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Note

Rapid gas chromatographic method for the determination of carbamazepine and unrearranged carbamazepine-10,11-epoxide in human plasma

ANGELO RANISE

Istituto di Chimica Farmaceutica, University of Genoa, Genoa (Italy)

and

ENRICO BENASSI* and GABRIELLA BESIO

Centro per l'Epilessia, University of Genoa, 16132 Genoa (Italy)

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Several gas—liquid chromatographic (GLC) methods for the determination of carbamazepine in plasma have been proposed [1—4]. Prolonged extraction procedures or carbamazepine derivative formation, in consequence of its thermal instability, are needed with these methods. Moreover, carbamazepine-10,11-epoxide (CBZ-E) is always degraded to 9-acridine carboxaldehyde [5]. We describe here a rapid GLC method for the routine assay of carbamazepine and CBZ-E, without the necessity of derivatization.

EXPERIMENTAL

Materials

Solvents used were: methylene chloride, acetone, petroleum ether (b.p. 40—70°C), diethyl ether (without further purification). N,O-Bis(trimethylsilyl)acetamide (BSA) or hexamethyldisilazane (HMDS) (both from Carlo Erba, Milan, Italy) were the silylating agents for the sorbent.

Standards

Internal standard. Dissolve 20 mg of 10,11-dihydrocarbamazepine (99%; Aldrich, Milwaukee, WI, U.S.A.) in 100 ml of acetone and keep the solution frozen at −20°C in screw-capped tubes.

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Drug standards. Carbamazepine (Tegretol) was from Ciba-Geigy (Basel, Switzerland). Carbamazepine-10,11-epoxide was synthesized according to the method of Baker et al. [5]. The stock drug standards were prepared in the following concentrations: carbamazepine 10 mg and CBZ-E 5 mg per 100 ml of acetone. These standards were stored in the freezer at −20°C.

Standard curves. These were prepared for each drug. Each serum plus standard was extracted and chromatographed in duplicate as if it were a patient's sample. The mean value of the relative peak area ratios of each standard was plotted against the concentration of the respective standard (Fig. 1).

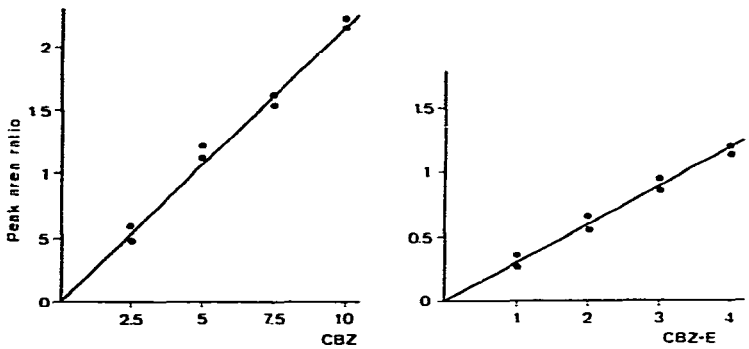

Fig. 1. Calibration curves relating peak area ratios (carbamazepine/internal standard and CBZ-E/internal standard) to drug concentrations in the extracted plasma.

Instrumentation and analysis conditions

Chromatography was performed on a Fractovap 2400 T instrument (Carlo Erba) equipped with a flame ionization detector. Mass spectra were performed on an A.E.I./MS 902 spectrometer (Varian Instruments, Turin, Italy).

The column was 1.80 m × 4 mm I.D. borosilicate glass tubing. It was packed with 3% OV-17 on 80—100 mesh (or 100—120 mesh) silanized Varaport 30 (Varian). The column was conditioned at 270°C for 12 h, without nitrogen flow. The oven temperature was maintained at 270°C. Detectors and injection ports were heated, respectively, to 280°C and 290°C. Gas flow-rates were adjusted at 70 ml/min (nitrogen), 35 ml/min (hydrogen) and 300 ml/min (air). The electrometer was operated in the range of 10^{-10} A/mV and the amplifier output was attenuated at 2. The peaks were recorded at a chart speed of 10 mm/min.

