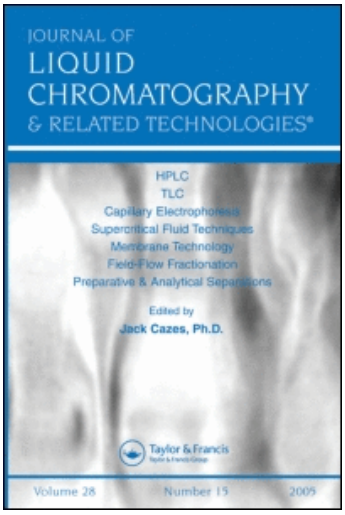

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REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CEFIXIME IN BULK DRUGS

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ABSTRACT

A new technique for the quantitative determination of cefixime trihydrate in bulk drugs by high performance liquid chromatography was developed using simple reagents. Finally, chosen conditions of analysis were as follows: LiChrospher 100 RP - 18 (250 x 4 mm I.D.) column, mobile phase consisting of phosphate buffer pH 7.0 and acetonitrile (93:7, v/v), flow rate of 0,8 mL/min, loop of 20 μL and UV detection at 287 nm. The prospective validation of this technique showed that it is linear in a range of 0.1 to 0.6

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mg/mL (r = 0.9997), sensitive (0.3 %), precise (within-a-day repeatability, RSD = 1.0 %, day-to-day repeatability RSD = 1.3 %), accurate and selective (cefixime can be determined in presence of its related compounds) . The limits of detection and quantitation are 37 ng (0.3 %) and 128 ng (1.1 %), respectively, relative to a 0.6 mg/mL solution.

INTRODUCTION

Cefixime (**I**), the trihydrate of (6R,7R)-7-[[(Z)-2-(2-aminothiazol-4-yl)-2-[(carboxymethoxy)imino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]pct-2-ene-2carboxilic acid (see Fig. 1), is an orally active semisynthetic cephalosporin. Remarkable antibacterial activity of **I** against both Gram positive and Gram negative bacteria has been reported(1). Cefixime possesses excellent efficacy against a broad spectrum of pathogens, including Haemophilus influenzae, Streptococcus pneumoniae, and Moraxella catarrhalis, and excellent activity against beta-lactamase-producing strains as well(2). An excellent efficacy is found in the treatment of urinary (UTI) and respiratory (RTI) infections with **I** in children and adults. In fact, very good efficacy of **I** was demonstrated microbiologically, with eradication in 35 of 36 isolates from children, including all Streptococcus pneumoniae isolates, 40 of 45 isolates from patients with respiratory tract infections, and 64 of 71 isolates from patients with UTI(3). Presently, antibacterial agents, which contains **I**, and exerting excellent effects in preventing or treating respiratory mixed infection with two or more bacteria belonging to different genera selected from among Streptococcus, Moraxella, Haemophilus, Klebsiella, have been developed(4).

For the quantitative assay of **I** in bulk drugs and pharmaceutical dosage forms, an Ion-Pair HPLC (IP HPLC) technique is the current official method pre-

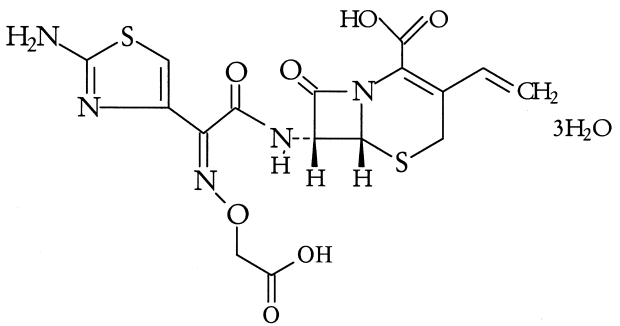

Figure 1. Chemical structure of cefixime trihydrate.

scribed by the United States Pharmacopoeia (USP)(5) and European Pharmacopoeia (Ph. Eur.)(6). Only a slight difference in the pH of mobile phase was perceived between both techniques (the USP prescribes a pH of 7.0, the Ph. Eur. – 6.5). On the other hand, Reversed Phase High Performance Liquid Chromatography (RP-HPLC) has been used only to determine **I** in biological fluids(7-12). Recently, a high performance-TLC method for the determination of **I** in the presence of ceftriaxone and cefotaxime is described(13).

When the ion pair RP HPLC official method was used, it was found that after the analysis of approximately 30 samples, the column efficiency was considerably diminished. This is probably due to the interaction of the ion pair reagent (tetrabutylammonium hydroxide) at pH 7.0 with the stationary phase. For this reason, this method could lessen the time of life of columns used and effects the field of its application, since as it is known, this reagent modifies its selectivity permanently(14).

In order to avoid the use of the ion pair reported in the chromatographic official methods, the development and validation of a new RP HPLC technique to assay I in bulk drugs using simple reagents is proposed in this paper.

