International Journal of Engineering Research-Online
A Peer Reviewed International Journal
Articles available online http://www.ijoer.in

Vol.2., Issue.6, 2014

RESEARCH ARTICLE

ISSN: 2321-7758

A NEW VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF ZIFIRLUKAST IN PURE AND PHARMACEUTICALS

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Article Received: 24/10/2014 | Article Revised on: 08/11/2014 | Article Accepted on:10/11/2014

ABSTRACT

A validated stability indicating RP HPLC method for the estimation of atazanavir in pure and phramaceuticals on ODS,C_{18} RP-Column (4.6mmx250mm) 5 µ column using mobile phase composition of 0.02M potassium dihydrogen phosphate buffer (pH-3.5) and Acetonitrile in the ratio of 60:40v/v. The Flow rate was maintained at 1.0 mL/min with 230nm UV detection. The retention time obtained for atazanavir was at 2.907min. The detector response was linear in the concentration range of 2.0 – 10.0 µg/mL. The linear regression equation for the calibration curve of zafirlukast (ZFK) was found to be Y= 504283x + 18359 with a coefficient of regression r^2=0.9999 respectively. This proposed RP-HPLC method has been validated and shown to be specific, sensitive, precise, linear, accurate, rugged, robust and fast and Hence, this method can be applied for routine quality control of atazanavir in capsule dosage forms as well as in bulk drug.

Keywords: Zifirlukast; RP-HPLC; validation

INTRODUCTION

Zafirlukast[1] is a synthetic, selective peptide leukotriene receptor antagonist (LTRA), with the chemical name 4(5-cyclopentyloxy-carbonylamino-1-methyl-indol-3ylmethyl)-3-methoxy-N-o-tolylsulfonylbenzamide used for the treatment of asthma and is often used in conjunction with an inhaled steroid and/or long-acting bronchodilator. It blocks the action of the cysteinyl leukotrienes on the CysLT1 receptors, thus reducing constriction of the airways, build-up of mucus in the lungs and inflammation of the breathing passages.

Zafirlukast, a fine white to pale yellow amorphous powder, is practically insoluble in water. It is slightly soluble in methanol and freely soluble in tetrahydrofuran, dimethylsulfoxide, and acetone. The molecular weight of zafirlukast is 575.7 and the structural formula is represented in **Figure.1**. The empirical formula is: $C_{31}H_{33}N_3O_6S$. It sold in the market in the trade name **ACCOLATE** supplied as 10 and 20 mg tablets for oral administration and administered orally.

It was quite evident from the literature, that few analytical methods[2-7] and only one RP-HPLC[8] method have been reported for the estimation of Zafirlukast (ZFK) in pure and in pharmaceutical dosage forms. The present paperr describes a sensitive, precise, accurate and stability indicating RP-HPLC method developed by the author for the assay of Zafirlukast (ZFK) which could be employed for the routine analysis in pure and in pharmaceutical dosage forms using mobile phase as diluent that made the developed method cost effective and economical.

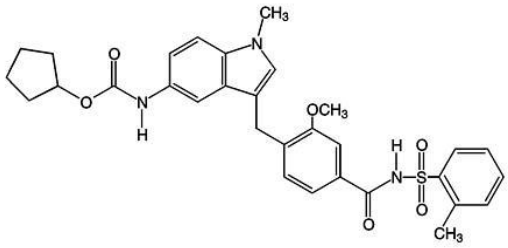

Figure.1: Structure of Zifirlukast

EXPERIMENTAL:

INSTRUMENTATION: The present analysis was performed using HPLC system (Waters Alliance 2695 separations module) equipped with 600e controller pump, 776 auto sampler, 2487 dual variable wavelength UV detector equipped with Empower software on Dell computer. A stainless steel ODS, C_{18} RP-Column (4.6mmx250mm) purchased from Waters Corporation (Bedford, MA, USA) was used in the present assay.

