

UV Spectrophotometric Determination of Zafirlukast in Pharmaceutical Formulations

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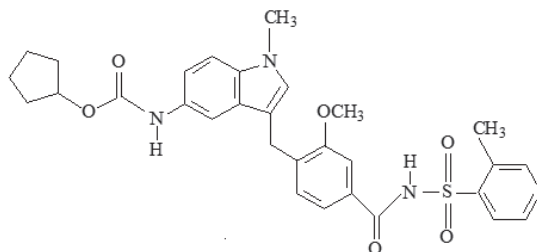
Introduction

The cysteinyl leukotrienes (C₄, D₄ and E₄) are lipid mediators which are implicated in producing an array of effects such as bronchoconstriction, increased vascular permeability, mucous secretion, inflammatory cell recruitment and airway smooth muscle proliferation. Antagonism of the effects of cysteinyl leukotrienes can be achieved by drugs preventing their synthesis using a 5-lipoxygenase inhibitor, or blocking specific leukotriene receptors¹⁻³. Zafirlukast (ZAF), 4 - (5 cyclopentylloxycarbonylamino - 1 - methylindol - 3 - ylmethyl) - 3 - methoxy - o - toylsulphonylbenzamide (Figure 1) is a selective and competitive orally administered inhibitor of the cysteinyl leukotrienes⁴. ZAF is indicated for the prophylaxis and treatment mild to moderate persistent and chronic asthma⁵. ZAF effectively improved asthma symptoms and exacerbations and improved pulmonary functions in patients with asthma⁶.

Because ZAF is a novel drug, only a few analytical methods for its determination in pharmaceutical formulations and biological fluids have been described in the literature, including high performance liquid chromatography (HPLC)⁷⁻¹⁰, derivative spectrophotometry⁸, capillary zone electrophoresis (CZE)¹¹ and electrochemical methods such as square - wave voltammetry (OSWV)¹², square - wave adsorptive stripping voltammetry (OSWAdSV)¹² and differential adsorptive stripping voltammetry (DPAdSV)¹³.

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**Figure 1**

Chemical formula of ZAF.

There is no UV spectrophotometric method for the analysis of ZAF in pharmaceutical formulations has been reported in literature.

The aim of this study was to develop a fast, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of ZAF in bulk drug and commercial pharmaceutical formulations as tablet. The proposed method was developed and validated according to the evaluation of the validation parameters¹⁴. The developed method was applied to the determination of ZAF in pharmaceutical formulations without the necessity of sample pre-treatment. The results obtained from this developed method were compared with those obtained by using derivative spectrophotometric method in literature⁸.

Experimental

Apparatus

The spectrophotometric measurements were carried out using an Agilent 8453 model UV-VIS spectrophotometer with a diode array detector (DAD) (190 - 1100 nm). UV spectra of standard and sample solutions were recorded in 1 cm quartz cells at the wavelength ranges of 190 - 350 nm.

The statistical analysis was performed with SPSS software (Version 10.7).

Chemicals and reagents

ZAF was kindly provided from Dr. Reddy's Laboratories (Hyderabad, India) and it was used without further purification. Melting point, UV and IR spectra of ZAF were evaluated to check purity and no impurities were found. Accolate Tablets® (20 mg ZAF per tablet) were kindly supplied by Astra Zeneca A.Ş. Milli - Q water was used for the preparation of

solutions. All solvents and other chemicals were analytical reagent grade. Acetonitrile was purchased from Merck.

Standard solutions

Standard stock solutions of ZAF ($1000 \mu\text{g mL}^{-1}$) was prepared in acetonitrile and kept in the dark and at $+4^\circ\text{C}$ maximum for 2 months. Working standard solutions were daily prepared by diluting stock solutions at the concentrations of $0.50 - 20.00 \mu\text{g mL}^{-1}$ in acetonitrile:water (80:20, v/v). Then the absorbance of these solutions was measured. In measurements acetonitrile:water (80:20, v/v) was used as a blank solution.