EXPERIMENTAL

Materials

Cefixime trihydrate (99.7% with 9.8% of water) was provided by the Laboratory of Organic Synthesis of the Center of Pharmaceutical Chemistry, Cuba. The content was verified by titration in non-aqueous medium and the water content by K. Fischer titration.

Crude cefixime (used for the selectivity test), was also obtained from the above cited laboratory.

Degradation compounds of **I**, used for the selectivity test, were obtained in aqueous acid (HCl 0.1 M solution), neutral (phosphate buffer pH = 7.0) and basic (borate buffer pH = 9) media by heating during 45 min of 5 mg/mL solutions of **I.**(15) The contents of **I,** remaining following the degradation, were 62.3%, 67.1%, and 56.1% in the neutral, acid, and basic solutions, respectively.

Acetonitrile HPLC – grade, sodium hydroxide analytical grade, and phosphoric acid 85% were from Merck (Germany). Potassium dihydrogen phosphate analytical grade, was from Fluka (Switzerland).

Preparation of Solutions

All cefixime solutions, which are described below in the results and discussion section, were prepared taking into account the water content of the solids.

0.1 M phosphate buffer pH 2.5 was prepared using potassium dihydrogen phosphate and phosphoric acid, while 0.1 M phosphate buffer pH 7.0 was prepared using phosphoric acid and sodium hydroxide.

Apparatus

pH values were measured using a 682 Metrohm titroprocessor.

The liquid chromatographic system consisted of an isocratic pump (Model L-6200), an injector (Rheodyne 7725), a UV-Vis detector (Model 4250) (Germany, Japan). The sensitivity of the detector was set to 1.000 AUF. A PC with BioCROM Software (CIGB, Cuba) was used to record chromatograms from the UV-Vis detector, determine peak areas, record UV spectra, and calculate chromatographic parameters.

Method

For the assay of **I**, samples (about 8 μg) were analyzed using the column, mobile phase, flow rate, and wavelength, as discussed under results and discussion.

Validation

For the linearity test, 5 standard solutions (0.1, 0.2, 0.4, 0.5, and 0.6 mg/mL) were prepared from a 1 mg/mL stock solution. These solutions were analyzed twice during three days. A Cochran statistical test was carried out to evaluate homogeneity of variances and regression analysis was performed to evaluate linearity. Experimental data obtained in this test were used to calculate calibration and analytical sensitivity, and discriminator capacity of **I**.

The limits of detection and quantitation were determined by extrapolation to the zero concentration method.

In the precision test, within-day and day-to-day repeatabilities were achieved by means of the analysis of six 0.4 mg/mL solutions by two analysts during two different days.

For the selectivity test, homogeneity of chromatographic peaks was accomplished by recording UV spectra at the beginning, in the maximum, and at the end of each peak assigned to **I** in the chromatograms obtained from solutions containing related compounds (degradation products and impurities from the synthesis). This study was correlated by results obtained in the accuracy test. The latter was carried out preparing two sets of six solutions. Set A consisted of

0.4 mg/mL standard solutions and set B of 0.4 mg/mL solutions spiked with related compounds, obtained as explained above. Comparison of variances and averages between both sets was completed by means of the statistical Fisher test and t Student test, respectively.

RESULTS

Development of the Method

Initially, a 250 mm x 4.6 mm in internal diameter LiChrospher C18 column (5 μm), a phosphate buffer with pH = 2.5 as the aqueous component of the mobile phase, acetonitrile as organic modifier, and the detection at 280 nm, were selected. The best separation using theses conditions was achieved with 10% of acetonitrile at a flow rate of 2 mL/min, but the analysis time was 35 min and the pressure was about 300 bar. The analysis time influenced, considerably, the practicability of the new technique, while the high presure value affected the equipment.

Considering these results some changes were introduced. Thus, pH of the aqueous component in mobile phase was increased to 7.0. Several flow rates (from 1.5 up to 0.5 mL/min), and different ratios of aqueous and organic components in the mobile phase (80:20, 85:15, 90:10, 95:5, and 93:7, v/v), were also tested. The best separation was reached at a ratio of 93:7 (v/v) and at a flow rate of 0.8 mL/min. The UV spectrum of **I** recorded in this mobile phase, led to the choice of 287 nm as the maximum giving the best sensitivity (extinction coeficient at 287 nm, 672 000 l $mol^{-1}cm^{-1}$, is greater than those achieved at 254 nm, 439 500, and 280 nm, 635 500). Figure 2 shows the chromatograms of the separation of **I** from its related compounds. Repeatability test (n = 5) of a 0.4 mg/mL standard solution under these conditions showed that with 95% probability, the number of theoretical plates was 1615, the asymmetry of the peak of **I** – 1.02, the k' value – 3.1, and the relative standard deviation (RSD) of the analytical response (peak areas) – 0.7%. The resolution among **I** and related compounds, especially the resolution between **I** and its E-isomer, which is the compound more closely retained to **I**, was greater than 1 at high concentrations (5 mg/mL) (Fig. 2). This presupposes an even better resolution at working concentrations used for assay (all below 1 mg/mL).

