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Note

Simple and reliable gas chromatographic assay for the determination of carbamazepine in plasma

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Therapeutic levels of carbamazepine in plasma are now determined routinely in many laboratories, and gas–liquid chromatography (GLC) is the technique generally employed[1–5]. Unfortunately, carbamazepine has low thermal stability and in many GLC systems readily undergoes acid-catalysed hydrolysis to iminostilbene and rearrangement to 9-methylacridine[6], resulting in multiple peaks and poor reproducibility. To overcome this problem the use of derivatives, which may have greater thermal stability, has been suggested[7–9] but this introduces into the procedure an additional step which may be difficult to control.

There is therefore a need for a simple and reliable GLC procedure in which carbamazepine can be chromatographed underivatised and give a single reproducible peak. In this paper, a system that fulfils these criteria is described.

EXPERIMENTAL

Materials and equipment

Carbamazepine, iminostilbene and 9-methylacridine were kindly supplied by Geigy (Macclesfield, Great Britain) and amitriptyline was obtained from Roche (London, Great-Britain). Diethyl ether and acetone were redistilled before use. GLC analyses were carried out with a Pye Unicam Series 104 chromatograph equipped with an alkali flame ionization detector, and infrared (IR) spectra were measured in chloroform with a Pye Unicam SP 4000 spectrophotometer using NaCl optics. Mass spectra were obtained with a VG Micromass MM16B mass spectrometer *via* a direct inlet system.

Method

Carbamazepine together with added internal standard (amitriptyline, 0.02 μmol) was extracted from plasma (1 ml) by shaking with diethyl ether (8 ml) for 5 min. After the addition of ammonium sulphate (2 g, diethyl ether-washed) and further shaking for 30 sec, the organic phase was decanted into a conical centrifuge tube and evaporated to dryness under nitrogen at 40°. The residue was dissolved in acetone (50 μl)

and an aliquot (3 μl) injected into the chromatograph. Chromatograms were run at 235° with a glass column (1.0 m × 4 mm i.d.) packed with 5% Apiezon L–1% KOH on diatomite CLQ (JJ's Chromatography, King's Lynn, Great Britain) and a carrier gas (argon) flow-rate of 45 ml/min. The injection port temperature was 250° and the detector temperature was 260°. The concentration of carbamazepine was calculated by measuring the peak height ratio of carbamazepine to internal standard in each chromatogram and relating it to a calibration graph (Table I).

TABLE I

PREPARATION OF CALIBRATION GRAPH

The drugs were added to 1-ml aliquots of drug-free plasma and then assayed as detailed in the Method section

Tube no.	*Carbamazepine (μmol/l)*	*Amitriptyline (μmol/l)*
1	2	20
2	5	20
3	10	20
4	20	20
5	40	20
6	60	20
7	80	20
8	100	20

Reproducibility

Within-batch precision was determined from replicate ($n = 20$) analyses made simultaneously on a plasma pool to which carbamazepine (64.0 μmol/l) had been added. Between-batch precision was determined from serial analyses ($n = 20$) of the plasma pool made over three months. The coefficient of variation (standard deviation divided by the mean) was then calculated in each case.

Stability of carbamazepine during GLC

The GLC column was connected to an effluent splitter (ratio 100:1) and the effluent containing the carbamazepine peak condensed in a glass trap at room temperature. The product thus collected was examined by mass spectrometry (ionization beam energy 70 eV, ion source temperature 220°, accelerating voltage 4 kV) and IR spectroscopy and the results compared with spectra obtained from authentic carbamazepine, iminostilbene and 9-methylacridine.

RESULTS

Typical chromatograms obtained with this GLC system are shown in Fig. 1. Fig. 1A is an extract of drug-free plasma and Fig. 1B is an extract of plasma from a patient undergoing anti-epileptic therapy with carbamazepine. It can be seen that carbamazepine (peak 1) gives a single, sharp and symmetrical peak (retention time 3.2 min) which is adequately separated from the internal standard (amitriptyline, peak 2, retention time 4.6 min.). No interfering plasma peaks occur in the same region of the chromatogram (2–6 min) as carbamazepine and amitriptyline and no subsequent components are eluted when the chromatogram is run for up to 3 h.

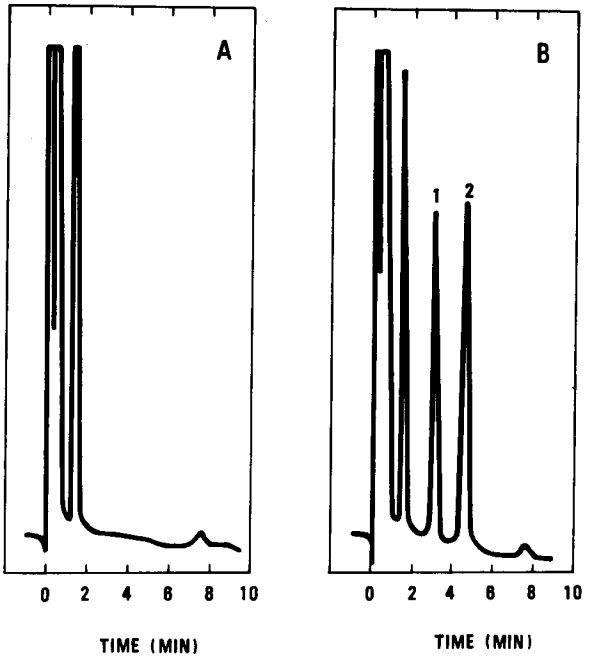

Fig. 1. Chromatograms (A) from an extract of drug-free plasma and (B) from plasma containing 17 μmol/l carbamazepine (peak 1) and 20 μmol/l amitriptyline (peak 2).

