# Kinetic Spectrophotometric Determination of Zafirlukast in Bulk and in Drug Formulations

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**Abstract:** Simple, accurate and reliable kinetic spectrophotometric method was developed for the determination of zafirlukast (ZAF) in bulk powder and in pharmaceutical formulation. The proposed method depends on hydrolysis of zafirlukast using 1M NaOH at 100°C for 2.5 hours and subsequent reaction of the formed degradation product which contains primary amino ($NH_2$-) group with 4-Chloro-7-nitrobenzo-2-oxa-1,3 diazole (NBD-Cl) at 90 ± 5 ºC to form a yellow colored chromogen. The formed color is spectrophotometrically measured after 10 min at 466 nm. The fixed time method was adopted for constructing the calibration curve. The linearity range was found to be 0.5-3 µg $mL^{-1,}$ and the limit of detection is 0.139 µg $mL^{-1}$. The proposed method has been successfully applied to the determination of hydrolyzed zafirlukast in pharmaceutical dosage forms with no interference from the excipients. Statistical comparison of the results shows that there is no significant difference between the proposed and official methods.
[Amal Mahmoud Abou Al Alamein. **Kinetic Spectrophotometric Determination of Zafirlukast in Bulk and in Drug Formulations.** *Life Sci J* 2012;9(4):2693-2701]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 398

**Keywords:** Zafirlukast; alkaline hydrolysis; kinetic spectrophotometry; 4-Chloro-7-nitrobenzo-2-oxa-1,3 diazole (NBD-Cl).

## 1. Introduction

Zafirlukast (ZAF), chemically [4-(5-cyclopentyloxy-carbonylamino-1-methyl-indol-3-yl-methyl)-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 are inhibited and also decrease the bronchial hyper responsiveness to inhaled histamine. 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 hours and the onset of action was seen in 1 hour and take 3 hours to reach peak plasma concentration [4 and 5].

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

NBD-Cl (4-Chloro-7-nitrobenzo-2-oxa-1,3 diazole) is a highly sensitive chromogenic and fluorogenic reagent that reacts with molecules containing primary and secondary amino group [15]. NBD-Cl has been abundantly used as a coloring reagent for the spectrophotometric determination of certain cephalosporins [16], fluvastatin [17], praziquantel [18], ascorbic acid [19] and trimetazidine dihydrochloride [20].

No kinetic spectrophotometric methods have been reported in the literature for the assay of ZAF. So in the present work, a kinetically based method is proposed for the determination of zafirlukast. The method is based on the fact that alkaline ZAF degradate molecule possesses a typical primary amino group which readily and quantitatively reacts with NBD-Cl to form a yellow colored condensation product which can be spectrophotometrically measured at $\lambda_{max}$ 466nm.

Generally, some specific advantages that the kinetic methods possess are as follows [21 -23] : simple and fast methods because some experimental steps such as filtration , extraction, etc. are avoided prior to absorbance measurements, high selectivity since they involve the measurement of the absorbance as a function of reaction time instead of measuring the concrete absorbance value, other active compounds and common tablet excipients present in the commercial dosage forms may not interfere if they are resisting the chemical reaction conditions established for the proposed kinetic method and colored and/or turbid sample background may possibly not interfere with the determination process.

So, the aim of this study was to develop a simple, sensitive and stability indicating kinetic colorimetric method for the quantitative determination of zafirlukast in bulk powder and in pharmaceutical preparations after its alkaline hydrolysis and subsequent reaction

with 4-Chloro-7-nitrobenzo-2-oxa-1, 3 diazole (NBD-Cl).

## 2. Experimental

### 2.1. Apparatus

Dual-beam UV-visible spectrophotometer, UV Probe 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.

### 2.2. Materials and reagents

All materials used were of analytical reagent grade.

- Reference zafirlukast was kindly provided by DELTA PHARMA S.A.E, Tenth of Ramadan City, Egypt. 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 each one tablet; manufactured by DELTA PHARMA S.A.E, Tenth of Ramadan City, Egypt.
- 4- Chloro-7-nitrobenzo-2-oxa-1, 3 diazole (NBD-Cl) (E. Merck, Darmstadt-FRG): 0.1%w/v, freshly prepared in 95% ethanol.