Validation

Tables 1, 2, 3, and 4 show the results obtained in the tests of linearity, sensitivity, and limits of detection and quantitation, precision and accuracy, respectively.

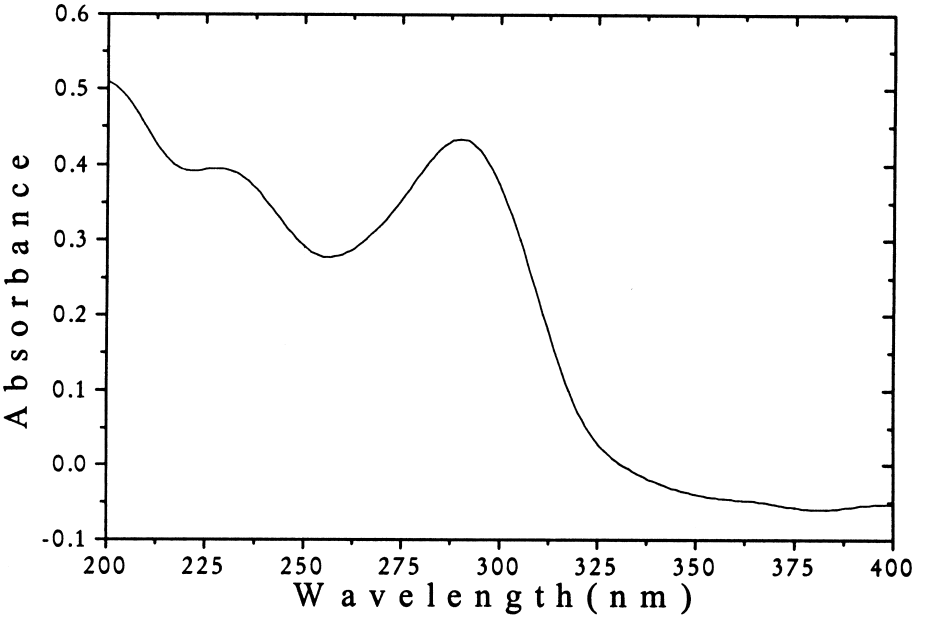

Figure 2. UV spectrum of the peak cefixime recorded from the chromatographic analysis of a 1 mg/mL solution under finally chosen conditions.

DISCUSSION

Development of the Method

When the pH value of the aqueous component of the mobile phase was changed from 2.5 to 7.0, the starting analysis time (35 min) was diminished to 20 min. Thus, the retention time of **I** and its related compounds was reduced. This is probably due to the shift of the equilibrium to more polar ionized forms in neutral aqueous medium, which are less retained in the column. Under the finally chosen conditions at a relatively low flow rate (0.8 mL/min), the high pressure in the chromatographic system was eliminated and the resolution among **I** and its related compounds was kept unaltered, as can be seen from Figure 3.

Validation

The homogeneity test (Cochran test), completed before the linearity test, showed that the quantity of **I** does not influence the variance of the responses

Table 1. Linearity of the RP-HPLC Technique for the Determination of Cefixime Trihydrate in Bulk Drugs

Parameter	Value	Acceptance Criteria (15)
Linear range (μg)	2–12	-
N	30	-
Homogeneity test	G exp = 0.3208	< G tabled = 0.5065 (p = 0.05, m = 5, f = 5)
Equation of the fitted model[a]	y = −0.695 + 24.329 x	-
Slope, b	24.329	-
S_b	0.109	-
RSD of b	0.4 %	< 2 %
Intercept, a	−0.695	-
S_a	0.884	-
CL of a ($a \pm t\, S_a$)	(1,116 ; −2,506)	should include 0
R	0.9997	> 0.99
r^2	99.94 %	> 98 %
RSD of response factors	1.6 %	< 5 %
Significance of a	t exp = 0.7857	< t tabled = 2.048 (p = 0.05, f = 28)
Significance of b	t exp = 222.8181	> t tabled = 2.048 (p =0.05, f = 28)

[a]y – Peak area and x – quantity of analyte in μg; S_b and S_a – standard deviations of the slope and the intercept, respectively; CL – confidence limits; r – coefficient of correlation; r^2 – coefficient of determination.

(peak areas) and, therefore, the variances of the test results are homogeneous. For this reason, the parameters of the model were estimated by the method of ordinary least squares.(16) The r-squared statistic indicates that the model, as fitted, explains 99.94% of the variability in response.

