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Determination of zafirlukast by stability indicating LC and derivative spectrophotometry

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Abstract

Two analytical methods have been developed for the determination of zafirlukast, a novel anti-asthmatic drug: high performance liquid chromatography (HPLC) and derivative spectrophotometry (DS). HPLC with ultraviolet detection at 225 nm is carried out with a Symmetry Shield RP18 column and a mobile phase constituted of acetonitrile and 0.01 M potassium dihydrogen phosphate buffer, adjusted the pH to 3.5 with 0.1 M KOH. The LC method is simple, rapid, selective and stability indicating. Indole was used as internal standard for the purpose of quantification of zafirlukast in HPLC. Spectrophotometry uses the third order derivative of the UV spectrum at 251.1 nm ($\Delta\lambda$ value 2.1 nm) for determination. Both methods were fully validated and a comparison was made. The results confirm that the methods are highly suitable for its intended purpose.

Keywords: Zafirlukast; HPLC; Derivative spectrophotometry; Assay; Formulation; Validation

1. Introduction

Anti-leukotrienes drugs have recently become available for the clinical management of asthma and they functioning either by blocking the interaction of leukotrienes with receptors or by inhibiting leukotriene synthesis [1].

Zafirlukast, a specific cysteinyl leukotriene receptor antagonist, developed by Zeneca Pharmaceuticals, with the chemical name 4-5(-cyclopentyl-oxy-carbonylamino-1-methyl-indol-3-ylmethyl) -3-methoxy-*n*-*o*-tolylsulfonylbenzamide (Fig. 1). FDA has approved it, for use in the prophylaxis and chronic treatment of asthma in adults and children above 12 years [2].

Only a few chromatographic methods have been appeared in the literature. Furthermore, Khanh H. Bui et al. reported a normal phase HPLC method for the determination of zafirlukast in human plasma using fluorescence detection [3]. Ficarra et al. described a LC method for the analysis zafirlukast in a pharmaceutical formulation [4]. Quantitative determination of zafirlukast using HPLC in human plasma during the comparison of zafirlukast (Accolate) absorption after oral and colonic administration was

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published by Fisher Jeff D et al. [5]. According to International Conference on Harmonization (ICH), the analytical test procedures for stability samples should be fully validated and the assays should be stability indicating [6]. However, none of the above reported methods is stability indicating and therefore cannot be used for the analysis of stability batches.

Derivative Spectrophotometry (DS) [7–9] is an analytical technique of great utility for extracting both quantitative and qualitative information from spectra composed or unresolved. It has proved particularly useful in eliminating matrix interference in the assay of many drugs [10]. DS able to enhance the resolution of overlapping absorption bands and to discriminate sharp bands over large bands [11]. This ability has been conveniently applied to the analysis of active ingredients in formulations. These methods posses advantage of simple operation, fast analysis, low consumable costs and high sample throughput. Hence derivative spectrophotometric methods can be used for the determination of assay in content uniformity tests, in dissolution tests and in other similar in-process tests of pharmaceutical formulations. Hitherto, there is no reported literature for the assay determination of zafirlukast in their commercial formulations using derivative UV spectrophotometric method.

Hence an attempt has been made to develop a stability indicating LC procedure for the determination of zafirlukast in bulk drug and its commercial formulations; and also to find simple, in-expensive and precise derivative spectrophotometric method, equivalent to more time consuming HPLC method for release testing of zafirlukast.

2. Experimental

2.1. Chemicals

Sample of zafirlukast was received from Dr Reddy's laboratories, Hyderabad, India. HPLC grade acetonitrile was obtained from Merck, USA. Tablets of Accolate (equivalent to 20 mg of zafirlukast) of Zeneca pharmaceuticals were purchased through local market. Analytical grade potassium dihydrogen phosphate were obtained from Pro analysis, E Merck (India) Ltd Ortho phosphoric acid was obtained from Qualigens, India. High purity water was prepared by using Waters Milli -Q plus purification system.

2.2. Instrumentation

2.2.1. High performance liquid chromatography

The LC system consisted of a Waters 510 pump, a Rheodyne injector equipped with a 10 μl sample loop, and a Photodiode array detector (Waters 996). The output signal was monitored and integrated using MILLENIUM 2010 Chromatography Manager software (Waters).

