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ORIGINAL ARTICLE

Validated stability-indicating methods for the determination of zafirlukast in the presence of its alkaline hydrolysis degradation product

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Abstract Three simple stability-indicating methods for the analysis of Zafirlukast (ZAF) in the presence of its alkaline degradation products were developed and validated as per the International Conference on Harmonization (ICH) guidelines to evaluate the stability-indicating power of the proposed methods. The developed high-performance liquid chromatographic technique was achieved on ZORBAX–ODS (5 μm, 150 × 4.6 mm, i.d.) by isocratic elution with a mixture of acetonitrile/0.05 M phosphate buffer, pH 5.0, (50:50; v/v) as a mobile phase at flow rate of 1.0 mL min^{-1}, followed by UV detection at 240 nm. The method could determine ZAF in the range of 2–40 μg mL^{-1} with a mean percentage recovery of 99.73 ± 0.903. The proposed HPLC method was utilized to investigate the kinetics of alkaline degradation of ZAF. First derivative of the ratio spectra (^{1}DD) method was applied to analyze the drug under investigation without any interference from its degradation product with a linearity range of 4–32 μg mL^{-1} and with a mean percentage recovery of 99.85 ± 0.608. A chemometric method was also developed using the partial least squares (PLS) model for selective determination of ZAF in the range of 4–40 μg mL^{-1}, the mean percentage recovery was found to be 100.00 ± 0.336.

1. Introduction

Zafirlukast (ZAF), chemically [4-(5-cyclopentyloxy-carbonylamino-1-methyl-indol-3-ylmethyl)-3-methoxy-*n*-*o*-tolylsulfonylbenzamide] is a novel selective peptide leukotriene receptor antagonist,[1] used as an antiasthmatic drug in the prophylaxis and treatment of mild-to-moderate chronic asthma in adults and children.[2] ZAF is a competitive orally administered inhibitor of the cysteinyl leukotriene LTC_4, LTD_4 and LTE_4 in respiratory tracts.[3] By using zafirlukast as a single dose, bronchoconstriction caused by foreign allergens is inhibited and also decreases the bronchial hyper responsiveness to the inhaled histamine. Bioavailability is reduced when administrated after a high fat or protein meal. It is highly bounded to plasma proteins especially albumin (99%). It is metabolized in the liver by the cytochrome P450 enzyme. Its half life is about 10 h and the onset of action was seen in 1 h and takes

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3 h to reach peak plasma concentration. ZAF is eliminated mainly through feces (90%) and 10% only in the urine.[4,5]

Till date there are only few analytical methods reported for the estimation of ZAF in pharmaceutical preparations and in biological fluids. These methods include high performance liquid chromatography,[6–10] capillary electrophoresis,[11] spectrophotometry,[7,12] and electrochemical methods.[13,14]

Nowadays, investigation on the chemical stability and kinetics of decomposition of drugs is an essential matter to the quality control of pharmaceuticals and to understand the degradation pathway of drugs which is important to evaluate the product's shelf-life period. Up to now no literature review is reported for kinetic studies of the degradation process of ZAF, so the presented work is concerned to investigate the kinetics of the alkaline degradation process of the cited drug using the proposed HPLC method.

The aim of the present work is to develop simple, sensitive and selective stability-indicating methods for the quantitative determination of Zafirlukast in the presence of its alkaline degradation product and in pharmaceutical formulations. This was achieved by developing different techniques including HPLC, first derivative of the ratio spectra and PLS mathematic methods.

2. Experimental

2.1. Instrumentation

High performance liquid chromatography composed of a quaternary pump (1200 series, G 1311A) with an ultraviolet variable wavelength detector, 1200 series (Agilent Technologies, Waldbronn, Germany) and equipped with a 20-μl injector loop manual injector (model 7725 I USA), Dual-beam UV–visible spectrophotometer, (UVProbe 1800 version 2.32 Shimadzu, Kyoto, Japan) with matched 1-cm quartz cells, connected to an IBM compatible personal computer (PC) and a HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software version 3.7, was used to process the absorption and the derivative spectra. The data were then exported into MICROSOFT EXCEL program. The chemometric calculations were performed in Matlab for Windows™ version 7 Mathworks Inc. 2004. The PLS procedure was taken from PLS Toolbox 2.1, Eigenvector Research Inc. 2001 created by B.M. Wise, N.B. Gallagher for use with Matlab.

2.2. Materials and reagents

Pure samples of Zafirlukast were kindly supplied by DELTA PHARMA S.A.E, Tenth of Ramadan City, A.R.E.

The purity of the samples was found to be 100.43 ± 0.735% ($n = 5$) according to the reported method.[7]

Ventair® tablets, Batch No. 06093, labeled to contain 20 mg of Zafirlukast per tablet; manufactured by DELTA PHARMA S.A.E, Tenth of Ramadan City, Egypt.

