



Spectrophotometric Quantification of Anti-inflammatory Drugs by Application of Chromogenic Reagents

Anti-enflamatuvar İlaçların Kromojenik Reaktifler Kullanılarak Spektrofotometrik Miktar Tayinleri

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ABSTRACT

Objectives: Simple, specific, accurate, precise, sensitive, and cost effective spectrophotometric methods were developed and validated for quantification of the drugs lornoxicam (LOR) and mesalamine (MES) in pure form and in pharmaceutical formulations.

Materials and Methods: A Shimadzu UV-1800 double-beam UV-Visible spectrophotometer having a spectral bandwidth of 0.1 nm with wavelength accuracy ± 0.1 nm with a pair of 1 cm path length matched quartz cells was used to measure the absorbance of the resulting solution.

Method I was used for the quantification of LOR, based on measurement of the absorbance of bluish green chromogen complex at 760 nm, which is formed by the reaction of LOR with ferric chloride and potassium ferricyanide (redox technique). Method II was used for the quantification of MES, based on measurement of the absorbance of yellow chromogen at 400 nm, which is formed by the condensation reaction of the primary amino group of MES with salicylaldehyde reagent (SA) (Schiff base formation).

Results: Both methods obeyed Beer's law in the concentration range of 0.5-4.5 $\mu\text{g/mL}$ and 0.2-1.7 $\mu\text{g/mL}$ with good correlation coefficients of 0.9974 and 0.998 for methods I and II, respectively.

Conclusion: The developed method is simple, sensitive, and specific, which was validated statistically as per ICH guidelines, and can be used in the routine analysis of LOR and MES in pharmaceutical dosage forms.

Key words: Ferric chloride, lornoxicam, mesalamine, salicylaldehyde reagent, visible spectrophotometry

ÖZ

Amaç: Lornoksikam (LOR) ve mesalaminin (MES) hem saf formda hemde farmasötik formülasyonlarda tayini için basit, spesifik, doğru, duyarlı ve düşük maliyetli spektrofotometrik yöntemler, geliştirmek ve valide etmektir.

Gereç ve Yöntemler: Elde edilen çözeltilerin absorbanları Shimadzu UV-1800 çift ışıklı spektrofotometrede 0,1 nm spektral bant genişliği ve $\pm 0,1$ nm hassasiyetle 1 cm'lik kuvars küvetler kullanılarak ölçüldü.

Bulgular: Metot I, LOR'nin demir III klorür ve potasyum ferrisiyanür (redoks tekniği) ile reaksiyonu sonucu oluşan mavimsi yeşil renkli kromojen kompleksinin absorbanının 760 nm'de ölçümüne dayanan yöntemdir. Metot II, MES'nin primer amino grubunun salisilaldehit reaktifi (SA) (Schiff baz oluşumu) ile kondensasyon reaksiyonundan oluşan sarı renkli kromojenin absorbanının 400 nm'de ölçümüne dayanan yöntemdir. Metot I ve II için; her iki yöntemde de, sırasıyla 0,5-4,5 $\mu\text{g/mL}$ ve 0,2-1,7 $\mu\text{g/mL}$ 'lik konsantrasyon aralıklarında, Beer yasasına sırasıyla 0,9974 ve 0,9980'lık korelasyon katsayıları ile iyi bir uygunluk göstermişlerdir.

Sonuç: Geliştirilen yöntemler; LOR ve MES'nin farmasötik dozaj formlarının rutin analizinde kullanılabilecek ve ICH yönergelerine göre valide edilmiş basit, hassas ve spesifik yöntemlerdir.

Anahtar kelimeler: Demir III klorür, lornoksikam, mesalamin, salisilaldehit reaktifi, görünür spektrofotometri

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INTRODUCTION

Lornoxicam (LOR) has the IUPAC name 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno [2, 3-e]-1,2-thiazine-3-carboximide 1,1-dioxide. It belongs to the class of oxicams and it is a nonsteroidal anti-inflammatory drug with analgesic properties (Figure 1).¹ Mesalamine (MES) has the IUPAC name 5-amino-2-hydroxy benzoic acid (Figure 2). It is an anti-inflammatory drug used to treat inflammation of the digestive tract (Crohn's disease) and mild to moderate ulcerative colitis.

A literature survey revealed that numerous analytical methods have been published for the analysis of both LOR and MES by ultraviolet (UV) spectrophotometric and high performance liquid chromatography (HPLC) methods.²⁻¹⁸ Most of the reported procedures are not simple for routine analysis and require expensive or sophisticated instruments. Hence, it is always required to develop simple, fast, and inexpensive analytical methods that can be readily adopted for routine analysis at a relatively low cost for the different requirements of analytical problems.

