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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF ZILEUTON IN BULK AND ITS DOSAGE FORM

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ABSTRACT

Two simple and sensitive visible spectrophotometric methods were developed for the estimation of zileuton in bulk and tablet dosage form. Method A is based on the oxidation of zileuton with ferric chloride followed by complex formation with potassium ferricyanide that is a green coloured complex, which shows maximum absorption at 710 nm and obeys Beer's law in the concentration range of 0.5-3 μg/mL. In method B zileuton reacts with hetero polyacid Folin-Ciocalteu reagent (FC) in presence of alkaline condition undergoes reduction and converts to molybdenum blue having absorption maxima at 727 nm and obeys Beer's law in the concentration range of 1-4 μg/mL. The proposed methods were simple, sensitive and accurate for routine quality control of zileuton in bulk and pharmaceutical formulation.

Key words: Zileuton, Potassium ferricyanide, Folin-Ciocalteu reagent (FC).

INTRODUCTION

Zileuton[1] is chemically N-[1-benzo (b) thien-2-ylethyl]-N-hydroxyurea. The structure is shown in Fig. 1. It is official in USP[2]. It is indicated for the prophylaxis and chronic treatment of asthma in adults and children 12 years of age and older. It is an orally active inhibitor of 5-lipoxygenase, and thus inhibits leukotrienes (LTB_4, LTC_4, LTD_4, and LTE_4) formation.

According to literature, zileuton and its inactive N-dehydroxylated metabolite in plasma is determined by HPLC[3] and LC/MS-MS[4]. An UV spectrophotometric method[5] also

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reported for analysis of bulk and tablet formulation. Literature study reveals that so far there are no methods for the estimation of zileuton by visible spectrophotometry in bulk and tablet formulation.

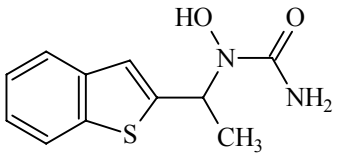

Fig. 1: Chemical structure of Zileuton

Present study describes two new simple visible spectrophotometric methods for zileuton with reagents such as ferric chloride with potassium ferricyanide in method A and Folin-Ciocalteu (FC) reagent in the Method B.

EXPERIMENTAL

Instrument

An Elico UV-Visible spectrophotometer SL210 having spectral bandwidth of 1 nm and a pair of 10 mm matched quartz cells were used for the absorbance measurements.

Reagents and materials

Zileuton pure drug was procured as gifted sample from RA Chem Pharma Ltd., Hyderabad and formulation brand name GRILUTO CR was used. All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. A 0.9% w/v aqueous solution of ferric chloride was prepared by dissolving 900 mg of ferric chloride in 100 mL of distilled water.

Aqueous solution of 0.04% w/v potassium ferricyanide was prepared by dissolving 40 mg of potassium ferricyanide in 100 mL distilled water. FC reagent (1:3) ratio was prepared by diluting 1 mL of FC with 3 mL of distilled water and aqueous sodium hydroxide 1% w/v was prepared by dissolving 1 g of sodium hydroxide in 100 mL of double distilled water.

Preparation of standard stock solution (100 µg/mL)

Accurately weighed quantity of 100 mg of zileuton was transferred to 100 mL volumetric flask and dissolved in 10 mL of methanol by shaking manually for two minutes and volume was made upto 100 mL. This solution was then diluted to get working concentration of 100 µg/mL with methanol.

Determination of λ_{max}

A 10 µg/mL solution of zileuton was prepared by addition of reagents in method A and B. The resulting solution was scanned in UV-VIS spectrophotometer from 400-800 nm to determine the λ_{max} against the reagent blank. The λ_{max} of Zileuton was found to be 710 nm and 727 nm for method A and B, respectively. The absorbance spectra were shown in Fig. 2 and 3.

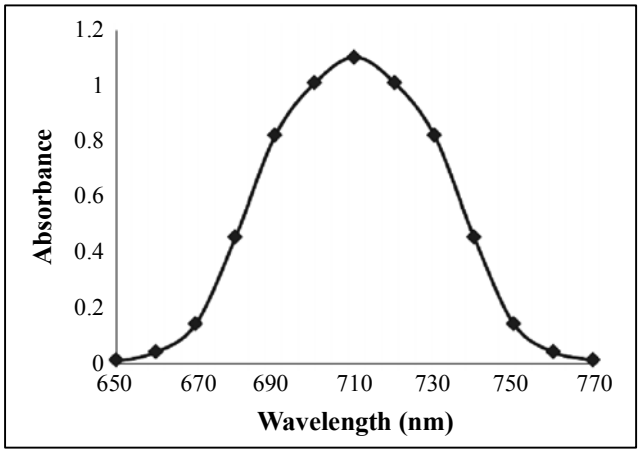

Fig. 2: Visible spectra of zileuton (Method A)

