Open Access Contents
Int. J. Drug Dev. & Res.
| January - March 2015 | Vol. 7 | Issue 1 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Development and Validation of RP-HPLC method for the estimation of Zileuton in bulk and its dosage form

Vara Prasada Rao K*

Tanuja M

Srinivasa Rao Y

Hemant Kumar T

Department of Pharmaceutical Analysis and Quality Assurance
Vignan Institute of Pharmaceutical Technology
Beside VSEZ, Duvvada,
Visakhapatnam-530 049

Corresponding Authors:
K. Vara Prasada Rao
Department of Pharmaceutical Analysis and Quality Assurance
Vignan Institute of Pharmaceutical Technology
Beside VSEZ, Duvvada,
Visakhapatnam-530 049
Andhra Pradesh, India
Ph.No:0891-2511222, 2589777
E-mail:varaprasadvipt@gmail.com

Abstract:

A simple, economical, sensitive, specific, precise and accurate RP-HPLC method was developed and validated for determination of Zileuton in bulk and pharmaceutical dosage form. Chromatography was carried on an Enable C18 G 250 x4.6 mm column using filtered and degassed mixture of methanol and water (70: 30 v/v) as mobile phase at a flow rate of 1.0 ml/min in isocratic mode and effluent was monitored at 229 nm. The retention time for zileuton was found to be 3.5 min. The method was linear over the concentration range of 5-30 µg/ml with correlation coefficient 0.999. Proposed method was validated for specificity, precision, accuracy, linearity, robustness.

Keywords: Zileuton, RP-HPLC, Pharmaceutical dosage form.

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.

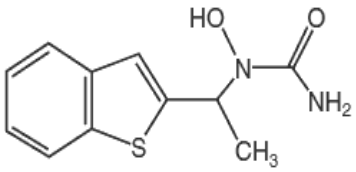

Figure 1: Chemical structure of Zileuton

According to literature, zileuton and its inactive N-dehydroxylated metabolite in plasma was determined by HPLC [3] and LC/MS-MS [4]. An UV spectrophotometric method [5] also reported for analysis of bulk and tablet formulation. Literature study reveals that so far there are no simple RP-HPLC methods for the estimation of zileuton in bulk and tablet formulation.

The present study was aimed at developing simple, specific, accurate and precise RP-HPLC method for determination of zileuton in bulk and tablet formulation.

Materials and Methods

Instrumentation

HPLC analysis was performed on Shimadzu Prominence Liquid Chromatograph comprising a LC-20AT pump, Shimadzu SPD-20A Prominence

UV-VISIBLE detector and a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "LC solutions" software. An electronic analytical weighing balance (1mg sensitivity, Keeroy), a sonicator (sonica, model 2200 MH).

Reagents and Chemicals

Methanol and Water of HPLC grade were procured from E. Merck Limited (India). Zileuton standard was obtained from RA Chem Pharma Ltd, Hyderabad. Zileuton tablets label claim 60 mg brand name GRILUTO CR manufactured by Cadila Health Care Ltd, Goa was procured from local pharmacy.

Selection of Wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Zileuton. Suitable wavelength selected was 229 nm.

Optimised Chromatographic conditions

Mobile phase : Methanol : Water (70:30 v/v)
Elution type : Isocratic
Column : Enable C18 G 250 x 4.6mm
U.V. detection : 229 nm
Flow rate : 1.0 ml/min
Injection volume : 20 µl
Temperature : Ambient
Runtime : 10 min.

Preparation of Mobile Phase

The mobile phase was prepared by mixing methanol and water in the ratio of 70:30 v/v and later it was sonicated for 15 minutes for the removal of air bubbles and filtered using 0.45 µ filter under vacuum filtration.

Preparation of standard stock solution (100 µg/ml)

Accurately weighed quantity 10 mg of Zileuton was transferred to 100 ml volumetric flask and dissolved in 10 ml of methanol by shaking manually for 2 minutes. The volume was made up to the mark with methanol to give concentration of 100 µg/ml.

Procedure for Calibration curve

Aliquots of (0.5-3 ml) standard stock solution (100 µg/ml) of Zileuton were transferred into a series of 10 ml calibrated volumetric flasks and volume was made up to mark with methanol. The calibration curve was plotted with the six concentrations of 5, 10, 15, 20, 25, and 30 µg/ml of standard solutions. Each solution was filtered through 0.2 µ membrane filter paper and sonicated prior to injection.

