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Note

Rapid determination of anticonvulsant drugs by isothermal gas—liquid chromatography

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Several gas chromatographic methods are available for measuring anticonvulsant drugs in serum. A large number of these techniques use chromatography in the form of their derivatives; methylation is the most commonly used procedure [1—4], another possibility is silylation [5,6]. Recently, two special column packings, GP 2% SP 2510 DA and 2% SP 2110/1% SP 2510 DA on Supelcoport 100—120 mesh have been developed for the separation of anticonvulsant drugs as their metabolites in the underivatized form [7]. Temperature programming was used with the packing 2% SP 2510 DA [7], but working in a routine laboratory it is necessary to use a fast, simple and accurate technique.

In this paper some further experience with the packing 2% SP 2110/1% SP 2510 is reported in the determination of all the more commonly used anticonvulsant drugs without derivatization under isothermal conditions.

Phenobarbital (PB), carbamazepine (CZ), primidone (PD) and phenytoin (DPH) were analysed at 240°C on a column 2 m × 2 mm I.D. and at 225°C on a column 1 m × 2 mm I.D.; ethosuximide (Etx) was analysed at 120°C and at 110°C on the respective columns.

MATERIALS AND METHOD

Reagents

The drugs and internal standards were obtained from Supelco (Bellefonte, PA, U.S.A.). Methylene chloride was analytical grade (E. Merck, Darmstadt, G.F.R.).

The combined internal standard was prepared by convenient dilution in

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methanol of individual stock solutions (1 mg/ml in methanol) to make a solution of 100 μg/ml for 5-methyl-5-phenylhydantoin (MPH) and for 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH), and 200 μg/ml for α,α-dimethyl-β-methylsuccinimide (MS).

A reference mixture solution of anticonvulsant drugs was formed from individual stock solutions (1 mg/ml in methanol) to contain the following concentrations (μg/ml in methanol): 40.0 Etx, 20.0 PB, 10.0 CZ, 10.0 PD and 20.0 DPH; these solutions were stable at −20°C.

Procedure

Groups of extraction tubes were prepared by adding 100 μl of combined internal standard solution to 10-ml screw-capped glass tubes; the methanol was evaporated with nitrogen at room temperature. These tubes were capped and stored at 4°C until use. A 0.5-ml aliquot of a patient's serum was added to the extraction tubes, followed by one drop of hydrochloric acid. After each addition the mixture was mixed for 30 sec in a rotamixer. Then 5 ml of methylene chloride were added and the tube contents vortex-mixed for 5 min at 25 rpm. After centrifugation at 3400 *g* the aqueous layer was aspirated carefully and the organic layer transferred to 8-ml PTFE-lined screw-capped tubes (Kimax 13 × 100 mm 45066), and evaporated carefully to dryness with nitrogen at room temperature. The dried extract was dissolved in 60 μl of methylene chloride and 1.5 μl were injected into the column.

A reference mixture and a serum blank were treated in the same way as the patient's serum. For the reference mixture, 0.5 ml of reference mixture solution is added to the extraction tube and mixed for 30 sec; the methanol is then evaporated to dryness and 0.5 ml of drug-free serum is added to the tube without internal standard.

Chromatography

Two glass columns (2 m × 2 mm I.D. and 1 m × 2 mm I.D.) were filled with 2% SP 2110/1% SP 2510 DA on Supelcoport 100—120 mesh and were mounted in a Perkin-Elmer gas chromatograph Model 3920B equipped with flame-ionization detectors. Nitrogen (50 ml/min for each column) was the carrier gas. Hydrogen (30 ml/min) and air (400 ml/min) were supplied to the detector.

Injector and detector temperatures were 250°C. The analysis was run isothermally at 240°C and 225°C on the 2 m and 1 m columns, respectively, for MPH, PB, CZ, PD, DPH and MPPH, and at 120°C and 110°C on the two columns, respectively, for Etx and MS; later the column temperature was elevated to elute the other drugs. Amplifier settings were: range 10; attenuation 16.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms obtained with a drug-free serum supplemented with the reference mixture solution, and a serum blank. Phenylmethyl malonamide (PEMA), a metabolite of Primidone, elutes before MPH. Cholesterol did not interfere with any of the drugs — it has a retention time longer than those of the drugs studied — and under our conditions it is not detected. In the blank no peaks at the retention times of the drugs were observed. The only problem