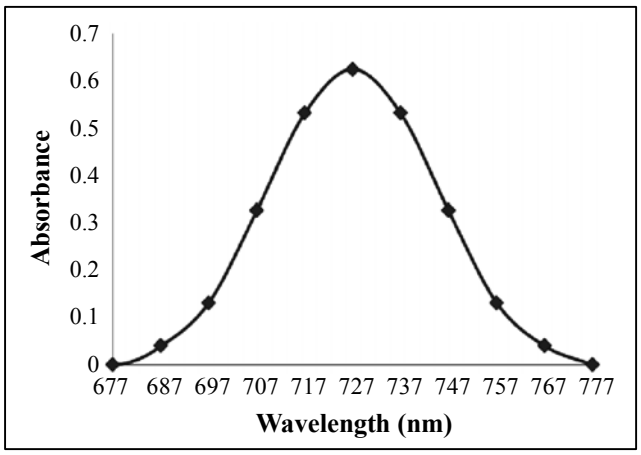

Fig. 3: Visible spectra of zileuton (Method B)

Procedure for method A

Aliquots of (0.05-0.3 mL) standard stock solution (100 µg/mL) of zileuton were

transferred into a series of 10 mL calibrated volumetric flask. To each of the aliquots, 1.0 mL (0.9% w/v) of ferric chloride was added followed by addition of 1.0 mL of (0.04% w/v) potassium ferricyanide and kept aside for 15 min with occasional shaking for the completion of reaction at room temperature. The volumes were made upto 10 mL with double distilled water and the absorbance of each solution was measured at 710 nm against the reagent blank. The calibration curve is shown in Fig. 4. The calibration table is shown in Table 1.

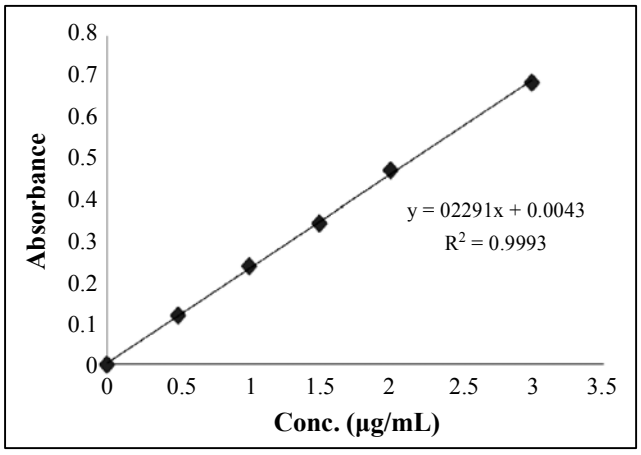

Fig. 4: Calibration curve of Zileuton (Method A)

Table 1: Linearity of Zileuton for Method A

Concentration (µg/mL)	Absorbance
0.5	0.1181
1	0.2402
1.5	0.3424
2	0.4719
3	0.6858

Procedure for Method B

Aliquots of (0.1-0.4 mL) standard stock solution (100 µg/mL) of zileuton were transferred into a series of 10 mL calibrated volumetric flask. To each of the aliquots 0.5 mL of (1:3 ratio) FC reagent was added followed by addition of 2 mL of (1% w/v) sodium hydroxide, and kept aside for 10 min at room temperature. The volumes were made upto

10 mL with double distilled water and the absorbance of each solution was measured at 727 nm against the reagent blank. The calibration curve is shown in Fig. 5. The calibration table is shown in Table 2.

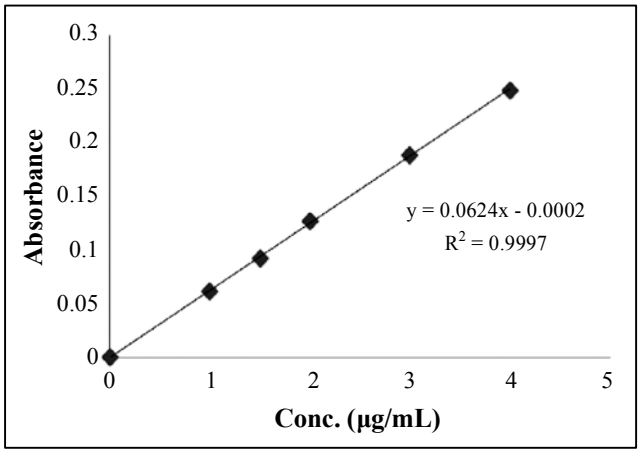

Fig. 5: Calibration curve of Zileuton (Method B)

