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Note

Simultaneous gas chromatographic analysis for the seven commonly used antiepileptic drugs in serum

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Modern antiepileptic therapy frequently involves the simultaneous administration of two or more anticonvulsant drugs[1,2]. The importance of anticonvulsants and the development of methods adds a new dimension to the control of epilepsy[3].

Gas–liquid chromatography appears to be the method of choice for such analyses[1,4–6]. Its sensitivity and specificity allows simultaneous measurement of anticonvulsant drugs. Here, we describe a simple method for simultaneously determining phenobarbital, diphenylhydantoin, primidone, carbamazepine, ethosuccinimide, methsuccinimide and phensuccinimide in 1 ml of plasma or serum in less than one hour.

EXPERIMENTAL

Drugs used were: diphenylhydantoin (Dilantin; Parke-Davis, Detroit, Mich., U.S.A.); carbamazepine (Tegretol; CIBA Pharmaceutical, Summit, N.J., U.S.A.); phenobarbital (Eli Lilly, Indianapolis, Ind., U.S.A.); primidone (Ayerst Laboratory, New York, N.Y., U.S.A.); 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH; Aldrich, Milwaukee, Wisc., U.S.A.); ethosuccinimide (Zarontin; Parke-Davis); methsuccinimide (Celontin; Parke-Davis); phensuccinimide (Milontin; Parke-Davis); and Fluorene (Eastman-Kodak, Rochester, N.Y., U.S.A.). Trimethylphenylammonium hydroxide (TMPAH, 0.4 moles/l) was prepared as described by Abraham and Joslin[4].

As internal standard, 90 μg of MPPH and 60 μg of fluorene were added to the 5 ml of chloroform used for the extraction. All seven drugs were added to a drug-free pool of serum to obtain five standards with the concentrations listed in Table I. Each serum standard was extracted and chromatographed in duplicate as if it were a patient sample.

The instrumental conditions were the same as explained by Abraham and Joslin[4]. In the chromatographic conditions two temperature modes were used: (a) isothermal heating at 150° for 1.0 min, increased to 300° at a rate of 20°/min; (b) temperature-programmed from 180° to 300° at a rate of 10°/min. Gas flow-rates were: nitrogen, 160 ml/min; hydrogen, 30 ml/min; air, 300 ml/min.

Serum or plasma (1 ml) was combined with 0.5 ml of 0.25 *M* hydrochloric acid in a 150 × 13 mm test tube (PTFE-lined screw cap). Chloroform (5 ml) con-

TABLE I

CONCENTRATIONS OF WORKING STANDARDS (mg/l)

Standard	*Pheno-barbital*	*Diphenyl-hydantoin*	*Primidone*	*Carbama-zepine*	*Etho-succinimide*	*Meth-succinimide*	*Phen-succinimide*
1	5	2.5	2.5	1.0	12.5	3.1	3.1
2	10	5.0	5.0	2.5	25.0	6.2	6.2
3	20	10.0	10.0	5.0	50.0	12.5	12.5
4	40	20.0	15.0	7.5	100.0	25.0	25.0
5	80	40.0	20.0	10.0	200.0	50.0	50.0

taining the internal standards was added to the screw-cap tube and shaken in a vortex-type mixer. The aqueous (upper) phase was aspirated and the organic phase filtered through a Whatman No. 1 filter paper into a conical centrifuge tube and evaporated under a stream of nitrogen.

The dried residue in the centrifuge tube was reconstituted with 50 μl of methanol. This extract (1–2 μl) was chromatographed as explained in chromatographic condition (a). After this first injection, 50 μl of TMPAH were added to the residue and mixed well in a vortex mixer. This extract (1–2 μl) was chromatographed as explained in chromatographic condition (b). The peaks were identified by comparing their relative retention times (relative to internal reference peak) to known standards. Drug concentrations were calculated from the standard curve.

