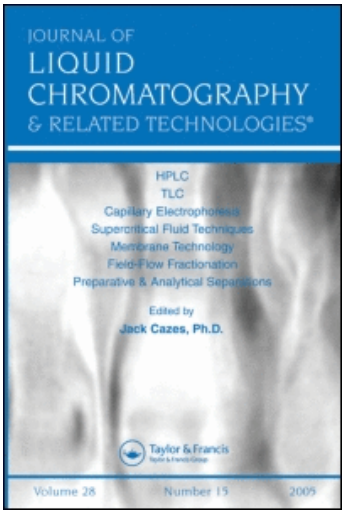

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DETERMINATION OF CEFTIZOXIME IN HUMAN ABSCESS FLUID BY PAIRED ION REVERSED-PHASE HPLC

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ABSTRACT

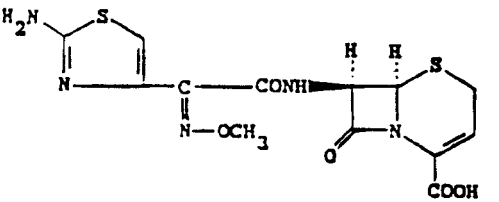
An ion paired reversed - phase high performance liquid chromatographic assay for ceftizoxime in human abscess fluid using barbital as the internal standard is described. Sample preparation consists of precipitating the proteins in diluted abscess fluid with methanol, and centrifuging. Decanted supernatant after evaporation to dryness is reconstituted with the HPLC mobile phase for injection. The HPLC separation was carried out on an octyl C_8 column using a mobile phase composed of potassium phosphate buffer, tetrabutylammonium dihydrogen phosphate IP reagent and 20% methanol (v/v). UV detection was at 254 nm and the concentration range for the assay was 2.0μg/mL to 50.0μg/mL. The results of the full validation of the assay gave coefficients of variation for inter-assay variability of 8.4-12.6% and intra-assay variability of 4.3 - 9.3% for three levels of concentration. This isocratic HPLC method is relatively simple, rapid and shows good reproducibility for the determination of ceftizoxime in human abscess fluid.

INTRODUCTION

Ceftizoxime (FK749), [(6R,7R)-7-[(Z)-2-(2-amino-4-thiazolin-4-yl) - 2-methoxy-iminoacetomido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid] (Figure 1) is a broad spectrum, semi-synthetic, third generation cephalosporin antibiotic (1) that is administered parenterally. Ceftizoxime possess activity against both aerobic and anaerobic, gram positive and gram negative microorganisms and it is widely used in the treatment of bacterial infections including abscesses.

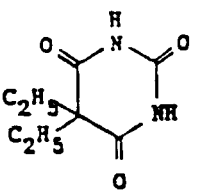
Various analytical methods including HPLC have been used to determine cephalosporin drugs in different biological matrices. Ceftizoxime concentrations have been determined in human plasma (2), serum (3,4,5), urine (6,7), blister fluid (8) and rat serum, bile and urine (9). However, an assay to determine the ceftizoxime concentrations in human abscess fluid was necessary and none of the available methods was found suitable. Some of the difficulties in the previous methods were the lack of an internal standard or the use of another cephalosporin drug as the internal standard. Thin layer chromatographic methods even with fluorescence detection were not considered satisfactory. HPLC methods frequently applied for determination of cephalosporins in aqueous dosage forms did not perform well in the presence of abscess-matrix. The method by Moore et al (10) using microbore HPLC column and mass spectrometry was only applied to aqueous media. This method could not possibly be applied to clinical assays due to matrix interference and high costs of operation and instrumentation. Accordingly, the method developed by us is the first HPLC assay suitable for determination of cephalosporins in abscess fluid. In this method an octyl (C-8) column with an ion pairing mobile phase system, very similar to that used for assaying iothalamate in plasma was used (11). The main difference was a more freely available ion-pairing reagent tetrabutylammonium dihydrogen phosphate used instead of dodecyltriethylammonium phosphate Q-12 ion- pairing reagent.

A complete assay validation, reported in this paper was performed. This was followed by determination of ceftizoxime in a set of clinical samples.



(A) CEFTIZOXIME

$C_8H_{13}N_5O_5S_2$
MW.383.40



(B) BARBITAL

$C_8H_{12}N_2O_3$
MW 184.2

FIGURE 1. Chemical structures of (A) ceftizoxime and (B) 5,5 diethyl barbituric acid (barbital, internal standard).