The mass spectrometer was used under the following conditions: ionization energy 70 eV, injector temperature 270°C, oven temperature 265°C, helium flow-rate 40 ml/min. The sample introduction was either direct or by GLC procedure (column and packing as described above).

Silylation was performed on coated support by five injections of 10 μl of silylating agent repeated every 5 min, with slow carrier flow-rate. The oven temperature was maintained at 80—100°C for HMDS, or at 140—160°C for BSA. During this procedure the end of the column was disconnected from the detector, to avoid silica deposits.

Extraction

Forty microlitres of internal standard solution were placed in a glass-stoppered centrifuge tube. After evaporation of the solvent under nitrogen stream, 1 ml of plasma was added and alkalinized by 0.2 ml of 1.5 *N* NaOH (or 3 *N* NaOH when primidone was present in plasma). To the sample was added 1 ml of methylene chloride and, after shaking, 2 ml of petroleum ether. Three layers were formed. Then the sample was gently swirled for a few minutes without mixing the layers. After discarding the petroleum ether by aspiration, 6 ml of methylene chloride were introduced into the glass tube, which was subsequently shaken on a horizontally rotating device (18 rotations/min) for 15 min. The sample was then centrifuged at 314 *g* for 15 min, at −4°C. Occasionally an emulsion formed, but it was easily dispersed by stirring with a glass rod and recentrifugation. The upper layer was removed by aspiration, whereas the rest of the solution was transferred to a conical tube and evaporated in a water bath (35°C) by a gentle nitrogen stream.

Chromatography and mass spectrometry

The dried residue was reconstituted with 20 μl of acetone. This solution (1—1.5 μl) was injected (for chromatography or mass spectrometry) following the analysis conditions described above. Plasma peaks were identified by comparing their retention times with those obtained by injection of authentic samples of carbamazepine or CBZ-E. Drug concentrations were calculated from the standard curve.

RESULTS

Results are summarized in Figs. 1—3 and in Table I. The peak area ratio of carbamazepine and CBZ-E to internal standard (cyheptamide) was plotted against the peak areas obtained from the same anticonvulsants, as added to a

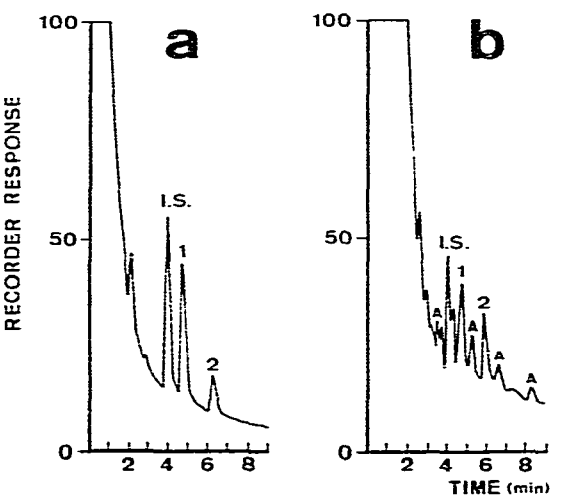

Fig. 2. Typical chromatograms from plasma, (a) obtained with this method; (b) obtained with a different extraction procedure. 1 = Carbamazepine; 2 = CBZ-E; I.S. = cyheptamide; A = artifacts due to lipidic contamination.

