GLC Determination of Carbamazepine in Plasma

HARVEY JEROME KUPFERBERG

Abstract □ This report describes a GLC assay for carbamazepine in plasma. The procedure involves the selective extraction of carbamazepine from phenobarbital and diphenylhydantoin. Carbamazepine is stabilized for quantitation in GLC through the formation of its trimethylsilyl derivative. The sensitivity of the method is sufficient to quantitate blood levels in patients receiving carbamazepine for seizure disorders.

Keyphrases □ Carbamazepine in plasma—GLC determination, trimethylsilyl derivative formation □ Plasma levels—carbamazepine, GLC determination □ GLC—assay, carbamazepine

Carbamazepine (I), 5*H*-dibenz[*b*,*f*]azepine-5-carboxamide, is used for the treatment of convulsive disorders and the pain of trigeminal neuralgia (1–4). Its structure is not related to any of the commonly used anticonvulsants. The action of the anticonvulsants, both therapeutic and toxic, appears to be related to their concentration at the site of action; this, in turn, is related to the concentration of free drug in plasma. Absorption, distribution, metabolism, and excretion control the concentration in plasma, and these parameters vary from individual to individual. Effective treatment of patients with seizure disorders can be significantly improved by determining anticonvulsant blood levels and adjusting the dosage when they are outside of the therapeutic blood level range. Plasma levels of 15 mcg./ml. of diphenylhydantoin are required for seizure control in a significant number of patients (5–7).

A sensitive, specific method of analysis of carbamazepine is needed to determine the parameters controlling blood levels. Spectrophotometric methods have been reported (8, 9), but these methods are not sensitive or require TLC purification and separations before quantitation. Larsen (10) reported in abstract form a GLC assay for carbamazepine; however, a complete description of the method was not published.

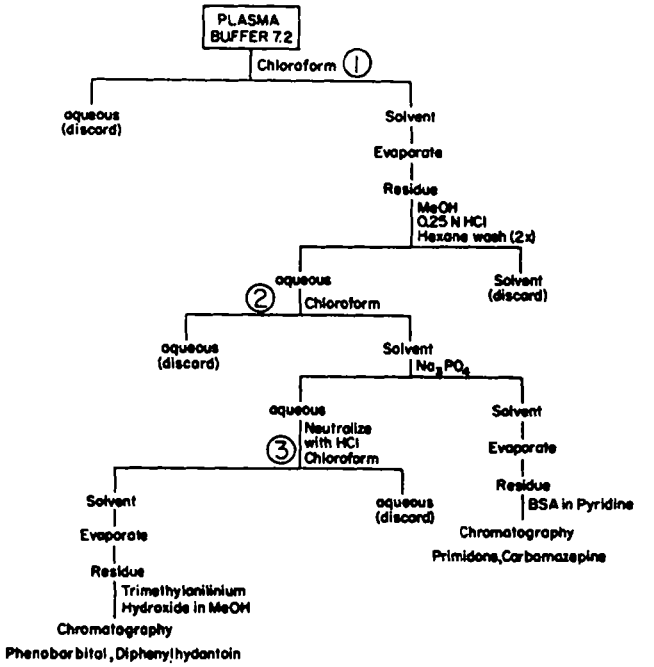
This report describes a GLC method of analysis for carbamazepine which requires the formation of its trimethylsilyl derivative. Diphenylhydantoin, phenobarbital, and primidone can also be assayed in the same sample.

EXPERIMENTAL

Reagents—Chloroform and *n*-hexane[1] were used without further purification. Carbamazepine[2] and cyheptamide[3] melted at reported temperatures, and formation of the trimethylsilyl derivative gave one chromatographic peak. TRI-SIL/BSA in pyridine[4] was supplied by a commercial source. Stock solutions of the anticonvulsants were prepared by dissolving the drugs in absolute methanol and storing them under refrigeration.

Apparatus—A gas chromatograph[5] with flame-ionization detectors was used. The column packing was 3% OV-17 on 80/100 mesh Chromosorb W (HP)[4]. The columns were 1.83-m. (6-ft.) long, U-shaped, borosilicate glass tubing (4.0 mm. i.d.). The conditioning of the columns was carried out as follows: "no flow" at 300° for 4 hr. and then with nitrogen flow overnight at 275°. The analysis of carbamazepine alone was performed under the following conditions: injection port temperature, 250°; column temperature, 235°; and detector temperature, 300°. The gas flows were: hydrogen, 25 ml./min.; oxygen, 100 ml./min.; and nitrogen as a carrier gas, 80 ml./min. The attenuation of the electrometer ranged between 2.0 and 8.0 $\times 10^{-10}$ A.