Table 2: Linearity of Zileuton for Method B

Concentration (µg/mL)	Absorbance
1	0.0610
1.5	0.0922
2	0.1269
3	0.1881
4	0.2481

Estimation of zileuton in tablet formulation

Weighed accurately about 20 tablets and triturated to fine powder. Tablet powder equivalent to 100 mg of zileuton was weighed and dissolved in 10 mL of methanol with shaking and final volume is made upto 100 mL with methanol. This was then filtered through whatmann's filter paper No. 41 to get concentration of 1 mg/mL solution. This was then diluted to make the working concentration of 100 µg/mL with methanol and used for method A and B, respectively. The amount of drug in the tablet was determined from calibration curve.

Method validation[6]

The proposed method has been validated for linearity, sensitivity, precision, accuracy, solution stability study.

Linearity, Limit of Detection and Limit of Quantitation

The linearity of the proposed methods was assessed at concentration ranges 0.5-3 µg/mL and 1-4 µg/mL for method A and Method B, respectively. Each concentration level was independently analyzed repeatedly for five times. The absorbances obtained at each concentration were plotted against the concentration of zileuton in µg/mL. The linear regression equation was evaluated by least square treatment of the calibration data. LOD and LOQ were determined based on statistical calculation from the calibration curves, where LOD = (3.3 × σ)/m; LOQ = (10.0 × σ)/m (σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves.

Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed condition. The system precision was analysed by six different solutions of same concentration and absorbances were noted. The result was indicated by % RSD. Intra-day precision was investigated on 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. Both inter day and intraday precision was expressed as % RSD.

Accuracy

Accuracy was determined by recovery studies. The recovery studies were carried out by adding the known amount of standard zileuton drug to the sample solution of the tablets.

Solution stability study

The developed colored solutions for both methods were under gone stability study at different time intervals for test preparation. The absorbance's were measured at every ten minutes interval.

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits and molar absorptivity values, together with other analytical performance characteristics such as LOD, LOQ, regression

equation parameters, system precision are given in Table 3. To evaluate intra-day and inter-day precision of the methods, pure zileuton was analyzed at three different concentration levels, each determination being repeated six times.

Table 3: Regression parameters and optical parameters for Method A and B

S. No.	Parameter	Method A	Method B
	Regression parameters		
1	Regression Equation*	Y = 0.229X + 0.004	Y = 0.062X – 0.000
2	Slope (b)	0.229	0.062
3	Intercept (a)	0.004	0.000
4	Correlation coefficient (r)	0.999	0.999
5	% RSD**	0.565	0.547
	Optical parameters		
1	Absorption maxima (nm)	710	727
2	Linearity range (µg/mL)	0.5-3	1-4
3	Molar absorptivity (Lit.mol^{-1} cm^{-1})	5.5254 x 10^4	1.4680 x10^4
4	Sandell's sensitivity (µg/cm^2/0.001 abs unit)	0.00427	0.01609
	% range of errors		
5	0.01 level	0.472	0.676
	0.05 level	0.699	0.457
6	Limit of detection (µg/mL)	0.106	0.088
7	Limit of quantification (µg/mL)	0.322	0.267

*Y = bX+a, where X is the concentration of Zileuton in µg/mL and Y is the absorbance at respective λ_{max}

**For six replicate samples

The intra-day precision of zileuton by two methods was between 0.96-1.1% for method A and 0.86-0.96% for method B. The inter-day precision of zileuton by two methods was between 0.88-1.04% for method A and 0.86-1.1% for method B. The % RSD for both intra-day and inter-day were < 1.9% indicating good precision of the methods. The results are shown in Tables 4 and 5. The accuracy of the methods were evaluated by recovery studies by adding pure zileuton to the pre-analyzed formulation. The results are summarized in Tables 6 and 7, respectively.

Table 4: Interday and intraday precision for method A

Con. taken (µg/mL)	Intraday		Interday	
	Con. found* (µg/mL)	%RSD	Con. found* (µg/mL)	%RSD
1.0	1.02	0.96	0.98	0 .98
1.5	1.46	0.86	1.47	0.88
2.0	2.04	1.1	1.98	1.04

*Average of six determinations

Table 5: Interday and Intraday precision for method B

Con. taken (µg/mL)	Intra-day		Inter-day	
	Con. found* (µg/mL)	%RSD	Con. found* (µg/mL)	%RSD
1.5	1.45	0.94	1.47	0.98
2.0	2.04	0.86	1.98	0.86
2.5	2.49	0.96	2.47	1.1

