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Note

Simultaneous determination of carbamazepine, oxcarbazepine and their main metabolites in plasma by liquid chromatography

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Carbamazepine (5*H*-dibenz[*b,f*]azepine-5-carboxamide) (IV, Fig. 1), the active ingredient of Tegretol, is an anticonvulsant that is widely used in the treatment of both partial and generalized tonic-clonic seizures [1]. Many methods have been published for the analysis of carbamazepine and its epoxide metabolite (V). However, only one liquid chromatographic (LC) method has been described that allows the simultaneous determination of carbamazepine, its epoxide metabolite and its *trans*-diol metabolite (III) [2]. Under steady-state conditions, the *trans*-diol metabolite can reach plasma concentrations even higher than those of the epoxide metabolite.

Oxcarbazepine (10,11-dihydro-10-oxo-5*H*-dibenz[*b,f*]azepine-5-carboxamide) (I), the ketohomologue of carbamazepine, is a new anticonvulsant in development [3, 4]. Three LC methods have been described for the simultaneous determination of oxcarbazepine and two of its metabolites [5-7]. No analytical method was available to measure oxcarbazepine, carbamazepine and their metabolites simultaneously.

The need to monitor the plasma concentrations of both oxcarbazepine, carbamazepine and their respective metabolites arose during clinical trials in which carbamazepine was to be replaced by oxcarbazepine and vice versa. Therefore, the specific and sensitive LC assay described was developed to allow the

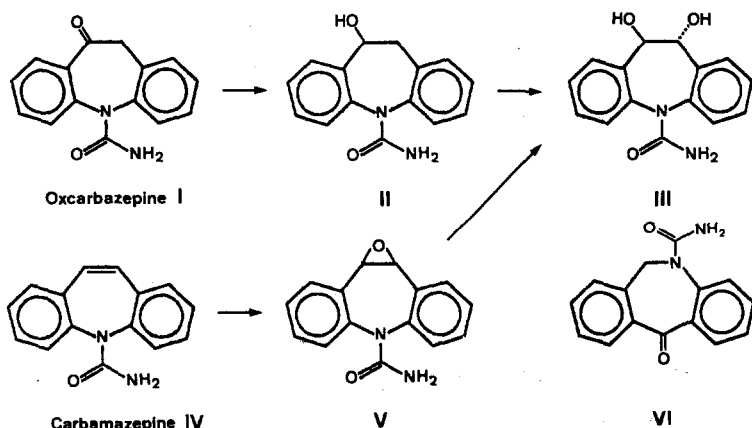


Fig. 1. Metabolic pathways of oxcarbazepine and carbamazepine and the chemical structure of the internal standard. (I) oxcarbazepine; (II) 10,11-dihydro-10-hydroxycarbamazepine, the main metabolite of oxcarbazepine; (III) 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine, the common metabolite of oxcarbazepine and carbamazepine; (IV) carbamazepine; (V) 10,11-epoxycarbamazepine, the metabolite of carbamazepine; (VI) internal standard.

simultaneous determination of oxcarbazepine (I) and its main metabolite, 10,11-dihydro-10-hydroxycarbamazepine (II), and of carbamazepine (IV) and its metabolite, 10,11-epoxycarbamazepine (V), as well as of their common metabolite, 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (III) (Fig. 1).

EXPERIMENTAL

Chemicals

All solvents and reagents were of analytical grade (Fluka, Buchs, Switzerland, and Merck, Darmstadt, F.R.G.) and were used without further purification. Water was deionized and distilled in a glass apparatus and filtered through a 0.45- μm Millipore[®] filter before use. The following compounds originated from Ciba-Geigy (Basle, Switzerland): oxcarbazepine (I); 10,11-dihydro-10-hydroxycarbamazepine (II); 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (III); carbamazepine (IV); 10,11-epoxycarbamazepine (V) and the internal standard 5,6-dihydro-11-oxo-11*H*-dibenz[*b,e*]azepine-5-carboxamide (VI) (Fig. 1).

Ethosuximide, primidone, phenobarbital, phenytoin and valproic acid were from the antiepileptic drug mix No. 1 (Supelco, Gland, Switzerland).

Apparatus

A Hewlett-Packard liquid chromatograph (HP 1090) equipped with a programmable variable-volume injector was used. The Spectroflow SF.773 (Kratos) variable-wavelength detector was operated at 210 nm and the Hewlett-Packard integrator (HP 3392) was used for plotting and peak-area integration. A Bischoff Hypersil column (125 mm \times 4.6 mm I.D.), filled with Shandon MOS Hypersil 5 μm C₈ sorbent, protected by a guard column of 20 mm length filled with the same material, was used. The mobile phase, acetonitrile-water (22:78, v/v), was

pumped at a flow-rate of 1 ml/min and the column was kept at a constant temperature of 30°C. Large series of clinical samples were analysed on a Hewlett-Packard liquid chromatograph (HP 1082 B) equipped with a Model 79850 B LC terminal, which served for system control, plotting and peak-area integration. All other conditions were similar to those used with the HP 1090 system.

