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DETERMINATION OF CARBAMAZEPINE IN PLASMA

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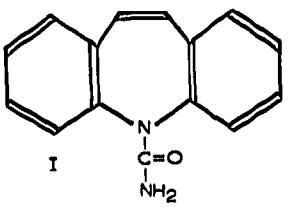
SUMMARY

A description is given of a simple gas chromatographic method for assay of plasma levels of carbamazepine. This uses OV-1 as stationary phase and avoids high column temperatures. The calibration curve is linear and the results are reproducible. Plasma levels in patients under treatment with carbamazepine alone ranged from 1.1 μg/ml to 22.4 μg/ml.

INTRODUCTION

Carbamazepine (I) has found increasing application as an anti-convulsant drug but there have been few attempts to relate clinical actions and plasma levels. Most of the early methods for assay and some recent ones[1,2] use spectrophotometry.



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The indirect measurement of carbamazepine by spectrophotometric measurement of 9-methylacridine produced by the acid hydrolysis of carbamazepine has been reported[2]. Recently, gas chromatographic methods for carbamazepine have been described[3–7]. These have various drawbacks such as tedious extractions, necessity to form derivatives and consequent cost or the use of high column operating temperatures. This paper describes a gas chromatographic method for the assay of plasma levels of carbamazepine which avoids these problems.

EXPERIMENTAL

Instrumental

In the method finally adopted, analyses are performed on a Varian Aerograph 1400 gas–liquid chromatograph equipped with an alkali flame ionization detector (AFID). The carbamazepine is extracted from plasma into an organic solvent and,

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after concentration, is subsequently chromatographed without derivatization on a 3% OV-1 column operated isothermally at 175°. The stationary phase, OV-1 (Varian Aerograph), is dissolved in chloroform and stirred with the appropriate amount of the support material Gas-Chrom Q (80–100 mesh, AW-HMDS, Applied Science Labs.). The solvent is evaporated overnight and the coated support packed into a 2 m × 1.5 mm I.D. borosilicate glass coil. The carrier gas is nitrogen at a flow-rate of 25 ml/min. Other flow-rates are 35 ml/min for hydrogen and 235 ml/min for air.

Extraction from plasma

The extraction method is summarized in Fig. 1.

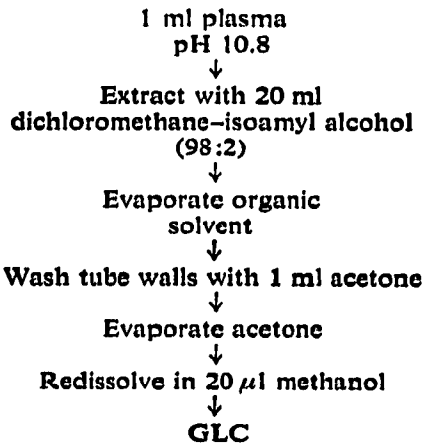
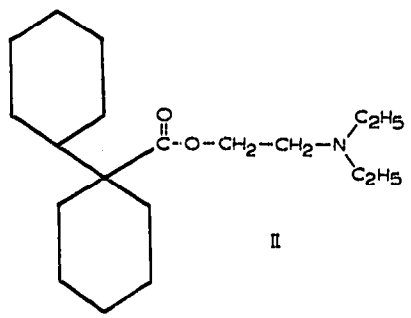


Fig. 1. Flow diagram of extraction procedure.

One millilitre of plasma is placed in a 40-ml glass centrifuge tube and the internal standard, dicyclomine (II), is added prior to extraction. The sample is made alkaline with one ml of bicarbonate buffer (pH 10.8), prepared by combining 87.9 ml of 0.1 *M* $NaCO_3$ and 12.1 ml of 0.1 *M* $NaHCO_3$, and extracted with 20 ml of dichloromethane–isoamyl alcohol (98:2) by shaking for 10 min. The aqueous layer is removed by aspiration. The organic layer is evaporated at 40° in a water-bath aided by a stream of clean compressed air. To avoid excessive dispersion of the dried deposit on the wall of the vessel, this is done in a 10-ml tapered centrifuge tube by repeated additions of 5-ml aliquots.

The deposit is washed from the walls of the tube with 1 ml of acetone and evap-



II

orated again at 60° in a water-bath using air as the evaporative aid, to give a concentrated deposit, which is redissolved in 20 μl of methanol. 1–2 μl of this solution are injected into the gas–liquid chromatograph.

Quantitation

Peak heights measured from the baseline proved to be as satisfactory as areas measured with a planimeter and are preferred for routine use. Known ratios of carbamazepine/dicyclomine are added to drug-free plasma and the resulting peak height ratios used to construct a calibration curve. Table I shows the typical ratios used. From this relationship the peak height ratios obtained from the chromatograms are converted directly to weight ratios.

TABLE I

CONSTRUCTION OF CALIBRATION CURVES

Tube No.	*Carbamazepine (μg/ml)*	*Dicyclomine (μg/ml)*
1*	0	20
2*	15	0
3	4	20
4	6	20
5	8	20
6	9	20
7	10	20
8	15	20

* Tubes 1 and 2 are used as retention time markers for dicyclomine and carbamazepine, respectively.

