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ANALYSIS OF CEFOXITIN, CEPHALOTHIN AND THEIR DEACYLATED METABOLITES IN HUMAN URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Cefoxitin, cephalothin and their deacylated metabolites, decarbamylcefoxitin and deacetylcephalothin, have been quantitatively analyzed in whole human urine using high-performance anion-exchange liquid chromatography with UV detection. The rate of excretion and extent of deacylation for the two compounds were determined after intravenous injection with or without probenecid and after intramuscular injection. Recoveries of intact cefoxitin were considerably higher than those of cephalothin in all the cases studied, owing to the almost total resistance of cefoxitin to inactivation by deacylation. Cephalothin was found to be deacylated rapidly and to a relatively large extent.

INTRODUCTION

Cefoxitin is a semi-synthetic cephamycin antibiotic[1], characterized by the presence of a methoxyl group in the 7α-position of the 7β-aminocephalosporanic acid nucleus. Its structure and that of cephalothin are shown in Table I. The presence of the 7α-methoxyl group has been found to confer a high degree of resistance to destruction by bacterial cephalosporinases[2]. In a series of human clinical bioavailability studies comparing cefoxitin and cephalothin[3], it was noted that the urinary excretion of intact drug was considerably higher for cefoxitin than for cephalothin as measured by microbiological assay. It was considered desirable to develop an analytical method capable of detecting and determining the amount of deacylated metabolite in the presence of unchanged drug in urine.

This paper describes a high-performance anion-exchange liquid chromatographic (HPLC) analysis for cefoxitin, decarbamylcefoxitin, cephalothin and deacetylcephalothin in whole urine. The method is sensitive, reproducible and rapid. While this work was in progress, a similar analysis for cephalothin and deacetylcephalothin was published[4], using a different anion-exchange resin packing and elevated temperatures. The present procedure, which can be carried out at ambient temperatures, lessens the possibility of degradative effects on the column.

EXPERIMENTAL

Equipment

A Nester-Faust Model 1200-H liquid chromatograph (Perkin-Elmer) was used. A Valco loop injector with a volume of 74 μl was fitted to the inlet of a 316 stainless-steel column (100 $\times$ 0.3 cm) packed with Zipax SAX strong anion-exchange resin (DuPont, Wilmington, Del., U.S.A.) and operated at ambient temperature. The column effluent was monitored at 254 nm with a Nester-Faust Model 250 or a DuPont Model 410 UV detector. The chromatograms were recorded on a Honeywell Electronik 193 6-in. strip-chart recorder.

A second chromatographic system, consisting of a Milton Roy (Philadelphia, Pa., U.S.A.) mini-pump, a jacketed column fitted with a septum injector, and the same UV detection and recording system as above, was used for the flow-rate and temperature studies described later.

Materials

The eluent buffer used for chromatography was 0.25 *M* acetic acid (Merck, Rahway, N. J., U.S.A., reagent grade) in water adjusted to pH 5.0 with sodium hydroxide. Cefoxitin, decarbamylcefoxitin and deacetylcephalothin were prepared in the Merck Sharp & Dohme Research Laboratories. Cephalothin (Keflin; Lilly, Indianapolis, Ind., U.S.A.) was obtained from the supplier.

Urines were obtained from healthy subjects dosed with cefoxitin or cephalothin by intravenous infusion, either directly or after pre-infusion of probenecid, or intramuscular injection. Samples were collected before dosing and at intervals of 1, 2, 3, 4, 6 and 12 h. Separate aliquots were taken for microbiological assay* and liquid chromatographic analysis, and stored frozen.

Liquid chromatographic analysis procedure

The eluent buffer, 0.25 *M* sodium acetate at pH 5.0, was pumped through the column at a flow-rate of 0.82 ml/min (750 p.s.i.g.).

Stock solutions of the known compounds were prepared in the buffer and were used for the standard curves. Stability studies at 1 mg/ml showed that not more than 2–3% decomposition occurred in 3–4 days at refrigerator temperatures, compared with losses of 5–7% per day at room temperature. Degradation was manifested by a diminution in the height of the UV peak in the chromatogram. No UV absorption was detected at the elution volumes of the deacylated or lactonized products, indicating that the decomposition products either were not eluted from the column or that they no longer contained the chromophore characteristic of the intact molecule. Standard solutions were prepared at 3-day intervals and stored in a refrigerator. Standard curves were run before and after each series of urine analyses.

