Journal of Chromatography, 573 (1992) 65–68
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6113

Automated microanalysis of carbamazepine and its epoxide and *trans*-diol metabolites in plasma by column liquid chromatography

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(First received November 15th, 1990; revised manuscript received June 11th, 1991)

ABSTRACT

A fully automated high-performance liquid chromatographic procedure for the simultaneous determination of carbamazepine and its main metabolites, epoxycarbamazepine and dihydroxycarbamazepine, in plasma is described. Liquid–solid extraction on disposable C_{18} columns and reversed-phase chromatography on a 3 μm particle size C_{18} column were combined and automated by using the Automatic Sample Preparation with Extraction Columns system. Ultraviolet detection was performed at 210 nm. 5,6-Dihydro-11-oxo-11*H*-dibenz[*b*,*e*]azepine-5-carboxamide was used as internal standard. A small plasma volume (100 μl) was required. The total run time for the assay of one sample was about 10 min. The assay demonstrated good reproducibility. The limit of quantitation was 0.1 μmol/l (about 25 ng/ml).

INTRODUCTION

Several methods have already been published for the simultaneous determination of the anticonvulsant, carbamazepine, and its epoxide and *trans*-diol metabolites in plasma [1–3]. These methods involve manual liquid–liquid extraction from plasma followed by reversed-phase high-performance liquid chromatography (HPLC). The aim of this work was to develop an automated assay which requires a plasma volume smaller than in the previously described methods. Liquid–solid extraction via disposable extraction columns (DECs) was applied in conjunction with reversed-phase chromatography on a short 3 μm particle size column. Both sample preparation and injection were fully automated by the new Automatic Sample Preparation with Extraction Columns (ASPEC) system [4].

EXPERIMENTAL

Materials

Carbamazepine (5*H*-dibenz[*b*,*f*]azepine-5-carboxamide), its epoxide metabolite (10,11-epoxycarbamazepine), its *trans*-diol metabolite (10,11-dihydro-10,11-transdihydroxycarbamazepine) and the internal standard (5,6-dihydro-11-oxo-11*H*-dibenz[*b*,*e*]azepine-5-carboxamide) were provided by Ciba-Geigy (Basle, Switzerland). The structures of these compounds are shown in Fig. 1.

Reagents

Potassium dihydrogenphosphate and dipotassium hydrogenphosphate were purchased from Merck (Darmstadt, Germany). Acetonitrile (HPLC quality) and methanol (Royal Society–American Chemical Society quality) were from Carlo Erba France (Puteaux, France). Water was purified and deionized using a Milli-Q reagent-

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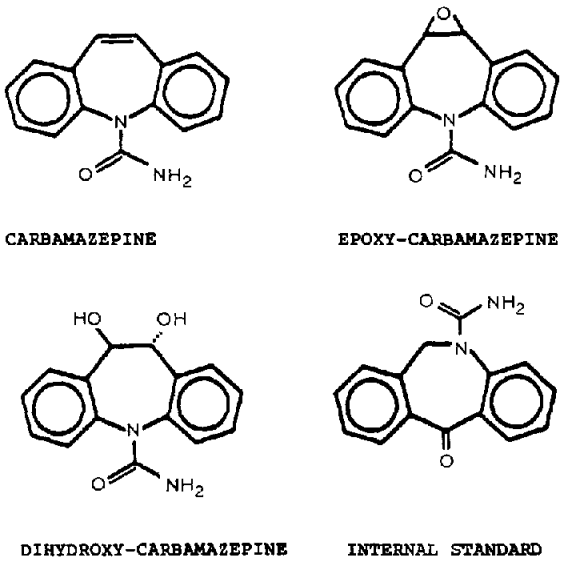

Fig. 1. Structures of the compounds investigated.

grade water system (Millipore, Bedford, MA, USA).

Apparatus

The chromatographic system consisted of a Model 303 pump (Gilson, Villiers-le-Bel, France), an ASPEC system (Gilson) and a Model Spectroflow 783 variable-wavelength UV detector (Kratos, Ramsey, NJ, USA) set at 210 nm with a response time of 1 s. A Model C-R3A integrator recorder (Shimadzu, Kyoto, Japan) was used for data capture.

Columns and filter

A guard column (stainless-steel tube, 3.3 cm × 4.7 mm I.D.) was placed between the pump and the injector. It was filled in our laboratory. A 0.8-g aliquot of Partisil 10 ODS-3, 10 μm particle size (Whatman, Clifton, NJ, USA), was suspended in 27 ml of isopropanol–methanol (80:20, v/v). Methanol was used as the pressurizing solvent. The filling pressure was set at 450 bar for 30 min with a Haskel pneumatic pump.

