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(RESEARCH ARTICLE)



UV spectrophotometric method for estimation of zileuton in pharmaceutical formulation

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Abstract

A simple, rapid, sensitive and accurate UV-spectrophotometric method has been developed for the estimation of zileuton in pharmaceutical formulation. The method was developed by using 0.1 N Sodium hydroxide as a solvent and absorbance was measured at 230 nm. The drug exhibited the linearity in the concentration range of 1-6 µg/ml with correlation coefficient of 0.9993. The % recovery of the drug was found to be 98.62 % - 100.5 %. The method was validated as per ICH guidelines. The proposed methods are economical and sensitive for the estimation of zileuton in bulk and tablet dosage form.

Keywords: Zileuton; 0.1 N NaOH; UV-spectrophotometric method; ICH guidelines

1. Introduction

Zileuton [R,S(±)N-(1-(benzo [b]-thien-2-yl) ethyl)-N-hydroxyurea] (Fig. 1) is a racemic mixture having approximately equal therapeutic activities which selectively and reversibly inhibits 5-lipoxygenase potentiating leukotrienes (LT's – LTA₄, LTB₄, LTC₄, LTD₄ and LTE₄); mostly indicated in inflammatory diseases akin to psoriasis, rheumatoid arthritis, asthma, multiple sclerosis, uveitis and inflammatory bowel syndrome[1]. Zileuton is a very slightly soluble compound without any ionizable functional group. Zileuton is used for the prophylaxis and chronic treatment of asthma in adults and children 12 years of age and older. Contraindications are active liver disease or resistant elevation in transaminase at least 3 times the history of allergic reactions to zileuton or any of its inactive ingredients. Zileuton is a minor substrate of CYP1A2, 2C8/9, 3A4, and a weak inhibitor of CYP 1A2[2]. The drug has been shown to increase the serum concentration or effects of theophylline, propranolol and warfarin, although significant increase in prothrombin time is not obvious. It is advised that the doses of each medication be monitored and/or reduced accordingly. Literature survey reveals analytical methods reported for estimation of zileuton in API, pharmaceutical dosage form and biological fluid includes spectrophotometry[3-9], RP-HPLC[10-12] and LC-MS[13-14]. The HPLC and LC-MS analytical techniques are quite expensive and not easily available in every labs. So the alternate choice is the spectrophotometric technique in which UV spectrophotometric methods[15-17] are widely used because of its cost effectiveness and easy availability. Here authors attempts to develop and validate a simple, rapid and sensitive zero order UV spectrophotometric method for the estimation of zileuton in API and pharmaceutical formulation. The developed method is validated as per the ICH guidelines[18] and results are statistically interpreted

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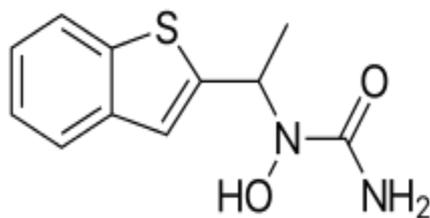


Figure1 Structure of Zileuton

2. Material and methods

2.1. Instrumentation

All the absorbance and spectral measurements were made by using ELICO (Hyderabad, India) double beam model SL 244 UV/VISIBLE Spectrophotometer. One cm matched quartz cells were used for absorbance measurements. The pH measurements were performed with an LI 1120(Elico,India).All weighing were carried out on AUX220(Shimadzu, Japan).Ultra sonicator (Citizen ultra sonicator) were used for solubilisation purpose.

2.2. Reagents and materials

Zileuton raw material was obtained from Yarrow Chem. products, Mumbai, India. Analytical grade sodium hydroxide pellets was obtained from Alpha chem. manufacturer. Tablet formulation GRILUTO-CR (Cadila Healthcare Limited, Goa, India) containing Zileuton 600 mg was purchased from local pharmacy. All reagents and solvents used were analytical grade. Double distilled water was obtained from a water purification unit.

2.3. Preparation of standard solution

A standard stock solution was prepared by weighing 10 mg of zileuton pure , dissolved with little quantity of 0.1 N NaOH and transferred into 10 ml volumetric flask. The volume was made upto the mark with 0.1N NaOH. This solution produces 1000 µg/ ml zileuton concentration. Working standard solution equivalent to 100 µg/ ml of zileuton was obtained by appropriate dilution of stock solution with 0.1 N NaOH.

2.4. Preparation of working standard solution

From the standard stock solution 0.3 ml was transferred to 10 ml volumetric flask and diluting to 10 ml with 0.1 N NaOH to get a concentration of 3 µg/ml. Working standard solution of zileuton was scanned between 200-400 nm. The wavelength maximum exhibited for zileuton was at 230 nm (Fig.2).