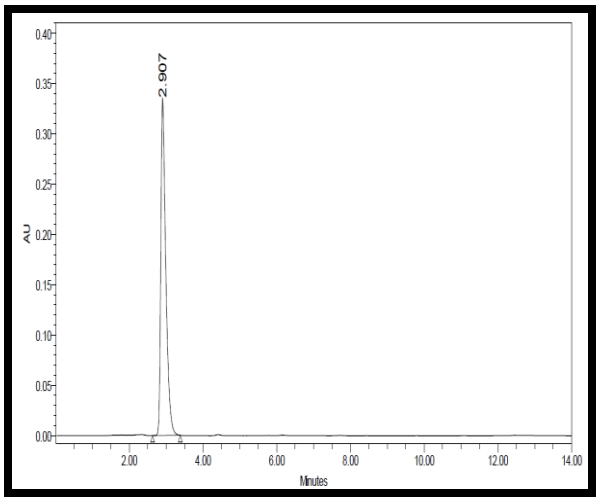

Figure.2: Chromatogram of Zafirlukast (ZFK)

CHEMICALS AND REAGENTS: Pure Zafirlukast (ZFK) was received as a gift sample from Msn labs,Hyd and Zafirlukast (ZFK) tablet (Accolate:10mg of zifirlukast) was purchased from local drug market. Analytical grade Hydrochloric acid, Sodium hydroxide pellets and Hydrogen peroxide solution 30% (v/v), Potassium dihydrogen phosphate Triethylamine methanol and Orthophosphoric acid (AR grade), Acetonitrile (HPLC grade) and Milli-Q water were obtained from Ranbaxy Fine

PREPARTION OF BUFFER SOLUTION: Dissolve 68.0 g of potassium dihydrogen phosphate in water and dilute to 1000.0 ml with the same solvent. Adjust the pH (3.5) with phosphoric acid. This buffer solution was filtered and degassed prior to the assay.

PREPARATION OF MOBILE PHASE: The mobile phase in the present assay is prepared by dissolving 0.02M potassium dihydrogen phosphate buffer (pH-3.5) and Acetonitrile in the ratio of 60:40v/v. This Mobile phase is filtered and degassed prior to the assay.

PREPARATION OF DILUENT: Mobile phase is used as diluent in the present assay.

STANDARD STOCK SOLUTION: An accurately weighted sample of 100mg of zafirlukast (ZFK) was dissolved in methanol to give standard stock solution of 100µg/ml. A series of working standard solutions (2.0µg/ml - 10µg/ml) were obtained by diluting the aliquots of stock solution with the same diluent. All the above volumetric flasks of working standard solutions were wrapped with aluminium foil and stored in the dark.

RESULTS AND DISCUSSIONS:

A)METHOD DEVELOPMENT: In developing the new stability indicating RP-HPLC method a systematic study of the effect of various factors [i.e, the influence of column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluent, concentration of analyte and other chromatographic parameters] was carried out by varying one parameter at a time and keeping all other conditions constant.

From these studies it was revealed that in the current study ODS,C_{18} RP-Column (4.6mmx250mm) column having 5µm particle size was used among the other columns because of its advantages of high

degree of retention, high resolution capacity, better reproducibility, ability to produce lower back pressure and low degree of tailing. A good symmetrical peak for zafirlukast (ZFK) was obtained, when water was replaced by phosphate buffer (adjusted to acidic pH-3.5 by orthophosphoric acid) as aqueous phase in mobile phase. Preliminary trials on mobile phase proportion were carried to provide good resolution for zafirlukast (ZFK) using different compositions of mobile phase. From these trails the proportion of phosphate buffer (pH-3.5) and Acetonitrile in the ratio of 60:40v/v was finalized as it gave good symmetrical peak for zafirlukast (ZFK).

The appropriate wavelength for determination of zafirlukast (ZFK) was scanned by UV-visible spectrophotometer and was observed that the maximum absorbance (λ_{max}) was obtained at 230nm. At this wavelength zafirlukast (ZFK) offered high response with good linearity. The best separation with adequate resolution and symmetric peak of zafirlukast (ZFK) was obtained when the injection volume was fixed to 20µL with a flow rate was set to 1.0 mL/min for the mobile phase respectively.

On this finalized chromatographic conditions, obtained chromatogram of zafirlukast exhibited good peak symmetry with higher theoretical plates. The representative chromatogram of zafirlukast (ZFK) is shown in **Figure.2**.