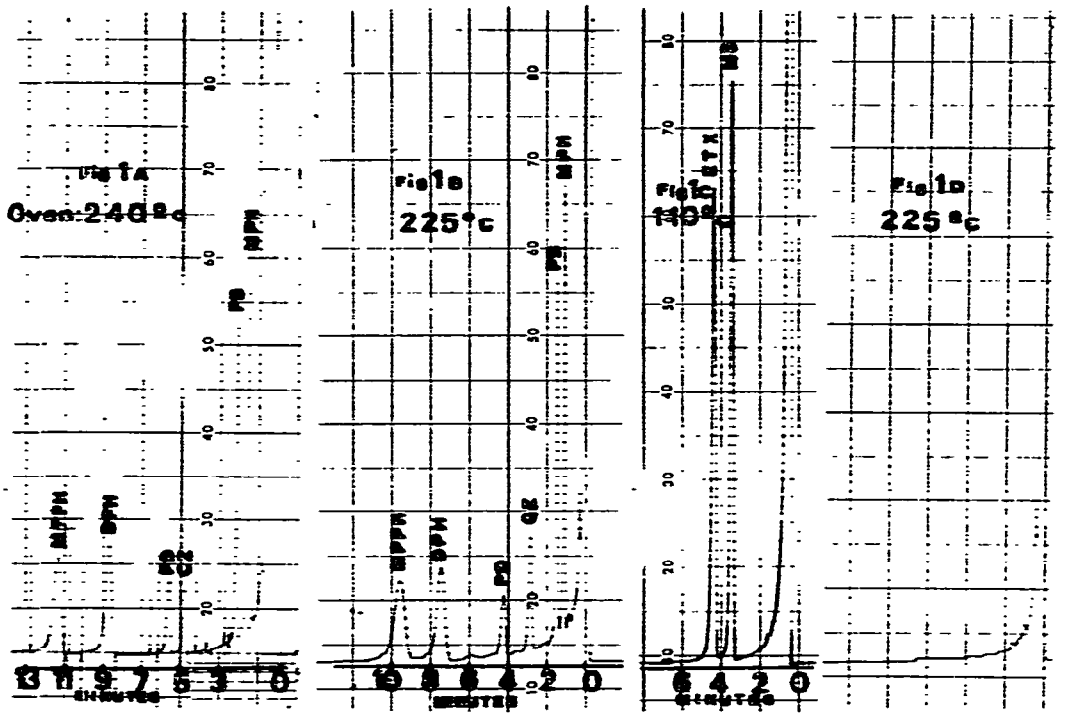

Fig. 1. Chromatograms of a drug-free serum supplemented with the reference mixture solution (A, B, C) and a serum blank (D). Column measurements: 2 m × 2 mm (A), and 1 m × 2 mm (B, C, D).

encountered with the blank was an unidentified interfering substance which co-eluted with CZ and originated from a single lot of methylene chloride.

MPH was used as internal standard for PB, CZ and PD, MPPH for DPH, and MS for Etx. The peak areas were measured with an integrator M2 (Perkin-Elmer). To calculate the concentrations the peak area was compared with that obtained for the reference compound. Linearity of response versus concentration was determined for all drugs studied (Fig. 2). The calibration factors were similar for PB, PD, DPH and Etx on the columns of both lengths, but for CZ it was about 0.94 on the 1-m column.

The analytical recovery was evaluated by spiking drug-free serum with reference mixture solution, and comparing the results with those obtained by direct injection of the same amount of reference mixture solution; the percentages recoveries were about 97 (PB), 104 (CZ), 85 (PD), 98 (DPH) and 104 (Etx).

The precision of the analysis was determined using a pool of drug-free serum spiked with PB, CZ, PD, DPH, and Etx, which was mixed and divided into aliquots and frozen at −20°C; twelve samples were analysed simultaneously and twelve at different times. The results are given in Table I.

The accuracy was established by analysis of serum samples from the Anti-epileptic Drug Level Control Program.

The column of 1 m length was preferred, the analysis time being 11 min and the column temperature was lower than when using the 2-m column. This

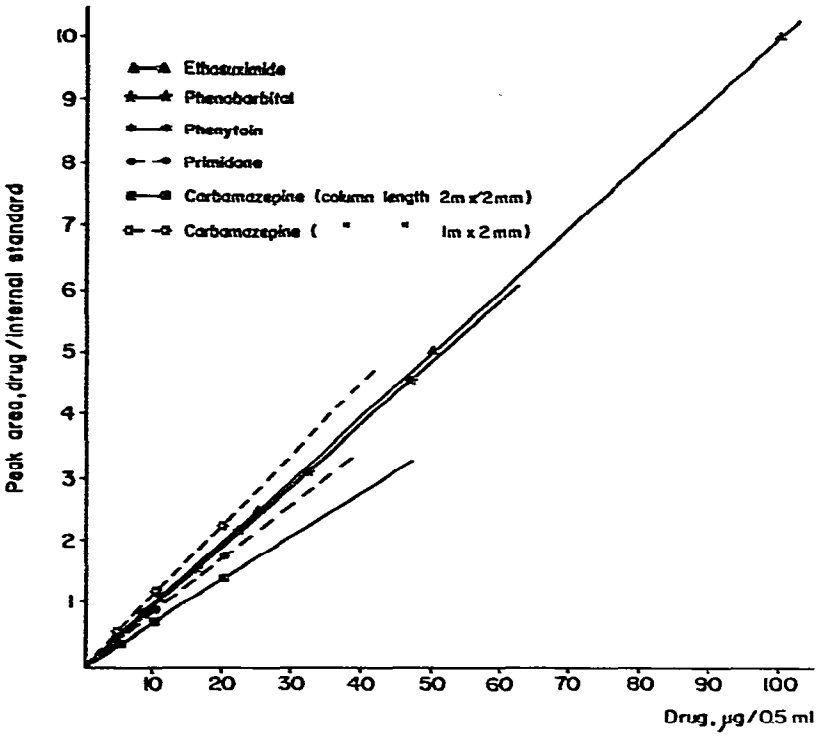

Fig. 2. Calibration curves for each drug studied after extraction and GLC. The area ratio value for ethosuximide is multiplied by 2.

TABLE I

PRECISION OF ANTIEPILEPTIC DRUG ASSAY IN SERUM

Measurements were made after extraction and GLC on a column 2 m × 2 mm I.D. (n = 12).

Drug	Mean concentration (µg/ml)	C.V. (%)	
		Within-day	Between-day
Ethosuximide	49.4	5.2	5.8
Phenobarbital	24.2	2.6	3.4
Carbamazepine	8.4	5.7	6.0
Primidone	9.2	7.0	7.4
Phenytoin	16.0	4.3	6.7

is better for the life of the stationary phase and also the calibration factor for CZ is closer to 1.

Bredensen [8] recently reported the analysis of PB, CZ, PD and DPH without derivatization, with a mixture GP 2% SP 2510 DA and 3% OV-17 under isothermal conditions, but he used only one internal standard for quantitation of the four drugs and the packing is not available commercially. With pure 3% OV-17 the isothermal separation of anticonvulsant drugs is not satisfactory [9].

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