ATATURK UNIVERSITY PUBLICATIONS

Research Article

DOI: 10.5152/Pharmata.2023.23008

Development and Validation of HPLC-UV Method for Determination of Meloxicam in Tablet Dosage Formulation

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ABSTRACT

Objective: The development and validation of a novel, simple, and quick high-performance liquid chromatography–ultraviolet detection (HPLC-UV) technique for measuring meloxicam in pharmaceutical formulations was made.

Methods: The technique parameters were tuned to be 0.8 mL/min flow rate, variable column temperature, 290 nm wavelength, 10 µL injection volume, and a mobile phase combination of water (with 0.6% triflour acetic acid—pH:2.6) and methanol (30 : 70 v/v) to carry out this study. In this study, valsartan was used as ınternal standard (IS).

Results: Specificity, the limit of quantitation (LOQ), linearity, accuracy, precision, stability, recovery, and ruggedness were all tested. The technique was linear between 1.0 µg/mL and 50 µg/mL, with precision (relative standard d (RSD) %) and accuracy (relative error %) of less than 3.9% and 0.7%, respectively. The LOQ and LOD values of method were 1.00 and 0.25 µg/mL, respectively. Analytical recovery from pharmaceutical preparations was performed according to the standard addition method, and the average analytical recovery value was determined as 100.4%. The developed and validated HPLC-UV method was successfully applied to 4 commercial tablet dosage formulations obtained from a local pharmacy store in Turkey (Zeloxim, Melox, Meksun, Exen).

Conclusion: It has been concluded that the developed HPLC-UV method is sensitive, accurate, and precise and can be successfully applied in quality control studies in the pharmaceutical industry.

Keywords: HPLC-UV, meloxicam, tablet dosage formulation

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs), which are non-narcotic analgesics, are also referred to as simply anti-inflammatory drugs, which better suit their pharmacological profile. They are also known as non-opioid analgesics. The anti-inflammatory efficacy of this group of drugs is weaker compared to the most potent synthetic or natural anti-inflammatory steroid drugs known as glucocorticoids. Their analgesic activity is generally weaker compared to strong analgesics that do not possess anti-inflammatory effects, such as narcotic analgesics. However, they are preferably used in most painful conditions due to their non-addictive properties and their lack of causing narcotic-like effects such as sedation and clouding of consciousness.[1-3]

Meloxicam is one of the NSAIDs and is commonly used for pain, inflammation, and fever control. Meloxicam is an yellow crystalline powder. Its molecular weight is 351.39 g/mol. The melting point is 242-250°C. Meloxicam has a chemical structure of $C_{14}H_{13}N_3O_4S_2$ and a molecular weight of 351.403 g/mol. Its IUPAC name is (8E)-8-hydroxy-[(5-methyl-1,3-thiazol-2-yl)amino]methylidene]-9-methyl-10,10-dioxo-10-thia-9-azabicyclo [4.4.0]dec - 1,3,5-triene-7-one. The chemical structure is shown in Figure 1.[4]

Meloxicam is insoluble in water but soluble in dimethyl sulfide, dimethyl sulfoxide, and alcohols. It is partially and slowly absorbed from the gastrointestinal tract. It reaches its peak in plasma approximately 5-6 hours after a single dose.[5] The elimination half-life is about 20 hours. Comparative trials lasting between 23 days and 1 month have shown that gastrointestinal side effects occur at the frequency seen with placebo and at a lower rate than those who took piroxicam 20 mg per day or diclofenac 100 mg per day.[6] However, it has been reported that the analgesic effect may be slightly lower. It is given orally at a dose of 7.5 mg once a day during the meal, increasing the daily dose to 15 mg if necessary. It is mainly used for osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis.[7]

Received: 11.07.2023
Accepted: 03.07.2023
Publication Date: 31.07.2023

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Cite this article as: Çelik RS, Bayrak B, Kadıoğlu Y. Development and validation of HPLC-UV method for determination of meloxicam in tablet dosage formulation. *Pharmata* 2023;3(3):59-63.