EXPERIMENTAL

Apparatus

The HPLC system used consisted of a Waters M-510 HPLC pump, a model 712 WISP autoinjector, and a model M441 variable UV detector (Waters Assoc., Milford, MA). An HP 3392A-integrator (Hewlett

Packard, Avondale,PA) was used for collecting the chromatographic data. The detector wavelength was 254 nm and the absorbance was set at 0.05 aufs. Separation was achieved with an Ultrasphere, octyl (C-8), 5μm particle size, 4.6 mm (i.d.) x 25cm reversed-phase column (Beckman Instruments Inc., San Ramon, CA).

Reagents

All solvents were of HPLC grade [Fischer Scientific Co., Fair Lawn, NJ]. Tetrabutylammonium dihydrogenphosphate [(Ion pair reagent, 1M solution), Aldrich Chemical Company Inc., Milwaukee, WI 53233], potassium diacid phosphate, potassium mono acid phosphate [Fischer Scientific Co., Fair Lawn,NJ], ceftizoxime sodium [Fujisawa Pharmaceutical Co., Osaka, Japan], and barbital [Sigma Chemicals Co., St. Louis, MO], were used as received. Deionized distilled water was obtained from Barnstead Nanopure purification system (Barnstead Co., Boston, MA). For blank samples, human abscess fluid from patients who were not administered ceftizoxime was used and the clinical samples assayed for ceftizoxime were obtained from patients at UC San Francisco-Long Hospital, who were administered ceftizoxime (1g, iv, Q8) prior to surgery.

Mobile Phase

The mobile phase was composed of 20% methanol, and 10mM IP reagent in 40 mM potassium phosphate. This was prepared by dissolving 6.44 g KH_2PO_4, 7.04 g K_2HPO_4 and 10 mL of 1M ion-pairing reagent solution in 1 L of deionized water and the pH was adjusted to 7.0. The prepared buffer was filtered through a 0.22μm filter and mixed with 250 mL of methanol. The mixture was degased by sonication under vacuum. The isocratic flow rate of the mobile phase was 1 mL/min.

Sample preparation

Calibration curve samples were prepared by spiking 1:2 diluted abscess fluid with ceftizoxime and the internal standard (barbital). The proteins in these samples, quality assuarance (QA) controls and clinical samples (50 µL aliquots) were precipitated out with 500 µL aliquots of HPLC grade methanol. Samples were vortexed for 20 seconds and centrifuged for 10 minutes at 2500 rpm. The supernatants were concentrated under nitrogen and residues were reconstituted in running buffer (500µl). Twenty five to thirty microliter samples were injected onto the column for analysis.

All clinical samples, QA samples and stock solutions of compounds were stored at -80°C until analysis. Fresh stock solutions of ceftizoxime were prepared every week. Spiked samples for calibration curves were prepared immediately prior to the assay and frozen controls at three different drug concentrations were prepared every week. The frozen control samples were prepared in human abscess fluid which were relatively ceftizoxime free, and were spiked with separately prepared drug solutions.

RESULTS

Typical chromatograms of the internal standard in blank abscess fluid and the abscess fluid spiked with ceftizoxime and internal standard, are presented in Figure 2A and 2B respectively. The mean retention times of ceftizoxime and barbital are 16.2, and 18.0 minutes respectively. (However, fluctuations of these retention times are observed due to the variation of temperature, pH and column performance). The concentrations used for the calibration curve are 2.0, 5.0, 10.0, 20.0, 35.0 and 50.0 µg/mL.

A typical calibration graph used for the calculation of ceftizoxime concentration is presented in Figure 3. Linear regression of peak height ratio vs. drug - concentration gives typical coefficient of determination (r^2) of 0.999.

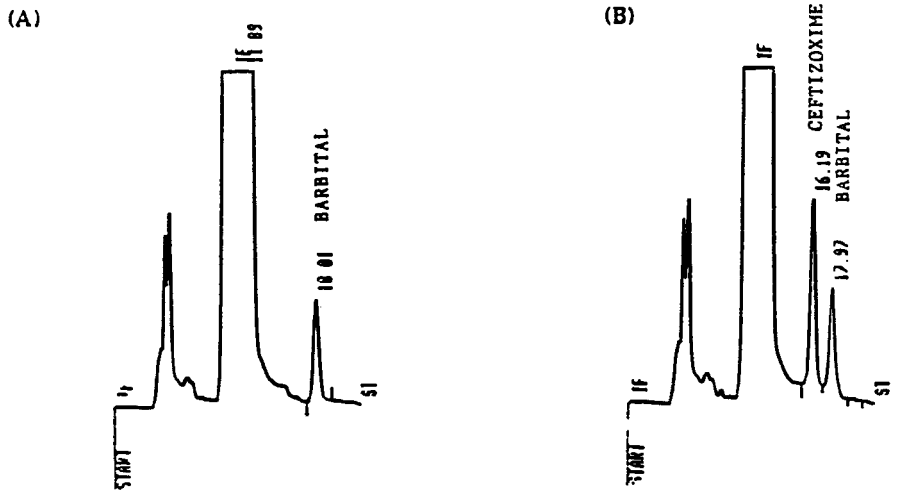


FIGURE 2 (A) Chromatogram of blank abscess fluid with internal standard. (B) Chromatogram of abscess fluid spiked with ceftizoxime and the internal standard.