### 2.3. Preparation of standard solution of degraded zafirlukast (D-ZAF):

Accurately weighed 20 mg of ZAF were dissolved in 20 mL acetonitrile. Subsequently, 25 mL of 1M sodium hydroxide were added and the solution was heated under reflux or in a temperature controlled oven at 100 $^0$C for 2.5 hours. The solution was concentrated nearly to dryness under vacuum, cooled to room temperature (~25 $^0$C), then quantitatively transferred into a 100-mL calibrated flask and the volume was completed with acetonitrile. Aliquot portion of this solution was diluted with distilled water to prepare a working standard solution of D-ZAF (0.02mg mL$^{-1}$). Complete alkaline degradation of the studied drug was confirmed by the reported HPLC method [7], where no peaks corresponding to intact drug were detected in case of the degraded samples.

### 2.4. General assay procedure

Aliquot portions of degraded ZAF working solution (0.02mg mL$^{-1}$) over the concentration range (0.5-3µg mL$^{-1}$) were transferred separately to a series of test tubes. 1 mL of 0.1 % NBD-Cl was added and then the mixture solutions were heated in a thermostatically controlled water bath at 90 $^0$C for 10 minutes. The test tubes were cooled to room temperature and quantitatively transferred to 10-ml calibrated flasks. The volume was completed with distilled water and the absorption spectrum of the reaction product was recorded at 466 nm against the corresponding reagent blank. The concentration of D-ZAF was then computed from the corresponding equation of the calibration graph for the fixed-time method.

### 2.5. Procedure for pharmaceutical formulation

From the average weight of 20 crushed Ventair® tablets into fine powder, an accurately weighed quantity of the mixed powder containing an equivalent to 20 mg of ZAF was extracted with 2 x 30 mL of acetonitrile with vigorous shaking for ~ 10 minutes. The solution was filtered through a Whatman filter paper (No. 42) and then diluted to volume with acetonitrile in a 100-mL calibrated flask.

The solution samples were prepared as described under 2.3 by heating with 1 N NaOH for 2.5 hours. An aliquot of this hydrolyzed degraded solution was diluted with distilled water as required and analyzed according to the suggested procedure.

## 3- Results and discussion

### 3.1. The reaction mechanism

The hydrolytic degradation of zafirlukast was used as a preliminary step in the analytical procedure used for its determination. The proposed spectrophotometric method was based on the alkaline hydrolysis of the drug and subsequent reaction of the formed amino group with chromogenic reagent. Zafirlukast is completely hydrolyzed with 1M sodium hydroxide after 2.5 hours at 100 $^0$C, through the splitting of the ester group. The method is based on the feasibility of hydrolysis of amide ester linkage of zafirlukast in alkaline medium [24 -27]. The expected major degradation product (D-ZAF) is obtained according to the suggested mechanism for alkaline degradation process of zafirlukast , Figure (1).

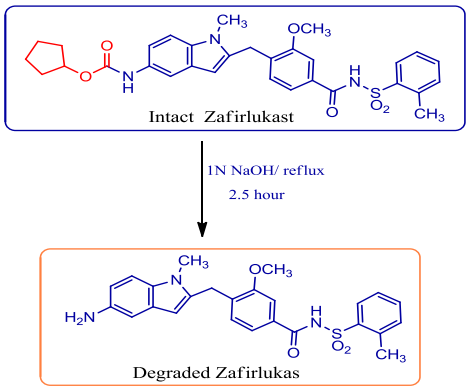
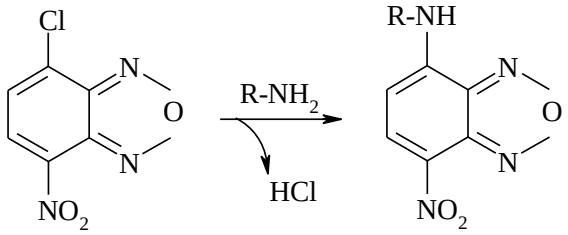



**Figure (1): Scheme of alkaline degradation of ZAF**

The assignments and structure elucidation of the degradation product were confirmed by mass spectral data. The mass spectrum of (D-ZAF) was characterized by the appearance of the molecular ion peak at 463 m/z & 465m/z ( M & M$^{+2}$) which confirms the molecular weight of the suggested degradation product. The proposed reaction mechanism between

NBD-Cl and primary amines [15] is illustrated in Figure (2).




