ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 241–248

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Analysis of ceftazidime and related compounds by micellar electrokinetic chromatography[1]

R. Porrà *, A. Farina, V. Cotichini, R. Lecce

Istituto Superiore di Sanita', Laboratorio di Chimica del Farmaco, V.le Regina Elena 299, 00161-Rome, Italy

Received 4 December 1997; received in revised form 9 March 1998; accepted 21 March 1998

Abstract

A micellar electrokinetic chromatographic method for the separation and quantification of ceftazidime, its Δ^2-isomer and pyridine (two ceftazidime related impurities) was developed and validated. Optimised conditions were obtained using an electrolyte system consisting of 25 mM sodium tetraborate, at pH 9.2, and 75 mM sodium dodecylsulphate. A limit of detection of 0.2 μg ml^{-1} and a limit of quantitation of 0.6 μg ml^{-1} were estimated for pyridine and Δ^2-isomer; this means that levels of $< 0.1\%$ of pyridine and Δ^2-isomer in ceftazidime can be determined. Calibration curves for all analytes were linear over the studied ranges with correlation coefficients > 0.999. Good reproducibility for migration times and corrected peak areas were achieved (RSD % 0.3 and 1.0, respectively). The results demonstrate that the method is reproducible, accurate and appropriate for ceftazidime assay in pharmaceutical samples.

Keywords: Ceftazidime; Related impurities; Pyridine; Capillary electrophoresis; MEKC; Pharmaceuticals

1. Introduction

Capillary electrophoresis (CE) is an high efficiency separation technique for both ionic and neutral species [1]. In addition, with its different operational modes, CE can be advantageously applied for either qualitative and quantitative analytical methods [2,3].

Although high performance liquid chromatography (HPLC) has always been the conventional method applied for the analysis of antibiotics, the use of CE technique has now assumed a considerable importance.

Ceftazidime (CTZ), in its syn conformation, is a well known β-lactam antibiotic of the cephalosporin family [4]. As a consequence of its poor oral bioavailability it is used in clinical chemotherapy for parenteral administration. In solid form this antibiotic can contain some impurities, namely pyridine and Δ^2-isomer (see Fig. 1) a compound deriving from synthesis; since pyridine is a toxic product, its content has to be controlled by manufactures [5].

* Corresponding author. Tel.: + 39 6 49902400; fax: + 39 6 49387100.

[1] Presented at the 7th Meeting on Recent Developments in Pharmaceutical Analysis, Island of Elba, Italy, September 16–20, 1997.

