Journal of Pharmaceutics & Drug Delivery Research

Research Article A SCITECHNOL JOURNAL

Development and Validation of Zafirlukast by UV Spectroscopy

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Received date: 16 December, 2019, Manuscript No. JPDDR-19-5589;

Editor assigned date: 18 December, 2019, PreQC No JPDDR-19-5589(PQ);

Reviewed date: 01 January, 2020, QC No. JPDDR-19-5589;

Revised date: 25 August, 2022, QI No. JPDDR-19-5589, Manuscript No. JPDDR-19-5589(R);

Published date: 22 September, 2022, DOI: 10.4172/Jpddr.1000018

Abstract

A simple, new and advantageous spectroscopic analysis by U.V was estimated in bulk formations for the Zafirlukast and method development also performed. Zafirlukast was estimated at 240 nm in 25% acetonitrile. Limits for range of linearity (2 µg/ml^{-1} to 10 µg/ml^{-1}) (r2=0.078 x+0.046; r2=0.999), molar absorptivity noticed to be (5.7 × 10^{5} mol^{-1} cm^{-1}) in 25% ACN. According to ICH guidelines and USP this method was validated and tested for different validation parameters. Range for quantitation limits was noticed as (0.0740 µg/ml^{-1}) and 0.0244 µg/ml in 25% ACN, respectively. By demonstating the results method was found to be precise, reproducible (related standard deviation 2%) and accurate.

Keywords: Zafirlukast; Acetonitrile; UV Spectrophotometry; Validation

Introduction

Chemically Zafirlukast (ZFT) 4-(5 Cyclopentyloxycarbonylamino-1-methyl-1H-indol-3methoxy-N-O tolylsulfonylbenzamideCyclopentyl3-(2-methoxy-4-((o-tolylsulfonyl) carbamoyl) benzyl)-1methylindole-5 carbamate. Zafirlukast is employed in treatment of asthma; it is an oral Leokotriene Receptor Antagonist (LTRA). Zafirlukast is often used in combination with long acting bronchodilator and steroids. Methods include high performance liquid chromatography.

Electrochemical methods, traditional spcetrophotometric methods and capillary electrophoresis. Hence the present work is focused on stability of Zafirlukast followed by analytical method development and validation by UV spectrophotometry at 240 nm. Main aim of study is to estimate Zafirlukast in bulk in formulations through sensitive, stability indicating and simple process [1].

Materials and Methods

Absorption maximum (λ max)

To determine (λ max) absorbance, drug 10 µg/ml in solution with appropriate concentration in 25% Acetonitrile scanned with a wavelength range of 200 nm to 400 nm against a reagent blank [2]. Curve of absorption with appropriate (λ max) at 240 nm was shown by resulting spectrum. The absorption spectrum and overlay spectrum of Zafirlukast is given in Figures 1-3.

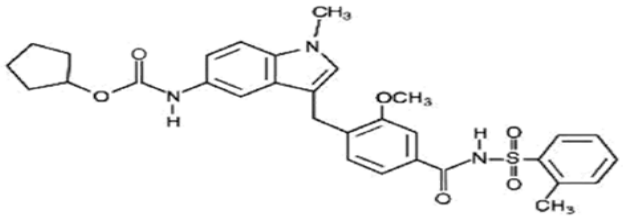

Figure 1. Comparison of RBC count, according to serum UA tertiles in males.

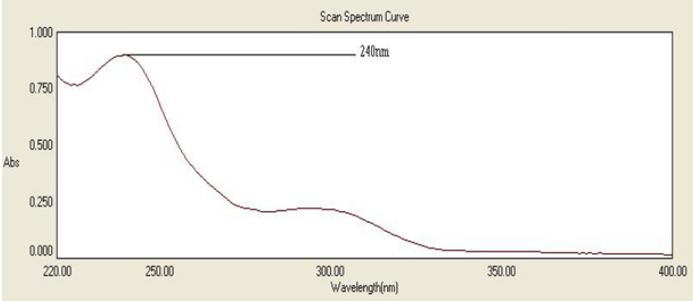

Figure 2. Absorption spectrum of Zafirlukast.

