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Note

Rapid gas chromatographic determination of carbamazepine for routine therapeutic monitoring

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The tricyclic drug carbamazepine (Tegretol, Geigy, Summit, N.J., U.S.A.) is widely prescribed for the treatment of trigeminal neuralgia and of several forms of epilepsy. Its pharmacology, particularly with respect to epilepsy, has been reviewed [1—3].

Several methods for its determination have been published; the spectrophotometric and fluorometric methods have been reviewed [1, 3]. More recently, gas—liquid chromatographic [4—13] and high-pressure liquid chromatographic [14] methods have been introduced, as well as enzyme immunoassay [15]. Each technique has its advantages and disadvantages with respect to sensitivity, potential interference, speed, and cost and availability of reagents and equipment. At present, gas—liquid chromatographic methods using flame ionization detection appear to be most widely used.

Several such procedures have been described for determining carbamazepine along with other anticonvulsant drugs [4—6, 8, 10]. Many of these procedures are lengthy and fairly complicated, mainly because of carbamazepine, which differs chemically from the other generally administered anticonvulsants. In most laboratories, phenobarbital, primidone, and phenytoin determinations are requested much more frequently, and more urgently in many cases, than is that of carbamazepine. It appeared, therefore, that a more practical approach was to use a quicker routine methodology for the determination of the former three drugs and do a separate rapid determination of carbamazepine when that analysis was required.

Several methods are available for chromatography of carbamazepine in the underivatized form [7, 8], or after conversion to trimethylsilyl [9—11] or methyl [4—6] derivatives. Underivatized carbamazepine is prone to decomposi-

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tion during chromatography. Procedures involving use of the other derivatives generally are lengthy, requiring several extraction and evaporation steps and, in the case of the trimethylsilyl derivatives, rigid exclusion of moisture.

Perchalski and Wilder [12, 13] introduced the use of dimethylformamide dimethyl acetal as a carbamazepine derivatizing reagent. The reagent appeared to have good potential in the determination of this drug and of other compounds as well. The procedure, however, presented several problems. Cyheptamide [9] subsequently had been withdrawn from commercial availability (it has only very recently become available again), and we preferred to use a column packing that is more generally useful and already in routine use for other analyses. The use of carbon disulfide in the final step and as injecting solvent, while minimizing the size of the solvent front, also was considered objectionable.

The method described here has been used successfully in this laboratory for over two years. In addition to avoiding many of the problems discussed, the present method allows: (1) the use of reagents, glassware, and chromatography column common to other analyses a laboratory may be called upon to do; (2) the preparation in advance of extraction tubes containing internal standard; and (3) the reporting of results promptly after receipt of specimens with a minimum number of extraction and derivatization steps.

EXPERIMENTAL

Materials

Chemicals used in the routine method, and their sources, were: carbamazepine, a generous gift from Geigy Pharmaceuticals (Summit, N.J., U.S.A.), and used as a stock solution (50 μg/ml ethanol); *p*-amino-N-(2-dipropylaminoethyl)-benzamide hydrochloride, generously provided by E.R. Squibb and Sons, Inc. (New Brunswick, N.J., U.S.A.), and used as internal standard as a stock solution (100 μg/ml ethanol); N,N-dimethylformamide diethyl acetal, from Aldrich Chemical Co. (Milwaukee, Wisc., U.S.A.). Glass distilled benzene and ethyl acetate were from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.).

Sodium phosphate buffer (0.2 *M*) was prepared with Na_2HPO_4 and the pH adjusted to 12.5 with NaOH.

Other chemicals used in the development of the method were phenytoin (grade I) and phenobarbital (crystalline), purchased from Sigma (St. Louis, Mo., U.S.A.), mephenytoin from Sandoz Pharmaceuticals (Hanover, N.J., U.S.A.), primidone from Ayerst Labs. (New York, N.Y., U.S.A.), and ethotoin and trimethadione from Abbott Labs. (North Chicago, Ill., U.S.A.). The latter four compounds were the generous gifts of their manufacturers.

Glassware included 12-ml heavy-duty conical centrifuge tubes with PTFE-lined screw caps (Pyrex) and 5-ml Reacti-Vials (Pierce, Rockford, Ill., U.S.A.). A Lab-Line Temp-Blok heater with a Pierce Reacti-Blok (5-ml vial heating block) were used for heating. A laboratory-improvised multiple evaporator with adjustable-height glass capillary tubes was used for solvent evaporation with nitrogen.

Gas chromatography

A Hewlett-Packard 7611A flame-ionization gas chromatograph was used with a 6 ft. × 2 mm I.D. silanized glass column packed with 3% OV-1 on Gas-Chrom Q, 100—120 mesh (Applied Science Labs., State College, Pa. U.S.A.). Nitrogen (20 ml/min) was the carrier gas. Injector and detector temperatures were 240° and 245°, respectively. The analysis was run isothermally at 230°. Any residual material on the column could be eluted by raising the oven temperature to 240° after completion of a run.