Tablet solutions

Ten tablets of Accolate[®] were accurately weighed and finely powdered and mixed. A portion of the powder equivalent to the average weight of one tablet was transferred into a 50 mL volumetric flask and 25 mL of acetonitrile was added. The content of the flask was sonicated for 15 min and diluted to volume with acetonitrile. This solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with acetonitrile:water (80:20, v/v) to give final concentration ($10 \mu\text{g mL}^{-1}$). Then the absorbance of these solutions was measured. The amount of ZAF per tablet was calculated using the calibration curve method.

Synthetic tablet solutions

For preparing the synthetic tablet some common excipients (e.g. croscarmellose sodium, lactose, microcrystallin cellulose, povidone, magnesium stearate, hydroxypropylmethyl cellulose and titanium dioxide) and standard ZAF equivalent amount to one tablet (20 mg) were weighed and finely powdered and mixed. This mixture was transferred into a 50 mL volumetric flask and dissolved and analyzed as explained in tablet solutions section.

Results and Discussion

ZAF is very poorly soluble in acidic media. Thus, basic solutions and different solvents were used to prevent its possible precipitation. In order to optimize the condition, different solutions such as acetonitrile, water, acetonitrile:water and sodium hydroxide were investigated and the UV

spectrum of ZAF was measured. The UV spectra of ZAF standard in these solutions are given in Figure 2. In sodium hydroxide solutions at different concentrations, well-defined peak was not observed for determination of ZAF. As results, well-defined peak was obtained in acetonitrile:water solutions. Therefore, the effect of acetonitrile in the solution was evaluated over the range 50-80 %. But, in these solutions peak shape was not changed and almost identical maximum absorbance at wavelength of 242 nm (Figure 3). At the end of these studies, acetonitrile:water (80:20, v/v) was chosen for the working solution. The spectrum shows a well-defined peak at 242 nm in the measuring wavelength range 190-350 nm. This wavelength was used for the determination of ZAF.

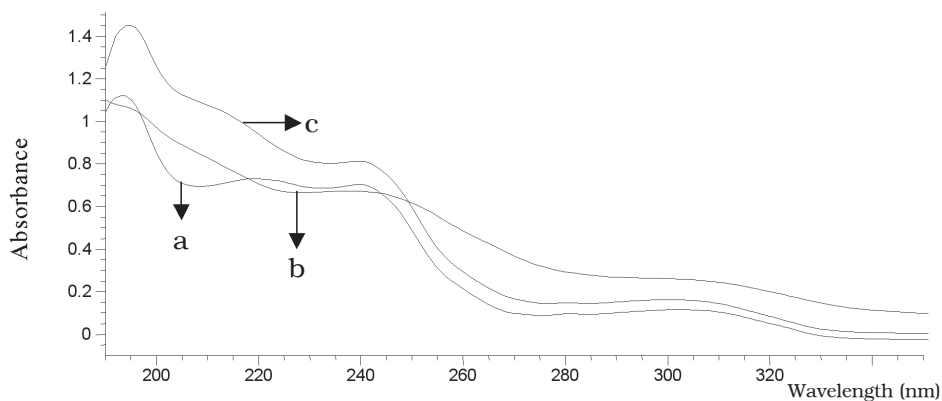


Figure 2

UV spectra of ZAF ($10.00 \mu\text{g mL}^{-1}$) in different solutions, a) acetonitrile, b) water, c) acetonitrile:water (80:20, v/v).