0731-7085/98/$ - see front matter
PII S0731-7085(98)00178-2

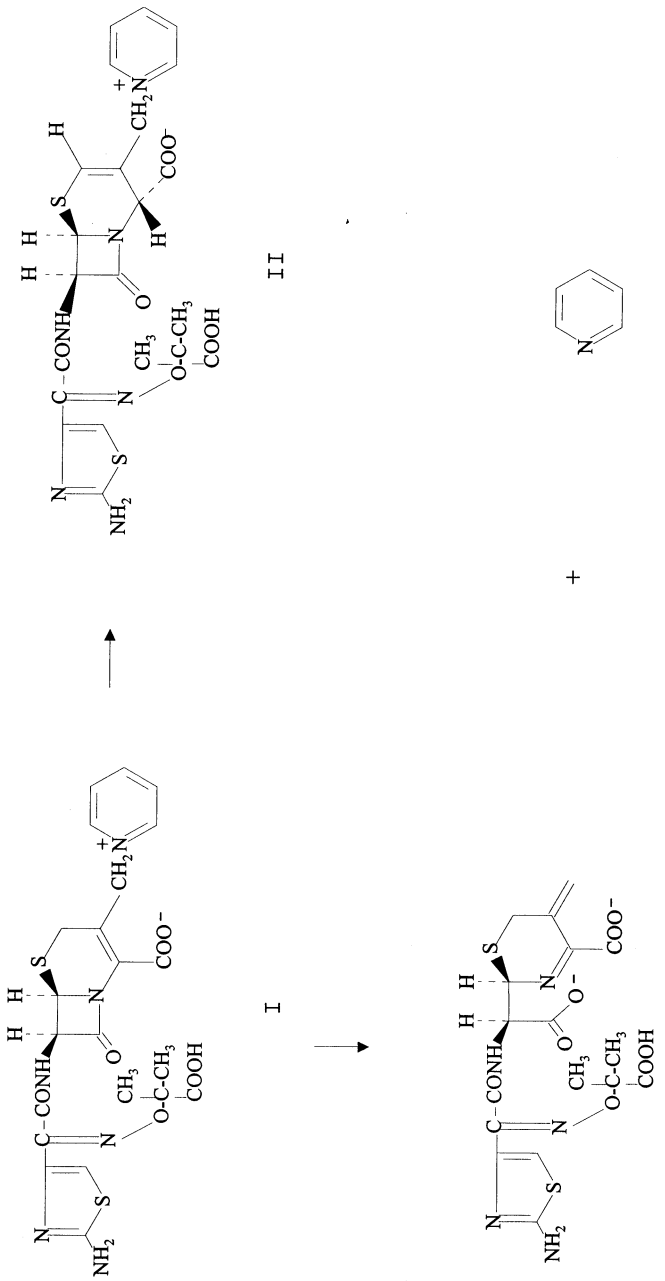

Fig. 1. Chemical structure of (I) CTZ, and its main impurities: (II) Δ^2-isomer, (III) pyridine and (IV) 3-methylidene compound.

In aqueous solution ceftazidime is unstable and this leads to the formation of a variety of degradation products. The primary decomposition pathway involving the scission of the C–(3) side chain leads to the formation of pyridine and the remaining moiety i.e. a 3-methylidene compound.

HPLC techniques for the analyses of CTZ have been used [6], and specific methods for stability studies [7,8] have been developed. However, new analytical methods characterized by high selectivity, sensitivity and time saving are still required to monitor ceftazidime and related impurities.

In this work we report the development and validation of a micellar electrokinetic capillary chromatography (MEKC) method that is capable:

- to resolve ceftazidime (CTZ) from its two major related impurities, pyridine and Δ^2-isomer;
- to assay CTZ and pyridine content in commercially available samples.

2. Experimental

2.1. Instrument

All experiments were carried out using a Hewlett Packard^{3D} capillary electrophoresis system equipped with a Diode array detector (from 190 to 600 nm), and a chemstation for system control, data collection and data analysis. (Hewlett Packard, Waldbronn, Germany).Online UV detection at 205 nm was used.

2.2. Electrophoretic conditions

Uncoated silica capillary tube with extended pathlight (Hewlett Packard) 64.5 cm length × 50 μm I.D. was used; the effective length to the detector was 56 cm. The running buffer consisted of 25 mM sodium tetraborate at a pH value of 9.2. Sodium dodecylsulphate (SDS) as surfactant was used. The running buffer was prepared daily and SDS was added just before use. The buffer was degassed by sonication and filtered using 0.45 μm filters (Millipore).

Before CE separation the capillary was cleaned using 0.1 M NaOH for 5 min followed by an H_2O rinse of 5 min. After a washing cycle the capillary tube was filled with the running buffer. Injection was done by pressure and it was fixed for all experiments at 50 mbar × 8 s; the applied voltage was 20 kV (constant) and the temperature of the containing capillary cartridge was maintained at 25°C; as marker of electrosmotic flow (eof) and micelles elution, methanol and Sudan III respectively were used.

2.3. Chemicals and reagents

Standard ceftazidime pentahydrate (84.7% anhydrous ceftazidime), Δ^2-isomer and bulk samples of commercial batches were generously supplied by Glaxo–Wellcome (Verona, Italy), pyridine was obtained from Merck.