Fig. 2, which is a calibration graph prepared as detailed in Table I, shows that the method is linear for carbamazepine concentrations in the range 2–100 μmol/l and that the standard curve passes through the origin. The within-batch precision as determined from the plasma pool (theoretical value 64.0 μmol/l) was 63.5 $\pm$ 2.2 μmol/l (coefficient of variation 3.5%) and the between-batch precision was 63.7 $\pm$ 4.2

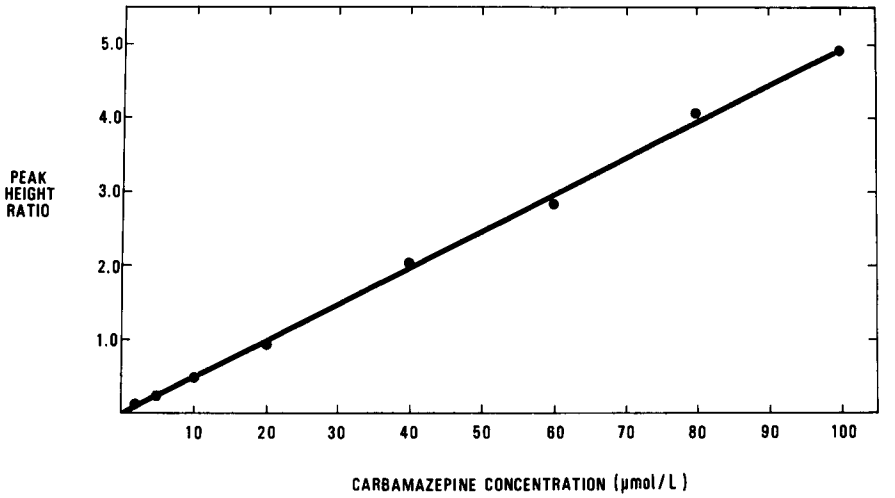

Fig. 2. Calibration graph obtained by plotting the peak height ratio of carbamazepine to internal standard against carbamazepine concentration. Each point represents a single estimation.

μmol/l (coefficient of variation 6.6%). The recovery of carbamazepine and amitriptyline from plasma was 80% and 85%, respectively.

Fig. 3 displays the IR spectra for the ranges 4000–2900 cm^{-1} and 1800–400 cm^{-1} of the three standard compounds (i) carbamazepine, (ii) 9-methylacridine and (iii) iminostilbene, together with the single column effluent (iv).

