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Short communication

Enantioselective analysis of praziquantel in plasma samples

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Abstract

A direct enantioselective high-performance liquid chromatography method is described for the quantitative determination of praziquantel enantiomers in plasma samples. The method involves two-step extraction of plasma with toluene, evaporation of the solvent and chromatography on a Chiralcel OD-H column using hexane–ethanol (85:15, v/v) as the mobile phase and detection at 220 nm. The assay satisfies all of the criteria required for use in clinical pharmacokinetic studies. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Praziquantel

1. Introduction

Schistosomiasis, a disease caused by three species of the trematode *Schistosoma* (*mansoni*, *japonicum* and *haematobium*), is a serious public health problem in tropical countries where it affects approximately 200 million people. In Brazil, infection with *Schistosoma mansoni* involves more than 10 million people and is endemic from the state of Rio Grande do Norte to north of Paraná [1].

Praziquantel [2-(cyclohexylcarbonyl)-1,2,3,6,7-11b-hexahydro-4H-pyrazino[2,1-a] isoquinolin-4-one, PZQ] is the drug of choice for the treatment of this endemic disease because it is administered orally, it is effective in single dose schedules for the three major *Schistosoma* species, because it is of low toxicity, which makes it well tolerated by patients, and finally because there are indications that treatment with this drug confers a certain resistance to reinfection. The drug also reduces the mortality associated with periportal fibrosis and other complications [2–5].

PZQ is a chiral compound (Fig. 1) marketed as racemate, although its *S*-(+)-enantiomer has no proven pharmacological activity [6–9]. Westhoff and Blaschke [10] observed higher plasma concentrations

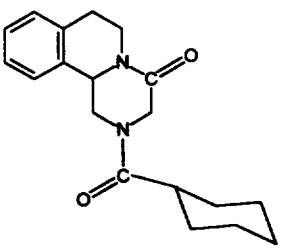

Fig. 1. Structure of praziquantel.

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of *S*-(+)-PZQ in humans, i.e., of the enantiomer with no pharmacological activity, and of the active metabolite *R*-(−)-*trans*-4-hydroxypraziquantel [7], indicating a possible stereoselective presystematic metabolism.

In the study by Westhoff and Blaschke [10], the enantiomers were separated using a Chiralcel OD column. The authors reported quantitation limits of 5 ng/ml and the method was used for analysis of PZQ and its monohydroxylated metabolites in samples from volunteers treated with a single dose of PZQ. The procedure for sample preparation included a previous separation of the compounds of interest on a reversed-phase column, fraction collection, solvent evaporation and later analysis on the chiral column.

More recently, the same column was used by Kelly et al. [11] to develop a method for analysis of PZQ enantiomers in serum using solid-phase extraction (SPE) for sample preparation, a procedure with quantitation limits of 25 ng/ml.

In the present report we propose a method for the analysis of PZQ enantiomers in plasma with a sensitivity, simplicity and selectivity useful for studies of kinetic disposition.

2. Experimental

2.1. Chemicals and reagents

PZQ was kindly supplied by Merck. The standard solutions of *rac*-PZQ were prepared in methanol at the concentration range of 1.0 to 100.0 μg/ml and were stable at least for three months when stored at −20°C.

The solvents used as mobile phase and in the extraction procedure were chromatographic grade supplied by Merck and EM Science.

2.2. Apparatus

Chromatographic analysis was performed with a modular liquid chromatograph (Shimadzu, Kyoto, Japan) consisting of an LC10AS solvent pump, a Rheodyne Model 7125 injection valve equipped with a 50-μl loop, a Model SPD-10A variable-wavelength UV detector and an electronic integrator Model CR-6A.

2.3. Chromatographic analysis

The direct resolution of PZQ enantiomers was performed on a 150×4.6 mm Chiralcel OD-H 5-μm column (Daicel, Chiral Technology) using hexane–ethanol (85:15, v/v) as the mobile phase. A pre-column (CN 4×4 mm, Merck) was used to protect the analytical column. The mobile phase flow-rate was 1.0 ml/min, and the eluted species were detected at 220 nm.

The columns Chiralcel OJ (10 μm particle size, 250×4.6 mm, Daicel, Chiral Technology) and Chiralpak AD (10 μm particle size, 250×4.6 mm, Daicel, Chiral Technology) were also evaluated using hexane–ethanol (90:10, v/v) and hexane–isopropyl alcohol (75:25, v/v) as mobile phases, respectively.