2.2.2. Derivative UV spectrophotometry

Spectrophotometric analysis was performed on Chemito UV 2500-a micro computer controlled double beam recording UV Visible spectrophotometer using 10 mm quartz cells with a slit width of 2 nm and scan speed of 480 nm min^{-1}. The mean response time is 0.02 s. in spectrum mode. The $\Delta\lambda$ values were selected in accordance with nature of the analyte. The Smoothing routine supplied by the manufacturer uses 17 data points.

2.3. Solutions

2.3.1. Liquid chromatography

2.3.1.1. Mobile phase preparation. Mobile phase was prepared by mixing 300 ml of aqueous 0.01 M potassium dihydrogen phosphate buffer with 700 ml of acetonitrile and pH of the resultant mixture was adjusted to 3.5 with *o*-phosphoric acid. The mobile phase was filtered and degassed.

2.3.1.2. Standard preparations. Standard solutions of zafirlukast were prepared using its mobile phase. The working concentration of zafirlukast for the determination of assay was 150 μg ml^{-1}. Internal standard concentration was maintained at 200 μg ml^{-1} in each solution of zafirlukast that was used for validation studies.

2.3.1.3. Sample preparations. Twenty weighed tablets of Accolate (each tablet contains 20 mg of zafirlukast) were ground to a fine powder. The amount of powder equivalent to 20 mg of the active compound was extracted with acetonitrile and centrifuged. The supernatant was diluted with mobile phase to required concentrations and analyzed.

2.3.2. Derivative spectrophotometry

2.3.2.1. Preparation of standard solutions of zafirlukast. Stock solution of zafirlukast was prepared in water and acetonitrile mixture (20:80) by dissolving about 15 mg in 50 ml volumetric flask. Standard solutions were prepared by diluting the stock solution with same solvent.

2.3.2.2. Preparation of zafirlukast samples from accolate tablets. About 20 tablets of Accolate (each tablet contains 20 mg of zafirlukast as API) were weighed and thoroughly powdered. The amount of powder equivalent to about 15 mg of zafirlukast was placed in a 50 ml volumetric flask. To it around 40 ml of solvent (acetonitrile–water, 80:20 v/v) was added and the flask was placed in an ultrasonic bath for 15 min. The solution was then cooled and diluted to volume with the same solvent. The solution was filtered though a 0.45 μm filter and then the filtrate were used to prepare sample solutions of different concentrations.

2.4. Conditions

2.4.1. Chromatographic conditions

HPLC separations was carried out by a Symmetry Shield RP18, 5 μm, 250 × 4.6 mm column (Waters make). The mobile phase flow rate was 1.0 ml min^{-1}. The analysis was carried out at ambient temperature (~ 22 °C). The sample injection volume was 10 μl. The photodiode array detection was made at 223 nm for the determination of zafirlukast.

2.4.2. Measurement of derivative spectra

For zafirlukast solutions, the third derivative spectra were recorded in the wavelength range 200–300 nm using acetonitrile–water mixture (in the ratio 80:20, v/v) as reference. The instrument settings were optimized to produce a spectrum with about 80% full-scale deflection and acceptable noise level. Each spectrum was recorded in triplicate. For each replicate measurement the cell was refilled with fresh solution.

3. Results and discussion

3.1. HPLC

3.1.1. Method development

The HPLC procedure was optimized with a view to develop a stability indicating method so as to resolve the degraded products from the drug. Various mobile phase compositions, columns of different packing materials (C_{18}, C_8, phenyl), and configurations (10, 15, and 25 cm columns) were tried so as to obtain a sharp peak and also resolve the peaks of degraded products (Section 3.1.2.1) from the peak of drug. The mobile phase consisting of 0.01 M aqueous KH2PO4 buffer and acetonitrile in the ratio 30:70 (v/v), pH adjusted to 3.5 was found to be an appropriate mobile phase on Symmetry Shield RP18, 250 mm column at a flow rate of 1.0 ml min^{-1}. In the proposed system zafirlukast peak was eluted with a capacity factor (k') 4.58, tailing factor (T) 1.02 and number of theoretical plates (N) 12715.

