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Note

Simultaneous gas chromatographic determination of diphenylhydantoin, carbamazepine (tegretol), phenobarbital and primidone in presence of kemadrin (procyclidine) and prolixin (fluphenazine) in plasma of psychiatric patients

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Patients with convulsive disorders are commonly treated by simultaneously administering anticonvulsants such as diphenylhydantoin, carbamazepine (tegretol), phenobarbital and primidone. Some of the epileptic patients also receive in addition to the four anticonvulsants, the anticholinergic blocking agent kemadrin (procyclidine) and the tranquilizer prolixin (fluphenazine). The monitoring of the blood levels of the antiepileptic drugs during therapy of the patients is essential for proper treatment[1].

Gas–liquid chromatography (GLC) offers a convenient and sensitive method for simultaneous determination of several anticonvulsant drugs. Hydantoins and barbiturates[1–5] and carbamazepine[6–9] have been determined by GLC. The methylated derivatives are the most commonly used derivatives for GLC analysis of anticonvulsants. Several recently published GLC procedures[10–14] provide a satisfactory separation of diphenylhydantoin, carbamazepine, phenobarbital and primidone, as their methylated derivatives. However, when a patient is receiving both kemadrin and primidone, the two drugs interfere and elute together as one peak.

This paper describes a simple, rapid and sensitive procedure for simultaneous determination of diphenylhydantoin, carbamazepine, phenobarbital and primidone in presence of kemadrin and prolixin in human plasma as their methylated derivatives. This procedure overcomes the interference between primidone and kemadrin, since primidone elutes just before kemadrin. Also, no analytical interference is observed in psychiatric epileptic patients who are on prolixin therapy while also receiving the four anticonvulsant drugs and kemadrin. An internal quality control program for the analysis of the four anticonvulsants is also described.

METHODS AND MATERIALS

Standards and reagents

Methylene chloride and methanol were of "Baker Instra-Analyzed" Grade, supplied by J. T. Baker (Phillipsburg, N.J., U.S.A.) Trimethylphenylammonium hydroxide was purchased from Eastman-Kodak (Rochester, N.Y., U.S.A.).

The pure drug standards, phenobarbital, primidone, diphenylhydantoin and the internal standard 5-(*p*-methyl-phenyl)-5-phenylhydantoin were supplied by Ap-

plied Science Labs. (State College, Pa., U.S.A.). Carbamazepine (tegretol) was obtained from Theta (Media, Pa., U.S.A.). Kemadrin was supplied by Burroughs Wellcome (Research Park, N.C., U.S.A.) and prolixin by Squibb and Sons (Princeton, N.J., U.S.A.).

Plasma pool for plasma blanks and internal quality control were made from plasma samples of the hospital employes left over from their routine clinical check-ups. The plasma pool was divided into 1-ml aliquots in stoppered centrifuge tubes and kept frozen for use as plasma blanks. The quality control plasma consisted of the plasma pool supplemented with known concentrations of all the four anticonvulsants and frozen as 1-ml aliquots.

Procedure

Standard solutions. All standards were dried at room temperature under vacuum prior to weighing. All the solutions were prepared in methanol and were preserved in a freezer. The combined anticonvulsant drugs standard solution containing 1 mg of each of these drugs was prepared in 1 ml of methanol. A 5-ml volume of the internal standard solution was prepared at a concentration of 1 mg/ml. For standard curves, 1 ml of a standard solution (1 mg/ml) of each of the four anticonvulsants was also prepared, separately. For kemadrin and prolixin interference checks, two separate solutions (1 mg/ml) of each of these were prepared.

Extraction

After thawing 1 ml of blank plasma sample, 20 μl (= 20 μg) of the combined standard solution (1 mg/ml) containing all the four anticonvulsants and 20 μl of the internal standard solution were added. The solution was buffered with 1 ml of phosphate buffer (pH 6.5, 0.1 mole/l) and 5 ml of methylene chloride were added. The extraction was carried out by carefully inverting the stoppered centrifuge tube 8–10 times, without vigorous shaking or vortexing to avoid emulsion or gelling of the mixture. Following centrifugation for 2 min, the top aqueous layer was removed by aspiration and 2.5 ml of the clear methylene chloride layer were pipetted into another centrifuge tube. The solution was evaporated to dryness with a stream of nitrogen in a water bath at 40°.

