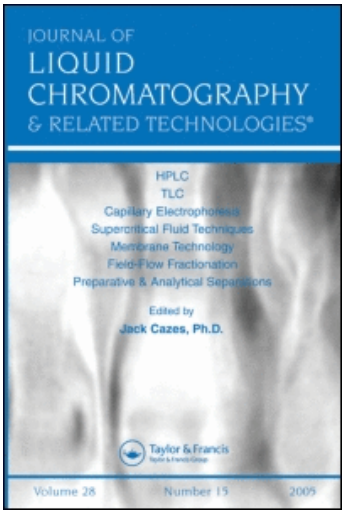

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SIMULTANEOUS HPLC DETERMINATION OF OXCARBAZEPINE, CARBAMAZEPINE AND THEIR METABOLITES IN SERUM.

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ABSTRACT

We propose a simple procedure for the simultaneous determination of the anticonvulsants oxcarbazepine, carbamazepine and three of their metabolites (10-hydroxy-10,11-dihydro-carbamazepine, trans-10,11-dihydroxy-10,11-dihydro-carbamazepine and 10,11-epoxy-carbamazepine) in serum or plasma. The alkalinized sample is extracted with ethyl acetate. The extract is evaporated to dryness and taken up with the mobile phase. An aliquot is injected into the liquid chromatograph and eluted with water/methanol/acetonitrile (55/40/5, by vol.) on a 5-μm C-18 reversed-phase column. Eluent is monitored at 254 nm. No interference by other anticonvulsants or by endogenous constituents from the sample is observed. Owing to its good precision, specificity, sensitivity, and selectivity, this method is well adapted to the therapeutic monitoring of oxcarbazepine or carbamazepine treated patients, as well as for pharmacokinetic studies.

INTRODUCTION

Carbamazepine (CBZ) is an effective anticonvulsant drug mainly used against psychomotor and generalized tonic-clonic seizures. Oxcarbazepine (OCZ), the 10,11-dihydro-10-keto derivative of CBZ, has shown anticonvulsant properties in animals and humans (1) and is now under clinical evaluation. In man, CBZ is partially metabolized to 10,11-epoxy-carbamazepine (ECBZ), iminostilbene, and trans-10,11-dihydroxy-10,11-dihydro-carbamazepine (DHCBZ) (2,3).

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OCZ is extensively converted into 10-hydroxy-10,11-dihydro-carbamazepine (HCBZ), which is its main metabolite in serum, and into DHCBZ (4-6).

The clinical interest of management of CBZ serum levels is now well established (2). Determination of its metabolites is also of interest in therapeutic monitoring and, more evidently, in pharmacokinetic studies. The clinical usefulness of the quantitation of OCZ and/or its metabolites in serum is under evaluation. Yet, pharmacokinetic studies needs for determination of both parent drug and metabolites. Our aim was to develop a single method for the determination of OCZ, CBZ and their respective metabolites in serum.

EXPERIMENTAL

Reagents and Standards

Ethyl acetate R.G., methanol R.G., acetonitrile R.G. and NaOH 1 mol/l were obtained from Merck (Darmstadt, GFR). Water was deionised and distilled in a glass apparatus. CBZ, ECBZ, DHCBZ, OCZ, HCBZ, and 9-hydroxymethyl-10-carbamoylacridane were kindly supplied by Ciba-Geigy (Basel, Switzerland).

Extraction solvent: ethyl acetate containing 0.5 mg 9-hydroxymethyl-10-carbamoylacridane per liter, as internal standard. This solution is stable at 4°C for at least 4 months.

Mobile phase: water/methanol/acetonitrile (55/40/5, by vol.) degassed by helium sparging.

Apparatus

The liquid chromatograph consisted of a Pye-Unicam 4010 dual piston pump (Cambridge, UK), a Rheodyne 7125 injection valve with a 500- µl loop (Berkeley, CA, USA), a fixed wavelength LKB Uvicord SII 2338 detector (Bromma, Sweden), and a Kipp and Zonen BD 40 recorder (Delft, The Netherlands).

