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

Kromojenik Reaktiflerin Uygulanmasıyla Antiinflamatuar İlaçların Spektrofotometrik Kantitasyonu

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ABSTRACT:

OBJECTIVE: Simple, specific, accurate, precise, sensitive and cost effective spectrophotometric methods have been developed and validated for quantification of Lornoxicam (LOR) and Mesalamine (MES) drugs in pure form and in pharmaceutical formulations.

MATERIALS AND METHODS: Shimadzu Double – beam UV – Visible Spectrophotometer 1800 having spectral bandwidth of 0.1 nm with wavelength accuracy ±0.1 nm and a pair of 1 cm path length matched quartz cells were used to measure absorbance of the resulting solution. Method (I) is used for the quantification of LOR which is based on the measurement of absorbance of bluish green coloured chromogen complex at 760 nm which is formed by reaction of LOR with ferric chloride and potassium ferricyanide (redox technique). Method (II) is used for the quantification of MES that is based on measurement of absorbance of yellow coloured 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 the methods obeyed Beer's law in concentration range of 0.5-4.5 µg/mLand 0.2-1.7 µg/mL with good correlation coefficients of 0.9974 and 0.998 for Methods (I) and (II) respectively.

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

Keywords: Ferric chloride, Lornoxicam, Mesalamine, Salicylaldehyde reagent, visible spectrophotometry.

ÖZET:
AMAÇ:
Basit, özgün, doğru, hassas, hassas ve uygun maliyetli spektrofotometrik yöntemler, Lornoxicam (LOR) ve Mesalamine (MES) ilaçlarının saf formda ve farmasötik formülasyonlarda ölçülmesi için geliştirilmiş ve onaylanmıştır.
MALZEMELER VE YÖNTEMLER
Shimadzu Çift ışınlı UV - Görünür Spektrofotometre 1800 dalga boyu hassasiyetinde 0.1 nm'lik spektral bant genişliğine ve 0.1 mm'lik bir dalga boyuna sahip bir çift kuvars hücresi kullanılarak elde edilen çözeltinin absorbansını ölçmek için kullanıldı.
SONUÇLAR:
Metot (I), LOR'un ferrik klorür ve potasyum ferrisiyanit (redoks tekniği) ile reaksiyonu sonucu oluşan 760 nm'de mavimsi yeşil renkli kromojen kompleksinin absorbansının ölçümüne dayanan LOR'un ölçümü için kullanılır. Metot (II), MES'in primer amino grubunun salisilaldehit reaktifi (SA) (Schiff baz oluşumu) ile kondensasyon reaksiyonundan oluşan 400 nm'de sarı renkli kromojenin absorbansının ölçümüne dayanan MES ölçümü için kullanılır. . Her iki yöntem de, Beeryasasini sırasıyla 0.5-4.5 µg / mL ve 0.2-1.7 µg / mL'lik konsantrasyon aralığında, sırasıyla Metotlar (I) ve (II) için 0.9974 ve 0.998'lik iyi korelasyon katsayısına uymuştur.
SONUÇ:
Geliştirilen yöntem, ICH yönergelerine göre istatistiksel olarak doğrulanan ve LOR ve MES farmasötik dozaj formlarının rutin analizinde kullanılabilen, basit, hassas, spesifiktir.
Anahtar Kelimeler Ferrik klorür, Lornoksikam, Mesalamin, Salisilaldehit reaktifi, görünür spektrofotometri.

INTRODUCTION

Lornoxicam 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 non- steroidal anti-inflammatory drug with analgesic properties (Fig. 1).[1]Mesalamine has the IUPAC name as 5 – amino – 2 – hydroxy benzoic acid (Fig.2). It is an anti-inflammatory drug used to treat inflammation of the digestive tract (crohn's disease) and mild to moderate ulcerative colitis.

