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Determination of ciprofloxacin and its impurities by capillary zone electrophoresis

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Abstract

Capillary zone electrophoresis (CZE) has been elaborated for separation, identification and determination of ciprofloxacin and its impurities. The separation, phosphate buffer pH 6.0 was supplemented with 0.075 M pentane-1-sulfonic acid sodium salt. The elaborated method was validated. The selectivity, linearity, limits of detection (LOD) and quantification (LOQ), precision, and accuracy of capillary zone electrophoresis were evaluated. The results obtained by CZE were also compared with those obtained by liquid chromatography. Regarding the validation results the CE method fulfils the current European Pharmacopoeia (Eur. Ph.) requirements. The evaluated CE method could be applicable to the analysis of different medicinal products containing ciprofloxacin.

Keywords: Pharmaceutical analysis; Ciprofloxacin; Quinolones; Antibiotics

1. Introduction

Ciprofloxacin—the first 1-cyclopropyl fluoroquinolone antibiotic—was synthesised in 1981. It has a broad spectrum of antimicrobial activity and remains effective in a wide variety of indications. Its role is important in bacterial infections treatment. Recently, ciprofloxacin significance as effective drug in *Bacillus anthracis* infection treatment, essentially increased, because of bacteriological (anthrax) terrorists' attack threats.

Structures of ciprofloxacin and related compounds are presented in Fig. 1. Impurities A, B, and D are by-products from the synthesis route. Other impurities C, E, and F, are probably degradation products of ciprofloxacin. Impurity C is a potential photolysis product, impurity E is possibly a degradation product resulting from the decarboxylation of the drug, and the last, impurity F, is a hydroxylation product.

Most of published analytical methods concerning ciprofloxacin and its impurities determination are based on high-performance liquid chromatography (HPLC), which is also recommended by European Pharmacopoeia (Eur. Ph., TLC assay suitable for analysis of impurity A) [1,2]. Some of the authors described the determination of ciprofloxacin in various biological matrices [3,4]. However, some methods have been developed with the usage of capillary electrophoresis (CE). The conditions for a simultaneous determination of series of fluoroquinolone antibiotics under a variety of buffer compositions in pharmaceutical formulations or in biological material were established [5–12]. Other studies have concentrated on the quinolones structure and pK_a values. For instance, Barron et al. [13,14] studied the variation of quinolones dissociation constanses in mixtures of non-aqueous–water media. The application of CE techniques with laser-induced fluorescence to determine ciprofloxacin and its main photodegradation product were also described [15,16].

However, so far only one study has been reported on the determination of ciprofloxacin and its related compounds [17]. In this work low-pH CE method at 272 nm has been validated for the determination of impurities (unspecificated) in a quinolone antibiotic drug substance.

Looking for alternative to HPLC new method (without usage of considerable amounts of expensive and environmentally hazardous organic solvents), which will be easy, quick, cheap, safe, and selective, specific and repeatable, led us to

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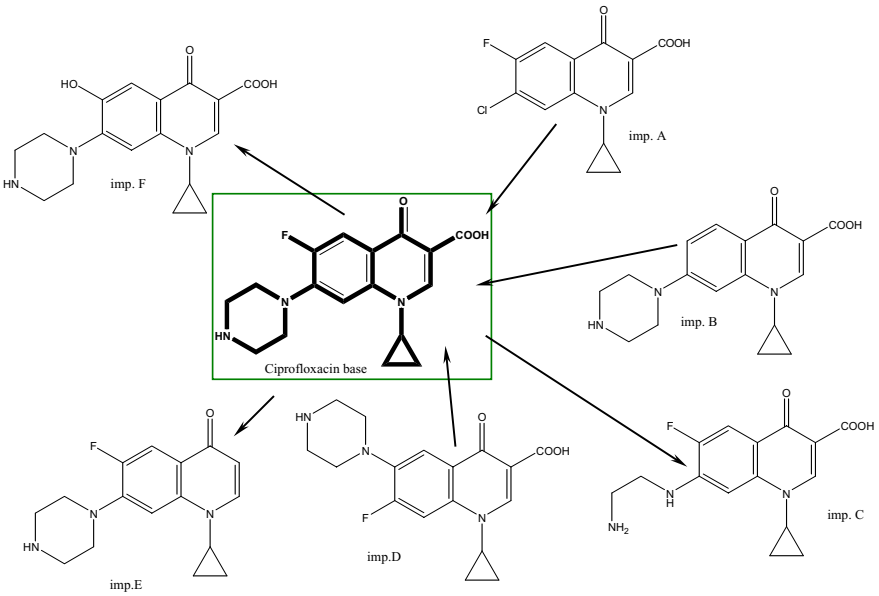