Analytical grade sodium tetraborate was obtained from Fluka (Buchs, Switzerland); sodium dodecylsulphate was 99% pure from Aldrich (Buchs, Switzerland) and was used without further purification; buffer, sample and standard solutions were prepared with doubly distilled deionized water (Milli-Q, Millipore, Molsheim, France). The pH of the running buffer, if necessary, was adjusted with 0.1 M NaOH or phosphoric acid 85% (Fluka); all other chemicals were of analytical grade.

2.4. Standard solutions

Three standard solutions containing anhydrous CTZ (1 mg ml^{-1}), pyridine (100 μg ml^{-1}) and Δ^2-isomer (100 μg ml^{-1}), respectively, were prepared daily in distilled water.

Appropriate amounts of these solutions were combined and diluted to 10 ml with water in order to obtain a standard mixture containing 100 μg ml^{-1} CTZ, and 5 μg ml^{-1} of pyridine and Δ^2-isomer, respectively. Calibration curves were obtained for the following concentration ranges: 0.9–0.02 mg ml^{-1} for CTZ, 0.5–35.0 μg ml^{-1} for Δ^2-isomer and 0.5–100.0 μg ml^{-1} for pyridine; appropriate dilution of the standard solutions were used.

2.5. Sample preparation

Sample solutions were prepared just before the electrophoretic analyses by dissolving a sample of a bulk of CTZ pentahydrate blended with sodium carbonate (containing from 73.4 to 80.4% of anhydrous ceftazidime) corresponding to 1 g of anhydrous CTZ in water. The final concentration of the sample was ~ 0.1 g per ml; the obtained solution was then diluted with water to the appropriate concentration.

The bulk samples were the same as those used in the preparations of pharmaceutical forms.

3. Results and discussion

3.1. Method development and optimisation

The first steps in the method development process were the selection of the optimum pH value, the determination of solutes charge and the subsequent choice of capillary electrophoresis mode (CZE, MEKC, etc.).

Buffer solutions in the pH range 2.5–9 in the absence of SDS were investigated; the optimum pH value for adequate analyte peak shapes equalled 9.2.

Sodium tetraborate buffer ionic strength was also studied in the range 10–100 mM and the best concentration was fixed at 25 mM, the resulting current was 40 μA. Under this conditions the analysis was completed in a reasonable time (ca. 7 min). As expected neutral pyridine eluted strongly close to the eof while ionic CTZ and Δ^2-isomer, moved in a single peak slower than pyridine (Fig. 2a).

In order to shift the peak of pyridine from eof and to try to resolve CTZ from Δ^2-isomer, MEKC using SDS as surfactant, was investigated. A concentration range 0–150 mM of SDS added to the buffer was studied; the electropherograms relative to increasing SDS concentration are shown in Fig. 2(b–f). A slight shift of neutral pyridine from eof resulted from the addition of SDS at a concentration a little greater than its CMC. With the increase of SDS concentration above 50 mM the migration time of pyridine increased, as a consequence of its interaction with the micellar pseudo-stationary phase; simultaneously a slight resolution ($R = 1.78$) between Δ^2-isomer and CTZ was achieved. Such resolution, automatically calculated at half height of the peak, was improved increasing the SDS concentration; a baseline resolution at 75 mM SDS ($R = 2.55$) was consequently obtained (Fig. 2d). Increasing the SDS concentration resulted in a greater resolution between Δ^2-isomer and CTZ. The elution order of the solutes did not follow a linear trend; in fact, when 100 mM of SDS was added to the buffer, an inversion of the elution order was noted as shown in Fig. 2(e); pyridine migrated to the cathode slower than Δ_2 -isomer but faster than CTZ. Pyridine, strongly solubilized by the micelles, had the greatest migration time at a 150 mM SDS concentration (see Fig. 2f).