Visible spectrophotometry, because of its simplicity and cost effectiveness, sensitivity and selectivity, fair accuracy, precision, and easy access in most quality control laboratories, has remained competitive in the area of chromatographic techniques for pharmaceutical analysis. Visible spectrophotometric methods based on diverse reactions have been reported for the determination of LOR and MES in pharmaceutical dosage forms.^{19,20} However, most of the reported visible spectrophotometric methods suffer from disadvantages like narrow range of determination, poor sensitivity, and temperature and pH maintenance. In the present work, two simple and sensitive extraction-free spectrophotometric methods based on the redox reaction and

condensation reaction are proposed for the determination of LOR and MES in bulk drug and pharmaceutical dosage forms.

MATERIALS AND METHODS

Preparation of reagents and solutions

Ferric chloride solution (3% w/v) was prepared by dissolving 3 g in 100 mL of 0.1 N hydrochloric acid. Potassium ferricyanide (0.3% w/v) was prepared by dissolving 300 mg in 100 mL of distilled water. Salicylaldehyde reagent (5% v/v) was prepared by diluting 0.5 mL to 10 mL using ethanol.

LOR stock solution was prepared by weighing 10.0 mg of LOR and dissolving it in a few milliliters of 0.01 M NaOH and the volume was made up to 100.0 mL with 0.01 M NaOH to acquire 100 µg/mL solution. Further dilutions were made from stock solution to obtain the required concentration for method I.

MES stock solution was prepared by weighing 10.0 mg of MES and dissolving it in a few milliliters of 0.1 M NaOH and the volume was made up to 100.0 mL with 0.1 M NaOH to acquire 100 µg/mL solution. Further dilutions were made from stock solution to obtain the required concentration for method II.

Preparation of sample solutions

Redox-complexation method

Aliquots of standard drug solution of LOR ranging from 0.05 to 0.45 mL were taken into a series of 10.0-mL volumetric flasks. Then 0.5 mL of 3% w/v ferric chloride, 0.5 mL of 0.3% w/v potassium ferricyanide, and 0.5 mL of 1 N hydrochloric acid were added. The volume was made up to the mark with water to prepare a series of standard solutions containing 0.5-4.5 µg/mL. The solutions were kept aside for 30 min and later the absorbance was measured at 760 nm against the corresponding reagent blank.

Condensation method

Aliquots of standard drug solution of MES ranging from 0.02 to 0.17 mL were prepared in a series of 10.0 mL volumetric flasks. To this 1 mL of 5% v/v salicylaldehyde was added. The volume was then made up to the mark with ethanol to prepare a series of standard solutions containing 0.2-1.7 µg/mL. The complete color development was attained after 45 min. Then the absorbance of the colored chromogen was measured at 400 nm against the corresponding reagent blank.

In both methods (I and II), calibration curves were prepared and the linearity in pure solution was checked over concentration of 0.5-4.5 µg/mL for LOR and 0.2-1.7 µg/mL for MES. The relative standard deviation (RSD) and correlation coefficient of the standard curve were calculated.

Assay of pharmaceutical dosage form

Method I

The contents of 20 tablets (Lornoxi 4 and 8; Lorsaid 4 and 8) were weighed and powdered. The equivalent quantity to 4 mg of active ingredient was dissolved in 0.01 N NaOH and the volume was made up to 10.0 mL and was filtered using Whatman filter paper. Appropriate dilutions of the prepared

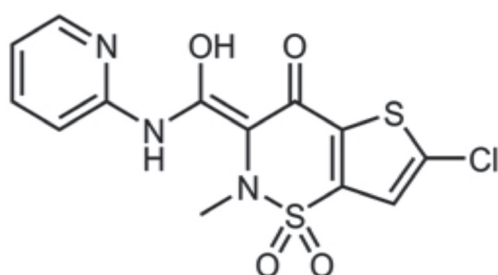


Figure 1. Structure of lornoxicam

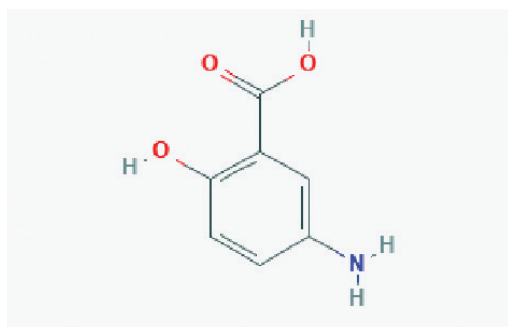


Figure 2. Structure of mesalamine

solution were made to prepare its working solution and the procedures under linearity were followed. The absorbance of the colored chromogen was measured at 760 nm against the corresponding reagent blank.

