihydrogen orthophosphate, pH adjusted to 4 with orthophosphoric acid (filtered): acetonitrile (50:50) respectively. The flow rate is 1.0 ml/min on detecting wavelength 220 nm. **Results:** 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 6.0 min. respectively. All parameters were found to be within the acceptance limit. The calibration curve was linear in ranges of 3-6, 6-9, and 15-18 mg/ml for Meloxicam. The R^2 of Meloxicam was found to be 0.996 respectively. **Conclusion:** A novel simple, simple, sensitive, precise, rapid, accurate and economical and reliable RP-HPLC method was developed and validated for the Meloxicam suppository.

Key words: Meloxicam, RP HPLC, Method Development, Validation, Suppository, Novel.

INTRODUCTION

Meloxicam is chemically designated as 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide [Figure 1] The molecular weight is 351.4 gm/moles. Its empirical formula is $C_{14}H_{13}N_3O_4S_2$. Meloxicam is highly soluble in strong acids and bases. It has pKa values of 1.1 and 4.2.[1-3]

Meloxicam is an NSAID of the oxicam class that acts by inhibiting the prostaglandin synthesis and inducible COX-2, thereby exerting anti-inflammatory, analgesic and antipyretic effects. The molecule is highly plasma protein bound when circulating in the body (95-99%). It has a long plasma half-life, enabling less frequent dosage schemes.[4-7]

The detailed literature survey divulges bio analytical method for the analysis of Meloxicam individually and in various combinations in biological matrices.[8] and few RP- HPLC methods for the determination of assay of Meloxicam in bulk and in tablet and capsule dosage form .[9-10]

Method validation is an important issue in drug analysis according to conventional regulations such as FDA, EMEA and ICH. The process confirms that the analytical procedure employed for the analysis is suitable for its intended use and to show reliability of the results produced by any method. Therefore method validation is essential in drug analysis.

However, to best of our knowledge, no reported RP-HPLC method have ever been reported in literature for the development and validation of Meloxicam suppository. The aim of present study, the authors report a simple, sensitive, sensitive, precise, rapid, accurate and economical and reliable RP-HPLC method was developed and validated for the Meloxicam suppository.

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MATERIAL AND METHOD

Chemicals and reagents

Working standards of pharmaceutical grade Meloxicam were obtained as generous gifts sample from Swati Pantose, Alkan bioscience Pvt. Ltd. Mumabi. They were used without further purification. Fixed dose tablet was purchased from local market. All the chemicals were of HPLC grade, purchased from Thomas Baker, Loba chemical laboratory reagent and fine chemicals, S D Fine chem. limited, water used was double distilled and filtered through 0.45 µm filter paper.

Instrumentation

The HPLC system consisted of Shimadzu (LC-20AD) Gradient System UV detector. Spinchrom software used. The chromatographic separations were carried out on a reverse phase phenomenex Luna C_{18} column (4.6 x 250 mm i. d., particle size 5 µm).

Chromatographic Conditions

The HPLC method was optimized with a view to develop a reversed-phase HPLC method for Meloxicam in pharmaceutical dosage form. A well-defined symmetrical peak was obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials that can be summarized.

Columns used for the study was Phenomenex Luna C18 (250 mm X 4.6 mm, 5 µ) column. It produced symmetrical peaks with high resolution. The UV detector response of Meloxicam was studied and the best wavelength was found to be 220 nm showing highest sensitivity.

Development studies revealed that a mobile phase containing 0.02M Potassium dihydrogen orthophosphate (pH adjusted to 4 with Orthophosphoric acid) and Acetonitrile in the ratio of 50:50at the flow rate of 1 ml/min was suitable for simultaneous estimation of Meloxicam.

Objectives in chromatographic method development were to achieve a peak tailing factor not more than 2. Under the optimized conditions Meloxicam gave sharp peaks with minimum tailing and good resolution.

Preparation of standard stock and sample solution

Preparation of standard stock solution

15 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 [Figure 2].

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

Experiments previously suggest use of C_{18} stationary phase of (250mm, 4.5 mm i. d., and particle size 5µm) hence for the study a reverse phase C_{18} column made by core shell technology was utilized. Parameter such as mobile phase composition of buffer was exhaustively studied so as to achieve a reasonable degree of separation of analyte. Several binary or ternary eluants were tested using different proportions of solvent, such as acetonitrile, methanol, water and buffer at different pH conditions. Initially isocratic mode of separation was experimented and was found insufficient to resolve the mixture with good peak characters but after many trial methods developed in isocratic system. Method selected so as to achieve separation of analytes with good peak characters. The mean retention time of analyte was 6 min. Peak identification was done by injecting individual analyte in developed chromatographic conditions.

Method Validation

Accuracy

Standard drug solution was added to the pre-analyzed suppositories sample solution at the three different concentration levels (80%, 100%, and 120%) within the range of linearity of the drug. Results are shown in Table 1.

Acceptance criteria: Accuracy should be between 98% - 102% and % RSD should not be more than 2.0.

