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Determination of sertaconazole in pharmaceutical preparations by capillary zone electrophoresis

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Abstract

A capillary electrophoresis assay for the analysis of sertaconazole has been 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 a 20 mM phosphate buffer (pH 4.0) containing 40% acetonitrile, capillary temperature 30 °C, applied voltage 30 kV and 3 s hydrodynamic injection at 50 mbar. The detection wavelength was set to 205 nm. Verapamil was used as internal standard. The method showed good selectivity, repeatability, linearity and sensitivity according to the evaluation of the validation parameters. The method was applied to the determination of sertaconazole in pharmaceutical cream formulations.

Keywords: Sertaconazole; Capillary zone electrophoresis; Validation; Pharmaceuticals

1. Introduction

Sertaconazole (SER) (*RS*)-1-[2-[(7-chloro-1-benzothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole nitrate (Fig. 1) is a broad spectrum antifungal agent with excellent activity against yeasts, dermatophytes and opportunistic fungi. It has a good safety profile, sustained cutaneous retention, and low systemic absorption, all of which make it ideal for topical applications [1,2].

In the literature, only one method was reported for the analysis of SER in pharmaceuticals by liquid chromatography (LC) [3]. Capillary electrophoretic (CE) analysis of SER has not been reported in the literature in any matrix.

CE is an analytical tool that has shown great promise in replacing many conventional methods, especially LC. The main attraction of CE was that it was fast, used small amounts of sample and reagents, and was extremely versatile, being able to separate large and small analytes, both neutral and charged [4,5].

The aim of this study was to demonstrate method development and validation strategies of CE for the analysis of SER and to describe a capillary zone electrophoretic (CZE) method for the determination of SER. For this purpose, the influence of buffer concentration, buffer pH, acetonitrile (ACN) as organic modifier, capillary temperature, applied voltage and injection time was systemically investigated and the method validation studies were performed. The validated method was applied to the pharmaceutical cream formulations.

2. Experimental

2.1. Apparatus

All experiments were performed using an Agilent 3D CE (Waldbronn, Germany) system equipped with a diode-array UV detector, an auto sampler, a temperature controller and

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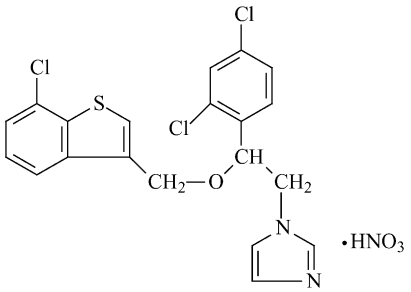

Fig. 1. Chemical structure of SER.

30 kV high voltage power supply. A CE Chemstation software was used for instrument control, data acquisition and data analysis. A fused-silica capillary of 80.5 cm total length (72.0 cm effective length) and 50 μm i.d. was used.

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

The pH of solutions was measured by a pH meter (Mettler Model MA 235, Switzerland).

2.2. Chemicals and reagents

SER was kindly supplied by Adeka Drug Company (Turkey). Verapamil (IS) was purchased from Sigma. All other chemicals were of analytical reagent grade from Merck. Milli-Q water system (Barnstead, USA) was used for the preparation of buffer and other aqueous solutions. Pharmaceutical preparations of SER were obtained from local pharmacies.

2.3. Standard and sample solutions

2.3.1. Standard solutions

Standard stock solutions of SER (1000 μg mL^{-1}) and verapamil (IS) (1000 μg mL^{-1}) were prepared in methanol and water, respectively. These solutions were kept at +4 °C.

Various aliquots of standard solution were taken, the IS added and then diluted to 1 mL with 20 mM phosphate buffer (pH 4.0) containing 40% ACN to give a final analyte concentration of desired.

2.3.2. Running buffer

Potassium dihydrogenphosphate (200 mM) and ACN were mixed at the volumes giving the desired concentration and percentage of organic modifier. The exact pH of running buffer was adjusted by the addition of 0.1 M phosphoric acid. The final optimum running buffer consisted of 20 mM phosphate buffer (pH 4.0) and 40% (v/v) ACN.

2.3.3. Preparation of the pharmaceutical cream formulation solution

A 2 g mass of pharmaceutical cream (label amount: 2% SER) was weighed into a 25 mL beaker and dispersed with methanol using a stirring rod. The resulting solution was transferred to a 50 mL flask, the beaker was washed three times with the same solvent and the washings added to the flask. The flask was sonicated for 15 min and diluted to the mark with methanol. Then an aliquot was centrifuged at 5000 rpm for 15 min, 1 mL of clear supernatant was transferred to a 5 mL flask and diluted to the mark with methanol. Appropriate solutions were prepared by taking suitable aliquots of this latter solution and diluting them with 20 mM phosphate buffer (pH 4.0) containing 40% ACN. Then pharmaceutical cream formulation solutions were analyzed by CZE.

