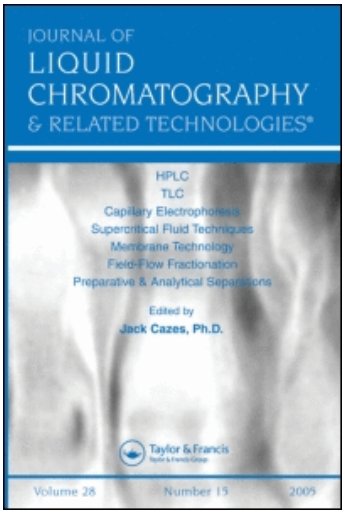

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Capillary Electrophoresis as an Alternative Method for the Determination of Cefotaxime

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CAPILLARY ELECTROPHORESIS AS AN ALTERNATIVE METHOD FOR THE DETERMINATION OF CEFOTAXIME

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ABSTRACT

Capillary Zone Electrophoresis (CZE) was evaluated for the determination of cefotaxime in the presence of its major decomposition products. The relative standard deviation of the migration times and peak areas were 0.26 and 0.31%, respectively. These figures and those obtained in the assay of cefotaxime were comparable with results of an HPLC method and comply with the requirements for drug quality control.

INTRODUCTION

This work is part of a study involving the investigation of new methods for the determination of cephalosporins in pharmaceutical formulations and biological samples (1-3), or following their degradation kinetics in aqueous solutions (4-6). Since capillary electrophoresis has proved to be a valuable

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method in the quality control of drug substances (7-8), its performance in the determination of cefotaxime sodium was evaluated in this study. This paper investigates the possibilities offered by capillary zone electrophoresis (CZE) for the routine analysis of this drug. The results were compared with those obtained by HPLC -UV which is the official method (9) for the assay of cefotaxime.

MATERIALS, REAGENTS AND SOLUTIONS

Milli-Q water and analytical grade chemicals were used throughout and all solutions were freshly prepared before use.

Cefotaxime sodium (C) and related compounds, deacetylcefotaxime lactone (L), desacetoxycefotaxime (DO), desacetylcefotaxime (DA), thiazoximic acid (TH), N-formyl cefotaxime (F), cefotaxime *anti*-isomer (AN), cefotaxime dimer (DIM) (Table I) were kindly donated by Roussel UCLAF laboratories (Romainville, France) and were used as received.

Aqueous solutions of the working standard and samples (both at 100 mg l^{-1}) were used for the cefotaxime assay in CZE. For HPLC, solutions of working standard and cefotaxime were 50 mg l^{-1}.

The electrolyte for separation in CZE was 40 mM potassium dihydrogen phosphate, pH 8.0 prepared daily by diluting a 100 mM stock solution of KH_2PO_4. The stock solution was stored at 4°C and used within fifteen days of preparation.

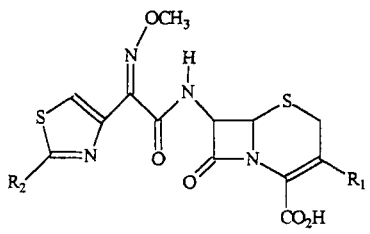
The mobile phase for HPLC was a phosphate buffer-methanol mixture (80 : 20, v/v). The buffer was prepared by dissolving 3.5 g of potassium dihydrogenophosphate and 11.6 g of disodium hydrogenophosphate. 12 H_2O in 1000 ml water.

INSTRUMENTATION AND OPERATING CONDITIONS

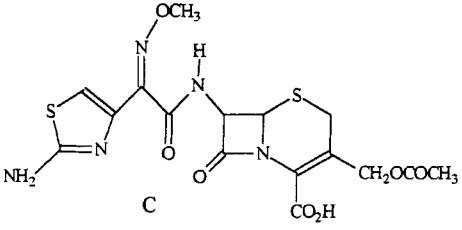
CE

A Beckman P/ACE 5500 (Palo Alto, CA, USA) capillary electrophoresis system equipped with a UV filter detector at 254 nm was used. A diode array

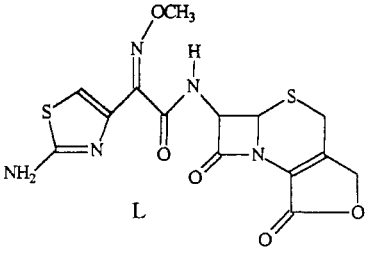
TABLE 1. Structure of cefotaxime and related impurities.



