Journal of Chromatography, 132 (1977) 140–144

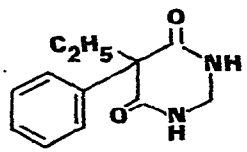
CHROM. 9668

Note

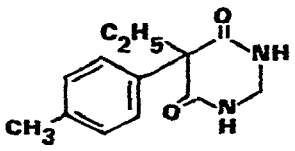
Gas–liquid chromatographic determination of primidone in plasma

R. N. GUPTA, K. DOBSON and P. M. KEANE
Department of Laboratory Medicine, St. Joseph's Hospital, Hamilton, Ontario (Canada)
(First received May 20th, 1976; revised manuscript received August 10th, 1976)

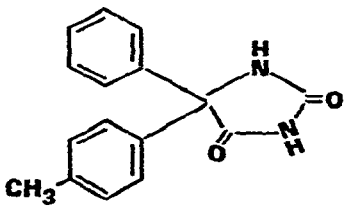
Primidone (1) is a commonly used antiepileptic drug. Gas–liquid chromatography (GLC) is the method of choice for its assay in biological matrix[1]. However, the reproducibility of primidone estimations by GLC is poor[2]. In many of these procedures, primidone is estimated simultaneously with other antiepileptic drugs[3–7] and the conditions for the assay of primidone are less than ideal[5]. We have improved the GLC estimation of primidone by using 5-ethyl-5-(*p*-methyl)-phenyl-2-desoxybarbituric acid (2), an analogue of primidone as internal standard, by separating primidone from other drugs by solvent partition and by carrying out the GLC analysis isothermally using alkali flame ionization detector without any derivatization of primidone.



1



2



3

MATERIALS AND METHODS

All reagents and solvents are of analytical grade and are used without further purification*. Primidone was obtained from Ayerst Labs. (Montreal, Canada). 5-Ethyl-5-(*p*-methyl)-phenyl-2-desoxybarbituric acid was obtained from I.C.I.

* The quality of each batch of hexane and dichloromethane is tested by running reagent blanks. Solvents giving peaks after the initial solvent peak are not used.

(Macclesfield, Great Britain). Stock primidone: 100 mg of compound 1 was dissolved in 100 ml of methanol. Its concentration was determined by measuring its absorbance at 258 nm* ($\lambda_{max.} = 258$ nm, $A^{1\%}_{1cm} = 9.9$) (ref. 8).

Plasma standards of 5, 10 and 15 mg/l of compound 1 were prepared from drug-free plasma and alcoholic solution of 1, divided into 2-ml aliquots and frozen. Stock internal standard: 100 mg of 2 was dissolved in 100 ml methanol and stored at 4°. Working internal standard: 0.5 ml of stock solution of 2 was diluted with water to 100 ml.

To 0.5 ml of standards or tests in duplicate, in 25-ml extraction tubes with PTFE-lined screw caps, 0.5 ml of working internal standard and about 7–8 ml of hexane were added. The tubes were shaken on a mechanical shaker and centrifuged. The upper hexane layer was removed by aspiration. Approximately 10 ml of dichloromethane were added and the tubes were shaken at a low speed on a mechanical shaker. The upper aqueous phase was removed by aspiration and 0.5 ml of tribasic sodium phosphate (1 *M*) was added to each tube. The tubes were shaken, centrifuged and the upper aqueous phase was either collected for isolation of acidic drugs (appropriate internal standard was added in the beginning) or removed by aspiration. About 5 g of anhydrous sodium sulphate was added, the contents were briefly mixed and allowed to stand for 5 min. After centrifugation, the dichloromethane extract was poured into disposable glass tubes (16 × 100 mm) and evaporated in a water-bath at 45–50°. The residue in each tube was dissolved in 20 μl of methanol by vortex mixing. The tubes were kept well stoppered until aliquots were analyzed by GLC.

A Varian Model 2700 gas chromatograph equipped with dual alkali flame ionization detectors was used. A 2-m glass column (2 mm I.D.) was packed with 3% OV-17 on Gas-Chrom Q (80–100 mesh) (Applied Science Labs., State College, Pa., U.S.A.). The column was conditioned overnight at 260° with a carrier gas (nitrogen) flow-rate of 10 ml/min. The analysis of primidone is carried out isothermally with the oven temperature at 240°, the injector and detector at 260° and a carrier gas flow-rate of about 25 ml/min. The hydrogen and air flow-rates were optimized for the selected oven temperature and carrier gas flow-rate. The areas of the desired peaks are computed with an Autolab System IV integrator (Spectra Physics).

Optimisation of extraction of primidone

To select a solvent for optimum extraction of primidone, 1.0 ml of its solution (10 mg/l) in 1 *M* sodium chloride was extracted with 10 ml each of a number of water-immiscible solvents. To 5 ml of each extract, 0.5 ml of internal standard (10 mg/l) in methanol was added. After evaporation and reconstitution of residue in 20 μl methanol, the ratio of primidone/internal standard for each solvent was determined by GLC. These ratios were compared with the ratio of 0.5 ml of primidone solution (10 mg/l) in methanol to the same amount of internal standard as was used for the extraction of primidone. The results are summarised in Table I.