All solutions were filtered through a 0.45 μm syringe filter and degassed with ultrasonic bath for 5 min before injection to the CE system.

2.4. Electrophoretic procedure

Electrophoretic separations were carried out using fused silica capillary having 50 μm i.d. and 80.5 cm total length (72 cm effective length), in a positive mode using constant voltage (30 kV). At the beginning of each working day, the capillary rinsed with 0.1 M NaOH for 15 min. Between each injection, the capillary was rinsed with 0.1 M NaOH (2 min), water (2 min) and running buffer (4 min). Injection was performed hydrodynamically at the anodic side by pressure (50 mbar) for 3 s and capillary temperature was 30 °C. SER and IS were detected using a diode-array detector at 205 nm (bandwidth 10 nm).

3. Results and discussion

3.1. Optimization of electrophoretic conditions

Manipulation of buffer pH is a key strategy for optimizing the separation of ionizable analytes in CE because buffer pH determines the degree of ionization of the solutes and their electrophoretic mobility [6,7]. The effect of pH was investigated in the range from 3.0 to 5.0 (20 mM phosphate buffer and 40% ACN) (Fig. 2). When the pH increased, the

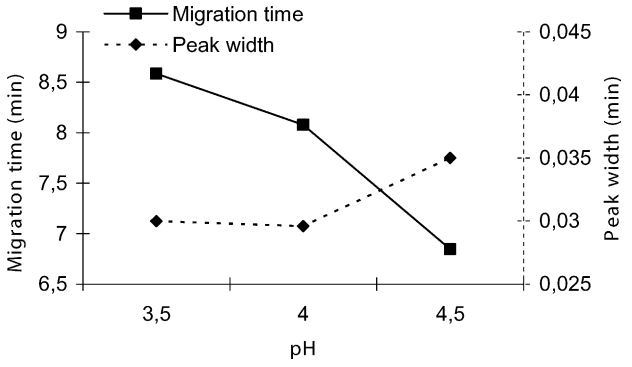

Fig. 2. Effect of buffer pH on migration time and peak width. Operating conditions: 20 mM phosphate buffer, 40% ACN, hydrodynamic injection (3 s at 50 mbar) 30 kV, 25 °C, 205 nm (bandwidth 10 nm) (SER and IS: 20 μg mL^{-1}).

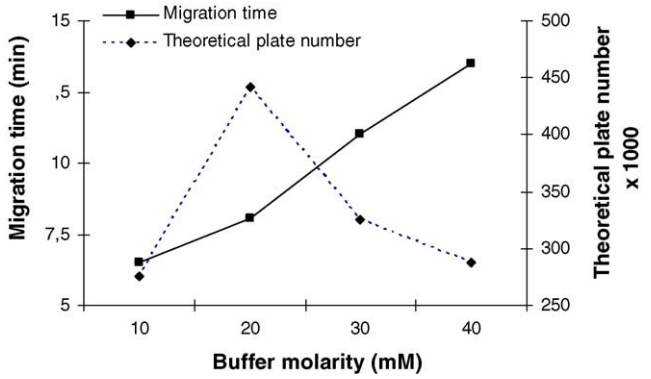

Fig. 3. Effect of buffer molarity on migration time and theoretical plate number. Operating conditions: phosphate buffer (pH 4.0), 40% ACN, hydrodynamic injection (3 s at 50 mbar) 30 kV, 25 °C, 205 nm (bandwidth 10 nm) (SER and IS: 20 μg mL^{-1}).

migration times of SER and IS were decreased but peak tailing occurred above pH 4.0. Therefore, pH 4.0 was selected as the optimum pH value of the running buffer for short analysis time and good peak shape.

The effect of phosphate concentration of running buffer was examined by varying the concentration from 10 to 40 mM (pH: 4.0 and 40% ACN) (Fig. 3). When the phosphate concentration increased, the migration times of SER and IS were increased but the resolution between them remained without any change. In considering the efficiency, migration time and peak symmetry, 20 mM was chosen as the optimum concentration of the running buffer.

