Journal of Reports in Pharmaceutical Sciences 2017, 6(2), 96-104

Analytical and Physicochemical Characterization of Cefquinome Using Capillary Electrophoresis

Leila Behbood[a]*, Yahya Mrestani[b], Ralf Warrass[c], Reinhard Neubert[b]

[a] Pharmaceutical Science Research Center, Faculty of Pharmacy, Kermanshah University of Medical Science, Kermanshah, Iran.
[b]Institut of Pharmacy, Martin Luther University, Wolfgang Langenbeck Str. 4, 06120 Halle-Wittenberg, Germany.
[c]Intervet Innovation GmbH, Zur Propstei, 55270 Schwabenheim, Germany.

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 2017-02-20
Revised: 2017-04-27
Accepted: 2017-06-02
ePublished: 2017-06-10

***Keywords*:**
Capillary Electrophoresis
Cefquinome Sulfate
Cephalosporins

A B S T R A C T

The Capillary Electrophoresis (CE) is a very useful technique to determine and to quantify pharmaceutical materials. In this work, a capillary electrophoresis method has been developed for the determination of Cefquinome sulfate in aqueous solution and in biological media (urine, milk and plasma). The ion mobility was measured at the various pH values. The detection limit and the relative standard deviation of migration times of the peak area were determined. The influence of the pH on the separation of Cefquinome sulfate in plasma and in urine was investigated. The SDS micellar elecrokinetic chromatography (SDS-MEKC) was used for the determination of Cefquinome sulfate in water, plasma and urine. The solubility and stability of Cefquinome sulfate were considered at the different pH values. The best stability was found at pH 7.4. Also the hydrophilic/lipophilic properties of Cefquinome sulfate (partition coefficient and permeation, in vitro) were investigated. The hydrophilic properties of cefquinome affected the results; the partition coefficient was 0.01 at pH 7.4. In addition, the permeation coefficient was 1.65 at pH 7.4. Acid the dissociation constant of cefquinome was calculated using CZE. The low amount of sample required and the relatively short analyze time were the main advantages of this method.

*Corresponding Author: Leila Behbood E-mail: Leila_behbood@yahoo.com

Introduction

Most of drugs are either acidic or/and basic groups in their molecular structure. The basis for determination and separation in Capillary Electrophoresis (CE) is the differences between the analytes' electrophoretic mobility, which are related to their charge and size [1]. Consequently, determination of many drugs is possible by this method, since the measurement of physicochemical properties of drug is important to ensure the safety and efficacy of the final product. Although high performance liquid chromatography (HPLC) is the most common technique used for analysis of drugs, the CE method has some advantages over HPLC such as less required sample and consuming very little organic solvent. Micellar electrokinetic chromatography (MEKC) provides a composition technique of CE and chromatography which has been used to analysis of drugs due to some values such as high efficacy, simplicity of operation, rapidity and low cost [2, 3]. On the other hand, in recent years a number of β-lactamase stable, highly- active, broad-spectrum cephalosporin have been developed [4-6]. Until now, these antibiotics were measured using various analysis methods such as HPLC, MEKC and Capillary zone electrophoresis (CZE) [7-9]. Cefquinome sulfate (Fig.1) is a board-spectrum fourth generation cephalosporin antibiotic, which has been developed exclusively for veterinary infections in animals [10-12].This antibiotic has determined using High performance liquid chromatography (HPLC) [13, 14]. Therefore, in this paper, using CZE some quantitative properties of Cefquinome sulfate has been considered, in aqueous solution and biological media (urine, milk and plasma). Sodium dodecyl sulfate-Micellar Electrokinetic Cromatography (SDS-MEKC) was used for the separation of Cefquinome sulfate from biological media. Furthermore, the physicochemical parameters of Cefquinome sulfate using Capillary Zone Electrophoresis were studied.

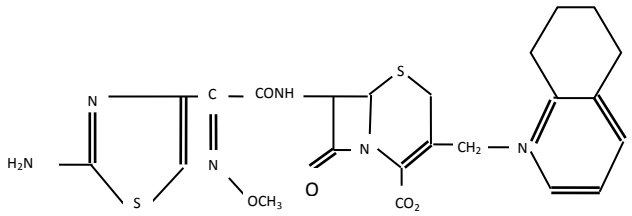

Fig.1. Molecular Structure of Cefquinome sulfate

Materials and Methods

Chemicals

Cefquinome sulfate was obtained from Intervet Innovation GmbH (Intervet, Germany). Sodium dodecyl sulfate (SDS) was obtained from Fluka chemie GmbH (Buchs, Switzerland). Dimethylsulfoxid (DMSO), sodium hydroxide, boric acid and Citric acid were obtained from Roth (Karlsruhe, Germany). Hydrochloric acid was obtained from KMF Laborchemi Handels GmbH (Lohmer, Germany). Potassium hydrogenphosphate and potassium dihydrogenphosphate were obtained from Merck (Darmstadt, Germany). Dodecanol and n-octanol were obtained from Caesar & Loretz (Hilden, Germany).