Analysis were performed on a reversed-phase 5-µm spherical C-18 Resolve column (150 mm x 3.9 mm id) (Waters Ass., Milford, MA, USA).

Operating Conditions

Mobile phase flow rate: 0.9 ml/min; temperature: ambient; detector wavelength : 254 nm, time constant: 2 s, sensitivity: 0.01 and 0.05 A full scale.

Procedure

Into a 10-ml stoppered glass centrifuge tube, pipette 500 µl of serum or plasma, add 50 µl of NaOH 1 mol/l and mix. Add 2.50 ml of extraction solvent. Shake on a rotary mixer for 5 min at 20 rpm and centrifuge. With a 1-ml Eppendorf pipette, transfer 2 ml of the organic layer into a 10-ml conical glass centrifuge tube. Evaporate to dryness at 50°C under a stream of nitrogen. Add 100 µl of mobile phase to the dry residue, vortex and inject 40 µl.

Quantitation

Each drug or metabolite was quantitated by measuring the ratio of its peak height to that of the internal standard, and by comparing with the ratio obtained for a calibration serum analysed under identical conditions.

The calibration serum was prepared by mixing 99.4 volumes of a drug-free human serum with 0.6 volume of a fresh ethanolic solution of CBZ, HCBZ, DHCBZ, OCZ, and ECBZ at 2000, 2000, 1000, 500, and 500 mg/l, respectively. Frozen aliquots of this serum are stable for at least 4 months.

RESULTS

Figure 1 shows the chromatograms of a drug-free serum, the calibration serum, and sera from patients treated with OCZ and CBZ, respectively. Retention times are 4.2 (DHCBZ), 5.2 (HCBZ), 6.1 (ECBZ), 7.6 (OCZ), 9.2 (internal standard), and 13.8 min (CBZ).

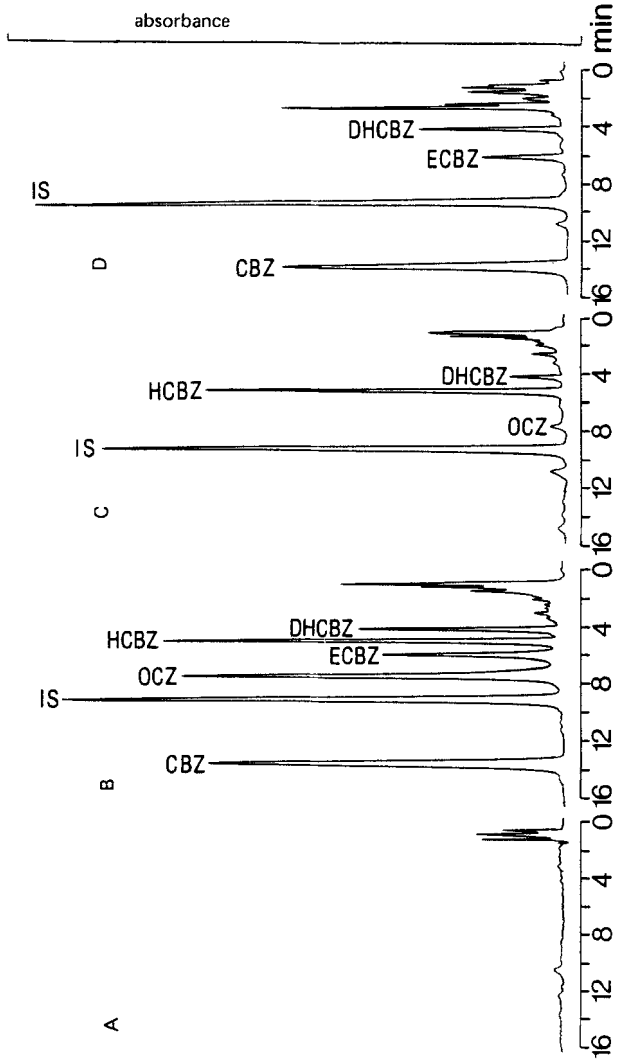

FIGURE 1. Chromatograms of (A) a drug-free serum extracted without internal standard; (B) the calibration serum containing DHCBZ (6.0 mg/l), HCBZ (12.0 mg/l), ECBZ (3.0 mg/l), OCZ (3.0 mg/l), and CBZ (12.0 mg/l); (C) a serum from a patient treated with OCZ; (D) a serum from a patient treated with CBZ.
Sensitivity: 0.01 A full scale, except for CBZ (0.05 A). IS: internal standard.

