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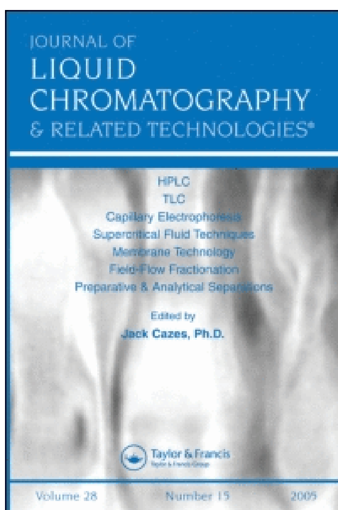

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Stereoselective Bioanalysis of Oxcarbazepine and the Enantiomers of Its Metabolites by High-Performance Liquid Chromatcgraphy

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STEREOSELECTIVE BIOANALYSIS OF OXCARBAZEPINE AND THE ENANTIOMERS OF ITS METABOLITES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The simultaneous liquid chromatographic determination of oxcarbazepine and the enantiomers of its metabolites 10,11-dihydro-10-hydroxycarbamazepine and trans-10,11-dihydroxycarbamazepine in spiked human plasma is described. The compounds are subjected to solid phase extraction before chromatography. The separation of the analytes is achieved using chiralcel OD column coupled on line with chiralcel ODH column and a mobile phase consisting of *n*-hexane-ethanol (70/30, v/v). The compound were detected by ultraviolet absorbance at 220 nm. The limit of quantification for each compound was 5 ng/ml.

INTRODUCTION

Oxcarbazepine (OXC) (10,11-dihydro-10-oxo-5*H* dibenz (*b,f*) azepine-5-carboxamide) is an antiepileptic drug structurally related to carbamazepine (CBZ). Unlike CBZ, which is metabolized by an oxidation, OXC appears in the human blood in

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trace amounts and undergoes rapid reduction to the 10-oxo function to the chiral 10-hydroxy derivative (10,11-dihydro-10-hydroxycarbamazepine, MHC or GP47779) which is eliminated in the urine as unchanged and as glucuronide conjugate. A small amount of MHC is converted to the chiral trans-10,11 dihydroxyderivative (trans-10,11-dihydroxycarbamazepine, DHC or CGP10000) (Fig.1) [1-3]. Conversely, in animal species such as rat, guinea-pig, rabbit, dog and rhesus, the metabolic pattern is different from that seen in man. Indeed, preliminary studied showed plasma concentration of the parent compound higher than those of the metabolite [4].

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Recently, it has been shown that in humans the metabolism of OXC is stereospecific. Indeed, after an oral dose of 600 mg oxcarbazepine,the area under the plasma concentration-time curve (AUC) of the (+)-S-MHC represented 81% of the total AUC, whereas that of the (-)-R-MHC represented only 19% [5].

High-performance liquid chromatographic (HPLC) methods with ultraviolet detection have been developed for the determination of oxcarbazepine and the sum of both enantiomers of MHC and DHC in plasma after administration of the parent drug [6-9] but only Flesch et al. reported an HPLC method for the measurement of the two enantiomers of MHC in plasma samples [5]. Neither oxcarbazepine, nor the enantiomers of DHC were detected at that time, although the authors admitted the co-elution of the standards of these latter analytes with the enantiomers of MHD.

In this paper we describe an enantioselective HPLC method to determine oxcarbazepine simultaneously with the enantiomers of the monohydroxylated and dihydroxylated metabolites using solid phase extraction and a spectrophotometric detection.

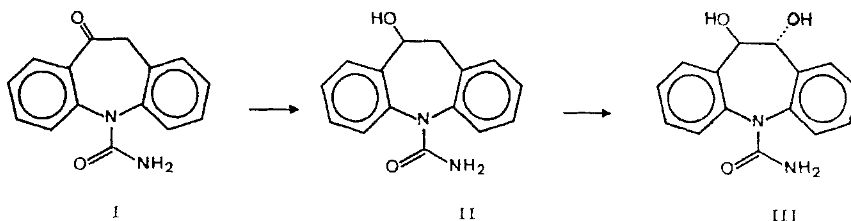

Figure 1. Structures of oxcarbazepine (I), racemic 10,11-dihydro-10-hydroxy-carbamazepine (II), racemic 10,11-dihydro-10,11-trans-dihydroxy-carbamazepine (III).