Acetonitrile and methanol (HiPer Solv®, HPLC grade, E. Merck, Darmstadt, FRG).

All other chemicals were of analytical grade.

2.3. Standard solutions for the drug

Standard stock solutions of ZAF (800 μg mL^{-1}) were prepared by dissolving the pure sample in acetonitrile.

For the HPLC-method, a working standard solution (80 μg mL^{-1}) was prepared in the described mobile phase by 10-times dilution of the stock solution. While, other working standard solutions (80 μg mL^{-1} and 40 μg mL^{-1}) were prepared in acetonitrile for the first derivative of ratio spectra method (^{1}DD) and chemometric method, respectively.

2.4. Preparation of the alkali-induced degradation product

Accurately weighed 80 mg of ZAF was dissolved in 20 mL acetonitrile. Subsequently, 25 ml 1 M sodium hydroxide was added and the solution was heated in a temperature controlled oven at 100 °C for 2.5 h. The solution was concentrated nearly to dryness under vacuum, cooled to room temperature (~25 °C), then quantitatively transferred into a 100-mL measuring flask and the volume was completed with acetonitrile. Complete alkaline degradation of the studied drug was confirmed by the proposed HPLC method, where no peaks corresponding to intact drug were detected in case of the degraded samples.

Structural elucidation of the obtained degradation product was achieved by IR and Mass spectrophotometry.

2.5. Analytical techniques

2.5.1. Solution stability

The solution stability of zafirlukast was evaluated by leaving the standard solutions (20 μg mL^{-1} in acetonitrile) in tightly capped volumetric flasks, protected from light on a laboratory bench and in the refrigerator. The stability of studied compound solutions was checked by the proposed HPLC method.

2.5.2. Calibration curve for HPLC method

A series of standard solutions containing 2–40 μg mL^{-1} ZAF, were prepared by suitably diluting aliquots of the working standard solution of ZAF using acetonitrile/water (50:50; v/v).

The chromatographic separation was carried out at ambient temperature on a ZORBAX–ODS (150 × 4.6 mm, i.d.), particle size (5 μm), (Agilent Technologies, Waldbronn, Germany), isocratically at 1.0 mL min^{-1} with a mobile phase consisting of a mixture of acetonitrile/0.05 M phosphate buffer, pH 5.0, (50:50; v/v). The mobile phase was filtered through a 0.45 μm Millipore membrane filter and was degassed for ~15 min. in an ultrasonic bath prior to use. To reach equilibrium, the analysis was usually started after the passage of 50–60 mL of the mobile phase. The eluted analytes were detected at 240 nm, with a sensitivity of 0.001 AUFS (Absorbance Unit Full Scale).

Triplicate 20-μL injections were made for each solution and the peak area ratios of the cited drug to 12 μg mL^{-1} ZAF as an external standard were plotted against the corresponding concentrations to obtain the calibration graph.

2.5.3. Calibration curve for first derivative of ratio spectra method (^{1}DD)

Accurately measured volumes of ZAF stock solution (0.5–4 mL) were transferred separately into 10-mL calibrated flasks, diluted to volume with acetonitrile/water (50:50; v/v) to reach the concentration range of 4–32 μg mL^{-1}.The zero-order spectra of ZAF standard solutions were recorded using the same solvent as a blank and divided by the 8 μg ml^{-1} spectrum of the completely degraded drug substance. The first derivative

was then calculated for the obtained spectra with $\Delta\lambda = 2$ nm and scaling factor = 10 and the values at a maximum (232.4 nm) were measured. The calibration curve was constructed by relating the peak amplitudes to the corresponding concentrations and the regression equation was computed.

2.5.4. Training and validation sets for the PLS method

Different 20 mixtures of ZAF and its alkaline degradation product were prepared by transferring different volumes of their corresponding standard working solutions (40 μg mL^{-1}) into 10 ml measuring flasks, completing the volume with acetonitrile. Ten samples were used for calibration and the other ten samples were used as external validation set. The concentration ranges and the composition of the calibration and validation samples are given in Table 1.

The absorbances of these solutions were scanned between 200 and 400 nm and were exported to MATLAB® 7 for subsequent data manipulation. The suggested model was applied to predict the concentrations of ZAF in the validation samples.

2.5.5. Laboratory prepared mixtures

Aliquot portions (1–9 mL) of the intact ZAF working solution (40 μg mL^{-1}) were accurately transferred into a series of 10-mL measuring flasks. Different portions of the alkaline degradation product (40 μg mL^{-1}) were also added to prepare different mixtures containing 10–90% of the degradates, then the volume was completed with acetonitrile/water (50:50; v/v). The samples were analyzed by the proposed HPLC and ^{1}DD methods and the concentrations of the intact drug were calculated from the corresponding regression equations.

