Journal of Chromatography, 342 (1985) 469—471
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2642

Letter to the Editor

Simultaneous determination of anticonvulsant drugs and metabolites in plasma by high-performance liquid chromatography

Sir,

The simultaneous determination of phenobarbital (PB), phenytoin (DPH), carbamazepine (CBZ) and its metabolite 10, 11-epoxycarbamazepine (ECBZ) by high-performance liquid chromatography (HPLC) is well documented [1—6]. However, with the exception of the procedure of Wad [6], none of the published methods includes *trans*-10,11-dihydroxy-10,11-dihydrocarbamazepine (DHCBZ), another metabolite of CBZ. Although mean plasma levels of DHCBZ are greater than those of ECBZ [7, 8], only two papers have dealt with its HPLC quantitation together with CBZ and ECBZ [6, 8]. Moreover, a new anticonvulsant, oxcarbazepine (OCZ), is under clinical evaluation. This last compound, found at very low levels in plasma, is extensively metabolized to DHCBZ and 10-hydroxy-10,11-dihydrocarbamazepine (HCBZ), which is its main metabolite in plasma [9, 10]. Methods for the determination of OCZ and its two metabolites have been described [8, 10—13].

We propose here a simple liquid chromatographic method for the simultaneous quantitation of PB, DPH, CBZ, ECBZ, DHCBZ and HCBZ in plasma.

A 500-μl aliquot of serum or plasma and 500 μl of a 0.3 mol/l phosphate buffer (pH 6.7) are mixed in a 10-ml stoppered glass centrifuge tube, then 2.50 ml of ethyl acetate R.G. containing 1.5 mg/l 9-hydroxymethyl-10-carbamoylacridane (HMCA) (Ciba-Geigy, Basle, Switzerland) and 5 mg/l 5-allyl-5-cyclopentenylbarbituric acid (ACB) (Siegfried, Zofingen, Switzerland) as internal standards are added. The tube is shaken on a rotary mixer for 5 min at 20 rpm and centrifuged. A 2-ml volume of the organic layer is transferred into a 10-ml conical glass centrifuge tube. The extract is evaporated to dryness at 50°C under a stream of nitrogen. The mobile phase described below (100 μl) is added to the dry residue. After vortex mixing, 40 μl are injected.

As the liquid chromatographic system, a Pye Unicam (Cambridge, U.K.) 4010 dual-piston pump, a Rheodyne (Berkeley, CA, U.S.A.) 7125 injection valve with a 500 μl-loop, an LKB Uvicord SII 2338 fixed-wavelength detector

0378-4347/85/$03.30

(Bromma, Sweden) and two Kipp and Zonen (Delft, The Netherlands) BD 40 recorders are used.

Analyses are performed on a reversed-phase 5-μm Spherisorb ODS column (150 × 4.6 mm I.D.) protected by a guard column packed with a pellicular reversed-phase material (Chrompack 28984 and 28623, Middelburg, The Netherlands). The mobile phase is water—methanol (R.G.)- acetonitrile (R.G.) (64:22:14, v/v), degassed by helium sparging. The operating conditions are as follows: mobile phase flow-rate 1.4 ml/min; ambient temperature; detector wavelength, 254 nm; time constant, 2 sec; sensitivity, 0.01 and 0.05 a.u.f.s. (simultaneous dual recording).

The following peak height ratios are calculated for each sample: PB/ACB, DPH/HMCA, CBZ/HMCA, DHCBZ/HMCA, HCBZ/HMCA and ECBZ/HMCA. The concentration of each compound is calculated by comparison with the ratio obtained for a calibration serum analysed under identical conditions. The latter is a drug-free human serum pool spiked with known amounts of the six compounds assayed.

The chromatograms show good resolution. The retention times are 3.4 (DHCBZ), 3.8 (PB), 4.4 (HCBZ), 5.6 (ACB), 6.2 (ECBZ), 7.9 (HMCA), 9.0 (DPH) and 13.4 min (CBZ). The plate heights range from 0.020 to 0.028 mm and the selectivities between two consecutive peaks (α) from 1.12 to 1.52.

Salicylate, theophylline, phenylethylmalonamide, ethosuximide, caffeine, primidone, oxcarbazepine and iminostilbene elute at 1.1, 1.7, 1.9, 2.1, 2.5, 2.8, 7.6 and 10.5 min, respectively. None of these substances interfere. Valproic acid is not detected. Endogenous constituents of the samples do not interfere.

The detection limits (mg/l) are 0.2 (PB), 0.4 (DPH), 0.05 (CBZ), 0.1 (ECBZ), 0.2 (DHCBZ) and 0.2 (HCBZ). The within-run and between-run coefficients of variation (%) estimated under routine conditions are 3.6 and 5.8 (PB), 2.3 and 3.7 (DPH), 2.1 and 3.0 (CBZ), 5.6 and 8.4 (ECBZ), 4.8 and 9.1 (DHCBZ) and 2.9 and 4.1 (HCBZ). This good precision results from the use of two internal standards, a barbiturate for PB [14] and an acridane derivative for the tricyclic substances and for DPH [13]. Of the extraction solvents tested (ethyl acetate, dichloromethane, chloroform, methyl isobutyl ketone, diethyl ether and diisopropyl ether), ethyl acetate yields the best compromise between drug recovery and extraction specificity [8, 13].

Compared with the only other procedure described for determining these six compounds [6], our method has two advantages, namely that lower retention times are obtained and that gradient elution and column heating are unnecessary, thus ensuring greater practicability and rapidity.

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(First received November 27th, 1984; revised manuscript received March 23rd, 1985)