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Note

Determination of oxcarbazepine in human plasma by high-performance liquid chromatography

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Oxcarbazepine (I), a potent anticonvulsant [1], is the keto-homologue of carbamazepine, a well-known antiepileptic drug (Fig. 1). After the administration of single oral doses of oxcarbazepine to volunteers, only low plasma concentrations of the unchanged compound have been observed [2]. Therefore, in pharmacokinetic studies the concentrations of both the parent compound

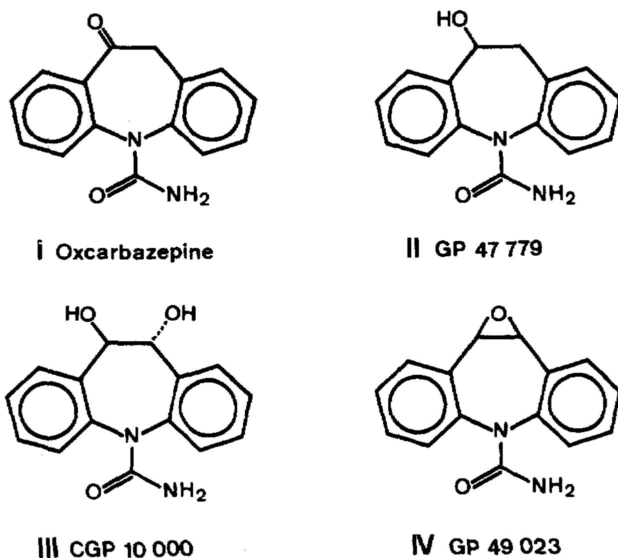

Fig. 1. Structures of: I, oxcarbazepine (10,11-dihydro-10-oxo-carbamazepine); II, GP 47 779 (10,11-dihydro-10-hydroxy-carbamazepine); III (10,11-dihydro-10,11-*trans*-dihydroxy-carbamazepine); and IV (10,11-epoxy-carbamazepine).

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and the main metabolite 10,11-dihydro-10-hydroxy-carbamazepine, GP 47 779 (II), as well as of a minor metabolite 10,11-dihydro-10,11-*trans*-dihydroxy-carbamazepine, CGP 10 000 (III), need to be measured. For the simultaneous determination of these three compounds a sensitive high-performance liquid chromatographic (HPLC) assay has been developed using 10,11-epoxy-carbamazepine (IV) as the internal standard.

EXPERIMENTAL

Chemicals

All solvents and reagents were of analytical grade (Fluka, Buchs, Switzerland, and Merck, Darmstadt, G.F.R.) and were used without further purification. Oxcarbazepine, GP 47 779 (II), CGP 10 000 (III) and the internal standard originated from Ciba-Geigy Ltd., Basle, Switzerland. Water was deionised, distilled in a glass apparatus and was filtered through a 0.45-μm Millipore® filter before use.

Apparatus

A Hewlett-Packard isocratic liquid chromatograph (Model 1082 B) equipped with an automatic sampling system (Model 79842 A) was used. The variable-wavelength detector (Model 79875 A) was operated at 210 nm and an LC terminal (Model 79850 B) was used for programming and peak area integration.

The column (25 cm × 3.2 mm I.D.) was packed with LiChrosorb RP-18, 10 μm (Merck). The mobile phase (acetonitrile—water, 20:80) was used at a flow-rate of 1 ml/min and at a column temperature of 30°C.

Preparation of standard solutions

Stock solutions of oxcarbazepine and the metabolites were prepared by dissolving 10 mg of the compound in 100 g of water containing 20% of ethanol (10% of ethanol for the metabolites).

Aliquots of these stock solutions were combined and diluted with water to yield a concentration of 2 μg/g for each of the three compounds. This solution served to prepare spiked plasma samples for calibration curves and recovery analyses. Weighing of aliquots was preferred because of higher precision and better documentation.

Preparation of the internal standard solution

A stock solution was prepared by dissolving 10 mg of 10,11-epoxy-carbamazepine in 100 ml of water containing 10% of ethanol. An aliquot was diluted with water to yield a concentration of 2 μg/ml. To each analytical plasma sample 1 μg of 10,11-epoxy-carbamazepine was added using a Repipette® sampler. The working solutions, when kept at 5°C, were found to be stable for at least four weeks.