Preparation of standard solutions

Stock solutions of all compounds were prepared by dissolving 10 mg of each compound in 100 g of water containing 10% of ethanol (20% of ethanol for oxcarbazepine). Aliquots of these stock solutions were combined and diluted with water to yield a concentration of 2 µg/g (ca. 8 nmol/g) for each of the compounds. This solution served to prepare spiked plasma samples for calibration curves and recovery analyses. Weighing of aliquots was preferred for greater precision and better documentation.

Preparation of the internal standard solution

A stock solution was prepared by dissolving 10 mg of VI in 100 ml of water containing 10% of ethanol. An aliquot was diluted with water to yield a concentration of 4 µg/ml (15.856 nmol/ml). The working solutions kept at 5°C were stable for at least four weeks.

Procedure

Samples of 0.5 g of plasma were weighed (Mettler AC 100 balance) into 70×16 mm disposable glass ampoules and diluted with 1 g of water. For calibration curves and recovery analyses, known amounts of the five compounds were added instead of 1 g of water. Using a Repipette® sampler, 0.25 ml of the internal standard solution were added to the plasma sample, which was shaken for 5 min (Heidolph DSG 304 vertical mixer). To saturate the aqueous phase, 1.8 g of solid sodium carbonate decahydrate were added before 7 ml of the extraction solvent, diethyl ether-dichloromethane (2:1, v/v). The ampoule was sealed with a polyethylene cap and shaken for 30 min (Infors TR 1 horizontal shaker at 200 rpm). After centrifugation for 5 min (MSE Multex at 940 g), the aqueous phase was frozen in a mixture of dry ice-ethanol. The organic layer was transferred into a 40×16 mm disposable glass ampoule and the solvent was evaporated by gently blowing nitrogen into the ampoule at 40°C. The ampoule was placed in a desiccator and evacuated for 10 min to completely eliminate the dichloromethane. The extraction residue was reconstituted in 0.2 ml of the mobile phase and the solution was transferred into a micro-injection vial. The sealed vial was centrifuged for 1 min to ensure that no air bubbles or particulate matter would be injected into the chromatograph.

RESULTS AND DISCUSSION

Extraction yield

Solvent extraction after dilution of the samples was found to be a suitable method of isolating the compounds to be analysed from plasma. Besides offering

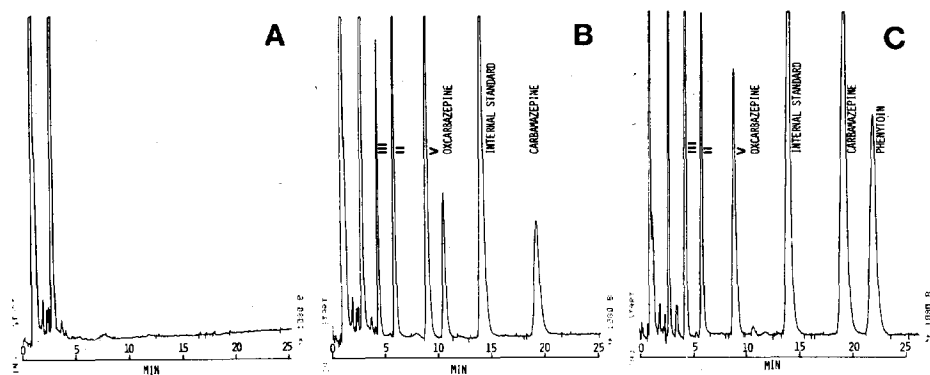


Fig. 2. Chromatograms of an extract of 0.5 g of blank plasma (A), 0.5 g of plasma spiked with oxcarbazepine, carbamazepine and three of their metabolites at concentration levels of 4 nmol/g, and 8 nmol/g of internal standard (B), and 0.5 g of plasma from a patient treated with carbamazepine, oxcarbazepine and phenytoin (C).

a high degree of purification, the single-step solvent extraction permits the extract to be concentrated before injection.

The extraction yield of all the compounds to be analysed was determined by comparison of the peak areas after direct injection of known amounts of the compounds with those after injection of the reconstituted extract of spiked plasma samples that had undergone the full work-up procedure. The extraction yields (mean \pm S.D.), determined at eight to eleven different concentration levels in the range 0.8–16 nmol/g, were $79.5 \pm 3.6\%$, $89.9 \pm 3.6\%$, $67.4 \pm 4.0\%$, $91.2 \pm 3.0\%$, $93.7 \pm 5.8\%$ and $88.7 \pm 4.8\%$ for I–VI, respectively. For VI, only a concentration of 15.856 nmol/ml (4 μ g/ml) was tested.