Fig. 1. Structure of ciprofloxacin and its related compounds.

elaborating a capillary zone electrophoresis (CZE) technique for ciprofloxacin and its related substances in pharmaceutical formulations.

The aim of this study was to elaborate and validate the capillary zone electrophoresis method for separation of ciprofloxacin from impurities and to establish the ciprofloxacin content and its purity.

2. Experimental

2.1. Apparatus

Capillary electrophoresis experiments were carried out on Quanta 4000E CE system (Waters, Milford, MA, USA) suitable to perform both hydrodynamic and voltage injection, equipped with 30 kV power supply and UV spectrophotometric detector, connected to data collection system. The selected detection wavelengths, using a mercury lamp with appropriate filter, were 280 nm for monitored ciprofloxacin and B, C impurities and 254 nm for ciprofloxacin and A, D impurities. Separations were performed in an uncoated fused-silica capillary [60 cm (52 cm from inlet to detector) × 75 μm i.d.] Accu-Sep (Waters), thermoregulated at 25 °C. The voltage of 15 kV and hydrodynamic 10 s injections were applied. The capillary was reconditioned every morning before analysis with following solutions: 0.1 M potassium hydroxide for 5 min, water for 5 min and running buffer for 10 min.

The HPLC experiments were carried out on a chromatograph series LC-10Avp Shimadzu (Kyoto, Japan) with UV detector set at 278 nm. Separation was achieved on a LiChroCart RP-18 endcapped column, 5 μm particle size (250 mm × 4.0 mm i.d.) (Merck, Darmstadt, Germany).

2.2. Standards and reagents

Ciprofloxacin hydrochloride certified reference standard (CRS) and its impurities CRS: A (fluoroquinolic acid), B (desfluoro compound), C (ethylenediamine compound), D (by-compound A) were obtained from European Pharmacopoeia (Strasbourg, France). Ciprofloxacin hydrochloride substance (Quimica Sintetica, Madrid, Spain) and Serviflox 500 mg film coated tablets containing ciprofloxacin, from Biochemie Co. (Kundl, Austria) were used in this study.

Disodium tetraborate decahydrate, sodium dihydrogen phosphate, disodium hydrogen phosphate and potassium hydroxide were of analytical reagent grade from POCh (Gliwice, Poland). Pentane-1-sulfonic acid sodium salt (PS) was purchased from Merck (Darmstadt, Germany). Acetonitrile (MeCN), HPLC Far-UV grade, was supplied by Lab-Scan Analytical Sciences (Dublin, Ireland), ammonium hydroxide (25% NH_3 in water) from Odczynniki (Lublin, Poland). Tetrabutylammonium dihydrogenphosphate (TBA) and orthophosphoric acid (85%) were obtained from Fluka (Buchs, Switzerland). Diluted phosphoric acid (7%) was prepared by dilution of the concentrated phosphoric acid solution with water. The water used to prepare standards and samples solutions as well as running buffers, was obtained from Labconco System (Kansas City, MO, USA).

2.3. Preparation of running buffers and mobile phase

The running buffers for CE experiments were prepared as follow: 7.80 g of sodium dihydrogen phosphate (0.1 M) and 4.48 g of disodium hydrogen phosphate (0.025 M) were dissolved in about 480 ml of water, adjusted to pH 6.0, if necessary with diluted phosphoric acid and finally filled up to 500 ml with water (this solution was kept in an amber

bottle). Then, about 50 ml of this solution was supplemented with 0.65 g of pentane-1-sulfonic acid sodium salt and again pH was adjusted to 6.0.