Apparatus

Capillary electrophoresis experiments were performed on a Hewlett Packard Model G1600A (Waldbronn, Germany) ^{3D} CE system with a diode-array detector from 190 to 600 nm. The detection wavelength was at 200 nm. The CE silica fused capillary was obtained from Chromatographie Service GmbH (Langerwehe, Germany). The pH of buffers was measured at 25 ºC using a Testo® 252 pHmeter from testo (Lenzkirch, Germany)

Sample preparation

Aqueous solution

Standard solutions of Cefquinome sulfate in distillation water (10-500µg/ml) were prepared and filtered through a 0.45 µm syringe filter from Roth (Karlsruhe, Germany), before injection.

Plasma *solution*

Plasma was separated by centrifugation at 2000 g for 10 min at room temperature. Heparin was used as anticoagulant agent in plasma preparation. Standard solutions of Cefquinome sulfate were prepared from 20-800µg/ml in Plasma. For deproteinization of plasma 3 ml of acetonitrile was added to 1 ml of plasma. The solution was then centrifuged for 10 min at 2000 g at room temperature. Plasma samples were filtered through a 0.45 µm syringe filter and measured using CE.

Urine solution

Standard solutions of Cefquinome sulfate were prepared from 10 to 500µg/ml in Urine. These solutions were diluted with 10 mM phosphate buffer at pH 7.4 (1:1- v: v) .
The samples were filtered through a 0.45 µm polypropylene syringe filter.

Milk solution

Standard solutions of Cefquinome sulfate were prepared from 20-400µg/ml in Milk.1 ml of acetonitrile was added to 1 ml of Milk. The solution was then centrifuged for 10 min at 2000 g at room temperature. The milk samples were filtered through a 0.45 µm polypropylene syringe filter and measured using CZE.

Buffer preparation

50 mM citrate buffer (Sörensen, pH 3, 3.5, 4, 4.5,5 and 5.5), 50 mM phosphate buffer (KH_2PO4-K_2HPO_4, pH 6, 6.5, 7 and 7.5) and 50 mM borate buffer (Sörensen and Clark, pH 8, 8.5,9, 9.5, 10, 10.5 and 11) were prepared like Rauscher et al. [15]. The buffer solutions were filtered through a 0.45 µm syringe filter.

Recovery study

Samples of plasma and milk were spiked with 10 and 100µg/ml of Cefquinome sulfate. The resulting peak areas were compared with peaks resulting from aqueous solution at the same concentration.

Analytical Conditions

A new capillary was rinsed for 10 min with 1M sodium hydroxide solution and for 5 min with water at 25 ºC. Before each injection, the capillary was flushed with 0.1 M sodium Hydroxide solution for 3min, with water for 2 min and with the actual buffer solution for 5 min at 25 ºC. The separation potential of 30 kV was used. Samples were injected at 50 mbar pressure for 10 s (hydrodynamic injection). The wavelengths of detection were 200 and 270 nm. Detailed experimental conditions are listed in the figures.

Quantification of partition coefficients (P_{ow})

The partition coefficient of Cefquinome sulfate was determined between water and n-octanol. These two phases were saturated with each other. The Cefquinome sulfate was dissolved in the water phase (200 µg/ml). The n-octanol/buffer solutions were filled into suitable vials and shaken for 12 h at 37 °C. After separation of the samples into both phases, the drug content was analyzed by CZE.

Permeation model

The transport model system has been described by Neubert et al. [16]. The donor and the acceptor compartments were separated by a dodecanol collodium membrane. The effective permeation area of dodecanol collodium membrane was 15.8 cm^2. For permeation, cells were simultaneously used at 37 °C. 20 ml of solution of Cefquinome sulfate in phosphate buffer at pH 7.4 (200 µg/ml of drug) were placed in the donor compartment and 20 ml of the buffer (phosphate, pH 7.4) were filled into the acceptor compartment. The samples (2.0 ml) have been periodically removed from the acceptor compartments over 4 hours and after 4 hours from the donor compartments. The drug content in these samples was analysed. P_G was calculated according to literature [17].