TABLE 1

Precision, Detection Limit and Linearity for CBZ, OCZ and their Metabolites in Serum.

	within-run precision CV% (*)	detection limit (**)	linearity (***) range	slope	intercept	S_{yx}
CBZ	2.2% (2N=40) (8.50)	0.05	0.2-25	0.2694	0.0130	0.0901
ECBZ	4.6% (2N=40) (1.16)	0.1	0.2-10	0.1077	-0.0054	0.0214
DHCBZ	12.5% (2N=78) (1.83)	0.2	0.5-20	0.0643	-0.0061	0.0230
OCZ	5.6% (2N=28) (2.60)	0.05	0.2-20	0.2170	0.0679	0.1647
HCBZ	2.2% (2N=40) (12.9)	0.2	0.2-30	0.0605	0.0062	0.0080

(*) estimated from duplicates of patients'sera analysed under routine conditions, with the exception of OCZ, for which precision is estimated from calibration or control sera.
The mean levels (mg/l) of all these sera are in parentheses.

(**) for a signal to baseline noise ratio of 3 (in mg/l)

(***) the calibration curve regression is estimated over the levels range indicated (6 points, mg/l). All correlation coefficients are more than 0.996. S_{yx} : standard error of estimate.

Salicylate, theophylline, caffeine, ethosuximide, primidone, phenobarbital, phenytoin, and iminostilbene elute at 1.8, 2.0, 2.6, 2.6, 3.4, 4.6, 10.3, and 10.4 min, respectively. Valproic acid is not detected. None of these compounds interfere. Table 1 gives precision, detection limit and linearity for the five compounds analysed.

DISCUSSION

In order to achieve simultaneous determination of OCZ, CBZ and three of their metabolites without interference by other anticonvulsants or endogenous constituents from the sample, we carefully selected the mobile phase, the column packing and the extraction procedure.

TABLE 2

Mean Serum Levels ± SD (mg/l) found in OCZ or CBZ treated Patients.

	OCZ treated patients (N=20)	CBZ treated patients (N=20)
CBZ	—	8.50 ± 2.78
ECBZ	—	1.16 ± 0.54
DHCBZ	1.15 ± 1.14	2.50 ± 1.35
OCZ	0.18 ± 0.15	—
HCBZ	12.87 ± 7.37	—

The 5-µm C-18 stationary phase was chosen for its capacity to adequately resolve the compounds analysed (resolution from 1.63 to 6.13; plate heights from 0.070 to 0.038 mm). The excellent separation of the 5 compounds and of possible interfering drugs such as phenobarbital and phenytoin was also obtained by recourse to a ternary mobile phase which is composed of commonly used solvents, avoiding column equilibration with mobile phase additives.

The sample is alkalinized to decrease the recovery of phenobarbital and phenytoin, high concentrations of which might cause quantitation problems. The following extraction solvents were tried: ethyl acetate, methyl-isobutylketone, dichloromethane, ethyl ether, di-isopropylether, and several mixtures of these solvents. Methyl-isobutylketone and dichloromethane yield clean chromatograms and do not extract phenobarbital and phenytoin. However, lesser extraction efficiency of the compounds to be analysed was observed. Overall best results were obtained with ethyl acetate. This solvent was first proposed by Dörhöfer (7), whose extraction procedure was applied here with some modifications.

Our procedure provides two advantages over published determinations of OCZ (5-8): 1°) interference by phenobarbital, phenytoin and other anticonvulsants is not encountered, 2°) CBZ and its metabolites may also be simultaneously assayed.

Preliminary results of OCZ, CBZ and metabolites quantitation in serum of chronically treated patients are given in table 2.

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