Journal of Chromatography, 389 (1987) 312–316
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 182

Note

In vitro stability of cefixime (FK-027) in serum, urine and buffer

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(Received October 15th, 1986)

Cefixime (FK-027, Merck) is a β-lactamase-resistant third-generation cephalosporin antibiotic for oral use. It exhibits a good *in vitro* activity against most gram-negative pathogens and a wide range of gram-positive organisms[1,2]. The present study describes the development of a rapid and sensitive high-performance liquid chromatographic (HPLC) method for the determination of cefixime in serum and buffer, involving direct injection of diluted serum samples onto column. The purpose of this study was to investigate the *in vitro* stability of cefixime in serum and buffer during storage for 3 months at various temperatures. Furthermore its stability in serum, buffer and urine at 37°C was analysed over 24 h.

EXPERIMENTAL

Reagents

Cefixime was kindly provided by E. Merck (Darmstadt, F.R.G.). Methanol, dipotassium hydrogenphosphate and phosphoric acid were obtained from Riedel de Haen (Seelze, F.R.G.).

Apparatus

HPLC was performed with a Constametric III-G pump, a vaiable wavelength UV detector Spectromonitor D and a LDC computing integrator 301 (LDC-Milton Roy, Hasselroth, F.R.G.) and an SP 8780 XR autosampler (Spectra Physics, Darmstadt, F.R.G.). The column 200 mm × 4 mm I.D.) was packed with the reversed-phase material Nucleosil 5 C_{18} (Macherey & Nagel, Düren, F.G.R.).

HPLC procedure

The solvent system was methanol–phosphate buffer (15:85, v/v; 43 mmol dipotassium hydrogenphosphate and 1 litre water) adjusted to pH 5.20 with phosphoric acid. The mobile phase was delivered at a flow-rate of 1 ml/min at room temperature. The absorbance of the column effluent was monitored at 230 nm (0.2 a.u.f.s.).

Study design

Serum specimens were obtained from five healthy donors. Cefixime (250 μg/ml) was added to three buffer and five serum samples, divided into 100-μl portions and

0021-9673/87/$03.50

stored at different temperatures for 3 months: 4°C (only for 1 month), −20°C and −70°C. HPLC analysis was performed at 0, 1, 3, 7, 14 days and after 1, 2 and 3 months.

For kinetic studies in serum, buffer and urine, cefixime-containing samples were incubated under sterile conditions at 37°C in a water-bath. Aliquots of the samples were analysed before incubation, directly after addition of cefixime or piperacillin and after 15, 30, 60 min, 2, 4, 8 and 24 h.

Sample preparation for HPLC

This required only dilution of the serum, buffer (1:10) and urine (1:10–100) samples in Soerensen buffer (0.06 mol dipotassium hydrogenphosphate adjusted to pH 7.40 with 0.05 mol potassium dihydrogenphosphate). The diluted samples were centrifuged at 9300 *g* for 4 min; the resulting supernatants (20 μl) were then subjected to HPLC. The amount of serum required for the assay ranged between 50 and 100 μl.

RESULTS

Chromatography

Fig. 1 shows representative chromatograms of a standard solution, control serum and of a cefixime-containing serum sample. Interference with the cefixime quantitation caused by coeluting peaks of the biological matrix was not observed. Under the above conditions, cefixime was eluted with a retention time of 12 min.

Standards (5, 25, 50, 100 and 250 μg/ml) of cefixime were freshly prepared

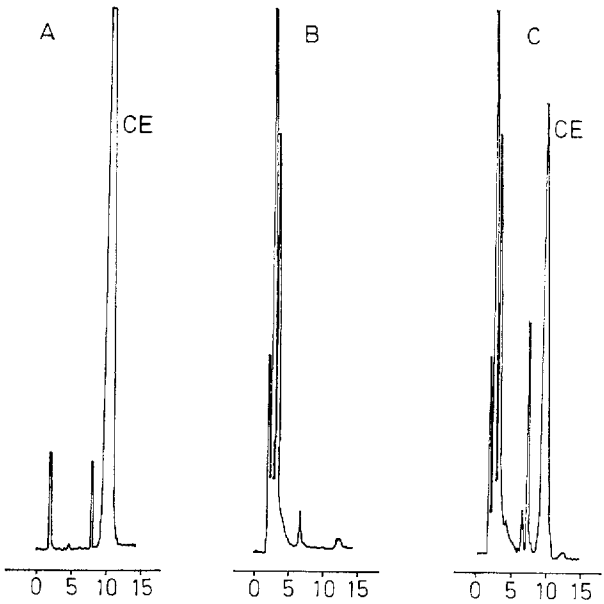

Fig. 1. Representative chromatograms. (A) Buffer control to which cefixime (CE) (125 μg/ml) was added; (B) blank serum (diluted 1:10 in Soerensen buffer); (C) serum sample to which 250 μg/ml cefixime were added.

TABLE I

CONCENTRATIONS (μg/ml) OF CEFIXIME AFTER STORAGE AT DIFFERENT TEMPERATURES IN SERUM AND BUFFER

Given as mean and standard deviation of $n = 5$ serum and of $n = 3$ buffer samples; N.D. = not determined.