3.1.1.1. Purity estimation of zafirlukast. For purity estimation of a real sample of zafirlukast, a high–low chromatography technique [12] was employed. In this technique a high concentrated (3.5 mg ml^{-1}) sample was injected without ignoring about exceeding the linear range of main component (API). The sensitivity towards low level impurities was thus increased by more than five times and the response of each impurity was recorded. Weight percentage of each impurity present in the sample was calculated by comparing its response to response of dilute zafirlukast (0.5%), prepared from concentrated sample (3.5 mg ml^{-1}). A chromatogram of zafirlukast using high–low chromatography and its corresponding spectrum index plot using PDA detection is shown in Fig. 2. The impurities from UK1

to UK6 eluted at retention times 5.8, 6.1, 7.3, 18.9, 25.1 and 37.8 min, respectively, were not characterized (Fig. 2). UV absorption spectra of these impurities are shown in the same figure. The detected impurities and zafirlukast show significant UV absorbance at wavelength 223 nm. Hence this wavelength was selected for analysis.

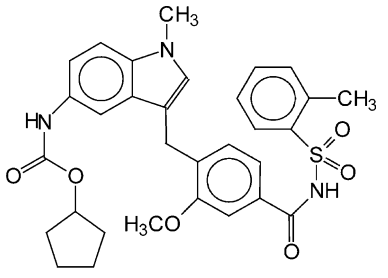
3.1.2. Method validation

The described method has been extensively validated for assay determination of zafirlukast using the following parameters. 5-methyl 2-nitrophenol was used as internal standard for the purpose of quantification of zafirlukast.

3.1.2.1. Specificity. Complete resolution of zafirlukast from its related compounds, with no apparent shoulders (Fig. 2) confirms the specificity of the described method.

The use of photodiode array detection technique in the analysis itself is an evidence of the specificity of the method. Chromatographic peak purity was determined using wavelength comparisons at 223 versus 238 nm [13]. The plot with a flat top indicates homogeneity and no detectable impurities embedded in it.

Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Zafirlukast samples were refluxed separately with 0.1 N NaOH and 0.1 N HCl at 60 ± 1 °C for 12 h and then neutralized with HCl or NaOH. The solutions were further diluted to required concentration with mobile phase and analyzed. The sample refluxed with 0.1 N HCl was degraded to several products as shown in Fig. 3. But with 0.1 NaOH, zafirlukast was mainly converted to a degradation product that was eluted at 2.7 min in Fig. 3. In both cases the degradation products were well resolved from zafirlukast peak.



zafirlukast

Fig. 1. Structure of zafirlukast.

In another experiment, two separate solutions of pure zafirlukast (0.2 mg ml^{-1} in mobile phase) were prepared. One solution was exposed to ultraviolet light (254 nm) for 24 h, and the other was kept at 60 °C temperature for 12 h. The samples were analyzed. The samples exposed to UV light or subjected to high temperature did not give any degradation products. All the degraded samples were subjected to photo-diode array analysis for peak purity of zafirlukast. The plot reports in all instances showed that zafirlukast peak had no detectable impurity peaks embedded in and are free of co-eluting degradation compounds. From above results, it is clear that the method can be used for determining the stability of zafirlukast in bulk and pharmaceutical formulations.

3.1.2.2. Linearity. Linearity was checked at five different concentration levels ranging from 30 to 175 μg ml^{-1}. The linearity was also checked for 3 consecutive days for the solutions of same concentrations prepared from the stock solution. The equation for calibration curve is $y = 0.01975x - 0.0107$ with a correlation coefficient equal to 0.999. The cross-validated R_2 was also calculated and found to be more than 0.996, indicating good linearity.

3.1.2.3. Precision

System repeatability. The system repeatability was determined from six replicate injections of a sample solution of zafirlukast at the analytical concentration of about 150 μg ml^{-1}. The relative standard deviation (R.S.D.) for the active compound was found to be 0.3%.

Method repeatability/intermediate precision. Method repeatability/intermediate precision was assessed by the assay of two, six-sample sets by two different analysts using different chromatographic systems on different days. The results are summarized in Table 1. The R.S.D. values of repeatability/intermediate precision shows that the proposed method provides acceptable precision.