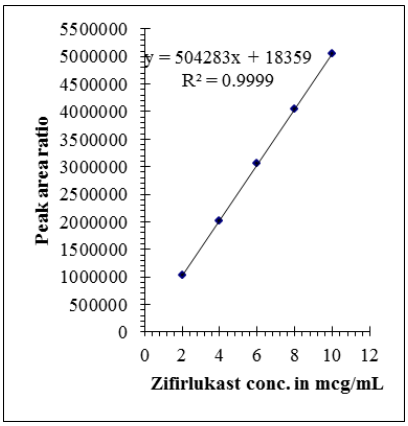

Figure.3:Calibration Curve of Zifirlukast

METHOD VALIDATION: After fixing the optimization studies the developed method was validated as per ICH guidelines which include system suitability, specificity, linearity, accuracy test, precision, robustness, ruggedness, sensitivity, limit of detection and quantification.

Table.1:System Suitability Parameters

PARAMETERS	ZAFIRLUKAST
Retention time	**2.907**
USP Plate count	**2250.8**
USP Tailing	**1.6**
Linearity Range (µg/ml)	**2.0-10.0**
Limit Of Detection (LOD) (µg/ml)	**0.0071**
Limit Of Quantitation (LOQ) (µg/ml)	**0.023**

Table.2: Results of Regression analysis of the proposed RP-HPLC method

Concentration (µg.mL)	Area (mAU)
2	1025845
4	2024941
6	3063452
8	4049804
10	5056245
Regression equation; Intercept (a)	18359
Slope (b)	504283.15
Correlation coefficient	0.9999
Standard deviation on intercept (S_a)	1202.0171
Standard deviation on slope (S_b)	2083.716
Standard error on estimation (S_e)	13178
LOD, µg/mL	0.0071
LOQ, µg/mL	0.023

Table.3:Results of Intra-Day Precision

S No	Name	Area
1	Injection-1	5055789
2	Injection-2	5078989
3	Injection-3	5154567
4	Injection-4	5076321
5	Injection-5	5037826
6	Injection-6	5095437
Avg		5083155
Std Dev		40294.41
*% RSD		0.792

Table.4:Recovery studies of the Proposed RP-HPLC method

Labled amount $\mu g.mL^{-1}$	Amount added $\mu g.mL^{-1}$	Total amount $\mu g.mL^{-1}$	*Amount found $\mu g.mL^{-1}$	% of Recovery
10	5	15	14.89	99.26
10	10	20	19.91	99.55
10	15	25	24.98	99.92

*All the values are the averages of three determinations

Table.5:Recovery studies of tablet containing Zafirlukast (ZFK)

Pharmaceutical formulation	Amount of Zafirlukast (ZFK)		% Recovery
	Labeled	Found*	
ACCOLATE[a](Tablet)	10.0 mg	9.98 mg	99.80

*Average of three determinations

3.2.3.2.1.SYSTEM SUITABILITY: The present HPLC system was equilibrated initially with the above said mobile phase, followed by six injections of the same standard **Figure.2**. These six consecutive injections were used to evaluate the system suitability. Parameters of system suitability studies include the peak symmetry (symmetry factor), no of theoretical plates of the column, resolution, mass distribution ratio (capacity factor) and relative retention and the results of these studies were summarized in **Table.1**. The number of theoretical plates was higher than 2000, making the proposed method acceptable for the assay of zafirlukast (ZFK) in dosage forms as reported in **Table.5**.

3.2.3.2.2.FORCED DEGRADATION STUDIES: In the present study above said drug was submitted to various stress degradation studies as per the ICH recommended guidelines. As zafirlukast (ZFK) is soluble in methanol all solutions of zafirlukast (ZFK) for use in forced degradation studies were prepared in methanol. This is done by subjecting zafirlukast (ZFK) powder to acidic (0.1N HCl), basic (0.1N NaOH), oxidizing (30% H_2O_2), and photo stability stress conditions.

