Journal of Chromatography, 276 (1983) 129—137
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1699

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CEFOPERAZONE IN HUMAN SERUM AND URINE

J. DOKLADALOVA*, G.T. QUERCIA and J.P. STANKEWICH

Quality Control Division, Pfizer Inc., Eastern Point Road, Groton, CT 06340 (U.S.A.)

(First received June 22nd, 1982; revised manuscript received March 10th, 1983)

SUMMARY

A gradient high-performance liquid chromatographic (HPLC) procedure has been developed for the determination of microgram amounts of cefoperazone in human serum and urine. The method employes a μBondapak C_{18} column and gradient elution with two mobile phases. Excellent separation of the drug from potential degradation products as well as from representative penicillins (sodium ampicillin, sodium methicillin, potassium penicillin G) and aminoglycosides (tobramycin, gentamicin, kanamycin) has been demonstrated. Coefficients of variation of 7.3% or less were obtained for 25—100 μg/ml cefoperazone in both serum and urine. Average recoveries of the drug from spiked serum and urine samples corresponded to 97.6% and 98.6%, respectively. Amounts as low as 1 μg cefoperazone per ml of sample can be estimated using sample volumes corresponding to 0.1 ml serum or 1 ml urine.

Good correlation between the HPLC assay and a microbiological cylinder—plate assay employing *Micrococcus luteus* ATCC 9341 has been demonstrated for human serum and urine of patients treated with cefoperazone. While the microbiological method is less time-consuming, it lacks specificity in the presence of other antibiotics. The HPLC method can be used to analyze cefoperazone in the presence of penicillins and aminoglycosides which can potentially be co-administered with cefoperazone.

INTRODUCTION

Cefoperazone (I) is a recently developed cephalosporin which has been demonstrated to be bactericidal, not only against gram-positive bacteria but also against gram-negative bacteria, especially *Pseudomonas* and *Enterobacter* species [1].

A selective assay was required to determine bioavailability of this drug and to monitor its levels in serum and urine of subjects to whom cefoperazone had been administered. A recent paper published by Dupont and DeJager [2] describes an isocratic high-performance liquid chromatographic (HPLC) procedure for cefoperazone quantitation in serum. After clean-up of samples

0378-4347/83/$03.00

with Sep-Pak cartridges containing μBondapak C_{18}/Porasil (R/B), cefoperazone is injected onto the reversed-phase μBondapak C_{18} (10 μm particle size, 250 mm × 4.6 mm I.D.) HPLC column. A mobile phase consisting of methanol—water (1:1) is used. The HPLC portion of the procedure is very rapid, the retention time of cefoperazone being 2.3 min. However, the selectivity was not discussed.

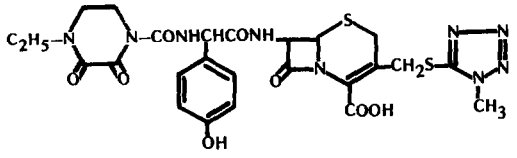
Using slower gradient HPLC described in this paper, the preliminary sample clean-up is not needed. In addition, cefoperazone can be separated from known degradation products as well as from representative penicillins and aminoglycosides.

The HPLC method has been compared to a cylinder—plate microbiological assay procedure employing *Micrococcus luteus* ATCC 9341 as the test organism.

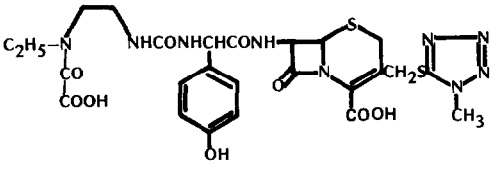
HPLC METHOD

Materials

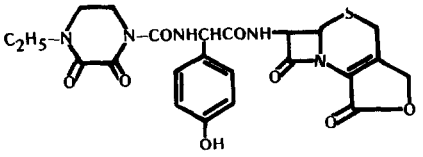
Cefoperazone (I), cefoperazone degradation products A, B, D, E, F, (II—VI), sodium ampicillin and potassium penicillin G were supplied by Central Research of Pfizer (Groton, CT, U.S.A.). Tobramycin was obtained from Eli Lilly (Indianapolis, IN, U.S.A.). Sodium methacillin and kanamycin were



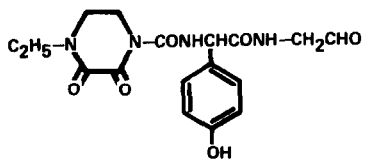
(I)



