DEVELOPMENT OF A REVERSED PHASE - HPLC METHOD FOR DETERMINATION OF MELOXICAM IN TABLET FORMULATION AND HUMAN SERUM

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Abstract

Developing a simple, economic, sensitive and rapid isocratic RP-HPLC method for determination of meloxicam (MX) in bulk drug, tablet formulation and human serum. The retention time observed for meloxicam was just 2.35 minutes using C_{18} column (150 x 3.0 mm, 5 µm) with a mobile phase consisting of acetonitrile: 0.2% formic acid (70:30 v/v%) at a flow rate of 1 ml/min with UV detector set at 355 nm. Linearity in concentration range of 0.05 – 50 µg/ml, with coefficient of determination, R^2 = 0.9956; slope= 25464 and intercept= -8872. The limit of detection and the limit of quantification were found to be 0.011 and 0.173 µg/ml, respectively. The precision and accuracy of method were checked by calculating RSD% and relative error E%, which were found to be reasonable. A RSD% (0.06 and 0.021%) for marketed brand and human serum, respectively and E% (0.64 and -0.47 %) for marketed brand and human serum, respectively. The method was found to be applicable for the analysis of MX in bulk, tablet formulation and human serum.

Keywords: Meloxicam, Isocratic RP-HPLC, Human serum, tablet formulation

Introduction

Meloxicam, 4- hydroxy-2- methyl-N- (5- methyl- 2- thiazolyl)- 2H- 1, 2- benzothiazine-3- carboxamide- 1, 1-dioxide (Figure 1) commonly

prescribed as non-steroidal anti-inflammatory drug with analgesic and antipyretic properties. Prostaglandins are substances that contribute to inflammation of joints; it is very efficient for the treatment of rheumatoid arthritis, osteoarthritis, and other joint diseases[1-3].

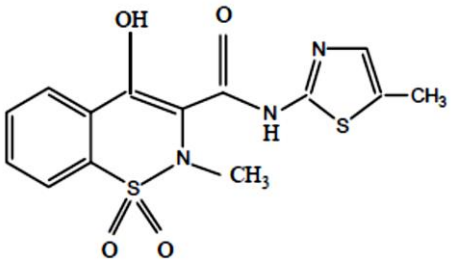


Figure 1. Structure of meloxicam

Literature survey indicated that there are several methods reported for the determination of MX in pharmaceuticals like, UV-spectrophotometry [4, 5], capillary electrophoresis [6], pulse polarography [7], turbidimetry [8], FI-chemiluminescence [9] and voltammetry [10]. HPLC is the most commonly used method for analysis of MX in pharmaceutical dosage forms as well as in biological fluids; some of them make use of buffer in the mobile phase and they are cumbersome, they required more time for the analysis and also expensive [11-14]. There are some papers in the literature reporting HPLC determination of meloxicam in plasma or serum. All the methods used apply extraction procedures[15-17]. The present study, reports development of an accurate, simple, precise, rapid and economical HPLC method with UV detection for the quantification of MX in tablet formulation and in human serum.

Experimental Section

Materials and solvents

Meloxicam (99% purity) was provided by Awamedica Company for Drug Industries and Medical Applications: Awa, Erbil, Iraq. All chemicals used were of analytical grade reagents and deionized water was used throughout. Acetonitrile (HPLC-grade) was purchased from (Hayman, England). Formic acid (analytical grade) was from (BDH, England).

Instruments

The HPLC system (Perkin Elemer series 200, USA) consisted of a (Perkin Elemer series 200 LC) pump with a high-pressure range 6000 psi; a (Perkin Elemer series 200) UV/VIS LC detector; a C_{18} column (Perkin Elemer series 200, USA) (150 mm×3.0 mm, 5 μm) were used. aAuto sampler type (Perkin Elemer series 200, USA) and a (Perkin Elemer series 200, USA) vacuum degasser were used. Data integration was done using a

(Sync Master 793MG, Samsung) software connected with (HP Laser Jet 1018, China) printer. Detection wavelength was set at 355 nm. Other equipment used in this study (Cecil CE3021, UV-VIS spectrophotometer, England) was used for spectral measurement with matched 1 cm quartz cell. A pH-meter type (HI 931401, Korea) was used for pH reading and electronic balance type (Sartorius AG Gottingen B2- 2105 Germany). A centrifuge (Jouan Quality System, France) B4i (30002303) was used for blood sample.