Urines were filtered, and diluted with buffer, if necessary, to an estimated concentration of 50–150 μg/ml based on bioassay data. Whole diluted urines were injected directly on to the column. Peak height measurement was used for calculating concentrations relative to the standard curves. Recoveries were calculated as a percentage of the administered dose in the urine.

* The microbiological assays were performed at the West Point, Pa., laboratories of the Merck Sharp & Dohme Research Laboratories under the supervision of Mrs. Helen Skeggs.

RESULTS AND DISCUSSION

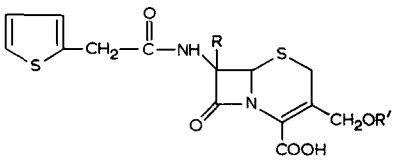
Chromatography of standards

The elution times and volumes, capacity factors and effective number of plates for cefoxitin, cephalothin, their deacylated analogs and the lactone of decarbamylcefoxitin using the analytical conditions outlined above are shown in Table I.

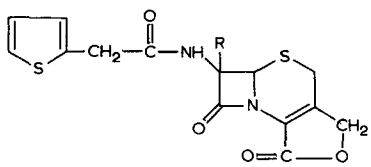
TABLE I

CAPACITY FACTORS (k') AND EFFECTIVE NUMBERS OF PLATES (r) PER 100 cm FOR STRONG ANION-EXCHANGE RESIN

Chromatographic conditions: column, 100 × 0.3 cm, Zipax SAX; eluent, 0.25 *M* sodium acetate buffer, pH 5.0; flow-rate, 0.82 ml/min (750 p.s.i.g.); sample charge, 74 μl via sample loop; detector, UV at 254 nm.



I–IV



V

No.	*Name*	*R*	*R'*	t_e	V_e	k'	r
I	Cefoxitin	CH_3O–	H_2N–C(=O)–	19.0	15.6	4.49	280
II	Decarbamylcefoxitin	CH_3O–	H–	16.3	13.4	3.72	310
III	Cephalothin	H–	CH_3–C(=O)–	33.0	27.1	8.55	290
IV	Deacetylcephalothin	H–	H–	21.5	17.7	5.90	306
V	Decarbamylcefoxitin lactone	CH_3O–	–	6.0	4.92	0.73	294

The equations for the calculations and the definitions of the terms in Table I are given below[5,6].

$$\text{Capacity factor, } k' = \frac{V_e - V_0}{V_0} \tag{1}$$

$$\text{Effective plates, } r = \left(\frac{V_e}{V_b}\right)^2 \cdot 16 \tag{2}$$

where

V_e = elution volume to UV peak (ml);
V_0 = elution volume for an unretarded solute (ml);
V_b = volume at the peak base measured between the intersections of tangents to the peak with baseline (ml);
t_e = elution time to the UV peak (min).

The lactone of decarbamylcefoxitin was essentially unretained ($k' = 0.73$) under these chromatographic conditions. Deacetylcephalothin lactone would be

expected to have similar properties although it was not investigated. It was therefore not possible to detect their presence in urine because of interfering UV absorption.

Standard curves

Stock solutions of the standards dissolved in the eluent buffer at concentrations of 2–500 μg/ml gave results that were linear to within $\pm$ 2%. As the sample charge was 74 μl, the amounts chromatographed covered the range from 0.148 to 37 μg. A standard curve for cefoxitin is shown in Fig. 1, as representative of the results obtained for the reference compounds.

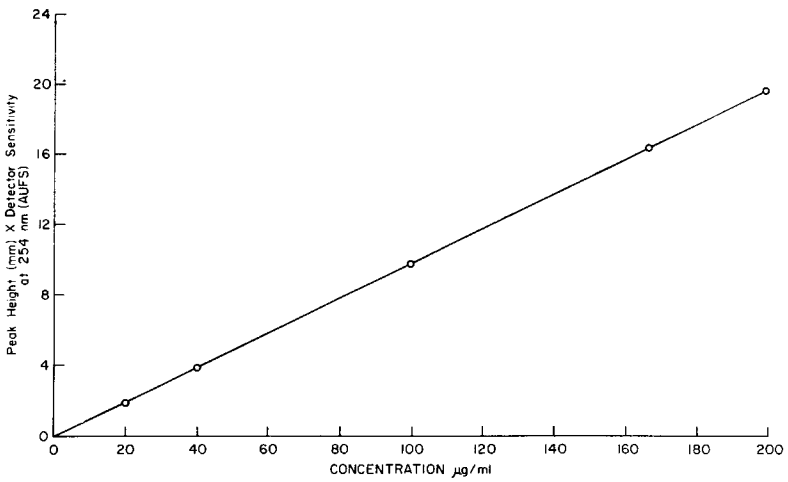


Fig. 1. Standard curve for cefoxitin. Conditions as in Table I.