2.4. Sample preparation

Plasma aliquots (1 ml) were acidified with 300 μl of 1 *M* H_3PO_4 solution and extracted with 4 ml toluene. After mechanical shaking for 20 min and centrifugation (1800 *g*), 3.5 ml of the organic phases were transferred to clean tubes to which 1 ml water and 200 μl of the 1.5 *M* NaOH solution were added. After shaking in a mixer for 1 min and centrifugation, 3 ml of the organic phases were recovered and evaporated dry under an air flow at room temperature. The resides were dissolved in 100 μl of the mobile phase and immediately submitted to chromatographic analysis.

2.5. Validation of the method

The calibration curves were prepared by the addition of 25 μl of the standard *rac*-PZQ solutions at concentrations of 1.0, 4.0, 10.0 and 20.0 μg/ml to 1-ml plasma aliquots resulting in plasma concentrations of 12.5, 50, 125 and 250 ng/ml of each enantiomer, respectively. Sample preparation and chromatographic analysis were then carried out as described earlier. The linearity of the method was obtained in a similar manner for the 12.5 to 1250 ng/ml range for each enantiomer.

Recovery was evaluated by analyzing plasma aliquots ($n=3$) spiked with *rac*-PZQ in the plasma concentration range of 12.5 to 250 ng/ml of each

enantiomer. The precision and accuracy of the method were evaluated for plasma concentrations of 24 and 200 ng/ml of each enantiomer. The quantitation limit, a parameter used to assess the sensitivity of the method, was obtained by the analysis of five plasma aliquots supplemented with 5 and 10 ng/ml of each enantiomer. The selectivity of the method was evaluated by analyzing approximately forty drugs under the chromatographic conditions established. In cases in which the drug interfered with PZQ analysis, the experiment was repeated after submitting the drug to the sample preparation procedure.

2.6. Application

The method developed and validated was used for the analysis of a sample obtained from a female patient (G.S.F., 11 years old, 37 kg) with schistosomiasis and treated with a single dose of 1480 mg PZQ. Blood samples (5 ml) were collected into heparinized flasks before and 2 h after drug administration (Cisticid, Merck) and immediately centrifuged for plasma separation. Plasma samples were then stored at −20°C until the time for analysis.

3. Results and discussion

Fig. 2 shows the resolution of the PZQ enantiomers using the Chiralcel OD-H, Chiralcel OJ and Chiralpak AD columns. Although the three columns proved to be adequate, the Chiralcel OD-H column was selected because of its higher efficiency which was due to smaller particle size. Although previous studies [10,11] have demonstrated the applicability of the Chiralcel OD column (10 μm particles) for the resolution of PZQ enantiomers, the present study demonstrated that the use of a column with smaller size particles (5 μm) permitted the development of a more sensitive method with a simpler procedure for sample preparation.

The order of elution of the enantiomers in the Chiralcel OD-H column was established by the methods of Westhoff and Blaschke [10] and Kelly et al. [11]. For the remaining columns, the order of elution was determined by analysis of the individual enantiomers obtained by previous separation on the

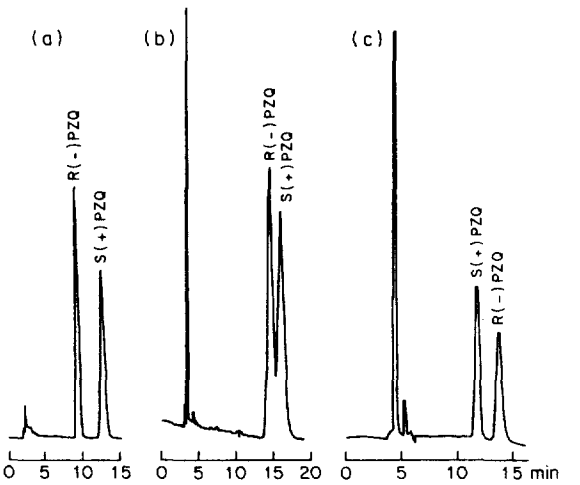

Fig. 2. Comparison of resolution of PZQ enantiomers on three chiral stationary phases. (a) Chiralcel OD-H column (dp=5 μm); mobile phase, hexane–ethanol (85:15, v/v); flow-rate, 1 ml/min. (b) Chiralcel OJ column (dp=10 μm); mobile phase, hexane–ethanol (90:10, v/v); flow-rate, 1 ml/min. (c) Chiralpak AD column (dp=10 μm); mobile phase, hexane–isoproanol (75:25, v/v); flow-rate, 1 ml/min. UV detection at 220 nm.

Chiralcel OD-H column. The elution order was inverted in the Chiralpak AD column.