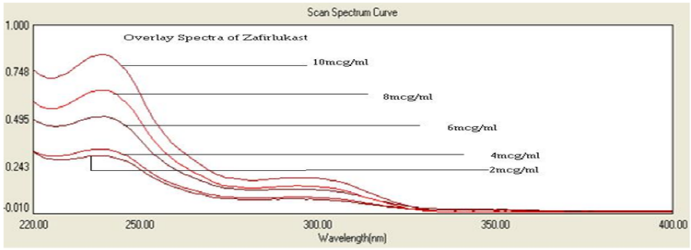

Figure 3. Calibration curve of Zafirlukast.

Stock solutions preparation

In 100 ml of 25% Acetonitrile 10 micrograms of Zafirlukast was weighed accurately and dissolved in 100 ml volumetric flask to get about 100 µg/ml concentration [3].

Preparation of calibration curve

Sample preparations (0.2 ml to 1 ml) from the standard solution and shifted to 10 ml volumetric flasks [4]. To obtain the range of concentrations from 2 µg/ml to 10g/ml respectively dilutes it with 25% acetonitrile [5]. The absorbance was measured at λmax 240 nm

by using blank. (25% Acetonitrile) (Table 1) showing results and (Figure 4) shows calibration curve [6].

S no	Con: (mcg/ml)	Absorbance at 240 nm ± (SD)	%RSD
1	2	0.211 ± 0001	0.4739
2	4	0.354 ± 0.001	0.2824
3	6	0.513 ± 0.001	0.1949
4	8	0.680 ± 0.00057	0.0848
5	10	0.835 ± 0.002	0.2395

Table 1: Calibration data of the approached method.

Figure 4. Calibration curve of Zafirlukast.

Specificity

In 25% Acetonitrile, Zafirlukast solution (6 µg/ml) was prepared including and excluding casual additives (Dextrose, starch, magnesium stearate, benzalkonium chloride indIvidually [7]. Within the range of 200 nm to 400 nm wavelength the solutions were scanned and examined at respective wavelengths for alterations in the absorbance [8]. In another study, from genuine drug stock in 25% Acetonitrile, 6 µg ml–1 drug concentration was prepared and analyzed individually (N=5) (Table 2).

Parameters	Zafirlukast
Molar absorptivity (l mol^{-1} cm^{-1})	5.75 x 10^{4}
Linearity range (mg/ml)	44836
Correlation coefficient	0.999
R E (Y)*	
Slope (a)	0.0078
Intercept (b)	0.046
Slope(s.e)	0.0078
Calculated F-value (critical F-value) a	2.07 (2.305)
selectivity and Specificity- tCal (tCrit) b	0.366 (2.353)
DL (mcg/ml)	0.0244
QL .02000……..0(mcg/ml)	0.7401
Robustness (mean% recovery ± S.D.)	0.284

Table 2: Regression equation and optical characteristics of Zafirlukast.

Accuracy

From standard stock solution, sample drug concentrations were prepared and examined (N=9). As a part of achieving accuracy of the projected method. This was assessed as mean percentage recovery (Table 3).

S.no	Concn mcg/ml	Absorbance of mean	S.D	%R.S.D	% recovery
1	2	0.211	0.001	0.4739	100.42
2	4	0.354	0.001	0.2824	100.32
3	6	0.513	0.001	0.1949	99.59

4	8	0.68	0.00057	0.0848	100.13
5	10	0.835	0.002	0.2395	99.4

Table 3: Accuracy data of the approached method.

Precision

Repeatability was determined. By testing three alternate range of sample drug concentrations (from standard stock solution (N=9) prepared and analyzed) (Table 4).

S.no	Concn µg/ml	Repeatability %R.S.D (n=9)			Inter day repeatability %R.S.D n=27
		First day	Second day	Third day	
1	10	0.396	0.398	0.392	0.631
2	30	0.867	0.846	0.821	2.23
3	50	1.212	1.237	1.238	0.978

Table 4: Precision data of the approached method.

Results and Discussion

To decide the intermediate precision of the projected methods, instrument variations, Inter-day and intra-day variation were choose. For calculating intra-day variation, range of drug sample concentrations were prepared in triplicates three various times in a day. The rule of conduct was followed for three alternate days to calculate inter-day variation (N=27) [9].