Extraction Procedure—One milliliter of plasma, 1 ml. of 0.25 *M* phosphate buffer (pH 7.2), 10 mcg. cyheptamide (1 mcg./μl. in methanol), and 12 ml. chloroform were combined in a 37-ml. ground-glass-stoppered centrifuge tube. The mixture was shaken for 15 min. and centrifuged, and 10 ml. of the organic phase was then removed. Chloroform extract No. 1 (Scheme I) was dried in a 13-ml. ground-glass-stoppered centrifuge tube on a rotary evaporator[6] under water vacuum. The residue was dissolved in 3 ml. of absolute methanol, and then 2 ml. of 0.25 *N* HCl and 5 ml. *n*-hexane were added. The mixture was shaken for 10 min. and centrifuged. The *n*-hexane layer was carefully aspirated off and another 5 ml. of *n*-hexane was added to the aqueous mixture. The organic phase was again aspirated off after shaking and centrifugation. Four and one-half milliliters of the aqueous layer was combined with 12 ml. of chloroform, and the mixture was shaken for 10 min. The aqueous layer was aspirated off and as much of chloroform extract No. 2 (Scheme I) was transferred to a 37-ml. ground-glass-stoppered



Scheme I—Extraction scheme for carbamazepine, primidone, phenobarbital, and diphenylhydantoin from plasma

[1] Spectral grade, Fisher Scientific Co., Fairlawn, N. J.
[2] Ciba-Geigy Pharmaceuticals, Ardsley, N. Y.
[3] Ayerst Laboratories, New York, N. Y.
[4] Pierce Chemical Co., Rockford, Ill.
[5] Barber-Coleman model 5000.
[6] Buchler Instruments Co., Fort Lee, N. J.

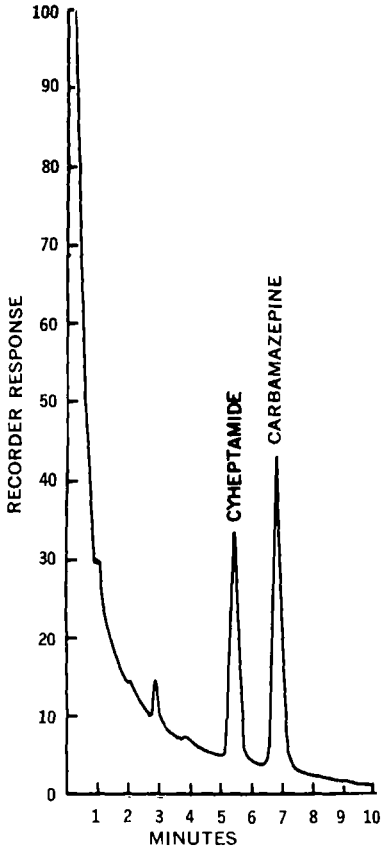


Figure 1—*Gas chromatogram of carbamazepine and cyheptamide following derivatization with TRI-SIL/BSA in pyridine. Conditions: 3% OV-17 on Chromosorb W (HP); nitrogen flow rate, 80 ml./min.; column temperature, 235°; recorder chart speed, 60 cm./hr.; and attenuation of electrometer, 8.0 × 10^{-10} A.*

centrifuge tube containing 4.5 ml. of 0.2 *M* Na_3PO_4. The aqueous phase was removed and saved for further analysis of the acidic anticonvulsants. Ten milliliters of the chloroform extract was then evaporated to dryness in a 13-ml. ground-glass-stoppered centrifuge tube. Forty microliters of TRI-SIL/BSA reagent was added to the residue, and the reaction was allowed to proceed for 20 min. One to three microliters of the reaction mixture was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Formation of Trimethylsilyl Derivative of Carbamazepine—The formation of the trimethylsilyl derivative of carbamazepine was required due to its instability at the elevated temperatures of GLC. Carbamazepine partially decomposes to iminostilbene and the cleavage varies from injection to injection. The chromatograph resulting from the trimethylsilyl derivatives of carbamazepine and cyheptamide is shown in Fig. 1. These derivatives are stable at the operating conditions used in this method, and the peaks are symmetrical with little or no tailing. Table I gives the peak height ratio of carbamazepine to cyheptamide as a function of time following the initiation of the silylation reaction. The ratio reached the maximum value within 15 min. and remained constant for at least 3 hr. Subsequent experiments indicated that the ratio did not change for 5 hr.

Cyheptamide (II) was chosen as the internal standard because of its similarity to carbamazepine (I) in structure, in its extraction characteristics, and in its rate of trimethylsilyl formation and stability.