Procedure

Weigh (Mettler AC 100 balance) 0.5 g of plasma into a 16 × 70 mm disposable glass ampoule and dilute with 1 g of water. (For calibration curves and recovery analyses add known amounts of the three compounds instead of 1 g of

water.) Add 0.5 ml (Repipette® sampler) of an aqueous solution of the internal standard and shake for 5 min (Heidolph DSG 304 vertical mixer). Then add 7 ml of extraction solvent (diethyl ether—dichloromethane, 2:1) and 1.0 g of solid sodium carbonate-10-hydrate to saturate the aqueous phase. Seal the ampoule with a polyethylene cap and shake for 30 min (Infors TR 1 horizontal shaker at 200 rpm). Centrifuge for 5 min (MSE Multex at 940 *g*) and freeze the aqueous phase in a dry ice—ethanol mixture. Transfer the organic layer into a 16 × 40 mm disposable glass ampoule and evaporate the solvent by gently blowing nitrogen into the ampoule at 40°C. Place the ampoule in a desiccator and evaporate for 10 min to eliminate completely the dichloromethane. Reconstitute the extraction residue in 0.2 ml of the mobile phase. Transfer the solution into a micro injection vial. Seal with a septum cap and centrifuge for 1 min to ensure no air bubbles or particulate matter will be injected into the chromatograph.

Calibration

To establish calibration curves, plasma samples with known concentrations were prepared by adding oxcarbazepine and the two metabolites to 0.5 g of blank human plasma. After addition of 1 μg of the internal standard, the samples were processed as described above.

A 20-μl volume of the reconstituted extract of each sample was injected and peak area ratios of the compound to the internal standard plotted against the

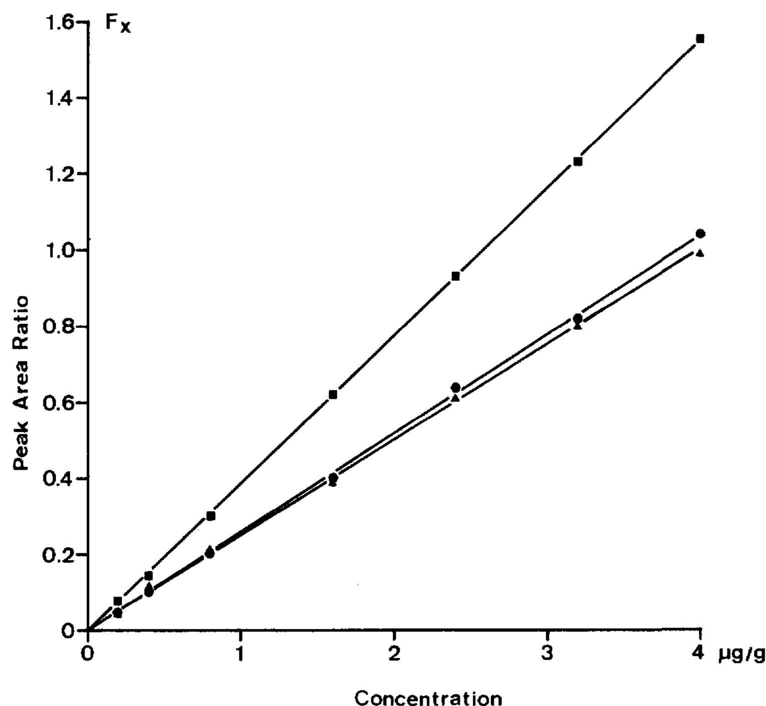

Fig. 2. Peak area ratios of test compounds to internal standard versus concentration. Calibration curves for oxcarbazepine (●——●), II (■——■) and III (▲——▲).

compounds concentrations given. Calibration curves for all three compounds were linear (r=0.999) in the range 0.2—4.0 μg/g (Fig. 2).

In the routine analysis of large series of plasma samples single-point calibration was used to calculate the concentrations of oxcarbazepine and the metabolites.

RESULTS

Extractability

The extractability of the three compounds and of the internal standard from plasma was determined by comparison of the peak area after direct injection of known amounts to that resulting after injection of the reconstituted extracts of spiked samples that underwent the whole work-up procedure. Covering the concentration range 0.2—2.0 μg/g the extractabilities (mean ± S.D.) were 80 ± 4% for oxcarbazepine, 90 ± 4% for II, 67 ± 4% for III and 94 ± 6% for the internal standard.