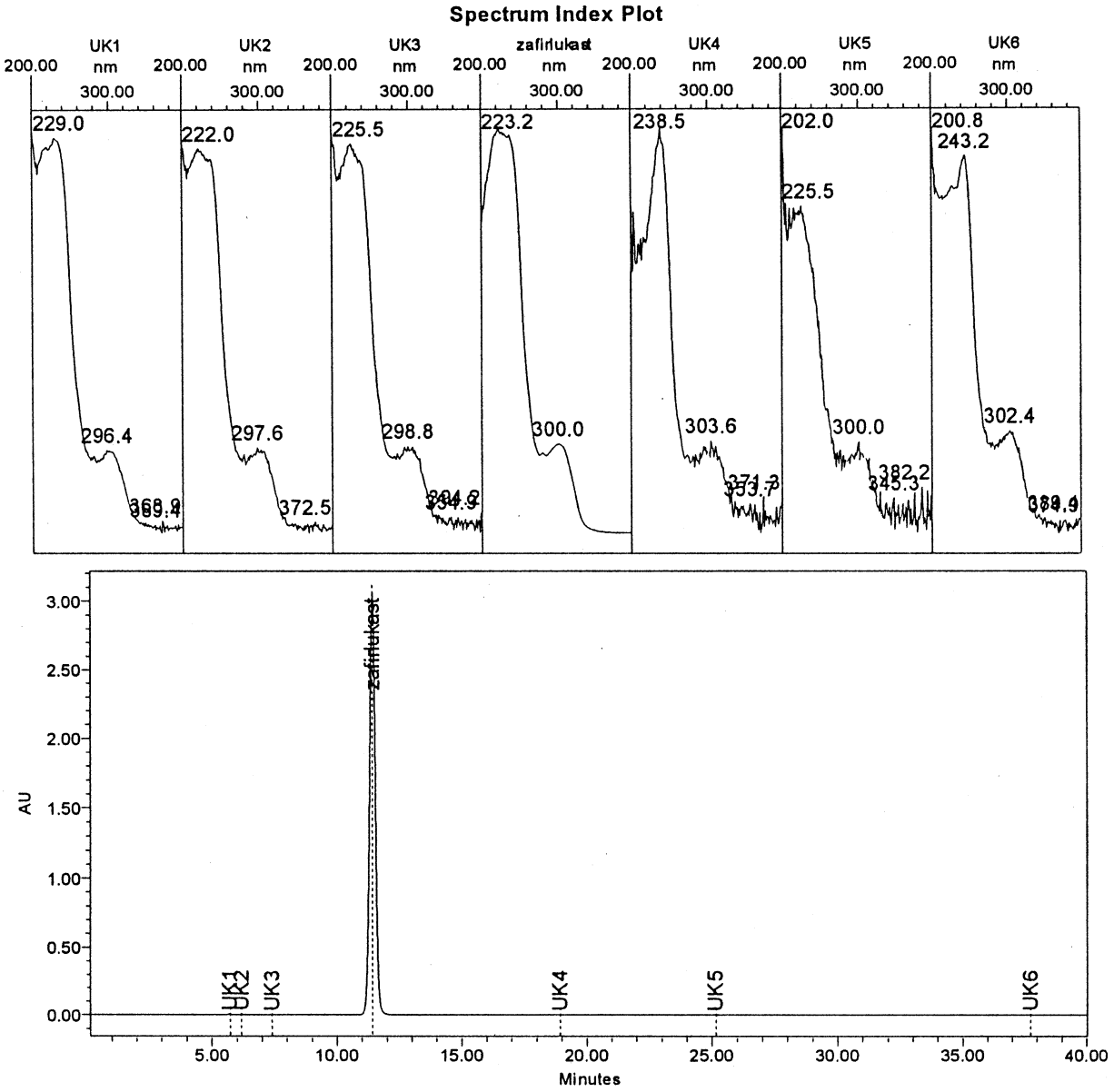


Fig. 2. High-low chromatogram of zafirlukast with spectrum index plot for all components using PDA detection.

3.1.2.4. Accuracy/recovery. Accuracy of the method for assay determination was checked by spiking pre-analyzed samples with three different concentration levels, i.e. at 40, 80, and 160 μg of standard zafirlukast and the mixtures were reanalyzed by proposed method ($n = 3$). The mean recovery data (at 95% confidence limits) obtained for each level as well as its percentage recoveries are tabulated in Table 2. Further the accuracy of the method was checked by F-test using a reported method [4] as reference. The calculated value of F-test of variance ratio less than the table value indicates that the developed method and reference methods not to differ significantly in terms of precision.