2.5.6. Application to pharmaceutical preparation

Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to about 20 mg of ZAF was accurately weighed, dissolved and diluted to 50 mL with acetonitrile. The sample solution was filtered. Further dilutions of the sample solution were carried out with acetonitrile/water (50:50; v/v) to reach the linearity range specified for the studied drug. The general procedures described under the construction of calibration curve were followed and the concentration of intact ZAF was calculated.

Table 1 The concentration of different mixtures of Zafirlukast and degradation product used in the training and validation sets.

Mixture number[a]	Percentage of degradation (%)	Intact ZAF (μg ml^{-1})	Degraded Z AF[b] (μg ml^{-1})
1	0	40	0
2	8.05	36	3.22
3	16.1	32	6.44
4	24.15	28	9.66
5	32.2	24	12.88
6	40.25	20	16.1
7	48.3	16	19.32
8	56.35	12	22.54
9	64.4	8	25.76
10	72.45	4	28.98
11	5.63	37.2	2.25
12	14.5	32.8	5.80
13	22.55	28.8	9.02
14	30.6	24.8	12.24
15	38.65	20.8	15.46
16	41.85	19.2	16.74
17	52	14	20.8

[a] Mixtures number 1–10 are those used for training set, while mixtures number 11–17 are those used for validation set.

[b] Concentrations of degraded samples are calculated on the basis of 1 M of intact drug that produces 1 M of degradation product.

2.5.7. Kinetic studies on the alkaline degradation of ZAF by the proposed HPLC method

Into a series of 10 test tubes, 2.5 mL of ZAF working solution 80 μg mL^{-1} was transferred and mixed with 2 mL of 1.0 M sodium hydroxide. The test tubes were allowed to stand in a thermostatically controlled oven at 100 °C and then were removed from the oven, one by one at 15 min time intervals up to 180 min. The test tubes were immediately inserted into an ice-bath to terminate the degradation reaction and then were put in another water bath set at room temperature.

The contents of the test tubes were transferred into 10 mL volumetric flasks and diluted to volume with acetonitrile/water (50:50; v/v). The proposed HPLC method was applied for the determination of the remaining intact ZAF at each time interval from its corresponding regression equation. A plot of log of the remaining concentration versus time in minutes was then performed to determine the kinetic order of alkaline degradation process.

For studying the effect of sodium hydroxide concentration on the reaction rate, the above procedure was followed using 0.5 and 1.0 M sodium hydroxide at 100 °C.

The effect of temperature on the reaction rate was also studied by following the above procedure using 1 N NaOH at different temperatures; 40, 60, 80, 90, and 100 °C.

3. Results and discussion

3.1. Identification of the degradation product

Zafirlukast is smoothly hydrolyzed with 1 M sodium hydroxide after 2.5 h at 100 °C, through the splitting of the ester group. The expected major degradation product (DG) is obtained according to the suggested mechanism for the alkaline degradation process of Zafirlukast,[15–18] (Fig. 1). The assignments and structural elucidation of the degradation product, were confirmed by the IR and mass spectral data. The IR spectrum (KBr) of DG was characterized by the absorption frequency of NH_2-band as a doublet at 3434.6 cm^{-1}. The mass spectrum of DG was characterized by the appearance of the molecular ion peaks at 463 m/z and 465 m/z (M & M^{+2}) which confirm the molecular weight of the suggested degradation product.

3.2. Method development and optimization

The quantitative determination of ZAF in the presence of its alkaline degradation product by conventional zero order spectrophotometry is completely hindered due to the strong spectral overlap throughout the wavelength range (Fig. 2).

The suggested methods used to resolve a complex mixture of such compounds are mainly HPLC, first derivative of ratio spectra and chemometric methods.

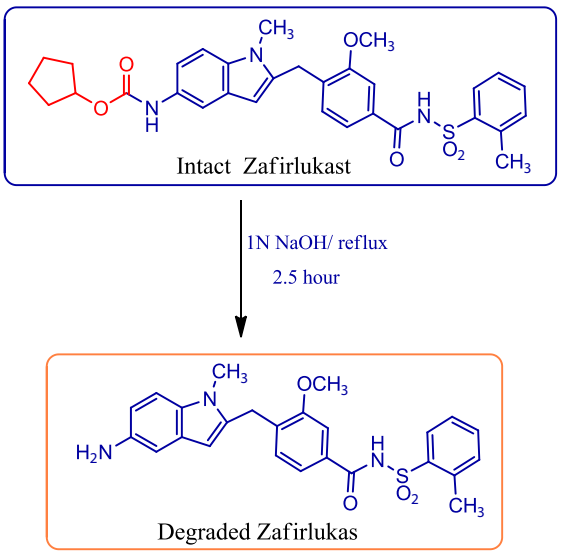

Figure 1 Scheme of alkaline degradation of ZAF.