The HPLC mobile phase was prepared as described in the Ciprofloxacin Hydrochloride of Eur. Ph. Monographie [1].

2.4. Preparation of sample and standard solutions

For CE experiments the impurity A was dissolved in a mixture of diluted ammonia–water (0.1:99.9, v/v), while the other impurities B, C, and D were prepared in a mixture of MeCN–water (13:87, v/v) and then were sonicated until dissolved. During investigations different concentrations (0.1–0.004 mg/ml) of these impurities were prepared. Ciprofloxacin HCL raw material and crushed Serviflox 500 mg tablets water solutions were prepared. Solutions: 2 mg/ml (calculated to ciprofloxacin) for purity tests and 0.5 mg/ml for assay, were prepared. All reference and sample solutions, which were used to compare CZE and HPLC methods, were dissolved in HPLC mobile phase, according to Eur. Ph.

3. Results and discussion

3.1. Development of CE method for ciprofloxacin and its impurities analysis

Capillary electrophoresis technique has been applied for some quinolone compounds analysis [5–12,15–17], although HPLC method is crucial. In the present study an attempt was made to elaborate CE method for separation and determination of ciprofloxacin and its impurities.

UV absorption spectra of ciprofloxacin hydrochloride and its related substances were estimated, to select an optimum wavelength for CZE measurement. Each of the analysed compounds possesses several absorbance maxima. Absorbance maxima of ciprofloxacin HCL and impurity B and C were nearly similar at 280 nm (275.6, 282.0, 272.4, respectively) and maxima of impurity D and A were close to 254 nm (255.8 and 254.6, respectively). From these results, two wavelengths: 280 and 254 nm were chosen as the best for analysis. Then several screening experiments were performed to study the influence of some electrophoretic parameters on separation efficacy of ciprofloxacin hydrochloride and its related compounds. The aim of our experiments was to obtain good peaks resolution of ciprofloxacin and available impurities (A, B, C and D). Two types of buffers: phosphate and borate, according to required pH value were applied. The phosphate buffer contained 0.100 M sodium dihydrogenphosphate and 0.025 M disodium hydrogenphosphate in the pH range from 5.0 to 7.0 was used. This buffer was chosen from different compositions containing sodium dihydrogenphosphate and disodiumhydrogen phosphate in ratio: 0.100 M/0.025 M, 0.050 M/0.025 M, 0.050 M/0.0125 M, and 0.025 M/0.0125 M. For better resolution of ciprofloxacin and impurity D, which is very similar to ciprofloxacin, different amounts of PS were added to phosphate buffer. These amounts varied between 0.010 and 0.075 M, keeping the phosphate concentration at 0.125 M (in ratio 0.100 M/0.025 M) and the pH at 6.0. Finally 0.075 M PS was selected for further experiments, because best selectivity was ensured.

The tetraborate buffer: 0.04 M disodium tetraborate supplemented with 0.075 M TBA (added to improve resolution between ciprofloxacin and impurity B) in the pH range from 8.6 to 9.0 was applied.

The influence of different, mentioned above, compositions of the buffer and pH, on ciprofloxacin and its impurities migration time is shown in Table 1.