Addition of organic solvent affects migration time, peak symmetry and selectivity [8,9]. In this study, ACN was selected to obtain short analysis time and to improve peak shape. ACN was added at various concentrations (20, 30, 40%, v/v) to the running buffer of 20 mM phosphate buffer pH 4.0 (Fig. 4) and 40% ACN was chosen to get short analysis time and to prevent peak tailing.

Injection time affects on the peak width and peak height. Analysis was performed in changing injection times from 1 to 5 s at 50 mbar. After 3 s the peak widths of SER and IS were

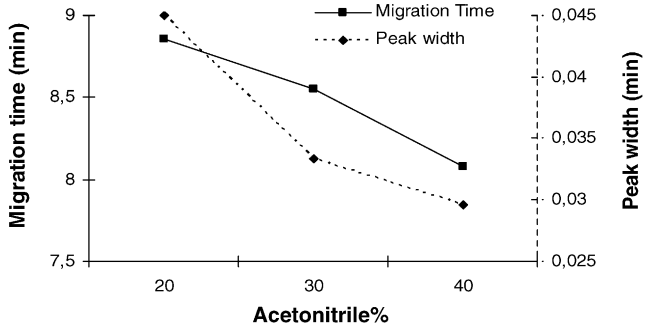

Fig. 4. Effect of ACN percentage on migration time and peak width. Operating conditions: 20 mM phosphate buffer (pH 4.0), hydrodynamic injection (3 s at 50 mbar) 30 kV, 25 °C, 205 nm (bandwidth 10 nm) (SER and IS: 20 μg mL^{-1}).

increased and the peak shapes were deformed. Therefore, 3 s was chosen as the optimum injection time.

The influence of the temperature on analysis was investigated at 20, 25 and 30 °C. A 30 °C was chosen as the optimum temperature because of the shorter analysis time.

The effect of voltage on the migration time of the analytes was studied. As expected, higher voltage gives shorter migration time for the analytes. At 30 kV, the analysis time was the shortest and the currents were not excessive (14.0 μA), so this voltage was selected as the optimum run voltage.

The detection wavelength was selected as 205 nm (bandwidth 10 nm) in which SER and IS had the maximum absorption so that the sensitivity of the method was increased.

Through the experiments above, the optimum conditions for the determination of SER were decided: 20 mM phosphate buffer at pH 4.0 containing 40% ACN, applied voltage 30 kV (current ca. 14.0 μA), hydrodynamic injection for 3 s at 50 mbar, working temperature 30 °C and detection at 205 nm. Under these conditions, SER and IS were eluted in 8.08 ± 0.04 and 8.69 ± 0.05 min (n = 10), respectively. The typical electropherogram of a standard solution of SER and IS is shown in Fig. 5a.

3.2. Validation

Numerous studies have shown that the use of internal standard is crucial for reproducibility in CZE in order to compensate injection errors and minor fluctuations of the migration time [10]. In this study, verapamil, a potent antihypertensive agent, was selected as an internal standard because of its suitable migration time. The assay of SER was validated with respect to linearity, precision, accuracy, selectivity and robustness [11,12].

3.2.1. Linearity

The CZE method developed was linear at least in the range of 1.0–50.0 μg mL^{-1} SER. The ratio of peak normalization technique was chosen to calculate SER concentration because of lower relative standard deviation (R.S.D.) (0.87%) and the best linearity (r = 0.9997).

The regression equation was $y = 0.0473(\pm 0.0012)x - 0.0065(\pm 0.0024)$ where y is the ratio of peak normalization (SER/IS) and x is the concentration (n = 6).

Limit of detection (LOD) is the lowest concentration that can be distinguished from the noise level, the concentration of SER at a signal-to-noise ratio of 3:1 was 0.40 μg mL^{-1}.

The limit of quantitation (LOQ) is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. The LOQ was found as 1 μg mL^{-1} with a R.S.D. 5.16% (n = 6).

3.2.2. Precision

The assay was investigated with respect to repeatability and intermediate precision. The repeatability of the system (while keeping the operating conditions identical) was ex-