Selectivity

With the described procedure, a good separation of all the compounds from each other and from the plasma components was obtained (Fig. 2). The following drugs were tested for interference in the present assay method: ethosuximide, primidone, phenobarbital, phenytoin and valproic acid. None of these anticonvulsants interfered with either carbamazepine or oxcarbazepine, or their metabolites. For phenytoin this is illustrated by a chromatogram of a plasma sample from a patient treated with carbamazepine, oxcarbazepine and phenytoin (Fig. 2).

Calibration

Eleven plasma samples in the concentration range 0.8–16 nmol/g were prepared by adding known amounts of the compounds to 0.5 g of blank human plasma. After addition of 3.964 nmol (1 μ g) of the internal standard, the samples were processed as described above. Volumes of 20 μ l of the reconstituted extracts were injected into the LC system, and the resulting ratio of the peak area of the compound divided by the peak area of the internal standard (F_x) was plotted against the initial concentration.

TABLE I

WITHIN-DAY PRECISION AND ACCURACY FOR THE DETERMINATION OF I-V IN SPIKED HUMAN PLASMA SAMPLES

Compound	Given (nmol/g)	Found (mean, $n=4$) (nmol/g)	Coefficient of variation (%)	Accuracy (%)
I	3.96	3.78	2.1	-4.6
	7.93	7.77	3.1	-2.0
	11.89	11.78	0.7	-0.9
II	3.93	3.98	2.0	+1.3
	7.87	7.77	3.0	-1.3
	11.80	11.59	0.7	-1.8
III	3.70	3.50	2.1	-5.4
	7.40	7.01	3.2	-5.3
	11.10	10.53	0.7	-5.1
IV	4.23	4.22	2.0	-0.2
	8.47	8.47	2.0	± 0.0
	12.70	12.73	0.7	+0.2
V	3.96	3.98	2.0	+0.5
	7.93	7.85	3.0	-1.0
	11.89	11.74	0.7	-1.3

By least-squares linear regression analysis the following parameters for the calibration curves ($F_x = a + bx$) in the range 0.8–16 nmol/g were obtained:

$$F_x = 0.04 + 1.31 x, \quad S_y = 0.06, \quad r = 0.9994 \text{ (I)}$$

$$F_x = 0.00 + 2.01 x, \quad S_y = 0.14, \quad r = 0.9985 \text{ (II)}$$

$$F_x = 0.12 + 1.29 x, \quad S_y = 0.12, \quad r = 0.9973 \text{ (III)}$$

$$F_x = -0.05 + 1.77 x, \quad S_y = 0.07, \quad r = 0.9995 \text{ (IV)}$$

$$F_x = -0.09 + 2.48 x, \quad S_y = 0.11, \quad r = 0.9994 \text{ (V)}$$

S_y denotes the estimated standard deviation and r the correlation coefficient.

Single-point calibration. For analysis of large series, the single-point calibration method was used. The calibration line was constructed using the origin and the mean peak-area ratio obtained from three spiked plasma samples containing the five compounds at a concentration of 16 nmol/g.

Limit of quantitation

The limit of quantitation was estimated to be twice the standard deviation (S_y) of the calibration curve and ranged between 0.1 and 0.3 nmol/g for the five compounds.

Precision and accuracy

Together with the clinical samples, spiked plasma samples were analysed to determine the recovery (Tables I and II).

Within-day precision and accuracy. The coefficients of variation (C.V. = S.D. $\times 100/\text{mean}$) of four samples each at concentrations of 4, 8 and 12 nmol/g were 2, 3, and 0.7%, respectively, for all five compounds analysed. Deviations from the

TABLE II

DAY-TO-DAY PRECISION AND ACCURACY FOR THE DETERMINATION OF I-V ON FIVE DIFFERENT DAYS

Compound	Given (nmol/g)	Found (mean, $n=20$) (nmol/g)	Coefficient of variation (%)	Accuracy (%)
I	3.96	3.92	2.0	-1.0
	7.93	7.89	2.4	-0.5
II	3.93	4.08	5.6	+3.8
	7.87	8.06	2.7	+2.4
III	3.70	4.17	11.0	+12.7
	7.40	7.63	3.8	+3.1
IV	4.23	4.16	3.1	-1.7
	8.47	8.39	1.0	-0.9
V	3.96	4.02	2.2	+1.5
	7.93	8.02	1.0	+1.1

concentrations given were between -5.4 and $+1.3\%$ at 4 nmol/g, between -5.3 and $\pm 0.0\%$ at 8 nmol/g and between -5.1 and $+0.2\%$ at 12 nmol/g.