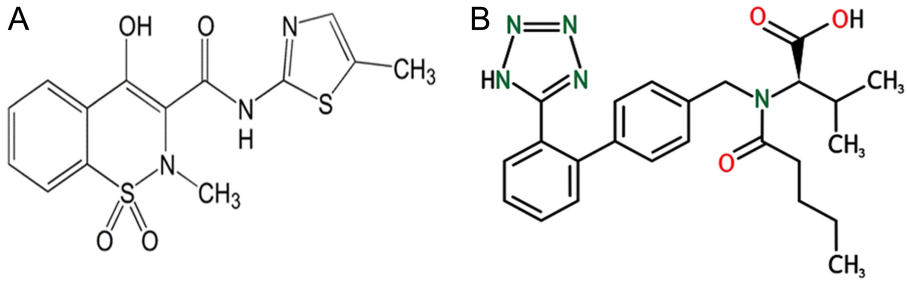

Figure 1. The chemical structures of meloxicam (A) and valsartan (B).

High-performance liquid chromatography (HPLC) is an analytical method used to separate and quantify components in a mixture. An HPLC device basically consists of a pump, column, and detector. The separation is carried out by using various mobile phases over the stationary phase used. The detector can be a variety of types, including UV/visible absorbance detectors, fluorescence detectors, or mass spectrometers. Among the advantages of the method are parameters such as wide usage areas and high sensitivity.

In the literature, several analytical methods have been reported for the quantification of meloxicam in bulk and tablets. These methods include spectrophotometric techniques,[8-11] near infrared spectrometry,[12] capillary zone electrophoresis,[13] HPLC,[14-20] and HPTLC/TLC.[21,22] Based on the methods reported above, it was aimed to develop a simple, fast, and accurate HPLCUV method for the determination of meloxicam in tablets. In this study, HPLC-UV method was developed and validated in the analysis of meloxicam in tablets without derivatization. The developed and validated method was successfully applied to 4 different commercial tablets for meloxicam analysis.

METHODS

Reagents and Chemicals

Meloxicam and valsartan [internal standard (IS)] were obtained from Novagenix Bioanalytical Pharmaceutical Research and Development Center San. ve Tic. Inc. The trifluoro acetic acid (TFA, analytical grade) and methanol (LC grade) were purchased from Merck (Germany). The deionized water that was made fresh every day, filtered (0.45 m) was used. Four commercial tablets (Meksun, Exen, Zeloxim, and Melox) containing the active ingredient meloxicam were obtained from the Turkish pharmaceutical market.

Instrumentation and Conditions for Chromatography

The HPLC System (Agilent Technologies 1200 Series) with a UV detector (Agilent Technologies), degasser (Agilent Technologies), pump (Agilent Technologies), auto-sampler (Agilent Technologies), and computer (HP).

Table 1. Method Conditions Used Proposed Study

Conditions	Meloxicam
Column	C_{18} (250 × 4.6 mm, 5 µm)
Detector	UV
Wavelength	290 nm
Mobile phase	Methanol : water with 0.6 TFA (70 : 30, v/v; pH: 2.6)
Flow rate	0.8 mL/min
Column temperature	Variable temperature
Injection volume	10 µL
Internal standard and concentration	Valsartan and 5 µg/mL

TFA, trifluoro acetic acid.

The most important aspect to focus on when developing a liquid chromatographic method is to determine if sufficient separation has been achieved. The selectivity of the chromatographic system reflects all interactions between the solutes, mobile phase components, and stationary phase. These interactions can be managed by modifying experimental conditions such as temperature, flow rate, column, and mobile phase composition. In the HPLC study, the chromatographic method conditions applied for the active ingredient meloxicam are provided in Table 1.

Preparation Stock, Standard, and Quality Control Solutions

The meloxicam stock solution was prepared by weighing the meloxicam standard on a sensitive balance and dissolving it in a 100 mL volumetric flask with methanol. All prepared solutions were kept in the refrigerator at +4°C until analysis. From this prepared stock solution, appropriate amounts were taken and diluted with methanol to prepare standard working solutions at concentrations of 1, 5, 10, 20, 30, 40, and 50 µg/mL, and quality control solutions at concentrations of 2, 25, and 45 µg/mL. The internal standard working solution at a concentration of 5 µg/mL was prepared from the valsartan standard substance.

