Journal of Chromatography, 581 (1992) 147–151
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6479

Short Communication

Determination of the *R*-(−) and *S*-(+) enantiomers of the monohydroxylated metabolite of oxcarbazepine in human plasma by enantioselective high-performance liquid chromatography

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(First received April 9th, 1992; revised manuscript received June 5th, 1992)

ABSTRACT

An enantioselective liquid chromatographic assay for the simultaneous determination of the *S*-(+) and *R*-(−) enantiomers of the monohydroxylated metabolite of oxcarbazepine in human plasma is described. The metabolite is the active principle. The method is based on the extraction of plasma with diethyl ether–dichloromethane (2:1, v/v), separation of the organic phase, evaporation of the solvent and dissolution of the residue in the mobile phase. The two enantiomers were resolved on a Chiralcel OD (250 mm × 4.6 mm I.D.) high-performance liquid chromatographic column. The separation was achieved by isocratic elution with *n*-hexane–2-propanol (77:23, v/v). The flow-rate of the mobile phase was 1.0 ml/min and the two enantiomers were detected by ultraviolet absorbance at 210 nm. The analytical method is suitable for the quantitative and simultaneous determination of the two enantiomers in plasma at concentrations down to 0.4 μmol/l after administration of oxcarbazepine.

INTRODUCTION

Oxcarbazepine (Fig. 1), 10,11-dihydro-10-oxo-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (GP 47 680), is an anti-epileptic drug. In humans, oxcarbazepine is rapidly and virtually completely converted into the monohydroxylated metabolite (racemic compound), 10,11-dihydro-10-hydroxycarbamazepine (GP 47 779 or MHD) (Fig. 1). A small amount of MHD is transformed into *trans*-10,11-dihydroxycarbamazepine (CGP 10 000 or DHD) (Fig. 1) [1].

An analytical method that allows the determination of the sum of both enantiomers as well as the metabolite DHD in plasma after administration of oxcarbazepine has already been developed [2]. In order to evaluate the pharmacokinetics of each of the two enantiomers of MHD, an enantioselective analytical method was needed.

This paper describes an assay for the simultaneous determination of the two enantiomers, *S*-(+)-MHD and *R*-(−)-MHD, in plasma samples, using liquid–liquid extraction, separation on a chiral column and spectrophotometric detection.

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0378-4347/92/$05.00

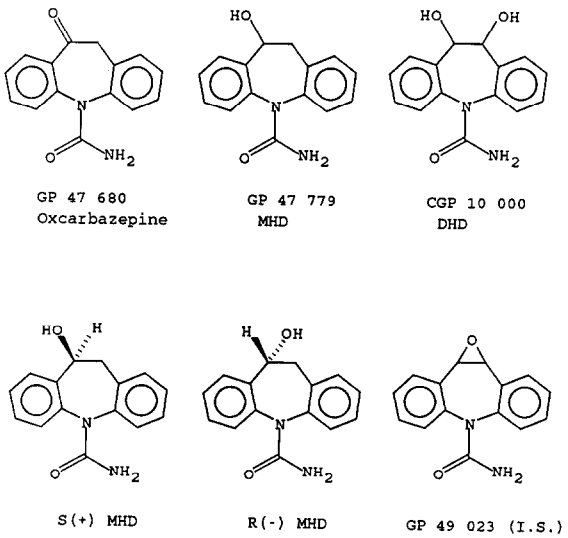

Fig. 1. Structures of oxcarbazepine, MHD (monohydroxylated metabolite of oxcarbazepine), DHD (dihydroxylated metabolite of oxcarbazepine), *S*-(+)-MHD and *R*-(−)-MHD (the two enantiomers of MHD) and the internal standard GP 49 023.

EXPERIMENTAL

Chemicals

All solvents were of analytical grade (Fluka, Merck) and were used without further purification. MHD, $C_{15}H_{14}N_2O_2$, M_r 254.29, Batch No. 800 188, *S*-(+)-MHD, $C_{15}H_{14}N_2O_2$, M_r 254.29, Batch No. 2, *R*-(−)-MHD, $C_{15}H_{14}N_2O_2$, M_r 254.29, Batch No. 2, and the internal standard GP 49 023, $C_{15}H_{12}N_2O_2$, M_r 252.27, Batch No. 800 185, orginated from Ciba-Geigy (Basle, Switzerland).

