Journal of Chromatography, 528 (1990) 473–479
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5244

Note

Simple and rapid micro-analytical high-performance liquid chromatographic technique for the assay of oxcarbazepine and its primary active metabolite 10-hydroxycarbazepine

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(First received December 5th, 1989; revised manuscript received January 30th, 1990)

Oxcarbazepine (10,11-dihydro-10-oxo-5*H*-dibenz[*b,f*]azepine-5-carboxamide), a keto derivative of carbamazepine, is presently undergoing clinical evaluation and has been found useful in the management of epilepsy [1–4], trigeminal neuralgia [5,6], affective disorders [7] and spasticity [8]. The efficacy and therapeutic spectrum of action of oxcarbazepine is similar to carbamazepine but it has a lower propensity to produce neurological side-effects and allergic reactions [9] and has shown no significant hepatic enzyme induction effects [10].

Oxcarbazepine is rapidly absorbed after oral ingestion [11,12] and in man it is quickly and extensively metabolized to 10,11-dihydro-10-hydroxycarbamazepine (10-hydroxycarbazepine) and *trans*-10,11-dihydro-10,11-dihydroxycarbamazepine. The primary metabolite, 10-hydroxycarbazepine, is pharmacologically active [5,13] with an elimination half-life of 10–12 h [14,15].

0378-4347/90/$03.50

Since after dosing oxcarbazepine is present at low concentrations for only 3–5 h, oxcarbazepine can be considered a prodrug.

As a potential first-line antiepileptic drug it is essential to determine concentration–effect relationships, to undertake pharmacokinetic studies and to evaluate the usefulness of therapeutic drug monitoring in patients with epilepsy. A number of methods are presently available for the measurement of oxcarbazepine and 10-hydroxycarbazepine by high-performance liquid chromatography (HPLC) and gas chromatography (GC) [12,14,16–20]. However, there are a number of disadvantages to these methods as they involve complex buffer extraction procedures and require large sample volumes (0.5–1.0 ml). Additionally some of these methods use as internal standard, compounds such as carbamazepine [16], carbamazepine-10,11-epoxide [12,17] or hexobarbital [19], all of which may be present in the plasma of patients with epilepsy.

We report a simple, rapid reversed-phase HPLC micro-procedure for the simultaneous measurement of oxcarbazepine and 10-hydroxycarbazepine using 10-methoxycarbamazepine as internal standard, a compound not normally administered, nor present as a metabolite in plasma of patients with epilepsy.

EXPERIMENTAL

Materials

Oxcarbazepine, 10-hydroxycarbazepine and 10-methoxycarbamazepine were obtained from Ciba Geigy Pharmaceuticals (Horsham, U.K.). All solvents were of HPLC grade. Water and acetonitrile were obtained from FSA (Loughborough, U.K.) and methanol and dichloromethane from BDH (Poole, U.K.).

Samples

Blood samples were obtained from the National Hospital for Nervous Diseases blood bank (drug-free plasma) or collected from patients prescribed oxcarbazepine for the management of their epilepsy. Plasma was stored at $-20\,^{\circ}C$ until required for analysis.

Apparatus

The liquid chromatograph used comprised of a Spectra Physics 8440 UV–visible detector, a Hewlett Packard integrator (HP3396A), Gilson 302 and 305 pumps and a dynamic mixer (Model 811B). Chromatograms were run at ambient temperature on a steel column (25 cm $\times$ 4.9 mm I.D.) packed with LiChrosorb RP8, 10 μm (Hichrom, Reading, U.K.). A mobile phase comprising of acetonitrile–water (32:68) at a flow-rate of 1.8 ml/min at 56.7 ± 6.7 bar was used. The column eluate was monitored at 215 nm with a sensitivity range of 0.04 a.u.f.s. and a chart speed of 0.5 cm/min.

Preparation of standard solutions

The stock solutions of oxcarbazepine and 10-hydroxycarbazepine were made up in acetonitrile to a concentration of 1 mg/ml and the stock solution of 10-methoxycarbamazepine as internal standard was made up in methanol to a concentration of 1 mg/ml. Working standards of oxcarbazepine and 10-hydroxycarbazepine were made by diluting 200 and 400 μl of stock solutions to 10 ml with drug-free plasma. Further dilutions were made to give a concentration range of 4.0–63.0 μmol/l for oxcarbazepine and 7–118.0 μmol/l for 10-hydroxycarbazepine. The amount of 10-methoxycarbamazepine in each extraction tube was 2 μg. Oxcarbazepine and 10-hydroxycarbazepine were determined by the ratio of the peak areas of each drug to the peak areas of internal standard plotted against concentration of the drug. Working standards were always made up on the day of analysis.