Other chromatographic conditions were also investigated in addition to those used for the urine analyses. Using the apparatus equipped with the Milton Roy pump and the jacketed column, the effects of slower flow-rates and elevated temperatures on column performance were studied. The results for cefoxitin are shown in Table II. It was found that the number of effective plates (r) was significantly increased by a reduction in flow-rate and by increasing the column temperature, indicating that maxi-

TABLE II

EFFECT OF FLOW-RATE AND ELEVATED TEMPERATURE ON THE EFFECTIVE NUMBER OF PLATES (r) FOR CEFOXITIN

Column and eluent as in Table I. Sample charge, 5 μg in 10 μl of eluent solvent.

Temperature (°C)	*Flow-rate (ml/min)*	t_e *(min)*	V_e *(ml)*	r
24	0.76	22.6	17.6	224
24	0.10	162.0	16.2	465
50	0.76	15.5	11.7	272
60	0.76	13.0	9.9	282
60	0.456	22.0	10.0	430
60	0.098	100.0	9.8	658
60	0.056	138.0	7.8	727

mum resolution was being approached only at the highest temperature and the lowest flow-rates (less than 0.1 ml/min). Neither of these conditions was considered suitable for routine analyses, as the time required was excessive, and the column packing proved to be unstable at 60°, showing signs of deterioration in performance after 3 or 4 days (decrease in V_e and increased pressure drop). When the column was dismantled after such exposure to elevated temperatures, the packing was found to be partially sintered. The elution volume was not greatly changed by variation in flow-rate at a given temperature, except for the last example at 60° in Table II, which was attributed to column changes.

The separation of cefoxitin and decarbamylcefoxitin at room temperature (24°) using a flow-rate of 0.10 ml/min was almost complete owing to the enhanced resolution. The k' values were 4.71 and 4.0, respectively ($\alpha = 4.71/4.0 = 1.18$). These conditions were employed in the analysis of a typical 0–1-h urine (subject 3, Table III; 2.0-g dose) for the presence of decarbamylcefoxitin. The amount detected was less than 1% of the dose.

Urine analysis

The results of HPLC analyses of 86 urines from cefoxitin- and cephalothin-dosed subjects, together with the bioassay values, are given in Tables III and IV. In Fig. 2, some examples of chromatograms for urine are shown. The generally good correlation between the two methods of measurement for intact drug was in agreement with the observations that urine gave no significant interference in the HPLC analysis, and that the deacylated metabolites were essentially devoid of activity in the bioassay. The occasional discrepancies encountered were found to be due to real differences in the separate aliquots used for measurement. Some possible causes of these differences could be variations in sample handling, such as exposure to relatively high temperatures during thawing, or higher urinary pH in some samples. Both of these conditions have been found to accelerate decomposition. The reproducibility of the HPLC assay was established with a series of thirteen replicate determinations on a single sample. The standard deviation for the series was 1.2%. This consistency was attributable to the extremely constant flow-rate delivered by the Nester-Faust screw-drive pumps and the use of loop injection, which is not subject to the variability of syringe injection. There was therefore no need for internal standards.

Both drugs were most rapidly excreted after direct intravenous infusion. With one exception (subject 2, Table III; 0.5-g dose), the largest fraction was found in the first hour. Pre-infusion of probenecid, or dosage by the intramuscular route, repressed the excretion significantly, thus prolonging the residence time in the subjects.