The identification of the three analytes in the electropherograms was made by spiking each peak with its relative standard and detecting on line the corresponding UV spectra. The change in elution order was determined by spectrically monitoring the analysis in the range 50–150 mM of SDS, (see Fig. 2d, e, f). The observed resolution between CTZ and Δ^2-isomer in such experimental conditions allows the following considerations: at each SDS increment, the migration time of CTZ and Δ^2-isomer presented only a small increase; since the eof was almost constant in all SDS ranges, the small increasing of their migration times should be due to an interaction with micelles. Assuming that syn-ceftadizime and Δ^2-isomer have the same lipophilicity, the observed variation of their relative migration time should be attributed to a variation of the interaction between the negative charge of the anionic surfactant and the dipole of the two zwitterionic analytes. Indeed the shifts of the double bond in the dihydrothiazine ring induces a perturbation of the electronic charge density at the carboxylic group at –C(3); consequently, the value of the electrical dipole moment of the Δ^2-isomer should be different from the corresponding value of the syn-ceftazidime; the observed shift consequently sup-

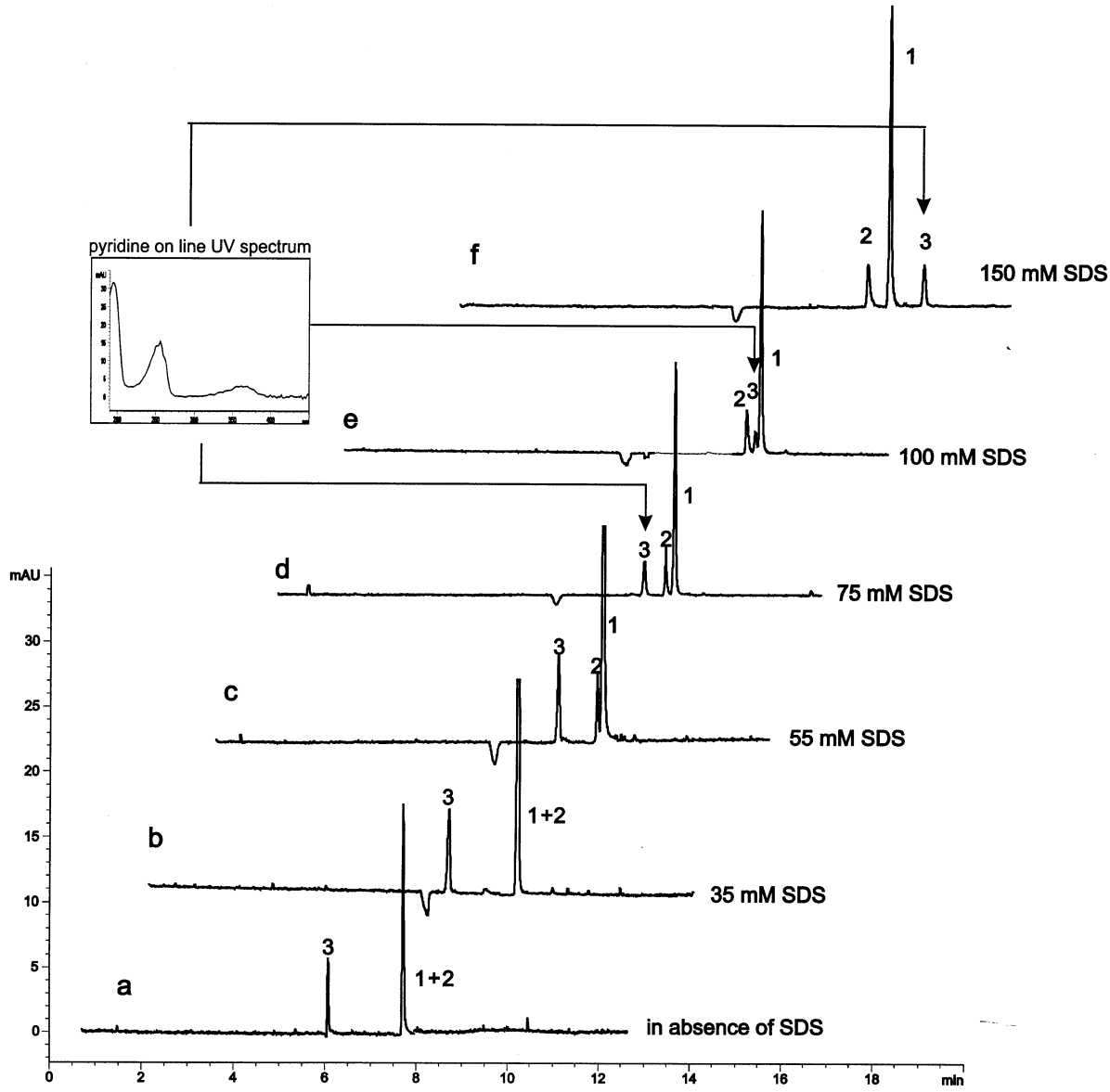

Fig. 2. Electropherograms of a standard mixture containing (1) CTZ, (2) Δ^2-isomer and (3) pyridine at different SDS concentrations. Electrophoretic conditions: 25 mM sodium tetraborate pH 9.2, 20 kV, temperature 25°C.

port a stronger interaction between SDS-micelles and syn-ceftazidime with respect to SDS micelles and Δ^2-isomer; this means a decrease of the dipole moment in the process syn-ceftazidime $\rightarrow \Delta^2$-isomer.

3.2. Performance of the method

3.2.1. Selectivity

Since one of our targets was to resolve CTZ from its two major degradation products, pyridine

