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Optimisation, validation and application of a capillary electrophoretic method for the determination of zafirlukast in pharmaceutical formulations

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Abstract

Zafirlukast is a selective and competitive orally administered inhibitor of the cysteinyl leukotrienes and currently indicated for the prophylaxis and treatment chronic asthma. A simple, rapid, reliable capillary zone electrophoresis method for the determination of ZAF in pharmaceutical formulations was developed and validated. The influence of buffer concentration, buffer pH, organic modifier, capillary temperature, applied voltage and injection time was systemically investigated in a fused silica capillary (i.d. 50 μm, total length 80.5 cm and effective length 72.0 cm). Optimum results were obtained with 50 mM borate buffer at pH 8.50, capillary temperature 25 °C and applied voltage 30 kV. The samples were injected hydrodynamically for 3 s at 50 mbar. Detection wavelength was set at 240 nm. Meloxicam was used as internal standard. The method was suitably validated with respect to linearity, limit of detection and quantification, accuracy, precision, selectivity, robustness and ruggedness. The linear calibration range was 2.00–80.00 μg mL^{-1} and the limits of detection and quantification were 0.75 and 2.00 μg mL^{-1} with R.S.D. of 3.88 and 2.75%, respectively. The proposed method was applied for the determination of ZAF in its pharmaceutical formulations. The results obtained from developed method were compared with a HPLC method reported in the literature and no significant difference was found statistically.

Keywords: Zafirlukast; Capillary zone electrophoresis; Optimisation; Validation; Tablet analysis

1. Introduction

Leukotriene receptor antagonists, such as montelukast and zafirlukast, have been demonstrated in a number of studies to possess bronchodilating and anti-inflammatory properties, that make these drugs ideal candidates for the treatment of asthma [1,2].

Zafirlukast (ZAF), 4-(5-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-*o*-toylsulphonylbenzamide (Fig. 1) is a cysteinyl leukotriene which used in the prophylaxis and treatment mild to moderate persistent and chronic asthma [3–5]. ZAF has shown effective in the inhibition of allergen, exercise, sulphur dioxide and aspirin induced asthma [6–8]. ZAF effectively improved symptoms and benefited lung function with asthmatic patients [9–11].

There are very few analytical techniques in the literature used for determination of ZAF in dosage forms and human biological material. In the high-performance liquid chromatography (HPLC) method, ZAF reference standard was obtained from extracted Accoleit tablet and different HPLC systems with diode array and UV detector were used at the two laboratories involved [12]. Derivative spectrophotometry and HPLC with UV detection methods have been used for the determination of ZAF in pharmaceutical formulation [13]. In the another HPLC method described, ZAF was extracted from deproteinated plasma samples using solid phase extraction columns and analyzed by normal phase liquid chromatography with fluorescence detection [14]. The other HPLC method with UV detection has been used for the determination of ZAF in pharmaceutical formulation [15]. Three voltammetric methods, such as square-wave voltammetry (OSWV), square-wave adsorptive stripping voltammetry (OSWAdSV) and differential adsorptive stripping voltammetry (DPAdSV) methods used for the direct determination of ZAF in its bulk form and pharmaceutical formulations [16,17]. To our knowledge, no capillary electrophoresis (CE)

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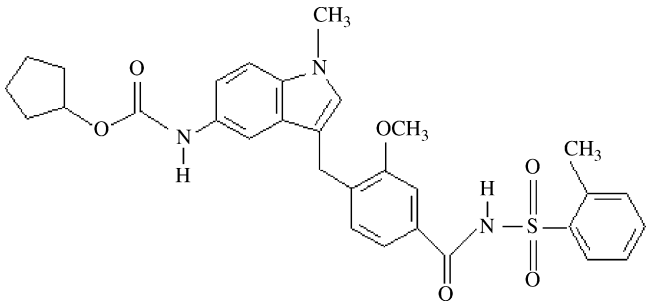

Fig. 1. Chemical structure of ZAF.

has been published for the determination of ZAF from the pharmaceutical formulation.

CE has emerged in recent years as a powerful analytical technique for the separation and quantitation of a large variety of substances including pharmaceutical compounds. CE possesses many unique advantages, such as small sample volumes, high separation efficiency, low consumption of reagents, short analysis time and easy conditioning of column. Therefore, CE method is an alternative technique to HPLC method.