Cefoxitin was found to be only slightly metabolized to decarbamylcefoxitin under any of the conditions of dosing that were studied. Deacetylation of cephalothin, on the other hand, was rapid, and consequently relatively large amounts of deacetylcephalothin were seen even during the first hour after dosing. In Table V, comparisons of the total recoveries of the two drugs for the dose levels and methods of administration investigated are shown. Recoveries of intact cefoxitin were consistently considerably higher than those for cephalothin. Pre-infusion of probenecid or intramuscular injection resulted in increases in the amounts of deacylated metabolites in the urine, presumably as a result of the slower excretion rates. Combined recoveries were lower for cefoxitin after intramuscular injection.

TABLE III

HPLC ANALYSIS OF CEFOXITIN AND DECARBAMYLCEFOXITIN IN URINES

Conditions as in Table I. Numbers in parentheses are bioassay values.

Subject No.	*Dose*	*Cefoxitin (% of dose)*						*Decarbamylcefoxitin (% of dose)*					
		0–1 h	*1–2 h*	*2–3 h*	*3–4 h*	*4–12 h*	*Total*	*0–1 h*	*1–2 h*	*2–3 h*	*3–4 h*	*4–12 h*	*Total*
1	0.5 g intravenous, 3 min infusion	66 (64)	15.2 (15.8)	3.9 (3.8)	2.5 (1.7)	—	87.6 (85.3)	0.4	1.1	1.2	1.4	—	4.1
2		12.5 (11.6)	59 (63)	2.6 (3.6)	1.4 (1.6)	—	75.5 (79.8)	<0.1	1.1	1.2	1.2	—	3.5
3		57 (56)	8.4 (11.0)	4.8 (4.6)	1.9 (1.6)	—	72.4 (73.2)	1.0	2.5	1.1	1.4	—	6.0
							Mean ± S.E.: 78.5 ± 4.6 (79.4 ± 3.5)						Mean ± S.E.: 4.5 ± 0.75
1	1.0 g intravenous, 3 min infusion	72 (70)	11.4 (10.9)	4.0 (3.7)	1.7 (1.6)	—	89.1 (86.2)	0.2	0.4	0.8	0.5	—	1.9
2		46 (43)	12.6 (12.5)	3.2 (3.6)	Sample missing	2.3 (2.9)	61.8 (59.1)	<0.1	<0.1	0.1	Sample missing	0.6	0.7
3		63 (60)	10.9 (16.4)	3.8 (3.5)	1.7 (1.6)	—	79.4 (75.5)	0.5	0.5	1.1	1.4	—	3.5
4		60 (50)	—	—	2.2 (1.4)	—	—	1.0	—	—	1.9	—	—
							Mean ± S.E.: 76.8 ± 8.0 (73.6 ± 7.9)						Mean ± S.E.: 2.0 ± 0.81

1	2.0 g intravenous, 3 min infusion	63 (68)	16.3 (15)	4.7 (4.4)	1.8 (1.7)	—	85.8 (89.1)	<0.1	0.2	0.4	0.5	—	1.2
2		57 (46)	15.4 (15.8)	4.9 (5.1)	2.1 (2.1)	—	79.4 (69.0)	0.4	0.6	0.5	0.5	—	2.0
3		66 (49)	14.6 (11.8)	5.2 (4.8)	2.1 (1.7)	—	87.9 (67.3)	0.7	0.2	0.7	0.6	—	2.2
5		69 (56)	14.4 (10.6)	5.0 (4.3)	2.2 (1.8)	—	90.6 (72.7)	<0.1	0.3	0.3	0.3	—	0.9
							Mean ± S.E.: 85.9 ± 4.77 (74.5 ± 5.0)						Mean ± S.E.: 1.6 ± 0.31
6	1.0 g intravenous, 3 min infusion, after 1 h pre-infusion of 1.0 g probenecid	37 (33)	15.8 (14.9)	12.5 (9.9)	4.7 (5.0)	8.5 (7.3)	78.5 (70.1)	1.1	0.5	0.8	0.6	4.4	7.4
7		32 (28)	15.6 (6.2)	12.8 (10.4)	6.0 (5.9)	13.8 (13.3)	80.2 (63.8)	<0.1	<0.1	<0.1	<0.1	2.2	2.2
							Mean ± S.E.: 79.4 ± 0.9 (66.9 ± 3.2)						Mean ± S.E.: 4.8 ± 2.6
8	0.5 g intramuscular	34 (34)	22.6 (21.7)	11.0 (10.3)	5.2 (5.2)	—	71.3 (71.2)	0.5	1.6	1.9	1.7	—	5.7
9		26 (30)	20.2 (25.2)	9.2 (9.1)	3.9 (2.1)	—	59.3 (63.4)	0.2	0.2	0.2	0.2	—	0.8
							Mean ± S.E.: 65.3 ± 6.0 (67.3 ± 3.9)						Mean ± S.E.: 3.3 ± 2.5
8	1.0 g intramuscular	31 (32)	20.9 (21.0)	8.5 (9.7)	5.4 (4.8)	—	65.8 (67.5)	<0.1	<0.1	0.2	0.2	—	0.4
9		48 (49)	19.1 (18.3)	6.4 (6.3)	2.9 (3.0)	—	76.4 (76.6)	0.6	0.4	0.2	0.2	—	1.4
							Mean ± S.E.: 71.1 ± 5.3 (72.1 ± 4.6)						Mean ± S.E.: 0.9 ± 0.5