Linearity

Nine individual alternate solutions of the drug (2 µg ml–1 to 10 µg ml–1 in 25% Acetonitrile medium) were prepared from the standard solutions and examined, for obtaining the data, square regression analysis was employed. Thus the linearity is fixed for the projected method [10].

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) and Limit of Quantitation (LOQ) for the projected method was determined by using perfect calibration standards [11]. LOQ were calculated as 3.3 r/S and 10 r/S, respectively, where r is the standard deviation of y-intercept and S is the slope of the calibration curve of regression equation (Table 5) [12].

S no		Calibration curve mean (N=9)	S D	DL	QL
1	Slope	0.079	0.0256	0.0244	0.074019
2	Intercept	0.049			
3	R2	1			

Table 5: limit of Detection and Quantification limit data for the approached method.

Robustness

Determination of robustness of current projected method is done by stability of the Zafirlukast in the medium for 8hrs at room temperature and changing strength of the medium by ± 2%. With various strengths, different concentrations (MQC, LQC and HQC) were prepared in medium [13]. Mean percentage recovery was determined (Table 6).

S.NO	Con: (mcg/ml)	Absorbance of mean (N=9)	S.D	%Recovery	%RSD
1	3	0.2822	0.00025	100.422	0.284
2	7	0.5964	0.00015	99.89	
3	9	0.7572	0.00021	100.35	

Table 6: Robustness data for the developed method.

Stress degradation studies

The stress degradation studies were performed as per the ICH guidelines [14]. Q1A (R2) stability test of new drug entity, using the projected analytical validated procedure and the obtained results were presented in Table 7.

S.no	Stress conditions	Time	%Purity	%Degradation
1	Acid degradation	30 min	91.021	8.979
2	Base degradation	30 min	90.012	9.988
3	Peroxide degradation	30 min	94.243	5.757
4	UV degradation	7 days	93.521	6.479

Table 7: Results of Stress degradation studies of Zafirlukast.

Acid stress induced studies

Reflux of Zafirlukast standard solution (1 ml) and 2N HCl (1 ml) was processed for 30 min. at 60°C. The spectrum was recorded against 200 nm to 400 nm [15].

Alkali stress induced studies

Reflux of Zafirlukast standard solution (1 ml) and 2N HCl (1 ml) was processed for 30 min. at 60°C. The spectrum was recorded against 200 nm to 400 nm (Figure 5).

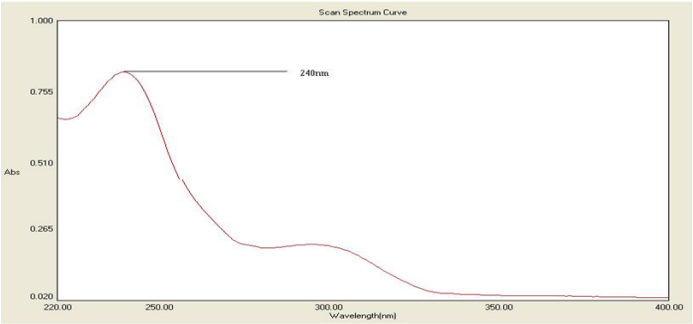

Figure 5. Base degradation.

Oxidation stress induced studies

Reflux of Zafirlukast standard stock solution (1 ml) and 20% H_2O_2 (1 ml) was processed for 30 min. at 60°C. The spectrum was recorded against 200 nm to 400 nm (Figure 6).

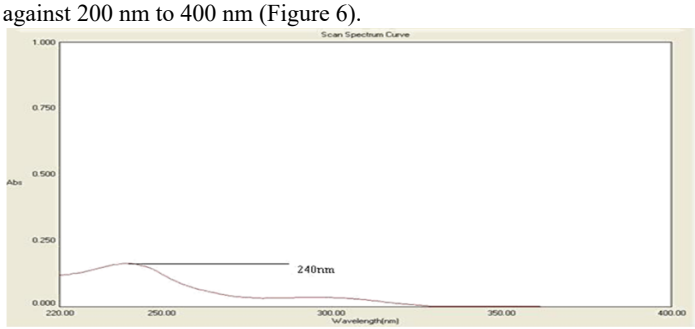

Figure 6. Peroxide degradation.