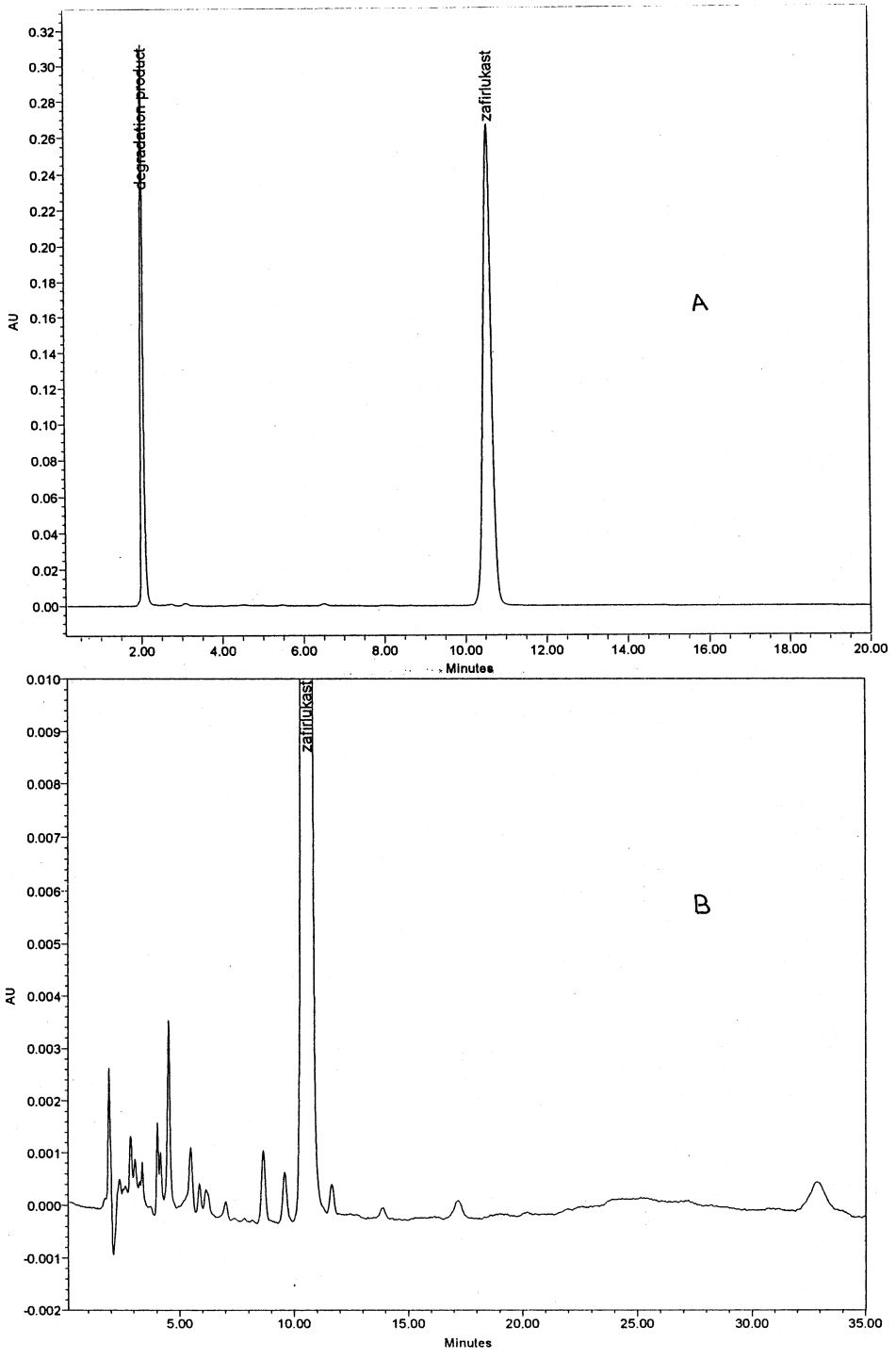


Fig. 3. (A) Separation of zafirlukast from its base degradation product. (B) Chromatogram of zafirlukast and its acid degradation products.