3.2.1. HPLC method

3.2.1.1. System suitability. The developed HPLC method has been applied for the separation and determination of ZAF in the presence of its alkaline degradation product. To optimize the HPLC assay parameters, the mobile phase composition was studied. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile/0.05 M phosphate buffer, pH 5.0, (50:50; v/v) at an ambient temperature with a flow rate of 1.0 mL min^{-1}, followed by UV detection at 240 nm. All peak parameters of resolution efficiency were calculated to ensure that the system is working correctly during the analysis. In the proposed system zafirlukast peak was eluted with a resolution of 20.62, a capacity factor (k) 2.50, tailing factor (T) 1.05, a relative retention time 5.89 and number of theoretical plates (N) 5282.[19,20]

HPLC chromatogram of a mixture of intact and degraded ZAF sample is shown in Fig. 3, where complete baseline separation of ZAF and its degradation product was noticed. The average retention time for ZAF and DG, were found to be 5.6 ± 0.2 and 1.2 ± 0.3, respectively for 10 replicates.

3.2.1.2. Kinetic investigation. Investigation into the chemical stability of pharmaceutical products is a matter of growing concern in many analytical laboratories. This is because systematic kinetic studies of the decomposition of drugs using stability testing techniques are essential for the quality control of such products. In stability studies, drugs co-exist with their degradation products; thus it is necessary to use an appropriate analytical method to determine one of them in the presence of the other.[21,22]

In this work, we report a kinetic investigation of alkaline degradation of ZAF. Calculations have been based on the measurement of the concentration of the remaining intact drug using the previously described HPLC method.

The advantages of using HPLC over spectrometric methods in the kinetic study are its separation capabilities, identification, quantification and purification of individual components of the mixture providing higher specificity, reproducibility and sensitivity. In addition, HPLC is an automated process that takes only a few minutes to produce results (~6 min in the present work). The results produced are of high resolution and are easy to read, and the tests are easily reproduced *via* the automated process.

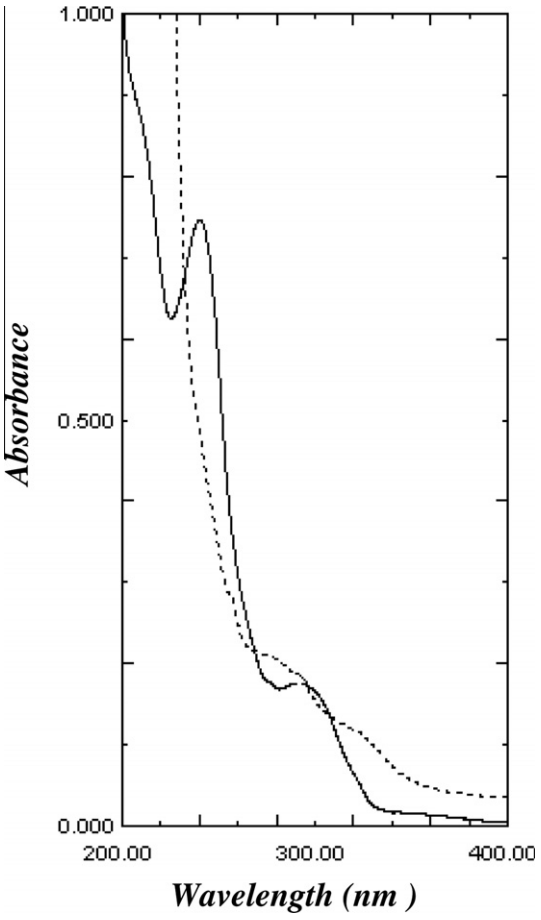

Figure 2 Zero-order spectra of pure Zafirlukast (—) and its degradate (- - -), each of 8 μg ml^{-1}.

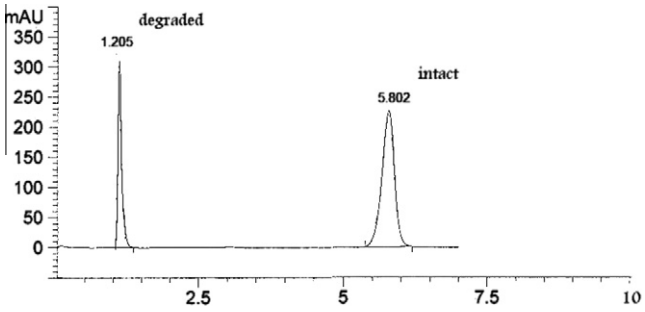

Figure 3 HPLC chromatogram of intact and degraded Zafirlukast, each of 20 μg ml^{-1}.