The method was developed using peak height ratios for quantitation. Subsequently, a Hewlett-Packard 3352B Laboratory Data System was used for this purpose. Statistical analyses also were done with this system.

METHOD

Batches of extraction tubes were prepared in advance by adding 100 μl of internal standard solution to 12-ml screw-capped centrifuge tubes; the solvent was carefully evaporated with nitrogen. These tubes, each containing 10 μg internal standard, were capped and stored until used.

Serum, 1.0 ml or 0.5 ml, was added to an extraction tube, followed by 0.5 ml phosphate buffer. Each addition was followed by brief vortex mixing. Five milliliters of benzene—ethyl acetate (4:1, v/v) were then added and the tube contents vortex-mixed for 2 min. (Pipetting and evaporation of the solvent mixture were done in a fume hood.) After centrifugation in an IEC clinical centrifuge for 5 min at 550 *g*, the organic layer was transferred to a 5-ml Reacti-Vial. The solvent was evaporated to dryness under a gentle stream of nitrogen and 30 μl of dimethylformamide diethyl acetal were added, followed by brief mixing. The vial was then heated at 100° for 10 min. A 1-μl aliquot was injected into the chromatograph.

RESULTS

A representative chromatogram of a serum extract is shown in Fig. 1. Retention times of carbamazepine and internal standard were 6 and 8.5 min, respectively. No peak at the retention time of carbamazepine was observed when serum containing no carbamazepine was extracted and derivatized.

Calibration curve

A calibration curve was constructed by adding varying amounts of carbamazepine, in duplicate, to drug-free serum and processing in the usual manner. A plot of carbamazepine:internal standard peak height ratio vs. carbamazepine concentration was linear to at least 15 μg/ml.

Recovery

Varying amounts of carbamazepine were added to drug-free serum and processed. The results were compared with those obtained with a derivatized carbamazepine—internal standard mixture. As shown in Table I, average recoveries ranged from 94 to 100%.

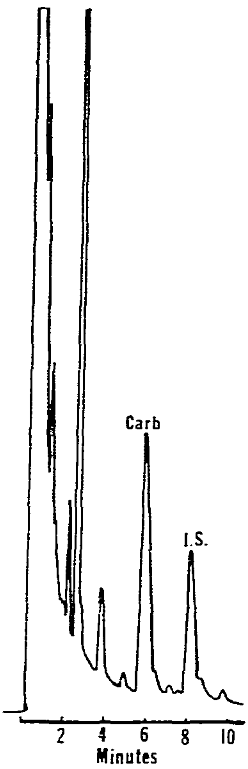

Fig. 1. Chromatogram of an extracted, derivatized serum specimen. Chromatographic conditions are as described in the text. Times shown are minutes after injection. Carb = carbamazepine, I.S. = internal standard, *p*-amino-N-(2-dipropylaminoethyl)benzamide, both as derivatives with dimethylformamide diethyl acetal. Chart speed, 0.25 in./min. Range and attenuation, 10^2, 4.

TABLE I

RECOVERY OF CARBAMAZEPINE ADDED TO SERUM

Amount added (μg/ml)	Amount recovered (μg/ml)	Recovery (%)
0	0	—
2.5	2.4 (2.3—2.6)	98 (92—104)
5.0	4.7 (4.7—4.7)	94
10.0	9.8 (9.6—10.0)	98 (96—100)
15.0	15.0 (15.0—15.1)	100.4 (100—100.7)

Interference

Since a patient's drug regimen frequently includes several anticonvulsant drugs, some of the more frequently administered potentially interfering drugs were tested in the assay procedure. Phenobarbital, primidone, phenytoin, mephenytoin, ethosuximide, ethotoin, and trimethadione were added to drug-free serum in amounts corresponding to their respective therapeutic ranges. None interfered with the analysis.

Precision

To establish the relative response of the flame ionization detector to carbamazepine and to the internal standard, a mixture of equal amounts of the two was extracted, derivatized, and injected. For ten determinations, the mean peak height ratio was 2.7, the standard deviation 0.024, and the coefficient of variation 0.9%.

To determine the within-assay and between-assay reproducibility of the method, carbamazepine was added to drug-free serum which was then mixed, divided into portions, and frozen. Twenty samples were processed simultaneously and fifteen were processed at different times. The mean level determined and the standard deviation and coefficient of variation are given in Table II.

TABLE II

PRECISION OF METHOD: WITHIN-RUN AND RUN-TO-RUN VARIATION

Variation	n	X (μg/ml)	s	C.V. (%)
Within run	20	17.2	0.31	1.8
Run-to-run	15	17.2	0.59	3.5

DISCUSSION

The method described has been in use in this laboratory for over two years and has given reliable results with the patient specimens received from a large neurology service.

The use of prepared extraction tubes containing dried internal standard, an approach we also follow with our other anticonvulsant drug analyses, appears to have several advantages over the more conventional procedures. The internal standard can be dispensed accurately and uniformly to a large batch of tubes whenever it is convenient to do so, not necessarily during the analysis. By gentle evaporation with nitrogen, the internal standard dries as a thin film in the lower part of the tube, and is readily dissolved when the added sample is mixed on a vortex-type mixer.