The method was applied to human blank serum spiked with oxcarbazepine, its metabolites and carbamazepine as an internal standard.

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MATERIAL AND METHODS

Chemicals

Oxcarbazepine, racemic MHC, (+)-S and (-)-R-enantiomers of MHC, racemic DHC and carbamazepine were obtained from Ciba-Geigy (Basle, Switzerland). It was not possible to obtain or characterize the two enantiomers of DHC, which were simply named as the first (DHC enantiomer n^o1) and the second enantiomer (DHC enantiomer n^o2) depending on their HPLC retention times.

Extrelut-1 extraction columns were from Merck (Bracco, Milan, Italy). All solvents were of analytical reagent grade.

Chromatographic instrumentation and conditions

The HPLC system consisted of a Merck-Hitachi L6200 intelligent pump, a Merck-Hitachi L4200 UV-VIS detector set to 220 nm and a Merck-Hitachi D2000 chromato-integrator (Bracco,

Milan, Italy). The columns used, from Daicel, Inc. (Schilling, Milan, Italy) were Chiralcel OD and Chiralcel ODH, both 25 cm x 4.6 mm i.d. and 10 um particle size. The Chiralcel OD column was coupled on line with the Chiralcel ODH column, which was heated at 40°C.

Resolution of the substances was achieved with n-hexane-ethanol (70/30, v/v) as the mobile phase at a flow rate of 0.9 ml/min. The mobile phase was left to equilibrate at least 2 hours before injections.

<u>Solutions and sample preparation</u>

Solutions of stock reference standards of OXC, racemic MHC, (+)-S and (-)-R-enantiomers of MHC, racemic DHC and CBZ (1 mg/ml, 10 ug/ml and 1 ug/ml) were prepared in ethanol and stored below 0°C. Dilutions were made fresh daily for each analysis. Serum standards were prepared daily by adding known amounts of the stock standards to blank human serum.
A 1 ml aliquot of spiked serum, with 100 ul of carbamazepine as internal standard (2 ug/ml ethanolic solution) added, was vortex-shaken for 30 sec and transferred to an Extrelut-1 glass column, which was preconditioned with 5 ml of dichloromethane-isopropyl alcohol (9/1, v/v) just before extraction and dried under nitrogen. After 10 min, the analytes were eluted under gravity with 5 ml of dichloromethane-isopropyl alcohol (9/1, v/v). The organic phase was evaporated to dryness under a stream of nitrogen and redissolved in 100 ul of HPLC mobile phase. A 20 ul volume was injected into the HPLC column.

<u>Calibration, analytical recovery and precision</u>

Spiked sera carried through the entire procedure were used to create calibration curves and to determine analytical

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recoveries, intra-day and inter-day variabilites. The linearity of the calibration curves was studied in the range of 5-2000 ng/ml for each analyte. Analytical recoveries were perfomed at three different concentrations (10, 100, and 500 ng/ml for each substance) with 5 samples for each concentration. The same concentrations were used to test the analytical imprecision, performing analyses of serum samples for up to six days.

Drugs Interferences

Several drugs commonly administered to epileptic patients were examined for their possible interference with the determination of oxcarbazepine and its metabolites. The substances tested were: phenobarbital, diphenylhydantoin, valproic acid, carbamazepine.

One microgram of each drug was added to blank serum and to serum spiked with OXC, MHC and DHC carried through extraction procedure and analyzed by HPLC.

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RESULTS AND DISCUSSION

The chiral separation of the enantiomers of MHC and DHC, and oxcarbazepine was achieved only using an on line-coupled two column system as shown in Fig. 2a.

Indeed, when Chiralcel OD column was used alone, with a mobile phase consisting of *n*-hexane-2-propanol, the peak of the second enantiomer of DHC completely overlapped that of oxcarbazepine (Fig.2b).

Modifications of the mobile phase and the addition of an achiral silica column did not improve the separation.

On the other hand, when Chiralcel ODH was used alone with a

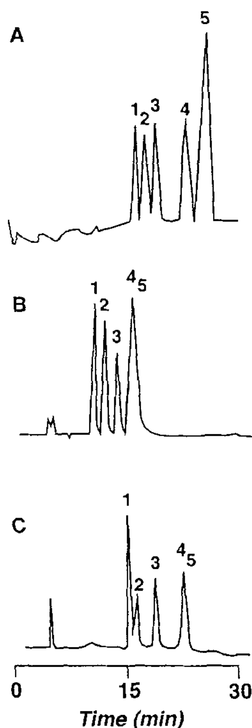

Figure 2. Chromatographic separation of oxcarbazepine and its metabolites using: a) Chiralcel OD column coupled on line with Chiralcel ODH column; b) Chiralcel OD column ; c) Chiralcel ODH column.

