Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (6):239-243
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Validated RP-HPLC with UV detection method for the estimation of meloxicam in bulk and tablet formulations

Anil Kumar Veeragoni[1]*, Vasudeva Murthy Sindgi[2] and Shoba Rani Satla[3]

[1]*Pathfinder Institute of Pharmacy Education and Research, Mamnoor, Warangal, Telangana,* [2]*Jayamukhi College of Pharmacy, Narsampet, Warangal, Telangana*
[3]*Center for Pharmaceutical Sciences, Institute of Science and Technology, JNTUH, Hyderabad, Telangana*

ABSTRACT

New, simple, sensitive, inexpensive and reproducible RP-HPLC method was developed and validated for the estimation of meloxicam in bulk and tablet formulations. Meloxicam was estimated at 361 nm using methanol - water (65:35, v/v) as solvent system. This HPLC analytical method was validated and analyzed for various parameters as per ICH guidelines.

Key words: Meloxicam, analytical method validation, RP HPLC method, Reverse phase, ICH

INTRODUCTION

Meloxicam (MEL) (Fig.1) (4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide) is a NSAID used for treating various conditions like pain, fever and inflammation[1]-[2]. Literature review did not reveal any simple and inexpensive methods for determination of MEL in bulk and pharmaceutical formulations and high performance liquid chromatography (HPLC) method and its estimation in biological samples [3] and stability indicating method [4], LC determination [5] were reported. A simple, inexpensive and precise RP-HPLC method can be essential for the analysis of bulk and tablet dosage forms.

The objective of the the study is to develop inexpesive, simple analytical method for estimation of MEL in bulk and tablet dosage forms. The developed method was validated as per ICH guidelines [6]. Statistical tests at 5% level of significance were performed on validation data [7].

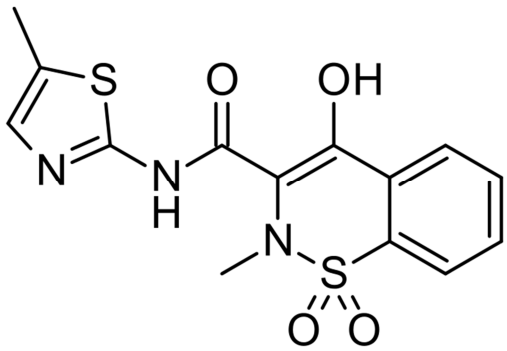

Fig. 1. Structure of meloxicam

MATERIALS AND METHODS

1. Experimental Procedures

Instruments

The HPLC consists of Binary LC-20AD pump and SPD-20A UV/VIS detector (Shimadzu, Kyoto, Japan), C-18 (5 µm, 250 mm × 4.6 mm) the reverse phase column and LC solution software. Methanol: water (65:35, v/v) as the mobile phase, flow rate was 1.0 ml/min, 361 nm parameters were used in analysis. Mobile phase was filtered by means of0.22 µm membrane filter and degassed using bath sonicator for 1 hr. A 20 µL of sample to be analyzed was injected into HPLC at 25 °C. The retention time of meloxicam was about 2.05 ± 0.3min, the linear regression equation was Area = 68.81 × Conc. (ng/mL) + 9888 ,*(r^2 = 0.9997)* and linear in the concentration range of 500-15000 ng/ml with coefficient of variance was found to be < 2%.

2.2 Materials & Reagents

Formulations containing meloxicam: Meloxicam was obtained as gift sample from Apex Healthcare Limited, Gujarat, India. Two marketed tablet formulations were selected to perform assay using developed method. Methanol was purchased from SD Fine Chemicals, Mumbai. Common formulation excipients such as starch, lactose, microcrystalline cellulose, crosscarmellose sodium, dicalcium phosphate, talc, polyvinyl pyrrolidine, aerosol, hydroxyl propyl methyl cellulose, magnesium stearate, ethyl cellulose and iron oxide were of analytical grade.

Analytical method development

Various solvents were explored to develop HPLC method for the determination of meloxicam in tablet formulations. For the selection of solvent system, the criteria considered were sensitive, simple, inexpensive, number of steps involved in sample preparation, solubility of meloxicam in diverse solvent systems, stability in various solvents and applicability of method to various analytical purposes. Area of meloxicam in the selected solvent system at respective wavelength (λ max) was determined and statistical parameters were calculated according to standard formulae (Table 2).

2.4 Calibration standards

Three stock solutions of 100 ng/mL of meloxicam were prepared by dissolving 10 mg in 100 ml of methanol-water (65:35, v/v). For the preparation of calibration standards, aliquots of stock solutions of MEL were transferred into a series of 10 ml standard volumetric flasks and final volume made with selected mobile phase.