A 1-ml sample of the patient's plasma, to which 20 μg of the internal standard had been added, was extracted exactly by the same steps as described above for the standards.

To show the separation of the four anticonvulsants (phenobarbital, carbamazepine, primidone, diphenylhydantoin), internal standard, kemadrin and prolixin, another 1 ml of blank plasma sample containing 20 μg of each of these drugs was processed by the present extraction procedure.

Gas chromatography

The residue containing the drugs extracted from plasma was dissolved in 75 μl of trimethylphenylammonium hydroxide, mixed by vortexing and after 5 min 1 μl of the solution was gas chromatographed.

GLC analyses were carried out on a Series 1200 Varian Aerograph equipped with a flame ionization detector and a 3380 A Hewlett-Packard Integrator. A glass column (4 ft. $\times$ 1/8 in.) packed with 3% OV-17 on Chromosorb W HP (60–80 mesh)

was used. The oven temperature was 140° for 2 min and programmed to 230° at a rate of 4°/min. Nitrogen was used as the carrier gas at a flow-rate of 20 ml/min. The injector and detector temperatures were maintained at 230° and 280°, respectively.

RESULTS AND DISCUSSION

Fig. 1 shows the standard curves for phenobarbital, carbamazepine, primidone and diphenylhydantoin using the peak-height ratio method. All the curves are linear to approximately twice the toxic concentrations of the anticonvulsants. The recovery of the four anticonvulsants and their retention times are given in Table I. The reproducibility of the present method was determined by carrying out analyses by setting up an internal quality control program and by analyzing one sample daily for a month from a frozen plasma pool supplemented with known concentrations of all the four anticonvulsant drugs. The reproducibility of the present procedure was good as shown by the standard deviation of the four anticonvulsants which varied from 0.5 to 1.3 μg/ml (Table I).

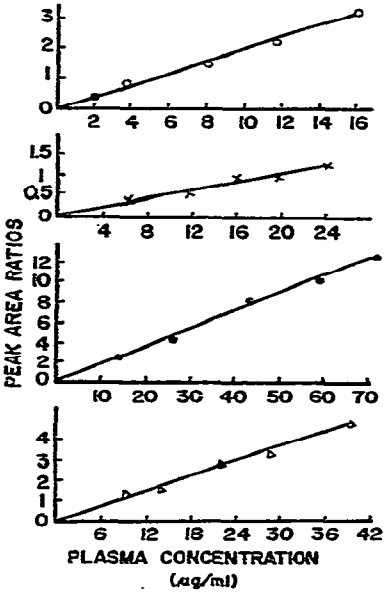

Fig. 1. Calibration curves for the anticonvulsant drugs, carbamazepine (○), primidone (×), phenobarbital (●) and diphenylhydantoin (△). The toxic levels of each of the aforementioned drugs is beyond 8, 12, 35 and 20 μg, respectively.

TABLE I

RETENTION TIMES AND RECOVERIES OF ANTICONVULSANT DRUGS

Drug	*Retention time (min)*	*Recovery (%) (mean ± S.D., n = 30)*
Phenobarbital	7.75	98 ± 0.9
Carbamazepine	10.52	103 ± 0.7
Primidone	12.16	100 ± 1.3
Diphenylhydantoin	16.21	99 ± 0.5