Day-to-day precision and accuracy. The C.V. of the mean values observed on five days were between 11.0% (III) and 2.0% (oxcarbazepine) at a concentration of 4 nmol/g; at 8 nmol/g they were between 3.8% (III) and 1.0% (V and carbamazepine). Deviations from the concentrations given were between -1.7% (carbamazepine) and $+12.7\%$ (III) at a concentration of 4 nmol/g and between -0.9% (carbamazepine) and $+3.1\%$ (III) at 8 nmol/g.

Application of the method

The method was used to measure the plasma concentrations of carbamazepine, oxcarbazepine and their metabolites in patients receiving therapeutic doses of both anticonvulsants (Fig. 3). Blood samples were collected before intake of the morning dose at intervals of at least one week.

Patient No. 1, who was on chronic treatment with phenobarbital [100 mg three times a day (t.i.d.)], started the titration phase with 100 mg twice a day (b.i.d.) of carbamazepine. In weekly intervals the daily dose was increased to 200 , 300 and 400 mg b.i.d. From week 6 to week 19 the dose was kept at 500 mg b.i.d. During weeks 20–23 the carbamazepine tablets were gradually replaced by the same number of oxcarbazepine tablets, corresponding to a dose of 750 mg b.i.d.

Patient No. 2 was on chronic treatment with clonazepam (2 mg t.i.d.) and phenytoin (200 , 100 , 200 mg per day). The patient started the titration phase of the trial with a dose of 150 mg b.i.d. of oxcarbazepine. In weekly intervals the daily dose was increased to 300 , 400 , 600 and 750 mg b.i.d. of oxcarbazepine. During weeks 21–25 the oxcarbazepine tablets were gradually replaced by the same number of carbamazepine tablets corresponding to a daily dose of 500 mg b.i.d.

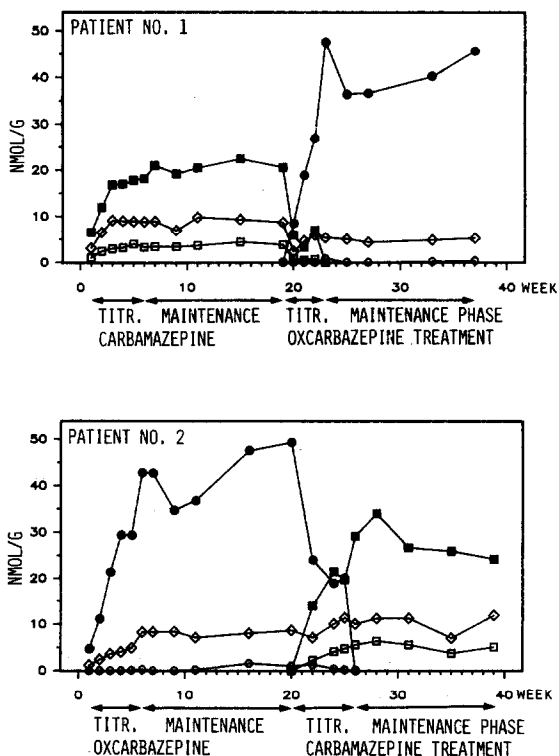


Fig. 3. Steady-state concentrations of carbamazepine (■), 10,11-epoxycarbamazepine (□), oxcarbazepine (○), 10,11-dihydro-10-hydroxycarbamazepine (●), and 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (◇) in two patients. Each dot represents the concentration of the drug just before intake of the morning dose.

CONCLUSIONS

The LC method described for simultaneous analysis of carbamazepine, oxcarbazepine and three of their metabolites allows monitoring of plasma concentrations in samples of epileptic patients receiving carbamazepine, oxcarbazepine and other anticonvulsants. Chromatographic peaks from common comedications are separated and do not interfere with the assay.

REFERENCES

- 1 R.J.Porter, in D.M. Woodbury, J.K. Penry and C.E. Pippenger (Editors), *Antiepileptic Drugs*, Raven Press, New York, 2nd ed., 1982, pp. 167-175.
- 2 N. Wad, *J. Chromatogr.*, 305 (1984) 127-133.
- 3 V. Baltzer and M. Schmutz, in H. Meinardi and A.J. Rowan (Editors), *Advances in Epileptology*, 1977, Swets & Zeitlinger, Amsterdam, Lisse, 1978, pp. 295-299.
- 4 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, Amsterdam, Lisse, 1978, pp. 290-294.
- 5 G. Menge and J.P. Dubois, *J. Chromatogr.*, 275 (1983) 189-194.
- 6 A. Noirfalise, A. Collinge, *J. Chromatogr.*, 274 (1983) 417-420.
- 7 N.A. Kristensen, B. Klitgaard, B. Jönsson and S. Sindrup, *Acta Neurol. Scand.*, 68 (1983) 145-150.