The chromatogram in Fig. 3a, corresponding to a plasma sample obtained from a patient immediately before PZQ administration, demonstrates that by applying the extraction procedure in two steps it was possible to fully eliminate endogenous plasma interferents. Fig. 3b shows the baseline resolution of the enantiomers. The remaining peaks appearing in the chromatogram in Fig. 3c, corresponding to a sample collected 2 h after administration of the drug, are due to the PZQ metabolites that are also found in plasma. The peaks eluting after 15 min were identified as *trans*-4-hydroxypraziquantel enantiomers.

The procedure for sample preparation showed a mean recovery of 87% regardless of concentration within the range studied. The method proved to be linear in the plasma concentration range of 12.5 to 1250 ng/ml of each enantiomer, with correlation coefficients of 0.999 being obtained for both enantiomers.

In the studies of precision and accuracy (Table 1) the method proved to be highly reproducible, with coefficients of variations of less than 10% being obtained in all cases.

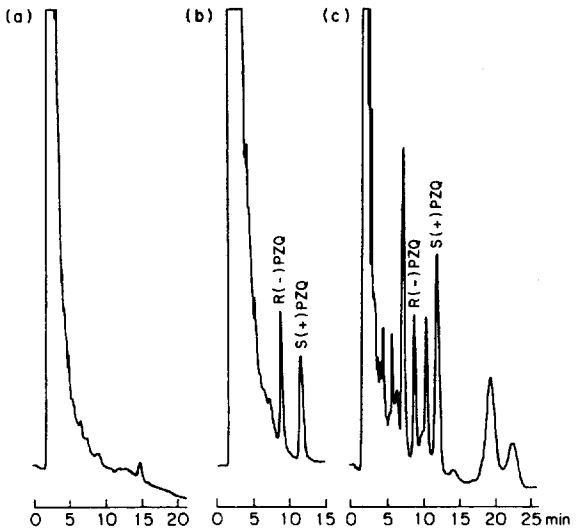

Fig. 3. Chromatographic analysis of PZQ enantiomers in plasma samples. (a) Plasma sample obtained from a patient before administration of PZQ; (b) plasma spiked with 50 ng/ml of *R*-(−)-PZQ and *S*-(+)-PZQ; (c) plasma sample obtained from a patient 2 h after administration of PZQ. Chromatographic conditions: Chiralcel OD-H column (150×4.6 mm, dp=5 μm); mobile phase: hexane–ethanol (85:15, v/v); flow-rate: 1 ml/min; UV detection at 220 nm, (a) and (b) at 0.008 auf, (c) at 0.032 auf.

The quantitation limit, considered to be the lowest concentration quantified with an error of less than 10%, was 5 ng/ml for *R*-(−)-PZQ and 10 ng/ml for *S*-(+)-PZQ. It should be pointed out that *S*-(+)-PZQ predominates in human plasma and therefore the quantitation limit observed for this enantiomer does not represent a disadvantage.

Approximately forty commonly used drugs were tested to determine the selectivity of the method. Carbamazepine, clonazepam, triazolam, lorazepam and albendazole sulfone, an albendazole metabolite, presented retention times close to those of the PZQ enantiomers. This interference is not eliminated by the extraction procedure and should be considered when samples from patients are analyzed.

4. Conclusions

We reported a method for the direct determination of PZQ enantiomers in plasma samples. The quantitation limit of 5 ng/ml for *R*-(−)-PZQ and 10 ng/ml for *S*-(+)-PZQ, the reproducibility, selectivity and wide linear range (12.5 to 1250 ng/ml for each

Table 1
Precision and accuracy of the method for analysis of PZQ enantiomers in plasma

Amount added	Amount obtained (ng/ml)	Precision (% C.V.)	Accuracy (%)
Within-day (n=10)			
24.0 ng/ml			
R-(−)-PZQ	24.6	4.7	97.5
S-(+)-PZQ	25.6	3.4	97.5
200.0 ng/ml			
R-(−)-PZQ	207.3	3.4	102.6
S-(+)-PZQ	203.2	3.2	100.6
Between-day (n=5)			
24.0 ng/ml			
R-(−)-PZQ	25.2	7.1	95.2
S-(+)-PZQ	25.9	7.7	91.3
200.0 ng/ml			
R-(−)-PZQ	210.1	3.3	104.0
S-(+)-PZQ	207.3	3.2	102.7

n=Number of determinations.
C.V.=Coefficient of variation.

enantiomer) of the method guarantee its usefulness for studies of kinetic disposition.

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