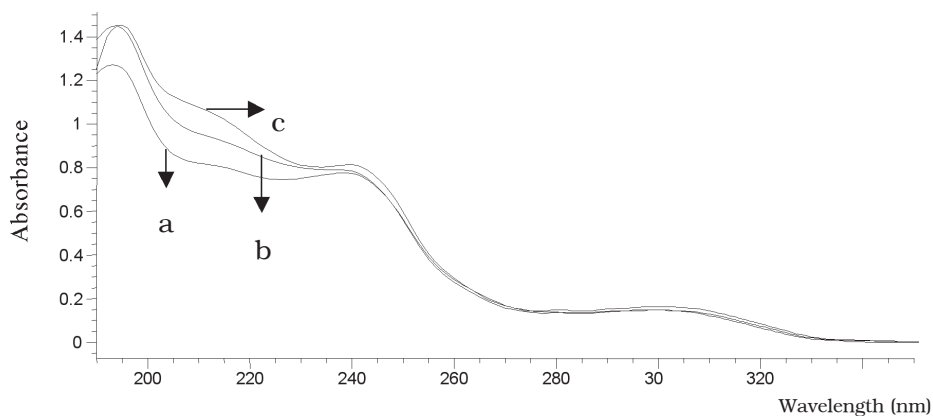


Figure 3

UV spectra of ZAF ($10.00 \mu\text{g mL}^{-1}$) in different ratio of acetonitrile:water (v/v) solutions, a) 50:50, b) 70:30, c) 80:20.

Validation

Validation is one of the most important steps in method development for analytical determinations. The main validation parameters such as stability, linearity, sensitivity, precision, accuracy, recovery, specificity, robustness and ruggedness were evaluated in developed method¹⁴.

Stability

The standard stock solutions of ZAF were stored +4 °C for 2 months. During this period, the solutions were analyzed with UV spectrophotometric method, the spectrum was compared with the spectrum of daily prepared standard solution and no difference was obtained between them as peak shape and maximum absorbance of ZAF. Therefore, ZAF is highly stable in the mentioned conditions.

Linearity range

Under the experimental conditions, the calibration graphs of the absorbance versus concentration were found to be linear over the range of 0.50 - 20.00 $\mu\text{g mL}^{-1}$ for proposed method. The calibration graphs were constructed after analysis of 7 different concentrations with each concentration was measured three times. Each point of the calibration graph corresponded to the mean value obtained from 7 independent measurements. The regression equations (with standard error of intercept and slope) and correlation coefficients of the mean of 7 consecutive calibration curves are given in Table I. The regression equation was $y = (0.0836 \pm 0.88 \times 10^{-3})x - 0.0099 \pm 2.74 \times 10^{-3}$ where y is the absorbance and x is the concentration in $\mu\text{g mL}^{-1}$ ($r = 0.9998$).

TABLE I

Analytical Characteristics of Proposed UV Spectrophotometric Method (n=7)

Parameters	UV Spectrophotometric Method
Regression equation (y) ^a	$y = 0.0836x - 0.0099$
Standard error of slope	0.88×10^{-3}
Standard error of intercept	2.74×10^{-3}
Correlation coefficient (r)	0.9998
Linearity range ($\mu\text{g mL}^{-1}$)	0.50 - 20.00
Number of data points	7
Limit of quantification (LOQ) ($\mu\text{g mL}^{-1}$)	0.50

^a $y = bx + a$ where x is the concentration in $\mu\text{g mL}^{-1}$, y is amplitude for UV spectrophotometry

Sensitivity

The limit of quantification (LOQ) is the lowest concentration of ZAF on the calibration curve that can be quantified with acceptable precision and accuracy^{15, 16}. The LOQ was found as $0.50 \mu\text{g mL}^{-1}$ (RSD = 3.51 %) (n=7) for proposed method.

Precision

The assay was investigated with respect to repeatability and intermediate precision^{17, 18}. Repeatability is based on the results of the method operating over a short time interval under the same conditions. The repeatability of the method was evaluated by performing 10 repeated measurements for $10.00 \mu\text{g mL}^{-1}$ of ZAF solution. The amount of ZAF was found to be 9.95 ± 0.01 with RSD of 0.35 % in the proposed method. Percentage recovery of ZAF was calculated as 99.47 ± 0.11 % with RSD % of 0.35. These values indicated that the proposed method have high repeatability and precision for the ZAF analysis.