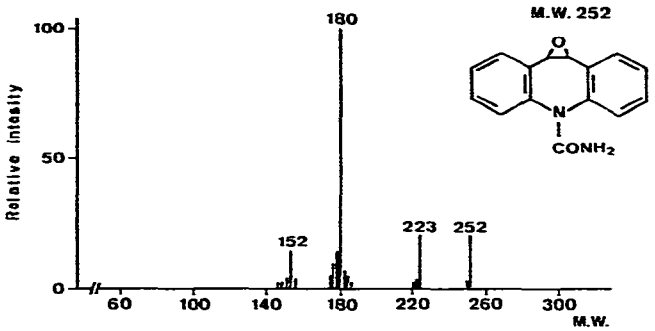

Fig. 3. GLC—mass spectrum of CBZ-E obtained from plasma. This spectrum is identical to that obtained from an authentic sample of CBZ-E and to the mass spectrum of CBZ-E obtained using the direct inlet system.

TABLE I

ANALYTICAL RECOVERIES

$n = 20$.

Drug added to plasma	conc. (μg/ml)	Mean conc. found (μg/ml)	S.D. (μg/ml)	Recovery (%)
Carbamazepine	3	2.9	0.3	97
	6	5.8	0.2	96
	9	8.7	0.1	96
	12	11.8	0.4	98
	15	15.1	0.3	101
Carbamazepine epoxide	1	0.95	0.1	95
	1.5	1.4	0.2	93
	2.5	2.3	0.2	92

plasma pool in a known amount. The resulting regression slope was linear, both for direct drug injection and after drug extraction from plasma or water. The recovery of the two compounds varied between 96 and 101% for carbamazepine and from 92 to 95% for CBZ-E. The standard deviation among recovered amounts of the two drugs in different determinations varied from 0.1 to 0.4 μg/ml. The GLC—mass spectrum of compound 2 (Fig. 2) extracted from plasma was identical to both the GLC—mass spectrum of an authentic CBZ-E sample and the mass spectrum of compound 2 obtained by the direct inlet system (Fig. 3). Plasma free from carbamazepine or CBZ-E was carried through the entire analytical procedure and no normally occurring interferences were seen. On the other hand, primidone, when present in a patient's plasma, produced interference only when the plasma was not sufficiently alkaline.

DISCUSSION

There are some points deserving discussion because of their importance in obtaining good reliability for this method.

Packing of the column must be very carefully performed. Bad packing causes the conversion of carbamazepine to an unknown compound quantitatively cor-

related and showing a retention time longer than 17 min. This partial conversion may depend on chemical interaction between carbamazepine and support occurring in poorly filled spaces of a column that is imperfectly packed. Naturally occurring impairment of the packing can be avoided by conditioning the column without carrier flow.

The residual active sites on the coated support are blocked by "in place silanizing" [6, 7]. We avoided degradation of the studied compounds, seen with chlorinated silanizing agents (trimethylchlorosilane, dimethyldichlorosilane), by using HMDS or BSA. Chlorinated agents would produce the primary chlorosilyl ether with residual active silanol groups, while HMDS and BSA would produce, respectively, NH_3 and acetamide, undergoing complete elimination by the carrier gas. Therefore, the good results obtained using HMDS or BSA should depend on non-acidic columns: it is well-known, in fact, that carbamazepine or CBZ-E easily react in acidic conditions.

Lipidic contamination of the dry residue, responsible for artifacts (see Fig. 2), is completely avoided without making the routine procedure impracticable, by our three-phase extraction.

One of the most frequent problems is degradation of carbamazepine and CBZ-E molecules. This may occur because of contact with metallic parts of the injector, or of combination with residual molecules remaining in the column after the previous determination of other compounds, or from use of chloride solvents that may contain trace amounts of acids, or from choosing the wrong support. Therefore, we recommend the use of glass columns long enough to permit a direct "in column" injection. Columns should be reserved exclusively for the determination of carbamazepine and CBZ-E and supports should be analogous to Varaport 30. Finally, it is advisable to use cyheptamide as internal standard, because of its structural analogy with the studied compounds. We suggest the use of analytical grade cyheptamide, to avoid interfering peaks due to impurities.

In conclusion, this method is reliable, rapid and suitable for the routine determination of carbamazepine and its major metabolite, without derivatization, which could be important for pharmacokinetic studies [8].

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