Results and Discussion

Characterization of dissociation behaviors of Cefquinome sulfate

The influence of the pH on the ionic mobility of Cefquinome sulfate was investigated from pH 3 to pH 11 using CE (Fig. 2).

Cefquinome sulfate exhibited different ionic mobility at various pH values. The effective mobilities of Cefquinome sulfate were determined at different pH values using equation (2) [18].

$$\mu_e = \left(\frac{L_G \times L_D}{V}\right)\left(\frac{1}{t_R} - \frac{1}{t_0}\right) \quad (2)$$

Where μ_e is the effective mobility, V is the applied voltage, L_G is the effective capillary length, L_D is the total capillary length, t_R the migration time of the Cefquinome sulfate and t_0 the migration time of neutral marker.

At low pH (3.0-5.9, $t_R < t_0$) Cefquinome sulfate had a positive electrophoretic mobility. From pH 6 to pH 8 ($t_R = t_0$) Cefquinome sulfate showed a zero electrophoretic mobility and moved with the EOF. At pH 8.5 to pH 11($t_R > t_0$) Cefquinome sulfate has negative electrophoretic mobility and migrated in the direction to the cathode.

Also using μ_e and according equation (3), acid dissociation constant (pK_a) of Cefquinome sulfate was calculated.

$$\mu_e = \frac{\left[H^+\right]/K_a}{1+\left[H^+\right]/K_a}\mu_b \quad (3)$$

Where μ_b is the electrophoretic mobility of the fully protonated species.

The pK_a of Cefquinome sulfate was determined like literature [19]. Eq. 3 was used in nonlinear regression to determine the p*Ka* value. Fig. 2 shows plots of the electrophoretic mobilities of Cefquinome sulfate against pH. The interpolated curve was obtained from Eq. (3) using weak bases. The pK_a of Cefquinome sulfate is 3.85 ±0.5.

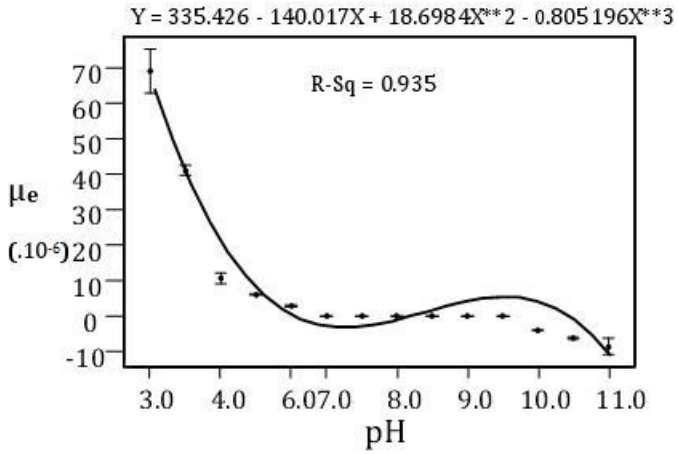

Fig. 2. Ion mobility (μ_e) of Cefquinome sulfate at different pH

Stability of Cefquinome sulfate

The stability of Cefquinome sulfate was investigated in three buffers at two different pH values (6.5, 7.4) using CZE. The results showed that Cefquinome sulfate had good Stability at pH 6.5 and 7.4 until 48 h. At pH 6.5 and after 72 h, 20% of Cefquinome sulfate was decomposed in the buffer solution. Table 1 exhibited the Stability of Cefquinome sulfate at different pH values.

Table 1. Stability of Cefquinome sulfate (Percent of peak area) at different pH (n=3)

Time(hour) / pH	0	24	48	72
6.5	100 ± 1.36	100 ± 3.73	98.5 ±0.27	82.6 ± 0.67
7.4	100 ± 0.69	99.8 ± 0.4	100 ±0.74	96.2 ± 0.66

Solubility of Cefquinome sulfate

Although Cefquinome sulfate exhibited very hydrophilic properties, the solubility of it in aqueous solution and in buffers (pH 6.5, 7.4) was not sufficiently. The results show that Cefquinome sulfate had small solubility at this pH values (Table 2). Good solubility was obtained at the biological pH value (pH=7.4) in compare to another pH value.