NBD-Cl Reaction colored product

**Figure (2): The proposed reaction mechanism between NBD-Cl and primary amines**

### 3.2. Absorption spectra

The method is based on the fact that D-ZAF possesses a typical primary amino group which readily and quantitatively reacts with NBD-Cl at 90 $^0$C for 10 minutes to form a yellow colored condensation product.

The formed colored product can be measured spectrophotometrically at $\lambda_{max}$ 466 nm against a reagent blank, Figure (3). This indicates formation of a new compound of higher sensitivity compared to the spectrum of D-ZAF which gives a very weak and very broad absorption maximum at 283.8 nm.

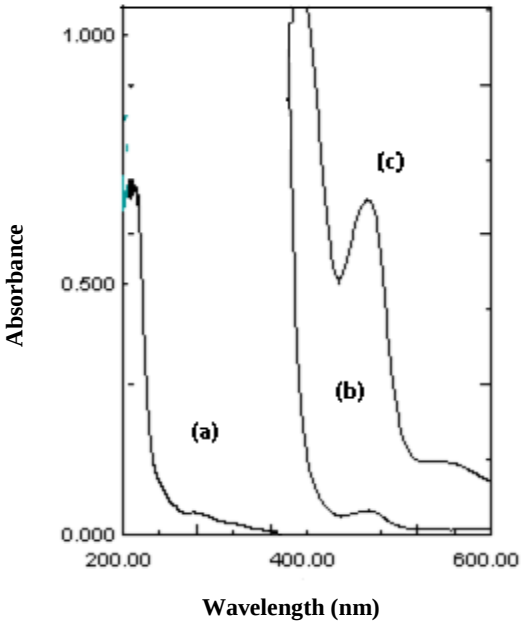



**Figure (3): Absorption spectra of (a) zafirlukast hydrolysate (2 µg mL$^{-1}$), (b) reagent blank and (c) the reaction colored product.**

### 3.3. Optimization of reaction conditions

The optimum conditions for the alkaline degradation and subsequently the development of method were established by varying the parameters one at a time and keeping the others fixed and observing the effect produced on the absorbance of the colored product. It was found that complete hydrolysis of ZAF and optimum yield of amino group were obtained after reflux or heating with1 M NaOH at 100 $^0$C for about 2.5 hours. In order to establish experimental conditions, various factors affecting the reaction conditions were studied such as, temperature, concentration of NBD-Cl and time of heating.

Borate buffer solutions of different pH values ranging from 7.8-10 were used and form an orange yellow color. This will decrease the absorbance of the sample solution when using it as a blank reagent. Therefore, maximum color intensity was attained with no buffer reagent.

The influence of volume of 0.1% NBD-Cl was studied and optimized to be 1± 0.25mL for maximum sensitivity, Figure (4). Increasing the volume of NBD-Cl leads to decrease in the absorbance; this may be due to the high background absorbance of the reagent.