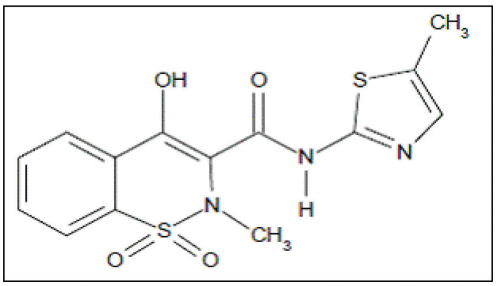

Figure 1: Structure of Meloxicam

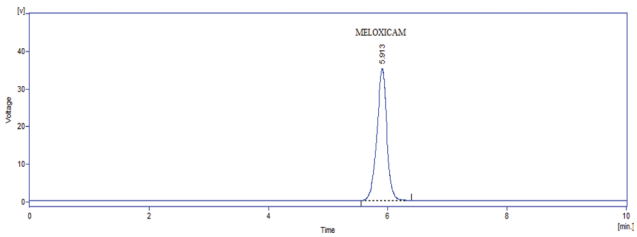

Figure 2: Chromatogram of Meloxicam (150µg/ml) in 0.02M potassium dihydrogen orthophosphate (pH adjusted to 4 with orthophosphoric acid) and acetonitrile (50:50) at 220 nm

Table 1: Data for accuracy of Meloxicam

Level no/ spike level in %	% Recovery	Mean	SD	% RSD
Level – 1 (80%)	101.32	100.29	1.106	1.10
	99.12			
	100.42			
Level – 2 (100%)	99.64	99.93	0.376	0.38
	100.36			
	99.81			
Level – 3 (120%)	99.55	99.16	0.418	0.42
	99.23			
	98.72			
Overall mean % recovery		99.79		
Overall SD		0.633		
Overall % RSD		0.633		

*mean and % relative standard deviation of six replicates

Table 2: Data for system precision of Meloxicam

Sample No.	Peak area of Meloxicam
1	202.077
2	202.591
3	199.781
4	203.157
5	201.230
6	203.949
Mean	202.130
SD	1.476
% RSD	0.73%

Table 3: Linearity data for Meloxicam

Concentration of Meloxicam in µg/ml	Peak areas
03	49.051
06	77.331
09	120.824
12	161.495
15	202.332
18	231.637
Slope	189.7
Intercept	7.598
R^2	0.996

Precision

System precision: A six replicates injection of the standard solution was injected into the HPLC system. The mean, SD and % RSD for peak areas of Meloxicam were calculated. Results are shown in Table 2.

Acceptance criteria: % RSD should not be more than 2.0.

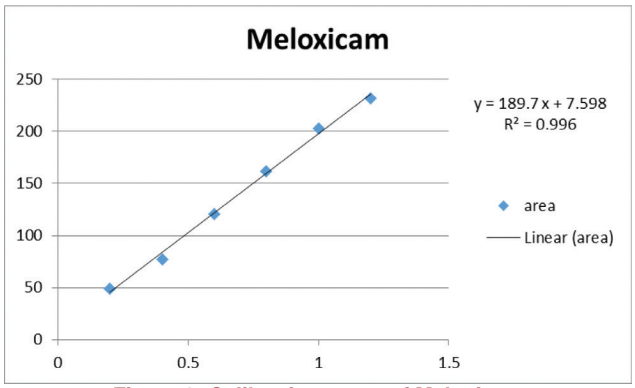

Figure 3: Calibration curve of Meloxicam

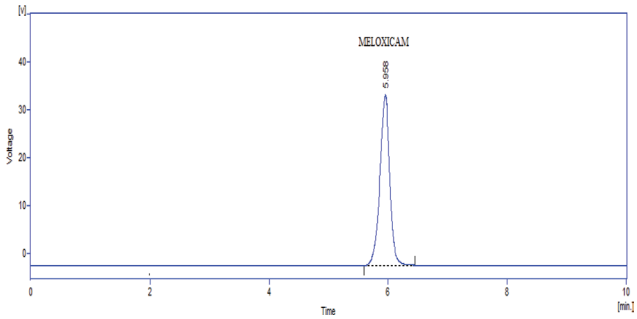

Figure 4.1: Chromatogram of standard solution in plus flow

Specificity

Specificity of the method was evaluated by injecting the blank (mobile phase), standard and sample solution prepared as per the proposed method and injected into the HPLC system to check interference if any at the retention time of Meloxicam.

Acceptance criteria: No peaks shall be eluted at the retention time Meloxicam in blank.

Linearity

Linearity of Meloxicam was performed using a standard solution in the range of 3 – 18 µg/ml respectively. Results are shown in Table 3 and Figure 3.

Acceptance criteria: Correlation coefficient (R^2) should be not less than 0.99.

Range: Range is inferred from the data of linearity, recovery and precision experiments.

Acceptance criteria: The range of the method based on the results from the linearity, accuracy and precision studies.

