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Research Article

VISIBLE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF ZILEUTON IN BULK DRUG AND PHARMACEUTICAL FORMULATION

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ABSTRACT

In the present work two simple and novel visible spectrophotometric methods (A&B) are described for the assay of Zileuton in bulk and tablet dosage form. Method A (Ferric Chloride) and Method B (Ferric Nitrate) are based on the oxidation of Zileuton with ferric ions to form a greenish-yellow coloured complex having absorption maxima at 397 nm for Method A and 390 nm for Method B. These two methods obey beers law in the same concentration range of 10-80 µg/ml. The proposed methods can be applied successfully for the quantitative determination of Zileuton in pure and tablet formulation without any interference from additives.

Keywords: Zileuton ,Visible Spectrophotometry, Oxidation, Ferric chloride, Ferric nitrate.

INTRODUCTION

Zileuton[1] is chemically N-[1-benzo (b) thien-2-ylethyl]-N-hydroxyurea. 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. The structure was shown in Figure 1. According to literature, zileuton and its inactive N-dehydroxylated metabolite in plasma is determined by HPLC[3] and LC/MS-MS[4]. An U.V spectrophotometric method[5] is reported for analysis in 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.

Present study describes two new simple visible spectrophotometric methods involving direct reaction of Zileuton with ferric chloride and ferric nitrate.

MATERIALS AND METHODS

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 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 2% w/v aqueous solution of ferric chloride was prepared by dissolving 2 g of ferric chloride in 100 mL double distilled water. Aqueous solution of 2% w/v ferric nitrate was prepared by dissolving 2 g of ferric nitrate in 100 mL double distilled water.

Procedure for 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 2 minutes and volume was made up to 100 mL. This solution was then diluted to get working concentration of 100 µg/mL with methanol and used for two methods A and B to construct calibration curve.

Determination of λ_{max}

From 100 µg/mL stock solution 1 mL was pipette out into a 10 mL volumetric flask followed by addition of 1 mL of (2% w/v) ferric chloride (Method A) and 1 mL of (2% w/v) ferric nitrate (Method B) and diluted to get 10 µg/mL solution. The resulting solution was scanned in UV-Visible spectrophotometer from 300-800 nm to determine the λ_{max} against the reagent blank. The λ_{max} of zileuton was found to be 397 nm and 390 nm for method A and B respectively. The spectra for method A and method B was shown in Figure 2 and Figure 3 respectively.

Procedure for Method A

Aliquots of (1-8 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 of (2% w/v) ferric chloride was added which produce greenish-yellow colour. The volumes were made up to 10 mL with double distilled water to get concentrations of 10, 20, 30, 40, 60, and 80 µg/mL and the absorbance of each solution was measured at 397 nm against the reagent blank. The calibration curve and linearity table was shown in Figure 4 and Table 1 respectively. The stability graph for coloured graph was shown in Figure 5.

Procedure for Method B

Aliquots of (1-8 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 of (2% w/v) ferric nitrate was added which produce greenish-yellow colour. The volumes were made up to 10 mL with double distilled water to get concentrations of 10, 20, 30, 40, 60, and 80 µg/mL and the absorbance of each solution was measured at 390 nm against the reagent blank. The calibration curve and linearity table was shown in Figure 6 and Table 2 respectively. The stability graph for coloured graph was shown in Figure 7.

Procedure for 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 was made up to 100 mL with methanol. This was then filtered through whatmann's filter paper No.41 to get concentration of 1mg/mL solution. This was then diluted to get the working concentration of 100 µg/mL with methanol. From this 30 µg/mL was prepared as per procedure given for method A & B. The percentage assay was calculated from the calibration curve.

METHOD VALIDATION[6]

The analytical method was validated with respect to parameters such as linearity, limit of detection(LOD), limit of quantitation(LOQ), precision, accuracy, robustness and recovery in accordance with the current ICH guidelines.

Linearity and range

Linearity was established by least squares linear regression analysis of the calibration curve.

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. It was analysed by 6 different solutions of same concentration and absorbances were noted. Both inter-day and intra-day precision were performed and results are expressed by %RSD.