Seven different concentrations were prepared in the range of 500 - 15000ng/mL of MEL in the methanol-water (65:35, v/v) for standard curve and estimated at 361 nm (Table 1).

2.5 Analytical method validation

2.5.1 Specificity and selectivity

MEL solutions (100ng/mL) was prepared using selected mobile phase along with and without general pharmaceutical excipients lactose, ethyl cellulose, crosscarmellose sodium, polyvinyl pyrrolidine, microcrystalline cellulose, starch, hydroxyl propyl methyl cellulose, talc, dicalcium phosphate, aerosol, magnesium stearate and iron oxide separately. The stock solution of 6000 ng/mL was scanned from 400 nm to 200 nm at a speed of 1 nm/sec and determined wavelength of maximum absorbance as 361 nm. Various concentrations of MEL in mobile phase were checked along with excipients to find any interference. The series of concentrations in selected media were analyzed (N = 9). (Table 2).

2.5.2 Accuracy

The accuracy of method was calculated by taking three dissimilar levels of drug concentrations-lower (LC), intermediate (IC) and higher concentration (HC) from stock solutions and analyzed (N = 9). Accuracy was measured as the percentage relative standard deviation and mean %recovery (Table 3). Furthermore, accuracy of the method was supported using standard addition method. In this study, various concentrations of pure drug (1000, 3000 and 6000 ng/mL) were added to a selected concentration of marketed formulation sample and the total MELconcentration was estimated using the proposed method (N = 9). The percent recovery of the added pure MEL was determined as, %Recovery = [(Cs-Cu)/Ca] × 100, where Cu, drug concentration in the formulation; Ca, drug concentration added to formulation, Cs is the total drug concentration measured after standard addition.(Table 4).

2.5.3 Precision

Repeatability was determined by taking different levels of concentrations, prepared from pure MELstandard stock solution and analyzed (N = 9) (Table 3). Intermediate precision was determined by taking the discrepancies of inter-day and intra-day response. The analytical calibration concentrations from stock in triplicates were prepared three variable times in a day and evaluated for intra-day and inter-day variation (N = 27). The coefficient of variance or

relative standard deviation (%R.S.D.) of the areas of the calibration standards were considered as precision (Table 5).

2.5.4 Linearity

Linearity of the proposed analytical method was evaluatedusing nine different series of solutions of MEL (500 – 15000ng/mL) in methanol-water (65:35, v/v) were made from the stock solution and analyses was performed. One-way ANOVA test and regression analysis was applied to the area values acquired for each level of MEL stock during the triplicate measurement of the standard solutions (Table 2).

2.5.5 Detection limit (DL) and quantitation limit (QL)

The DL and QL of MEL by intendedanalytical method was estimated using calibration standards. DL and QL was determined using 3.3σ/S and 10σ/S, respectively, where S is the slope of andσ (sigma) is the standard deviation of y-intercept inthe linear regression equation [5,6]. (Table 2).

2.5.6 Robustness

Robustness of the proposed HPLC analytical method was performed by (a) changing the concentration of methanol by ± 1% volume and (c) stability of MEL in the selected mobile phase at room temperature for 48 h. Mean percentage recovery was calculated (Table 2).

2.6 Estimation from tablet formulations

Accurately twenty tablets were weighed, powdered and amount equivalent to 10 mg of MEL was transferred to standard volumetric flask and volume was made with methanol and diluted to suitable quantity. The flask containing MEL was kept in bath sonicator for 30 min. These solutions were clarified and the filtrate was suitably diluted to prepare a 10000ng/mL concentration and the samples were analyzed using developed analytical method. The *t*-test and *F*-test was performed and the values were shown in Table 6.

RESULTS AND DISCUSSION

To select suitable mobile phase for estimation of MEL, various aqueous media like acetate buffers and phosphate buffers were explored. MEL was shown the stable HPLC chromatogram (Fig. 2). The final assessment of using methanol-water (65:35, v/v) as a mobile phase was based on criteria like: stability of drug, cost of analysis, sensitivity of the method, steps involved in the preparation of medium and suitability of the method to dissolution studies. The λ_{max} of MEL was found to be 361 nm.. Statistical parameters were shown in Table 2.