The pK_a values of the ciprofloxacin are: $pK_1 = 5.86$, connected with dissociation of carboxylic group and $pK_2 = 8.24$, associated with the deprotonation at N_4 of the piperazine ring [13,14]. Taking into considerations pK_a values and results of our experiments, the phosphate buffer containing 0.100 M $NaH_2PO_4 \cdot 2H_2O$, 0.025 M $Na_2HPO_4 \cdot 12H_2O$ and 0.075 M pentane-1-sulfonic acid sodium salt at pH 6.0 was used, as the best for separation and quantity determination of ciprofloxacin. The separation was carried out at 15 kV voltage and 25 °C temperature. In these electrophoretic conditions, impurities B, C, D, behaved as base compounds, and their mobility were higher than the electroosmatic flow (EOF), whereas impurity A with a negative net charge (had only one relevant ionisable group, corresponding to the carboxylic group) migrated slower than EOF. Figs. 2 and 3 show the separation obtained under described conditions, analysed at 254 and 280 nm, respectively (all solutions were of equal concentrations—about 0.017 mg/ml). The migration order was as follow: impurity C, then B, ciprofloxacin, compound D, EOF, and the last—impurity A. Differences of ciprofloxacin and its impurities peaks height were dependent on chosen wavelengths (it was taken into account during calculation of the amount of the related compounds).

3.2. Quantitative analysis

Determinations of ciprofloxacin and its impurities in raw material, as well as in Serviflox tablets, were performed.

In chosen CZE conditions specificity was confirmed by separation analysis of the ciprofloxacin alone and mixture of ciprofloxacin and available impurities (A, B, C and D) as well as placebo of Serviflox tablets. No interference with peak of ciprofloxacin was observed and all examined related compounds were well separated from each other. The linearity of the method was estimated by analysing ciprofloxacin and its impurity standards. In this study, six concentrations were chosen, ranging from 0.004 to 0.75 mg/ml for ciprofloxacin HCL, from 0.002 to 0.1 mg/ml for A and from 0.004 to 0.1 mg/ml for B, C and D impurities, respectively. Each sample concentration was injected twice. The high correlation coefficients—over 0.995 for A,

Table 1
Dependence on migration times from pH value

pH	Relative migration times (RMT) with reference to electroosmotic flow (EOF)																						
	0.72	0.73	0.74	0.76	0.80	0.82	0.85	0.90	0.93	0.98	1.0	1.05	1.13	1.18	1.22	1.26	1.28	1.30	1.38	1.54	1.59	1.67	1.93
5.0	C	B/Cyp		D							EOF				A								
5.5				C	B	Cyp	D				EOF								A				
6.0						C	B	Cyp	D		EOF									A			
6.6									C		B/EOF/D + Cyp											A	A
7.0											EOF	C	B + D	Cyp	C/D		B	Cyp			A		
8.6											EOF					C/D			B + Cyp			A	
9.0											EOF												

Where: CYP, ciprofloxacin·HCl; A, B, C, D are impurities A, B, C and D, respectively means that peaks were not separated to the baseline, + means that peaks were of the same migration time (comigrated). (*) pH range from 5.0 to 7.0—buffer consisted of 0.125 M appropriate ratios (0.100 M/0.025 M) sodium dihydrogenphosphate and disodium hydrogenphosphate, supplement with 0.075 M PS. pH range from 8.6 to 9.0—buffer consisted of 0.04 M tetraborate, supplemented with 0.075 M TBA.

D, and ciprofloxacin assay at 254 nm, as well as for B, C and ciprofloxacin assay at 280 nm, were calculated (Table 2).

The detection limit (LOD) and the quantification limit (LOQ) defined as signal to noise ratio of 3:1 and 10:1, respectively, were calculated according to the formulas: (*ch*/2*H*)3 and (*ch*/2*H*)10, where *c* is a concentration (mg/ml), *h* is height of noise signal and *H* is an analyte peak height. Instrumental precision was calculated from five consecutive injections of appropriate related substances at the concentration equal to LOQ. Repeatability of migration time and peak area, were very good. Summarised date are enclosed in Table 2.

In the following step of the study, quantitative analysis of ciprofloxacin raw material and Serviflox preparation, were performed.

For determination of ciprofloxacin content, solutions of sample and reference standard, containing 0.5 mg/ml of ciprofloxacin were prepared. The assay was performed with detection at 254 and 280 nm (wavelength was adjusted as required by the HPLC methods—manufacturer's or Ph. Eur.).