Robustness

Robustness of the method was evaluated by changing the flow rate by ± 10% and by changing the organic content by ± 2 % absolute. Results are shown in Table 4 and Figure 4.1-4.4

Acceptance criteria: % RSD should be not more than 2.0.[11-18]

Table 4: Robustness data for Meloxicam							
Sr. No.	Parameters	% Assay			Over all mean	Over all SD	Over all %RSD
		1	2	3			
I	Plus flow (1.1ml/min)	98.59	98.81	99.10	98.83	0.255	0.26
II	Minus flow (0.9 ml/min)	100.79	101.067	101.05	101.29	0.517	0.05
III	Plus Organic (+2%)	99.45	99.42	99.54	99.47	0.0624	0.06
IV	Minus Organic (-2%)	101.34	101.61	101.056	101.33	0.277	0.027

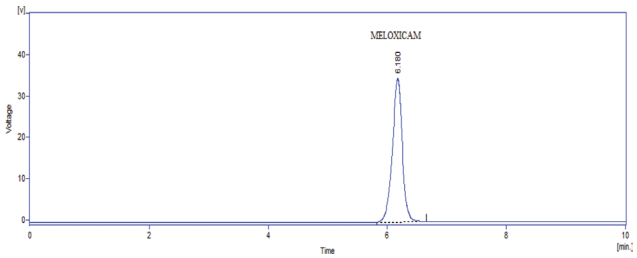

Figure 4.2: Chromatogram of standard solution in minus flow

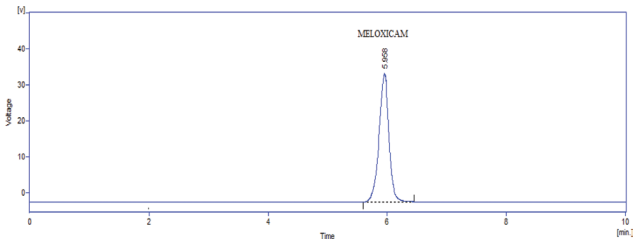

Figure 4.3: Chromatogram of standard solution in plus organic

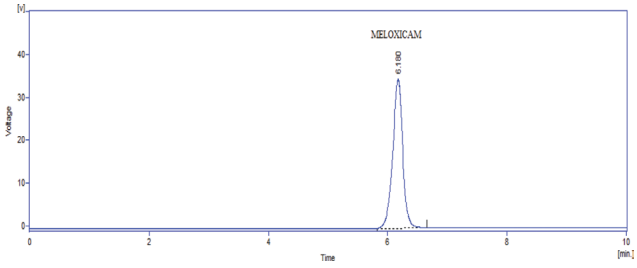

Figure 4.4: Chromatogram of standard solution in minus organic

CONCLUSION

Attempts were made to develop RP-HPLC method for simultaneous estimation of Meloxicam for the RP - HPLC method. Effective chromatographic separation achieved using a primesil C18 (4.6 mm, 250 mm, 5 µm) column with isocratic elution by the mobile phase composed of 0.02 M potassium dihydrogen orthophosphate (pH adjusted to 4 with orthophosphoric acid: acetonitrile (50:50) respectively. The flow rate is 1.0 ml/min on detecting wavelength 220nm. The proposed HPLC method was statistically validated with respect to linearity, ranges, precision, accuracy, selectivity, LOD, LOQ and robustness. The retention times (RT) of Meloxicam were found to be 6.0 min. respectively. All parameters were found to be within the acceptance limit. The calibration curve was linear in ranges of 3-6, 6-9, and 15-18 mg/ml for Meloxicam. The R^2 of Meloxicam was found to be 0.996 respectively.

Linearity study of Meloxicam was done successfully. The calibration curve yielded correlation coefficient (r^2) 0.996. Precision both intraday, inter-day study done. The results of the Precision study with in the acceptance limit. In system suitability study Retention time, Area, Theoretical plate number, Tailing factor etc parameters study done successfully.

In robustness study change in selected parameter like Flow rate, Mobile phase composition, Wave length. The results of robustness study shows less variability in retention time and tailing factor.

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CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

ABBREVIATION USED

HPLC: High performance liquid chromatography; **UV:** ultraviolet; **ICH:** International Conference on Harmonization; **LOQ:** Limit of quantitation; **LOD:** Limit of detection; **RSD:** Relative standard deviation; **MLX :** Meloxicam; **RT:** Retention time; **NSAID:** Nonsteroidal anti-inflammatory drugs; **COX-2:** Cyclooxygenase-2;

FDA: Food and Drug Administration; **SD:** Standard deviation.

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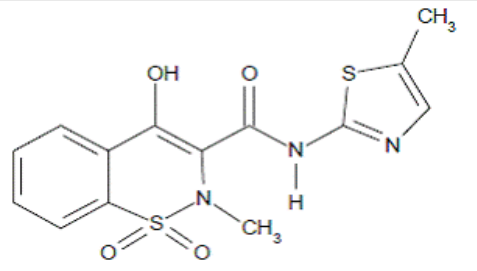
PICTORIAL ABSTRACT



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