Table 2. Sensitivity and Limits of Detection and Quantitation of the RP-HPLC Technique for the Determination of Cefixime Trihydrate in Bulk Drugs

Parameter	Value
Calibration sensitivity	24.3 mV/μg
Analytical sensitivity	26.8 μg^{-1}
Discriminator capacity	0.04 μg (0.3 %)[a]
Limit of detection	37 ng (0.3 %)[a]
Limit of quantitation	128 ng (1.1 %)[a] (RSD = 4.4 %, n = 6)

[a]Relative to a 0.6 mg/mL solution of cefixime.

Table 3. Precision of the RP-HPLC Technique for the Determination of Cefixime Trihydrate in Bulk Drugs

	Within-Day Repeatability (n = 6)	Day-to-Day Repeatability (n = 12)
$\bar{x}$ (mg/mL)	0.405	0.399
S (mg/mL)	0.004	0.006
RSD (%)	1.0	1.3

$\bar{x}$ - Average concentration and S – standard deviation.

The correlation coefficient equals 0.9997, indicating a relatively strong relationship between the variables. Since the estimated value of the intercept is not significant, systematic errors can be discarded. The estimated value of the slope is a significant value; therefore, there is a statistically significant relationship between analytical response (peak areas) and quantity of **I** at the 99% confidence level. The standard error of the estimate shows the standard deviation of the residuals to be 2.22059 (equals 1.2%, less than the accepted 1.5%)(17). According to these results, a linear behavior under the studied range is observed.

The smallest difference of the quantity of **I** that can be recorded using this technique at a 95% confidence level is 0.04 μg; about 0.3%, relative to a concentration of 0.6 mg/mL.

RSDs of precision are both within the limits of acceptance(17). These results predict a good precision for this technique.

No differences among UV spectra recorded at the beginning, in the maximum, and at the end of each peak assigned to **I** in all chromatograms were observed, therefore, they are homogeneous. This result supports the one obtained in the test of accuracy and make us conclude that the technique is selective.

Table 4. Accuracy of the RP-HPLC Technique for the Determination of Cefixime Trihydrate in Bulk Drugs

	Set A	Set B	Acceptance criteria
$\bar{x}$ (Peak area)	193.6	192.4	
S	2.679	2.711	
S^2	7.179	7.350	
Fisher	$F_{exp} = 1.01$		$< F_{tab} = 5.05$ ($p = 0.05$; $f_1 = 5$; $f_2 = 5$)
Student t	$t_{exp} = 0.76$		$< t_{tab} = 2.228$ ($p = 0.05$; $f = 10$)

Set A holds 0.4 mg/mL standard solution of cefixime and B – 0.4 mg/mL standard solution of cefixime spiked with related compounds, $\bar{x}$ - average, and S^2 - variance.

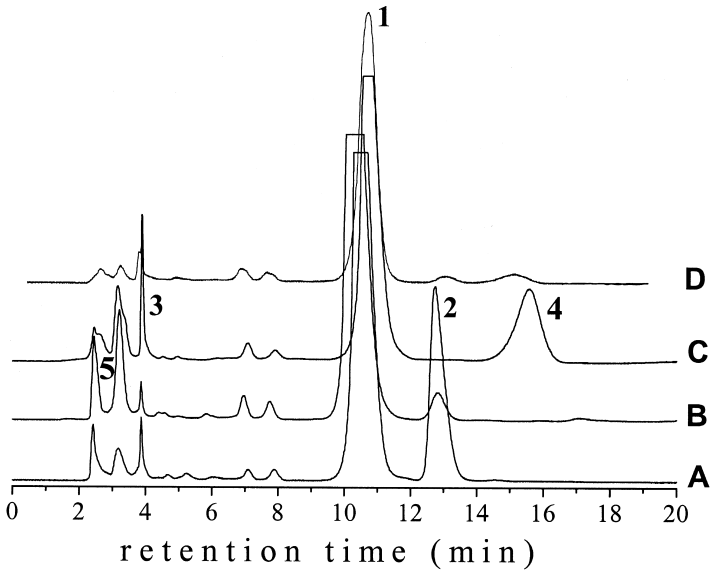

Figure 3. LC chromatograms of solutions of cefixime degraded in neutral (A), acid (B), basic aqueous media (C) and from a synthetic product without purification (D) (where: 1 – cefixime, 2 – cefixime E – isomer, 3, 4, and 5 – degradation products).

Since experimental F and t were lower than the respective values from tables, it can be stated that there were no significant differences between the variances and averages compared. For this reason, there are no significant differences between set A and B at a 95% confidence level, and, hence, this technique is accurate to determine **I** in the presence of its related compounds.

According to the results discussed above, we concluded that the technique developed using simple reagents, is valid for the quantitative determination of cefixime in bulk drugs.

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