Independent assays performed by two analysts on different days, confirmed good repeatability and intermediate precision (Table 3). To complete the analysis, the comparable assays by CZE and HPLC methods were performed. Results of ciprofloxacin content received by both methods did not

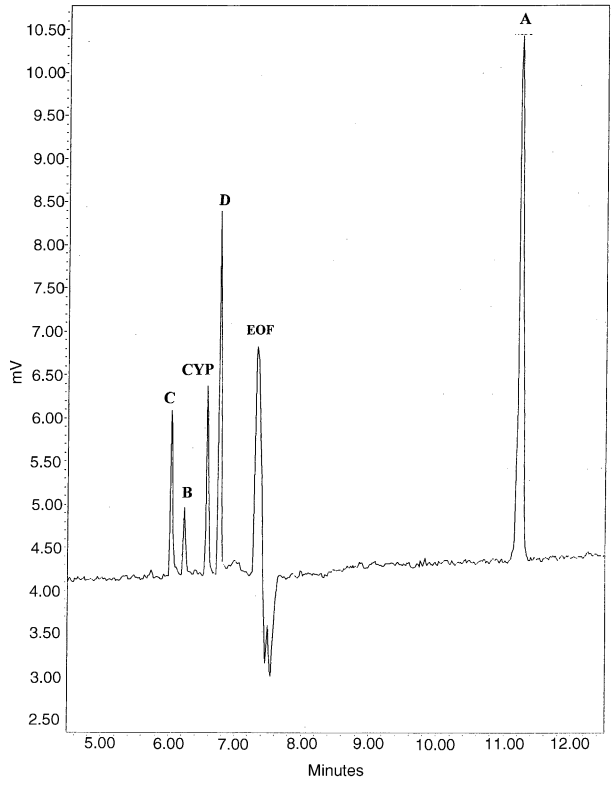

Fig. 2. Electrophoretic separation of ciprofloxacin (CYP) and its impurities A, B, C and D, obtained under the selected conditions: 0.100 M sodium dihydrogenphosphate and 0.025 M disodium hydrogenphosphate buffer supplemented with pentane-1-sulfonic acid sodium salt 0.075 M, pH 6.0; 15 kV; 25 °C; at 254 nm; EOF peak.

Table 2
The method validation results

Parameter	Detection at 254 nm			Detection at 280 nm		
	A	D	CYP	B	C	CYP
Repeatability of migration time (R.S.D.%)	1.25	0.52	0.44	0.19	0.20	0.18
Repeatability of corrected area (R.S.D.%)	1.40	1.56	0.98	1.68	1.13	0.95
Relative migration times (RMT)	1.91	1.03	1.0	0.97	0.94	1.0
Relative response factors[a] (RRF)	6.47	2.11	1.0	0.75	1.20	1.0
Reciprocal response factor (1/RRF)	0.15	0.50	1.0	1.30	0.80	1.0
Correlation coefficient	0.9998	0.9955	0.9992	0.9996	0.9970	0.9993
Detection limit (mg/ml)	0.0002	0.0006	0.002	0.001	0.0007	0.001
Quantification limit (mg/ml), and its R.S.D. (%)	0.0008	0.002	0.007	0.004	0.002	0.004
	2.22	2.49	5.72	4.96	2.45	4.98

[a] Relative response factor (RRF) was determined by calculation the ratio between the average response of each compound and the average response of ciprofloxacin at appropriate wavelength by: $(\text{area}_{\text{sample}}/\text{concentration}_{\text{sample}})/(\text{area}_{\text{ciprofloxacin}}/\text{concentration}_{\text{ciprofloxacin}})$.

differ significantly. The Student's *t*-values determined by the *t*-test, were lower than theoretical (tabular) value (Table 4).