Table 1
LC method repeatability/intermediate precision

Sample	Analyst-1 mg per tablet	Analyst-2 mg per tablet
Repeatability		
1	20.15	19.87
2	20.23	20.11
3	19.89	20.05
4	20.09	19.96
5	19.92	19.81
6	19.84	20.18
Mean ($n=6$)	20.02	19.99
(%) R.S.D.	0.79	0.71
Intermediate precision		
Mean ($n=12$): 20.01		
(%) R.S.D.: 0.72		

3.1.3. Assay determination of zafirlukast from accolate tablets

The assay results of samples from Accolate tablets at five different independent concentrations were comparable with claimed values (99.1–100.8%). Zafirlukast and the internal standard were well separated from the excipient peaks in the formulation sample. A typical LC chromatogram is shown in Fig. 4.

3.2. Derivative spectrophotometric method

The derivative order was selected by studying the first-, second-, third- and fourth order derivative spectra of zafirlukast. The study revealed that the third derivative spectra were simple, and gave results of highest accuracy with suitable precision and lowest detection limits at $\Delta\lambda$ value 2.1 nm. In the third derivative spectrum of zafirlukast (Fig. 5), amplitude from the valley at a wavelength of 251.1 nm to the zero-base line was chosen in the present work for quantitative studies of active drug.

3.2.1. Calibration graph

To obtain a calibration curve for zafirlukast, the third derivative spectra of standard solutions were recorded at five varied concentrations (3.2–16 μg ml^{-1}). The equation for calibration is $Y = 0.003344x + 0.0039$ with a correlation coefficient equal to 0.999. The cross-validated R_2 was and found to be more than 0.999, indicating good linearity.

3.2.2. Accuracy

Accuracy of the third derivative method for zafirlukast was checked at independently prepared concentration levels, i.e. at 4, 8 and 12 μg each in triplicate. The mean recovery data for each level (at 95% confidence limits) and its percentage recoveries are presented in Table 3.

3.2.3. Assay specificity

Recovery experiments were carried out by adding the active drug in solution to mixtures of commonly used pharmaceutical excipients and additives in the appropriate quantities used in the preparation of dosage forms such as starch, talc, stearic acid, gelatin, magnesium carbonate and sodium lauryl sulfate. Percent recoveries were ranged from 98.23 to 101.64. Mixture of excipients alone gave only a baseline spectrum at the detection wavelength of the compound.

3.2.4. Application to dosage forms

The final conditions established above for the assay of zafirlukast in pharmaceutical dosage forms gave results in excellent agreement with

Table 2
Accuracy in the assay determination of zafirlukast by LC method

Sample	Taken (μg)	Recovery (μg) ($n=3$) (mean $\pm$ $t(s/\sqrt{n})$[a]	Percentage of recovery
1	40.2	40.1 ± 0.74	99.7
2	80.3	80.0 ± 1.14	99.6
3	158.6	158.8 ± 1.38	100.1

[a] s, S.D.; $t = 4.30$ (at 95% confidence limit and $n-1$ degrees of freedom).