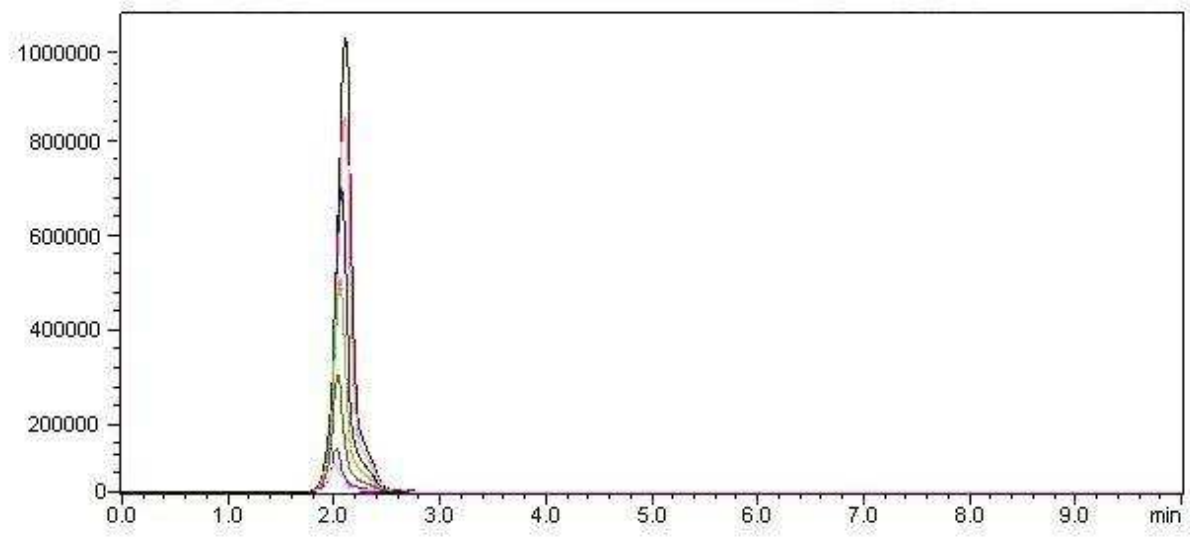

Fig.2. Comparison of chromatograms MEL in methanol-water (65:35, v/v) from 500 -15000 ng/mL

3.1 Calibration curve

The linear regression equation obtained was: area at 361 nm, Area = 68.81 × Conc. (ng/mL) + 9888; with a regression coefficient of 0.9997 (Table 2).

3.2 Analytical validation

3.2.1 Specificity and selectivity

The stable area values of MEL chromatogram was not varied in the presence of general pharmaceutical excipients in selected mobile phase. Chromatogram of pure MEL sample was equivalent with the marketed tablet formulation in the selected mobile phaseThet_{calc} assessments were found to be lower that of the t_{crit}value, indicating that there was no significant difference between areas of solutions prepared from pure MEL and marketed tablet formulation sample (Table 2). Therefore, developed analytical method is selective and specific for MEL determination.

3.2.2 Accuracy

Accuracy was observed from -0.27 to 0.32 in the selected mobile phase (Table 3). The exceptional mean %recovery (near to 100%) and low standard deviation (less than<2.5)corresponds to accuracy. The reliance on the developed HPLC method was evaluated by recovery studies using standard addition procedure (Table 4). The mean %recoveries (S.D) for LC, IC and HC were found to be 100.11 ± 1.32,100.07 ± 0.94 and 100.16 ± 0.61 respectively. These results have showed that any small variation in the MEL concentration was exactlyevaluated by the developed analytical method.

3.2.3 Linearity

The linearity range was found to be 500 -15000ng/mL at 361 nm in the selected mobile phase. Statistical parameters with lower values of standard deviationand coefficient of variance showed high precision of the developed HPLC analytical method. Also, the mean slope and intercept are within the 95% confidence interval. Best fit of the linear equation was maintained by lower calculated F_{calc}-value and high regression coefficient value.

3.2.4 DL and *QL*

DL and QL were found to be 368.81 ng/mLand 480 ng/mL in selected medium, respectively.

3.2.5 Robustness

Variation of contentsof the mobile phase by 1 % did not influence on area. The mean %recovery was found to be 100.14 ± 1.22 in the chosenmobile phase (Table 2). MEL in methanol-water compositionrevealed no areal change for 48 h when challenged at ambient temperature.

Table 1 Calibration data of the MEL in selected solvent system (*N* = 9) [†]

Drug concentration (ng/mL)	Mean Area at 361 nm (± S.D.[a])	% R.S.D.[b]
500	47933.56 ± 1054.56	2.20
1000	76711.78 ± 1540.78	2.00
3000	215405. 9 ± 3851.61	1.78
6000	417272.8 ± 1360.47	1.15
9000	640415.7 ± 9405.93	0.32
12000	825479.4 ± 13307.04	1.61
15000	1046072 ± 8475.52	0.81

[†] *Two standard stock solutions.; a Standard deviation.; b Relative standard deviation or Coefficient of variance.*

Table 2 Statistical data of MEL in selected mobile phase (*N* = 9)