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Three different concentrations of ZAF in the linear range (2.00 , 10.00 and $15.00 \mu\text{g mL}^{-1}$) were analyzed in 7 independent series in the same day (intra-day precision) and 7 consecutive days (inter-day precision) from three measurements of every sample in each series. The precision of the analysis was determined by calculating the relative standard deviation (RSD %). The RSD values of intra - day and inter - day studies varied from 0.96 to 1.90 % showed that the intermediate precision of the method was satisfactory (Table II).

TABLE II
Precision and Accuracy Data of the Developed UV Spectrophotometric Method for the Analysis of ZAF (n=7)

Intra - day				Inter - day		
Added ($\mu\text{g mL}^{-1}$)	Found ^a ($\mu\text{g mL}^{-1}$)	Precision RSD %	Accuracy ^b (Bias %)	Found ^a ($\mu\text{g mL}^{-1}$)	Precision RSD %	Accuracy ^b (Bias %)
2.00	2.05 ± 0.02	1.86	2.50	2.04 ± 0.02	1.90	2.00
10.00	10.05 ± 0.05	1.17	0.50	9.95 ± 0.06	1.69	-0.50
15.00	14.82 ± 0.05	0.96	-1.20	14.91 ± 0.06	1.14	-0.60

Found^a: \bar{x} : mean \pm standard error, RSD %: Relative standard deviation;
Accuracy^b: [(Found - Added) / Added] x 100

Accuracy and Recovery

The accuracy of a method was determined by calculating the percentage relative error (bias %) between the measured mean concentrations and added concentrations at the same concentration of ZAF^{19, 20}. Table 2 shows the results obtained for intra- and inter-day accuracy. The results obtained for intra and inter-day accuracy were between 0.50 - 2.50 %. Observed concentration values are in good agreement with the expected ones.

Recovery studies for the accuracy of the method were performed by spiking synthetic mixture with known amount of ZAF^{19, 21}. For this purpose, the determination of ZAF in a synthetic tablet samples (the mixture of excipients such as croscarmellose sodium, lactose, microcrystallin cellulose, povidone, magnesium stearate, hydroxypropylmethyl cellulose and titanium dioxide and labelled amount of ZAF as in pharmaceutical formulation) were prepared. The amount of 20 mg of ZAF was found to be 19.99 ± 0.08 with RSD of 1.10 %. The mean percentage recoveries were found as 99.94 ± 0.42 with RSD of 1.10 %. (Table III).

TABLE III
The Results of Percentage Recovery Value in Synthetic Mixture of ZAF for Proposed Method (Added ZAF for Tablet; 20 mg) (n=7)

Found (20 mg)	Recovery %
19.72	98.60
20.18	100.90
19.96	99.80
19.84	99.20
20.34	101.70
19.82	99.10
20.06	100.30

\bar{x} : 19.99 ± 0.08
SD: 0.22
RSD %: 1.10

\bar{x} : Mean \pm standard error. SD: Standard deviation. RSD %: Relative standard deviation.

Specificity

The spectra obtained from tablet and synthetic tablet solution were identical with that obtained spectrum from standard solution containing an equivalent concentration of ZAF. Comparison of the UV spectra of ZAF standard, tablet and synthetic solutions showed that the wavelength of maximum absorbance of ZAF did not change (Figure 4). It was concluded that the excipients did not interfere with quantification of ZAF in this method and the proposed method could be considered specific.

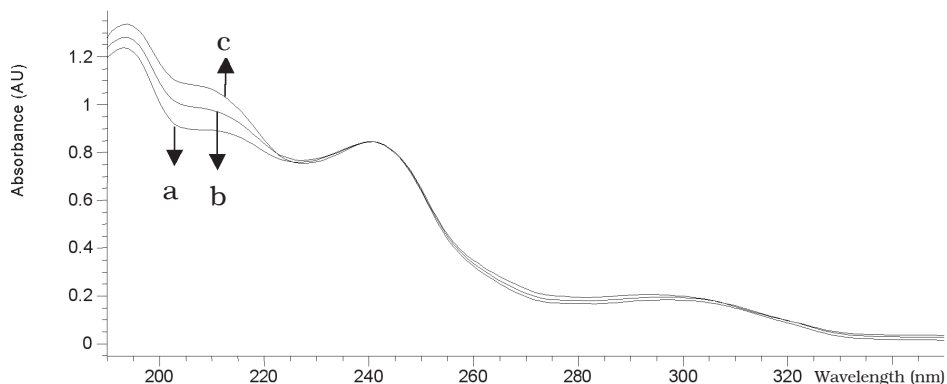


Figure 4

UV spectra of ZAF ($10.00 \mu\text{g mL}^{-1}$), a) standard ZAF solution, b) tablet solution, c) synthetic solution in acetonitrile:water (80:20, v/v).