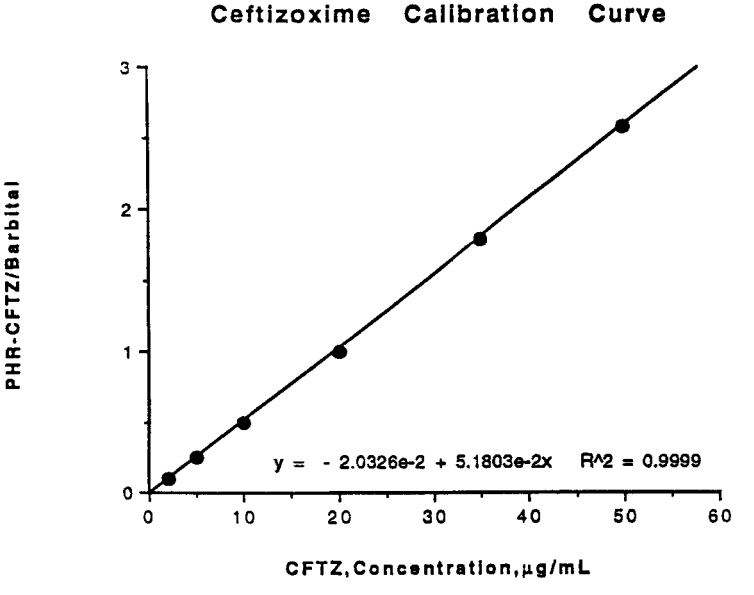


FIGURE 3. Calibration curve of ceftizoxime in abscess fluid.

Variability Studies

Inter-assay and intra-assay variability was studied using frozen controls at three concentrations, low, medium and high. Four to six samples from each concentration were assayed for both inter-assay and intra-assay studies. For inter-assay variability, six calibration curves on six different days were used and one calibration curve was used for the intra-assay variability study. The range for the coefficients of variation was 8.44 % to 12.60 % for the inter-assay study. Coefficient of variation ranged from 4.25 % to 9.31 % for the intra-assay study (Table 1).

Recovery

Assay recovery was measured by comparing the peak height ratios of ceftizoxime to barbital, at three different drug- concentrations in abscess fluid and in aqueous drug solutions spiked at the same concentration. The internal standard was added to the abscess fluid samples only after the supernatant was decanted from the precipitated proteins. Both sets of samples were then evaporated and reconstituted in running buffer for injection. The mean % recovery was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{peak height ratio of ceftizoxime in abscess fluid}}{\text{peak height ratio of ceftizoxime in water}} \times 100$$

The recovery for low (6.0μg/mL), medium (16.0μg/mL) and high (40.0μg/mL) controls were 87.0%, 74.1% and 74.4% respectively. The mean recovery of the ceftizoxime was 78.5 %.

Stability

The stability of ceftizoxime during storage at room temperature for 24 hours was evaluated in abscess fluid, plasma and in distilled -

TABLE 1
Inter-Assay and Intra-Assay Precision of Ceftizoxime in Abscess Fluid

		CONCENTRATION* (μg/mL)		
		LOW [6.0]	MEDIUM [16.0]	HIGH [40.0]
Inter-Assay				
	Mean	5.78	16.43	38.60
	SD	0.72	1.39	3.86
	%CV	12.60	8.44	10.04
Intra-Assay				
	Mean	5.93	16.16	38.55
	SD	0.25	1.22	3.59
	%CV	4.25	7.55	9.31

*Each value represents mean of n=6

deionized water (Table 2). Six samples each from three controls were used for this stability study. Further, the stability of ceftizoxime during three freezing and thawing cycles was investigated. These results showed that there was some loss of ceftizoxime up to 10.6% for the lowest concentration, after the third cycle (Table 3).