Standards and working standards

A stock solution, 100 µg/ml of MX was prepared by dissolving 10 mg in 100 ml methanol [18], and working standard solutions were prepared by suitable dilution of the stock solution with methanol.

Preparation of formulation sample solution

Ten tablets each containing 7.5 mg of MX purchased from (local pharmacy, Erbil, Iraq), were accurately weighed individually and finely powdered. Powdered sample containing 10.0 mg MX was weighed and dissolved in methanol. The solution was filtered and the clear solution was transferred to a 100 ml volumetric flask. This solution was diluted with mobile phase to yield concentrations in the range of working standard solution and the solutions were filtered through a 0.45 µm membrane then 10 µl of these solutions were injected into the HPLC system to analyze the MX content by the proposed procedure.

Preparation of serum sample solution

1.0 ml of human serum was added into 10 ml of acetonitrile. The mixture was vortexed for 1 min and then centrifuged for 15 min at 3500 rpm. The obtained supernatant was filtered by a 0.45-µm membrane[18]. The clear serum solution obtained was spiked with drug solutions to produce desired concentration of MX in human serum. The solution was filtered through a 0.45 µm membrane then 10 µl of this solution was injected into the HPLC system.

Chromatographic conditions

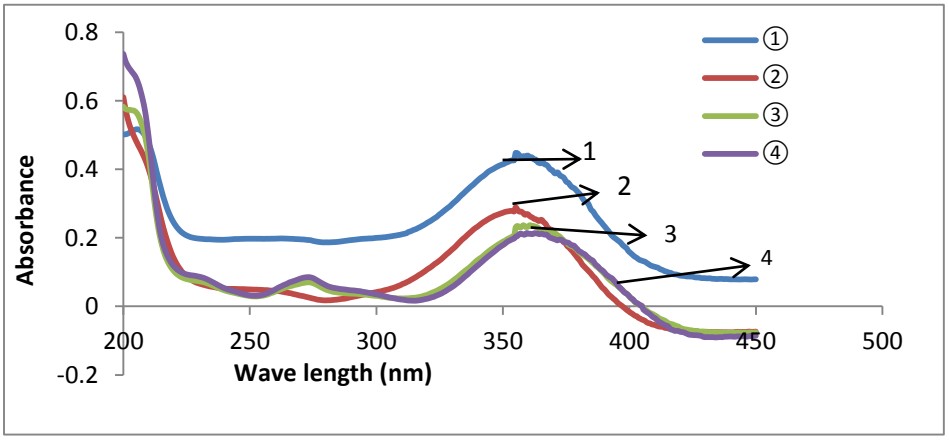
The mobile phase consisted of acetonitrile: 0.2% formic acid (70:30 v/v %) with pH of 3.5. The mobile phase was always freshly prepared and filtered through a 0.45 µm membrane filter. Chromatography was performed at ambient temperature by pumping the mobile phase at a flow rate of 1.0 ml/ min and with 10 µl of sample injection. The UV detector was monitored at a wavelength of 355 nm.

Results and discussion

HPLC method development and optimization.

Several trials were performed to optimize the chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of meloxicam in bulk drug, pharmaceutical dosage form and human serum. The UV absorption spectrum of meloxicam was observed maximum at wavelength 355 nm as shown in Figure 2.

Figure 2. Absorption spectrum of meloxicam (10 µg /ml) against mobile phase.



①Acetonitrile: 0.2%formic acid ②Acetonitrile: methanol: 0.2% formic acid ③Acetonitrile: methanol ④Acetonitrile: methanol: water.