Method II

The contents of 20 tablets (Mesacol 400 mg) were weighed and powdered. The equivalent quantity to 10 mg of active ingredient was dissolved in 0.1 N NaOH and the volume was made up to 10.0 mL and was filtered using Whatman filter paper. Appropriate dilutions of the prepared solution were made to prepare its working solution and the procedures under linearity were followed. The absorbance of the colored chromogen was measured at 400 nm against the corresponding reagent blank.

RESULTS AND DISCUSSION

To attain a sensitive and specific photometric method for quantification of LOR and MES, distinct experimental conditions were investigated such as concentration of chromogenic agent, strength of the medium, concentration of oxidizing agent, temperature conditions, and time for stability of the chromogenic complex.

Redox-complexation method

LOR exhibits a reducing property due to the presence of functional moieties vulnerable to oxidation selectively with oxidizing agents such as ferric chloride. Under controlled experimental conditions when treated with a known excess amount of oxidant, LOR undergoes oxidation, giving products of oxidation (inclusive of reduced form of oxidant Fe(II) from Fe(III)) besides unreacted oxidant. It is possible to estimate the drug content colorimetrically, which is equivalent to either reduced oxidant or reduced form of oxidant formed. The reduced form of Fe(III), i.e. Fe(II), has a tendency to give a blue green complex on treatment with potassium ferricyanide. The absorbance of the bluish green complex formed was measured at 760 nm (Scheme 1).

Condensation method

MES undergoes a condensation reaction with salicylaldehyde giving a yellow Schiff base product. MES contains a primary amine group, which reacts with an active carbonyl group in salicylaldehyde forming Schiff bases [compounds containing an imine or azomethine group ($-RCH=N-$)] of stable yellow exhibiting absorption maxima at 400 nm and the reaction proceeds in ethanol (Scheme 2).

Method validation

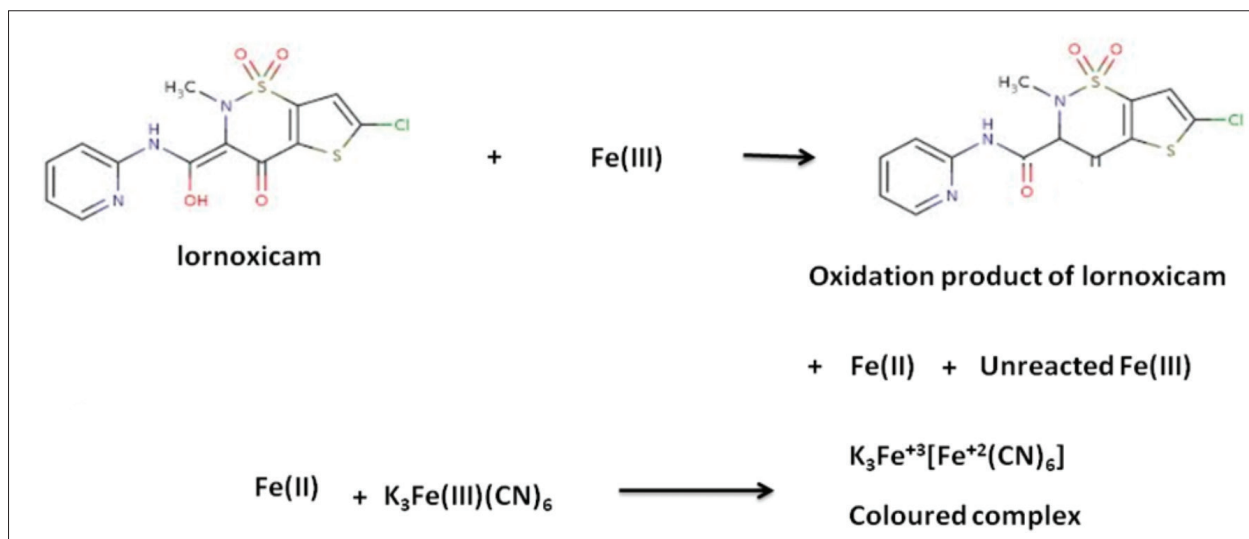
Validation of the analytical method was carried out according to International Conference on Harmonisation (ICH) recommendations [ICH, Q1A (R2), 2005].²¹

Linearity and range

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The calibration graph showed that a linear response was obtained over the range of concentrations used in the assay procedure. The linearity ranges are 0.5-4.5 $\mu\text{g/mL}$ and 0.2-1.7 $\mu\text{g/mL}$ for methods I and II respectively (Figures 3 and 4). The correlation coefficients of drugs in methods I and II were 0.9974 and 0.998, respectively. These data clearly demonstrate that the developed methods have adequate sensitivity to the concentration of the analytes in the sample. The optical characteristics of both methods such as absorption maxima, Beer's law limits, molar absorptivity, Sandell's sensitivity, and regression equation are reported in Table 1.