The analytical column (stainless-steel tube, 3.3 cm × 4.6 mm I.D.) was prepacked with Supelcosil LC-18, 3 μm particle size (Supelco France, St-Germain-en-Laye, France). A filter (No. FL 01, Société Française Chromato Colonne, Neuilly-Plaisance, France) with a replaceable 2-μm-pore frit was inserted between the injector and the analytical column.

Disposable extraction columns

C_{18} DECs of 1 ml capacity filled with 50 mg were used. They were manufactured by J. T. Baker (Deventer, Netherlands) and supplied by Sochibo (Vélizy-Villacoublay, France).

Calibration solutions

Stock solutions (50 μmol/l for the internal standard and 400 μmol/l for carbamazepine and its metabolites) were prepared in methanol–water (50:50, v/v). The reference solutions for calibration were prepared by dilution with the stock solution containing the internal standard.

Sample handling

Aliquots of 100 μl of plasma from healthy volunteers, 100 μl of water and 10 μl of internal standard (or calibration) solution were introduced into a vial and shaken on a vortex mixer for a few seconds. The vial was then placed on the appropriate rack of the ASPEC system.

Each of the following operations on the samples was performed automatically by the ASPEC system. The automatic sequences were as follows.

DEC conditioning: draw up 2 ml of methanol and dispense onto the 50-mg DEC, then dispense 2 ml of water (flow-rate 100 μl/s).

Extraction: draw up 200 μl of diluted plasma sample and dispense onto the DEC (flow-rate 25 μl/s); draw up 1 ml of 0.02 mol/l dipotassium hydrogenphosphate and dispense onto the DEC, then dispense 1 ml of water–methanol (95:5, v/v) (flow-rate 100 μl/s); shift the rack containing the DECs on top of the part of the rack containing the collection tubes; draw up 250 μl of methanol and dispense onto the DEC (flow-rate 50 μl/s) (the eluate is collected in the tube positioned under the DEC); pull back the rack containing the DECs.

Injection: draw up 950 μl of water and mix with the eluate by bubbling; draw up 1000 μl of the mixture and dispense through a 500-μl injection loop; inject.

Each plasma sample was prepared separately during the chromatography of the previous sample. After each liquid transfer, the needle was

rinsed with 1 ml of water. A 50-μl segment of air was created before pipetting the liquid to be transferred in order to avoid cross-contamination.

Chromatography

The chromatography was carried out at room temperature. The mobile phase was acetonitrile–methanol–pH 5 aqueous solution of 0.05 mol/l potassium dihydrogenphosphate (15:5:80, v/v/v). The flow-rate of the mobile phase was 2 ml/min and the total pressure drop across the guard column and the analytical column was around 100 bar.

RESULTS AND DISCUSSION

The ASPEC system and its use to carry out the full automation of both liquid–solid extraction and injection onto the analytical column have been previously described [4–6]. For the present procedure, two rinsing steps were necessary to eliminate interfering compounds. Before injection, the eluting strength of the eluate was decreased by addition of water in order to allow a direct automatic injection [6]. A large volume of diluted extract (500 μl) was injected in order to achieve sufficient sensitivity. The overall recovery of the extraction from plasma was 89% for carbamazepine, 90% for its epoxide metabolite and 89% for its *trans*-diol metabolite (concentration 10 μmol/l, $n = 2$), as compared with a direct injection. The extraction can be performed with 100-mg DECs, the volumes of the conditioning, rinsing and elution steps being doubled.

Carbamazepine, its metabolites and the internal standard were well separated from plasma components (Fig. 2). The time of chromatography and the time required by the ASPEC system for sample preparation and injection were similar, one sample being treated every 10 min. Diluted plasma samples spiked with the three compounds and the internal standard were stable for at least 15 h at room temperature on the rack of the ASPEC system, thereby allowing high sample throughput.

A guard column was inserted between the pump and the injector, and a filter was inserted between the pump and the analytical column in order to prevent column clogging. The filter frit was exchanged twice a week.

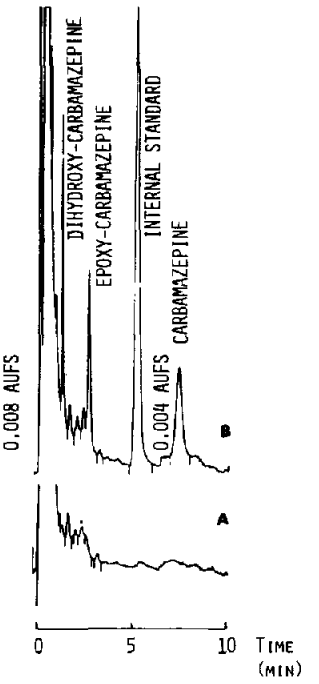

Fig. 2. Chromatograms of extracts of (A) blank plasma and (B) plasma containing 0.5 μmol/l carbamazepine and its metabolites and 5 μmol/l internal standard.