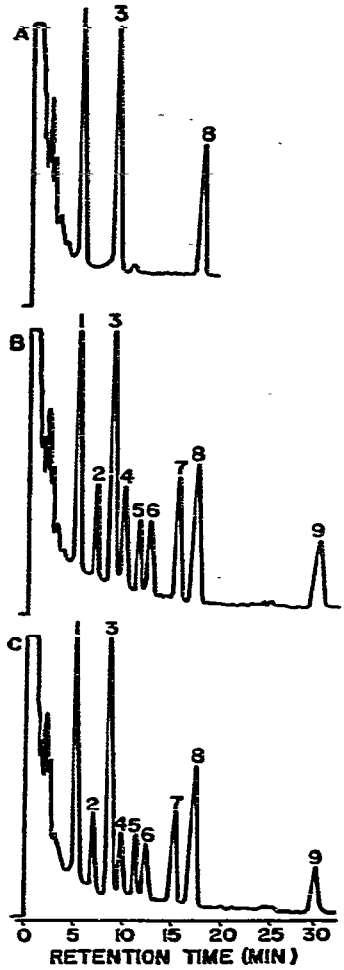

Fig. 2 (A). Chromatogram showing peaks in blank drug free plasma containing the internal standard only. (B). Chromatogram showing the separation of phenobarbital, carbamazepine, primidone, kemadrin, diphenylhydantoin, internal standard and prolixin after extraction of these drugs when added to 1 ml of blank plasma. (C). Chromatogram showing the separation of all the drugs described in (B) from 1 ml of plasma (to which the internal standard was added) of a psychiatric epileptic patient. 1 and 3 = plasma blanks; 2 = phenobarbital; 4 = carbamazepine; 5 = primidone; 6 = kemadrin; 7 = diphenylhydantoin; 8 = internal standard and 9 = prolixin.

The present procedure can determine as little as 0.5 μg/ml of anticonvulsant drugs and if needed, can be scaled down to as little as 0.25 ml of plasma.

Kemadrin and prolixin do not interfere with the determinations of the four anticonvulsant drugs, since kemadrin elutes at 12.9 min and prolixin elutes as the last peak at 29.8 min under the present conditions (Fig. 2B, C).

Fig. 2A shows a typical chromatogram for the blank plasma sample (from individuals not on anticonvulsant therapy) supplemented with the internal standard. The chromatographic separation of the four anticonvulsants: phenobarbital, carbamazepine, primidone, diphenylhydantoin and kemadrin, prolixin and internal stan-

dard added to and recovered from blank plasma is presented in Fig. 2B. Fig. 2C shows the chromatogram for a psychiatric epileptic patient who was on therapy with all the four anticonvulsant drugs, kemadrin and prolixin.

The procedure of Abraham and Joslin[10] for anticonvulsant drugs determination was found to be satisfactory and suitable for our routine determinations till it gave high levels of primidone in some of our psychiatric epileptic patients who had actually received no primidone at all for several months, but were on therapy with phenobarbital, diphenylhydantoin, carbamazepine, prolixin and kemadrin. Our investigation revealed that kemadrin and primidone eluted as one peak under the chromatographic conditions of Abraham and Joslin[10], which explained the discrepancy. To help an accurate determination of primidone levels, in addition to the three other anticonvulsants, the present procedure was worked out especially for analyses on those patients who were on therapy with a combination of primidone (an anticonvulsant) and kemadrin (an anticholinergic blocking agent).

Chromatography of pure prolixin and kemadrin standards (directly) without going through the steps of extraction from plasma, revealed that kemadrin had the same retention time (12.9 min) whether it was chromatographed in the presence or absence of the methylating agent. However, prolixin when chromatographed in the absence of the methylating agent appeared as a single peak at 38 min, while in the presence of the methylating agent it gave two minor peaks eluting with the plasma blank peaks at 5.86 min and 9.34 min, respectively, and a major peak at 29.8 min (Fig. 2B, C). Thus prolixin and kemadrin do not interfere with the determinationt of phenobarbital, carbamazepine, primidone and diphenylhydantoin by the present procedure. The extraction procedure is simpler and no filtration step is involved.

NOTE ADDED IN PROOF

This procedure has been extended for simultaneous determination of methsuximide (retention time 3.8 min) and mephenytoin (retention time 6.9 min) along with the four anticonvulsants; the recovery values are comparable to those of other anticonvulsants.

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