Initial trial experiments were performed to select a suitable solvent of mobile system for determination of MX. The suitability of the mobile phase was decided on the basis of the sensitivity of the method, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents. So, various mobile phase systems tried included: acetonitrile-methanol-water (40:40:20 v/v/v %), acetonitrile-methanol (50:50 v/v %), methanol-0.2%formic acid (50:50 v/v %), acetonitrile-0.2%formic acid (50:50 v/v %), acetonitrile-methanol- 0.2% formic acid (40:40:20 v/v/v %). A mobile phase system of (acetonitrile: 0.2% formic acid) as it get better chromatogram with a clear peak and at the lowest overlap with neighboring peaks. Then selection the ratio of the optimum mobile phase compositions has been studied. It was found that the best ratio of the mobile phase was (70:30 v/v %) from acetonitrile: 0.2% formic acid has got the best clear chromatogram and less interference with neighboring peaks. The determination of meloxicam was greatly affected by changing the pH values in the range of 1.3 to 5.5. The plots of the experimental data of capacity factor (Ќ) as a function of pH of the mobile phase (Figure 3) show that the maximum value has been at pH value of 3.5 and by increasing pH value, the

capacity factor (Ḱ) has been decreased, therefore the pH value of 3.5 for the mobile phase was recommended for the subsequent experiments.

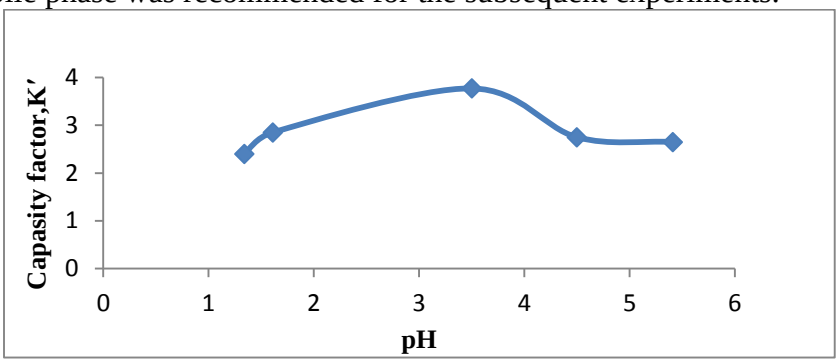


Figure 3. Effect of pH of the mobile phase.

Then effect of concentration of formic acid has been tried by different concentrations in the range (0.05-0.3 %) were tested for optimum ionization of the analyte and it was found that 0.2% formic acid gave the best result (Figure 4).

In order to investigate the effect of the flow rate on the retention times (t_R) of MX, flow rate in the rang (0.5-1.2 ml/ min) were studied. The aim of choosing the optimum flow rate was to obtain a short analysis time, which in turn prevents solute band brooding; this finally leads to increasing column efficiency [19]. Figure 5 showed that the retention time of MX decreased with increasing the flow rate. A flow rate of 1 ml/min was selected to obtain a maximum resolution in a suitable analysis time and in the allowed range of capacity factor (Ḱ). The optimum working conditions for the determination of meloxicam by HPLC isocratic elution systems were illustrated in Table 1.

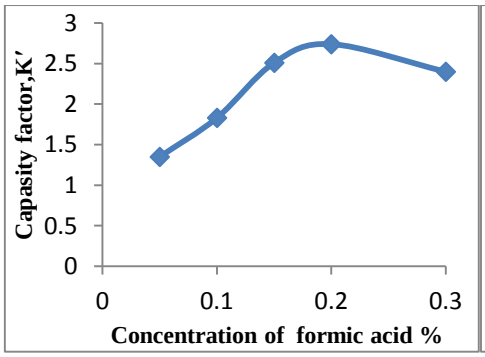


Figure 4. Effect of concentration of formic acid.