The aim of this study was developed and validated a capillary zone electrophoresis (CZE) method for the assay of ZAF in pharmaceutical formulation. For this purpose, the influence of buffer type, buffer concentration, buffer pH, organic modifier, capillary temperature, applied voltage and injection time was systemically investigated. The method validation studies of this method were performed according to the evaluation of the validation parameters such as linearity, sensitivity, selectivity, accuracy, precision, ruggedness and robustness according to the ICH Guidelines [18]. The developed and validated method was applied to the determination of ZAF in pharmaceutical formulation. The results obtained by the developed method were compared with the HPLC method in the literature [15].

2. Experimental

2.1. Apparatus

All CE experiments were performed using an Agilent ^{3D}CE (Waldbronn, Germany) system using ChemStation software, equipped with a diode array detector (DAD), an automatic sample injector, Peltier temperature controller and 30 kV high voltage power supply. Analysis was carried out in a fused-silica capillary (i.d. 50 μm, total length 80.5 cm and effective length 72.0 cm) in normal mode, applying a voltage of 30 kV. Sample injections were made in a hydrodynamic mode over 3 s under a pressure of 50 mbar.

For pH measurements, a pH meter (Mettler Toledo MA 235, Switzerland) calibrated with standard buffers was employed. Deionized water was prepared using a Barnstead NANOpure DIamond Analytical (USA) ultrapure water system. Running buffer and samples were filtered through a 0.45 μm Titan syringe filters (Sri Inc., Eaton Town, NJ, USA).

All HPLC experiments were carried out on Thermo Separation Products consisted of a P 2000 quarternary pump, an UV 2000 detector and SCM 1000 vacuum degazer. Operation, data acquisition and analysis were performed using ChromQuest programme. An Nucleosil C_{18} 100A (150 mm × 4.6 mm i.d., 5 μm) analytical column was used for chromatographic separation. The mobile phase consisted of acetonitrile and acetate buffer (pH 3.0) (70:30, v/v). The mobile phase was filtered through a 0.45 μm membrane filter and degassed ultrasonically before use. Separation was achieved by isocratic solvent elution at a flow rate of 0.8 mL min^{-1}. Analysis was performed at room temperature and detection wavelength was set at 240 nm. The injection volume was 20 μL.

2.2. 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. All solvents and other chemicals were analytical reagent grade.

2.3. Standard and sample solutions

2.3.1. Standard stock solutions

The stock solutions of ZAF (1000 μg mL^{-1}) was prepared in acetonitrile and kept in the dark and at 4 °C. The stock solution of meloxicam (M, internal standard) (250 μg mL^{-1}) was prepared in methanol. Various aliquots of standard solutions were taken, the M added and then diluted to 1 mL with running buffer to give a final analyte concentration (2, 5, 10, 20, 30, 40, 50, 60, 70 and 80 μg mL^{-1}). Standard solutions of ZAF were prepared daily by appropriate dilution of the stock solution with selected running buffer.

2.3.2. Running buffer

Optimized running buffer solution was 50 mM borate buffer adjusted to pH 8.50 with sodium hydroxide.

2.3.3. Sample preparation

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 solution was transferred to a vial, added 80 μL (250 μg mL^{-1}) M and diluted with 50 mM borate buffer at pH 8.50 to 1 mL. This solution was analyzed by CZE. All solutions were filtered through a 0.45 μm syringe filter before injection to the CE system.

2.4. Electrophoretic procedure

Electrophoretic separation was carried out using a fused-silica capillary having i.d. 50 μm, total length 80.5 cm and

effective length 72.0 cm, in a positive mode using constant voltage (30 kV). Before the first use, the capillary was conditioned by flushing with 1.0 M NaOH for 20 min, then with water for 20 min. At the beginning of each working day, the capillary rinsed with 0.1 M NaOH for 15 min, water for 10 min and then the running buffer for 10 min. Sample components can become adsorbed onto the capillary surface and change the effective charge on the wall. So, before each injection, the capillary was preconditioned with 0.1 M NaOH (2 min), water (2 min) and running buffer (4 min) to maintain proper reproducibility of run-to-run injections. Injection was carried out under hydrodynamic pressure at 50 mbar for 3 s and capillary temperature was 25 °C. A diode-array detector (DAD) was set at 240 nm with a bandwidth of 10 nm. The capillary temperature was kept constant at 25 °C and a voltage of +30 kV was applied.