The kinetics of degradation of ZAF was investigated in 1 N sodium hydroxide to obtain reliable kinetic data. A regular decrease in the concentration of intact drug with increasing time intervals was observed. At the selected temperatures (40–100 °C), the alkaline degradation processes followed pseudo first-order kinetics (Fig. 4). From the slopes of the

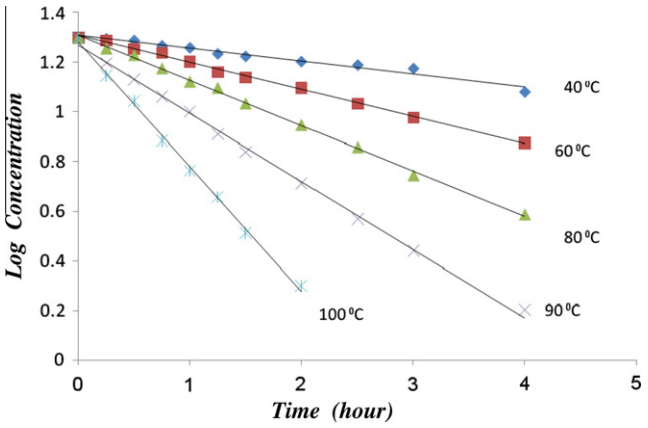

Figure 4 Pseudo first-order plot for the alkaline degradation of ZAF at various temperatures using the proposed HPLC method.

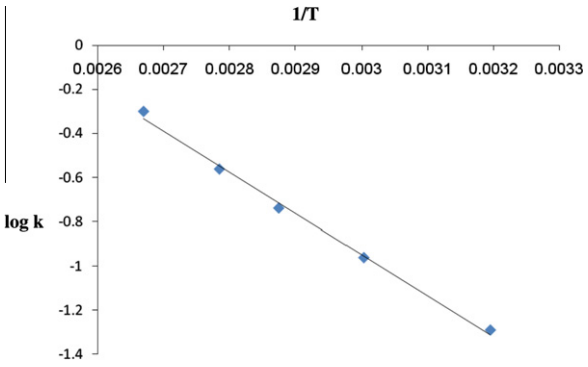

Figure 5 Arrhenius plot for the alkaline degradation of ZAF using the proposed HPLC method.

straight lines, it was possible to calculate the degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for alkaline degradation processes of ZAF (Table 2).

The effect of temperature was studied and Arrhenius plot (Fig. 5) was obtained by plotting log K_{obs} values versus $1/T$, which was found to be linear in the temperature range of 40–100 °C for alkaline degradation of ZAF. Effect of NaOH concentration (0.5, 1 N) was studied by plotting log of the remaining concentration of intact ZAF against time intervals in minutes. It was found that the reaction rate was increased by the effect of 1 N NaOH. The results of kinetic data and regression parameters are calculated and listed in Table 2.

3.2.2. ^{1}DD Spectrophotometric method

As shown from the zero-order spectra (^{0}D) of the drug and its alkaline degradates (Fig. 2); there is a great overlapping which makes the direct determination of the drug in the presence of its degradation product very difficult.

The technique of first derivative of the ratio spectra (^{1}DD) was proposed and applied as a sensitive, rapid and selective spectrophotometric method for the determination of zafirlukast in the presence of up to 90% of the degradation product, using a degradate spectrum of 8 μg ml^{-1} as a constant divisor and first derivative of the obtained spectra was done and measured at a maximum of 232.4 nm (Fig. 6).

Correct choice of the divisor concentration is playing an important role in the ^{1}DD method, regarding selectivity and sensitivity. Different degradate spectra (4, 8, 16, 32 μg mL^{-1}) were tried as a divisor, it was found that a degradate spectrum of 8 μg mL^{-1} is the best one which gives highest sensitivity and lowest peak noise.

3.2.3. Chemometric method

3.2.3.1. Method optimization. A stability indicating method based on multivariate calibration model namely Partial least squares (PLS) was investigated for the selective determination of zafirlukast in the presence of its alkaline degradation products and in pharmaceutical dosage forms.

A calibration set was designed with 10 calibration samples containing ZAF and DG and another seven samples were used for validation set in the ranges and concentrations shown in Table 1.

The UV spectra of the prepared solutions were recorded over the range 230–330 nm. Wavelengths (200–229 nm) dominated by noise and non informative spectral region after 330 nm are not included. Spectra were digitized each at 1.0 nm interval and the experimental data points were exposed to MATLAB® version 7.0 for calculations. The selection of the optimum number of factors for the PLS technique was a very important step before constructing the models because if the number of factors retained was more than the required, more noise will be added to the data. On the other hand, if the number retained was too small meaningful data that could be necessary for the calibration might be discarded. In this study the leave one out cross validation method was used[23,24] and the root mean square error of calibration values (RMSEC) of

Table 2 Kinetic results for the effect of temperature and NaOH concentration on the rate of alkaline degradation of 20 μg mL^{-1} of ZAF.

