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Short Communication

Sensitive high-performance liquid chromatographic assay for praziquantel in plasma, urine and liver homogenates

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ABSTRACT

A high-performance liquid chromatographic method for the determination of praziquantel in plasma, urine and rat liver homogenates has been developed. It requires 2 ml of biological fluid, an extraction using Sep-Pak cartridges, a 0.05 *M* phosphate buffer solution (pH 5.0) for equilibrating and washing and ethyl acetate–diisopropyl ether for drug elution. The analysis was performed on an Ultrasphere ODS C_{18} column with a mobile phase of acetonitrile–water with ultraviolet detection at 217 nm. The results showed that the assay is sensitive (31.2 ng/ml), linear between 0.125 and 4.0 μg/ml, precise (coefficient of variation = 10%) and selective with other drugs currently administered with praziquantel.

INTRODUCTION

Praziquantel (PZQ) has become the drug of choice in the treatment of schistosomiasis, most tissue flukes [1–3] and tapeworm infections [4–6] and it has proved of high efficacy in the treatment of brain cysticercosis [7,8]. Praziquantel is relatively non-toxic, well tolerated and can be given orally in single or multiple daily doses [9]. Despite extensive experimental studies in animals, the information about its pharmacokinetics and plasma levels in humans is limited [10–13]. There are some assays available for its measurement that include radiometric [10], fluorimetric [14], gas chromatographic [15], column liquid chromatographic [13,16] and bioassay [17] methods. The use of a radioactive drug is not suitable for routine clinical studies in patients and the other methods are limited by complex requirements for sample preparation or derivatization.

In this paper we describe a rapid and sensitive assay for PZQ determination using an analytical wavelength of 217 nm and the 2-cycloheptylcarbonyl analogue of PZQ as internal standard. This method was standardized for measurement of PZQ in plasma, urine and liver homogenates.

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EXPERIMENTAL

Chemicals and solutions

Praziquantel (2-cyclohexylcarbonyl-4-oxo-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinoline) and the internal standard (2-cycloheptylcarbonyl-4-oxo-1,2,3,6,7,11*b*-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinoline) were kindly donated by E. Merck (Darmstadt, Germany). Acetonitrile for the mobile phase was of chromatographic grade (J. T. Baker, Phillipsburg, NJ, USA). All other reagents were of analytical-reagent grade (J. T. Baker, Xalostoc, Mexico). The following aqueous solutions were prepared: 0.05 *M* phosphate buffer with potassium dihydrogenphosphate and potassium monohydrogenphosphate, pH adjusted to 5.0 with 0.8 *M* sodium hydroxide, and 0.2 *M* sodium hydroxide.

Chromatographic conditions

The instruments used were a Beckman (Fullerton, CA, USA) high-performance liquid chromatograph equipped with two solvent-delivery systems (Model 110B), an automatic gradient controller (Model 240), an injection valve fitted with 20-μl sampling loop, a variable-wavelength UV detector (Model 164) and a data module (Model 427). Extractions were made using Sep-Pak C_{18} cartridges (Waters–Millipore, Milford, MA, USA).

Analysis was performed on a Ultrasphere ODS C_{18} column (250 mm × 4.6 mm I.D., particle size 5 μm) (Beckman Instruments, San Ramon, CA, USA) with acetonitrile–water (50:50 for liver homogenate and 45:55 for plasma and urine) as the mobile phase. The column was kept at room temperature (20–24°C) and the flow-rate was kept constant at 1.5 ml/min. The absorbance at 217 nm was recorded at a sensitivity of 0.005 aufs.