3. Results and discussion

3.1. Optimization of the electrophoretic conditions

The pH of the running buffer has got a strong influence in the ionization of the compounds and the magnitude of the electroosmotic flow (EOF). ZAF is very low soluble at low pH values. Thus, basic buffers were used to prevent its possible precipitation. Because of that the effect of pH was investigated in the range from 7.0 to 9.0.

In this study, the running buffers, such as borate (50 mM, pH 8.0) and phosphate buffer (50 mM, pH 8.0) were studied to achieve the highest sensitivity and short analysis time. Different parameters such as migration time, peak area, peak shape, height, width, symmetry of ZAF and M were evaluated. Since, borate buffer of pH 8.0 provided the best results, this buffer was selected for the further studies.

The effect of the buffer pH was investigated within the range of 7.0–9.0 at a 50 mM borate buffer concentration (Fig. 2). When the pH of borate buffer increased, the migration times of ZAF and M increased and peak width of ZAF decreased. So pH 8.50 was chosen as the optimum pH value of the running buffer for suitable migration time (t) and peak width (w).

The effect of borate concentration of running buffer was examined by varying the concentration from 25 to 75 mM

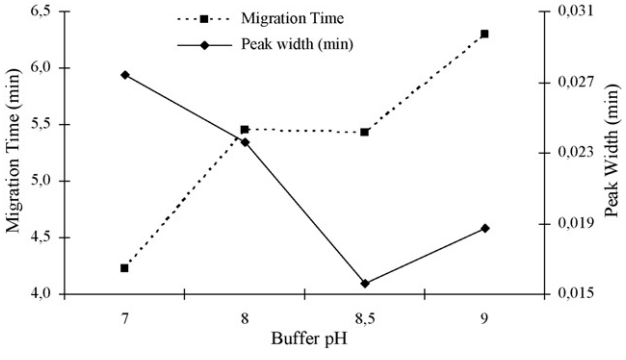

Fig. 2. Effect of buffer pH on migration time and peak width of ZAF. Operating conditions: 50 mM borate buffer, hydrodynamic injection (3 s at 50 mbar), 25 kV, 25 °C and 240 nm (bandwith 10 nm). ZAF and M: 20.00 μg mL^{-1}.

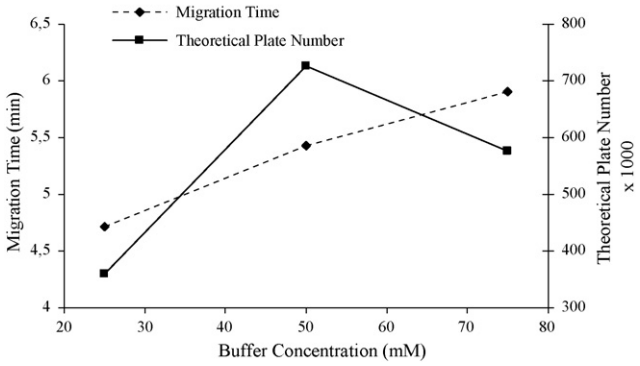

Fig. 3. Effect of buffer concentration on migration time and theoretical plate number (N) of ZAF. Operating conditions: pH 8.50 borate buffer, hydrodynamic injection (3 s at 50 mbar), 25 kV, 25 °C and 240 nm (bandwith 10 nm). ZAF and M: 20.00 μg mL^{-1}.

at pH 8.50. As shown in Fig. 3, when borate concentration was increased, the migration times of ZAF and M increased. Meanwhile the efficiency was increased until 50 mM buffer concentration and then decreased. Thus, a concentration of 50 mM borate buffer at pH 8.50 was selected as an optimum borate concentration for running buffer since it mains a good peak shape, short migration times, low peak width and higher efficiency.

The addition of organic modifiers to the running buffer was considered because they affect several parameters such as viscosity, dielectric constant, zeta potential and migration time, peak symmetry and resolution. Thus, methanol and acetonitrile were added at various concentrations (5, 10 and 15%, v/v) to the running buffer of 50 mM borate buffer pH 8.50. The migration times of ZAF and M increased significantly with the addition of organic modifiers, so no organic modifiers were added to the running buffer.