Extraction procedure

To plastic 2-ml screw-cap tubes (Sarstedt, Leicester, U.K.) were added 250 μl of plasma or standard, 2 μg of 10-methoxycarbamazepine, 25 μl of 1 *M* sodium hydroxide and 1.2 ml of dichloromethane. After mixing for 15 min (Janke and Kunkel VX2E electronic mixer, Epsom, U.K.), the mixture was centrifuged in a micro-centrifuge (Abbott, Maidenhead, U.K.). The solvent extract was then separated and evaporated to dryness at 40 °C under a stream of oxygen-free nitrogen. The residue was dissolved in 20 μl of acetonitrile, and 10 μl were injected into the chromatograph.

RESULTS AND DISCUSSION

Quantitation

Quantitation was achieved by the peak-area ratios of the drugs to internal standard. To determine the linearity of the method, various standards ranging from 4 to 65 μmol/l for oxcarbazepine and 7.0 to 118.0 μmol/l for 10-hydroxycarbazepine were prepared by adding known amounts of each drug to blank plasma, extracted and analysed. The concentrations and peak-area ratios (standard drug to internal standard) were linearly related over this range. Linearity for 10-hydroxycarbazepine was tested and confirmed up to a concentration of 200 μmol/l (data not shown).

Sensitivity

The limit of quantitation for both oxcarbazepine and 10-hydroxycarbazepine was 2 μmol/l in plasma. The limit of detection was 0.5 μmol/l.

Selectivity

Commonly prescribed antiepileptic drugs were analysed for possible chromatographic interference. Blank plasma spiked with antiepileptic drug con-

TABLE I

RETENTION TIMES OF COMMONLY PRESCRIBED ANTIEPILEPTIC DRUGS AND METABOLITES

Drug	Retention time[a] (min)
Phenobarbitone	2.3
Primidone	2.7
10-Hydroxycarbazepine	3.1
Carbamazepine-10,11-epoxide	4.2
Oxcarbazepine	4.8
Carbamazepine	7.0
10-Methoxycarbamazepine	9.3
Clobazam	No peak
Clonazepam	No peak
Diazepam	No peak
Ethosuximide	No peak
Phenytoin	No peak
Valproic acid	No peak

[a]From time of injection into column.

centrations commonly encountered in clinical practice were extracted and analysed. Chromatographic elution was undertaken for 20 min and Table I lists the retention times of the drugs tested. The UV detection at 215 nm, the use of dichloromethane and the fact that the extraction was at alkaline pH resulted in a number of drugs being preferentially not extractable or detectable (e.g. clobazam, clonazepam, diazepam, ethosuximide, phenytoin and valproic acid). Fig. 1 shows typical chromatograms of (A) drug-free plasma, (B) plasma of patient co-medicated with oxcarbazepine and primidone and (C) plasma of patient co-medicated with oxcarbazepine and carbamazepine.

Precision

Within-batch precision was determined from analysis of pooled plasma samples containing oxcarbazepine and 10-hydroxycarbazepine at three different concentrations (low, medium and high), covering the range commonly encountered in clinical practice. Between-batch precision was similarly determined over a period of two weeks. Coefficients of variation were less than 6% (Table II) which is acceptable for the routine measurement of oxcarbazepine and 10-hydroxycarbazepine.

Recovery

The relative analytical recovery (extractability) from plasma at three different concentrations for oxcarbazepine and 10-hydroxycarbazepine was determined in the following way. The drugs were added to drug-free plasma to