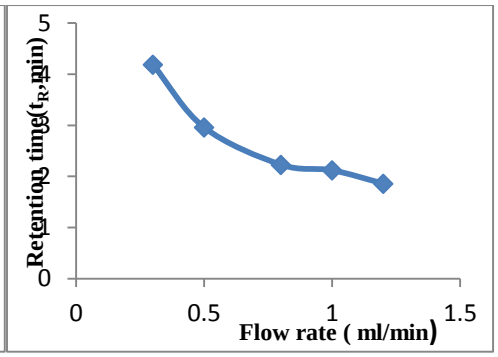


Figure 5. Effect of flow rate of the mobile phase.

Table 1. The optimum working conditions for the determination of meloxicam by HPLC isocratic elution systems.

Parameters	Value
Mobile phase	Acetonitrile: 0.2% formic acid (70:30 v/v %)
Sample injection volume	10 µl
pH	3.5
Concentration of formic acid	0.2%
Flow rate	1.0 ml/min
Column temperature	22°C
$\lambda_{maximum}$	355 nm
Retention time	2.35 min

Linearity of calibration curves

Under optimum conditions illustrated in Table 1, a calibration curve was plotted to construct a standard curve for MX between peak areas versus drug concentration. The calibration plot showed good linear relationship with coefficient of determination, R^2= 0.9956; slope= 25464 and intercept= -8872.2 over the concentration range studied. The range of linearity was 0.05-50 µg/ml. The linear regression data for the calibration plot was indicative of a good linear relationship between peak area and concentration over a wide range (Figure 6).

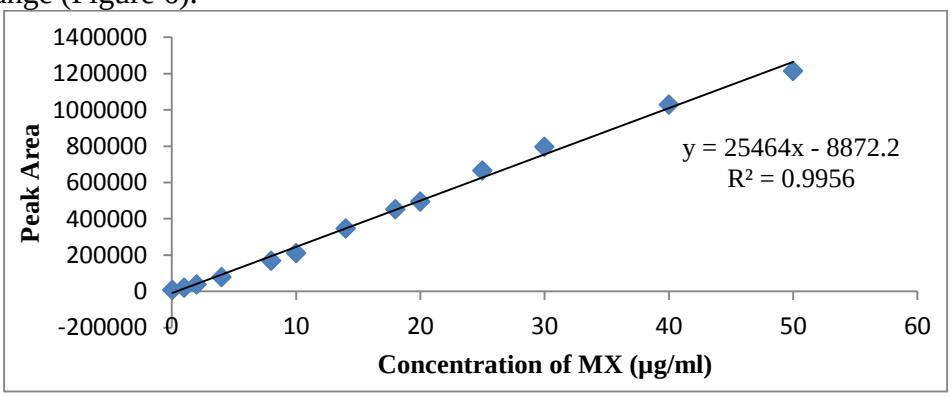


Figure 6. Calibration curve of meloxicam.

Limit of detection (LOD) and limit of quantification(LOQ)

Based on the standard deviation of the response and the slope, LOD and LOQ were estimated according to the International Union of Pure and Applied Chemistry [20] using the formulae:

$$LOD = 3.0\ \sigma/S$$

$$LOQ = 10\ \sigma/S$$

Where σ = the standard deviation of the response for the lowest concentration in the range, S = the slope of the calibration curve.

LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations. The limit of detection and limit of quantification were found to be 0.011 and 0.173 µg/ml, respectively. This

indicated the method can be used for detection and quantification of MX over a very wide range of concentrations.

Accuracy and precision

To check the accuracy and precision of the proposed method was evaluated by replicate analysis (n =5) of calibration standards at three concentration levels as low, medium and high (4, 14 and 30 µg/ml) were set as the control points of the proposed HPLC method. Precision and accuracy were based on the calculated relative standard deviation (RSD %) and relative error (E %) of the found concentration compared to the theoretical one, respectively. The results illustrated in Table 2 indicated that the method was satisfactory.

Table 2. Accuracy and precision for determination of meloxicam in serum and marketed brand.