DISCUSSION

This is the first method reported for the determination of ceftizoxime concentrations in abscess fluid. The stability of the drug in abscess fluid was unsatisfactory due to degradation of the drug. This decomposition was observed in abscess fluid, plasma and in distilled - deionized water. The manufacturers of ceftizoxime sodium for injection (Fujisawa Pharmaceutical Co.,) stated that the drug solutions are stable in most of parenteral fluids for 24 hours at room temperature and 96 hours under refrigeration (5°C) at 100mg/mL concentration (12). However, the

TABLE 2
Storage Stability (24 Hours at RT) of Ceftizoxime in Abscess Fluid, Plasma and DI water

	Percent Ceftizoxime Remaining*		
Conc.,µg/mL	Abscess Fluid	Plasma	DI Water
6.0	74.64	70.07	72.40
16.0	83.15	78.39	92.72
40.0	92.50	78.89	94.95

*Each value represents mean of n=5

TABLE 3
Freeze - Thaw Stability of Ceftizoxime in Abscess Fluid

	Percent Change CFTZ Conc.*	
Conc.,µg/mL	From Cycle 1 to 2	From Cycle 1 to 3
6.0	-9.51	-10.65
16.0	1.77	-0.77
40.0	-1.95	-6.28
Mean	**-3.23**	**-5.90**

* Each value represents mean of n=5

recent studies by A.B. Lesko et al revealed that the ceftizoxime sodium is stable in both NaCl solution and in 5Xdistilled water for 48 hours at RT and for seven days in NaCl solution and 5 days in 5Xdistilled water under refrigeration (5°C) at 20mg/mL and 40mg/mL concentrations (13). According to our experience the drug solutions were not stable in the range of 2.0-50.0 µg/mL for more than five days in plasma, in abscess fluid and in distilled - deionized water even at -20°C. Our results showed that the drug percent declined for these concentrations in the range of 7-26%, 21-30% and 5-28% at room temperature for 24 hours in abscess fluid, plasma and distilled-deionized water respectively (Table 2). Further, freeze-thaw stability results in abscess fluid showed that the

mean drug percent change, was in the range of -3.23 to -5.90% (Table 3). In order to maintain the stability of the drug in clinical samples, QA samples and stock solutions, they were stored at -80°C for 5 days.

The inter-assay and intra-assay variability results were satisfactory. The assay method has a lower limit of quantitation of 2.0μg/mL. The recovery of the drug during extraction was satisfactory and within acceptable limits.

Using this method, various abscess samples from the abdomen, pancreas, brain, gall bladder, thigh, cysts, and nodes from eighteen subjects were analysed for the ceftizoxime concentrations. The full range of concentration for these samples was 2.18-30.66 μg/mL.

The validation of this assay was carried out according to the suggestions offered by the conference on "Analytical Methods Validation; Bioavailability, Bioequivalence and Pharmacokinetics studies" (14). The IP-RP-HPLC method described here is simple, rapid, and reliable for the determination of ceftizoxime concentrations in various abscess fluids and hence is useful for clinical drug studies.

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REFERENCES

1. T.kamimura, Y.Matsumoto, N.Okada, Y.Mine, M.Nishida, S.Goto, and S.Kuwahara, Antimicrob. Agents Chemother., 16(5), (1979), 540-548.

2. C.M.Moore, K.Sato, H.Hattori and Y.Katsumata, Clinica Chimica Acta, 190, (1990), 121-124.

3. M.LeBel, J.F.Ericson and D.H.Pitkin, J. Liquid Chromatogr. 7(5), (1984), 961-968.

4. E.M.McCormick, R.M.Echols and T.G.Rosano, Antimicrob.Agents Chemother., 25(3), (1984), 336-338.

5. C.E.Fasching, L.R.Peterson, K.M.Bettin and D.N.Gerding, Antimocrob.Agents Chemother., 22(2), (1982), 336-337.

6. M.Blanchin, M.Rondot-Dudragne, H.Fabre and B.Mandrou, Analyst, 113, (1988), 899-902.

7. M.C.Rouan, F.Abadie, A.Leclerc and F.Juge, J. Chromatogr., 275, (1983), 133-144.

8. G.S.Jaresko, S.L.Barriere, and B.L.Johnson,Jr., Antimicrob. Agents Chemother., 36(10), (1992), 2233-2238.

9. A.Suzuki, K.Noda and H.Noguchi, J.Chromatogr., 182, (1980), 448-453.

10. C.M.Moore, K.Sato and Y.Katsumata, J.Chromatogr., 539, (1991), 215-220.

11. A.L.Jayewardene, A.K.Seneviratne and J.G.Gambertoglio, J.Liquid Chromatogr., 1994, in press.

12. The Package Insert, Cefizox (Sterile Ceftizoxime Sodium), SmithKline Beecham, Philadelphia, 1991.

13. A.B.Lesko, G.P.Sesin, R.J.Cersosimo, Ann. Pharmacother., 23, (1989), 615-618.

14. V.P.Shah, K.K.Midha, S.Dighe, I.J.McGilveray, J.P.Skelly, A.Yacob, T.Layloff, C.T.Viswanathan, C.Edgarcook, R.D.Mcdowall, K.A.Pittman and S.Spector, J.Pharm.Sci., 309(81),3,1992.

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