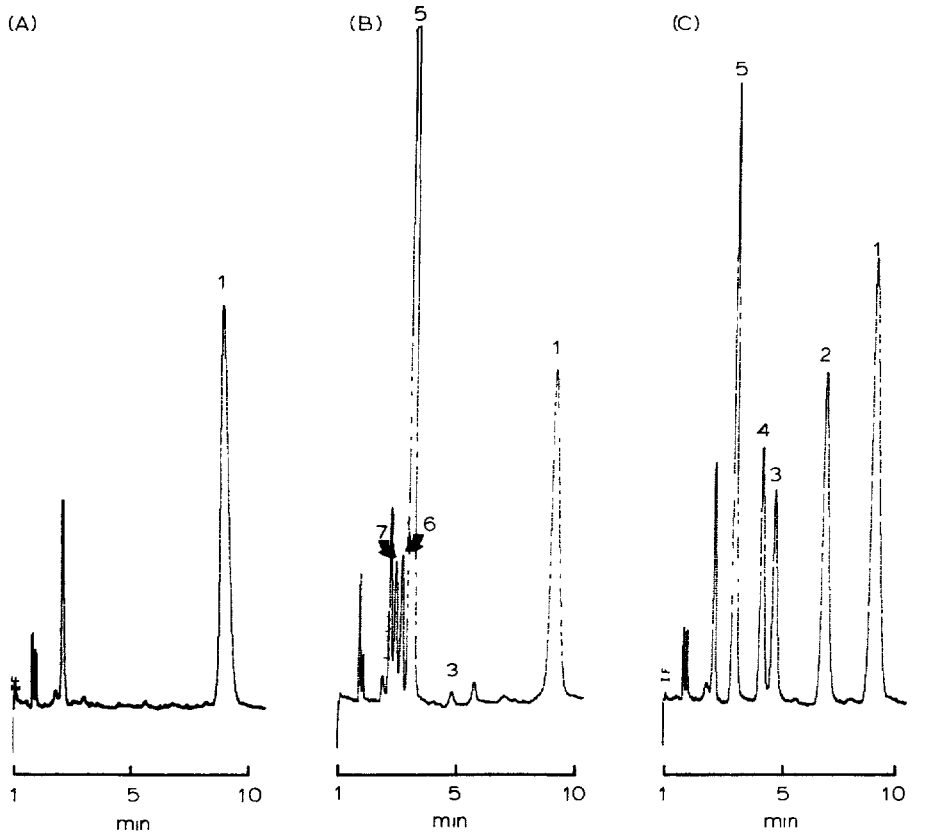

Fig.1. Chromatograms of (A) drug-free plasma, (B) plasma of a patient co-medicated with oxcarbazepine (1500 mg per day) and primidone (250 mg per day) and (C) plasma of a patient co-medicated with oxcarbazepine (1200 mg per day) and carbamazepine (800 mg per day). Peaks: 1 = 10-methoxycarbamazepine; 2 = carbamazepine; 3 = oxcarbazepine; 4 = carbamazepine-10,11-epoxide; 5 = 10-hydroxycarbazepine; 6 = primidone; 7 = phenobarbitone.

achieve the concentrations shown in Table III and after the internal standard was added, extracted and analysed as described above. The relative recovery of these drugs was calculated by comparing the concentrations of the drug-spiked plasma with the actual added concentration. Recoveries ranged from 74 to 78% for oxcarbazepine and 82 to 88% for 10-hydroxycarbazepine.

The method described for the measurement of oxcarbazepine and its primary pharmacologically active metabolite, 10-hydroxycarbazepine, in plasma is sufficiently sensitive and precise to determine both drugs over the entire therapeutic plasma concentration range reported to-date in patients [1–6,21]. Additionally, the method can be adapted to simultaneously measure plasma concentrations of carbamazepine and its active metabolite carbamazepine-10,11-epoxide, primidone and phenobarbitone.

TABLE II

WITHIN-BATCH AND BETWEEN-BATCH PRECISION FOR THE DETERMINATION OF OXCARBAZEPINE AND 10-HYDROXYCARBAZEPINE IN SPIKED HUMAN PLASMA

Values in parentheses are coefficients of variation (%).

Concentration added (μmol/l)	Concentration measured (mean ± S.D.) (μmol/l)	
	Within-batch ($n=10$)	Between-batch ($n=8$)
Oxcarbazepine		
6	6.3 ± 0.3 (4.8)	6.0 ± 0.2 (3.3)
26	27.1 ± 1.6 (5.9)	26.1 ± 1.1 (4.2)
50	49.6 ± 1.8 (3.6)	49.5 ± 2.0 (4.0)
10-Hydroxycarbazepine		
11	11.0 ± 0.6 (5.4)	11.2 ± 0.6 (5.3)
48	49.6 ± 2.0 (4.0)	47.1 ± 2.0 (4.2)
93	93.8 ± 2.5 (3.6)	92.7 ± 3.0 (3.2)

TABLE III

RECOVERY OF OXCARBAZEPINE AND 10-HYDROXYCARBAZEPINE FROM PLASMA

Drug	Concentration (μmol/l)	Recovery (mean ± S.D., $n=5$) (%)
Oxcarbazepine	7.9	74 ± 1
	15.8	77 ± 3
	63.4	78 ± 3
10-Hydroxycarbazepine	14.8	83 ± 4
	29.6	82 ± 2
	118.4	88 ± 3

The extraction procedure is simple, and quantitation is achieved by use of 10-methoxycarbamazepine as internal standard, a compound not normally present in patients' plasma. The use of an acetonitrile–water mobile phase, in contrast to the use of buffers, results in a short analysis time (10 min) and easier maintenance of HPLC equipment which would be important considerations if oxcarbazepine and 10-hydroxycarbazepine were to be monitored routinely.

Finally, the fact that other commonly prescribed antiepileptic drugs do not interfere with the analysis and that some of these antiepileptic drugs may be simultaneously assayed makes the method useful in the present clinical evaluation of oxcarbazepine. The small sample size required (250 μl) is particularly suitable for paediatric micro-samples.

ACKNOWLEDGEMENTS

We thank Miss Debbie Cleminson for her excellent secretarial assistance and Ciba-Geigy Pharmaceuticals (U.K.) for the supply of oxcarbazepine, 10,11-dihydro-10-hydroxycarbamazepine and 10-methoxycarbamazepine.

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