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Note

High-performance liquid chromatographic method for the determination of cefpimizole in tissue

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A high-performance liquid chromatographic (HPLC) method has been developed for the quantitative determination of cefpimizole, a third-generation cephalosporin, in tissue samples. The sample preparation procedure employs tissue homogenization and Waters C_{18} Sep-Pak® clean-up prior to HPLC analysis. The method has the necessary precision, accuracy and sensitivity to provide quantitative data. The lower level of assay detection sensitivity is approximately 30 μg cefpimizole per 250 mg tissue homogenate or 120 μg/g.

The method was utilized to determine cefpimizole levels in rat tissues collected from a study designed to evaluate the potential for cefpimizole kidney tissue accumulation. Cefpimizole has been shown to cause renal tubular degeneration in the rat [1]. Nephrotoxicity for a number of other cephalosporins is well documented in man [2].

EXPERIMENTAL

Apparatus

The isocratic HPLC system consisted of a ConstaMetric III pump and a SpectroMonitor III UV detector operated at 254 nm (Laboratory Data Control, Riviera Beach, FL, U.S.A.), a Rheodyne Model 7126 variable-loop injector with a 50-μl loop (Rheodyne, Cotati, CA, U.S.A.) mounted on an Upjohn autoinjector operated at 4°C, and a Linear Model 585 dual-pen recorder (Linear Instrument, Irvine, CA, U.S.A.). The column was an IBM octadecyl (C_{18}), 5-μm, 250 × 4.5 mm I.D. column (IBM Instruments, Danbury, CT, U.S.A.) fitted with a Co:Pell ODS, 35-μm, 50 × 2.1 mm I.D. guard column (Whatman,

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Clifton, NJ, U.S.A.). Peak-height data collection was by computer (Harris, Fort Lauderdale, FL, U.S.A.) using an in-house program.

Chemicals and reagents

Cefpimizole was supplied by The Upjohn Company (Kalamazoo, MI, U.S.A.). A stock solution was prepared by dissolving 10 mg of cefpimizole in 10 ml of water. The stock was stored at −20°C when not in use. Acetophenone was obtained from Aldrich (Milwaukee, WI, U.S.A.) and was diluted with water to prepare a 200 μg/ml stock solution. Tetrabutylammonium hydroxide (TBA), 0.4 *M* titrant grade, was obtained from Eastman-Kodak (Rochester, NY, U.S.A.). Ethylenediaminetetraacetic acid (EDTA) was obtained from Aldrich.

HPLC parameters

The HPLC eluent consisted of methanol—water (33:67) containing 0.001 *M* EDTA, 0.005 *M* TBA, adjusted to pH 6.5 with acetic acid. The prepared eluent was filtered through a 0.2-μm filter (Rainin Instrument, Woburn, MA, U.S.A.) and helium-degassed. The flow-rate was 1 ml/min.

Extraction procedure

A weighed tissue sample from each drug-treated rat was homogenized with 2.5 ml of water and 0.5 ml of 0.1 *M* EDTA solution in a small tissue grinder (Ace Glass, Vineland, NJ, U.S.A.). Blank rat tissues fortified with cefpimizole and homogenized were assayed to determine recovery. The homogenate was centrifuged and duplicate 100-μl aliquots of the supernatant were added to Waters C_{18} Sep-Pak cartridges pretreated with 3 ml acetonitrile, and then with 3 ml of 0.01 *M* EDTA and 0.05 *M* TBA in water (pH 5). The cartridges were washed with 3 ml of water and then with 2 ml acetonitrile–water (10:90), and cefpimizole was eluted with 2 ml acetonitrile—water (30:70). Acetophenone (10 μg) as a calculation standard was added to the eluate. The eluate was mixed and filtered through an Acrodisc® CR 0.45-μm filter (Gelman Filtration Products, Ann Arbor, MI, U.S.A.) into an autoinjector vial for HPLC analysis. The prepared samples were stored at 4°C to improve stability until analysis.

Protocol design

Rats received 1000 mg/kg cefpimizole intravenously daily for up to fourteen days. Groups of three experimental rats and control rats were necropsied 24 h after dosing on days 1, 7 and 14. Approximately 250 mg of rat liver, spleen, and kidney medulla and cortex were collected in sample vials containing 0.5 ml of 0.1 *M* EDTA as preservative. Tissues were stored at −30°C until analysis.