Sample preparation

A 2-ml volume of either human plasma, human urine or rat liver homogenate (10%) was spiked with various amounts of PZQ that varied from 0.125 to 4.0 μg/ml. To the mobile phase, 100 μl of a solution of the 2-cycloheptyl analogue of PZQ (10 μg/ml) (internal standard) were added, then 1.0 ml of 0.2 *M* sodium hydroxide solution was added, shaken on a vortex mixer for 15 s and extracted by passing it through a Sep-Pak C_{18} cartridge. The cartridge was prepared by flushing with 5.0 ml of methanol followed by 5.0 ml of 0.05 *M* phosphate buffer (pH 5.0). After spiked samples had been passed through the cartridge it was washed with 20.0 ml of phosphate buffer (pH 5.0) and 8.0 ml of methanol. The compounds were then eluted twice with 3 ml of ethyl acetate–diisopropyl ether [70:30 (v/v) for plasma or urine samples and 30:70 (v/v) for liver homogenates]. The two fractions were evaporated to dryness in pointed glass tubes in a water-bath at 25°C under nitrogen and the residues were dissolved in 100 μl of the mobile phase. Aliquots of 20 μl were injected into the HPLC system.

RESULTS AND DISCUSSION

Chromatograms of plasma, urine and liver homogenate are shown in Fig. 1. The retention times for PZQ and the internal standard were 6.19 and 8.74 min for plasma, 6.14 and 8.66 min for urine and 4.88 and 6.54 min for homogenate, respectively. No interfering peaks occurred at these times. A linear relationship ($r = 0.9999$) was found when the ratio of the peak height of praziquantel in homogenate supernatant to the peak height of the internal standard was plotted against concentration of praziquantel in the range 0.125–4.0 μg/ml. The same relationship ($r = 0.9997$ and 0.9998) was obtained for plasma and urine.

The average recoveries of praziquantel, assessed by comparison of peak heights from the biological fluids with those from standard solutions, were 87.45% in plasma, 102.55% in urine and 86.02% in homogenate. The reproducibility of the assay was determined by replicate analyses of spiked samples. Table I shows that the maximum within-day coefficient of variation (C.V.) was 8.83% at 1.0 μg/ml for plasma, 9.08% at 0.25 μg/ml for urine and 10% at 0.5 μg/ml for homogenate. It was found that samples were stable for at least four weeks when stored at −4°C. The

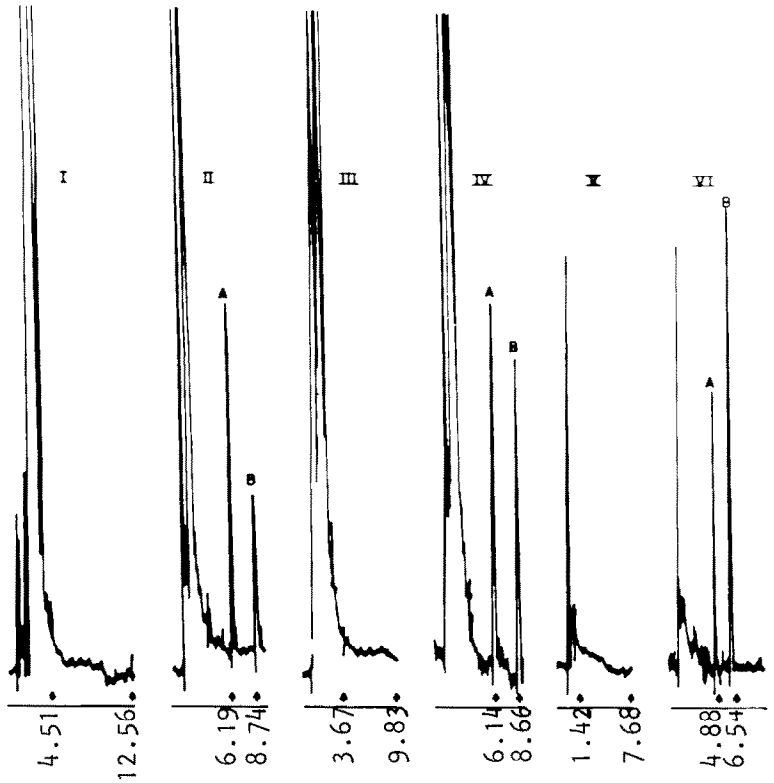

Fig. 1. Chromatograms of (I) plasma blank, (II) spiked plasma containing (A) 0.5 μg/ml praziquantel and (B) 0.5 μg/ml internal standard, (III) urine blank, (IV) spiked urine containing (A) 0.25 μg/ml praziquantel and (B) 0.5 μg/ml internal standard, (V) liver homogenate blank and (VI) liver homogenate containing (A) 0.125 μg/ml praziquantel and (B) 0.5 μg/ml internal standard. Time in minutes.