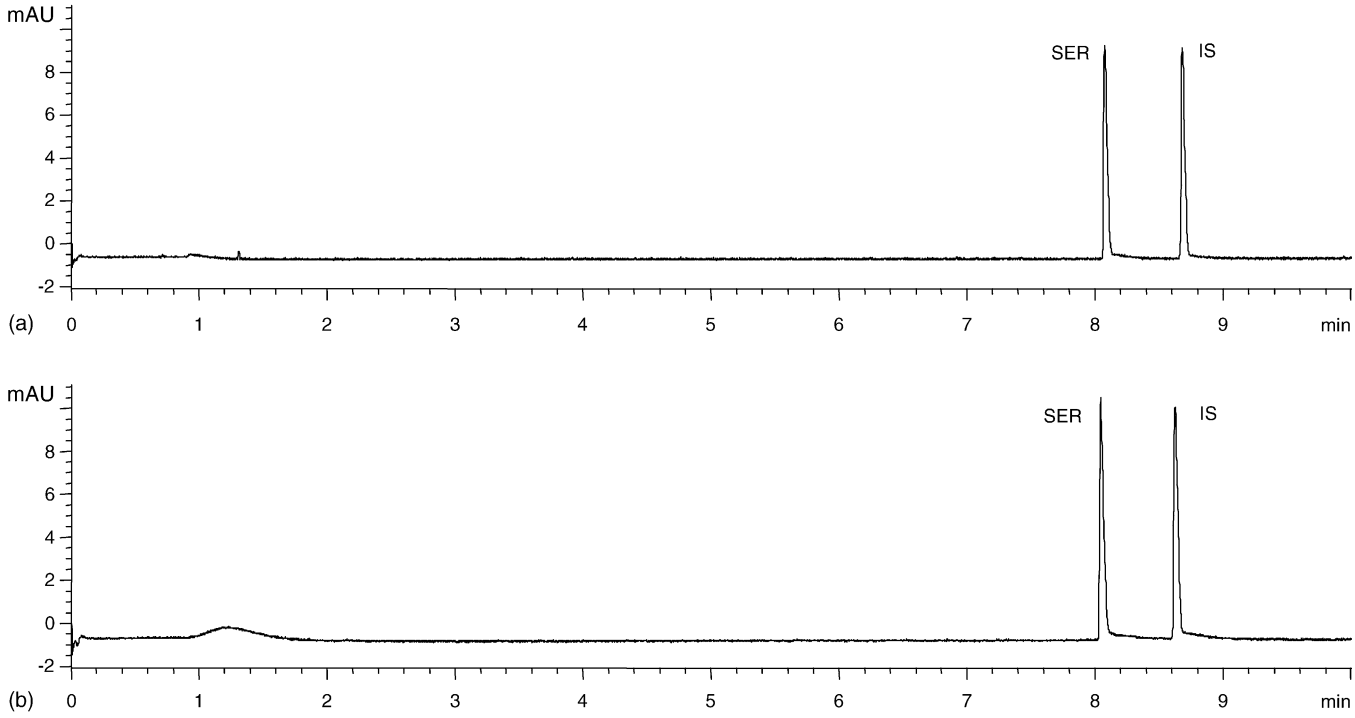

Fig. 5. The electropherograms of (a) 20 μg mL^{-1} SER standard solution, (b) pharmaceutical cream formulation solution including 20 μg mL^{-1} SER, in optimum conditions (IS: 20 μg mL^{-1}).

Table 1
Repeatability of peak area, peak normalization, ratio of peak area and ratio of peak normalization values (SER and IS: 20 μg mL^{-1}) ($n = 10$)

	SER			IS			Ratio of PN	Ratio of peak area
	Migration time (min)	Peak area	PN	Migration time (min)	Peak area	PN		
$\bar{x} \pm$ S.E.	8.20 ± 0.011	9.64 ± 0.129	1.18 ± 0.017	8.82 ± 0.012	11.60 ± 0.138	1.32 ± 0.017	0.89 ± 0.003	0.83 ± 0.003
S.D.	0.02	0.29	0.04	0.03	0.31	0.04	0.01	0.01
R.S.D. (%)	0.30	2.99	3.17	0.31	2.66	2.88	0.87	0.88

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

amined by injecting 20 μg mL^{-1} of SER and 20 μg mL^{-1} of IS with 10 replicate injections and they were evaluated by considering migration time, peak area, ratio of peak normalization and ratio of peak area values of SER and IS. The precision values with their R.S.D. are shown in Table 1.

Three different concentrations of SER (in the linear range) were analyzed in six independent series in the same day (intra-day precision) and six consecutive days (inter-day precision) within each series every sample was injected three times. The R.S.D. values of intra- and inter-day studies varied from 1.03 to 1.95% showed that the intermediate precision of the method was satisfactory (Table 2).