Table 2. Solubility of Cefquinome sulfate at different pH (n =3)

pH	Solubility (mg/ml)
6.5	2.869 ± 0.015
7.4	3.933 ± 0.330

Characterization of hydrophilic/ lipophilic properties

For the characterization of the hydrophilic/lipophilic properties of Cefquinome sulfate, a partition coefficient (P_{ow}) in the n-octanol/buffer system was used. The partition coefficient of Cefquinome sulfate was investigated in water and at pH 6.5, 7.4 and 8.5. The partitioning coefficient was calculated using equation (3):

$$P_{ow} = \frac{a_o}{a_w} \quad (3)$$

Where a_o and a_w are the concentrations of the drugs in the n-octanol and in the aqueous phases, respectively.

The results exhibited that Cefquinome sulfate is a very hydrophilic drug (very small partition coefficient).

Furthermore, the permeation of Cefquinome sulfate through artificial lipid membranes (dodecanol collodium membranes) was studied. Cefquinome sulfate showed very little transport via the lipid membranes and had very small permeation coefficient (Table 3).The results confirmed the results with the octanol/buffer system. The content of Cefquinome sulfate in the acceptor was between 0.5% and 1%.

Table 3. Characterzation of hydrophilic/ lipophilic properties of Cefquinome sulfate

pH	Ls (mg/ml)	PG cm/s	VK
6.5	2.869	1.26±0.64.10-3	0.02 ±0.0006
7.4	3.933	1.65±0.52.10-3	0.02 ±0.001
8.4	3.591	0.00±0.00	0.01 ±0.0009

Ls: solubility
P_G: permeation coefficient
V_K: partition coefficient

Quantitative analysis of Cefquinome sulfate

In this paper we studied the determination of Cefquinome sulfate in aqueous solution and in biological media.

Influence of pH in separation of Cefquinome sulfate in plasma and urine, was considered. In plasma, basic pHs over 10 are better for separation of Cefquinome sulfate from plasma compounds. However the optimal separation obtained using 25 mM SDS in 50 mM borate buffer at pH 11. In urine, we used buffer at pH 4-11, but it seems that the pH value of run buffer has no large effect on separation. Finally, the optimal run condition for separation was by adding 50 mM SDS to 10 mM phosphate buffer at pH 7.4. This method (SDS Micellar Electrokinetic Chromatography, SDS-MEKC) is a mode of CE in which the surfactant is added to the run buffer and is used as a separation method widely [20-22].

Aqueous solution

The determination of Cefquinome sulfate in aqueous solution was performed using standard capillary.

The calibration curves in water were made in concentration range from 10 to 500µg/ml using UV detector at 200 nm. Cefquinome sulfate lets to detect and to quantify easy in buffer solutions. In water, the determination of Cefquinome sulfate was very difficult. Cefquinome sulfate exhibited neutral ion mobility in the water and migrated with the EOF. Therefore we could not detect it directly using CZE. For the optimal separation we used the SDS-MEKC. For determination of Cefquinome sulfate in water, 25 mM SDS in 10 mM phosphate buffer at pH 7.4 was used.

Plasma

In plasma, Cefquinome sulfate was analyzed quantitatively at 200 nm. The determination of Cefquinome sulfate in plasma was performed using 25 mM SDS in 50 mM borate buffer at pH 11. For the separation of Cefquinome sulfate in plasma, it is necessary to precipitate the protein components. Good results were achieved through the treatment of the plasma samples with acetonitrile (1:3, v: v). Fig. 3 shows the electropherogram of a blank plasma standard and of Cefquinome sulfate in plasma. The calibration curve of the peak area was linear with a correlation of R= 0.95. Mean recoveries from spiked samples were ٧٠.78% at a Cefquinome sulfate concentration of 100 µg /ml and 63% at concentration of 10µg/ml by comparison with Cefquinome sulfate standard solution of equivalent concentration. To control the reproducibility of the peak areas and of the migration times, five injections of Cefquinome sulfate were mad for these samples.