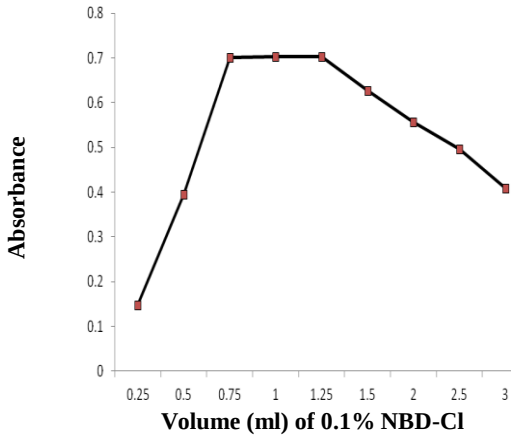



**Figure (4): Effect of volume of 0.1 % NBD-Cl on the reaction of zafirlukast (2 µg ml$^{-1}$) after alkaline hydrolysis**

The effect of temperature on the reaction was studied in the range of 40-100 ºC and it was found that 90 ºC is optimal for maximum color development; Figure (5).The influence of the time of heating was investigated in the range of 5-35 min. The experimental results show that heating in the range 5-25 min gave the optimal values in kinetic studies, Figure (6). The color product was stable for up to 2 hours at room temperature as shown in Figure (7).

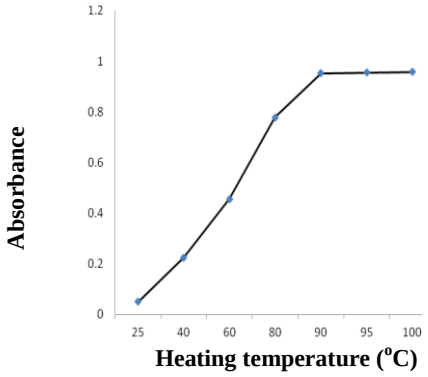



**Figure (5): Effect of heating temperature on the reaction of degraded zafirlukast (2 µg mL$^{-1}$) with NBD-Cl**

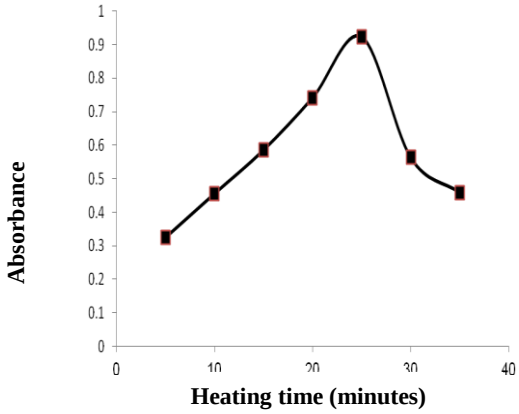



**Figure (6): Effect of heating time at 90 $^{O}$ C on the reaction of degraded ZAF (2 µg mL$^{-1}$) with NBD-Cl**

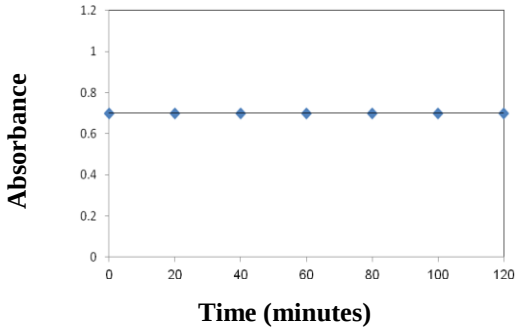



**Figure (7): Effect of time on the stability of color of D-ZAF- NBD-Cl complex (2 µg mL$^{-1}$)**

The molar ratio between the drug and NBD-Cl was determined by the limiting logarithmic method [28]. The slope of the curve in case of first solution set yields the number of moles of D-ZAF (0.9317) while that in case of second solution set gives the number of moles of NBD-Cl (0.9311). The stoichiometric ratio of 1:1 was revealed under the attained optimum conditions as shown in Figure (8).