The chromatograms of zafirlukast (ZFK) under acidic stress, basic stress and photo-stability stress conditions revealed that zafirlukast (ZFK) was found to be more stable did not showed any degradation and is eluted from the column respectively. The oxidative stress studies revealed that zafirlukast (ZFK) (Rt = 2.907 min) was not fully degraded and its degradation products were eluted separately at different retention times respectively.

From their respective chromatographs (not given), it is observed that the degradation products did not interfere in the detection analysis of zafirlukast (ZFK) establishing the high stability of the developed method.

LINEARITY: For linearity studies concentration levels corresponding to 25, 50, 75, 100 and 125% of test solution concentration were prepared separately and 20µL of each concentration was injected into the HPLC system and the response was read at 232nm and the corresponding chromatograms were recorded.

From these chromatograms a calibration curve was constructed by plotting the peak areas of the drug versus concentration of zafirlukast (ZFK) **Figure.3**. The linear regression equation for the calibration curve of zafirlukast (ZFK) was found to be Y= 504283x + 18359 with a coefficient of regression r^2=0.9999 respectively. The calibrated results of zafirlukast (ZFK) were tabulated in **Table.2** respectively.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION: The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the signal to noise (S/N) ratio. The LOD and LOQ values of zafirlukast (ZFK) by the proposed method was found to 0.0071µg/ml and 0.023µg/ml be respectively.

PRECISION: Precision of the proposed RP-HPLC method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision). It was expressed as % relative standard deviation (%RSD).

a.Intra-day precision: To study the intraday precision, six replicate standard solutions (10.0µg/mL) of zafirlukast (ZFK) were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.9, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in **Table.3**.

ACCURACY: The accuracy of this present proposed method was assessed by determination of recovery for three concentrations (corresponding to 50, 100, 150 % of test solution concentration) of zafirlukast (ZFK) covering the within the linearity range of the proposed method. Each concentration, were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and the results are compiled in **Table.4** and these results indicated the high degree of accuracy of the proposed RP-HPLC method for determination of zafirlukast (ZFK).

RUGGEDNESS & ROBUSTNESS: The ruggedness of the present RP-HPLC method was determined by carrying out the experiment by different analysts using different columns of similar types. The percentage of assay of different preparations assay values with two different analysts and columns were 99.5%, 98.7% respectively.

Robustness of the method was determined by small deliberate changes in flow rate, and temperature. The robustness limit for flow rate variation and temperature variation were well within the limit, revealing that the proposed method is robust under given set of defined experimental conditions.

ESTIMATION OF ZAFIRLUKAST (ZFK) IN TABLET DOSAGE FORM: The proposed RP-HPLC method has been validated for the assay of zafirlukast (ZFK) in tablet as per guidelines of ICH. Ten tablets(ACCOLATE;10mg of zafirlukast) are procured and powdered. An accurately weighed portion of powder equivalent to 25mg of zafirlukast (ZFK) was dissolved in 25ml of diluent and filtered through 0.45µm membrane filter. From this filtrate, suitable aliquots were pipetted in to 10ml graduated test tube and made up to volume with the mobile phase to obtain concentrations that obey in linearity limits. 20µL of this sample was injected into the column and the drug content in the tablet was quantified using the regression equation and the chromatogram and the results are reported in **Table.5** respectively.

CONCLUSIONS: In the present paper a simple, rapid, efficient, cost effective and reproducible stability indicating reverse phase high performance liquid chromatography method (RP-HPLC) has been developed and validated successfully by the author for the assay of zafirlukast in active pharmaceutical ingredient and in tablet dosages. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including linearity, accuracy, sensitivity, precision, ruggedness and robustness. This developed RP-HPLC method was found to be linear over a concentration range of 2.0 – 10.0µg/ml for zafirlukast respectively. The limit of detection and limit of quantitation was found to be as 0.0071µg/ml and 0.023µg/ml for zafirlukast respectively. The recovery was found to be in the range 99.26-99.92% and precision less than 1% revealing that the developed method was successfully applied for routine analysis of zafirlukast in pure and in pharmaceutical formulations.

ACKNOWLEDGEMENTS:

The authors would like to acknowledge the help of Department of Chemistry, A.N.U-Guntur for providig necessary lab facilities.

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