3.2.3. Accuracy

The accuracy of a method is expressed as the closeness of agreement between the found value and reference value. It is determined by calculating the percentage relative error between the measured mean concentrations and added concentrations at the same concentration of SER. The results

Table 2
Precision and accuracy of the developed CZE method for the analysis of SER ($n = 6$)

Added (g mL^{-1})	Intra-day			Inter-day		
	Found $\bar{x} \pm$ S.E.	Precision R.S.D. (%)	Accuracy bias (%)	Found $\bar{x} \pm$ S.E.	Precision R.S.D. (%)	Accuracy bias (%)
5	4.92 ± 0.02	1.03	−1.58	4.96 ± 0.04	1.94	−0.88
20	20.33 ± 0.11	1.30	1.66	20.13 ± 0.16	1.95	0.64
40	39.45 ± 0.24	1.48	−1.38	39.74 ± 0.16	0.97	−0.64

Bias (%) = [100 × (found − added)/added].

Table 3
Recovery data of the developed CZE method for the analysis of SER ($n = 6$)

Theoretical concentration ($\mu g\,mL^{-1}$)	Found concentration ($\mu g\,mL^{-1}$)	Recovery (%)	R.S.D. (%)
10	9.98 ± 0.15	99.82	3.63
20	19.92 ± 0.31	99.60	3.85
30	30.03 ± 0.31	100.10	2.55

obtained for intra and inter day accuracy were $\leq$1.66 and $\leq$0.64%, respectively (Table 2).

3.2.4. Recovery

According to official validation guidelines [8], in cases where it is impossible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product for determining recovery. For this reason, in order to know whether the excipients in the pharmaceutical cream formulation show any interference with the analysis, the recovery test was done by the standard addition method at three concentrations (10, 20 and 30 $\mu g\,mL^{-1}$). The results were summarized in Table 3. Closeness of the results to 100% showed that recovery of the method was very good.

3.2.5. Selectivity

The developed method was applied to the direct determination of SER in pharmaceutical formulations. The electropherogram obtained from commercial pharmaceutical cream formulation was identical with that obtained from standard solution containing an equivalent concentration of SER (Fig. 5).

3.2.6. Robustness

Robustness shows the reliability of an analyte with respect to deliberate variations in method parameters [12]. Only one parameter was changed in the experiments at a time. The determination of 20 $\mu g\,mL^{-1}$ SER standard solution under the various conditions was performed (Table 4). The statistically comparison was done with Friedman analysis and no difference was found between results ($P = 0.064 > P = 0.050$). Therefore, the method is robust to the small changes in experimental conditions.

Table 4
The robustness data of CZE method (SER 20 $\mu g\,mL^{-1}$) ($n = 6$)

	$\bar{x} \pm$ S.E.	R.S.D. (%)	Bias (%)
Standard	19.92 ± 0.24	2.96	0.41
pH 3.9	19.79 ± 0.23	2.84	−1.05
pH 4.1	19.65 ± 0.15	1.86	−1.73
39% ACN	19.85 ± 0.21	2.61	−0.75
41% ACN	20.01 ± 0.09	1.13	0.02
19 mM phosphate buffer	19.51 ± 0.18	2.27	−2.48
21 mM phosphate buffer	20.13 ± 0.22	2.63	0.62

Table 5
The data of cream formulation analysed by calibration and standard addition methods (theoretical amount: 20 $\mu g\,mL^{-1}$) ($n = 6$)

	Calibration	Standard addition
$\bar{x} \pm$ S.E.	20.03 ± 0.08	19.94 ± 0.23
S.D.	0.20	0.56
R.S.D. (%)	0.99	2.79
Recovery (%)	100.15	99.69

$P_c = 0.345 > P = 0.05$.

3.3. Analysis of pharmaceutical cream formulations

SER was analyzed through the procedure as given in Section 2.3.3. Analysis was performed under optimum conditions. Each pharmaceutical cream formulation solution was analyzed six independent determinations and each series were injected three times. The results obtained for SER from calibration method were compared with the data obtained from standard addition method. The statistical comparison of two methods was done by Wilcoxon paired test ($P = 0.345 > P = 0.050$). The results showed that there was no significant difference between them (Table 5).

4. Conclusion

A simple, fast, efficient, cheap and reliable CE method was developed for the analysis of SER in the pharmaceutical formulations. The CE assay was supported by method validation, which demonstrated good linearity, precision, accuracy and robustness.

The developed method when comparing with the reported LC method, offers several advantages such as shorter analysis time, lower LOQ and reagent consumption. In the existing LC method, analysis was performed on CN column at 20 min with higher LOQ (64 $\mu g\,mL^{-1}$). Also LC consumes a relatively large amount of organic solvent, which is expensive and harmful to the environment. It can be concluded that the proposed method provides an alternative procedure for the quality control of SER in pharmaceutical formulations.

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