Accuracy

Accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. Solutions were prepared in triplicates and accuracy was indicated by % recovery.

Limit of detection

It is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD = $3.3\sigma/s$.

Limit of quantification

It is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ = $10\sigma/s$ Where σ is the standard deviation of response and s is the slope of the calibration curve.

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 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.

The intra-day precision of Zileuton by two methods was between 0.85–0.96 % for method A and 0.54–0.62 % for method B. The inter-day precision of zileuton by two methods was between 0.92–0.98 % for method A and 0.61-0.66 % for method B. The % RSD for both intra-day and inter-day were < 1 % indicating good precision of the methods. The results were shown in Table 4 & 5.

The accuracy of the method was evaluated by recovery studies by adding pure zileuton to the pre-analyzed formulation. The results were summarized in Table 6 &7.

Commercial formulation of Zileuton was successfully analyzed and assay results for method A and method B was 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 and precise hence it can be used for the routine quality control of this drug in bulk as well as in pharmaceutical formulation.

CONCLUSION

Two visible spectrophotometric methods have been developed for assay of Zileuton. Proposed methods are simple, sensitive and reliable with good precision and accuracy. So, these methods can be used for the routine determination of Zileuton in bulk and pharmaceutical formulation.

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Table 1: Linearity of Zileuton with Ferric Chloride (Method A)

Concentration(µg/mL)	Absorbance
10	0.0824
20	0.1718
30	0.2635
40	0.3524
60	0.5324
80	0.7051

Table 2: Linearity of Zileuton with Ferric Nitrate (Method B)

Concentration(µg/mL)	Absorbance
10	0.0809
20	0.1591
30	0.2358
40	0.3026
60	0.4632
80	0.5914

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

S.No.	Parameter	Method A	Method B
Regression Parameters			
1	Regression Equation*	Y=0.008X-0.003	Y=0.007x+0.007
2	Slope(b)	0.008	0.007
3	Intercept(a)	-0.003	0.007
4	Correlation Coefficient(r)	0.999	0.998
5	%RSD**	0.362	0.188
Optical Parameters			
1	Absorption Maxima(nm)	397	390
2	Linearity Range(µg/mL)	10-80	10-80
3	Molar Absorptivity(lit.mol^{-1} cm^{-1})	$2.05x10^3$	$1.83x10^3$
4	Sandell's Sensitivity (µg/cm^2/ 0.001 abs unit)	0.1151	0.1288
5	**% range of errors** 0.01 level 0.05 level	 0.447 0.302	 0.232 0.157
6	Limit of Detection (µg/mL)	1.1	1.6
7	Limit of Quantification (µg/mL)	3.5	3.1

* 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.

Table 4: Inter-day and Intra-day precision for method A

Intra-day			Inter-day	
Con. taken (µg/mL)	**Con. found* (µg/mL)**	**%RSD**	**Con. found*(µg/mL)**	**%RSD**
20	20.02	0.96	19.89	0.98
30	29.98	0.85	29.91	0.96
40	39.89	0.95	39.65	0.92

*average of six determinations

Table 5: Inter-day and Intra-day precision for method B

Intra-day			Inter-day	
Con. taken (µg/mL)	**Con. found* (µg/mL)**	**%RSD**	**Con. found*(µg/mL)**	**%RSD**
20	19.87	0.54	19.86	0.64
30	29.90	0.58	29.87	0.61
40	39.95	0.62	39.98	0.66

*average of six determinations

Table 6: Accuracy for method A

% Spike level	Sample (µg/mL)	Amount added (Std.) (µg/mL)	Amount found (µg/mL)	% Recovery	Statistical parameters
80	30	24	23.96	99.83	Mean=99.69 SD=0.8228 %RSD=0.83
	30	24	23.91	99.62	
	30	24	23.92	99.63	
100	30	30	30.04	100.13	Mean=99.91 SD=0.818 %RSD=0.82
	30	30	29.97	99.90	
	30	30	29.91	99.70	
120	30	36	35.96	99.88	Mean=99.86 SD=0.300 %RSD=0.302
	30	36	35.94	98.86	
	30	36	35.95	99.87	

