Journal of Chromatography, 137 (1977) 206–209

CHROM. 10,052

Note

Gas–liquid chromatography of eight anticonvulsant drugs in plasma

ALOKANANDA SENGUPTA and MICHAEL ALLAN PEAT

Department of Forensic Medicine, Charing Cross Hospital Medical School, St. Dunstan's Road, London W6 8RP (Great Britain)

(First received November 5th, 1976; revised manuscript received February 16th, 1977)

The measurement of serum anticonvulsant drug levels greatly assists the management of epileptic patients by enabling a balance to be maintained between the therapeutic and toxic concentrations of these drugs. Several methods have been described for the determination of the anticonvulsant drugs in plasma[1–5]. Most of these, however, describe complicated and time-consuming extraction procedures or a separate analytical procedure for each drug.

This report presents a simple and rapid technique for the simultaneous extraction of eight commonly prescribed anticonvulsant drugs. Seven of these drugs are gas-chromatographed simultaneously using an alkali flame ionization detector. The remaining drug, sodium valproate, is analysed using a flame ionization detector.

EXPERIMENTAL

Reagents

For the extraction step diethyl ether AnalaR grade and 1 *M* hydrochloric acid were used.

As standards were used 1 mg/ml heptabarbitone (internal standard for ethotoin, ethosuximide, carbamazepine, pheneturide and phenobarbitone) in methanol; 1 mg/ml 5-(*p*-methylphenyl)-5-phenylhydantoin (internal standard for primidone and phenytoin) in methanol; 1 mg/ml cyclohexane carboxylic acid (internal standard for sodium valproate) in methanol; and 1 mg/ml ethotoin, ethosuximide, carbamazepine, pheneturide, phenobarbitone, phenytoin, primidone and sodium valproate in methanol.

Methanol AnalaR grade and tetramethylammonium hydroxide (TMAH) 20% in methanol, diluted 1:10 before use, were employed for gas–liquid chromatography.

Extraction

A 1-ml amount of plasma containing 20 μg each of heptabarbitone and 5-(*p*-methylphenyl)-5-phenylhydantoin and 100 μg of cyclohexane carboxylic acid is acidified with two drops of 1 *M* hydrochloric acid and extracted with 5 ml of diethyl ether. The organic phase is evaporated to dryness and dissolved in 100 μl of methanol.

Drug-free plasma containing 5–30 μg/ml phenobarbitone, phenytoin, primi-

done, pheneturide, carbamazepine, ethotoin, 10–50 μg/ml ethosuximide and 40–80 μg/ml sodium valproate is treated similarly to the tests to establish calibration curves.

Gas–liquid chromatography

Phenobarbitone, phenytoin, primidone, pheneturide, carbamazepine, ethosuximide and ethotoin are analysed using a Varian 1400 gas chromatograph equipped with an alkali flame ionization detector. The column used was a 4 ft. × ¼ in. glass tube containing 1% OV-17 on Gas-Chrom Q, 80–120 mesh. The column temperature is programmed from 110 to 240° at 8°/min. The other important gas–liquid chromatographic (GLC) conditions are: injector port temperature, 240°; detector temperature, 280°; carrier gas (nitrogen) flow-rate, 50 ml/min.