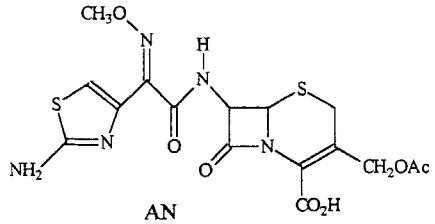
Name	R_1	R_2
DA	CH_2OH	NH_2
DO	CH_3	NH_2
F	CH_2OCOCH_3	NHCHO



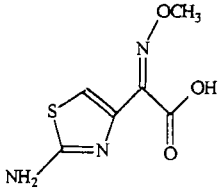
C



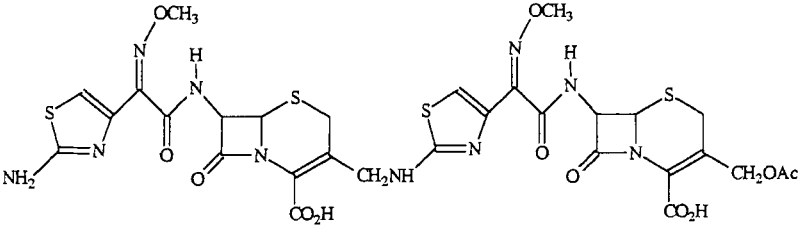
L



AN



TH



DIM

detector was employed for peak purity assessment of cefotaxime. The system was controlled by a Dell Optiplex 466/L with Gold® and Array view® software. Separation was carried out on a 75 μm i.d. x 57 cm (50 cm to detector) fused silica capillary (Beckman) maintained in a cartridge with a detection window of 100 x 800 μm.

The capillary was conditioned prior to its first use by rinsing with 0.25 M NaOH for 30 min and with water for 10 min. Before commencing a sequence, the capillary was washed with 0.25 M sodium hydroxide and then water both for 5 min.The capillary was filled with the separation buffer for 2 min, then a 5 s injection of the sample was performed, immediately followed by a 1 s injection of the separation buffer. The capillary was filled from separate vials of buffer for rinsing operations in order to keep the level of buffer constant in the anodic separation vial. The separation was performed at + 15 kV for 15 min, with a ramp voltage of 37.5 kV min^{-1} at 25°C.

Average peak areas (PA) were used for quantitation without correction with respect to the migration times, unless otherwise stated. The data generated from first two injections in a sequence were not used on account of the requirement for system equilibration.

HPLC

A Waters (Bedford, MA, USA) Model 510 pump was used to deliver the mobile phase. Sample introduction was via a Rheodyne, (Cotati, CA, USA) Model 7125 injection valve fitted with a 20-μl loop. Detection was by UV absorbance at 254 nm using Waters 920 diode array detector. This was connected to a Nec Powermate SX Plus with Waters PAD software. Separation was carried out on a 250 x 4 mm i.d. Lichrospher (Merck, Darmstadt, Germany) 100 C_{18} column (5 μm). A disposable guard column (4 mm x 4 mm i.d.) packed with the same material was fitted in advance of the analytical column. The mobile phase was delivered at a flow rate of 1 ml min^{-1}.

RESULTS AND DISCUSSION

Preliminary Studies.

On account of the structure of the analytes (wih the exception the lactone compound) basic or acidic buffers could be used to promote the ionisation of the carboxylic or amino functions, respectively. A basic buffer of pH 8.0 was chosen to avoid adsorption on the capillary wall, since both the analytes and the wall are negatively charged at this pH. In addition, since the capillary wall can be considered at this pH to be totally ionised at pH 8, the electroosmotic flow (e.o.f) is stable (10). A 40 mM borate buffer pH, 9.2 was found to yield similar resolution between the compounds, but the pH 8 buffer was chosen in preference to avoid artefacts arising from degradation of cefotaxime in the capillary during the separation; cefotaxime hydrolysis has been shown to be dramatically increased above pH 9.0 (4). A systematic study of the buffer ionic strength, voltage and temperature was conducted to optimise the electrophoretic separation. Under the selected conditions, the current was about 85 μA. Of the wavelengths available on the UV filter detector (200, 214, 254 and 280 nm) 254 nm was selected based on the UV absorption spectrum of cefotaxime.