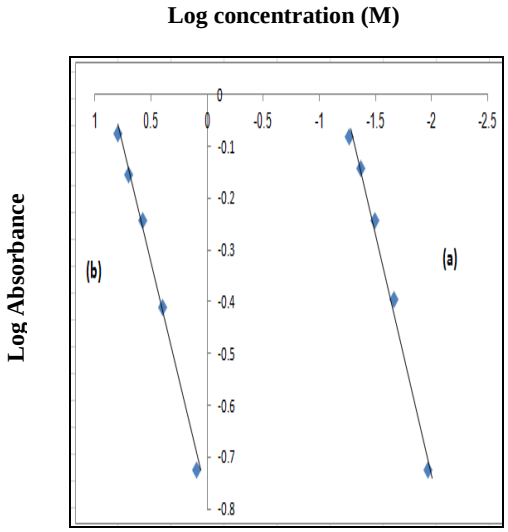



**Figure (8): Determination of the molar ratio between degraded zafirlukast (a) and NBD-Cl (b) by limiting logarithmic method**

### 3.4. Kinetics of the reaction

#### 3.4.1. Determination of reaction order

The rate of the reaction was determined using various concentrations of D-ZAF in the concentration range of 0.5-3 µg mL$^{-1}$, while keeping concentration of NBD-Cl constant at the optimized conditions. The rate of the reaction was found to be [drug] dependent. From the graphs shown in Figure (9), it was clear that rate of the reaction increases with increasing drug concentration, indicating that the reaction rate obeys the following equation [21,22]:

Rate = k' [drug]$^{n}$……equation (1), where,

k' = the pseudo-order constant of the reaction , n = order of the reaction.

The rate may be estimated from Figure (9) by the variable time method measurements as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds, Table (1).Taking logarithms of rates and concentrations, equation (1) is transformed into the following one:

Log (rate) = log $\Delta A/\Delta t$ = log k' + n log [drug] …. equation (2)

Regression of log [drug] versus log (rate) by least square method, Figure (10) yields the calibration equation:

Log (rate) = 1.0122 log C + 2.554 …….equation (3), with correlation coefficient (r) = 0.9996. So, k'= 358.096 $s^{-1}$ and the reaction is pseudo-first order (n ≈1) with respect to zafirlukast concentration after its alkaline hydrolysis.

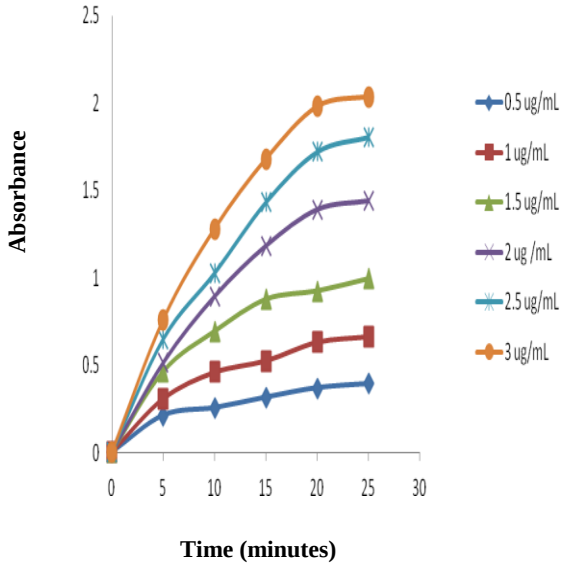



**Figure (9): Absorbance versus time for the reaction of different concentrations of D-ZAF with NBD-Cl**

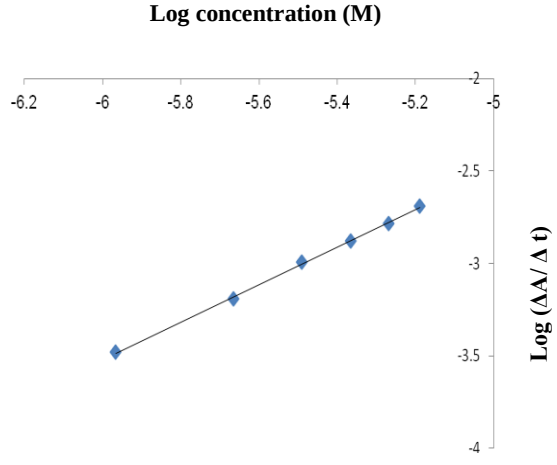



**Figure (10): Calibration plot of logarithm rate of the reaction versus molar concentrations of hydrolyzed ZAF**

**3.4.2. Evaluation of different kinetic methods**

Several experiments were run to obtain drug concentrations using the rate data, rate constant, fixed absorbance and fixed time methods and the most suitable analytical method was selected taking into account the applicability and the sensitivity (the slope of the calibration curve, the correlation coefficient (r) and the intercept).