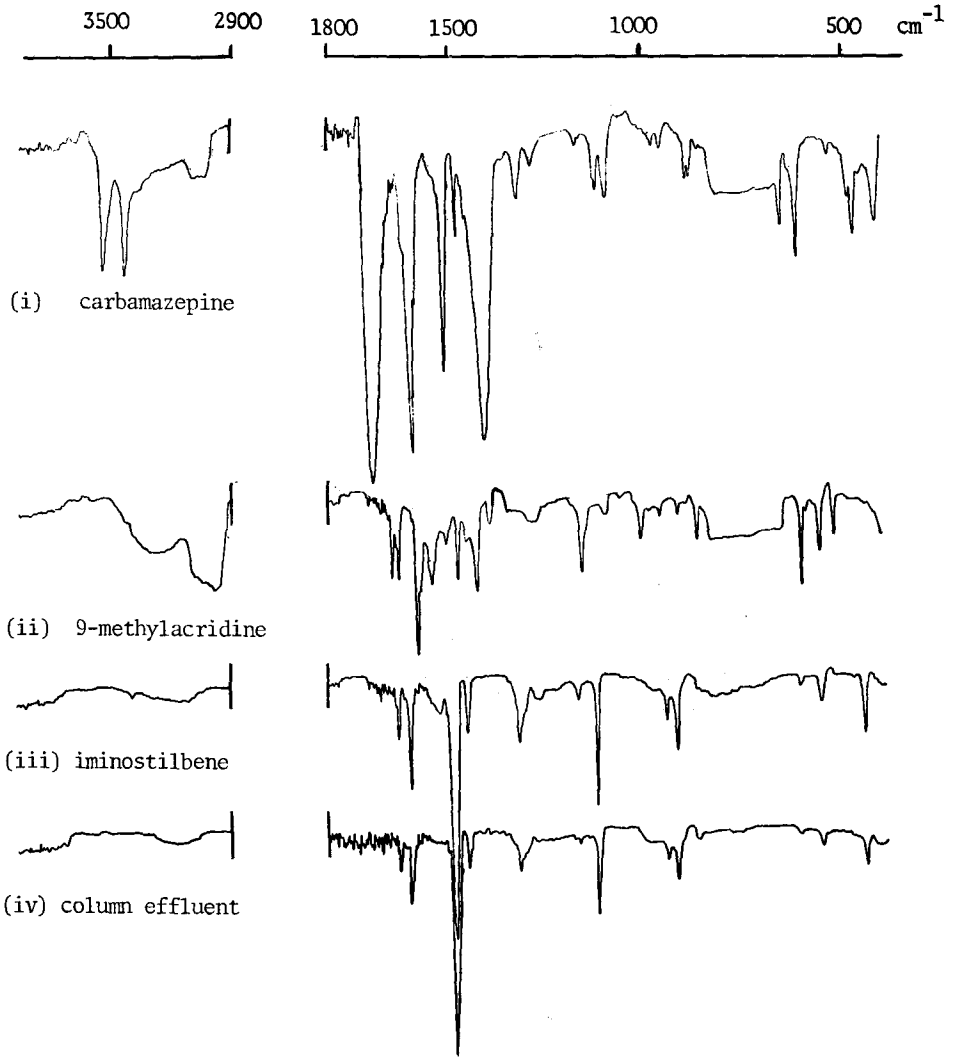

Fig. 3. IR spectra of (i) carbamazepine, (ii) 9-methylacridine, (iii) iminostilbene and (iv) column effluent measured in chloroform with NaCl windows.

DISCUSSION

The major problem associated with the GLC determination of therapeutic levels of carbamazepine in plasma is the weak thermal stability of the drug and the ease with which it undergoes on-column, acid-catalysed degradation and rearrange-

ment to multiple products[6]. Because the degree of decomposition is not constant, systems in which it occurs tend to suffer from poor precision. Although it is possible to stabilise carbamazepine prior to GLC by making the trimethylsilyl[7] or cyano[9] derivatives, or by reacting it with dimethylformamide dimethyl acetal[8], we have found that a more simple alternative is to chromatograph the underivatised drug on a column of Apiezon L incorporating KOH. Under such alkaline conditions, carbamazepine emerges as a single peak (Fig. 1B) which is sharp, symmetrical and reproducible from one injection to another. The mass spectrum of the column effluent shows a major ion at $m/e = 193$ corresponding to $M^+ - 43$, *i.e.* loss of the amide moiety from carbamazepine accompanied by a hydrogen migration. The mass spectrum of the carbamazepine standard, under the same conditions, shows no parent ion at $m/e = 236$ and variation of the ionising voltage down to 25 eV produces no significant effect. The major ion recorded occurs at $m/e = 193$. The mass spectra of both iminostilbene and 9-methylacridine display parent ions at $m/e = 193$ and the fragmentation pattern for all three compounds is essentially the same though some minor variations in intensity are noticable.

The IR spectra of carbamazepine, iminostilbene and 9-methylacridine together with that of the column effluent are shown in Fig. 3. Carbamazepine is characterised by the bands at 3543 and 3435 cm^{-1} which are assigned to ν_{N-H} and the very strong band at 1680 cm^{-1} indicating the presence of a carbonyl group. The spectrum of 9-methylacridine is more complex and displays significant strong bands at 1563, 1418 and 600 cm^{-1}. Strong bands at 1473, 1118 and 448 cm^{-1} characterise the spectrum of iminostilbene. The IR spectrum of the column effluent is clearly identified as that of iminostilbene and the absence of bands at 1563, 1418 and 600 cm^{-1} suggests that no significant amount of 9-methylacridine is produced. Neither does any carbamazepine remain. In this system therefore carbamazepine undergoes complete hydrolysis to yield a single stable product identified as iminostilbene. Subsequent reactions such as rearrangement to 9-methylacridine do not occur. Thus the method is entirely suitable for the routine measurement of carbamazepine. Not only is it linear (Fig. 2) over and beyond the therapeutic range (upper limit approximately 60 μmol/l), but also it is accurate and precise. Excellent agreement was obtained between the observed values of the serum pool (63.5 $\pm$ 2.2 μmol/l within-batch, coefficient of variation 3.5%; 63.7 $\pm$ 4.2 μmol/l between-batch, coefficient of variation 6.6%) and the weighed-in value (64.0 μmol/l). In addition, the method is simple to perform, involving only a single ether extraction followed by direct GLC analysis of the extract. This is made possible by the use of an alkali flame ionization detector which eliminates any interference that might otherwise occur from non-nitrogen containing, diethyl ether-soluble plasma constituents such as lipids and cholesterol. Similarly, interference from other anti-epileptic drugs such as phenobarbitone, primidone and diphenylhydantoin, one or more of which may be given simultaneously with carbamazepine, does not occur. Although these drugs are extracted by diethyl ether, being acidic in nature they are retained by the alkaline column and do not produce peaks on the chromatogram.

The method has now been used successfully for several months to monitor plasma carbamazepine levels, thereby assisting in the control of dosage. It has also been used to compare and correlate levels of carbamazepine in plasma with those in saliva[10].

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