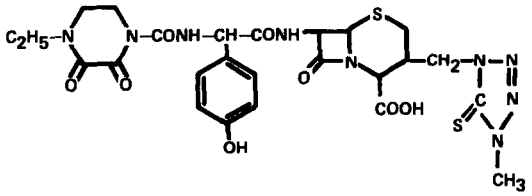
A(II)



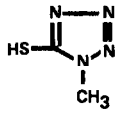
B(III)



D(IV)



E(V)



F(VI)

acquired from Bristol Laboratories (Syracuse, NY, U.S.A.). Gentamicin was supplied by Schering (Kenilworth, NJ, U.S.A.). Glass distilled acetonitrile was purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Triethylamine and acetic acid were reagent-grade quality and were used without further purification.

Apparatus and chromatographic conditions

Analyses were carried out using a μBondapak C_{18} column (10 μm particle size, 250 × 4.6 mm I.D.) attached to a UV detector at 254 nm. A dual-channel recorder, individual channels set at two different sensitivities (e.g. 0.01 a.u.f.s., 0.2 a.u.f.s.), was used in order to simultaneously record minor and major sample components. A sample injection system (e.g. Waters Assoc. U6K injector) was used to apply 20 μl of the sample or standard solution onto the column. A gradient programmer, Waters Model 660, was set using curve select-9. Two mobile phases were used. Mobile phase A was composed of 0.0012 *M* triethylamine and 0.042 *M* acetic acid in distilled water. Mobile phase B was composed of 0.0012 *M* triethylamine and 0.042 *M* acetic acid in acetonitrile—distilled water (24:76). The flow-rate of 1.5 ml/min mobile phase A and 0.5 ml/min mobile phase B was adjusted during 15 min to 1.2 ml/min mobile phase A and 0.8 ml/min mobile phase B. The assay was completed isocratically under the final conditions.

Preparation of samples and calibration curves

Serum analysis. Transfer a 1-ml aliquot of the serum sample to a 15-ml centrifuge tube and add 1 ml of methanol. Vortex the sample for 30 sec and allow it to stand at room temperature for 10 min; if necessary, dilute the sample with distilled water to obtain a cefoperazone concentration within the linearity range of 1—100 μg/ml serum and centrifuge the sample at 1022 *g* for 10 min. The procedure for a microsample is analogous. However, only 0.1 ml of sample and 0.1 ml of methanol are used.

Urine analysis. Centrifuge urine specimens at 1022 *g* for 10 min. If necessary, dilute the sample with distilled water to obtain a cefoperazone concentration within the range of 1—100 μg cefoperazone per ml urine.

Calibration for serum analysis. Prepare a series of cefoperazone standard solutions containing 25, 50, and 100 μg/ml in 1.0 ml of distilled water. Add 1.0 ml of methanol and vortex each sample for 30 sec. Inject 20 μl of each standard solution into the HPLC system. Measure peak heights of cefoperazone. Calculate the linear regression equation which characterizes the calibration curve. Prepare this curve daily.

Calibration for urine analysis. Prepare a series of cefoperazone standard solutions containing 25, 50, 100 μg in 1.0 ml of distilled water. Inject 20 μl of each standard solution into the HPLC system. Measure peak heights of cefoperazone. Calculate the regression equation which characterizes the calibration curve. Prepare this curve daily.

HPLC assay of serum and urine samples

Inject 20 μl of prepared sample into HPLC system. Measure peak heights of cefoperazone. Calculate μg cefoperazone per ml serum or urine, using regression lines obtained by the calibration of aqueous standards:

$$\mu g \text{ cefoperazone per ml} = \frac{\text{peak height of sample}}{\text{slope}} \times \text{dilution factor.}$$

MICROBIOLOGICAL ASSAY

Materials and equipment

The test organism used (*Micrococcus luteus* ATCC 9341) was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). The plastic Petri dishes (100 × 20 mm; Falcon Plastics, Cockeysville, MD, U.S.A.) contained approximately 12 ml of Antibiotic Medium A (BBL, Cockeysville, MD, U.S.A.). Sterile normal human serum (Bio-Bee, Boston, MA, U.S.A.) and 1% potassium phosphate buffer, pH 6.0, were used to dilute standard curves and samples for the serum and urine assays, respectively. The zones of inhibition were read by an automated zone reader (AZOR) manufactured by Digital Information Science (Silver Spring, MD, U.S.A.). The calculations were performed by a PDP8E (DEC, Maynard, MA, U.S.A.) computer hard wired to the reader.