In another experiment, 10-ml portions of a solution of primidone in dichloromethane were shaken separately with 0.5 ml each of 0.5 *M* sodium hydroxide, 0.5 *M*

* Because of the hygroscopic nature of primidone, solutions prepared by weighing may have a 10–20% error.

TABLE I

EFFICIENCY OF EXTRACTION OF PRIMIDONE BY DIFFERENT SOLVENTS

Solvent	*Extracted (%)*
Benzene	50
1-Chlorobutane	12
Chloroform	80
Dichloromethane	81
Dichloromethane + 1% isobutanol	75
Dichloromethane + 2% isobutanol	82
Diethyl ether	50
Ethyl acetate	88
Hexane	0
Toluene	47

sodium hydroxide saturated with sodium chloride and 1.0 *M* sodium phosphate. After removing the aqueous layer, the organic layer was dried over anhydrous sodium sulphate. A 5-ml aliquot was analyzed for primidone by GLC. The results are summarised in Table II.

TABLE II

EXTRACTION OF PRIMIDONE, PHENOBARBITAL AND DIPHENYLHYDANTOIN BY DILUTE ALKALI FROM ORGANIC SOLVENTS

Solvent (pH ≈ 14)	*Percent of drug extracted*		
	Primidone	*Phenobarbital*	*Diphenylhydantoin*
0.5 *M* Sodium hydroxide	50	95	95
0.5 *M* Sodium hydroxide saturated with sodium chloride	5	95	95
1.0 *M* Sodium phosphate	5	95	95

RESULTS AND DISCUSSION

As may be seen from Table I, ethyl acetate is the most efficient solvent for the extraction of primidone. However, in the present procedure, dichloromethane was used for the extraction of primidone for convenience. Dichloromethane has a low boiling point and has a higher density than that of plasma and sodium phosphate solution.

Pre-extraction of plasma samples with hexane removes some of the basic drugs such as diazepam and to a considerable extent neutral compounds such as lipids. These produce peaks even when an alkali flame ionization detector is used which is much less sensitive to non-nitrogenous lipids. These extraneous peaks are potentially interfering when a large number of specimens are chromatographed isothermally.

As seen in Table II, primidone dissolved in dichloromethane is poorly extracted into aqueous alkali (pH ≈ 14). Kupferberg[9] and Miyamoto *et al.*[10] have reported similar behaviour for this compound. In the present extraction procedure, barbiturates and hydantoins are separated by washing with 1 *M* sodium phosphate without any loss of primidone. When required, they may be recovered from the phos-

phate wash by making it acidic and extracting with dichloromethane. Treatment of primidone as an acidic drug results in poor recovery[3,5].

In many of the currently used procedures for the estimation of primidone, 5-(*p*-methyl)-phenyl-5-phenyl hydantoin (3) is used as an internal standard. However, compound 3, being an acidic compound, is an unsuitable internal standard for the present extraction procedure. The internal standard 2 used in this study has properties similar to those of primidone and has a convenient retention time in relation to that of primidone.

In the present sudy, primidone and its methyl analogue have been chromatographed without any derivatisation on a commonly used liquid phase (3% OV-17). Sharp peaks are obtained even when primidone is present in low concentration (Fig. 1). Unlike barbiturates and hydantoins, which tend to get adsorbed on the column due to their acidic character, primidone because of its neutral character shows no adsorption.

In the present extraction procedure, other neutral or basic drugs which are insoluble in hexane would be isolated with primidone. Their separation from primi-