Cefotaxime Assay Validation.

Specificity.

Fig. 1 shows the electropherogram of a cefotaxime sample at 500 mg l^{-1} and 100 mg l^{-1}. Cefotaxime is separated from its main decomposition products or synthesis intermediates, which were identified by co-injection of standard solutions (50 mg l^{-1}). The lactone, which is uncharged migrates with the e.o.f. ; all other compounds have a similar charge at the separation pH and migrate according to their size. DA + DO and F almost coelute (as they have a similar size) and migrate after C which has a higher molecular mass; TH has the longest migration time due to its small size. The dimeric compound was not available for this study, but based on the charge-to-size ratio it should migrate after

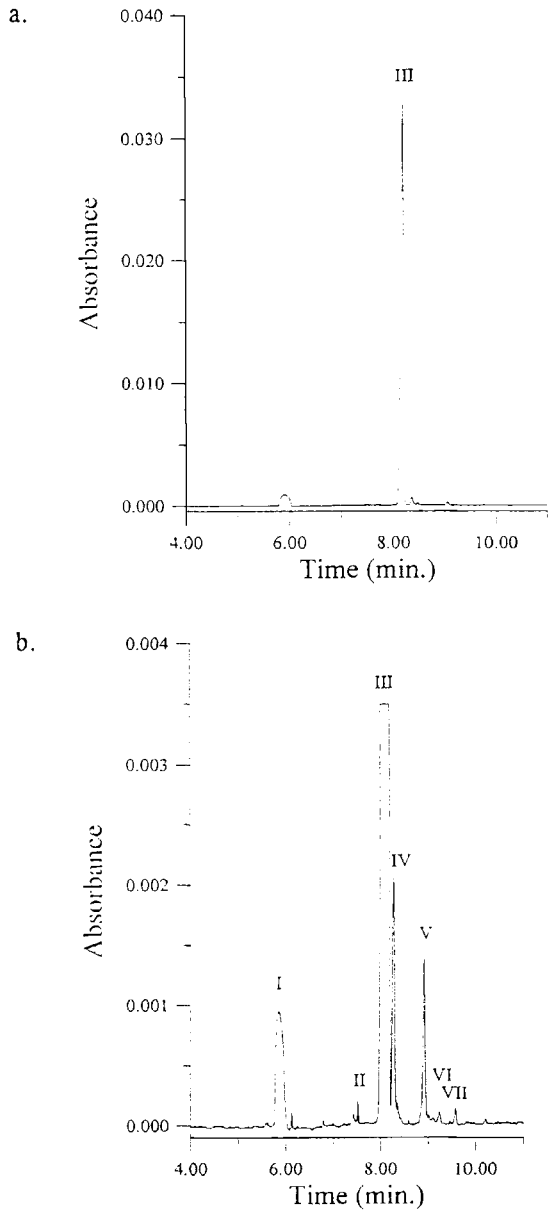


Figure 1: CZE separation of cefotaxime from its related impurities. I: e.f.o.+L; III: C; IV: DA+DO+F; V: DIM; II, VI and VII: Unknown.
a. Electropherogram with cefotaxime (100 mg l^{-1}) on scale.
b. Electropherogram with cefotaxime (500 mg l^{-1}) off scale

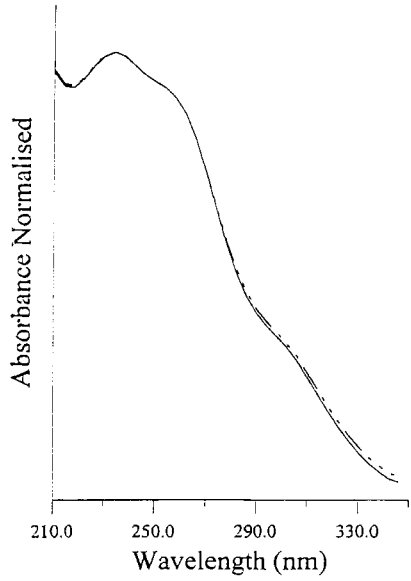


Figure 2: Spectral overlay (apex, upslope, downslope) for a 500 mg l^{-1} solution of cefotaxime.

cefotaxime. AN coelutes with C but this is not of major concern as it is not often present in the drug substance or, if so, only at trace levels. The critical resolution between C and the co-eluted DA + DO + F showed a high degree of repeatability throughout this study using different capillaries from the same supplier.