RESULTS AND DISCUSSION

HPLC methods for the analysis of plasma and urine concentrations of cefpimizole were described earlier [3]. The reported plasma linearity was between 0.33 and 17 μg/ml, and the detection limit was 0.05 μg/ml of plasma. The stability of cefpimizole at room temperature in EDTA-stabilized plasma reached 80% of the initial concentration (t_0) at 48 h, but at −30°C was greater

than 95% t_0 for 100 days. Plasma and urine concentrations of cefpimizole were determined by the relative weight response calculation method. This tissue method employs a unique extraction system while using similar HPLC parameters and calculation methods as the earlier report.

The linearity and recovery of the tissue method is reported in Table I. Control tissues were homogenized and centrifuged. Cefpimizole and 100-μl aliquots of the tissue supernatant were added to Sep-Pak C_{18} cartridges. Unfortified tissue aliquots were also assayed. The fortification range was 1.67—41.95 μg per 100-μl aliquot. Chromatographic interferences were not detected in the blank tissue homogenates. Recoveries varied according to tissue type and were probably an indication of the stability of cefpimizole in that tissue. Recoveries ranged from 81.3% in kidney cortex to 97.1% in liver, with the relative standard deviation of the mean recoveries being less than 5.4%. The lower level of assay sensitivity was 1 μg per 100-μl aliquot or 30 μg per tissue homogenate. The sensitivity can easily be increased ten-fold by assaying 1 ml of the tissue homogenate. The chromatography does not change with increased volumes assayed.

Good precision of the tissue method was observed by the similarity in the detected cefpimizole levels in the duplicate samples analyzed (Tables II and

TABLE I

LINEARITY AND RECOVERY OF CEFPIMIZOLE-FORTIFIED ALIQUOTS OF RAT TISSUE HOMOGENATES FROM WATERS C_{18} SEP-PAK CARTRIDGES

N.D. = not detectable, less than 1 μg per 100-μl aliquot.

Tissue type (100-μl aliquot)	Added (μg per cartridge)	Found (μg per cartridge)	Recovery (%)	Mean recovery ± S.D. (R.S.D.) (%)
Liver	0	N.D.		97.1 ± 3.2
	1.67	1.62	97.0	(0.033)
	4.19	4.21	100.5	
	8.39	8.23	98.1	
	25.17	23.35	92.8	
Spleen	0	N.D.		93.8 ± 4.8
	4.19	3.66	87.4	(0.051)
	8 39	7.83	93.3	
	20.97	20.11	95.9	
	41 95	41.41	98.7	
Kidney cortex	0	N.D.		81.3 ± 4.4
	1.67	1.30	77.8	(0.054)
	4.19	3.48	83.1	
	8.39	6.50	77.5	
	20.97	18.18	86.7	
Kidney medulla	0	N.D.		90.7 ± 3.7
	1.67	1.44	86.2	(0.041)
	4.19	3.79	90.5	
	8.39	7.62	90.8	
	20.97	19.99	95.3	

TABLE II

RECOVERY OF CEFPIMIZOLE FROM WHOLE RAT TISSUE HOMOGENATES

Tissue type	Added (μg/ml)	Found (μg/ml)	Recovery factor	Mean recovery factor ± S.D.
Liver	28.0	25.0	0.893	0.905 ± 0 015
	28.0	25.1	0.896	
	55.9	50.4	0.902	
	55.9	51.8	0.927	
Spleen	28.0	23.8	0.850	0.836 ± 0.016
	28.0	23.8	0.850	
	55.9	45.8	0.819	
	55.9	46.1	0.825	
Kidney cortex	28.0	20.2	0.721	0.724 ± 0.014
	28.0	20.7	0.739	
	55.9	39.4	0.705	
	55.9	39.7	0.710	
	139.8	101.9	0.720	
	139.8	103.3	0.739	
Kidney medulla	28.0	22.7	0.811	0.848 ± 0.039
	28.0	22.5	0.804	
	55.9	46.8	0 837	
	55.9	47.4	0.848	
	139.8	124.5	0.891	
	139.8	125.3	0.896	