**3.4.2.1. Rate constant method:**

It was performed by plotting graphs of logarithm of absorbance versus time for different D-ZAF concentrations in the range of 1.08 x $10^{-6}$ to 6.48 x $10^{-6}$M (0.5- 3 µg $mL^{-1}$). All graphs appeared to be rectilinear and the slope of the line is k / –2.303. Pseudo first order rate constants (K') corresponding to different drug concentrations (C) were calculated from slopes multiplied by –2.303, Table (2). Regression of (C) versus k' gave the following equation:

K' = - 60.855 C - 0.0005 (r = 0.8776)

The value of "r " indicated poor linearity which makes the method not applicable.

**3.4.2.2. Fixed absorbance method:**

A pre-selected value of absorbance was fixed (0.53). The time required for each drug concentration (2.16 x $10^{-6}$ to 4.31 x $10^{-6}$ M) to reach this value was measured in seconds. The reciprocal of measured time (1/ t) against the initial concentration of the drug, Table (3) was plotted and the following regression equation was obtained:

1 / t = 0.0011 C- 0.0002 (r = 0.9608)

The value of "r " indicated poor linearity and the range of drug concentrations that give satisfactory results, was limited therefore this method can't be used.

**3.4.2.3. Initial rate method:**

In this method, the calibration curves were constructed by plotting the logarithm of the initial rate versus the logarithm of molar drug concentration at the beginning of the reaction as a function of time between 0-5 minutes. Graphs were not easy to plot because the reaction was too fast to follow and so this method was abandoned.

**3.4.2.4. Fixed time method:**

Graphs of the absorbance versus initial concentrations of the drug were plotted at fixed times of 5, 10, 15, 20 and 25 minutes. The regression equations and correlation coefficient for each graph was calculated, Table (4). It was clear that slope is increasing while intercept is decreasing along with the best correlation coefficient obtained after 10 minutes so that it was chosen as the most suitable time for measurements and for constructing a calibration curve.

Therefore, the fixed time method was utilized to calculate the concentration of zafirlukast via its hydrolyzed form either in the pure powder form or in its pharmaceutical preparations.

**Table (1): Logarithms of the rates for different concentrations of degraded ZAF at the optimized experimental conditions.**

| Log rate (ΔA/ Δ t) | log [ drug] M |
|---|---|
| -3.481 | -5.967 |
| -3.189 | -5.666 |
| -2.994 | -5.491 |
| -2.881 | -5.366 |
| -2.785 | -5.268 |
| -2.691 | -5.189 |

**Table (2): Values of K' and molar concentrations of degraded ZAF**

| k' (sec $^{-1}$) | [ Drug] M |
|---|---|
| -5.34 x10 $^{-4}$ | 1.08 x10 $^{-6}$ |
| -6.22 x10 $^{-4}$ | 2.16 x10 $^{-6}$ |
| -6.06 x10 $^{-4}$ | 3.23 x10 $^{-6}$ |
| -8.38 x10 $^{-4}$ | 4.31 x10 $^{-6}$ |
| -8.56x10 $^{-4}$ | 5.39 x10 $^{-6}$ |
| -8.02x 10 $^{-4}$ | 6.48 x10 $^{-6}$ |

**Table (3): Values of reciprocal of time taken at fixed absorbance for different rates of various concentrations of D- ZAF at the optimized experimental conditions**

| [Drug] M | 1/ t (sec$^{-1}$) |
|---|---|
| 2.16 x10 $^{-6}$ | 1.11 x 10 $^{-3}$ |
| 3.23 x10 $^{-6}$ | 1.66x 10 $^{-3}$ |
| 4.31 x10 $^{-6}$ | 3.33 x 10 $^{-3}$ |