Chromatographic conditions

A Hewlett-Packard high-performance liquid chromatographic (HPLC) system (Model 1090) equipped with an automatic sampling system and a Kratos Spectroflow SF 773 detector was used. The UV detector was set at 210 nm. The peak heights were measured with a Merck–Hitachi computing integrator (Model D-2000). The column (250 mm × 4.6 mm I.D.) was a Diacel Chiralcel OD, purchased from Stehelin (Basle, Switzerland). Chromatography was performed at 40°C. The compounds were eluted with *n*-hexane–2-propanol (77:23; v/v). The flow-rate of the mobile phase was 1.0 ml/min. Under these conditions the retention times of the two enantiomers were 8.0 min for *R*-(−)-MHD and 10.5 min for *S*-(+)-MHD. The retention time of the internal standard was 20 min. The resolution (R_s) between the peaks of *R*-(−)-MHD and *S*-(+)-MHD was 1.7.

Solutions

The solutions of *S*-(+)-MHD, *R*-(−)-MHD and the internal standard (GP 49 023) were prepared by dissolving 1–4 mg of each compound in 100 ml of water–ethanol (8:2, v/v). Aliquots of these stock solutions served to prepare spiked plasma samples for calibration curves and validation analyses.

Sample preparation

The plasma samples (0.5 ml) containing *S*-(+)-MHD, *R*-(−)-MHD and the internal standard were extracted with 7 ml of diethyl ether–dichloromethane (2:1, v/v) on a mechanical horizontal shaker for 30 min at 200 rpm. After brief centrifugation (5 min at 1000 *g*), the tube was placed into a mixture of dry ice and ethanol until the aqueous phase was frozen. The organic phase was then decanted into a new tube and evaporated at 40°C under a stream of nitrogen. The residue was dissolved in 0.20 ml of *n*-hexane–2-propanol (77:23, v/v). An aliquot of 20 μl was injected onto the chiral HPLC column.

Calibration

To construct calibration curves, plasma samples with known concentrations were prepared by adding the two enantiomers to 0.5 ml of drug-free human plasma. The samples were processed as described above. A 20-μl volume of each sample was injected onto the column. The peak-height ratios of the compounds to the internal standard were plotted against the concentrations of *S*-(+)-MHD and *R*-(−)-MHD. Calibration curves for both enantiomers were calculated by linear least-squares regression ($y = a + bx$). The following terms for calibration curves in the range 0.4–16.4 μ*M* were obtained for *S*-(+)-

MHD: $y = -0.0152 + 0.0837x$, $r = 0.9999$; and in the same range for *R*-(−)-MHD: $y = -0.0161 + 0.1188x$, $r = 0.9999$.

RESULTS AND DISCUSSION

Selectivity

The chromatograms of a drug-free plasma sample (0.5 ml) and a plasma sample from a healthy male volunteer, 4 h after the intake of a single oral dose of 600 mg of oxcarbazepine and processed as described above are shown in Fig. 2. *S*-(+)-MHD and *R*-(−)-MHD are well separated from plasma components. The extract of the blank plasma sample contained no interfering substances. Neither the parent compound (oxcarbazepine) nor DHD was detected. Under the same chromatographic conditions, the retention times of the peaks corresponding to the two enantiomers of DHD would be 11 and 12 min and that of oxcarbazepine 14 min.

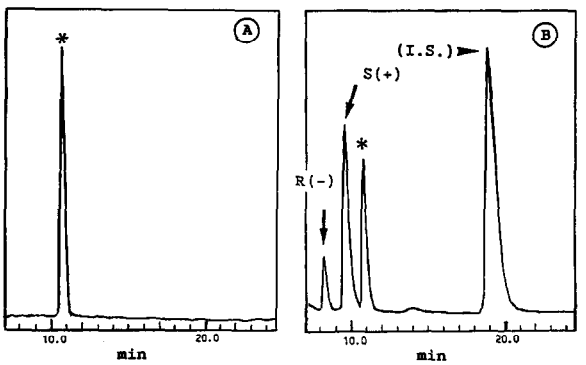

Fig. 2. Chromatograms of (A) extract of drug-free human plasma sample and (B) extract of 0.5 ml human plasma sample from a male healthy volunteer, 4 h after a single oral dose of 600 mg of oxcarbazepine and processed as described in Experimental. (*) Unidentified constituent of plasma.