Preparation of sample solution

Weighed accurately 20 tablets and crushed to a fine powder. Weight equivalent to 10 mg of Zileuton was transferred to a 100 ml volumetric flask and dissolved in mobile phase. The solution was made up to the mark with mobile phase and filtered through 0.45 µ membrane filter. Aliquots of solutions were prepared and injected into the system and the chromatograms were recorded. The peak area of the drug was calculated and the drug content in the tablets was quantified using the regression equation obtained from the pure sample.

Results and Discussion

Method Development

A reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of zileuton at 3.5 min. Figure 2 represent chromatogram of the standard solution (20 µg/ml).

The total run time is 10 minutes. System suitability tests are an integral part of method development

and are used to ensure adequate performance of the chromatographic system. Retention time (*Rt*), number of theoretical plates (*N*) and peak Asymmetric factor was evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1. In order to test the applicability of the developed method to a commercial formulation, GRILUTO CR was chromatographed at working concentration (20 µg/ml). The sample peak was identified by comparing the retention time with the standard drug . System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

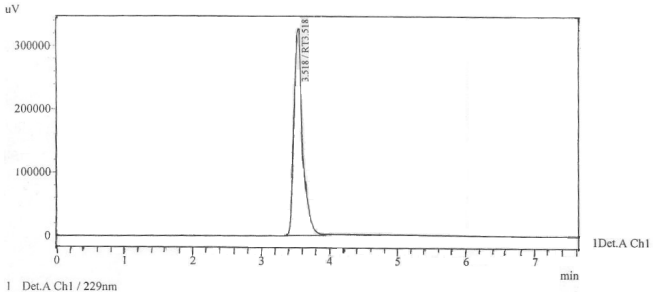

Figure 2: Chromatogram of standard zileuton

Table 1: System Suitability Parameters

Parameter	Result
Retention time	3.518 (mins)
Theoretical plates	4580
Tailing factor	1.1
HETP	34.67

Method validation [6]

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

Specificity

The specificity of was noticed in presence of tablet excipients. The specificity (selectivity) of the method was checked by a comparison of the chromatograms obtained from samples and the corresponding placebo. There was no any interference of excipient peaks at the retention time of zileuton.

Linearity and Range

Linearity was found by preparing six dilutions from the working standard solution and recording their responses at the optimized set of chromatographic conditions. The calibration plots were constructed between concentrations and peak areas which showed good linearity with acceptable correlation and regression. Range was obtained from the linearity of the calibration curve. Chromatograms show linearity up to concentration range of 5-30 µg/ml. The results are shown in Table 2 and Figure 3. The regression parameters were given in Table 3.

Table 2: Linearity of Zileuton

Concentration (µg/ml)	Peak area
5	242422
10	504845
15	1009690
20	1521008
25	2008530
30	2624220

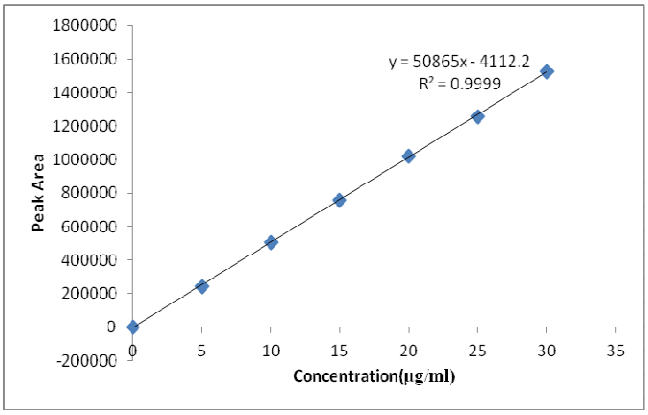

Figure 3: Calibration Curve of Zileuton

Table 3: Regression parameters of Zileuton

Parameters	Values
Concentration Range	5-30 µg/ml
Regression equation (Y)	Y = 50865x - 4112.2
Correlation Coefficient r^2	0.999
Slope (m)	50865
y-intercept (c)	4112.2

Precision

System precision

Six replicate injections of the standard solution at the working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 4.