The procedure for determination of related compounds was as follow: test solutions at concentration 2 mg/ml (in CZE, large amount of sample was necessary to analyse impurities on the level 0.2%) were injected and measured at 254 nm (for A and D) as well as at 280 nm (for B and C). The amount of impurities B and C in the test solution were calculated with relation to diluted (500 times) test solution containing ciprofloxacin at the 0.004 mg/ml concentration. This concentration corresponded to 0.2% content of impurity. Impurities A and D were assayed in comparison with reference standards of these substances at the concentration of 0.004 mg/ml. Determination of related substances at wavelength 254 nm (A, D), could not be calculated in relation to ciprofloxacin peak at the concentration of 0.004 mg/ml, because of its value lower than estimated LOQ for ciprofloxacin (0.007 mg/ml). The content of impurities were calculated by multiplication of the peak areas of appropriate impurities by corresponding reciprocal response factor (1/RRF). The estimated results of impurities are presented in Table 5. This table also contains comparable estimations of impurities by CE (test solutions

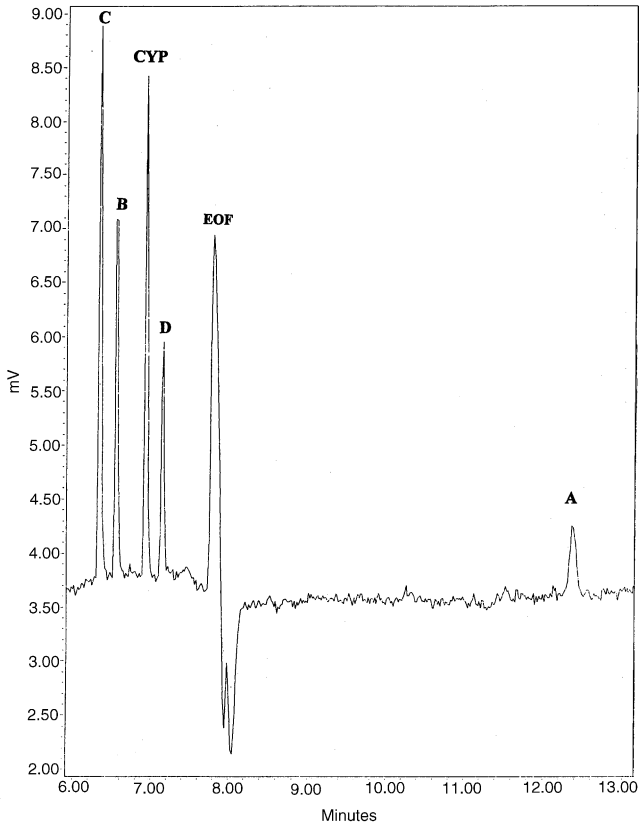

Fig. 3. Electrophoretic separation of ciprofloxacin (CYP) and its impurities A, B, C and D, obtained under the selected conditions: 0.100 M sodium dihydrogenphosphate and 0.025 M disodium hydrogenphosphate buffer supplemented with pentane-1-sulfonic acid sodium salt 0.075 M, pH 6.0; 15 kV; 25 °C; at 280 nm; EOF peak.

Table 3
Determination of ciprofloxacin in film coated tablets (Serviflox 500 mg), performed independently by two analysts in two CE assays

	Detection at 254 nm		Detection at 280 nm	
	Assay 1	Assay 2	Assay 1	Assay 2
Mean (mg, $n = 6$)	512.41	519.68	513.28	515.54
S.D.	3.14	11.89	8.80	10.54
R.S.D. (%)*	0.61	2.29	1.71	2.04
Mean from two assays (mg)	516.40	514.49		
S.D.	10.28	8.83		
R.S.D. (%)	1.99	1.72		

Table 4
Comparison of CE and HPLC methods for determination of ciprofloxacin HCL raw material (calculated as anhydrous substance)

	CE assay at 280 nm	HPLC assay at 278 nm[a]
Mean (%)	99.05	98.62
No. of samples	6	6
S.D.	1.89	0.79
R.S.D. (%)	1.91	0.80
Student's *t*-value for $P = 0.05$, $n = 10$, t tabulated $= 2.228$	0.514	

[a] The HPLC assay was performed according to Ph. Eur.