A 1-μl aliquot of the final residue in methanol is injected after flash methyl-

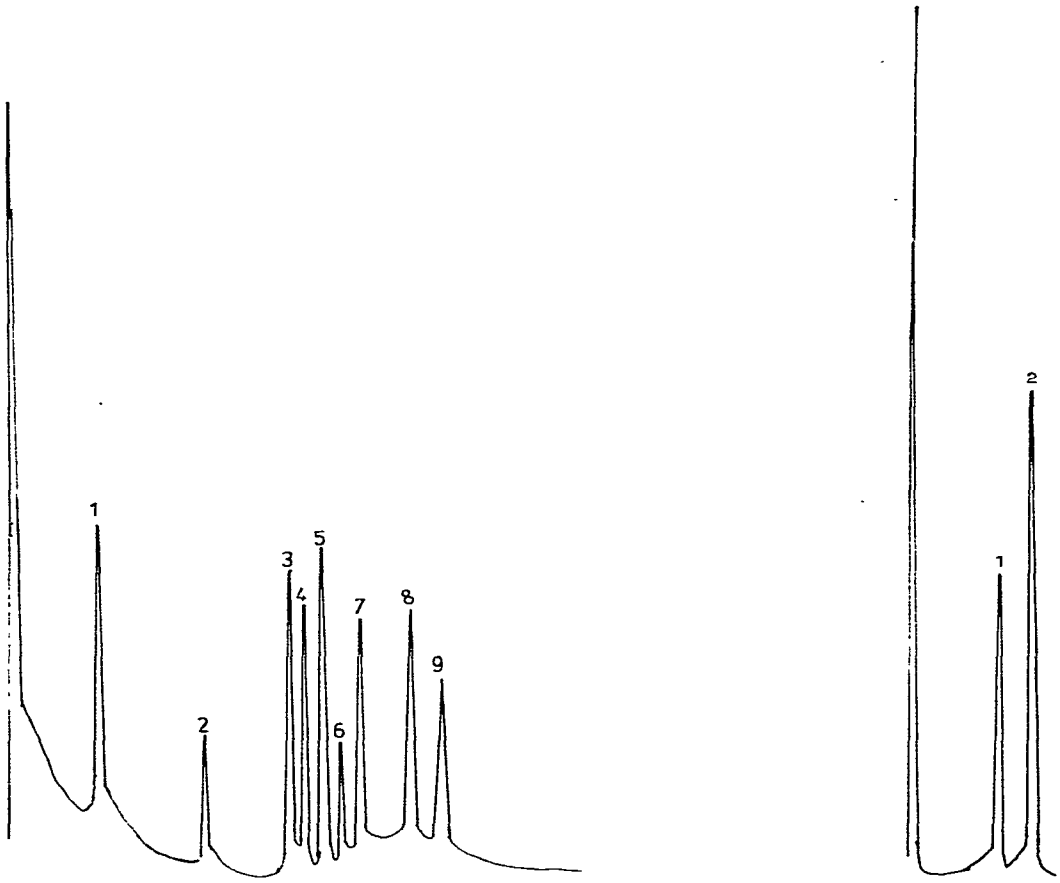

Fig. 1. Chromatogram of seven anti-convulsant drugs in plasma. Peaks: 1 = ethosuximide, 2 = pheneturide, 3 = ethotoin, 4 = phenobarbitone, 5 = heptabarbitone (internal standard), 6 = carbamazepine, 7 = primidone, 8 = phenytoin, 9 = 5-(*p*-methylphenyl)-5-phenylhydantoin (internal standard).

Fig. 2. Chromatogram of sodium valproate in plasma. Peaks: 1 = sodium valproate, 2 = cyclohexane carboxylic acid (internal standard).

ation with 1 μl of the freshly prepared TMAH. A typical chromatogram is presented in Fig. 1.

Sodium valproate is analysed using a Varian 2400 gas chromatograph fitted with flame ionization detectors and a 1 ft. $\times$ $\frac{1}{4}$ in. glass column containing 2% SP-1000 on Universal support, 85–100 mesh. The GLC conditions are: column temperature, 120°; injector port temperature, 200°; detector temperature, 240°; carrier gas (nitrogen) flow-rate, 40 ml/min.

A 2-μl aliquot of the underivatised final extract in methanol is injected on to the column and Fig. 2 shows a chromatogram of sodium valproate plus internal standard extracted from plasma.

In order to test the reproducibility of the method plasma samples spiked with known amounts of the eight anticonvulsant drugs, ranging from 5 to 100 μg/ml, were extracted and analysed by the technique described. Three independent analyses were carried out per sample of plasma and the results are presented in Table I.