Human serum (Sample 1)				**Marketed brand (Mobic)**		
Concentration taken (µg/ml)	Concentration found (µg/ml)	Accuracy (E %)	Precision (RSD %)	Concentration found (µg/ml)	Accuracy (E %)	Precision (RSD %)
4.0	3.96	-1.0	0.011	4.07	+1.75	0.025
14.0	13.89	-0.79	0.016	13.90	-0.71	0.071
30.0	30.12	+0.4	0.036	30.26	+0.86	0.083
Average		**-0.47**	**0.021**		**0.64**	**0.06**

*Average of five determinations.

Table 3. Optical, regression characteristics of the proposed HPLC method for assay meloxicam.

Parameters	**Value**
Linearity range (µg/ml)	0.05-50
Coefficient of determination, R^2	0.9956
Slope	25464
Intercept	-8872.2
Limit of detection, LOD(µg/ml)	0.011
Limit of quantification, LOQ(µg/ml)	0.173
Relative standard deviation, %	
Marketed brand	0.06
Human serum	0.021
Relative error, %	
Marketed brand	0.64
Human serum	-0.47

Application

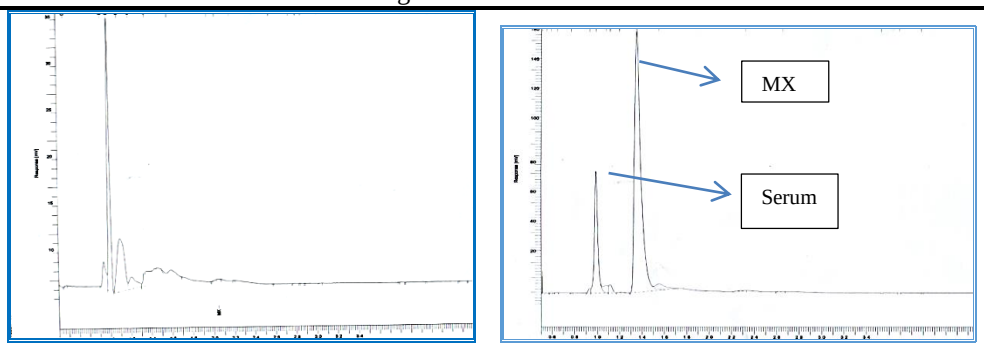
The proposed RP-HPLC system with the optimum conditions illustrated in Table 3 were used to determine meloxicam in human serum and tablet formulations by injecting 10 µl of human serum spiked with working solution of MX and solutions of formulations. The results obtained were

shown in Table 4. Also the chromatograms of serum sample and marketed brands were shown in Figure 7 and 8, respectively.

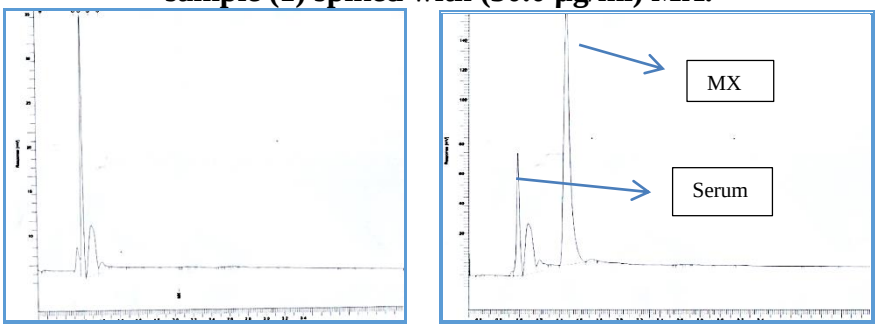
Table 4. Determination of meloxicam in marketed brands and human serum.

Samples	Drug/Brands	Sample peak area (µV.s)	Stated	Amount found	% Assay* ± SD
Marketed brands	Awamedica-Meloxicam Awa, Iraq.	391239.74	7.5(mg)	7.43(mg)	99.10± 0.047
	Boehringer Ingelheim-Mobic, Germany	388267.66	7.5(mg)	7.46(mg)	99.47± 0.012
Serum	Sample 1	453062.65	Spiked with MX (4µg)	3.96 µg	99.0±0.011
		690265.28	(30µg)	30.12 µg	100.4±0.036
	Sample 2	98033.45	Spiked with MX (4µg)	3.88 µg	97.0±0.022
		435101.65	(30µg)	29.56 µg	98.53±0.017

*Average of five determinations.