Linearity, accuracy and precision

Calibration graphs were obtained by plotting the peak-height ratio (carbamazepine or one of its metabolites/internal standard) *versus* the concentration of either compound in the plasma sample. Their equations were calculated by the least-squares method using weighted linear regression with a weighting factor of $1/(\text{concentration})^2$ [7]. The coefficient of correlation was about 0.9995.

The within-day accuracy and precision of the method were assessed by using series of six plasma samples spiked with different concentrations of carbamazepine and its metabolites. These samples were prepared and analysed on the same day. The precision was high, as shown by the low values of the coefficients of variation (C.V.) between 1 and 4% for concentrations between 0.5 and 10 μmol/l (Table I).

The between-day accuracy and precision were assessed by analysing on different days plasma samples spiked with different concentrations.

TABLE I

WITHIN- AND BETWEEN-DAY PRECISION AND ACCURACY OF THE ASSAY

To convert into μg/ml, multiply the data by 0.2363 for carbamazepine, 0.2523 for epoxycarbamazepine and 0.2703 for dihydroxycarbamazepine

Nominal plasma concentration (μmol/l)	Within-day (n = 6)		Between-day (n = 4)	
	Nominal/found (mean) (%)	C.V. (%)	Nominal/found (mean) (%)	C.V. (%)
Carbamazepine				
0.1	95	9	100	10
0.5	101	4	106	6
2	98	3	103[a]	4
10	101	2	103	3
Epoxycarbamazepine				
0.1	97	12	95[a]	6
0.5	101	2	103	3
2	102	1	100[a]	3
10	102	1	105	8
Dihydroxycarbamazepine				
0.1	101	4	98[a]	10
0.5	104	4	102	3
2	99	3	102[a]	3
10	96	3	100	3

[a] n = 3.

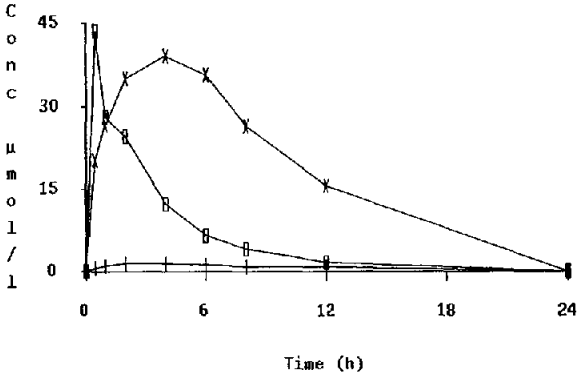

Fig. 3. Plasma profiles of carbamazepine and its epoxide and *trans*-diol metabolites in a rat following peroral single administration of a 50 mg/kg dose of carbamazepine. □ = Carbamazepine; X = epoxide metabolite; + = *trans*-diol metabolite.

The coefficients of variation were lower than 10% and the recoveries (amount found × 100)/(amount introduced) close to 100% (Table I).

The limit of quantitation was 0.1 μmol/l (about 25 ng/ml) for the three compounds. The sensitivity of the procedure described herein is similar to that of the previous methods [1–3] involving liquid–liquid extraction from plasma.

Application

Plasma samples were collected following peroral administration of a 50 mg/kg dose of carbamazepine to rats. The plasma profiles of carbamazepine and its epoxide and *trans*-diol metabolites for one rat are displayed in Fig. 3.

CONCLUSION

The described procedure demonstrated a good reproducibility. It permits the automated analysis of carbamazepine and its main metabolites with a total run time of 10 min and a sensitivity suitable for pharmacokinetic investigations. The method can be used to monitor carbamazepine and its metabolites in paediatric patients and small animals since only a 100-μl volume of plasma is required.

REFERENCES

1 G. P. Menge, J. P. Dubois and G. Bauer, *J. Chromatogr.*, 414 (1987) 477.
2 R. D. Chelberg, S. Gunawan and D. M. Treiman, *Ther. Drug Monit.*, 10 (1988) 188.
3 L. F. Riad and R. J. Sawchuk, *Clin. Chem.*, 34 (1988) 1863.
4 F. Vérillon, B. Pichon and F. Qian, *Int. Lab.*, July/August (1987) 50.
5 M. C. Rouan, J. Campestrini, J. B. Lecaillon, J. P. Dubois, M. Lamontagne and B. Pichon, *J. Chromatogr.*, 456 (1988) 45.
6 M. C. Rouan, F. Le Duigou, J. Campestrini, J. B. Lecaillon and J. Godbillon, *J. Chromatogr.*, 573 (1992) 59.
7 D. A. Schoeller, *Biomed. Mass Spectrom.*, 3 (1976) 265.