Precision and accuracy

Recovery analyses of spiked plasma samples were analysed together with each series of analytical samples. The results of recovery analyses from six independent series and the respective coefficients of variation (CV%, between-day) are given in Table I. Typical chromatograms are shown in Fig. 3.

TABLE I

MEAN VALUES OF RECOVERY ANALYSES PERFORMED ON SIX DIFFERENT DAYS USING SINGLE-POINT CALIBRATION

	Given (μg/g)	Found (μg/g) (mean ± S.D.)	Precision between-day (CV%)	Accuracy (%)
Oxcarbazepine	0.200	0.220 ± 0.016	7.3	110.0
	0.400	0.401 ± 0.035	8.7	100.3
	0.800	0.790 ± 0.049	6.2	98.8
	1.600	1.589 ± 0.037	2.3	99.3
	2.400	2.374 ± 0.077	3.2	98.9
	3.200	3.237 ± 0.080	2.5	101.2
II	0.200	0.218 ± 0.031	14.2	109.0
	0.400	0.419 ± 0.045	10.7	104.8
	0.800	0.803 ± 0.044	5.5	100.4
	1.600	1.612 ± 0.043	2.7	100.8
	2.400	2.384 ± 0.048	2.0	99.3
	3.200	3.188 ± 0.057	1.8	99.6
	4.000	3.935 ± 0.020	0.5	98.4
III	0.400	0.469 ± 0.034	7.2	117.3
	0.800	0.822 ± 0.056	6.8	102.8
	1.600	1.691 ± 0.143	8.5	105.7
	2.400	2.403 ± 0.109	4.5	100.1
	3.200	3.135 ± 0.233	7.4	98.0
	4.000	3.828 ± 0.043	1.1	95.7