Table I—Formation and Stability of the Trimethylsilyl Derivatives of Carbamazepine

Minutes after Addition of Trimethylsilyl Donor	Peak Height Ratio[a] 20.0 mcg.	Peak Height Ratio[a] 2.0 mcg.
5	1.23	0.052
20	1.47	0.159
30	1.52	0.150
60	1.48	0.140
90	1.51	0.160
120	1.51	0.141
180	1.52	0.147

[a] Peak height ratios of carbamazepine to 20 mcg. cyheptamide (as internal standard) as a function of time after addition of 40 μl. TRI-SIL/BSA in pyridine.

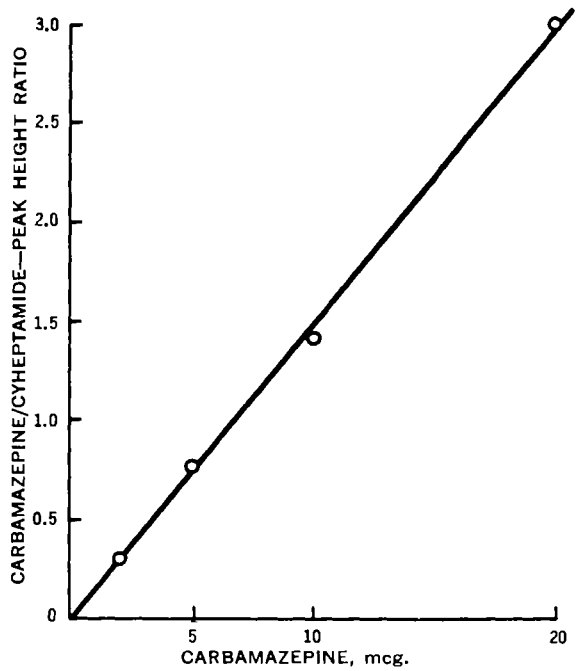
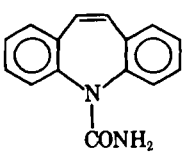


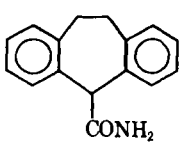
Figure 2—*Peak height ratio of carbamazepine to cyheptamide (as internal standard) as a function of carbamazepine added to 1 ml. of human plasma and then extracted. Each dot represents the mean of six determinations.*

Recoveries—Recoveries of carbamazepine and cyheptamide added to human plasma at concentrations from 2 to 20 mcg./ml. were 95 ± 5%; concentrations as low as 0.5 mcg./ml. were detectable. The peak height ratio of carbamazepine to cyheptamide, the internal standard, plotted against the amount of anticonvulsant added to plasma gave a linear line (Fig. 2). The variation in slope of the linear regression of the line of six experiments was 5.2%. The relative standard deviation of eight determinations of 2 mcg. of carbamazepine added to 1 ml. of oxalated plasma was 9%. The peak height ratios of carbamazepine to internal standard were the same whether the drug was extracted from plasma or water or was reacted directly with the trimethylsilyl reagent.

Specificity of Method—Phenobarbital, diphenylhydantoin, and primidone are commonly used in the treatment of seizures and, therefore, may be used in conjunction with carbamazepine. All three of these anticonvulsants form trimethylsilyl derivatives under the condition described previously. None of the trimethylsilyl derivatives has a retention time similar to carbamazepine. The simultaneous analysis of all the anticonvulsants using their trimethylsilyl derivatives is complicated because the formation and stability of the derivatives vary. The trimethylsilyl derivative of phenobarbital



I



II

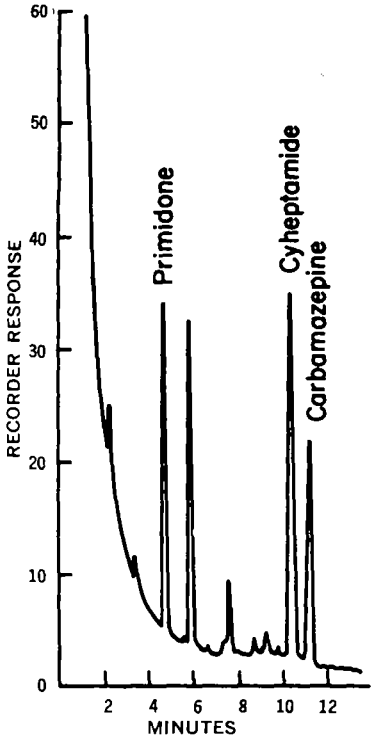


Figure 3—*Gas chromatogram of an extract of plasma taken from a patient receiving primidone and carbamazepine. Conditions: 3% OV-17 on Chromosorb W (HP); nitrogen flow rate, 80 ml./min.; initial column temperature, 160°; temperature program rate, 10°/min.; recorder chart speed, 30 cm./hr.; and attenuation of electrometer, 5.12 × 10⁻¹⁰ A.*