Figure 2 UV spectra of zileuton

2.5. Procedure for Calibration Curve

Appropriate volume of aliquot (0.1-0.6 ml) from standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with 0.1 N NaOH to give solutions concentrations in the range of 1-6 µg/ml. The absorbance measurements of these solutions were carried out against 0.1 N NaOH as blank at 230 nm. Calibration curve was constructed by plotting absorbance versus concentrations. Linear regression equation was obtained from this calibration curve.

2.6. Procedure for Pharmaceutical Formulation

Ten tablets (GRILUTO-CR) were weighed and average weight was calculated. Tablets were triturated to a fine powder in a mortar and pestle. Tablet powder equivalent to 10 mg zileuton was accurately weighed and transferred to 100 ml volumetric flask. To this 60 ml of 0.1 N NaOH was added and sonicated for 10 min. The flask was shaken and volume was made up to the mark with 0.1 N NaOH. The above solution was filtered through Whatman filter paper No.41. From the above solution 0.3 ml was transferred to 10 ml volumetric flask. Volume was made up to the mark with 0.1 N NaOH to give a solution containing 3 µg/ml. The content in the tablet was calculated from the calibration curve.

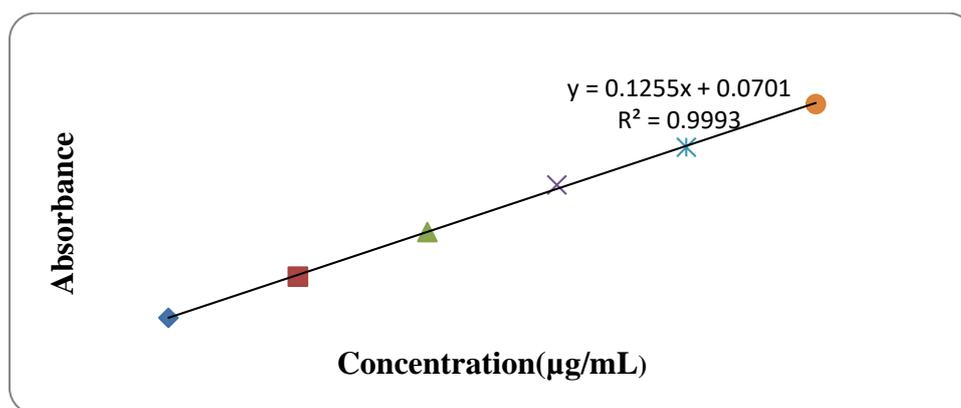
3. Results and discussion

3.1. Method Validation

The developed method was validated in terms of Linearity, precision, accuracy, Limit of detection (LOD) and Limit of Quantitation (LOQ), robustness and ruggedness.

3.1.1. Linearity

Six points calibration curve were obtained in a concentration range from 1-6 µg/ml for zileuton. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was $y=0.1255x+0.0701$ with correlation coefficient 0.9993 (Table 1 and 2, Fig. 3).



Figures 3 Calibration curve of zileuton

Table 1 Linearity Data for Zileuton

S.No	Concentration (µg/ml)	Absorbance
1.	1	0.196
2.	2	0.316
3.	3	0.446
4.	4	0.584
5.	5	0.694
6.	6	0.820

Table 2 Optical Characteristics of Zileuton

S.No	Parameters	Method
1.	λ_{\max} (nm)	230
2.	Beers law limit ($\mu\text{g/ml}$)	1-6
3.	Sandell's sensitivity($\mu\text{g/cm}^2/0.001$ A.U)	0.0051
4.	Molar absorptivity($\text{L mol}^{-1} \text{cm}^{-1}$)	4.6×10^4
5.	Correlation coefficient (r)	0.9993
6.	Regression equation($Y=mX+c$)	$Y=0.1255X+0.0701$
7.	Slope(m)	0.1255
8.	Intercept(c)	0.0701
9.	LOD ($\mu\text{g/ml}$)	0.24
10.	LOQ ($\mu\text{g/ml}$)	0.71
11.	Standard error of mean of Regression line	0.00494

3.1.2. Precision

Precision was checked in terms of repeatability, inter and intraday precision. It was expressed in percentage RSD.

3.1.3. Repeatability

The repeatability was evaluated by assaying six times of sample solution prepared for assay determination. Percentage RSD was calculated (Table 3).