Temperature (°C)	K_{obs} (h^{-1})	$t_{1/2}$ (h)	Regression equation parameters (n = 8)[a]		
			Slope ± SD	Intercept ± SD	Correlation r^2
40	0.051	13.59	−0.052 ± 0.002	1.309 ± 0.253	0.9699
60	0.109	6.36	−0.110 ± 0.009	1.310 ± 0.366	0.9972
80	0.182	3.81	−0.183 ± 0.012	1.310 ± 0. 445	0.9982
90	0.274	2.53	−0.274 ± 0.024	1.2691 ± 0.545	0.9975
100	0.502	1.38	−0.502 ± 0.005	1.2808 ± 0.679	0.9973
NaOH concentration 0.5 M	0.120	5.78	−0.002 ± 0.015	1.342 ± 0.701	0.8653
1 M	0.480	1.44	−0.008 ± 0.003	1.304 ± 0.255	0.9915

[a] n, Number of experimental points included in the linear fit (log conc. versus time).

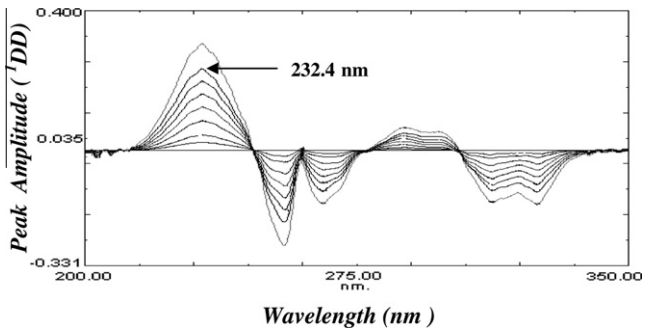

Figure 6 ^{1}D-ratio spectra of different concentrations (2–32 μg ml^{-1}) of Zafirlukast using a spectrum of 8 μg ml^{-1} of its degradate as a divisor.

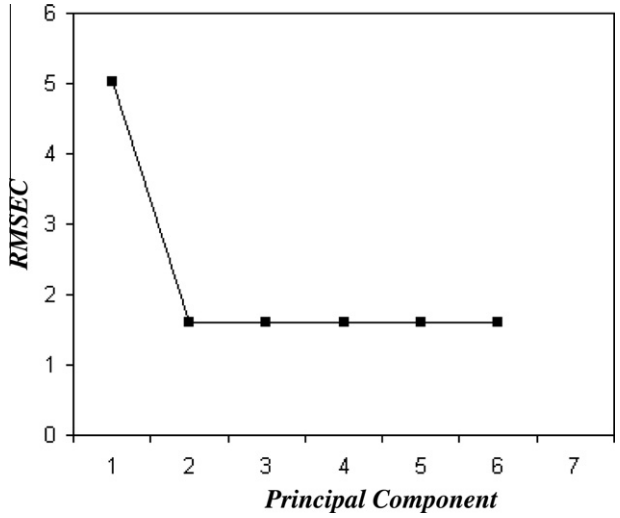

Figure 7 RMSEC plot of the cross validation results of the training set as a function of the number of principal components used to construct the PLS calibrations for Zafirlukast.

developed models was compared. Two factors were found suitable for PLS method (Fig. 7).

3.2.3.2. Validation of PLS- model. To assess the prediction ability of the suggested PLS- model, it was used to predict the concentration of ZAF in the presence of up to 52% of its degradation product in their laboratory prepared mixtures.

The validation of the suggested model was done using several diagnostic tools. These tools were grouped into two categories, which were the model diagnostic tools used to determine the quality of the model and the sample diagnostic tools used to study the relationship between the samples and to identify unusual samples. Also, the concentration residuals were plotted against the actual concentrations for the validation set samples. This tool was used to determine whether the model accounted for the concentration variation in the validation set and it also provides information about how well the method would predict future samples. The residuals for all samples appeared to be randomly distributed around zero.

The root mean square error of prediction (RMSEP) was another diagnostic tool for examining the errors in the predicted concentrations. It indicated both the precision and accuracy of predictions as it played the same role of standard deviation in indicating the spread of the concentration errors. It is found to be 0.0703. Also the Q^2 values of these models were calculated as 0.9998 which determined the variation in the sample prediction. So long as its value was closer to one, this indicated good prediction.