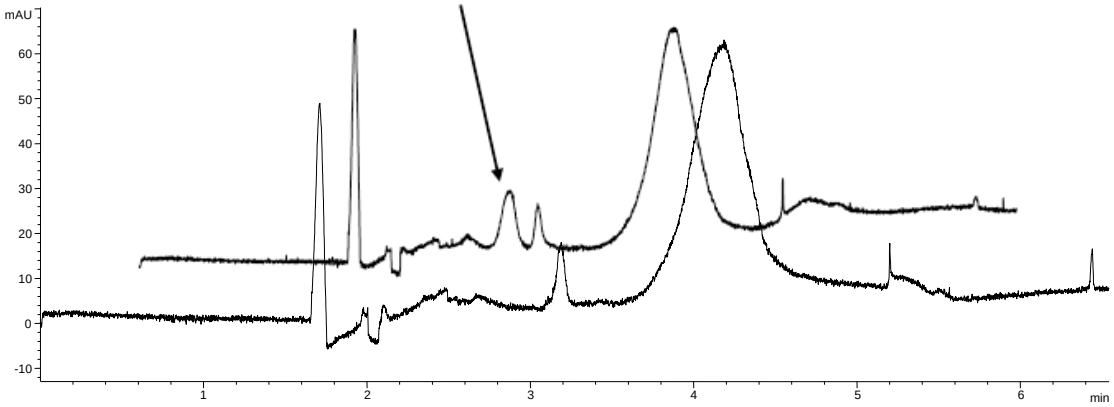

Fig. 3. Electropherogram of a blank plasma standard and a sample of plasma containing Cefquinome sulfate

Milk

The determination of Cefquinome sulfate in milk was performed at pH 11 using borate buffer. For precipitation the protein components, the milk samples were mixed with acetonitrile (1:1, v: v). Fig. 4 shows the electropherogram of a blank milk standard and of Cefquinome sulfate in milk. The calibration curve of the area was linear with a correlation of R= 0.999. Mean recoveries from spiked samples were 75.6% at a Cefquinome sulfate concentration of 100 µg /ml and 80% at concentration of 10µg/ml by comparison with Cefquinome sulfate standard solution of equivalent concentration.

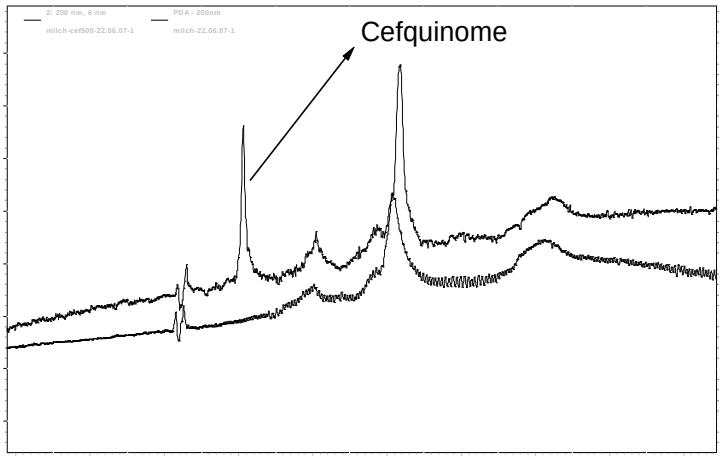

Fig. 4. Electropherogram of 100 µg/ml Cefquinome sulfate in Milk obtained by CE

Urine

The determination of Cefquinome sulfate in urine was performed using SDS-MEKC. For the determination in urine, 50 mM SDS in 10 mM phosphate buffer at pH 7.4 was used. Good detection limit and good repeatability was observed in urine using the SDS-MEKC method. Table 4 presents the analytical parameters for the determination of Cefquinome sulfate in urine.

Table 4. Analytical parameters for the determination of Cefquinome sulfate using CZE.

compound	Limit of detection (µg/ml)	R.S.D% (10-100µg/ml)	Regression equation	R-Sq
water	4.5	0.28-4.38	Y=0.99X+ 6.84	0.998
Plasma	8	0.1-2.3	Y=0.138X + 5.987	0.95
Milk	4.5	٠-7.09	Y=1.416X + 2.167	0.999
urine	3	0.26-6.25	Y=1.29X+ 6.22	0.994

R-Sq: correlation coefficient
R.S.D%: Relative standard deviation of the peak areas

Conclusion

In this work we have developed CZE and SDS-MEKC methods for the characterization of the physicochemical properties and of hydrophilic/ lipophilic properties of Cefquinome sulfate in water and at different pH values. Furthermore, we studied the determination of Cefquinome sulfate in aqueous solution and in biological media like Plasma, Urine and milk using the developed CZE and SDS-MEKC methods. The method described, is exact, easy and repeatable with relatively short analysis time. The results showed very hydrophilic properties of Cefquinome. According to these results it can be foresight that oral absorption of the drug is not great. Also challenge is expected with formulation of Cefquinome as liquid dosage forms, due to its low solubility. The data is useful in preformulation studies of Cefquinom.