**Table (4): Calibration equations at different fixed times for D- ZAF concentration range (1.08 x10 $^{-6}$ - 6.48 x10 $^{-6}$ M) at the optimized experimental conditions**

| Time (min) | Regression equation | Correlation coefficient (r) |
|---|---|---|
| 5 | A= 0.2393 C + 0.0679 | 0.9942 |
| 10 | A= 0.4001 C + 0.0008 | 0.9999 |
| 15 | A= 0.5100 C + 0.0697 | 0.9934 |
| 20 | A= 0.6391 C + 0.1433 | 0.9941 |
| 25 | A= 0.7297C + 0.1703 | 0.9966 |

### 3.5. Method validation

#### 3.5.1. Linearity and range

By applying the specified optimum conditions, the standard calibration curve was plotted between the absorption of the reaction product and the concentration of the studied drug. Beer-Lambert's law was obeyed and linear relationship was obtained over the range of 0.5-3 µg mL$^{-1}$, the linear regression equation was computed and found to be:

A= 0.4001 C+ 0.0008(r = 0.9999)

Where, A= the absorbance at 466 nm; C= the concentration in µg mL$^{-1}$; r = the correlation coefficient.

#### 3.5.2. Limits of detection and quantification

According to ICH recommendations [29], the approach based on the S.D. of the response and the slope of the calibration curve was used for determining the limit of detection (LOD) and limit of quantification (LOQ). For the proposed fixed time method, LOD and LOQ were determined and found to be 0.139 and 0.42 µg mL$^{-1}$, respectively.

#### 3.5.3. Precision

The precision of the proposed colorimetric method was carried out by five determinations at different concentrations of 0.5, 1 and 1.5 µg mL$^{-1}$ for D-ZAF. The percentage relative standard deviation (RSD %) for the intra-day and inter-day variations during 1 and 5 days was calculated.

Statistical analysis [30] of the small values of standard deviation of the intercept ($S_a$) and slope ($S_b$) also indicates high precision of the method. The results obtained are summarized in Table (5).

**Table (5): Regression and assay validation parameters for determination of zafirlukast by the proposed method**

| Parameter | D-ZAF |
|---|---|
| **Linearity ( µg mL$^{-1}$)** | 0.5-3 µg mL$^{-1}$ |
| **($S_a$) Intercept ± SD** | 0.0008 ± 0.0041 |
| **($S_b$) Slope ± SD** | 0.4001 ± 0.0013 |
| **Correlation coefficient (r)** | 0.9999 |
| **Mean recovery % ± RSD** | 99.96 ± 0.653 |
| **LOD* (µg ml$^{-1}$)** | 0.139 |
| **LOQ * (µg ml$^{-1}$)** | 0.42 |
| **Precision (RSD %)** ** **:** | |
| **RSD** $_a$ | 0.825 - 0.458- 0.625 |
| **RSD** $_b$ | 1.102 – 0.338 -0.776 |
| ***Robustness data* ***:** | |
| *-Standard ZAF(10 µg mL$^{-1}$)* | 100.25±0.285 |
| 1- wavelength | |
| 466.2 nm | 99.99 ± 0.558 |
| 465.8 nm | 100.05 ± 0.494 |
| 2- Volume of NBD-Cl | |
| 0.75mL | 100.45 ± 0.345 |
| 1.25 mL | 100.15 ± 0.602 |
| 3- Heating temperature | |
| 95°C | 99.98 ± 0.335 |
| 100°C | 100.45 ± 0.446 |

*Limit of detection and quantitation are determined via calculations:

LOD = (SD of the response/ slope) × 3.3

LOQ = (SD of the response/ slope) × 10

** RSD $_a$: The intra- day and RSD $_b$: inter- day relative standard deviations of 0.5, 1 and 1.5 µg mL$^{-1}$ ZAF, each of triplicate analysis. *** Mean recovery % ± RSD (n=5).

#### 3.5.4. Robustness

The robustness of the developed method was examined by detecting the effect of small but deliberate variations of some of the most important procedure parameters such as wavelength (466 ±0.2 nm) , heating

temperature (95 $^{O}$C , 100 $^{O}$C ) and volume of NBD- Cl reagent ( 0.75mL ,1.25 mL)

None of these variables significantly affected the assay of D-ZAF and the proposed method could be considered robust, Table (5).