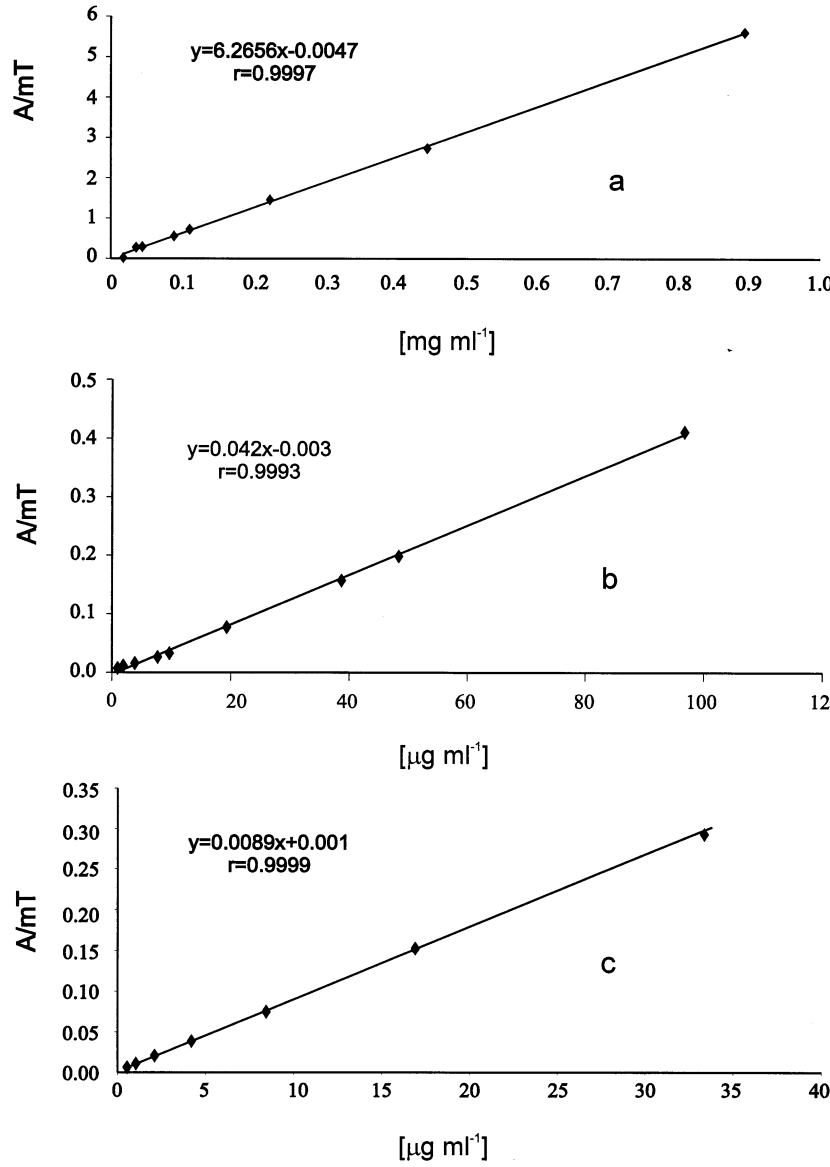

Fig. 3. Calibration graphs of (a) CTZ, (b) pyridine and (c) Δ2-isomer; each point represents the average of three replicated analyses.

and Δ^2-isomer, we optimised the method employing a electrolyte system consisting of 25 mM sodium tetraborate and 75 mM SDS. In fact, increasing the SDS concentration up to 150 mM a more satisfactory resolution of the three analytes (i.e. CTZ, its Δ^2-isomer and pyridine) was obtained but a lower specificity, particularly when the CTZ degradation process started to take place, was also observed; indeed, using SDS at 150 mM, the resolution of the peak due to pyridine was negatively affected by a number of interfering unknown peaks.

3.2.2. Linearity

Plots of corrected peak areas as a function of CTZ, pyridine and Δ^2-isomer concentrations are

Table 1
Relative standard deviation for migration times and corrected areas ($n = 10$) of a standard mixture containing ceftazidime, pyridine and Δ^2-isomer

Compound	RSD% (mT)	RSD% (*A/mT)
Pyridine	0.26	0.96
Δ^2-Isomer	0.20	0.96
Ceftazidime	0.23	0.87

*A/mT, Area/migration time.

shown in Fig. 3. The studied ranges were: 0.9–0.02 mg ml^{-1} for CTZ, 35–0.5 μg ml^{-1} for Δ^2-isomer and 100–0.5 μg ml^{-1} for pyridine. For all analytes good linearity was observed over these ranges with correlation coefficients > 0.999. RSD at the concentration of 1 μg ml^{-1} were 0.77 and 0.6 for pyridine and Δ^2-isomer, respectively.

3.2.3. Precision

Repeatability of migration times and corrected peak areas (A/mT) was determined on a standard mixture containing 100 μg ml^{-1} of CTZ, 2 μg ml^{-1} of Δ^2-isomer and 2 μg ml^{-1} of pyridine. RDS < 0.5 and 1% for intra-day migration times and corrected peak areas respectively were obtained. Table 1 shows the relative standard deviations obtained for ten replicated analyses.

3.2.4. Limit of detection and quantitation

The limit of detection defined as the lowest concentration of analyte that can be detected is estimated as three times the signal to noise ratio. The LOD for pyridine and Δ^2-isomer was determined separately analysing decreasing concentrations of both analytes, equalling ~ 0.2 μg ml^{-1}. The limit of quantitation for both pyridine and Δ^2-isomer was calculated to be three times the LOD value; this means that levels of $< 0.1\%$ of pyridine and Δ^2-isomer in ceftazidime can be determined.

3.2.5. Recovery of pyridine

The recovery of pyridine from a bulk of CTZ for injection (containing 77.3% of anhydrous CTZ) spiked with different concentrations of pyridine was studied.