Storage period	*4°C*		*−200°C*		*−70°C*	
	Serum	*Buffer*	*Serum*	*Buffer*	*Serum*	*Buffer*
0			252 ± 6	249 ± 4		
1 Day	248 ± 7	248 ± 4	255 ± 6	251 ± 3	255 ± 4	248 ± 3
3 Days	252 ± 4	249 ± 2	245 ± 7	250 ± 2	256 ± 4	248 ± 3
7 Days	139 ± 10	247 ± 3	256 ± 6	253 ± 4	246 ± 7	253 ± 3
14 Days	112 ± 8	251 ± 2	246 ± 9	258 ± 6	256 ± 9	253 ± 4
1 Month	90 ± 12	247 ± 2	258 ± 8	251 ± 3	258 ± 7	251 ± 3
2 Months		N.D.	248 ± 6	253 ± 3	249 ± 5	252 ± 2
3 Months		N.D.	249 ± 5	255 ± 3	253 ± 3	247 ± 2

every day by serial dilution in Soerensen buffer (pH 7.40) of a stock containing 1 mg/ml of the drug. The concentrations were determined by external standardization. Calibration curves were obtained by plotting the peak height against the concentrations of the standard solutions.

The cefixime recovery was 98.5% at a concentration of 5 μg/ml and almost 100% at a concentration of 250 μg/ml, calculated by comparing samples from cefixime-spiked serum with those of buffer solutions. The detection limits for cefixime are 0.1 μg/ml in serum and 0.05 μg/ml in buffer solution. A linear relationship between peak height and cefixime concentration was found between 2.5 and 250 μg/ml (correlation coefficient 0.999) with the following coefficients of variation: 2.30 at a cefixime concentration of 2.5 μg/ml, 1.40 at 100 μg/ml and 0.8 at 250 μg/ml.

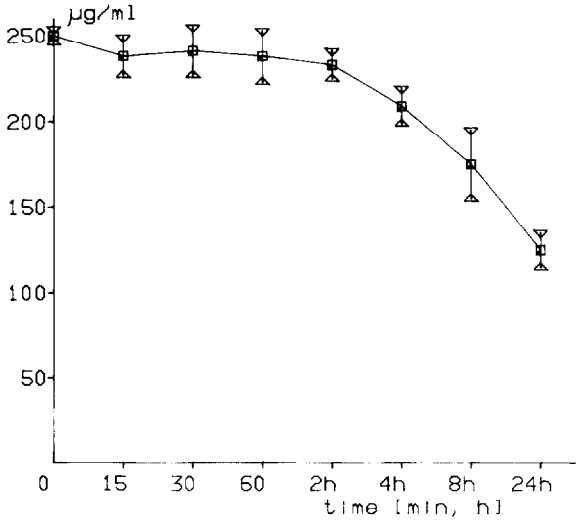

Fig. 2. Change in concentration of cefixime in serum after incubation at 37°C for 24 h.

Stability upon storage at various temperatures

Table I summarizes the stability data for cefixime after storage for 3 months at 4, −20 and −70°C. Degradation of cefixime in serum was observed only at 4°C: 44.6% of the serum-added cefixime was decomposed after 7 days and up to 64% after 1 month. Cefixime was stable in serum at −20 and −70°C. It also seemed to be stable in buffer solution (even at 4°C for 1 month), because no degradation could be observed during storage at these temperatures for 3 months.

Stability upon incubation at 37°C

Fig. 2 shows the change in concentration of cefixime during incubation in serum for 24 h: 15% of the cefixime were degraded in serum after 8 h, up to 50% after 24 h; no degradation was observed in buffer solution or in urine over this time period.

DISCUSSION

The data presented reveal an higher stability for the cephalosporin as compared to other β-lactam antibiotics such as the acylureidopenicillins[3,4]. While the latter penicillins are degraded in serum, *e.g.*, 34% of mezlocillin after 14 days, and buffer when stored at −20°C, cefixime remained stable in serum and buffer over 3 months. This higher stability might be due to an higher stability of the β-lactam ring of the new third-generation cephalosporins (most likely a chemical protection of the β-lactam binding by a *syn* configuration of the carboxymethoxyimino group which is an outstanding characteristic of the amino-thiazole-cephalosporins[5]). These results are of relevance to the storage and processing of cefixime-containing samples in pharmacokinetic studies. Thus these samples can be collected and stored at −20°C until analysis, in contrast to acylureidopenicillin-containing samples which have to be stored at −70°C or analyzed as soon as possible (storage at −20°C protects these samples from decomposition for only 1 week).

The determination of the *in vitro* stability of cefixime gave similar results to those obtained[4] for azlocillin which is the most stable acylureidopenicillin. These data showed an higher stability of cefixime in the first 4 h: 15% of the cephalosporin were decomposed after 8 h, whereas it was stable within the first 4 h. In contrast, 13% of the azlocillin were degraded after 4 h, up to 23% after 8 h and 50% after 24 h.

The HPLC method described allows a rapid and precise determination of cefixime in biological fluids. The high sensitivity and the low amount of serum required for the analysis demonstrate its applicability for pharmacokinetic studies. In contrast to most other HPLC methods available for the determination of cephalosporins in biological fluids[6,7], external standardization was used since an excellent recovery and a linear relationship between the cefixime concentration and the peak height were obtained.

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