3.3. Method validation

3.3.1. Solution stability

The solution stability of zafirlukast was evaluated by the suggested HPLC method, where the studied compound solutions exhibited no chromatographic changes for 48 h when kept at room temperature and for 4 days when stored at 5 °C, where the chromatogram plots in all samples showed only one peak corresponding to pure zafirlukast with no detectable peaks for degradation products or impurities.

3.3.2. Linearity and range

Calibration curves for the proposed methods were constructed and evaluated by their correlation coefficients. Linearity of HPLC method was obtained in the concentration range of 2–40 μg mL^{-1} of ZAF with mean percentage recoveries of 99.73 ± 0.90, (n = 10). While for ^{1}DD method the linearity was assessed in the range of 4–32 μg mL^{-1} with mean percentage recoveries of 99.85 ± 0.61, (n = 7).

In PLS-method, the predicted concentrations of the validation samples were plotted against the known concentration values of ZAF in the range of 4–40 μg mL^{-1}, this was used to determine whether the model accounted for the concentration variation in the validation set. Plots were expected to fall on a straight line with a slope of one and zero intercept. ZAF plot was on a straight line with a slope of 0.9979 ± 0.0005, an intercept of 0.0602 ± 0.0072, and correlation coefficient of (r) = 0.9998.

The regression equation parameters for all the proposed methods were computed and given in Table 3 which show a good linear relationship for the suggested methods as revealed by the correlation coefficients. Descriptive statistics of the regression showed low values of the standard deviation of intercept and slope which revealed high accuracy with minimum deviations and low scattering of the calibration points.

3.3.3. Detection and quantitation limits (sensitivity)

According to ICH recommendations,[25] the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits (LOD, LOQ). The theoretical values were assessed and given in Table 3.

3.3.4. Precision

Repeatability was evaluated by assaying freshly prepared solutions in triplicate on the same day having concentrations of 8, 12 and 16 μg mL^{-1} of ZAF by the different suggested methods.

Intermediate precision was evaluated by assaying freshly prepared solutions of the above mentioned concentrations in triplicate in three successive days. The recovery% and RSD% were then calculated (Table 3).

3.3.5. Specificity

In order to test the validity and applicability of the proposed methods as stability-indicating ones, recovery studies were

Table 3 Regression and assay validation parameters for determination of pure samples of Zafirlukast by the proposed methods.

Parameter	HPLC-method	^{1}DD-method	PLS-method
Linearity (μg mL^{-1})	2–40 μg mL^{-1}	4–32 μg mL^{-1}	4–40 μg mL^{-1}
Slope ± SD	0.0716 ± 0.0165	0.0097 ± 0.0044	0.9979 ± 0.0005
Intercept ± SD	0.0314 ± 0.0054	0.006 ± 0.0003	0.0602 ± 0.0062
Correlation coefficient (*r*)	0.9999	0.9999	0.9998
Mean recovery % ± RSD	99.73 ± 0.903	99.85 ± 0.608	100.00 ± 0.336
LOD[a] (μg ml^{-1})	0.460	1.021	1.167
LOQ[a] (μg ml^{-1})	1.397	3.093	3.537
Precision (RSD %)			
Repeatability[b]	0.875–0.358–0.775	1.524–0.457–0.885	1.265–0.684–1.205
Intermediate precision[b]	1.300–0.268–0.758	1.631–1.531–0.577	1.832–1.740–0.856
Robustness data[c]			
Standard ZAF(10 μg mL^{-1})	100.25 ± 0.322	100.56 ± 0.558	99.95 ± 0.675
Acetonitrile/buffer (48:52, v/v)	100.23 ± 0.399		
Acetonitrile/buffer (52:48, v/v)	100.56 ± 0.402		
Flow rate 0.8 mL min^{-1}	100.01 ± 0.556		
Flow rate 1.2 mL min^{-1}	100.15 ± 0.245		
Peak amplitude in ^{1}DD method			
at 232.2 nm		100.00 ± 0.706	
at 232.6 nm		99.99 ± 0.458	
PLS: spectra were digitized each at 0.8 nm and 1.2 nm			100.04 ± 0.822
			99.90 ± 0.715

[a] Limits of detection and quantitation are determined via calculations: LOD = (SD of the response/slope) × 3.3. LOQ = (SD of the response/slope) × 10.
[b] The intra-day and inter-day relative standard deviations of 8, 12 and 16 μg ml^{-1} ZAF, each of triplicate analysis.
[c] Mean recovery % ± RSD (n = 5).

Table 4 Determination of the studied drugs in the laboratory prepared (L.P.) mixtures with its degradation product and in tablets by the proposed methods.