An amount of sample of CTZ pentahydrate, blended with sodium carbonate, equivalent to 1 g of anhydrous CTZ was diluted in order to obtain a solution of anhydrous CTZ at a concentration of 10 mg ml^{-1}. 2, 5 and 8 ml of a solution containing 0.01 mg ml^{-1} of pyridine, were respectively added to three aliquots of 1 ml of CTZ solution and then diluted to 10 ml with water. The resulting solutions contained 2, 5 and 8 μg ml^{-1} of added pyridine, equivalent, respectively, to 0.2, 0.5 and 0.8% referred to anhydrous CTZ. For each spiked level, four replicated analyses were performed. The mean recoveries obtained are reported in Table 2.

3.3. Assay of bulk samples

To evaluate its reliability, the proposed method was applied to the quantitative determination of CTZ and pyridine in bulk samples of CTZ, blended with anhydrous sodium carbonate, from commercially available batches.

The results with respect to three bulk samples are summarised in Table 3. Satisfactory agreement was observed with respect to the anhydrous CTZ and pyridine content claimed in the certificates of the producer for every batch analysed.

4. Conclusion

The developed capillary electrophoresis method separates CTZ from its potential impurities and

Table 2
Percent recoveries of pyridine from spiked bulk samples of ceftazidime for injections ($n = 4$)

Sample solution (mg ml^{-1})[a]	Amount of pyridine added (μg ml^{-1})	Mean recovery %	SD
1	2	98.7	0.1
1	5	100.9	0.1
1	8	100.9	0.1

[a] Referred to anhydrous ceftazidime.

Table 3
Control analyses[a] of ceftazidime bulk samples[b]

Sample	Anhydrous ceftazidime		Pyridine[c]
	Claimed[d]	Found	Found
1	77.2	71.9 (±0.5)	0.10 (±0.01)
2	77.3	74.4 (±0.5)	0.12 (±0.01)
3	75.9	74.5 (±0.3)	0.14 (±0.02)

[a] Values (in %) represent the mean of four experiments; SD values are given in parentheses. The analysis of ceftazidime must be carried out immediately after the preparation of working and samples solutions because of the easy decomposition of ceftazidime when in solution.
[b] Ceftazidime pentahydrate blended with anhydrous sodium carbonate.
[c] Manufacturers specifications: $\not> 0.15\%$ initial and $\not> 0.5\%$ at end of life. USP specifications: $\not> 0.4\%$.
[d] Manufacturers specifications between 73.4 and 80.4 % w/w.

degradation products. The method shows good selectivity, repeatability, linearity and sensitivity and can be used for the assay and purity control of CTZ injections. The detection and quantitation limits for pyridine and Δ^2-isomer are adequate for the scope of this work. In addition this work demonstrates that capillary electrophoresis can be used to monitor the stability of CTZ when in solution and as a complementary method to the HPLC procedure typically used; indeed it was successfully applied to obtain stability data of CTZ reconstituted solutions. The results obtained will be the object of a further communication.

References

[1] H. Englehardt, W. beck, T. Schmitt, Capillary electrophoresis, Wiesbaden: Vieweg, Analytical Chemistry, 1996, pp. 1–215.
[2] K.D. Altria, S.D. Filbey, J. Liq. Chromatogr. 16 (1993) 228–292.
[3] K.D. Altria, J. Elgey, P. Lockwood, D. Moore, Chromatographia 42 (1996) 332–342.
[4] M.A. Abounassif, M.S. Mian, in: K.T. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 20, Academic press, New York, 1990, pp. 95–121.
[5] United States Pharmacopoeia XXIII, 4th suppl., United States Pharmacopoeial Convention, Rockville, MD, 1996.
[6] A.R. Barnes, J. Liq. Chromatogr. 18 (1995) 3117–3128.
[7] M. Zhou, R.E. Notari, J. Pharm. Sci. 84 (1995) 534–538.
[8] B. Vilanova, F. Muñoz, J. Donoso, F. Garcia Blanco, Helv. Chim. Acta 76 (1993) 2789–2802.