Preparation of Tablet Solutions

Eight tablets were taken from each of the Exen tablet formulation containing 15 mg meloxicam and the Meksun, Zeloxim, and Melox tablet formulations containing 7.5 mg meloxicam, and the weights of the tablets were determined. 8 tablets taken were ground in a mortar until they turned into powder and thoroughly mixed. From this mixture, an amount equivalent to the average weight of 1 tablet was weighed according to the sampling method. It was transferred to a 100 mL volumetric flask, and methanol was added to dissolve it while being mixed on a vortex mixer. After filtration, the volume was adjusted to 100 mL with methanol. Suitable volumes were taken from this solution to prepare tablet solutions at a concentration of 5 µg/mL, and they were injected into the HPLC system for analysis.

Mobil Phase Optimization

The composition of the mobile phase plays a significant role in the retention of compounds in reversed-phase liquid chromatography. The polarity of the solvent mixture used as the mobile phase is a measure of its eluting power and is a fundamental factor that affects the retention of the analyte in reversed-phase HPLC. In this study, solvent mixtures of methanol–water (with 0.6% TFA) and acetonitrile–water (with 0.6% TFA) were tested as mobile phases, and the methanol–water solvent mixture was selected as the suitable mobile phase. Subsequently, different compositions of the mobile phase mixture [water (with 0.6% TFA)—methanol ratios: 70 : 30, 80 : 20, and 90 : 10, v/v] were tested to achieve appropriate chromatographic separation. Based on the obtained

Table 2. Statistical Analysis Values of the Calibration Curve of Proposed Method (n=6)

Features	Meloxicam
Regression equation	$y = 0.1944x - 0.0956$
Linear range (µg/mL)	1-50
Wavelength λ(nm)	290
Standard deviation of slope	50.14
Standard deviation of intercept	0.51
Correlation coefficient	0.9992

results, the mobile phase composition with water (with 0.6% TFA)—methanol ratio of 70 : 30 was determined as the optimum value and used in the study.

Method Validation

The validation of method was carried out by establishing specificity, linearity, recovery values, limits of detection (LOD), limit of quantification (LOQ), and within- and between-day precision and accuracy according to International Conference on Harmonization guidelines (ICH)[23,24] for validation of analytical procedures.

RESULTS

Specificity (Selectivity)

The method was evaluated by examining the chromatograms obtained from the standard solutions. The retention times of meloxicam and IS were determined to be 3.4 minutes and 5.9 minutes, respectively. The chromatogram depicting the increased peak area of meloxicam in relation to the concentration while keeping the internal standard constant is presented in Figure 2.

Linearity and Working Range

The linearity of the method was determined by analyzing the repeated measurements of 5 standards at each concentration within the range of 1 - 50 µg/mL. The working range was selected as the concentration range where acceptable accuracy, precision, and linearity were achieved. Calibration curves were obtained by plotting the peak area ratios (meloxicam peak area/IS peak area) against the concentration of the solution within the specified concentration range (n=6). Regression analysis of the calibration curve was performed to obtain the equation of the standard curve and the correlation coefficient.

A calibration curve was obtained by plotting the peak area ratio (meloxicam peak area/IS peak area) against the meloxicam concentration (Figure 2). The statistical analysis results of the calibration curve are presented in Table 2.

Accuracy/Precision

Three different concentrations (2, 25, and 45 µg/mL) within the calibration curves of meloxicam were prepared. The accuracy and precision values were obtained by analyzing these solutions through intraday (6 times within the same method and laboratory conditions in a single day) and interday (6 times on different days using the same method) analyses. The mean and SD of the analysis results were determined. Accuracy was expressed as relative error (RE%= (found − added)/added × 100), and precision was expressed as relative standard deviation (% RSD = SD/mean × 100) (Table 3).