Parameter	Value
Regression analysis	
Slope	68.81
Regression coefficient (r^2)	0.9997
Calculated *F*-value (critical *F*-value) [a]	0.015 (2.1152)
Validation parameters	
Specificity and selectivity - t_{Cal} (t_{Crit}) [b]	1.15 (2.26)
Linearity (ng/mL)	500 - 15000
DL (ng/mL)	368.81 ng/mL
QL (ng/mL)	480 ng/mL
Robustness (mean % recovery ± S.D.)	100.14 ± 1.22

a Theoretical value of F(8, 54) based on one-way ANOVA test at P = 0.05 level of significance.
ctCal is calculated value and tCrit is theoretical value (at 9 d.f.) based on paired t-test at P = 0.05 level of significance.

3.3 Estimation of tablet formulations

The assay of MEL in various tablet dosage forms ranged from 100.33 ± 2.25to 101.33 ± 1.71 withrelative standard deviation is not more than 2.25%. Assay results of dosage forms were similar as label claim; this implied that the intervention of excipients is inconsequential in estimation of MEL by developed analytical method.

Table 3 Accuracy and precision data from single stock solution (*N* = 9)

Level	Predicted con. (ng/mL) [a]			Mean % recovery (± S.D.)	Accuracy (%) [b]
	Range	Mean (± S.D.)	% R.S.D.		
LC	987 - 1014	990.2 ± 0.14	1.24	100.11 ± 1.32	0.32
IC	2994 - 3011	3004 ± 0.23	1.30	100.07 ± 0.94	0.15
HC	5989 – 6018	6010 ± 0.11	1.18	100.16 ± 0.61	-0.27

aPredicted concentration of meloxicam.
bAccuracy as, % relative error = [(predicted concentration – nominal concentration)/nominal concentration)] × 100.

Table 4 Standard addition method (*N* = 9)

Concentration of MEL in formulations (ng/mL)	Concentration of pure drug added (ng/mL)	Total concentration of drug found (ng/mL)	%Analytical recovery (± S.D.)
1000	1000	2022	100.14 ± 1.00
1000	3000	3985	100.02 ± 1.24
1000	6000	7027	100.11 ± 0.21

Table 5 Intermediate precision study from single stock solution

Concentration (ng/mL)	(Intra-day repeatability ± S.D.) % R.S.D.[a] (*N* = 9)			Overall Inter-day repeatability % R.S.D. [a]
	Day 1	Day 2	Day 3	(*N* = 27)
1000	76761.74 ± 1240.78	76711.78 ± 1540.78	76711.78 ± 1540.52	1.56
3000	216701. 3 ± 3591.11	215405. 9 ± 3551.61	212567. 6 ± 3335.10	1.78
6000	414572.8 ± 1230.43	417272.8 ± 1360.47	418497.8 ± 1620.14	1.15

a Relative standard deviation.

Table 6 Estimation of meloxicam in marketed tablet formulations (*N* = 5)

Commercial products	Amount found	%Assay
Marketed Tablet Formulation - I (15 mg)		
Mean ± S.D. (mg)	15.2 ± 0.26	101.33 ± 1.71
Marketed Tablet Formulation - II (15 mg)		
Mean ± S.D. (mg)	15.05 ± 0.34	100.33 ± 2.25

CONCLUSION

The developed RP-HPLCanalytical method is simple, inexpensive, sensitive and decreased number of solvent preparation steps, hence can be used for the regular analysis of MEL in bulk, tablet formulations and for dissolution studies.

Acknowledgements
The authors are thankful to Apex Healthcare Limited, Gujarat, India for providing gift sample of MEL.

REFERENCES

[1] Engelhardt undefined G, Homma undefined D, Schlegel undefined K, Utzmann undefined R, Schnitzler undefined C. *Inflammation Research*. Springer; **1995** Oct 1;44(10):423–33.
[2] Luger P, Daneck K, Engel W, Trummlitz G, Wagner K. *European Journal of Pharmaceutical Sciences*. **1996**;4(3):175–87.
[3] Bae J-W, Kim M-J, Jang C-G, Lee S-Y. *Journal of Chromatography* B. **2007**;859(1):69–73.
[4] Bandarkar F, Vavia P. *Tropical Journal of Pharmaceutical Research*. **2009**;8.
[5] Dasandi B, Shivaprakash, Saroj H, Bhat KM. *Journal of Pharmaceutical and Biomedical Analysis*. **2002**; 28(5):999–1004.
[6] Validation of. Analytical Procedures: Text and Methodology. ICH Q2 R1
[7] Bolton S, Bolton S. Pharmaceutical statistics: practical and clinical applications. 5th ed. **2010**.