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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF ZAFIRLUKAST IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Zafirlukast in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade acetonitrile.[1] The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics.[2] It showed absorption maxima were determined in acetonitrile: water (80:20, v/v). The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity.[3,4,5,6] The UV spectroscopic method was developed for estimation of Zafirlukast in tablet dosage form and also validated as per ICH guidelines.[7] **Results:** The drug is freely soluble in tetrahadyofuran, acetonitrile and acetone. Slightly soluble in methanol and practically insoluble in water. So, the analytical grade acetonitrile: water (80:20, v/v) is used as a diluent in method. The melting point of Zafirlukast was found to be 139-140˚C (uncorrected). It showed absorption maxima 270 nm in analytical grade acetonitrile. On the basis of absorption spectrum the working concentration was set on 10µg/ml (PPM). The linearity was observed between 6-14 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 101.0 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.36%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Zafirlukast in tablet dosage form. The method could be considered for the determination of Zafirlukast in quality control laboratories.

KEYWORDS: Zafirlukast, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Zafirlukast is a tolyl compound and leukotriene receptor antagonist (LTRA), with anti-asthmatic and potential capsular contracture-preventing activities. Upon administration, Zafirlukast selectively and competitively binds to and blocks the cysteinyl leukotriene 1 receptor (CYSLTR1), thereby preventing the potent pro-inflammatory mediators leukotriene C4, D4 and E4 from binding. This prevents leukotriene-mediated actions, including enhanced migration of eosinophils and neutrophils, increased adhesion of leukocytes, increased monocyte and neutrophil aggregation, increased airway edema, inflammation, capillary permeability and bronchoconstriction. In addition, Zafirlukast may decrease collagen deposition, fibrosis, and capsular thickness after implantation, thereby preventing scar tissue. Zafirlukast is an orally available leukotriene receptor antagonist which is widely used for the prophylaxis and chronic treatment of asthma. Zafirlukast has been linked to rare, but occasionally severe cases of acute liver injury.

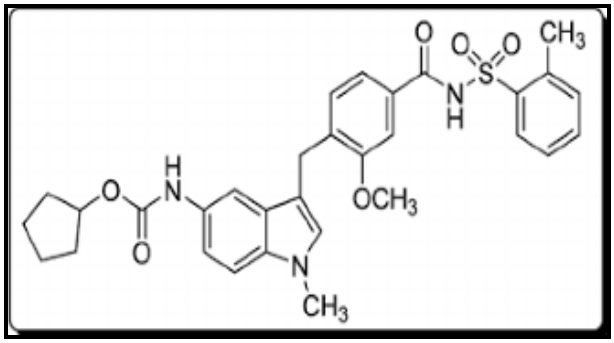

Fig. 1: Chemical Structure of Zafirlukast.

From literature review it's found that one method was reported on HPLC in Human plasma.[3,6] Also the method was reported on HPLC on Pharmaceutical formulation.[4] There is also work published on stability indicating LC and derivative spectrophotometry on Zafirlukast drug.[5] But no methods were reported on estimation of Zafirlukast in tablet dosage form for UV

spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Zafirlukast in tablet dosage forms.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair
Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

- **MATERIALS**

Zafirlukast was obtained as a gift sample from Dr. Reddy's Laboratories (Hyderabad). Zafirlukast tablets were procured from local pharmacy. Acetonitrile used was of analytical grade. Acetonotrile: Water (80:20) was used as a diluent throughout the experiment. Freshly prepared solutions were employed.

Method development

A. Determination of λ max (20 PPM)[10]

100 mg weighed amount of Zafirlukast was dissolved into 100 ml of volumetric flask with analytical grade acetonitrile. Pipette out 2 ml and added in 100 ml of volumetric flask dissolved and diluted up to the mark with diluent. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

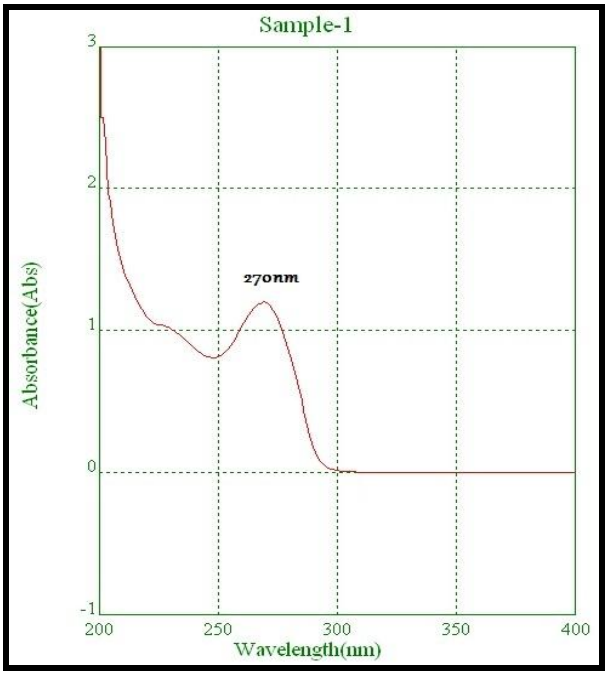

Fig. 2: Calibration Curve.