Conflict of Interests

Authors certify that there is no actual or potential conflict of interest in relation to this article.

References

[1] Altaria KD, Rogan MM. Introduction to quantitative applications of Capillary Electrophoresis in pharmaceutical analysis Beckman Coulter, 1994, p 1.

[2] Terabe S. Peer Reviewed: Micellar Electrokinetic Chromatography. Anal Chem. 2004; 76:240 A-6 A.

[3] Koji O, Shigeru T. Micellar electrokinetic chromatography. Molecular biotechnology. 1998; 9:253-271.

[4] Kavi J, Andrews J, Ashby J, Hillman G, Wise R. Pharmacokinetics and tissue penetration of cefpirome, a new cephalosporin. Journal of Antimicrobial Chemotherapy. 1988;22:911-916.

[5] Jacobson KL, Cohen SH, Inciardi JF, King JH, Lippert WE, Iglesias T, et al. The relationship between antecedent antibiotic use and resistance to extended-spectrum cephalosporins in group I β-lactamase-producing organisms. *CID*. 1995;21:1107-1113.

[6] Simon C, Stille W. Antibiotic therapy, Schattauer, Stuttgart, Germany, 1989, pp71-114

[7] Mrestani Y, Neubert RH, Härtl A, Wohlrab J. Determination of cephalosporins in urine and bile by capillary zone electrophoresis. Analytica chimica acta. 1997;349:207-213.

[8] Andrási M, Gáspár A, Klekner A. Analysis of cephalosporins in bronchial secretions by capillary electrophoresis after simple pretreatment. J Chromatography B. 2007;846:355-358.

[9] Fukutsu N, Kawasaki T, Saito K, Nakazawa H. Application of high-performance liquid chromatography hyphenated techniques for identification of degradation products of cefpodoxime proxetil. J Chromatogr A. 2006; 1129:153-159.

[10] Thomas E, Thomas V, Wilhelm C. Antibacterial activity of cefquinome against equine bacterial pathogens. Veterinary microbiology. 2006;115:140-147.

[11] Sader HS, Jones RN. Historical overview of the cephalosporin spectrum: four generations of structural evolution. Antimicrobic Newsletter. 1992;8:75-82.

Lang I, Rose M, Thomas E, Zschiesche E. A field study of cefquinome for the treatment of pigs with respiratory disease. Revue de Medecine Veterinaire. 2002;153:575-580.

[12] Sørensen LK, Snor LK. Determination of cephalosporins in raw bovine milk by high-performance liquid chromatography. J Chromatogr A. 2000;882:145-151.

[13] Ehinger A, Schmidt H, Kietzmann M. Tissue distribution of cefquinome after intramammary and "systemic" administration in the isolated perfused bovine udder. The Veterinary Journal. 2006;172:147-53.

[14] Raucher K, Voigt J, Wilke I, Wilke K.-Th Chemische Tabellen und Rechentafeln für analytische Praxis deutsche Verlag für Grundstoffindustrie Leipzig, pp 141-147.

[15] Neubert R, Fürst W. In vitro-Untersuchungen des Arzneistofftransportes.—Methoden, Möglichkeiten, Mechanismen—. Pharmazie in unserer Zeit. 1989;18:112-22.

[16] Mrestani Y, Mrestani-Klaus C, Bretschneider B, Neubert RH. Improvement of lipophilicity and membrane transport of cefuroxime using in vitro models. EJPB. 2004;58:653-657.

[17] Weinberger R. Practical capillary electrophoresis: Academic Press; 2000.

[18] Mrestani Y, Neubert R, Munk A, Wiese M. Determination of dissociation constants of cephalosporins by capillary zone electrophoresis. J Chromatogr A. 1998;803:273-278.

[19] Terabe S. Micellar Electrokinetic Chromatography, Beckman Coulter; 1994, p 1.
[20] Mrestani Y, Janich M, Rüttinger HH, Neubert RH. Characterization of partition and thermodynamic properties of cephalosporins using micellar electrokinetic chromatography in glycodeoxycholic acid solution. J Chromatogr A. 2000;873:237-46.
[21] Raggi MA, Pucci V, Maurizio A, Muzikar J, Kenndler E. Separation of carbamazepine and five metabolites, and analysis in human plasma by micellar electrokinetic capillary chromatography. J Chromatography B. 2002;770:217-225.