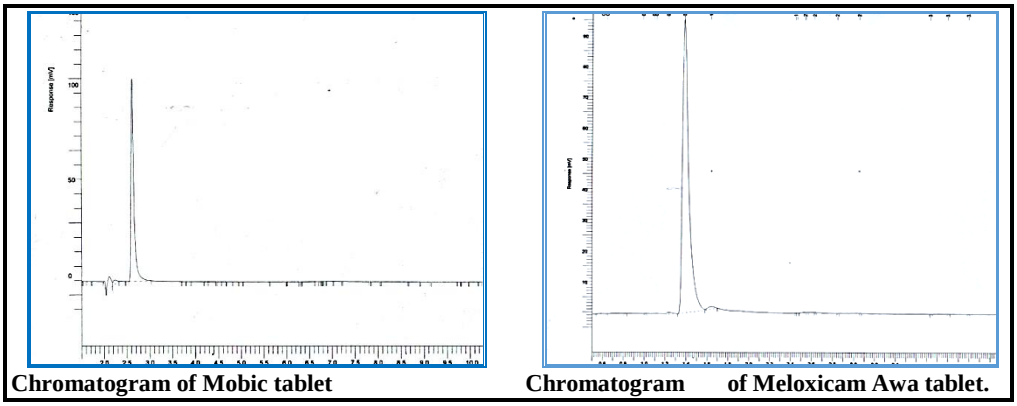


Chromatogram of blank treated serum sample (1). Blank serum sample (1) spiked with (30.0 µg/ml) MX.



Chromatogram of blank treated serum sample (2). Blank serum sample (2) spiked with (30.0 µg/ml) MX.

Figure 7. Chromatograms of serum sample.



Chromatogram of Mobic tablet **Chromatogram of Meloxicam Awa tablet.**

Figure 8. Chromatograms of marketed brands.

Comparison of the proposed method.

A comparison of the analytical performances of the previously reported methods and the proposed method for the determination of MX was summarized in Table 5. Compared with previous methods, the proposed method has relatively wide concentration range between 0.05-50 (µg/ml) and flow rate of the mobile phase as good as those methods previously reported. Also the present method didn't need controlling of temperature; it was done in the ambient temperature. The present method has a short retention time (2.35 min) compared with the two previous methods that have a long retention time (10.2 and 13.83 min), so this is meaning determined a large number of samples in a short time.

Table 5. Comparison between the proposed method and literature methods for determination of meloxicam by HPLC.

Analytical parameters	**Present method**	**Literature method[17]**	**Literature method[14]**
Column type	C_{18} (Perkin Elemer series 200) (150 x 3.0 mm,5 µm)	C_{18} (YMC Pack Pro)	C_{18} (Spherisorb ODS) (250 x 4.6 mm)
Mobile phase	Acetonitrile: 0.2% formic acid (70:30 v/v)	Methanol:phosphate buffer, pH 6.0 40:60(v/v)	Methanol: acetate buffer, pH 4.3 45:55(v/v)
$\lambda_{max,}$ nm	Uv- visible, 355	Uv-visible, 360	Diode array detector, 365
Temperature, ℃	20	50	R.T*
Flow rate (ml/min)	1.0	1.0	1.0
Retention time (min.)	2.35	10.2	13.83
Sample injection volume (µl)	10	50	20
Concentration range (µg/ml)	0.05- 50	0.05-2.5	100-/500

* R.T. is room temperature.

Conclusion

A simple, precise, accurate, having short chromatographic time, fast, and economical RP-HPLC method was developed and validated for the assay of meloxicam in serum and tablet formulations. The proposed method showed high recoveries with good linearity and precision. It can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of meloxicam formulations in quality control.

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