Table 5
Comparison of CE and HPLC methods for determination of related compounds in raw material

	HPLC					CZE[c]				
Impurities	A	B	C[a]	D	Unknown	A	B	C[b]	D	Unknown
Mean (%, $n = 6$)	n.d.	n.d.	0.053	n.d.	0.093	n.d.	n.d.	0.084	n.d.	0.096
S.D.			0.0013		0.0016			0.0058		0.0152
R.S.D. (%)			2.42		1.73			6.96		15.73

n.d., Not detected.
[a] For the calculation of contents impurity C peak area was multiplied by correction factor 0.6 (according to Ph. Eur.).
[b] For the calculation of contents impurity C peak area was multiplyed by reciprocal response factor 0.8 (evaluated during experiments).
[c] During CZE assay, both wavelenghts were used.

were at concentration 2.0 mg/ml) and HPLC (test solutions were at concentration 0.5 mg/ml). The results obtained from both methods confirmed the presence of two impurities in ciprofloxacin raw material: impurity C and unknown in the same level. The amount of unknown impurity obtained by CE, about 0.096%, corresponded to a concentration of about 0.002 mg/ml, which is in the range of the LOQ, while impurity C, 0.084%, corresponded to a concentration of 0.0017 mg/ml, which is lower than the LOQ (for impurity C LOQ = 0.002 mg/ml). Under our experimental conditions, UV-Vis absorbance detection in CZE, has a relatively lower sensitivity, when compared to that attainable by conventional UV-Vis in HPLC. The poorer sensitivity of the CZE method was caused by optical pathlength, which is restricted by the internal diameter of the capillary. This could be the reason why dates obtained from CZE and HPLC methods (all results obtained by HPLC were at LOQ value) were different.

4. Conclusions

The elaborated and validated CZE methods can be used for identification of ciprofloxacin and its impurities as well as for determination of ciprofloxacin·HCl and estimation its related substances, alternatively to HPLC method, routinely applied in analysis of medicinal products containing ciprofloxacin.

References

[1] European Pharmacopoeia 4.6 (2004) 3969.
[2] P.M. Lacroix, N.M. Curran, R.W. Sears, J. Pharm. Biomed. Anal. 14 (1996) 641.
[3] K. Borner, H. Lode, G. Hoffken, C. Prinzing, P. Glatzel, R. Wilej, J. Clin. Chem. Clin. Biochem. 24 (1986) 325.
[4] G.J. Krol, G.W. Beck, T. Benham, J. Pharm. Biomed. Anal. 14 (1995) 181.
[5] S.-W. Sun, A.-Ch. Wu, J. Liquid Chromatogr. Rel. Technol. 22 (1999) 281.
[6] M. Hernandez, F. Borull, M. Calull, J. Chromatogr. B 742 (2000) 255.
[7] C. Fierens, S. Hillaert, W. Van den Bossche, J. Pharm. Biomed. Anal. 22 (2000) 763.
[8] Ch.L. Flurer Reviev, Electrophoresis 22 (2001) 4253.
[9] T. Perez-Ruiz, C. Martinez-Lozano, A. Sanz, E. Bravo, Chromatographia 49 (1999) 419.
[10] D. Barron, E. Jimenez-Lozano, J. Barbosa, Chromatographia 52 (2000) 395.
[11] D. Barron, E. Jimenez-Lozano, J. Barbosa, Anal. Chim. Acta 415 (2000) 83.
[12] J. McCourt, G. Bordin, A.R. Rodriguez, J. Chromatogr. A 990 (2003) 259.
[13] D. Barron, E. Jimenez-Lozano, A. Irles, J. Barbosa, J. Chromatogr. A 871 (2000) 381.
[14] D. Barron, E. Jimenez-Lozano, J. Barbosa, J. Chromatogr. A 919 (2001) 395.
[15] K.H. Bannefeld, H. Stass, G. Blaschke, J. Chromatogr. B 692 (1997) 453.
[16] Ph. Schmitt-Kopplin, J. Burhenne, D. Freitag, M. Spiteller, A. Kettrup, J. Chromatogr. A 837 (1999) 253.
[17] K.D. Atria, Y.L. Chanter, J. Chromatogr. A 652 (1993) 459.