Preparation of standard and sample solutions

Serum analysis. The serum samples were analyzed against a compensatory standard curve diluted in human control serum. The standard curve ranged in

concentration from 1.5 to 6.0 μg/ml of cefoperazone. When necessary, the serum samples were diluted to contain approximately 3 μg/ml of cefoperazone using pooled human control serum.

Urine analysis. The standard curve was prepared in 1% potassium phosphate buffer, pH 6.0. The standard curve ranged in concentration from 0.3 μg to 1.2 μg/ml of cefoperazone. Samples that contained more than 1.2 μg/ml of cefoperazone were diluted with phosphate buffer to approximately 0.6 μg/ml.

Sample assay

The microbiological agar diffusion assay for cefoperazone employed a cylinder–plate technique essentially described in the United States Pharmacopeia [3]. The basic assay employed *Micrococcus luteus* ATCC 9341 as the test organism and antibiotic Medium A as the test medium. A single seeded 12-ml agar layer was used to enhance the sensitivity. After application of the standards and samples, the plates were incubated at 32–35°C for approximately 18 h. The resulting zones of inhibition were read by an automated zone reader (AZOR). The automated plate reader and computerized data handling system have been described [4]. A linear regression analysis was performed on the logarithm of the standard concentrations (μg/ml) versus their zone diameter (mm). The concentration of cefoperazone in the samples was calculated according to the following equation:

$$\text{Log } C_s = \log C_r - \Delta(m)$$

where C_s = sample concentration, C_r = reference concentration, Δ = average sample zone diameter − average reference zone diameter, and m = slope of standard curve. Potency is then calculated by the following equation:

$$\text{Potency } (\mu\text{g/ml}) = \frac{C_s \times \text{dilution}}{\text{ml}}.$$

RESULTS AND DISCUSSION

The first step in the development of the HPLC procedure was to establish selectivity. It was challenged with known cefoperazone (I) degradation products: A(II), B(III), D(IV), E(V) and F(VI).

While in the published procedure [2] cefoperazone co-eluted with cefoperazone degradation products A, E, and F, it was separated from these substances using gradient HPLC. A chromatogram depicted in Fig. 1 illustrates the good separation of all substances tested. In addition, potential interference of penicillins (e.g. sodium ampicillin, sodium methicillin, potassium penicillin G) and aminoglycosides (e.g. tobramycin, gentamicin, kanamycin) was investigated. Relative retention times summarized in Table I demonstrate that none of these substances interfere with the quantification of cefoperazone.

The HPLC response was found to be linear in the range of 1–100 μg/ml of sample. Since the calibration curves obtained by direct injection of cefoperazone standard solutions were identical with those obtained using spiked blank serum or urine, the more simple calibration using standard cefoperazone solutions directly is recommended for testing of clinical samples.

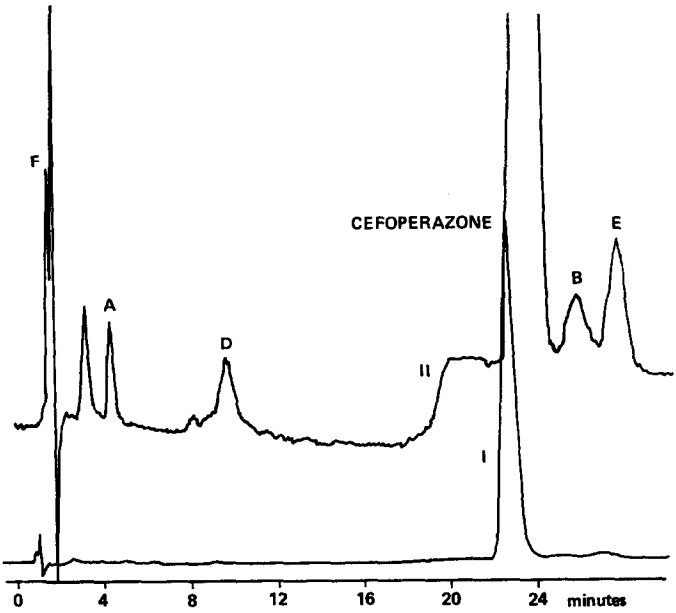


Fig. 1. Separation of cefoperazone (100 μg/ml) and cefoperazone degradation products (2 μg/ml of degradation products A, B, D and E and 0.5 μg/ml of degradation product F). I, 0.2 a.u.f.s.; II, 0.01 a.u.f.s.