Table 3 Repeatability

Concentration($\mu\text{g/ml}$)	Absorbance
3	0.445
3	0.445
3	0.445
3	0.449
3	0.445
3	0.445
Mean	0.445
Standard Deviation	0.0016
% RSD	0.035

3.1.4. Interday and Intraday precision

The intraday and interday precision study of zileuton was carried out by estimating different concentrations of zileuton six times on the same day (intraday precision) and on different days (interday precision) and the results were reported in terms of Percentage RSD(Table 4). The developed method was found to be précised as the average % RSD values for intraday and inter-day precision study was found to be 1.68 % and 1.51 % respectively (Table 4).

Table 4 Results for precision

Precision	Intraday		Interday	
	Morning	Evening	Day 1	Day 2
Mean (n=6)	0.432	0.446	0.432	0.447
SD	0.007305	0.006735	0.007305	0.006802
RSD	0.016878	0.015096	0.016878	0.015194
%RSD	1.68	1.50	1.68	1.51

3.1.5. Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the known amount of marketed formulation at three different concentration levels 80, 100 and 120 % taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed three times and average recoveries were measured (Table 5). Results of recovery study were within the range of 92.62%-100.5% indicating that the developed method is an accurate method for determination of Zileuton. The results are summarized in Table 3.

Table 5 Results for Accuracy

Recovery level (%)	Concentration ($\mu\text{g/ml}$)		% Mean Recovery* \pm SD	% RSD
	Test Con.	Std. added		
80	3	2.4	99.12 \pm 0.99	1.01
100	3	3	99.62 \pm 0.28	0.29
120	3	3.6	100.51 \pm 0.16	0.17

*Average of three determinations

3.1.6. Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ± 2 nm. The effect of variation in solvent strength was studied at ± 0.02 N NaOH. For changes of conditions, the sample was assayed in triplicates. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. Assay for all deliberate changes of conditions should be within 98.0–102.0 % for the proposed method (Table 6).

Table 6 Robustness Studies

Formulation	Amount of drug taken from tablet(mg)	At 238 nm	At 242 nm
		(n=3)%Assay \pm %RSD	(n=3)% Assay \pm %RSD
GRILUTO-CR (Tablets)	50	99.73 \pm 0.313	99.91 \pm 0.224
GRILUTO-CR (Tablets)	50	99.32 \pm 0.498	99.67 \pm 0.602

3.1.7. Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogeneous slot by two analysts using same operational and environmental conditions (Table 7).

Table 7 Ruggedness Studies

Formulation	Amount of drug taken from tablet(mg)	Analyst 1	Analyst 2
		(n=3)%Assay±%RSD	(n=3)%Assay±%RSD
GRILUTO-CR (Tablets)	50	99.83±0.243	99.86±0.324

3.1.8. 3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated according to below equation given by

$$\text{LOD} = 3.3 \sigma/s$$

$$\text{LOQ} = 10 \sigma/s$$

Where σ is the standard deviation of y intercepts of regression lines and s is the slope of the calibration curve (Table 2). The low value of LOD and LOQ indicate that the method is sensitive.

3.1.9. Application of method to formulation

The proposed was applied to pharmaceutical formulation of zileuton (Table 8).

Table 8 Assay

Formulation	Labelled Amount(mg)	Amount* Obtained(mg)	%Purity ± SD
GRILUTO-CR (Tablets)	100	99.86	99.86%± 0.685

*Average of three determinations

The % purity of zileuton presented in the table indicates that there is a good recovery of zileuton in tablet formulation by the developed UV spectrophotometric method.

4. Conclusion

A simple, economic, precise, accurate and sensitive UV spectrophotometric method for estimation of zileuton in bulk and in formulation was developed. This developed method was validated according to ICH guidelines. Beer's law was obeyed in concentration range of 1-6 $\mu\text{g/ml}$. The correlation coefficient (r^2) for zileuton was found to be 0.999. The % recoveries were found to be in the range of 99.84-100.18% for zileuton. The precision of method was determined by repeatability, intraday and interday precision whose % RSD < 1% indicates the precision of the method. The Limit of detection for zileuton was found to be 0.24 $\mu\text{g/ml}$. Limit of quantitation for zileuton was found to be 0.71 $\mu\text{g/ml}$. The proposed method was found to be simple, accurate, precise, reproducible and gave acceptable recovery of the analyte which can be applied to analysis of bulk and pharmaceutical capsule formulation of zileuton. Additionally the short analysis time and low cost is the other advantages of these methods for routine analysis. Its advantages over other existing methods are simplicity, speed and low cost. Compliance with ethical standards

Compliance with ethical standards

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Disclosure of conflict of interest

None

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