B. Preparation of Working concentration

Preparation of Standard stock solution

Standard stock was prepared by dissolving 100 mg of Zafirlukast in 100 ml of analytical grade acetonitrile to get concentration of 1000 µg/ml (PPM).

Preparation of Standard solution

Pipette out 1 ml from standard stock solution and diluted up to 100 ml with diluent to get concentration of 10 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with diluent. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[12,13]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 100 mg of Zafirlukast was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml analytical grade acetonitrile, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade acetonitrile. Filtered the solution through whatmann filter paper. Pipette out 1 ml of the above solution and diluted up to 100 ml with diluent. The absorbance was measured at 270 nm. The absorbance was recorded:

Table 1: Absorbance of Dosage Form.

AstraZeneca Pharmaceutical Limited (20 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.6672
3	Sample	0.6594

Table 2: Dosage Form Specifications.

Type	Brand Name	M.D.	E.D.	Batch No.	Average weight (g)	Assay (%)
1	**ACCOLATE® 20 mg**	07/2019	04/2022	GFC 08745	0.0302	99.15

E. Method of validation[7,15,16,18,19,20]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 6 - 14 PPM at 270nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	6 PPM	0.4215
2	8 PPM	0.5398
3	10 PPM	0.6495
4	12 PPM	0.7492
5	14 PPM	0.8554
Correlation coefficient	**0.999**	

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade acetonitrile and measuring the absorbance at 270nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75
100	1	1.01	101.00
120	1.2	1.19	99.17

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.6514
2	Sample Solution 2	0.6556
3	Sample Solution 3	0.6528
4	Sample Solution 4	0.6565
5	Sample Solution 5	0.6514
Mean		**0.6535**
SD		**0.0024**
% RSD		**0.3646**

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.6564	0.6543	98.07	0.2663
		0.6521			
2	Analyst 2	0.6574	0.6568	98.44	
		0.6562			

RESULTS

1. Solubility of Zafirlukast

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Acetonitrile, THF	Soluble
2	Methanol	Slightly soluble
3	Water	Practically insoluble

2. Melting point of Zafirlukast

The melting point of Zafirlukast was found to be 160-161˚C (uncorrected).

3. Results for linearity for assay method of Zafirlukast

The linearity of method was determined at concentration level ranging from 6 to 14µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.999**

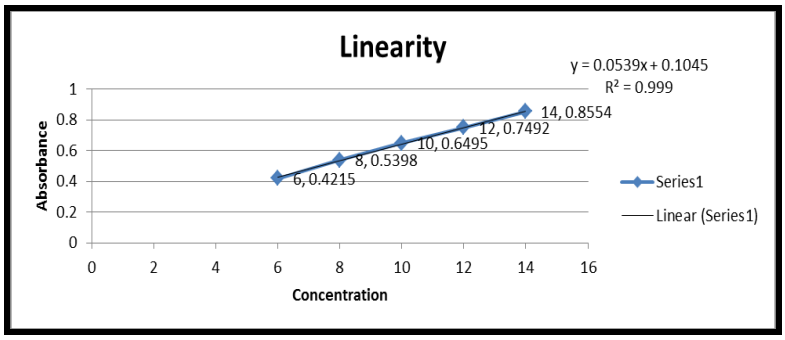

Fig. 3: Zafirlukast Standard Curve.

4. Results for accuracy for assay method of Zafirlukast

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Zafirlukast

The % RSD for different sample of precision was found to be 0.3646 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Zafirlukast

The %RSD for different sample of ruggedness was found to be 0.2663 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Zafirlukast in tablet form has been developed. From the spectrum of Zafirlukast, it was found that the maximum absorbance was 270 nm in analytical grade acetonitrile. A good linear relationship was observed in the concentration range of 6-14 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Zafirlukast in solid dosage forms. Hence, the method could be considered for the determination of Zafirlukast in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. LTRA - leukotriene receptor antagonist
6. THF - Tetrahydrofuran
7. ICH - International Council for Harmonization
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. °C - degree Celsius
12. M.D. - Manufacturing Date
13. E.D. - Expiry Date

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