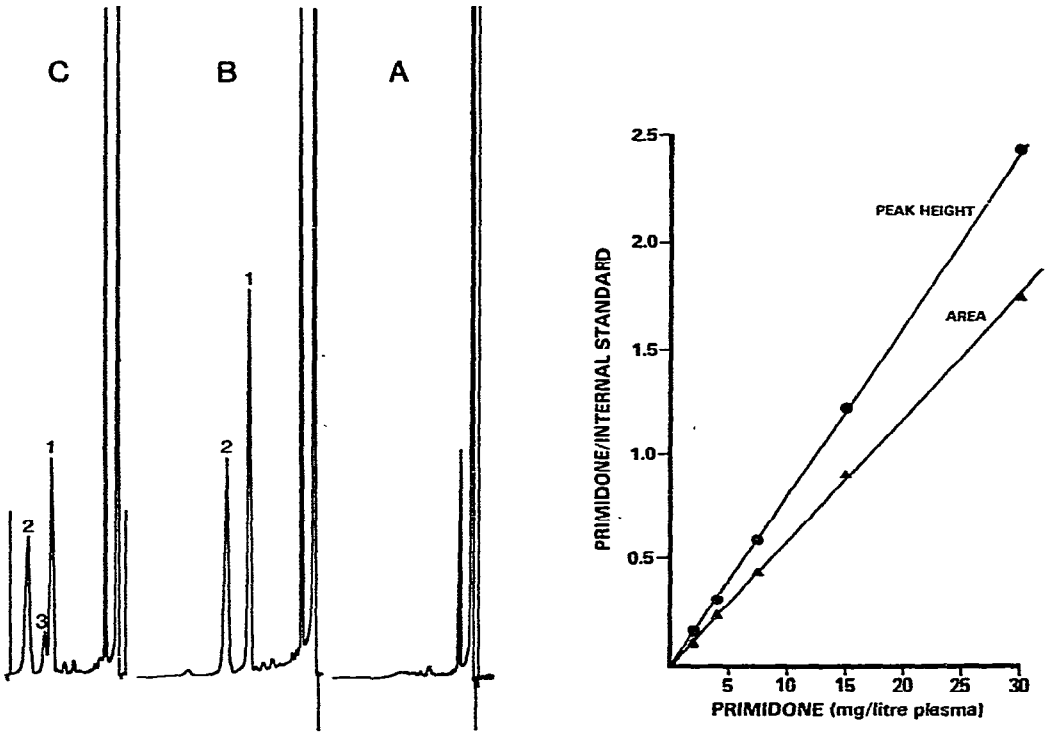


Fig. 1. Chromatograms of extracts of plasma. A, Extract of drug-free plasma; B, extract of plasma containing diphenylhydantoin (20 mg/l), phenobarbital (40 mg/l) and primidone (15 mg/l); C, extract of plasma containing drugs as in B and also carbamazepine (8 mg/l). 1 = Primidone; 2 = internal standard; 3 = carbamazepine (diphenylhydantoin and phenobarbital are removed during extraction).

Fig. 2. Standard curves (peak height or area ratios *versus* concentrations) for the estimation of primidone in plasma.

done or the internal standard is dependent on the differences in their retention times. Thus carbamazepine, which is sometimes prescribed simultaneously with primidone, gives a major peak (t_R = 320 sec) which appears between the peaks of primidone (t_R = 290 sec) and that of the internal standard (t_R = 390 sec) (Fig. 1) without interfering with their integration. However, quantitation of carbamazepine on the same chromatogram lacks precision due to its partial decomposition on the column and has to be estimated separately under optimum conditions for its estimation[11].

This procedure has been found to be linear for the range tested (2–30 mg/l) either by comparing the ratios of peak areas obtained by electronic integration or by comparing ratios of peak heights of primidone/internal standard for different concentrations of primidone (Fig. 2). A serum control containing 10 mg/l of primidone when analysed 10 times within a day showed a coefficient of variation (C.V.) of 7.0% and a between-batch C.V. of 10% (n = 27) using different technicians, different columns and different flame tips.

In our experience it is not more time-consuming or inconvenient to separate different drugs from the same specimen by solvent partition and analyse them successively under optimum GLC conditions for each drug than it is to analyse them simultaneously using temperature programming. However, the results obtained by optimised analysis of an individual drug are more accurate and reproducible than those obtained by simultaneous analysis of multiple drugs.

ACKNOWLEDGEMENT

Thanks are due to Dr. B. Scales of Imperial Chemical Industries (Limited) U.K. for his gifts of analogues of primidone.

REFERENCES

1 B. B. Gallagher and I. P. Baumel, in D. M. Woodbury, J. K. Penry and R. P. Schmidt (Editors), *Antiepileptic Drugs*, Raven, New York, 1973, Ch. 35, p. 353.
2 A. Richens, in H. Schneider, D. Janz, C. Gardner-Thorpe, H. Meinardi and A. L. Sherwin (Editors), *Clinical Pharmacology of Anti-Epileptic Drugs*, Springer, Berlin, 1975, p. 293.
3 H. J. Kupferberg, *Clin. Chim. Acta*, 29 (1970) 283.
4 R. J. Perchalski, K. N. Scott, B. J. Wilder and R. H. Hammer, *J. Pharm. Sci.*, 62 (1973) 1735.
5 E. B. Solow, J. M. Metaxas and T. R. Summers, *J. Chromatogr. Sci.*, 12 (1974) 256.
6 H. L. Davis, K. J. Falk and D. G. Bailey, *J. Chromatogr.*, 107 (1975) 61.
7 S. J. Least, Jr., G. F. Johnson and H. M. Solomon, *Clin. Chem.*, 21 (1975) 1658.
8 R. D. Daley, in K. Florey (Editor), *Analytical Profiles of Drug Substances*, Vol. II, Academic Press, New York, 1973, p. 409.
9 H. J. Kupferberg, *J. Pharm. Sci.*, 61 (1972) 284.
10 K. Miyamoto, M. Seino, Y. Ikeda and H. Yamagami, in J. W. A. Meijer, H. Meinardi, C. Gardner-Thorpe and E. van der Kleijn (Editors), *Methods of Analysis of Anti-Epileptic Drugs*, Excerpta Medica, Amsterdam, 1973, p. 106.
11 A. Gerardin, F. Abadie and J. Laffont, *J. Pharm. Sci.*, 64 (1975) 1940.