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mobile phase of n-hexane-2-propanol (88:12), not only the second enantiomer of DHC overlapped oxcarbazepine, but also the (-)-R isomer of MHC was poorly separated with the first enantiomer of DHC (Fig.2c).

The on-line coupling of Chiralcel OD and ODH column had a beneficial effect on the separation. Furthermore, when the ODH column was heated at 40°C both the tailing factors and peak widths decreased with an improving of the peak shape. Baseline resolution was achieved using ethanol instead of 2 propanol in the mobile phase. No loss of resolution was observed after more

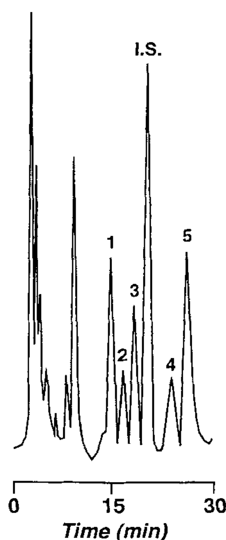

Figure 3. Chromatogram of extract of serum sample.
Peaks: 1= 1.9 ug/ml (-)-R-MHC; 2= 1.1 ug/ml first enantiomer of DHC; 3= 1.9 ug/ml (+)-S-MHC; 2 ug/ml carbamazepine (I.S.); 4= 1.1 ug/ml second enantiomer of DHC; 5= 1.1 ug/ml oxcarbazepine.

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than 100 chromatographic runs over a period of five months.
None of the other antiepileptic drugs, but phenobarbital which coeluted with oxcarbazepine, interfered with the assay. Carbamazepine, which was extracted with a good recovery and eluted between the (+)-S-MHC and the second enantiomer of DHC (retention time= 21.9 min), was chosen as internal standard of the assay when determing the analytes in spiked serum (Fig.3). The analytical recoveries of all the analytes, and the intra-day and inter-day variabilities are shown in Table 1.
The detection limit (signal-to-noise ratio of 3) and the linearity of the method are shown in Table 2.
The calibration curves were linear over the range 5-2000 ng/ml for OXC and its metabolites with correlation coefficients always higher than 0.99.

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TABLE 1

Recovery and Variability

Concentration (ng/ml)	Recovery (mean ±S.D.) (%)	Variability (%)	
		Intraday	Interday
Oxcarbazepine			
10	93.3 ± 1.1	1.1	1.8
100	94.1 ± 1.2	1.1	2.3
500	94.1 ± 1.4	1.6	2.4
(-)-R-MHC			
10	98.2 ± 1.0	1.1	1.4
100	98.4 ± 1.5	1.7	1.9
500	98.7 ± 1.7	1.7	2.1
(+)-S-MHC			
10	98.1 ± 1.0	1.2	1.8
100	98.4 ± 1.5	1.3	1.9
500	98.6 ± 1.6	1.5	1.9
DHC enantiomer n° 1			
10	89.5 ± 2.1	2.3	3.1
100	90.1 ± 2.3	2.6	3.3
500	91.8 ± 2.5	2.9	3.7
DHC enantiomer n° 2			
10	89.7 ± 2.0	2.2	2.9
100	90.1 ± 2.5	2.8	3.1
500	92.0 ± 2.5	2.8	3.4

TABLE 2

Detection Limit and Linearity

Compound	Retention time (min)	Detection limit (ng/ml)	Linearity
Oxcarbazepine	28.9	5	y=3.5x + 0.25
(-)-R-MHC	15.4	5	y=1.7x + 0.67
(+)-S-MHC	19.0	5	y=1.8x + 0.11
DHC enantiomer n° 1	17.6	5	y=1.5x - 0.13
DHC enantiomer n° 2	26.2	5	y=1.2x - 0.07

In conclusion, the above results demonstrate that this HPLC method permits quick and simple extraction and simultaneous determination of OXC and the enantiomers of its metabolites MHC and DHC. The development of this enantiospecific assay could be of great help in pharmacokinetic and pharmacodynamic studies of OXC both in human and animal models, taking into account the different metabolism in these species.

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