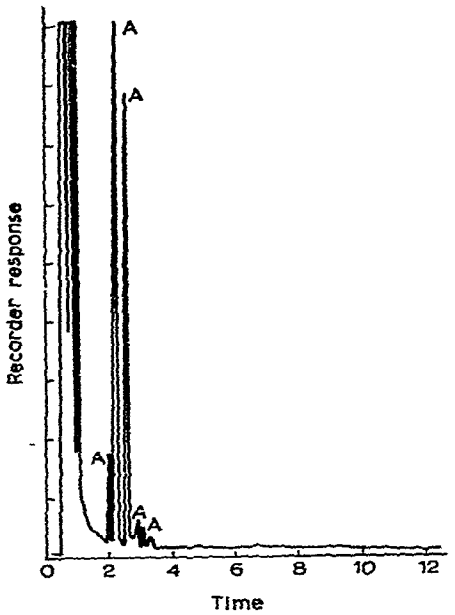
The precision of the proposed method was checked by using aliquots of spiked serum pools which were kept frozen. The serum pool contained all seven drugs.

RESULTS

Chromatograms of four specimens extracted and chromatographed according to our procedure are presented in Figs. 1–5. Fig. 1 is a typical pattern for serum from a normal individual not receiving anticonvulsant drugs when the program was run as explained in chromatographic condition (a). The peaks marked A are attributable to normal serum constituents. Fig. 2 with the internal standard MPPH is a typical pattern for serum from a normal individual not receiving anticonvulsants when the program was run as explained in chromatographic condition (b). The three prominent peaks marked S are attributable to normal serum constituents.

The chromatogram in Fig. 3 is a serum sample spiked with all three of the succinimide drugs. The concentrations calculated from the standard curve were: ethosuccinimide, 64 μg/ml; methsuccinimide, 91 μg/ml; phensuccinimide, 69 μg/ml. The spiked values for the drugs were 60, 90 and 65 μg/ml, respectively. Fig. 4 and 5 are from a patient who is receiving ethosuccinimide, carbamazepine and diphenylhydantoin; 75 μg/ml of primidone and 150 μg/ml of phenobarbital were added to the same sample. The calculated values for this sample were: phenobarbital, 142 μg/ml; carbamazepine, 7 μg/ml; primidone, 70 μg/ml; diphenylhydantoin, 36 μg/ml; ethosuccinimide, 76 μg/ml. All the drug peaks were well separated from one another and there were no interfering peaks.

The between-day precision of the proposed method was evaluated by analyzing the spiked serum sample for 31 consecutive days. The standard deviation varied from 0.4–1.2 mg/l. Analytical recoveries varied from 99–104%.



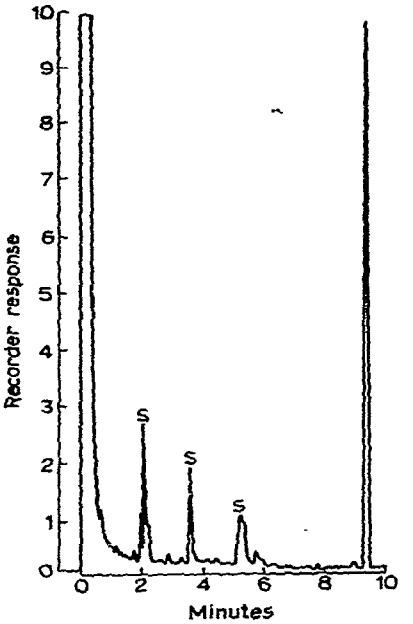
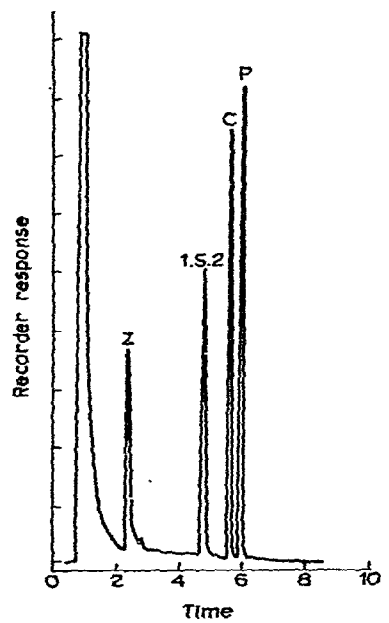


Fig. 1. Chromatogram obtained by injecting a serum extract from an individual who is not receiving any antiepileptic drugs. Chromatographic condition (a) was used. A = unknown constituent.