In order to evaluate the excipients in this method, the standard addition method was applied¹⁹. The regression equation of standard addition method was found to be $y = 0.0893x + 0.8500$, $r = 0.9973$. There was no difference between the slopes of two methods with calibration curve and standard addition methods. These data showed that there was no spectral interaction in the analysis of ZAF in pharmaceutical formulations by the proposed method. Therefore, the calibration curve method, which is easier and quicker than the standard addition method, was used in quantitative analysis of ZAF. These values showed that no significant excipients interference, thus the procedures was able to determination of ZAF in the presence of excipients. In the proposed method, there was no need for pre-separation and only centrifugation was applied to make the solution clear.

Robustness

The robustness of the proposed method was examined by evaluating the influence of small variations of some of the most important proce-

dition variables such as acetonitrile:water ratio (78:22 and 82:18, v/v) and wavelength (240 nm and 244 nm)^{17, 22}. Each deliberate small change was analyzed 7 independent series containing 10.00 $\mu\text{g mL}^{-1}$ of ZAF changed. Only one parameter was changed in the experiments at a time. The statistically comparison was done with Friedman analysis and no difference was found between results ($p = 0.062 > p = 0.05$) (Table IV). The results obtained from the various conditions were not different compared to the optimum conditions and none of these variables significantly affected the assay of ZAF and the proposed method could be considered robust.

TABLE IV
The Robustness Data of Developed Method (n=7)

	Found ($\mu\text{g mL}^{-1}$)	RSD %
Standard (10.00 $\mu\text{g mL}^{-1}$)	10.25 \pm 0.05	1.41
Acetonitrile:water (78:22, v/v)	10.25 \pm 0.07	1.71
Acetonitrile:water (82:18, v/v)	10.25 \pm 0.06	1.55
Wavelength (240 nm)	10.32 \pm 0.06	1.42
Wavelength (244 nm)	9.96 \pm 0.05	1.42
Friedman analysis : $p = 0.062 > p = 0.05$		

\bar{x} : Mean \pm standard error. RSD %: Relative standard deviation

Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10.00 $\mu\text{g mL}^{-1}$ of ZAF using the same instrument by two different analysts under the same optimized conditions at different days^{17, 19, 21}. The obtained results were found to be reproducible, since there was no significant difference between analysts ($p = 0.075 > p = 0.05$) (Table V). Thus, the proposed methods could be considered rugged.

Analysis of pharmaceutical formulations

The optimized spectrophotometric method was applied to the direct determination of ZAF in tablet using calibration curve method without any sample extraction or filtration. The results show that the proposed method was successfully applied for the assay of ZAF in its pharmaceu-

TABLE V
The Ruggedness of Proposed Method (Added of ZAF amount of 10.00 $\mu\text{g mL}^{-1}$) (n=7)

1. Analyst Found ($\mu\text{g mL}^{-1}$)	2. Analyst Found ($\mu\text{g mL}^{-1}$)
\bar{x} : 9.96 \pm 0.02 SD: 0.04 RSD %: 0.40	\bar{x} : 10.06 \pm 0.05 SD: 0.12 RSD %: 1.19

Wilcoxon Paired Test: $p = 0.075 > p = 0.05$

\bar{x} : Mean \pm standard error. RSD %: Relative standard deviation.

tical formulations (Table VI). The amounts of ZAF in tablets were calculated using calibration curve method. A derivative spectrophotometric method in literature was employed as a comparison to evaluate the validity of the developed methods⁸. The statistical comparison of methods was done by Wilcoxon Paired Test ($p = 0.735 > p = 0.05$). The experimental values did not exceed the theoretical ones indicating a good agreement with comparison method.