Accuracy and precision

The accuracy and precision were evaluated by analysing spiked plasma samples. Four plasma

TABLE I

ANALYSIS OF PLASMA SAMPLES, SPIKED WITH *S*-(+)-MHD

Expected concentration (μM)	Found concentration (μM)		Inter-assay precision (C.V., %)	Deviation from theory (%)
	Individual values	Mean ± S.D.		
1.848	1.957	1.880 ± 0.10	5.3	+1.7
	1.742			
	1.873			
	1.946			
10.862	10.857	10.753 ± 0.09	0.9	−1.0
	10.717			
	10.640			
	10.798			
22.640	22.870	22.761 ± 0.16	0.7	+0.5
	22.895			
	22.741			
	22.537			
31.815	32.200	32.215 ± 0.23	0.7	+1.3
	32.457			
	32.295			
	31.908			

TABLE II

ANALYSIS OF PLASMA SAMPLES, SPIKED WITH *R*-(−)-MHD

Expected concentration (μM)	Found concentration (μM)		Inter-assay precision (C.V. %)	Deviation from theory (%)
	Individual values	Mean ± S.D.		
1.766	1.792	1.750 ± 0.06	3.6	−0.9
	1.676			
	1.722			
	1.810			
10.296	10.338	10.201 ± 0.11	1.0	−0.9
	10.225			
	10.136			
	10.103			
21.826	21.749	21.862 ± 0.14	0.6	+0.2
	22.030			
	21.918			
	21.749			
30.895	30.745	31.029 ± 0.22	0.7	+0.4
	31.243			
	31.171			
	30.958			

samples spiked with *S*-(+)-MHD and *R*-(−)-MHD were analysed on four different days. The results are given in Tables I and II.

Limit of quantitation

The limit of quantitation in plasma, 0.4 μM for *S*-(+)-MHD and 0.3 μM for *R*-(−)-MHD, was calculated by taking twice the S_y values resulting from the linear least-squares regression analysis from the analysis of human plasma samples prepared each day (Tables I and II).

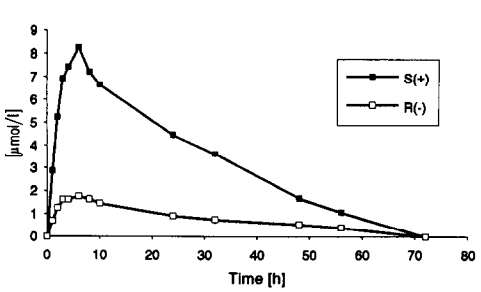

Fig. 3. Plasma concentration–time curves of *S*-(+)-MHD and *R*-(−)-MHD after a single oral administration of 600 mg of oxcarbazepine in a healthy volunteer.

Stability

The reference solutions of MHD, its two enantiomers, *S*-(+)-MHD and *R*-(−)-MHD, and the internal standard were stable for at least one week if stored at 4°C. No inversion of the enantiomers occurred. In samples from clinical trials both enantiomers were stable for at least six months if stored at −20°C.

Application

Fig. 3 shows plasma concentration–time curves of the two enantiomers of MHD in one volunteer after a single oral dose of 600 mg of oxcarbazepine. The $AUC_{0-72\ h}$ (area under the plasma concentration–time curve) of the *S* enantiomer was 240.6 h μmol/l and represented 81% of the total $AUC_{0-72\ h}$, whereas that of the *R* enantiomer was 57.5 h μmol/l, and represented only 19%.

CONCLUSION

The analytical method described is suitable for the simultaneous determination of the two

enantiomers *S*-(+)-MHD and *R*-(−)-MHD in human plasma at concentrations down to 0.4 μM after administration of oxcarbazepine.

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