The effect of applied voltage was studied in the range 10–30 kV. Using 50 mM borate buffer at pH 8.50 as running buffer, the increase of applied voltage led both to shorter analysis times and sharper peaks. However, higher voltages also exhibited higher currents and increased Joule heating. To limit this heating inside the capillary, the maximum applied voltage was chosen from an Ohm's plot (current versus voltage). This voltage was 30 kV.

Injection time effects on the peak width and peak height. In order to improve sensitivity, sample solutions were hydrodynamically injected at 50 mbar while the injection time was varied from 1 to 5 s. The peak area increased with increasing injection time. After 3 s, the peak shapes of ZAF and M were deformed, so 3 s was selected as the optimum injection time. The pressure used for injection was always 50 mbar.

Control of capillary temperature is important in capillary electrophoresis. Changes in capillary temperature can cause variations in EOF, efficiency, viscosity, electrophoretic mobilities, migration times, injection volume and detector response. The effect of temperature on analysis was investigated at 20, 25 and 30 °C. An increase of the capillary temperature resulted in a decrease of migration times for ZAF and M due to smaller electrolyte viscosity. The temperature giving the best compromise

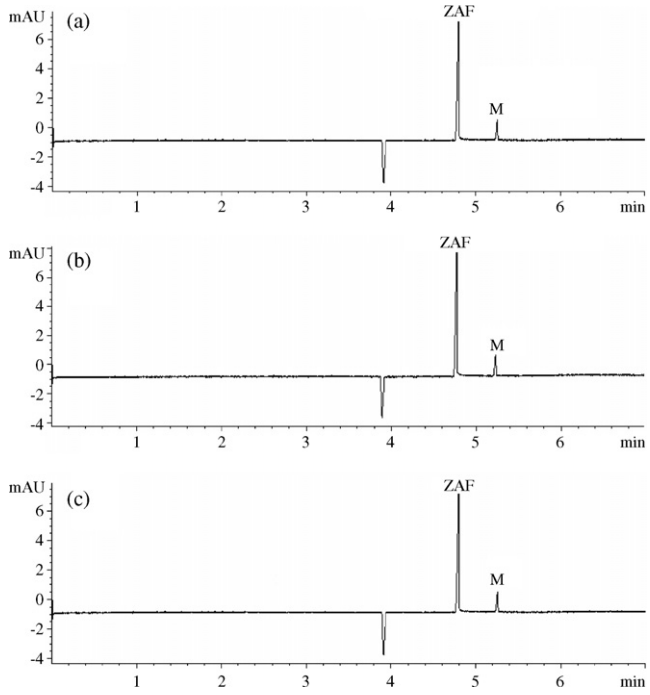

Fig. 4. The electropherograms of (a) standard solution, (b) synthetic tablet solution and (c) pharmaceutical tablet solution. Operating conditions: 50 mM borate buffer (pH 8.50), hydrodynamic injection (3 s at 50 mbar), 30 kV, 25 °C, 240 nm (bandwith 10 nm). ZAF and M: 20.00 μg mL^{-1}.

between resolution and run time, with a good level of baseline noise, was 25 °C, and it was selected as optimum temperature.

From these optimization studies, the following electrophoretic conditions were selected as optima:

- running buffer: 50 mM borate buffer at pH 8.50;
- capillary: fused silica capillary (i.d. 50 μm, total length 80.5 cm and effective length 72.0 cm);
- ınjection: hydrodynamically, 3 s at 50 mbar;
- voltage: 30 kV;
- temperature: 25 °C;
- detection wavelength: 240 nm.

Under these optimized conditions, the migration times of ZAF and M were 4.92 ± 0.04 and 5.33 ± 0.03 min, respectively. The total time of analysis was <6 min (Fig. 4a).

3.2. Validation of the method

Duplicate injections of the solutions were performed and ratios of average ratio of peak areas were used for quantitative analysis.

As the use of an internal standard is recommended for quantitative analysis to correct errors, which are introduced by variable injection volume, voltage, or EOF and improve injection precision [19,20], several drugs (meloxicam, diflunisal, piribedil and verapamil) were investigated as internal standard for validation procedures of ZAF. Meloxicam (M), a non-steroidal anti-inflammatory drug (NSAID), was found to be a suitable candidate since its migration time was close to the ZAF and it has smooth peak shape in optimized conditions.