Table 7: Accuracy for method B

% Spike level	Sample (µg/mL)	Amount added (Std.) (µg/mL)	Amount found (µg/mL)	% Recovery	Statistical parameters
80	40	32	31.79	99.34	Mean=99.80 SD=0.8228 %RSD=0.83
	40	32	32.08	100.25	
	40	32	31.94	99.81	
100	40	40	39.99	99.97	Mean=99.83 SD=0.818 %RSD=0.82
	40	40	39.94	99.85	
	40	40	39.87	99.67	
120	40	48	47.88	99.75	Mean=99.72 SD=0.300 %RSD=0.302
	40	48	47.79	99.56	
	40	48	47.93	99.85	

Table 8: Estimation of zileuton in pharmaceutical formulation

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

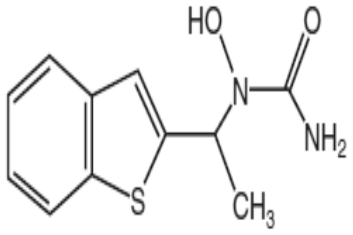

Fig. 1: Chemical structure of Zileuton

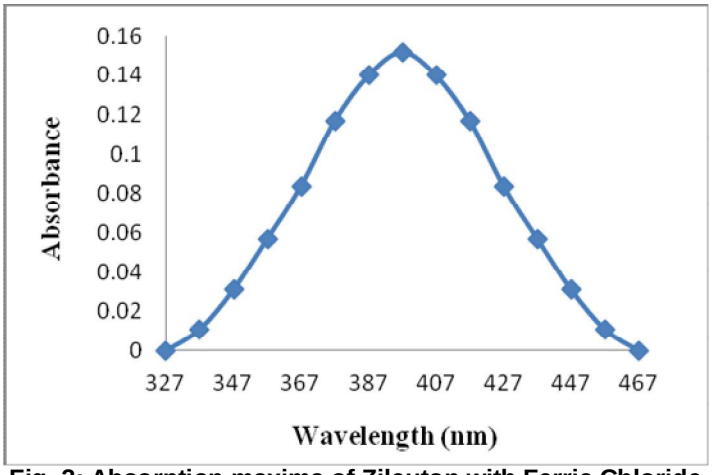