Limit of Detection and Limit of Quantitaion

In the meloxicam chromatograms, the signal-to-noise (S/N) ratio was determined to be 3 for the limit of detection (LOD), and 10 for the limit of quantification (LOQ). The LOD value was determined by preparing a series of standard solutions with concentrations lower than the lowest value on the calibration curve, which is 1 µg/mL.

Analytical Recovery

Analytical recovery studies from the pharmaceutical preparation were conducted using the standard addition method. Tablet solutions were prepared as described in the "2.4. Preparation of Tablet Solutions" section. Chromatograms were obtained for the tablet solutions at a concentration of 5 µg/mL, and the peak areas were determined. Then, standard working solutions at 3 different concentrations (2, 25, and 45 mg/mL) were separately added to these tablet solutions. Chromatograms were obtained, and the peak areas were determined. The analytical recovery values were obtained by subtracting the concentration values of the added standard solutions (2, 25, and 45 mg/mL) from the total solution concentration (tablet solution + standard solution) and relating them to the concentration of the tablet solution (5 µg/mL). The average analytical recovery value was determined as 100.2% (Table 4).

DISCUSSION

Chromatography is a collection of methods widely used for the separation, identification, and determination of chemical components in mixtures, including those of unknown quantity and containing other substances. Among this group of methods, HPLC stands out as a more advantageous technique compared to others due to its accuracy, precision, repeatability, selectivity, sensitivity, recovery, ability to analyze samples in low volumes, and rapid determination of results. Thanks to these features,

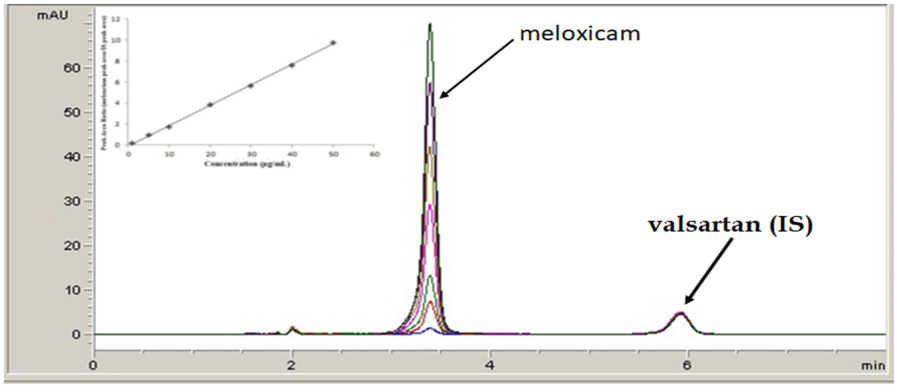

Figure 2. Calibration curve and chromatograms of meloxicam standard solutions.

Table 3. Accuracy and Precision Results of the Proposed Method

	Intra-day			Inter-day		
Added (µg/mL)	Found±SD (µg/mL)	Accuracy (RE%)	Precision (RSD%)	Found±SD (µg/mL)	Accuracy (RE%)	Precision (RSD%)
2	2.02 ± 0.07	1.0	3.5	2.05 ± 0.08	2.5	3.9
25	25.36 ± 0.62	1.4	2.4	25.17 ± 0.72	0.7	2.9
45	44.68 ± 0 .31	-0.7	0.7	44.61 ± 0.32	−0.9	0.7

RE%, relative error, RSD%, relative standard deviation; SD, standard deviation of 6 replicate determinations,

Table 4. Analytical Recovery values from tablets

Tablet	Tablet Solutions (µg/mL)	Added Standard Solutions (µg/mL)	Found±SD (µg/mL)	Analytical Recovery %	RSD %
Meksun	5	2	7.07 ± 0.10	101.4	1.41
		25	30.10 ± 0.45	102.0	1.50
		45	50.08 ± 1.01	101.6	2.02
Exen	5	2	7.04 ± 0.10	100.1	1.42
		25	30.08 ± 0.45	101.6	1.50
		45	49.94 ± 1.01	98.8	2.02
Zeloksim	5	2	6.99 ± 0.10	99.8	1.43
		25	29.96 ± 0.45	99.2	1.50
		45	50.01 ± 1.01	100.2	2.02
Melox	5	2	6.98 ± 0.10	99.6	1.43
		25	29.98 ± 0.45	99.6	1.50
		45	49.94 ± 1.01	98.8	2.02

SD, standard deviation, RSD%, relative standard deviation.