Precision

Precision of the method was determined by intraday and interday precision as per ICH guidelines. Intraday precision was investigated by preparing six replicate sample solutions on the same day. Interday precision was assessed by analyzing newly prepared sample solutions in triplicate over three consecutive



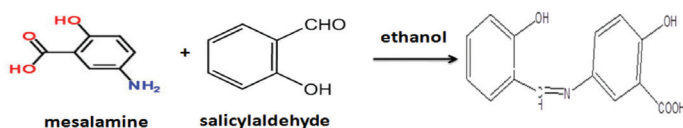
Scheme 1. Reaction mechanism of LOR with potassium ferricyanide in the presence of ferric chloride

LOR: Lornoxicam

days. The obtained RSD % was within the acceptable range. The results of this study are summarized in Table 2.

Accuracy

Accuracy of the methods was assured by applying the standard addition technique where good percentage recoveries were obtained, confirming the accuracy of the proposed methods. The average percentage recovery and RSD % were statistically calculated. The % recovery values for both the methods are shown in Table 3.



Scheme 2. Reaction mechanism of MES with salicylaldehyde

MES: Mesalamine

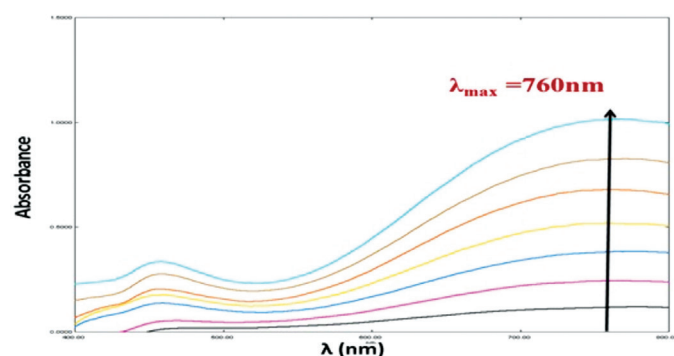


Figure 3. Absorption spectra of lornoxicam with potassium ferricyanide and ferric chloride

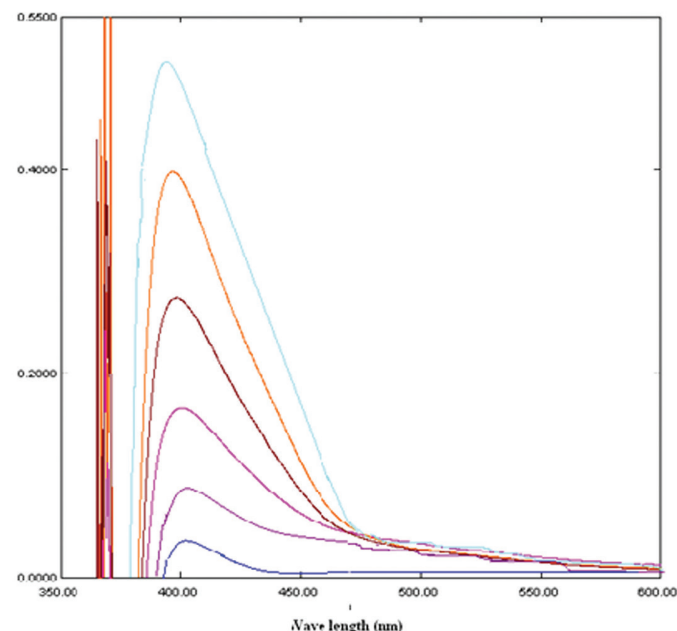


Figure 4. Absorption spectra of mesalamine with salicylaldehyde

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

The LOD and LOQ for methods I and II by the proposed method were determined using calibration standards. LOD and LOQ were calculated by using $3.3 \sigma/s$ and $10 \sigma/s$, respectively, where s is the slope of the calibration curve and σ is the standard deviation of the y intercept of the regression equation. The LOD and LOQ were 0.0094 $\mu\text{g/mL}$ and 0.0154 $\mu\text{g/mL}$ for method I and 0.0129 $\mu\text{g/mL}$ and 0.0392 $\mu\text{g/mL}$ for method II, respectively.