Preparation of drug stock solutions

Carbamazepine (Tegretol—Ciba-Geigy, Basel, Switzerland): 10 mg is dissolved in 50 ml of acetone.

Dicyclomine·HCl (Richardson-Merrell, Cincinnati, Ohio, U.S.A.): 50 mg is dissolved in 50 ml of water.

RESULTS

The best column performance was obtained with 3% OV-1 as the stationary phase (Fig. 2).

If more than 5 μg of carbamazepine is applied to a column packed with OV-1, two peaks emerge with markedly different retention times. The second peak is not seen if the column load is less than 5 μg. The reason for this has not been elucidated but since in the present method less than one tenth of the carbamazepine extracted from 1 ml of plasma is eventually injected, the mass injected is always much less than 5 μg and the second peak does not occur.

Internal standard is added to the plasma prior to extraction. This form of internal standardization has some advantages. It not only compensates for variations in column performance, injection volumes and for mechanical losses incurred during the extraction, but, provided that the internal standard chosen behaves in a similar

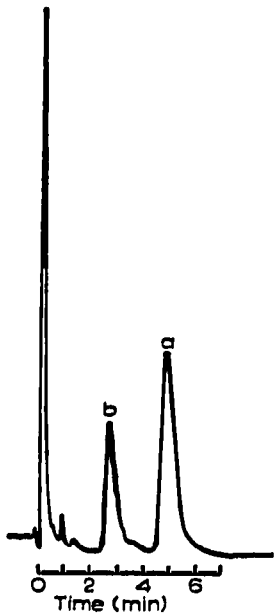


Fig. 2. Typical chromatogram trace of carbamazepine (a) and dicyclomine (b) on OV-1 (3%) at 175°.

fashion to the drug being assayed, it in part allows for variation in efficiency of the extraction procedure. With the present method, the average recovery from plasma of carbamazepine and dicyclomine relative to water blanks are 85.6% (S.E. 5.9%) and 78.0% (S.E. 5.1%), respectively. A typical calibration curve is shown in Fig. 3. It is linear over the range usually encountered in clinical samples.

Reproducibility of the method was estimated by analysis of variance of 23 duplicate estimations. The between injection standard deviation was 0.3 μg/ml and the between extraction standard deviation was 1.1 μg/ml.

Plasma levels of carbamazepine in patients being treated with it as the sole anti-convulsant ranged from 1.1 to 22.4 μg/ml.

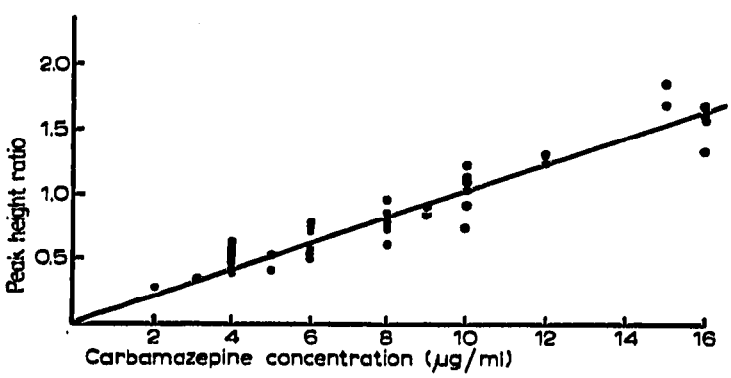


Fig. 3. Calibration curve relating peak height ratios (carbamazepine/dicyclomine) to carbamazepine concentration in the extracted plasma, derived from seven different extractions.

DISCUSSION

The present method utilizes the AFID with an $RbSO_4$ salt tip. Although its actual sensitivity is similar to that of the flame ionization detector (FID), *i.e.* of the order of 10^{-12} g, the AFID has the advantage of being 10,000 times less sensitive to hydrocarbons than is the FID[8] and in addition it can be tuned to specific heteroatoms, *e.g.* nitrogen, phosphorus or sulphur. The insensitivity to hydrocarbons produces very short solvent fronts which allows rapidly eluted peaks to be adequately detected. This increase in relative sensitivity of the AFID is utilized in the present method to permit adequate assay by the extraction of small volumes of plasma. Although 1 ml of plasma is routinely extracted in the method, successful assays can be accomplished using 0.5-ml samples.

Several recent methods for gas–liquid chromatographic analysis of carbamazepine[5,6] claim short analysis times. That of Meijer[4], using a QF-1/XE-60 column, has the considerable disadvantage of interference with the analysis by cholesterol. This was partially eliminated by a two-dimensional thin-layer chromatographic extraction step.

Although short retention times are reported by Larsen *et al.*[5] and Toseland *et al.*[6], using SE-52 and SP-1000, the high operating temperatures lead to rapid column breakdown. Another disadvantage is that the extraction is rather complex for routine application.

Kupferberg's method[7] involves a long extraction and derivatization, which increase both the time and cost of the assay. Another disadvantage is that phenobarbital and phenytoin interfere with the procedure and an extra extraction step is required to separate them from carbamazepine.

The plasma levels of carbamazepine obtained using the method described here are in agreement with the values found by Toseland *et al.*[6] (3–12 μg/ml), Larsen *et al.*[5] (4–11 μg/ml) and Kupferberg[7] (13.1 μg/ml). This method has been used for several months for routine assay to assist in the control of carbamazepine dosage.

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