HPLC is frequently employed in the pharmaceutical industry for the quantitative analysis of pharmaceutical preparations and the analysis of drug active ingredients in biological fluids.

In HPLC studies, parameters such as temperature, column type, stationary phase, composition of the mobile phase, and the percentages of components in the mobile phase can affect the absorbance values of the analyzed substance and the analysis time. Therefore, optimization of chromatographic conditions is necessary to improve separation and obtain acceptable results. The working parameters were determined as follows: a reverse-phase C18 column (5 µm, 250 × 4.6 mm), a mobile phase consisting of 0.6% TFA—methanol (30 : 70), variable column temperature, a mobile phase flow rate of 0.8 mL/min, a wavelength of 290 nm, and an injection volume of 10 µL. When determining these parameters, existing literature data were first examined, and based on these data, certain tests were conducted to establish the most suitable ranges. As detailed in the optimization section provided in the section 'Methods,' changes in mobile phase composition, pH, and other values were made in order to achieve the highest resolution and optimal retention times for the peaks. Additionally, the aim was to propose a new method that could serve as an alternative to existing methods in the literature.

In our study, there was no need for derivatizing agents commonly used in other methods. A highly linear calibration curve was obtained without any derivatization attempts, and the recovery values indicated a satisfactory performance.

When compared to other methods in the literature, the developed method has several advantages. In comparison to the study conducted by Arayne et al[15], our method does not require the use of a buffer solution, employs a lower flow rate (0.8 mL/min in our method compared to 2 mL/min in Arayne et al[15]), and demonstrates reduced plasma interference at 290 nm compared to the commonly used wavelength of 230 nm. Mahmood et al[19] employed a 0.2 N buffer and completed the analysis in 7.5 minutes. In contrast, our developed method has a shorter analysis time and does not require the use of a buffer solution, which can negatively affect column lifetime. Joseph-Charles and Betucat[25] performed their analysis with a flow rate of 1.5 mL/min and a 0.05M Tris and 0.05 M acetate buffer, while our method is more economical and less damaging to the column and other HPLC equipment due to the buffer-free application. In the study by Viqnaduzzo et al[17], the active ingredient meloxicam was analyzed at 225 nm using a phosphate buffer component at pH 5.9. Bandarkar et al[18], in their study on pharmaceutical preparations, reported a linear range of 4-20 µg/mL, while our method stands out as a more sensitive approach with a linear range of 1-50 µg/mL. Additionally, our method has a shorter analysis time compared to this method. Overall, the developed method offers several advantages compared to other methods in terms of reduced interference, improved efficiency, and shorter analysis time.

A new HPLC method was developed as an alternative to the existing methods in the literature for the quantitative analysis of the active ingredient meloxicam in standard solutions and pharmaceutical preparations. The validity tests demonstrated that this method is sensitive, selective, accurate, precise, and reproducible for meloxicam, thus indicating its applicability for the quantitative analysis of meloxicam in pharmaceutical preparations. The data obtained from this study are believed to provide guidance for future research endeavors.

Ethics Committee Approval: Since this study is an in vitro (quantification in pharmaceutical preparations) study, ethics committee approval is not required.

Informed Consent: This study is not about the patient.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.K., R.S.C.; Design – Y.K., R.S.C.; Supervision– Y.K., R.S.C.; Resources – Y.K.; Materials – Y.K.; Data Collection and/or Processing – Y.K.; Analysis and/or Interpretation – B.B.; Literature Search – B.B.; Writing Manuscript – Y.K., B.B.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

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