TABLE I

PRECISION AND ACCURACY FOR PLASMA, URINE AND TISSUE HOMOGENATE SAMPLES SPIKED WITH PRAZIQUANTEL (n = 6)

P = plasma sample; U = urine sample; H = homogenate sample.

Amount added (μg/ml)	Amount found (μg/ml)			Accuracy (%)			C.V. (%)		
	P	U	H	P	U	H	P	U	H
0.125	0.108	0.115	0.090	86.90	92.72	72.32	7.23	7.12	5.75
0.250	0.221	0.282	0.206	88.44	113.14	82.66	6.92	9.08	9.09
0.500	0.413	0.507	0.487	82.64	101.54	97.48	7.95	4.52	10.00
1.0	0.880	0.998	0.855	88.08	99.88	85.51	8.83	4.03	5.39
2.0	1.749	2.130	1.728	87.46	106.51	86.43	6.08	5.89	1.58
4.0	3.645	4.060	3.667	91.13	101.50	91.69	5.04	3.70	7.36
Average				87.45	102.55	86.02	7.0	5.72	6.53
C.V. (%)				3.17	6.67	9.9			

detection limit (signal-to-noise ratio = 2) was 31.2 ng/ml for praziquantel in all the biological samples.

The method is being used for the determination of PZQ in plasma and urine in patients with brain cysticercosis. Fig. 2 shows a typical chromatogram from a male patient following the administration of praziquantel at 50 mg/kg per day for fifteen days. Praziquantel levels at the steady state were 1.764 μg/ml in plasma and 0.4162 μg/ml in urine. There was no chromatographic interference from any endogenous compounds or from the drugs used in clinical practice (carbamazepine, dexamethasone). Although PZQ is metabolized extensively to hydroxylated derivatives [10,18], more polar metabolites should elute before the parent compound or remain in the aqueous phase [17]. There was no evidence for other substances overlaid in the PZQ region when samples from patients were analysed with a Waters Model 991 photodiode-array detector equipped with a Model 5200 printer plotter and an NEC Power Mate SX Plus computer with software version 5 (Waters–Millipore).

This HPLC method is simple, sensitive and fast (thirty samples can be analysed in one day). It can also be used as a reliable assay in the study of the biopharmaceutics and pharmacokinetics