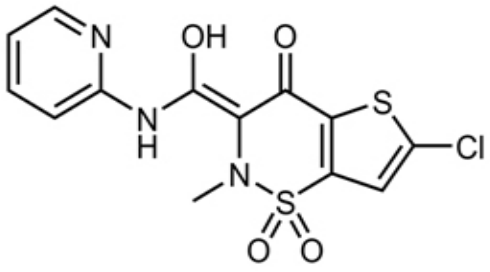


Fig.1 Structure of Lornoxicam

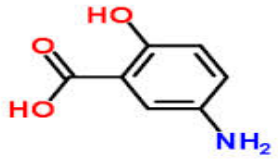


Fig.2 Structure of Mesalamine

Literature survey revealed that numerous analytical methods have been published for the analysis of both LOR and MES by UV spectrophotometric and HPLC method. [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, inexpensive analytical methods that can be readily adopted for routine analysis at a relatively low-cost to 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 one or the other disadvantage like narrow range of determination, poor sensitivity, temperature and pH maintenance etc. In this present work, two simple and sensitive extraction-free spectrophotometric methods based on redox reaction and condensation reaction are proposed for the determination of LOR and MES in bulk drug and pharmaceutical dosage forms.

EXPERIMENTAL:

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 to10 mL using ethanol.

LOR stock solution was prepared by weighing 10.0 mg of LOR and dissolved in few mL of 0.01 M NaOH and volume was made upto 100.0 mL with 0.01 M NaOH to acquire 100 µg/mLsolution. 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 dissolved in few mL of 0.1 M NaOH and volume was made upto 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

Standard drug solution's aliquots of LOR which are ranging from 0.05 – 0.45 mL were taken into a series of 10.0 mL volumetric flasks. To this 0.5 mL of 3 %w/v ferric chloride, 0.5 mL of 0.3 %w/v potassium ferricyanide and 0.5 mL of 1N hydrochloric acid were added. The volume was then made upto 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 corresponding reagent blank.

Condensation method

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

In both the methods (Method I and II), calibration curves were prepared and the linearity in pure solution was checked over concentration ranging 0.5-4.5 µg/mL for LOR and 0.2-1.7 µg/mL for MES. The RSD and correlation coefficient of standard curve was 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 upto 10.0 mL and was filtered using whatmann's 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 760 nm against the corresponding reagent blank.

Method II

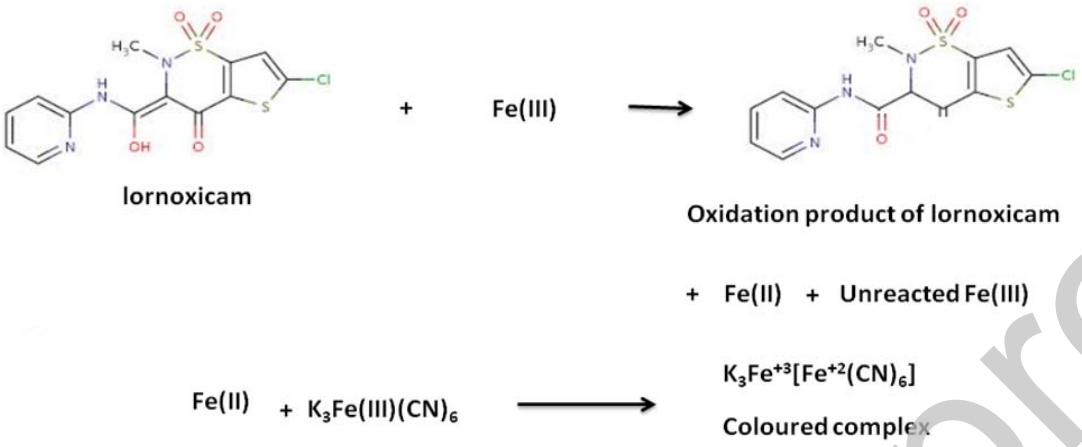
The contents of 20 tablets (Mesacol 400 mg) were weighed and powdered. The equivalent quantity to 10 mg of active ingredient was dissolved in 0.1 N NaOH and the volume was made upto 10.0 mL and was filtered using whatmann's filter paper. Appropriate dilutions of the prepared solution were made to prepare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