Table 1
Analytical characteristics of proposed method ($n = 7$)

Regression equation	$y = 0.1686x + 0.0167$
Standard error of slope	1.51×10^{-3}
Standard error of intercept	0.03
Correlation coefficient (r)	0.9998
Linearity range (μg mL^{-1})	2.00–80.00
Number of data points	10
LOD (μg mL^{-1})	0.75
LOQ (μg mL^{-1})	2.00

Validation of the proposed method was performed with respect to linearity, limit of detection (LOD), limit of quantitation (LOQ), repeatability, accuracy, precision, recovery, selectivity, robustness and ruggedsess according to the ICH Guidelines [18].

3.2.1. Linearity range

Under the optimum analysis conditions, linearity was studied in the concentration range of 2.00–80.00 μg mL^{-1} for ZAF [21]. In all cases 20.00 μg mL^{-1} of M was added as internal standard. The ratio of peak area (ZAF/M) was chosen for plotting calibration curve because of providing lower R.S.D. (1.30%) than the ratio of peak normalization technique (as the ratio ZAF peak area/migration time-M peak area/migration time) and the best linearity ($r = 0.9998$). Calibration curve was constructed with 10 different ZAF concentrations. Each point of the calibration graph corresponded to the mean value obtained from 7 independent measurements. The linearity curves were defined by the following equation: $y = 0.1686x + 0.0167$, where y is the ratio of peak area of ZAF to M and x is the concentration of ZAF expressed in μg mL^{-1}. The correlation coefficient was 0.9998. All raw data were calculated using calibration curve.

The r value found to be significant $t_c = 91.30 > t_t = 2.57$, $p < 0.05$ for proposed method. In Table 1, the analytical characteristics are summarized for proposed CZE method.

3.2.2. Sensitivity

A signal to noise ratio (S/N) of approximately 3:1 is generally considered to be acceptable for estimating the limit of detection (LOD), which is lowest concentration that can be detected. The calculated LOD values of ZAF was 0.75 μg mL^{-1} (R.S.D. = 3.88%) ($n = 7$) [22–24].

The limit of quantification (LOQ) is the lowest concentration of ZAF on the calibration curve that can be quantified with acceptable precision and accuracy [25,26]. The LOQ was found as 2.00 μg mL^{-1} (R.S.D. = 2.75% and bias = 3.00%) for proposed method.

3.2.3. Precision

The assay was investigated with respect to repeatability and intermediate precision [24,27–29]. In order to measure repeatability of the system (while keeping the operating conditions identical), 12 consecutive injections were made with a standard solution containing 40.00 μg mL^{-1} of ZAF and 20.00 μg mL^{-1} of M and the results were evaluated by considering migration time, peak area, peak normalization (peak area/migration time),

Table 2
Repeatability of peak area, peak normalization, ratio of peak area and ratio of peak normalization values (ZAF: 40.00 μg mL^{-1} and M: 20.00 μg mL^{-1}) (n = 12)

	ZAF			M			Ratio of peak area	Ratio of PN
	Migration time (min)	Peak area	PN	Migration time (min)	Peak area	PN		
$x \pm$ S.E.	4.92 ± 0.04	9.94 ± 0.07	2.02 ± 0.02	5.33 ± 0.03	1.44 ± 0.01	0.27 ± 0.003	6.90 ± 0.03	7.47 ± 0.06
S.D.	0.15	0.24	0.07	0.10	0.04	0.01	0.09	0.21
R.S.D.%	3.05	0.41	3.47	1.88	2.78	3.70	1.30	2.81

$\bar{x}$: mean ± standard error; S.D.: standard deviation; R.S.D.%: relative standard deviation; PN: (Peak Normalization): peak area/migration time.