forms rapidly, reaching the maximum value within 15 min., and remains stable for only 15 min. before decreasing rapidly. Diphenylhydantoin reacts slower than carbamazepine but forms a stable trimethylsilyl derivative for more than 2 hr. The formation and stability of the trimethylsilyl derivative of primidone are very similar to carbamazepine. Furthermore, the trimethylsilyl derivative of diphenylhydantoin and cyheptamide, the internal standard, cannot be completely resolved using either an OV-1 or OV-17 phase.

To facilitate the analysis of carbamazepine, diphenylhydantoin and phenobarbital were removed from chloroform extract No. 2 (Scheme I) by extracting them with 0.2 *M* Na_3PO_4. The organic phase now would contain carbamazepine, cyheptamide, and primidone. The acidic anticonvulsants can be quantitated by acidifying the Na_3PO_4, reextracting them into chloroform, and forming their more stable methyl derivative with trimethylanilinium hydroxide (11).

Quantitation of primidone can be accomplished by linear temperature programming the trimethylsilyl reaction mixture between 160 and 250° at a rate of 10°/min. Primidone was not recovered from plasma to the same extent as carbamazepine. Only 70–75% of the added drug was recovered in chloroform extract No. 2. The percent recovery of primidone was the same over the range of 2–20 mcg. added to plasma. There were no peaks with the same retention of

Table II—Carbamazepine Plasma Levels

Patient	Total Daily Dose of Carbamazepine, mg.	Concentration, mcg./ml. Plasma
R.S.	600	9.2 (5-6-71)
	800	13.1 (5-9-71)
K.M.	300	2.0
S.G.	800	3.8
L.D.	1200	7.9
T.C.	400	1.8

carbamazepine when plasma from a series of patients who were not receiving the drug was extracted and chromatographed.

Plasma samples taken from patients receiving various amounts of carbamazepine were analyzed (Table II). A chromatograph of Patient R.S., who received both primidone and carbamazepine, is shown in Fig. 3. Patient R. S. was maintained on 800 mg./day and had plasma levels varying between 12.8 and 16.2 mcg./μl. over a 10-day period. Patient S. G. also received 800 mg./day for several days and achieved a level of 3.8 mcg./μl. The plasma levels of Patient S. G. were one-fourth those of Patient R. S., even though S. G's body weight was two-thirds of R.S. The variability in plasma levels supports the need for blood level determinations in the treatment of epilepsy.

REFERENCES

(1) H. Doose, H. Helmchen, E. Ketz, H. Künkel, A. Matthes, G. Oberhoffer, H. Penin, F. Rabe, D. Scheffner, and U. Voigt, *Pharmakopsy. Neuro-Psychopharm.*, **3**, 227(1970).

(2) H. W. Kienast and L. D. Boshes, *Headache*, **8**, 460(1968).

(3) J. M. Killan and G. H. Fromm, *Arch. Neurol.*, **19**, 129(1968).

(4) C. A. K. Bird, B. P. Griffin, J. M. Miklaszewska, and A. W. Gailbraith, *Brit. J. Psychiat.*, **112**, 737(1966).

(5) F. Buchthal, O. Swensmark, and P. J. Schiller, *Arch. Neurol.*, **2**, 624(1960).

(6) H. Kutt and F. McDowell, *J. Amer. Med. Ass.*, **203**, 969 (1968).

(7) O. Swensmark and F. Buchthal, *Amer. J. Dis. Child.*, **108**, 82(1964).

(8) J. Fuhr, *Arzneim.-Forsch.*, **14**, 74(1964).

(9) K. H. Beyer and D. Klinge, *ibid.*, **19**, 1759(1969).

(10) N. E. Larsen, *Scand. J. Clin. Lab. Invest. (Suppl.)*, **110**, 35 (1969).

(11) H. J. Kupferberg, *Clin. Chim. Acta*, **29**, 283(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 1, 1971, from the *Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455*

Accepted for publication October 14, 1971.

Supported by National Institutes of Health Contract 70-2269.

The author thanks Mrs. Sharon Gammel and Mr. Wayne Yonakowa for their technical assistance in developing this method.

Present address: Applied Neurologic Research Branch, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD 20014