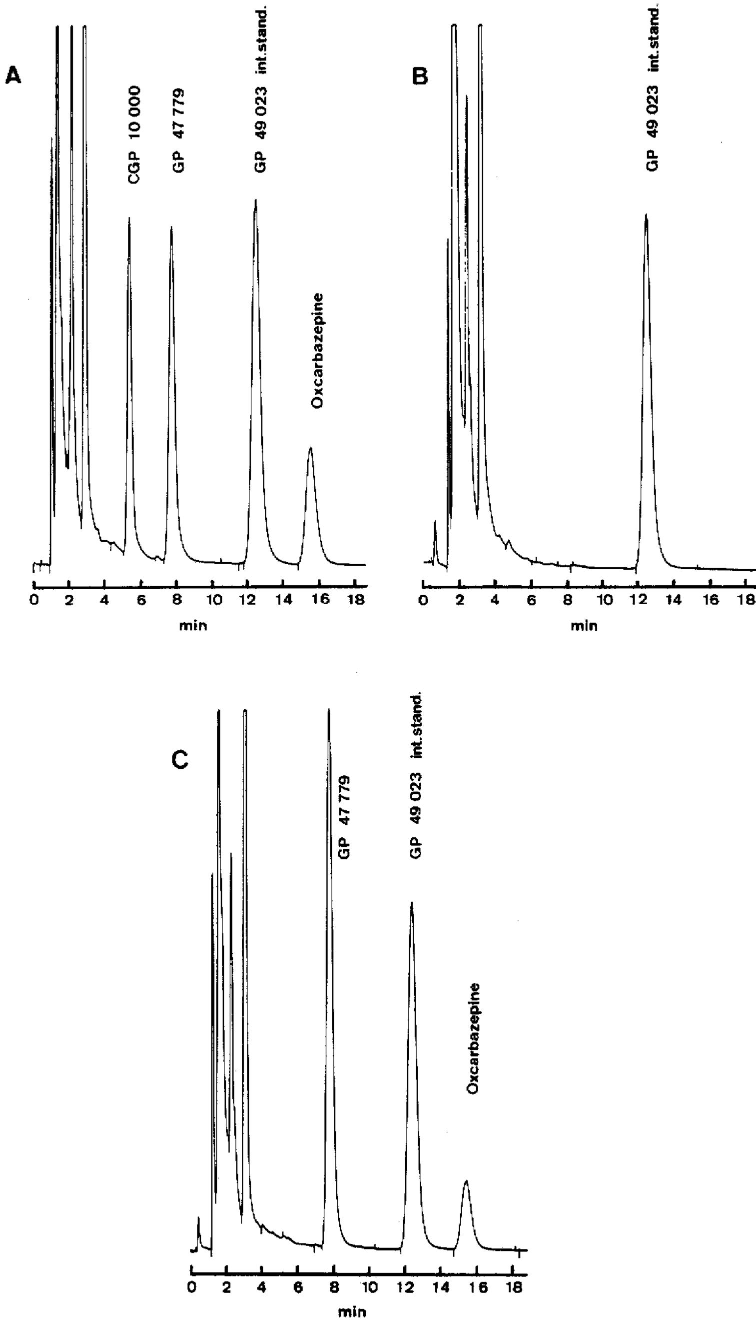

Fig. 3. (A) Chromatogram obtained from analysis of a spiked human plasma sample containing 0.8 μg/g each of oxcarbazepine, II and III. (B) Chromatogram obtained from analysis of a blank plasma sample. (C) Chromatogram obtained from analysis of a plasma sample withdrawn from a healthy volunteer after administration of one 300-mg tablet of oxcarbazepine.

Plasma concentrations of oxcarbazepine and its metabolites

After administration of one 300-mg tablet of oxcarbazepine to a healthy volunteer, the plasma concentrations of unchanged oxcarbazepine reached a maximum level of 0.4 μg/g 1 h after intake of the dose, and dropped below the limit of quantitation (0.1 μg/g) within 3 h.

The main metabolite II reached a maximum plasma concentration of 2.5 μg/g 4 h after administration.

The plasma concentrations of the minor metabolite III never exceeded the limit of quantitation (0.1 μg/g) after the 300-mg dose (Fig. 4).

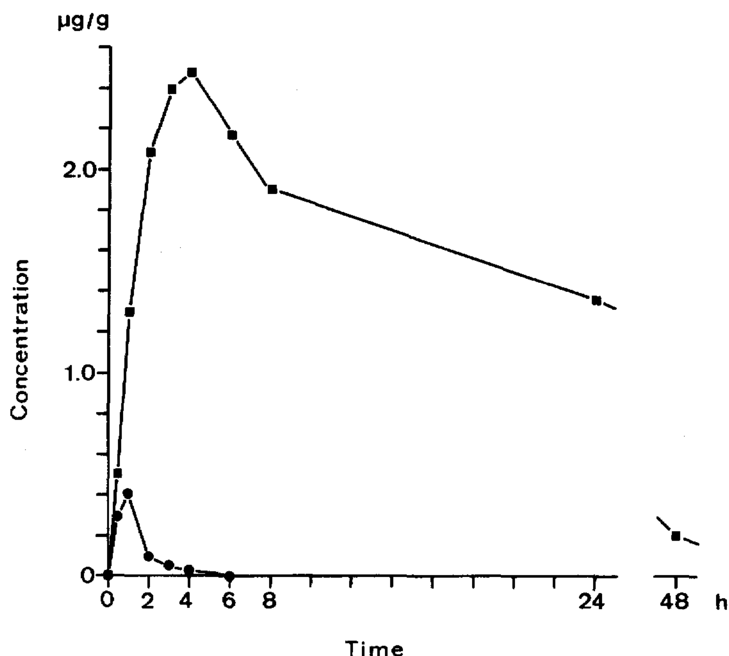

Fig. 4. Plasma concentrations of oxcarbazepine (•——•) and of the main metabolite II (▪——▪) after administration of 300 mg of oxcarbazepine to a healthy volunteer.

CONCLUSIONS

The HPLC assay described for routine analysis of oxcarbazepine and two of its metabolites using 10,11-epoxy-carbamazepine as internal standard is highly sensitive due to the good extractabilities and the use of optimum wavelength for the detection of all compounds. The procedure is able to quantitate plasma levels as they emerge after single oral doses of oxcarbazepine.

REFERENCES

1 V. Baltzer and M. Schmutz, in H. Meinardi and A.J. Rowan (Editors), Advances in Epileptology — 1977, Swets & Zeitlinger B.V., Amsterdam, Lisse, 1978, pp. 295—299.

2 K.F. Feldmann, S. Brechbühler, J.W. Faigle and P. Imhof, in H. Meinardi and A.J. Rowan (Editors), Advances in Epileptology — 1977, Swets & Zeitlinger B.V., Amsterdam, Lisse, 1978, pp. 290—294.