Table 3
Precision and accuracy data for proposed method (n = 7)

Added (μg mL^{-1})	Intra-day			Inter-day		
	Found[a] (μg mL^{-1})	Precision R.S.D.%	Accuracy[b] (bias%)	Found[a] (μg mL^{-1})	Precision R.S.D.%	Accuracy[b] (bias%)
10.00	10.04 ± 0.05	1.43	0.40	9.84 ± 0.06	1.60	−1.40
40.00	40.25 ± 0.18	1.16	0.63	40.57 ± 0.17	1.13	1.43
70.00	70.37 ± 0.21	0.78	0.53	70.57 ± 0.25	0.93	0.81

[a] Found—$\bar{x}$: mean ± standard error; R.S.D.%: relative standard deviation.
[b] Accuracy: [(founded − added)/added] × 100.

ratio of peak normalization and ratio of peak area values of ZAF and M. The precision values with their R.S.D. are shown in Table 2. The amount of ZAF was found to be 40.01 ± 0.16 with R.S.D. of 1.35% for CZE method. Percentage recovery of ZAF was calculated as 100.01 ± 0.39% with R.S.D.% of 1.36. 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 (10.00, 40.00 and 70.00 μg mL^{-1}) were analyzed in 7 independent series in the same day (intra-day precision) and 7 consecutive days (inter-day precision) within each series every sample was injected three times. The precision of the analysis was determined by calculating the relative standard deviation (R.S.D.%). The R.S.D. values of intra-day and inter-day studies varied from 0.78 to 1.60% showed that the intermediate precision of the method was satisfactory (Table 3). The precision around the mean value should not exceed 15% of the R.S.D.% [24,25,28,29].

3.2.4. Accuracy and recovery

The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value [24,25,28,30,31]. It is determined by calculating the percentage relative error (bias%) between the measured mean concentrations and added concentrations at the same concentration of ZAF [25]. Table 3 shows the results obtained for intra- and inter-day accuracy.

Recovery studies for the accuracy of the method were performed by spiking synthetic mixture with known amount of ZAF [28,31,32]. For this purpose, the determination of ZAF in a synthetic tablet samples (the mixture of excipients such as croscarmellose sodium, lactose, microcrystalline cellulose, povidone, magnesium stearate, methylhydroxypropylmethylcellulose and titanium dioxide and labelled amount of ZAF as in pharmaceutical formulation) were prepared. Samples were treated as described in the procedure for sample preparation. The amount of 20 mg of ZAF was found to be 20.03 ± 0.04. The mean percentage recoveries for 40.00 μg mL^{-1} of ZAF were found as 99.05–100.88% with R.S.D. of 0.60% (Table 4).

3.2.5. Selectivity

In order to evaluate the excipients in this method, the standard addition method was applied [28]. The regression equation of standard addition method was found to be $y = 0.1689x + 2.8694$, $r = 0.9974$. y is the ratio of peak area (ZAF/M) and x is the concentration of ZAF. There was no difference between the slopes of two methods with calibration curve and standard addition methods. These data showed that there was no interaction of excipients in the analysis of ZAF in pharmaceutical formulations by the proposed method. These values showed that no significant excipients interference, thus the procedures was able to determination of ZAF in the presence of excipients.

Comparison of the electropherograms of ZAF standard, synthetic and tablet solutions containing the equivalent concentrations of ZAF are identical (Fig. 4a–c) and showed that the migration time of ZAF and M did not change. Accordingly these results, the proposed methods can be considered selective.

Table 4
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
20.05	100.25
20.10	100.48
19.99	99.95
19.98	99.88
20.12	100.58
19.81	99.05
20.18	100.88

$\bar{x}$: mean ± standard error (20.03 ± 0.04). S.D.: standard deviation (0.12). R.S.D.%: relative standard deviation (0.60).

Table 5
The robustness data of proposed method ($n = 3$)

	Found ($\mu g\,mL^{-1}$)	R.S.D.%	Bias%
Standard (40.00 $\mu g\,mL^{-1}$)	40.11 ± 0.05	0.22	0.28
pH 8.4	39.99 ± 0.08	0.33	−0.03
pH 8.6	40.07 ± 0.05	0.20	0.18
Buffer molarity (40.00 mM)	39.97 ± 0.04	0.18	−0.08
Buffer molarity (50.00 mM)	40.09 ± 0.03	0.12	0.23
Applied voltage (29 kV)	40.11 ± 0.11	0.50	0.28
Applied voltage (29.5 kV)	40.11 ± 0.03	0.12	0.28
Wavelength (238 nm)	40.04 ± 0.07	0.30	0.10
Wavelength (242 nm)	40.02 ± 0.05	0.20	0.05

$\bar{x}$: mean ± standard error; R.S.D.%: relative standard deviation.