Application of the proposed method (analysis of commercially available formulations)

The proposed method was successfully applied to the analysis of both the drugs in their respective pharmaceutical formulations. The results obtained were in good agreement with the label claim as concluded from the satisfactory values of % assay and % RSD shown in Table 4. The assay values were compared with

Table 1. Optical parameters for methods I and II

Parameters	Method I	Method II
Absorption wavelength (nm)	760	400
Beer's law range ($\mu\text{g/mL}$)	0.5-4.5	0.2-1.7
Molar absorptivity (Lcm/mol)	1.1×10^4	1.2×10^4
Sandell's sensitivity ($\mu\text{g/cm}^2$)	0.0038	0.0048
Limit of detection ($\mu\text{g/mL}$)	0.0094	0.0129
Limit of quantification ($\mu\text{g/mL}$)	0.0154	0.0392
Correlation coefficient (r^2)	0.9974	0.9984
Slope (m)	0.2343	0.306
Intercept (c)	0.0083	0.011
Regression equation	$Y=0.2343x+0.0083$	$Y=0.306x-0.011$

Table 2. Precision for methods I and II

Concentration ($\mu\text{g/mL}$)	Intraday precision		Interday precision	
	Concentration estimated ($\mu\text{g/mL}$) AM \pm SD*	% RSD ^a	Concentration estimated (ng/mL) AM \pm SD*	% RSD ^a
Precision values for method I				
0.5	0.524 \pm 0.002	0.381	0.512 \pm 0.0012	0.234
2.5	2.428 \pm 0.004	0.164	2.524 \pm 0.0045	0.178
4.5	4.621 \pm 0.001	0.021	4.545 \pm 0.0026	0.057
Precision values for method II				
0.5	0.501 \pm 0.004	0.279	0.505 \pm 0.0012	0.237
1.1	1.14 \pm 0.0028	0.245	1.12 \pm 0.0032	0.285
1.7	1.73 \pm 0.0056	0.323	1.72 \pm 0.0026	0.151

*Mean value of 6 determinations. Relative standard deviation (%).

RSD: Relative standard deviation, SD: Standard deviation

Table 3. Accuracy studies for methods I and II

Brand name	Recovery level (%)	Amount taken (mg)	Amount of drug spiked (mg)	Theoretical amount of drug (mg)	Amount recovered mg \pm SD*	% Recovery	% RSD
Accuracy studies for method I							
Lornoxi 4	80	2	1.6	3.6	3.69 \pm 0.016	102.5	0.433
	100	2	2.0	4.0	3.82 \pm 0.024	95.0	0.628
	120	2	2.4	4.4	4.34 \pm 0.074	98.6	1.705
Accuracy studies for method II							
Mesacol -400	80	5	4	9	8.82 \pm 0.015	98	0.17
	100	5	5	10	10.03 \pm 0.022	100	0.21
	120	5	6	11	11 \pm 0.089	100	0.80

RSD: Relative standard deviation, SD: Standard deviation

Table 4. Assay of methods I and II

Formulation	Label claim (mg)	Amount found (mg) AM \pm SD*			% Assay	% RSD
		Proposed method	Reference method ^{6,15}	T-test value		
Lornoxi 4	4	3.92 \pm 0.015	4.02 \pm 0.008	0.882	99	0.377
Lornoxi 8	8	7.99 \pm 0.062	7.95 \pm 0.011	0.21	99.8	0.775
Lorsaid 4	4	3.96 \pm 0.045	3.93 \pm 0.012	0.09	99	0.303
Lorsaid 8	8	7.97 \pm 0.012	8.04 \pm 0.017	0.5	99.8	0.150
Mesacol	400 mg	399.4 \pm 0.6	399.5 \pm 0.017	0.01	99.8	0.15

*Mean of three determinations.

RSD: Relative standard deviation, SD: Standard deviation

reference method values using Student's t-test. The calculated values were less than the tabulated t-value ($t=2.571$ at $p\leq 0.05$), which revealed that there is no significant difference between the proposed method and the reference method (similarity of the methods).

CONCLUSION

The contemplated method was simple, sensitive, accurate, and precise for the quantification of LOR and MES in pharmaceutical dosage forms. The assay values were in good concord with their respective dosage form. The developed spectrophotometric methods were found to be enhanced because of their specificity, sensitivity, no extraction procedures, time saving nature, cost effectiveness, and involving very simple procedures. Besides the simplicity and sensitivity of the procedures, the relative inexpensive apparatus and cost effective reagents demonstrate their advantageous characteristics when compared to HPLC techniques. These advantages indicate that the contemplated method can be routinely used in quality control for analysis of LOR and MES in pharmaceutical dosage forms.

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