RESULTS AND DISCUSSION

To attain 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

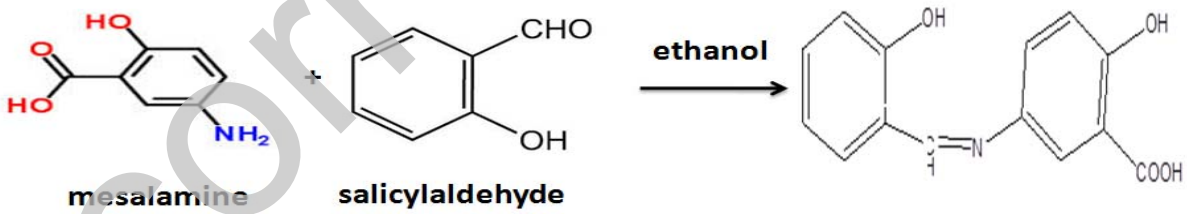
LOR exhibits reducing property due to the presence of functional moieties vulnerable to oxidation selectively with oxidizing agents such as ferric chloride. Under controlled experimental conditions when treated with known excess amount of oxidant, LOR undergoes oxidation, giving products of oxidation (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 coloured complex on treatment with potassium ferricyanide. The absorbance of bluish green coloured complex formed was measured at 760 nm (scheme-1).



Scheme-1: Reaction mechanism of LOR with potassium ferricyanide in presence of ferric chloride

Condensation method

MES undergoes condensation reaction with salicylaldehyde giving yellow coloured Schiff base product. MES contains 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 color exhibiting absorption maxima at 400 nm and the reaction proceeds in ethanol (scheme-2).



Scheme-2: Reaction mechanism of MES with Salicylaldehyde

Method validation

Validation of the analytical method has been carried out according to ICH recommendation (ICH, Q1A (R2), 2005). [21]

Linearity and range

Linearity of 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 µg/mL and 0.2-1.7 µg/mL for Method I and II respectively (Figure-3 & 4). The correlation coefficient of drugs in method I and method II were found to be 0.9974 and 0.998 respectively. These data clearly demonstrates 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 and Sandell's sensitivity and regression equation were reported in Table 1.

Table 1 Optical parameters for Method I and Method II

Parameters	**Method I**	**Method II**
Absorption Wavelength(nm)	760	400
Beers law range (µg/mL)	0.5-4.5	0.2-1.7
Molar absorptivity(Lcm/mol)	$1.1x10^4$	$1.2x10^4$
Sandell sensitivity, (µg/cm^2)	0.0038	0.0048
Limit of Detection (µg/mL)	0.0094	0.0129
Limit of Quantification (µg/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

Precision

Precision of the method was determined by intra-day and inter-day precision as per ICH guidelines. Intra-day precision was investigated by preparing six replicate sample solutions on the same day. Inter-day precision was assessed by analyzing newly prepared sample solutions in triplicate over three consecutive days. The obtained RSD% was within the acceptable range. The results of this study were summarized in Table 2.

Table-2 Precision for Method I and Method II

	Intra-day precision		Inter-day precision	
Concentration (µg/mL)	Concentration estimated(µg/mL) A.M± SD *	% RSD[a]	Concentration estimated (ng/mL)	% RSD[a]
	Precision values for			
0.5	0.524±0.002	0.381	0.512±0.0012	0.234
2.5	2.428±0.004	0.164	2.524±0.0045	0.178
4.5	4.621±0.001	0.021	4.545±0.0026	0.057
	Precision values for			
0.5	0.501 ±0.004	0.279	0.505 ±0.0012	0.237
1.1	1.14±0.0028	0.245	1.12±0.0032	0.285
1.7	1.73 ±0.0056	0.323	1.72±0.0026	0.151
*Mean value of 6 determinations				
Relative standard deviation (%)				

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 were shown in Table-3

Table-3 Accuracy studies for Method I and Method II

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

120	5	6	11	11±0.089	100	0.80

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

The LOD and LOQ for Method A and Method B by the proposed method were determined using calibration standards. LOD and LOQ are calculated by using 3.3 σ /s and 10 σ/s respectively, where s is the slope of the calibration curve and σ is the standard deviation of y- intercept of the regression equation. LOD and LOQ were found to be 0.0094 µg/mL and 0.0154 µg/mL for Method I and 0.0129 µg/mL and 0.0392 µg/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. Results obtained were in good agreement with the labeled claim as concluded from the satisfactory values of % assay and % RSD shown in Table 4. The assay values were compared with reference method values by using student t-test. The calculated values were less than the tabulated t-value (t=2.571 at p≤ 0.05), Which revealed that the there is no significant difference between proposed method and reference method (similarity of the methods) .