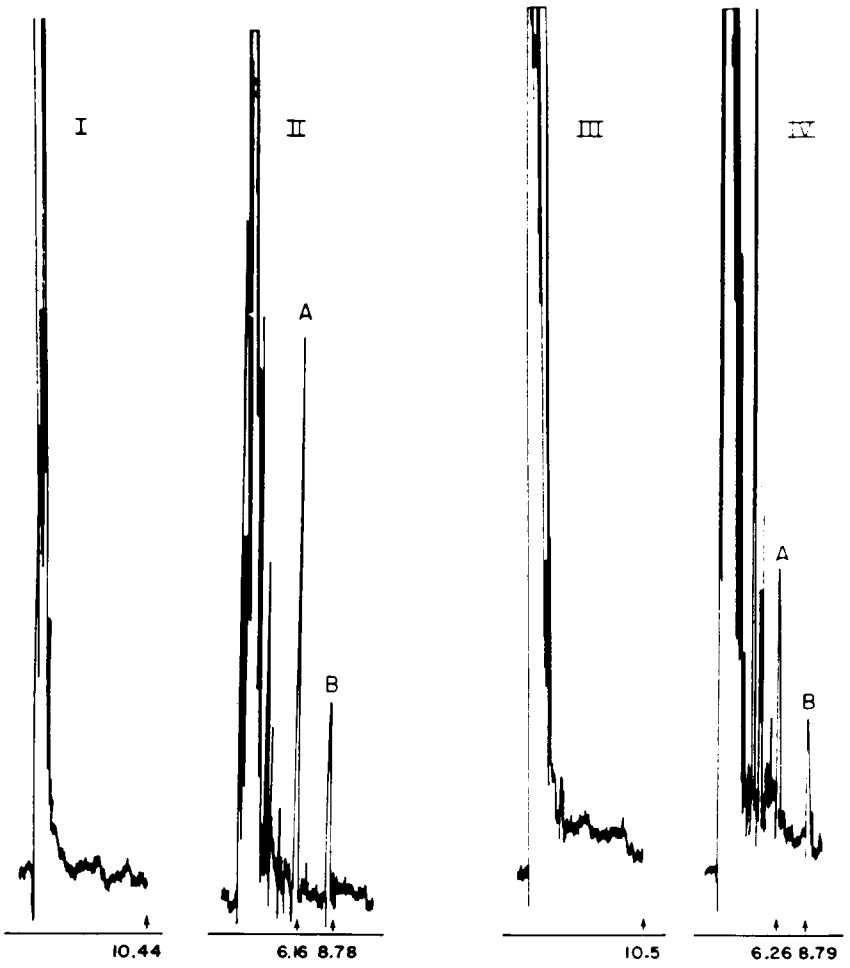

Fig. 2. Chromatograms of predose plasma and urine samples (I and III) from a male patient with brain cysticercosis and plasma and urine samples from the same patient receiving daily 50 mg/kg praziquantel (II and IV) (PZQ concentrations 1.764 and 0.4162 μg/ml, respectively). Peak A corresponds to PZQ and peak B to the internal standard. Time in minutes.

of praziquantel. The method has also been applied for *in vitro* metabolism studies of PZQ.

REFERENCES

1 *Med. Lett. Drugs Ther.*, 30 (1988) 15.
2 A. H. S. Omer, *Arzneim.-Forsch.*, 31 (1981) 605.
3 B. C. Yangco, C. De Lerma, G. H. Lyman and D. L. Price, *Antimicrob. Agents Chemother.*, 31 (1987) 135.
4 B. G. Weniger and P. M. Schantz, *J. Am. Med. Assoc.*, 251 (1984) 2391.
5 E. Groll, *Acta Trop.*, 37 (1980) 293.
6 H. Schenone, *Am. J. Trop. Med. Hyg.*, 29 (1980) 320.
7 J. Sotelo, F. Escobedo, J. Rodríguiz-Carbajal, B. Torres and F. Rubio-Donnadieu, *N. Engl. J. Med.*, 310 (1984) 1001.
8 J. Sotelo, B. Rorres, F. Rubio-Donnadieu, F. Escobedo and J. Rodríguez-Carbajal, *Neurology*, 35 (1985) 752.
9 H. Frohberg and M. S. Schenching, *Arzneim.-Forsch.*, 31 (1981) 555.
10 K. Patzschke, J. Putter, L. A. Wegner, F. A. Horster and H. W. Diekman, *Eur. J. Drug Metab. Pharmacokin.*, 3 (1979) 149.
11 A. Spina-Franca, L. R. Machado, J. P. S. Nobrega, J. A. Livramento, H. W. Diekmann, E. Groll and G. L. de Rezende, *Arq. Neuro-Psiquiatr.*, 43 (1985) 243.
12 H. Jung, M. L. Vásquez, M. Sánchez, P. Penagos and J. Sotelo, *Proc. West. Pharmacol. Soc.*, 34 (1991) 335.
13 M. El M. Mandour, H. El Turabi, M. M. A. Homeida, T. El Sadig, H. M. Ali, J. L. Bennet, W. J. Leahey and D. W. G. Harron, *Trans. R. Soc. Trop. Med. Hyg.*, 84 (1990) 389.
14 L. A. Pütter, *Eur. J. Drug Metab. Pharmacokin.*, 3 (1979) 143.
15 H. W. Diekmann, *Eur. J. Drug Metab. Pharmacokin.*, 3 (1979) 139.
16 S.-H. Xiao, B. Catto and L. Webster, *J. Chromatogr.*, 275 (1983) 133.
17 P. Andrews, *Vet. Med. Rev.*, 2 (1976) 154.
18 M. H. Ali, D. D. Fetterolf, F. P. Abramson and V. H. Cohn, *Biomed. Environ. Mass Spectrom.*, 19 (1990) 179.