Fig. 2: Absorption maxima of Zileuton with Ferric Chloride

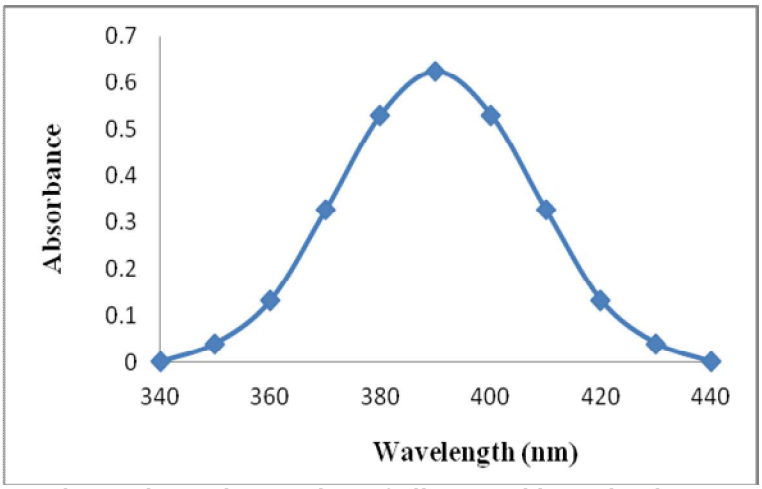

Fig. 3: Absorption Maxima of Zileuton with Ferric Nitrate

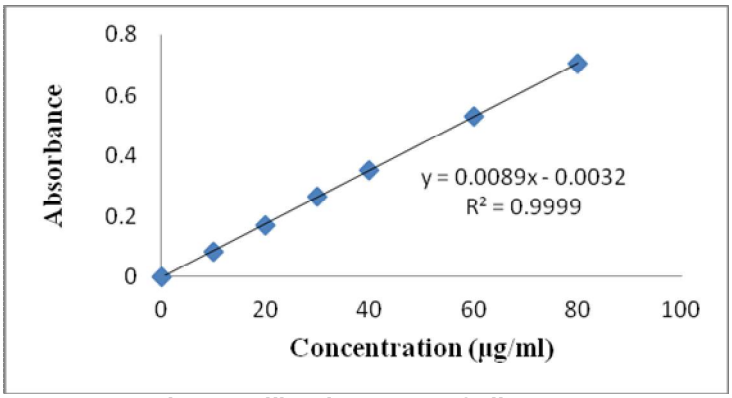

Fig. 4: Calibration curve of Zileuton with Ferric Chloride (Method-A)

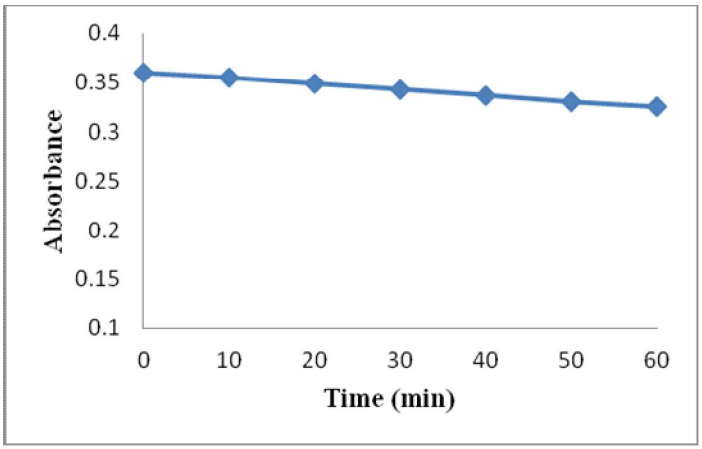

Fig. 5: Stability study of Zileuton with Ferric Chloride

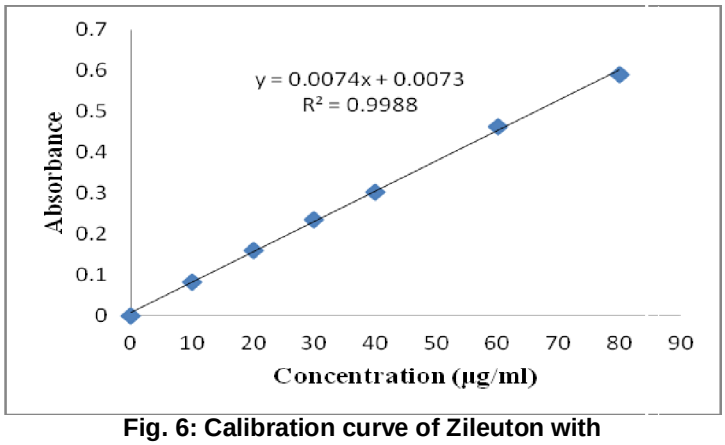

Fig. 6: Calibration curve of Zileuton with Ferric Nitrate (Method B)

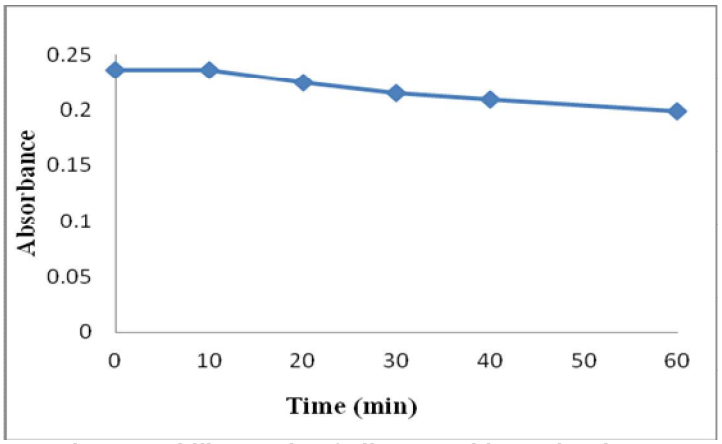

Fig. 7: Stability study of Zileuton with Ferric Nitrate

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