TABLE III

CONCENTRATIONS OF CEFPIMIZOLE IN DRUG-TREATED RAT KIDNEY CORTEX AND MEDULLA

N D = not detectable, less than 30 μg per tissue homogenate

Rat No	Study day*	Kidney cortex tissue weight (mg)	Concentration (μg/mg)		Concentration Mean ± S.D (μg/mg)	Kidney medulla tissue weight (mg)	Concentration (μg/mg)		Concentration Mean ± S.D (μg/mg)
			A	B			A	B	
1	2	283	0 95	0 97	2 09 ± 1 19	149	1 88	1 87	5 12 ± 3 98
2	2	499	1 99	1 99		250	3 93	3 91	
3	2	481	3 37	3 29		233	9 54	9 59	
4	8	389	3 34	3 32	3 29 ± 0 11	169	0 87	0.88	0 87 ± 0 25
5	8	433	3 35	3 41		194	1 11	1.12	
6	8	423	3 20	3 14		161	0 62	0 61	
7	15	523	1 81	1 82	1 81 ± 0 25	343	0 48	0 48	0 73 ± 0 22
8	15	380	2 07	2 03		230	0 82	0 81	
9	15	386	1 56	1 55		245	0 90	0 91	
Control	15	228	N D	N D		145	N D.	N D.	

*Tissues excised 24 h after dosing on days 1, 7 and 14

III). Control rat tissues fortified and homogenized with cefpimizole and extracted by the Sep-Pak method showed good recovery of the drug (Table II). A recovery factor was determined from the mean recovery of cefpimizole from fortified tissue homogenates. This value was used to calculate cefpimizole concentrations in drug-treated rat tissues. The volume contributed by the tissue

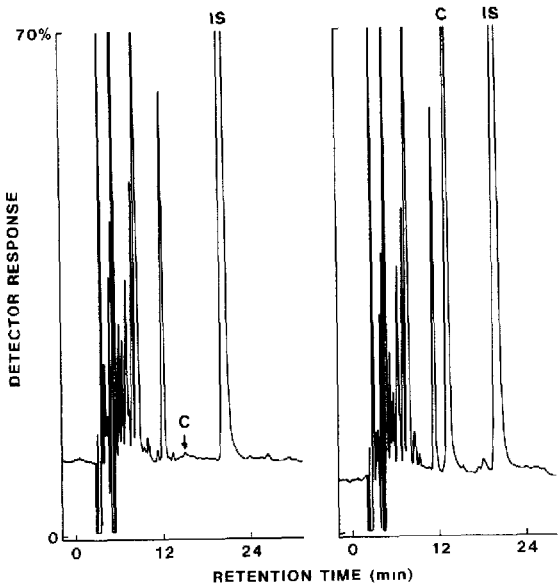

Fig. 1. Chromatograms of control and drug-treated rat kidney medulla. Chromatographic conditions are as given in Experimental. Detector, 254 nm, 0.064 a.u.f.s. Recorder, 10 mV. Peaks: C = cefpimizole; IS = internal standard, acetophenone. Drug-treated rat contains 7.9 μg cefpimizole per 100-μl aliquot of homogenate.

sample was not included in the total volume of the homogenate, which would cause the recovery factor to be less than 1.0.

Cefpimizole concentrations were not detectable in rat liver and spleen tissues (less than 30 μg per tissue homogenate) after multiple dosing with drug. Significant concentrations of cefpimizole were found in rat kidney cortex and kidney medulla (Table III). Kidney cortex concentrations averaged 2.09 ± 1.19 μg/mg of tissue after the first-day dose, 3.29 ± 0.11 μg/mg after the seventh-day dose and 1.81 ± 0.25 μg/mg after the fourteenth-day dose. Kidney medulla concentrations averaged 5.12 ± 2.98 μg/mg of tissue after the first-day dose, 0.87 ± 0.25 μg/mg after the seventh-day dose and 0.73 ± 0.22 μg/mg after the fourteenth-day dose. High standard deviations for some of the average tissue concentrations are indicative of a large variation in the tissue cefpimizole concentrations between rats. Fig. 1 presents typical chromatograms of control and drug-treated kidney medulla extracts. The data indicate that no apparent higher accumulation of cefpimizole occurred in the rat kidney cortex or medulla after repeated dosing. However, significant concentrations of drug are found in the kidney and not in comparator tissues.

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