Tubes can be kept for long periods and are ready for use whenever routine specimens are received, as well as day or night stat tests, at which time only one accurate pipetting, that of the specimen, is required. The 100-μl volumes of internal standard solution can be dispensed with greater accuracy and precision than can the 10—20 μl or smaller volumes used in some methods in which the internal standard is added to a serum aliquot just before extraction. Initial solution of the internal standard in the specimen allows a more uniform extraction of both drug and internal standard than is the case when the standard is not present in the sample at all but is already dissolved in the extracting solvent. In these latter procedures, preparation of large volumes of the internal standard solution is necessary, and accurate pipetting of solvent volumes of several milliliters is essential.

Cyheptamide, the internal standard used in several published methods [7, 9, 12], had been withdrawn from commercial availability before this method was developed; only very recently has it again become available from another source. Synthesizing an internal standard by chemically modifying a limited amount of carbamazepine did not appear to be a reasonable long-term solution for most clinical laboratories. We tested several possible internal standard solutions already in use for other analyses and found *p*-amino-N-(2-dipropylaminoethyl)benzamide hydrochloride, used in the determination of procainamide [16], to be satisfactory for this determination as well.

In the present method, the internal standard is dissolved in the sample aliquot at the outset, is extracted with the drug, and gives a peak well separated from potentially significantly interfering peaks, yet close to that of carbamazepine under the chromatographic conditions employed. Decomposition of the two compounds does not appear to be a problem, and removal of the derivatizing reagent and solution in another solvent before chromatography was found to be unnecessary. The use of carbon disulfide, which, while minimizing the solvent front in the chromatogram, is extremely unpleasant to use, is thereby avoided.

The use of the same internal standard solution in several analyses and of a generally useful column such as OV-1 has also been found economical and advantageous in a laboratory doing many different types of analyses. The use of the diethyl acetal of dimethylformamide gave a "cleaner" chromatogram than did the dimethyl acetal. Both acetals give the same derivative [17]. The less satisfactory performance of the dimethyl acetal may be due only to the particular reagent lot used.

Because of the convenience of saliva determinations in assessing levels of the non-protein bound, pharmacologically active form of anticonvulsant drugs [18], the present method also was tested for its suitability for saliva analyses. Using a 1:5 dilution of internal standard and varying concentrations of carbamazepine added to pooled, drug-free saliva, the method appears applicable to such analyses. Due to an insufficient number of saliva specimens, the clinical application of the method to saliva assays has not yet been thoroughly evaluated.

REFERENCES

1 H. Meinardi, in D.M. Woodbury, J.K. Penry and R.P. Schmidt (Editors), Antiepileptic Drugs, Raven Press, New York, 1972, p. 487.
2 P.L. Morselli, M. Gerna, D. de Maio, G. Zanda, F. Viani and S. Gerattini, in H. Schneider, D. Janz, C. Gardner-Thorpe, H. Meinardi and A.L. Sherwin (Editors), Clinical Pharmacology of Anti-Epileptic Drugs, Springer-Verlag, New York, 1975, p. 166.
3 H. Kutt, in C.E. Pippenger, J.K. Penry and H. Kutt (Editors), Antiepileptic Drugs: Quantitative Analysis and Interpretation, Raven Press, New York, 1978, p. 297.
4 H.M.H.G. Cremers and P.E. Verheesen, Clin. Chim. Acta, 48 (1973) 413.
5 J.-C. Roger, G. Rodgers, Jr. and A. Soo, Clin. Chem., 19 (1973) 590.
6 C.V. Abraham and D. Gresham, J. Chromatogr., 136 (1977) 332.
7 P. Friel and J.R. Green, Clin. Chim. Acta, 43 (1973) 69.
8 J.W.A. Meijer, Epilepsia, 12 (1971) 341.
9 H.J. Kupferberg, J. Pharm. Sci., 61 (1972) 284.
10 C.J. Least, Jr., G.F. Johnson and H.M. Solomon, Clin. Chem., 21 (1975) 1658.
11 G.L. Lensmeyer, Clin. Toxicol., 11 (1977) 443.
12 R.J. Perchalski and B.J. Wilder, Clin. Chem., 20 (1974) 492.
13 R.J. Perchalski and B.J. Wilder, J. Chromatogr., 145 (1978) 97.

14 R.F. Adams, Advan. Chromatogr., 15 (1977) 132.
15 L. Sun and I. Szafir, Clin. Chem., 23 (1977) 1753.
16 A.J. Atkinson, Jr., M. Parker and J. Strong, Clin. Chem., 18 (1972) 643.
17 R.J. Perchalski, B.D. Andresen and B.J. Wilder, Clin. Chem., 22 (1976) 1229.
18 J.J. McAuliffe, A.L. Sherwin, I.E. Leppik, S.A. Fayle and C.E. Pippenger, Neurology, 27 (1977) 409.