UV stress induced studies

The study of photochemical stability of the drug was examined by exposing the 10 µg/ml solution containing beaker in the UV light chamber. The spectrum was recorded (Figure 7).

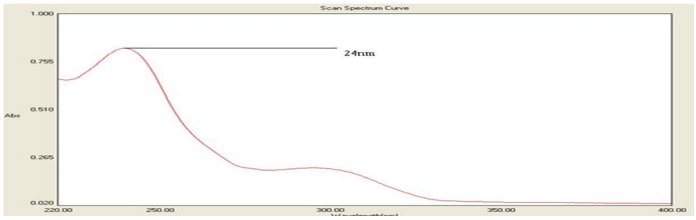

Figure 7. UV degradation.

Zafirlukast undergoes degradation in acidic, alkaline, oxidation and UV. Acidic degradation was found more. The results of the degradation studies of Zafirlukast in acidic, alkaline, oxidation and UV were found to be (8.979%, 10.988%, 5.757%, 6.479%). Agents like acetate and phosphate buffers of PH (3.6 to 5.8) (5.8 to 8.0) and 0.1N NaOH were employed for media optimization. In 25% in ACN the absorption spectra of Zafirlukast was seen. Finally establishment of 25% ACN using as the medium is on the basis of methods acuteness, price paid, simple development and its appositeness in kinetic studies. The absorption spectra of the drug in 25% ACN were demonstrated in the image2 in 25% ACN the lambda max of the drug was found to be 240 nm in 25% ACN the molar absorptivity of the drug was apparently 5.75 x 104 mol−1cm^{-1}. The obtained linear regression equation is absorbance at 240 nm=0.078 x +0.046 with a coefficient of 0.9999.

Specificity and selectivity

With the existence of casual enhancers in 25% ACN there occurred no changes in the UV absorption spectra of Zafirlukast. Hence the projected protocol is clear cut and choosy for the drug.

Accuracy

Low standard deviation values (S.D.1.5) and their wonderful restoration values (nearly 100%) represent accuracy. the mean percentage recoveries (%R.S.D.) of the small, medium, bigger concentrations in 25% ACN were found to be 100.42 (0.089), 99.89 (0.0256) and 100.351 (0.0274). This conclusion tells us that any minute alteration in the drug concentration of the solution can be obtained by the projected methods.

Precision

By examining intermediate precision and repeatability precision is determined. Repeatability (%R.S.D.) in 25% Acetonitrile, at each and every of the three ranges of concentration. Repeatability results signify the precision under the changeless administered environment over a concised time interval and inter-assay precision. Within the laboratory working on different days on various instruments, those variations

signify the intermediate precision. In this study, %R.S.D. values were almost not great than 2.5% in all the point. These methods have good intermediate precision and repeatability because the R.S.D values are within the acceptable range.

Linearity

The linearity range in 25% ACN was found to be 2-10 µg ml^{-1} at 240 nm. By lower values of standard error of slope and intercept parameters high precision of these projected methods can be indicated.

DL and QL

LOD, LOQ in 25% ACN were found to be 0.0244 µg ml^{-1} and 0.0740 µg ml^{-1} respectively.

Robustness

There is no significant effect on absorbance, even by ± 2% variation in the strength of the selected medium. In the 25% ACN the mean % recovery (± S.D.) were found to be 100.42 (± 0.089) respectively.

Conclusion

In summary, the projected method was clean and clear, quick, easy, definite, detailed and economical and can be used for conventional pharmaceutical formulations, dissolution studies, analysis of Zafirlukast in bulk. The developed derivative UV spectrophotometric method is a new, simple, precise, accurate and economical for simultaneous quantitative estimation of MTK and LCT. The method was validated (as per ICH guidelines) for various parameters, viz., linearity, accuracy, precision, limits of detection (LOD) and limit of quantification (LOQ). It could serve as an alternative method for determination of MTK and LCT simultaneously in marketed products and therefore, may be used for routine quality control analysis of MTK and LCT in multidrug products.

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