Table 4 Assay of method I and method II

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

*Mean of three determinations

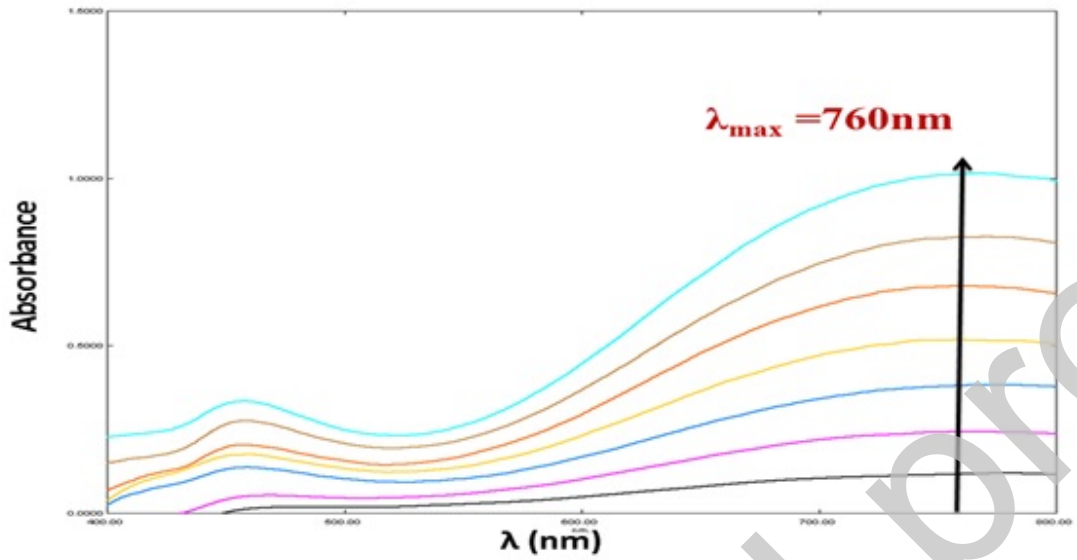


Fig.3 Absorption spectra of lornoxicam with potassium ferricyanide and ferric chloride

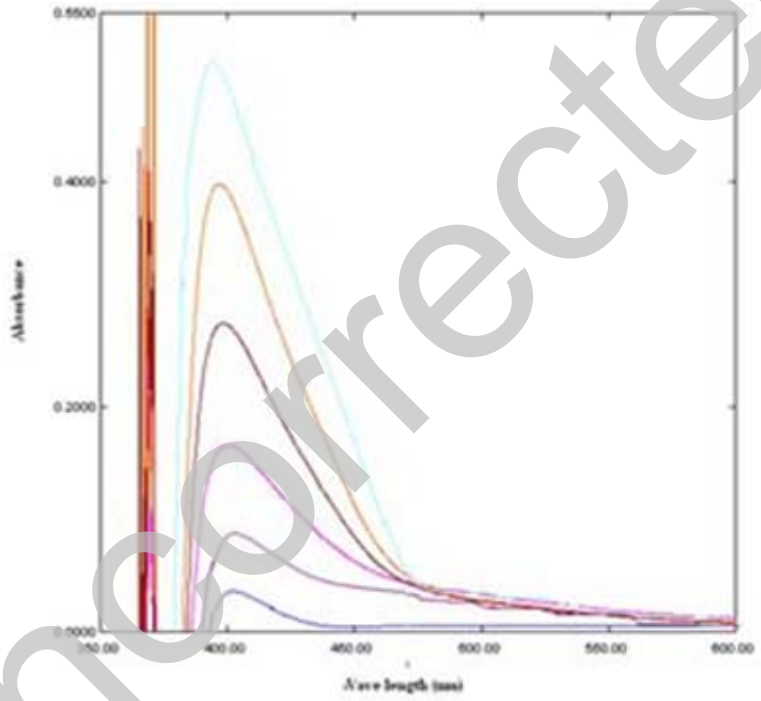


Fig.4 Absorption spectra of mesalamine with salicylaldehyde

CONCLUSION

It is concluded that the contemplated method was found to be 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 its specificity, sensitivity, no extraction procedures, time saving, cost effectiveness and involving very simple procedures. Besides the simplicity and sensitivity of the procedures, the relative inexpensive apparatus and cost effective reagents demonstrates their advantageous characteristics when compared to HPLC techniques. These advantages persuade that the contemplated method can be routinely used in quality control for analysis of LOR and MES in the pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are sincerely thankful to the management and Prof.C.V.S. Subramanyam, Principal, Gokaraju Rangaraju College of Pharmacy for supporting this work.

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