TABLE IV

HPLC ANALYSIS OF CEPHALOTHIN AND DEACETYLCEPHALOTHIN IN URINES

Conditions as in Table I. Numbers in parentheses are bioassay values.

Subject No.	*Dose*	*Cephalothin (% of dose)*						*Deacetylcephalothin (% of dose)*					
		0–1 h	*1–2 h*	*2–3 h*	*3–4 h*	*4–12 h*	*Total*	*0–1 h*	*1–2 h*	*2–3 h*	*3–4 h*	*4–12 h*	*Total*
10	1.0 g intravenous, 3 min infusion	39.5 (37.0)	2.3 (3.1)	0.9 (0.8)	3.8 (3.6)	—	46.5 (44.5)	13.8	3.5	1.0	4.0	—	22.3
11		48.2 (48.3)	2.8 (2.6)	0.3 (0.7)	0.3 (0.4)	—	51.6 (52.0)	17.3	4.5	0.7	0.5	—	23.0
							Mean ± S.E.: 49.1 ± 2.6 (48.3 ± 3.8)						Mean ± S.E.: 22.7 ± 0.4
10	1.0 g intravenous, 3 min infusion, after 1 h pre-infusion of 1.0 g probenecid	30.6 (28.7)	8.2 (9.8)	1.7 (3.0)	0.9 (1.3)	—	41.4 (42.8)	13.5	9.2	2.6	1.6	—	26.9
11		31.9 (35.7)	8.1 (8.3)	3.3 (4.4)	1.0 (1.5)	—	44.3 (50.0)	13.5	9.4	5.2	2.5	—	30.6
							Mean ± S.E.: 42.9 ± 1.5 (46.4 ± 3.6)						Mean ± S.E.: 28.8 ± 1.9
12	1.0 g intramuscular	35.4 (35.3)	11.9 (9.6)	2.9 (2.8)	1.6 (1.9)	—	51.5 (49.6)	15.2	10.6	3.8	1.8	—	31.4

