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Note

Simple and rapid gas—liquid chromatographic method for estimating carbamazepine in serum

D.A. COCKS* and T.F. DYER

Department of Biochemistry, Royal Infirmary, Blackburn BB2 3LR (Great Britain)

and

K. EDGAR

Division of Chemistry, Preston Polytechnic, Preston PR1 2TA (Great Britain)

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A simple and rapid gas—liquid chromatographic (GLC) method for estimating serum carbamazepine is presented. The method has been in continuous use for more than two years in this laboratory. The extraction and chromatography are based on the work of Gardner-Thorpe et al. [1], Toseland et al. [2] and Teasdale [3]. Carbamazepine is chromatographed underivatised and the resultant column effluent has been confirmed to be carbamazepine by infrared spectroscopy.

MATERIALS AND METHCD

The instrument used was a Perkin-Elmer F11 gas chromatograph equipped with dual flame ionisation detectors. A single 1 m × 2 mm I.D. glass column packed with 1% cyclohexane dimethanol succinate in methylene chloride on Diatomite C.Q. 100—120 mesh (Pye Unicam, Cambridge, Great Britain) was used. The column was conditioned for 15 h overnight at 230°C with a carrier flow-rate of 10 ml/min.

The following reagents are required for the extraction procedure: chloroform (A.R.), 5 *M* sodium hydroxide and an internal standard solution containing 0.7 mg dehydroepiandrosterone (BDH, Poole, Great Britain) in 100 ml chloroform. The extraction is performed as follows: 1 ml of internal standard solution is added to a glass-stoppered centrifuge tube (105 × 14 mm) and the contents evaporated to dryness; 1 ml of sample (or standard), 1 ml of 5 *M* sodium hydroxide and 5 ml chloroform were added to the tube. The contents

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were shaken mechanically for 10 min, then centrifuged at 650 *g* for 5 min. The upper aqueous layer including the protein precipitate was removed by suction. The chloroform layer was transferred to a 105 × 15 mm conical centrifuge tube and evaporated to dryness under a stream of nitrogen at 60°C. The side of the tube was washed with 1 ml chloroform and again evaporated to dryness. The residue was dissolved in 20 μl chloroform and a 3-μl aliquot was injected on to the column under the following conditions: flow-rates, carrier gas (helium), 45 ml/min; hydrogen, 18 ml/min; air, 29 ml/min; temperatures, injection port, 275°C; oven, 250°C and amplifier setting 1×10^2.

To facilitate effluent collection a Perkin-Elmer Sigma 2 gas chromatograph equipped with 10 : 1 effluent splitter was used. The column and analytical conditions were identical with the F11 operation and identical peaks were obtained on both gas chromatographs.

The effluent was examined by infrared spectroscopy using a Perkin-Elmer 720 infrared spectrometer. The samples were supported as Nujol mulls between sodium chloride plates.

RESULTS AND DISCUSSION

A typical chromatogram is shown in Fig. 1. The retention times of dehydroepiandrosterone and carbamazepine were 5.5 min and 6.8 min, respectively. Precision was assessed by analysing a pooled serum of unknown value in replicate ($n = 29$) within one day. The coefficient of variation was 8.66% (mean = 23.31 μmol/l, S.D. = 2.02 μmol/l). A further pooled specimen was analysed on each subsequent day of analysis to assess day-to-day variation. The coeffi-

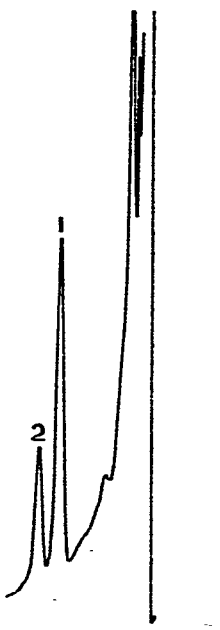

Fig. 1. Chromatogram of serum extract from a patient taking carbamazepine medication showing dehydroepiandrosterone (1) and carbamazepine (2). Chromatography conditions are as given in Materials and Method. The patient's carbamazepine value is 20.2 μmol/l.

cient of variation was 9.18% (mean 23.7 μmol/l; S.D. = 2.17 μmol/l; n = 40).

Participation in the International Quality Control Scheme run by Dr. Alan Richens, provided us with an assessment of accuracy of our results. There is a good correlation (r = 0.96, n = 23) between the authors' laboratory results and the mean of the international results. The equation of the regression line between the two sets of results is $y = 0.986x + 0.96$ (y = authors' result, x = inter-