TABLE I

ASSESSMENT OF THE REPRODUCIBILITY OF THE METHOD FOR ANALYSING ANTICONVULSANTS IN PLASMA

Drug	*Concentration of anticonvulsant present (μg/ml)*					
	5.5	*15*	*25*	*30*	*60*	*100*
	Recovery (mean $\pm$ S.D.)*					
Ethosuximide			25.4 $\pm$ 0.49	29.9 $\pm$ 0.95	59.0 $\pm$ 0.81	
Pheneturide	5.6 $\pm$ 0.43	15.5 $\pm$ 1.08	24.9 $\pm$ 0.68			
Ethotoin		14.7 $\pm$ 0.31	24.8 $\pm$ 0.61	28.5 $\pm$ 0.75		
Phenobarbitone		15.2 $\pm$ 0.71	23.8 $\pm$ 0.62	29.8 $\pm$ 1.5		
Carbamazepine	5.1 $\pm$ 0.56	14.6 $\pm$ 0.32	24.7 $\pm$ 0.9			
Primidone	5.7 $\pm$ 0.38	15.5 $\pm$ 0.35	25.6 $\pm$ 0.47			
Phenytoin	5.2 $\pm$ 0.2	15.3 $\pm$ 0.21	25.6 $\pm$ 0.54			
Sodium valproate			25.0 $\pm$ 1.0		61.3 $\pm$ 0.94	103.3 $\pm$ 1.2

* Mean of 3 values.

RESULTS AND DISCUSSION

The use of an alkali flame ionization detector to monitor seven different anticonvulsants is plasma is described. The method has also been applied to the analysis of several biological media, such as urine and liver homogenate.

The specificity of the alkali flame ionization detector results in a considerable reduction in extraction time as there is no need to remove naturally interfering plasma constituents, such as cholesterol, during the extraction procedure. The other major advantage of this type of detector is the enhanced sensitivity for nitrogen-containing compounds resulting in a lower detection limit than conventional flame ionization detectors and enabling less sample volume to be used if necessary.

Sodium valproate cannot be determined using the alkali flame ionization detector because there are no nitrogen-containing moieties present in the molecule. We found that the SP-1000 column was the most satisfactory column for the valproate analysis because, at the analytical temperature, none of the other commonly en-

TABLE II

DRUG CONCENTRATION IN *POST MORTEM* SPECIMENS FROM A FATAL CASE INVOLVING ANTICONVULSANT DRUGS

Male subject, age 30 years.

Drug	*Peripheral blood (μg/ml)*	*Liver blood (μg/ml)*	*Stomach content (μg/ml)*
Phenobarbitone	83	9	432
Phenytoin	15	23	256
Sodium valproate	32	28	32
Diazepam	0.6	0.4	1.04
Paracetamol	28	45	224
Thioridazine	—*	—*	0.35

* — = Not detected.

countered anticonvulsants or naturally occurring compounds interfered with the GLC analysis.

In addition to providing an efficient, reproducible and rapid procedure for the therapeutic monitoring of anticonvulsant drugs in plasma, the technique is used for simultaneous screening and quantitation in cases of suspected poisoning. The analytical results of the toxicological investigations of one such case where death resulted from anticonvulsant drug overdose is presented in Table II.

REFERENCES

1 G. H. Goudie and D. A. Burnett, *Clin. Chim. Acta*, 43 (1973) 423.
2 P. A. Toseland, M. Albani and F. D. Gauchel, *Clin. Chem.*, 21 (1975) 98.
3 K. K. Midha, I. J. McGilveray and D. L. Wilson, *J. Pharm. Sci.*, 65 (1976) 1240.
4 C. Hoppel, M. Garle and M. Elander, *J. Chromatogr.*, 116 (1976) 53.
5 R. F. Adams and F. L. Vandemark, *Clin. Chem.*, 22 (1976) 25.