3.2.6. Robustness

The robustness of the proposed method was examined by evaluating the influence of small variations of some of the most important procedure variables such as buffer pH (8.4 and 8.6), buffer concentration (40 and 50 mM), applied voltage (29 and 29.5 kV) and detection wavelength (238 and 242 nm) for 40.00 $\mu g\,mL^{-1}$ of ZAF in CZE method [29,30]. Analyses were carried out in triplicate and only one parameter was changed in the experiments at a time. The migration time ratios and peak area ratios under the various conditions were not different compared to the optimum conditions (Table 5). As shown in Table 5, none of these variables significantly affected the assay of ZAF and the proposed methods could be considered robust.

3.2.7. Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 40.00 $\mu g\,mL^{-1}$ of ZAF in CZE method using the same instrument by two different analysts under the same optimized conditions at different days [28–31] (Table 6). The obtained results were found to reproducible, since there was no significant difference between the results obtained by the two analysts ($t_c = 10.00 > t_t = 2.00$, $p > 0.05$). Thus, the proposed methods could be considered robust.

3.2.8. Stability

Stability of the standard solution of ZAF in acetonitrile, stored in the dark and at 4 °C, was evaluated at various time points over 2 months. The absorption spectrum of this solution was checked and ZAF solution was found to be unchanged within this period.

Table 6
The ruggedness of proposed method (added of ZAF amount of 40.00 $\mu g\,mL^{-1}$) ($n = 7$)

Analyst 1[a] found ($\mu g\,mL^{-1}$)	Analyst 2[b] found ($\mu g\,mL^{-1}$)
40.66	39.78
40.76	40.48
40.24	39.75
39.91	40.31
40.41	40.59
40.11	39.87
40.04	40.36

$\bar{x}$: mean ± standard error; R.S.D.%: relative standard deviation (t_c: calculated t value; t_t: tabulated t value; t_t: 2.00).
[a] $\bar{x}$: 40.30 ± 0.12; S.D.: 0.32; R.S.D.%: 0.79; t_c: 10.00 > t_t: 2.00; $p > 0.05$.
[b] $\bar{x}$: 40.16 ± 0.13; S.D.: 0.35; R.S.D.%: 0.87.

Table 7
The results of pharmaceutical preparations containing ZAF analyzed by proposed methods ($n = 7$)

Proposed method[a] (found (mg))	Compared method[b] [15] (found (mg))
20.15	20.09
20.26	20.04
20.08	19.99
19.96	20.02
20.13	20.10
19.90	20.06
20.23	19.97

$\bar{x}$: mean ± standard error; R.S.D.%: relative standard deviation. (t_c: calculated t value; t_t: tabulated t value; $t_t = 2.00$).
[a] $\bar{x}$: 20.10 ± 0.05; S.D.: 0.13; R.S.D.%: 0.66; t_c: 7.50 > t_t: 2.00; $p > 0.05$.
[b] $\bar{x}$: 20.04 ± 0.02; S.D.: 0.05; R.S.D.%: 0.25.

3.3. Analysis of Accolate® tablets

The optimized proposed CZE method was applied to the determination of ZAF in Accolate® tablets. The amounts of ZAF in tablets were calculated using calibration curve method. The obtained percentage recoveries and the relative standard deviations based on the average of 7 replicate measurements were found and the results are presented in Table 7. The obtained results were compared to those obtained by a reported HPLC method [15]. The statistical comparison of methods was done by Wilcoxon Paired Test ($t_c = 7.50 > t_t = 2.00$, $p > 0.05$). The experimental values (t_c) did not exceed the theoretical ones (t_t) indicating the good agreement with comparison method.

4. Conclusion

A simple, fast and reliable CZE method was developed and validated for the determination of ZAF. The method shows a good performance with respect to specificity, linearity, accuracy, precision and robustness and it offers a simple, fast, inexpensive and precise way for the determination of ZAF in pharmaceutical preparations. The proposed 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.

The developed method is compared with the reported method in the literature, offers several advantages such as lower LOQ and reagent consumption [12,13]. Also HPLC consumes a relatively large amount of organic solvent, which is expensive and harmful to the environment.

The proposed method can be employed for the routine quality control of ZAF in pharmaceutical formulations.

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