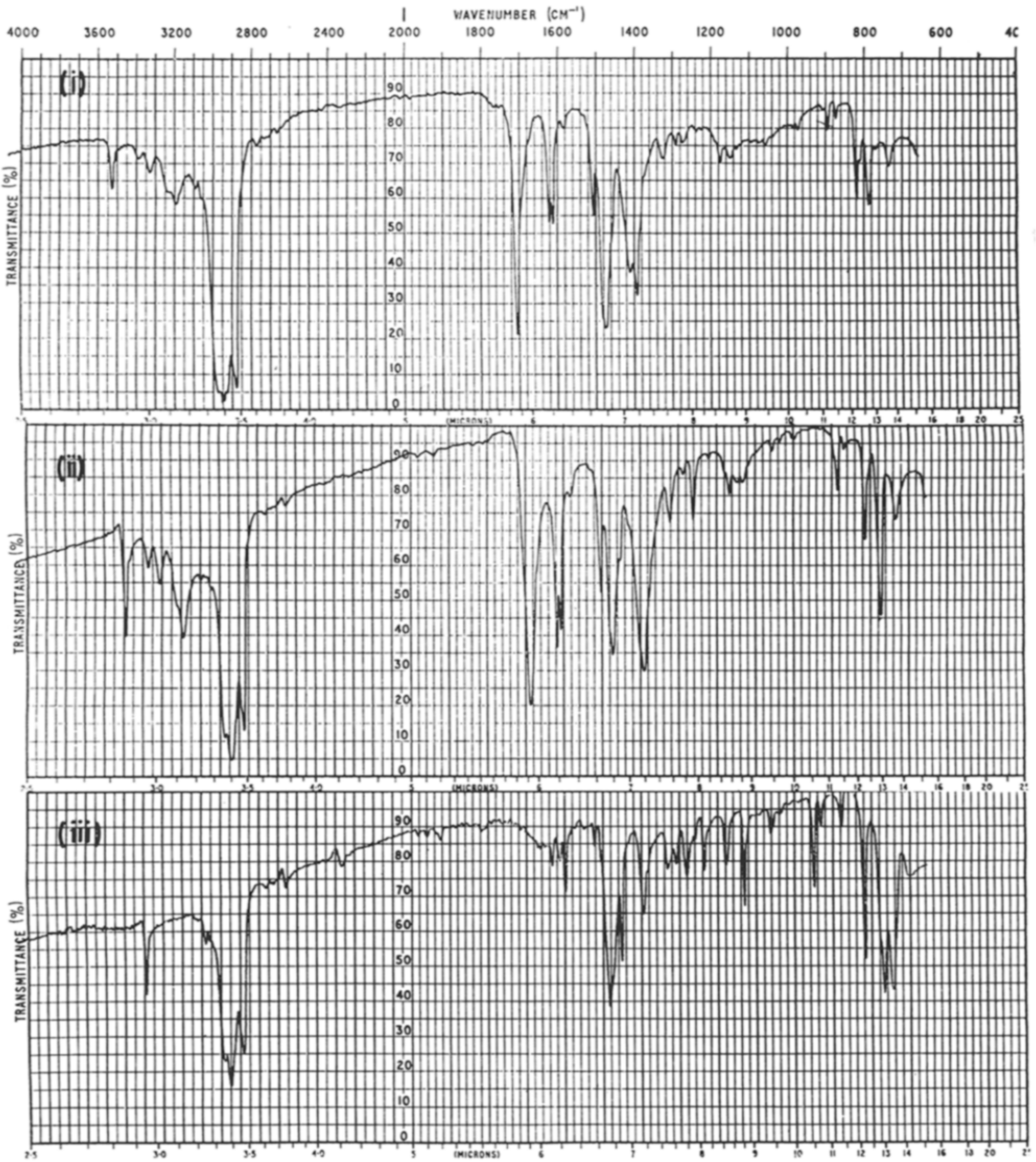

Fig. 2. Infrared spectra of (i) column effluent, (ii) carbamazepine and (iii) iminostilbene measured in Nujol with sodium chloride windows.

national mean). The method is linear up to at least 100 μmol/l, and recoveries for the method are between 97.5% and 108%.

Interference from other commonly prescribed antiepileptic drugs has not been encountered, indicating the method to be highly specific for carbamazepine.

Morselli and Frigerio [4] have shown that 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 rearrangement to multiple products. In the method described by Chambers and Cooke [5], carbamazepine is reported to undergo complete hydrolysis to iminostilbene. However, our work has shown that the extent of hydrolysis of carbamazepine was insignificant. The infrared spectra of carbamazepine, iminostilbene and column effluent are shown in Fig. 2, from which the column effluent is clearly identified as carbamazepine.

The numerous published methods for estimating carbamazepine in serum testify to problems which arise with various methods. The method of Friel and Green [6] has a rather long extraction step and the chromatogram is also longer than with our method. Derivative formation methods such as the method of Kupferberg [7] although overcoming the problem of degradation to iminostilbene usually have prolonged extraction times. Although Mashford et al. [8] report a rapid extraction procedure for their method, our attention is drawn to the rather low recoveries by this method. Sheehan and Beam [9] give an excellent account of the problems associated with iminostilbene formation, indicating the need to use the relative response of both iminostilbene and carbamazepine in calculating the blood levels of carbamazepine. Even so, their chromatograms are too long and also require temperature programming to a very high temperature. Chambers [10] relies on the complete conversion of carbamazepine to iminostilbene. His method is also used to estimate the metabolite carbamazepine-10,11-epoxide, but seems to have rather low recoveries.

The authors' method is simple, rapid, yields good recoveries and is interference-free from other antiepileptic drugs. However, first indications suggest that it may be necessary to temperature programme in order to accommodate the epoxide on the chromatogram. The authors are currently examining this situation in more detail. A further improvement in the method may result from using cyheptamide or possibly imipramine as the internal standard because the chemical structures of these compounds are very similar to carbamazepine. However, the method itself using dehydroepiandrosterone as the internal standard has proved to be very satisfactory and has enabled the laboratory to offer a very rapid and accurate service for estimating carbamazepine in serum.

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