### 3.5.5. Application to pharmaceutical formulation

The suggested method was successfully applied for determination of ZAF in Ventair® tablets through its alkaline degradation product. The results shown in Table (6) were satisfactory and with good agreement with the labeled amounts.

### 3.5.6. Accuracy

The accuracy of the suggested method was assessed by applying standard addition technique by spiking different amounts of zafirlukast samples in its degraded form to pre- analyzed tablet samples equivalent to 0.5µg mL$^{-1}$ of ZAF. The mean recoveries of the added drug and percentage relative standard deviations were calculated and illustrated in Table (7). The obtained results suggested that there is no interference from formulation excipients in the estimation.

**Table (6): Determination of zafirlukast in pharmaceutical formulation by the proposed colorimetric method**

| Preparation | Taken (µg mL$^{-1}$) | Found (µg mL$^{-1}$) | Recovery* (%) |
|---|---|---|---|
| Ventair® tablets, Batch No. 06093 | 2 | 2.021<br>1.998<br>2.01<br>2<br>1.99 | 101.05<br>99.9<br>100.5<br>100<br>99.5 |
| | | Mean ±SD<br>RSD | 100.19 ± 0.598<br>0.597 |

*Average of triplicate determinations

**Table (7): Standard addition method for the assay of the studied drug in pharmaceutical dosage forms by the proposed method.**

| Preparation | Authentic Added (µg mL$^{-1}$) | Found (µg mL$^{-1}$) | Recovery* (%) |
|---|---|---|---|
| Ventair® tablets, Batch No. 06093 | 0.5<br>1<br>1.5<br>2 | 0.498<br>1.008<br>1.489<br>2.01 | 99.6<br>100.8<br>99.267<br>100.5 |
| | | Mean ±SD<br>RSD | 100.04 ± 0.725<br>0.725 |

*Average of triplicate determinations

### 3.5.7. Statistical comparison to the reported method

Statistical analysis [31] of the results obtained by the suggested procedure and the reported HPLC method [7] were carried out. Table (8) showed that the calculated t - and F values were less than the theoretical ones, indicating no significant differences between the proposed procedure and the reported one. Moreover, the method is highly sensitive compared to the reported one [7] which needs not less than 30 µg mL$^{-1}$ from the drug.

**Table (8): Statistical analysis of the results obtained by determination of zafirlukast by the proposed colorimetric method and those obtained by the reference method.**

| Parameter | Proposed method | Reference method [7] |
|---|---|---|
| **Mean** | 99.96 | 100.43 |
| **S.D.** | 0.653 | 0.735 |
| **Variance** | 0.426 | 0.540 |
| **n** | 6 | 5 |
| **t *(2.262)** | 0.841 | |
| **F *(5.19)** | 1.268 | |

*Figures in parenthesis are corresponding theoretical t- and F- values at *p*=0.05 [31]

## 4. Conclusion

The kinetically based colorimetric method proposed in this work is the first kinetic method developed for determination of zafirlukast after its alkaline hydrolysis. The suggested method is found to be accurate, precise, selective, and reproducible and requires simple apparatus for its performance. Furthermore, the method can be used as a stability indicating assay due to the fact that the alkaline degradate of zafirlukast reacts quantitatively with NBD-Cl with formation of a new compound of higher sensitivity.While; intact zafirlukast doesn't react with the same reagent and gave a baseline spectrum under the optimized experimental conditions even in a very large quantity. From the previous discussion and results obtained in this work, we can conclude with 95% of confidence that the suggested method can be successively applied for quality control and routine analysis of D-ZAF in bulk and pharmaceutical formulation.

## Acknowledgements

The author would like to thank Dr. N. El Degwi and Dr. S. El Degwi for providing assistance and support. This study has been realized in the research laboratory in the Modern Science and Arts University (MSA), October city, Egypt.

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11/5/2012