TABLE I

RELATIVE RETENTION TIMES OF KNOWN CEFOPERAZONE DEGRADATION PRODUCTS, REPRESENTATIVE PENICILLINS AND AMINOGLYCOSIDES

Substance	Relative retention time
Gentamicin	0.00
Kanamycin	0.00
Tobramycin	0.00
Cefoperazone F	0.07
Cefoperazone A	0.19
Ampicillin sodium	0.23
Cefoperazone D	0.43
Methacillin sodium	0.95
Penicillin G potassium	0.95
Cefoperazone	1.00
Cefoperazone B	1.15
Cefoperazone E	1.23

The accuracy and precision were evaluated by adding known amounts of cefoperazone to blank serum and urine in both the presence and absence of penicillins and aminoglycosides. The results of the HPLC analyses are summarized in Tables II -IV. Coefficients of variation of 7.3% or less were obtained for 25—100 μg/ml cefoperazone in both serum and urine. Average recoveries of the drug from spiked serum and urine samples corresponded to 97.6% and 98.6%, respectively.

TABLE II

ACCURACY AND PRECISION OF THE HPLC ASSAY FOR CEFOPERAZONE IN URINE AND SERUM

Concentration range	*n*	Concentration of cefoperazone added	Average concentration found	Coefficient of variation (%)	Average recovery (%)
Urine 25—100 μg/ml	5	25.0	24.3	5.41	97.2
	5	50.0	50.0	0.66	100.0
	5	75.0	74.6	1.48	99.5
	5	100.0	99.8	0.42	99.8
Serum 25—100 μg/ml	5	25.0	24.9	2.04	99.6
	5	50.0	49.6	0.72	99.2
	5	100.0	98.3	1.03	98.3

TABLE III

ACCURACY AND PRECISION OF THE HPLC ASSAY FOR CEFOPERAZONE IN URINE AND SERUM IN THE PRESENCE OF SODIUM AMPICILLIN, SODIUM METHICILLIN AND POTASSIUM PENICILLIN G

Amounts of 100 μg sodium ampicillin, 100 μg sodium methicillin and 100 μg potassium penicillin G were added to 1 ml of each sample.

Concentration range	*n*	Concentration of cefoperazone added	Average concentration found	Coefficient of variation (%)	Average recovery (%)
Urine 25—100 μg/ml	5	25.0	23.6	0.93	94.4
	5	50.0	48.8	2.73	97.6
	5	100.0	98.6	4.09	98.6
Serum 25—100 μg/ml	6	25.0	24.4	3.86	97.6
	6	50.0	48.1	3.26	96.2
	6	100.0	94.6	3.67	94.6

TABLE IV

ACCURACY AND PRECISION OF THE HPLC ASSAY FOR CEFOPERAZONE IN URINE AND SERUM IN THE PRESENCE OF TOBRAMYCIN, GENTAMICIN AND KANAMYCIN

Amounts of 100 μg tobramycin, 100 μg gentamicin and 100 μg kanamycin were added to 1 ml of each sample.

Concentration range	*n*	Concentration of cefoperazone added	Average concentration found	Coefficient of variation (%)	Average recovery (%)
Urine 25—100 μg/ml	2	25.0	25.1	7.06	100.4
	2	50.0	49.5	7.29	99.0
	2	100.0	101.2	3.91	101.2
Serum 25—100 μg/ml	2	25.0	25.5	5.83	102.0
	2	50.0	48.5	7.29	97.0
	2	100.0	96.5	5.20	96.5

The HPLC method was compared to the microbiological assay by analyzing urine and serum samples of patients who had been administered only cefoperazone. A typical chromatogram of a patient serum is presented in Fig. 2. Results of parallel determinations by both methods are listed in Table V. The