Sample	HPLC-method[a]	^{1}DD-method[a]	PLS method[a]
L.P.-mixtures	100.23 ± 1.103	100.46 ± 1.166	99.89 ± 1.231
	(n = 5) [b]	(n = 5)[b]	(n = 7)[b]
	Up to 90% degradation.	Up to 90% degradation	Up to 52% degradation
Ventair tablets, Batch No. 06093	99.76 ± 0.644	100.08 ± 0.892	100.38 ± 0.621

[a] Recovery ± RSD.
[b] Sets each of 3 replicates.

performed by analyzing synthetic mixtures of different ratios of the intact drug and its degradation product. Results in Table 4 confirm the validity and specificity of the proposed methods.

3.3.6. Application to commercial tablets

The suggested methods were successfully applied for the determination of ZAF in Ventair® tablets. The results shown in Table 4 were satisfactory and with good agreement with the labeled amounts.

3.3.7. Accuracy

The accuracy of the suggested methods was assessed by applying standard addition technique by spiking different amounts of pure zafirlukast samples to the previously analyzed tablets equivalent to 8 μg mL^{-1} of ZAF. The mean recoveries of the added drug were calculated and illustrated in Table 5. No interference due to excipients was observed as shown from the obtained results.

3.3.8. Robustness

The robustness of the developed methods was examined by detecting the effect of small but deliberate variations of some of the most important procedure parameters such as percentage organic strength and flow rate of the mobile phase in the HPLC method and wavelength of the peak amplitude in ^{1}DD method (232.2 nm and 232.6 nm). In PLS method, spectra of analyzed mixtures were digitized each at 0.8 nm and 1.2 nm.

None of these variables significantly affected the assay of ZAF and the proposed methods could be considered robust (Table 3).

3.3.9. Statistical comparison to the reported method

Statistical analysis of the results obtained by the suggested procedures and the reported HPLC method[7] was carried out. Table 6 shows that the calculated *t*- and *F*-values were less than the theoretical ones, indicating no significant differences between the proposed procedures and the reported one.

Table 5 Application of the standard addition technique to the analysis of the studied drug by the proposed methods.

Method	HPLC-method			^{1}DD-method			PLS method		
Pharmaceutical preparation	Authentic added ($\mu g\ mL^{-1}$)	Authentic found ($\mu g\ mL^{-1}$)	Recovery** (%)	Authentic added ($\mu g\ ml^{-1}$)	Authentic found ($\mu g\ mL^{-1}$)	Recovery** (%)	Authentic added ($\mu g\ mL^{-1}$)	Authentic found ($\mu g\ mL^{-1}$)	Recovery** (%)
Ventair tablets*	8	8.09	101.13	4	3.98	99.50	4	4.05	101.25
(Batch No. 06093)	12	12.15	101.25	8	7.89	98.63	8	8.06	100.75
	16	15.86	99.13	12	12.15	101.25	12	11.85	98.75
	24	24.0–13	100.54	16	16.24	101.50	16	15.85	99.06
Mean ± SD			100.51 ± 0.973			100.22 ± 1.384			99.95 ± 1.233
CV			0.968			1.381			1.234

* Equivalent to 8 $\mu g\ mL^{-1}$ of ZAF.
** Average of at least 3 experiments.

Table 6 Statistical analysis of the proposed methods and the reported method of Zafirlukast in the pure powder form.

Parameter	HPLC method	^{1}DD-method	PLS method	Reported** method[7]
Mean	99.73	99.85	99.89	100.43
SD	0.901	0.607	0.336	0.735
N	10	7	7	5
Variance	0.812	0.368	0.113	0.540
Student's *t*-	1.386 (2.160)*	1.306 (2.228)*	0.599 (2.228)*	
F	1.504 (6.00)*	1.467 (6.16)*	2.796 (6.16)*	

* Figures in parenthesis are corresponding theoretical *t*- and *F*-values at $p = 0.05$.
** Reported HPLC method is carried out with a Symmetry Shield RP18, 250 × 4.6 mm column. The mobile phase constituted of 0.01 M potassium dihydrogen phosphate buffer and acetonitrile (30:70, v/v), pH adjusted to 3.5 with ultraviolet detection at 225 nm.

4. Conclusion

A simple isocratic HPLC method and newly developed ^{1}DD and PLS methods are presented as stability indicating methods for the determination of zafirlukast in the presence of its alkaline degradation product.

From the previous discussion and results obtained in this work, we can conclude with 95% of confidence that the three suggested methods are simple, sensitive, selective and can be applied for quality control and routine analysis of zafirlukast in pure form, in the presence of its alkaline degradation product and in the available dosage form without any interference from the excipients. In addition, the suggested HPLC method has an advantage over the reference one[7] of being used to investigate the kinetics of alkaline degradation process of zafirlukast in a detailed study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bfopcu.2012.07.005.

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