Table 4: System Precision

Concentration(µg/ml)	Peak area
20 µg/ml	1521100 1531219 1501012 1521008 1513146 1501027
Mean	1514752
Standard deviation	12085.85
%RSD	0.7978

Interday Precision

The Interday precision of the sample was measured on three concentrations of the drug on three different days .The measurement of the peak areas were expressed in terms of % RSD and were found to be <1%.The results are given in Table 5.

Table 5: Interday Precision

Concentration (µg/ml)	Mean Peak Area*	%RSD
15	1008540	0.56
20	1514752	0.75
25	2016530	0.53

*average of six determinations

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120 %). At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 6. The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 6: Accuracy results

% Spike level	Sample Con. (µg/ml)	Con. added (µg/ml)	Con. found (µg/ml)	% Recovery	Statistical parameters
80	20	16	15.89	99.31	Mean=98.85 SD=0.692 %RSD=0.703
	20	16	15.69	98.06	
	20	16	15.87	99.18	
100	20	20	19.97	99.85	Mean=99.90 SD=0.463 %RSD=0.474
	20	20	19.99	99.95	
	20	20	19.98	99.90	
120	20	24	23.88	99.50	Mean=99.66 SD=0.646 %RSD=0.653
	20	24	23.92	99.66	
	20	24	23.96	99.83	

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in wave length, composition of mobile phase and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the

RP-HPLC method developed is robust. The results were shown in Table 7.

Table 7: Robustness Studies

Condition	Modification	Peak area	Mean % RSD
Mobile phase composition (v/v)	75: 25	1518742	0.11
Flow rate (ml/min)	0.8	1589162	0.21
Wavelength (nm)	224	1592123	0.15

Sensitivity

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated from linear curve using formulae

LOD= 3.3 σ / S

LOQ= 10 σ / S

(Where σ = the standard deviation of the response and S = Slope of calibration curve).

The results were shown in Table 8.

Table 8: LOD AND LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Zileuton	0.41	1.27

Application of Proposed method

The assay of marketed sample (Tablet formulation) for Rosuvastatin calcium is summarized in Table 9.

Table 9: Assay results of marketed formulation

Tablet	Drug	Labeled Claim (mg)	Amount Found (mg)	% Recovery
Griluto CR	Zileuton	600 mg	598.11	99.680.3

Conclusion

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, limit of detection and limit of quantitation, for the quantitative estimation of zileuton in tablets. The precision is exemplified by relative standard deviation of 0.79 %. A good linear relationship was observed for the drug between concentration ranges of 5-30 µg/ml. The interday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries were between 98 and 102%, an indicative of accurate method. Accordingly it can be concluded that the developed reverse phase isocratic HPLC method is accurate, precise and linear and therefore the method can be used for the routine analysis of zileuton in tablets.

Acknowledgments

The authors are grateful to Dr.L.Rathaiah, Chairman of Lavu educational society for providing necessary facilities to carry out the above research work.

References

1) Martindale - The Complete Drug Reference, 34th edition, Pharmaceutical Press, London-2005, Page 807.
2) The United States Pharmacopeia. The United States Pharmacopoeial Convention, Inc., Rock Ville, MD, USA, 2010,3891-3893.
3) Granneman GR, Braeckman RA, Erdman KA. Determination of a new 5-lipoxygenase inhibitor, zileuton, and its inactive N-dehydroxylated metabolite in plasma by high performance liquid

chromatography. Clin Pharmacokinet 1995; 29(2): 1-8.

4) Ping Lu, Michael L. Schrag, Donald E. Slaughter, Conrad E. Raab, Magang Shou, A. David Rodrigues. Mechanism-based inhibition of human liver microsomal cytochrome p450 1a2 by zileuton, a 5-lipoxygenase inhibitor. The American Society for Pharmacology and Experimental Therapeutics 2003; 31 (11): 1352–1360.

5) Ananda Kumar K, Naresh Babu N. Development and validation of analytical method for the estimation of zileuton in bulk and pharmaceutical dosage form by U.V spectroscopy. International Research Journal of Pharmacy: 2012; 3(12):154-157.

6) ICH, Q2 (A), Validation of analytical procedures: Text and methodology International Conference on Harmonization, Geneva. 2005, pp 1- 13.

Article History: ----------

Date of Submission: 12-02-2015

Date of Acceptance: 24-02-2015

Conflict of Interest: NIL

Source of Support: NONE