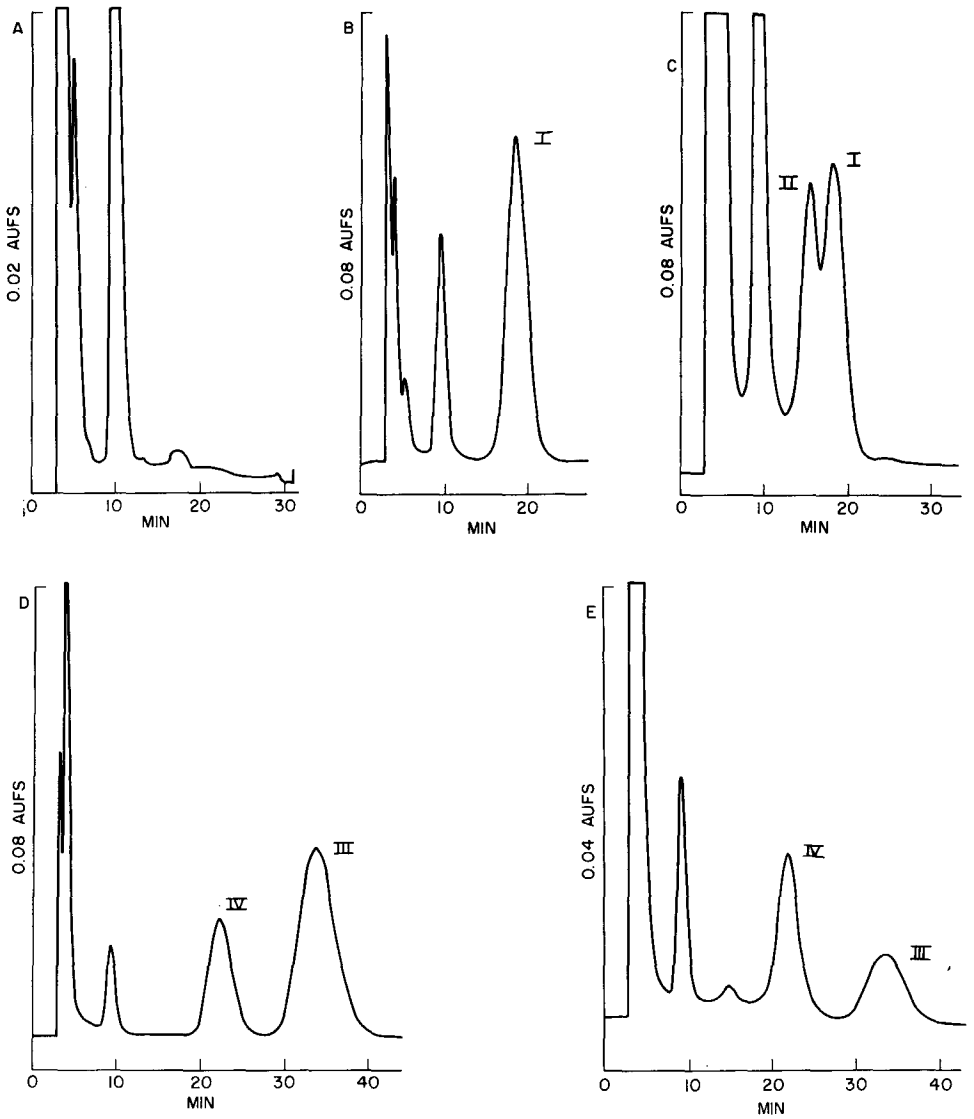


Fig. 2. High-performance liquid chromatograms of urines. Conditions as in Table I. Detector sensitivity indicated on chromatograms. Details for samples are given below:

	I.v. dose (g)	*Time after dose (h)*	*Dilution*	*% of dose*			
				Cefoxitin (I)	*Decarbamyl-cefoxitin (II)*	*Cephalothin (III)*	*Deacetyl-cephalothin (IV)*
A	Pre-drug	—	1:25	—	—	—	—
B	1.0	0–1	1:50	60	1.0	—	—
C	1.0	3–4	1:5	2.2	1.9	—	—
D	1.0	0–1	1:10	—	—	31.9	13.5
E	1.0	2–3	1:10	—	—	3.3	5.2

TABLE V

TOTAL RECOVERY OF CEFOXITIN, CEPHALOTHIN AND THEIR DEACYLATED METABOLITES IN URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS

Mean totals from Tables III and IV.

Compound	*Method of dose*	*Dose (g)*	*Time after dose (h)*	*% of dose*		
				Intact drug	*Deacylated metabolite*	*Combined totals*
Cefoxitin	Direct i.v.	0.5	0–4	78.5	2.7	81.2
		1.0	0–4*	76.8	1.7	78.5
		2.0	0–4	85.9	0.5	86.4
	I.v. after probenecid	1.0	0–12	79.4	3.6	83.0
	Intramuscular	0.5	0–4	65.3	3.6	68.9
	Intramuscular	1.0	0–4	71.1	0.2	71.3
Cephalothin	Direct i.v.	1.0	0–4	49.1	22.7	71.8
	I.v. after probenecid	1.0	0–4	42.9	28.8	71.7
	Intramuscular	1.0	0–4	51.5	31.4	82.9

* Includes 4–12-h urine, but not 3–4-h for subject 2, Table III.

CONCLUSIONS

A high-performance liquid chromatographic analysis has been developed for the determination of cefoxitin, cephalothin and their deacylated metabolites in whole human urine. The method is simple, sensitive and reproducible. It has certain advantages over microbiological assays, such as speed and specificity, and is capable of measuring total recovery of drug plus metabolite, which is not feasible with microbiological assay procedures. It could prove to be a useful method for clinical applications.

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