Fig. 2. Chromatogram obtained by methylation of serum extract from an individual who is not receiving any antiepileptic drugs. Chromatographic condition (b) was used. S = serum constituent; I.S. = internal standard (MPPH).



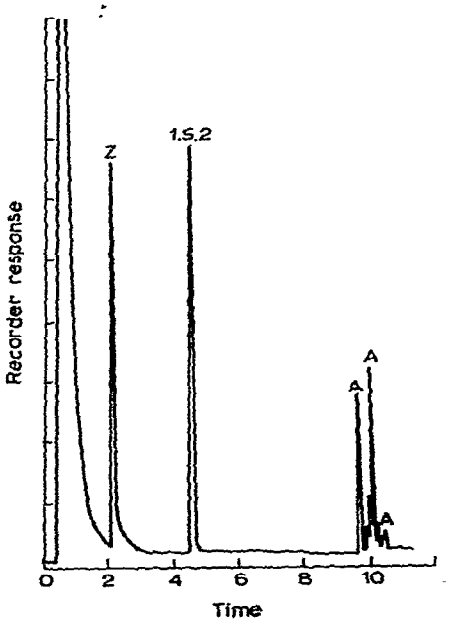


Fig. 3. Chromatogram obtained from a serum extract of a sample spiked with ethosuccinimide, methsuccinimide and phensuccinimide. Chromatographic condition (a) was used. Z = ethosuccinimide; C = methsuccinimide; P = phensuccinimide; I.S.2 = internal standard (fluorene).

Fig. 4. Chromatogram of a serum extract from a patient who is receiving ethosuccinimide, carbamazepine, diphenylhydantoin and the same serum spiked with primidone and phenobarbital. Chromatographic condition (a) was used. Z = ethosuccinimide; A = unknown constituent; I.S.2 = internal

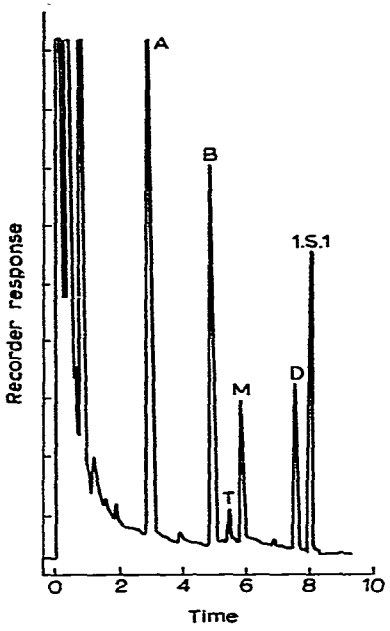


Fig. 5. Chromatogram of a methylated serum extract from a patient who was receiving ethosuccinimide, carbamazepine, diphenylhydantoin and the same serum spiked with primidone and phenobarbital. Chromatographic condition (b) was used. A = unknown constituent; B = phenobarbital; T = carbamazepine; M = primidone; D = diphenylhydantoin; I.S.1 = internal standard (MPPH).

The usual sample volume used was 1 ml of serum or plasma. In pediatric cases, similar results can be obtained by decreasing the amounts of serum, chloroform and reagent proportionately. In some instances we successfully used as little as 0.2 ml of serum.

The proposed method is simple and well suited for the clinical laboratory. A single extraction and chromatographic system is used to analyze all seven of the anticonvulsant drugs. Use of two internal standards in the same extracting solvent for both chromatographic conditions reduces the technical difficulties, the standardization procedure and the analytical time. The non-methylated derivatives of succinimide-type drugs and the methylated derivatives of other drugs were well resolved as were the two internal standards. The use of glass columns and SP 2250 gives good peak characteristics and a linear standard curve.

The five standards that contain all the seven drugs are prepared in pooled serum. In our proposed method the concentration in standard 5 is about twice the toxic concentration, standard 1 is less than a third of the therapeutic concentration.

The most advantageous aspect of the procedure is the capability to determine the most commonly used antiepileptic drugs in a single procedure within one hour.

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