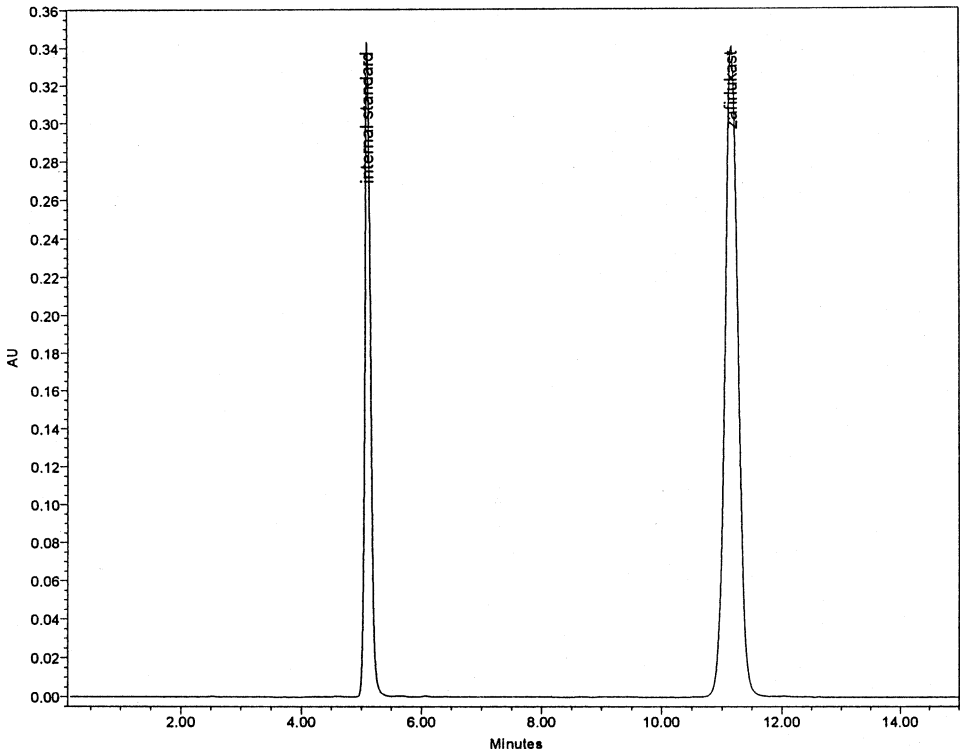


Fig. 4. Chromatogram of formulated zafirlukast (Accolate 20 mg) with internal standard.

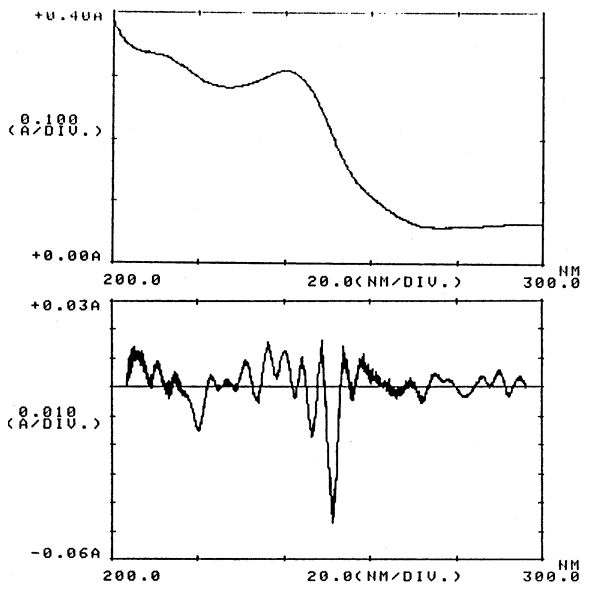


Fig. 5. Zero and third derivative spectra of zafirlukast.

those obtained using an independent referee method based on liquid chromatography (described above) are shown in Table 4. The results obtained from proposed method and from HPLC method were compared statistically with the student's t test and F-tests and were found that these methods not to differ significantly in precision and accuracy.

Table 3
Accuracy in the assay of zafirlukast by derivative spectrophotometry

Taken (μg)	Recovery (μg) ($n=3$) (mean $\pm$ $t(s/\sqrt{n})$[a]	Percentage of recovery
4.03	4.08 ± 0.09	101.2
7.95	7.92 ± 0.13	99.6
12.12	12.04 ± 0.13	99.3

[a] s = S.D.; $t = 4.30$ (at 95% confidence limit and $n-1$ degrees of freedom).

Table 4
Assay of zafirlukast in pharmaceutical formulation

Labeled amount (mg per tablet)	By derivative spectrophotometric method (mg)[b]	By HPLC method (mg)[b]	t-test[a]	F-test[a]
20	20.25	20.12	1.258	2.98
	20.31	19.95		
	20.13	20.14		
	19.94	20.06		
	20.09	19.98		
Mean	20.144	20.05		
S.D.	0.1445	0.0837		

[a] Theoretical values at 95% confidence limit, $t = 2.31$, $F = 9.6$.
[b] $n = 3$.

4. Conclusion

HPLC and derivative spectrophotometric methods have been developed and validated for the quantitative determination of zafirlukast in bulk and in pharmaceutical dosage form. The developed HPLC method was found to be selective, precise and stability indicating. The derivative spectrophotometric method serves as an alternative method for the determination of API in commercial samples of zafirlukast. The assay results obtained by these two methods are in fair agreement. These methods can be used for the routine determination of zafirlukast in release testing.

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