TABLE VI
The Results of Pharmaceutical Formulations Containing ZAF Analyzed by Proposed Method (n=7)

Proposed Method Found (mg)	Derivative Spectrophotometric Method (Compared Method) [8] Found (mg)
19.98	19.83
19.88	20.30
20.34	19.81
19.96	20.03
20.30	20.51
20.52	21.10
20.50	20.18
\bar{x} : 20.21 \pm 0.10 SD: 0.27 RSD %: 1.34	\bar{x} : 20.25 \pm 0.17 SD: 0.45 RSD %: 2.22

Wilcoxon Paired Test: $p = 0.735 > p = 0.05$

Conclusion

In this study a simple, fast and reliable UV spectrophotometric method was developed and validated for the determination of ZAF in pharmaceutical formulations. This method was applied directly to the analysis of pharmaceutical dosage forms without the need for separation or complex sample preparation such as extraction steps prior to the drug analysis.

As this proposed method has the lowest LOD value and wider linear range is more sensitive than the other published derivative spectrophotometric method⁸.

From the results obtained, we concluded that the suggested method showed high sensitivity, accuracy, reproducibility and specificity. Moreover, this method is simple and inexpensive and it can be employed for the routine quality control of ZAF in pharmaceutical formulations.

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Summary

UV Spectrophotometric Determination of Zafirlukast in Pharmaceutical Formulations

A simple, rapid, reliable and fully validated UV spectrophotometric method was developed for determination of zafirlukast in pharmaceutical formulations. The analysis was carried out in acetonitrile:water (80:20, v/v) solution. The absorbance of zafirlukast was measured at 242 nm in the wavelength range of 190 - 350 nm. The linear calibration range was found to be 0.50 - 20.00 $\mu\text{g mL}^{-1}$ and limit of quantification was 0.50 $\mu\text{g mL}^{-1}$ for the proposed UV spectrophotometric method. This method was validated and applied to the determination of zafirlukast in tablets. No interference was found from tablet excipients at the selected wavelength and analysis conditions. The obtained data from developed method was compared with the derivative spectrophotometric method in the literature and no significant difference was found statistically. It was concluded that the developed method was sensitive, accurate, precise, selective, robust and rugged.

Keywords: Zafirlukast, UV spectrophotometry, validation, tablet analysis

Özet

Farmasötik Formülasyonlarda Zafirlukast'ın UV-Spektrofotometrik Tayini

Bu çalışmada, farmasötik formülasyonlardaki zafirlukastın tayini için basit, hızlı, güvenilir ve tamamen valide edilmiş bir UV spektrofotometrik yöntem geliştirilmiştir. Analiz, asetonitril:su 80:20, h/h) çözeltisinde yapılmıştır. Zafirlukastın absorbansı 190-350 nm dalga boyu aralığında 242 nm'de ölçülmüştür. Önerilen UV spektrofotometrik yöntem için, doğrusal olduğu kalibrasyon aralığı 0.50 - 20.00 µg mL⁻¹ bulunmuştur, ve alt tayin sınırı 0.50 µg mL⁻¹'dir. Bu yöntem valide edilmiş ve tabletlerdeki zafirlukastın tayinine uygulanmıştır. Analiz koşullarında ve seçilen dalga boyunda tablet yardımcı maddelerinden gelen bir girişim bulunmamıştır. Geliştirilen yöntemden elde edilen veriler, literatürdeki türev spektrofotometrisinden elde edilenler ile karşılaştırılmış ve aralarında istatistiksel olarak anlamlı fark bulunmamıştır. Geliştirilmiş yöntemin duyarlı, doğru, kesin, seçici, sağlam ve tutarlı olduğu sonucuna varılmıştır.

Anahtar kelimeler: Zafirlukast, UV spektrofotometri, validasyon, tablet analizi

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