*Average of six determinations

Table 6: Accuracy results of Zileuton for Method A

% Spike level	Sample (µg/mL)	Amount added (Std.) (µg/mL)	Amount found (µg/mL)	% Recovery	Statistical parameters
80	1.0	0.8	0.79	99.85	Mean = 98.94
	1.0	0.8	0.78	98.24	SD = 0.8228
	1.0	0.8	0.79	98.75	%RSD = 0.83
100	1.0	1.0	0.99	99.2	Mean = 99
	1.0	1.0	0.98	98.1	SD = 0.818
	1.0	1.0	0.99	99.7	%RSD = 0.82
120	1.0	1.2	1.18	99.08	Mean = 99.09
	1.0	1.2	1.19	99.4	SD = 0.300
	1.0	1.2	1.18	98.8	%RSD = 0.302

Table 7: Accuracy results of Zileuton for Method B

% Spike level	Sample	Amount added (Std.)	Amount found (µg/mL)	% Recovery	Statistical parameters
80	2.0	1.6	1.56	97.5	Mean = 98.5
	2.0	1.6	1.59	99.3	SD = 0.916
	2.0	1.6	1.58	98.7	%RSD = 0.930
100	2.0	2.0	1.99	99.5	Mean = 99.5
	2.0	2.0	1.96	99.3	SD = 0.251
	2.0	2.0	1.97	99.8	%RSD = 0.252
120	2.0	2.4	2.36	98.3	Mean = 98.1
	2.0	2.4	2.34	97.5	SD = 0.633
	2.0	2.4	2.37	98.75	%RSD = 0.644

The stability of the coloured complex for method A & B are shown in Fig. 6 & 7, respectively.

Commercial formulation of Zileuton was successfully analyzed and result is shown in Table 8. There was no interference of additives or excipients in proposed analytical methods. The proposed methods were found to be simple, sensitive, accurate, precise and can be used for the routine quality control of this drug in bulk as well as in pharmaceutical formulation.

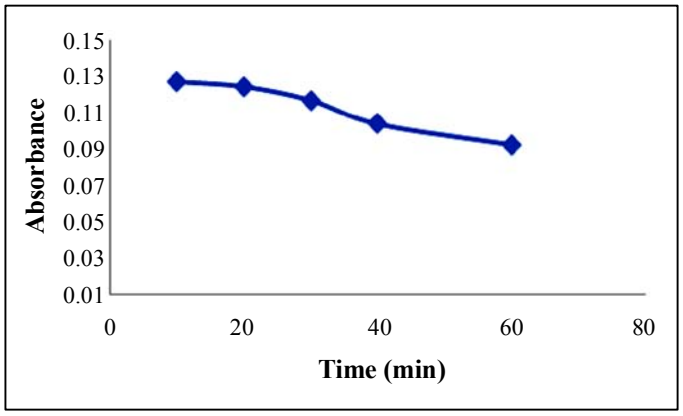

Fig. 6: Stability study of Zileuton (Method A)

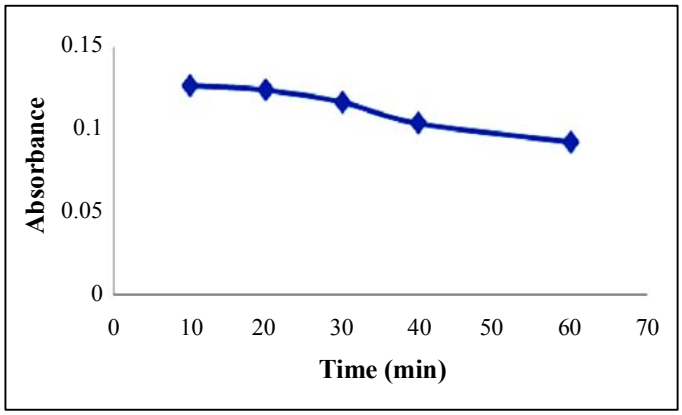

Fig. 7: Stability study of Zileuton (Method B)

Table 8: Estimation of Zileuton in pharmaceutical formulation

Formulation	Labelled amount	Amount found		% Recovery ± SD	
		Method A	Method B	Method A	Method B
Griluto CR (Tablet)	600 mg	599.98 mg	599.76 mg	99.98 ± 0.087	99.80 ± 0.075

CONCLUSION

Two visible spectrophotometric methods are developed for the determination of zileuton, which are fairly sensitive, simple and economical with reasonable precision and accuracy. Parameters and statistical comparison justify these methods for application in estimation of zileuton in pure and dosage form. Moreover, the methods are free from interference by common additives and excipients for the assay and evaluation of zileuton in pharmaceutical preparations.

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