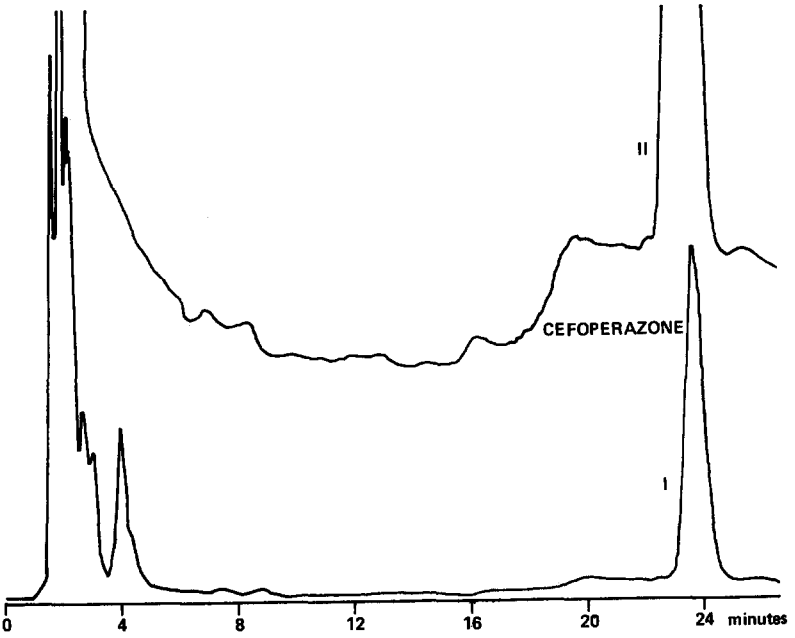


Fig. 2. Typical chromatogram of a serum of a patient who had been administered cefoperazone. I, 0.2 a.u.f.s.; II, 0.01 a.u.f.s.

TABLE V

EQUIVALENCY OF MICROBIOLOGICAL AND HPLC ANALYSES OF SERUM AND URINE OF PATIENTS ADMINISTERED CEFOPERAZONE

	Sample No.	Cefoperazone determined (μg/ml)				Sample No.	Cefoperazone determined (μg/ml)	
		HPLC assay		Bioassay			HPLC assay	Bioassay
Serum	1	166	167	156	157	8	288	277
	2	77	75	74	70	9	197	187
	3	46	43	42	40	10	126	123
	4	153	163	160	144	11	70	76
	5	72	71	67	65	12	33	37
	6	49	40	38	37	13	9	10
	7	243		235		14	2	3
Urine	1	317		333		4	67	57
	2	109		132		5	16	14
	3	101		103				

average percent agreement of the HPLC assay compared to the microbiological assay was +3.8% for the serum samples and −4.5% for the urine samples, differences which are acceptable for analysis of clinical specimens. The correlation between the serum and urine cefoperazone concentrations determined by the HPLC and microbiological methods was evaluated further using linear regression analysis. The correlation coefficients were 0.999 and 0.996 for the serum and urine determinations, respectively. The results support the conclusion regarding the excellent correlation between the two methods.

The microbiological method is less time-consuming than the HPLC method and is well suited for processing large numbers of clinical samples where sufficient sample exists and cefoperazone is the only antibiotic administered. The good agreement of the microbiological method with the HPLC technique, which specifically measures the intact drug, indicates that the microbiological method is relatively specific for the active parent compound. It further suggests that the degradation products and potential metabolites of cefoperazone do not substantially interfere with the microbiological assay.

For samples which potentially contain other penicillins or aminoglycosides, the HPLC method is clearly the method of choice due to its specificity. The HPLC method is also particularly useful in those instances where very low volumes of samples (e.g. 0.1 ml) are available as, for example, in monitoring cefoperazone levels in the serum of new born infants.

ACKNOWLEDGEMENT

The authors wish to acknowledge the technical assistance of Mr. G.H. Keeler.

REFERENCES

1 T. Yoneda, T. Shibata, H. Masatami, S. Sato, Y. Kawamura, S. Iwasaki, A. Nagai, Y. Takimoto, M. Nagasawa and A. Takai, Chemotherapy (Tokyo), 28 (Suppl. 6) (1980) 21.
2 D.G. Dupont and R.L. DeJager, J. Liquid Chromatogr., 4 (1981) 123.
3 The United States Pharmacopeia, 20th Revision, 1980, Mack Publishing Co., Easton, PA, pp. 882–888.
4 R.B. Dardas, J.H. Perry, Z.A. Coles and C.G. Iwanicki, Bull. Parenteral Drug Assoc., 27 (1973) 185.