Peak purity was assessed for both the working standard and samples of cefotaxime. A typical overlay of the spectra captured at the apex, upslope and downslope is presented in Fig. 2.

Linearity

Linearity was assessed both by injecting solutions of C in the concentration range 50-150 mg l^{-1} (n = 5) at a constant injection time (5 s), and by injecting a C solution (100 mg l^{-1}) with variable injection times (from 1 to 10 s, n = 5). The regression equations were :

Area = -0.042 ($\pm$ 0.178)+ 0.021 ($\pm$ 0.002) conc.(mg l^{-1}); r = 0.998 (constant injection time)

TABLE 2. Repeatability (RSD, %, n = 8 injections), for the migration times (MT), peak areas (PA) and PA corrected from the MT (CPA).

	M.T.	P.A.	C.P.A
	8.125	1.95336	0.240414
	8.135	1.95471	0.240284
	8.135	1.95015	0.239723
	8.131	1.94998	0.23982
	8.146	1.94257	0.238469
	8.152	1.95031	0.239243
	8.159	1.95393	0.239482
	8.173	1.9496	0.23854
Average	8.145	1.95057	0.239497
S.D.	0.016	0.00381	0.000722
R.S.D. %	0.198	0.19545	0.301474

Area = 0.132 (± 0.010) + 0.445 (± 0.002) time (s) ; r = 0.999 (constant volume)

The correlation coefficient values are satisfactory for both methods. The graph obtained with different concentrations passed through the origin,whereas that obtained with different injection times (p = 0.05) showed a slight bias. This may be related to the "spontaneous injection" which takes place even when no injection is carried out (11). However the bias was only 2 % of the response at the targeted concentration, which may be considered to be satisfactory for validation in pharmaceutical analysis (12). For routine use, it is possible to use one calibration point in addition.

Precision.

Repeatability was assessed under the following conditions, which have been shown to optimise the precision in quantitative assays (13): the capillary was rinsed with separation buffer held in a separate vial; an aliquot of buffer was introduced after the sample was injected; the sample and buffer vials were

TABLE 3. Comparison of CZE and HPLC results for the determination of cefotaxime content (%, m/m)

	RECOVERY (%)	
SAMPLE	HPLC	CZE
Batch 2G	99.3	98.9
Batch 3G	99.6	99.3

maintained at the same level at all times; a high sample concentration was used and water was used as a dissolution solvent; a constant injection of 5 seconds was used and the buffer was selected to have a high buffer capacity; the operating voltage was ramped and a constant temperature was maintained. Table II shows the migration times (MT), peak areas (PA) and PA corrected from the MT (CPA) resulting from 8 replicate injections of C using the same set of buffer vials. These results show that it is unecessary to use corrected PA since the repeatability of migration times is more than acceptable. Comparable MTs (8.23 and 8.15 min) were also obtained on two different capillaries with RSD values of 0.21 and 0.20 % (n = 8 injections).

Accuracy.

Two different batches of cefotaxime were analyzed against a reference standard of cefotaxime both by the proposed CE method and a HPLC method with UV detection at 254 nm. The results given in table III, obtained from duplicate preparations of standard and sample solutions, demonstrate a good agreement between the two methods.

CONCLUSION

The results obtained show than CZE is a valuable method for the determination of cefotaxime. Assay and repeatability results are comparable to

those obtained with an HPLC method and comply with the requirements of drug quality control. CZE is suitable for routine use and offers advantages of simplicity of operation, flexibility and low cost. The proposed method may allow a total evaluation of the related impurities present in the drug substance but individual quantitation of each compound is not possible in CZE because DA, DO and F co-elute. A MEKC method is presently under investigation in our laboratory for the individual determination of the related substances.

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