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Note

Gas chromatographic analysis of ethosuximide (2-ethyl-2-methyl succinimide) in plasma at therapeutic concentrations

D. J. BERRY and L. A. CLARKE

Poisons Unit, New Cross Hospital, Avonley Road, London SE14 5ER (Great Britain)

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Ethosuximide has been used in the treatment of petit mal epilepsy for nearly twenty years. It has been shown that regular monitoring of plasma ethosuximide concentration has significantly improved control of therapy[1] and optimum clinical effect has been demonstrated when plasma drug concentration is in the range 40–80 mg/l (ref. 2). It is useful, therefore, to have a rapid, simple and reliable method for the determination of plasma ethosuximide concentration.

A spectrophotometric method was reported by Hansen and Felburg[3], but this was not specific nor sufficiently sensitive. Various gas–liquid chromatographic (GLC) methods have been reported, but some involve extraction with large volumes of solvent[4–6] and subsequent lengthy evaporation. The method of Solow and Green[7] requires derivative formation which, in our opinion, is not necessary for ethosuximide. The method of Van der Kleijn *et al.*[8] includes a single chloroform extract without derivative formation, but requires a relatively large plasma sample (0.5–1 ml) and is reported to suffer from emulsification. Bonitati[9] has modified this procedure to perform a micro-scale extraction, but he includes an evaporation step which, in our experience, causes loss of ethosuximide.

Microextraction methods have been in use in this laboratory for several years[10–12] and the present paper describes the application of this procedure to ethosuximide. The extraction is rapid, requires only 100 μl plasma and does not include either an evaporation or derivatisation step. The method is quite specific, economic on reagents and time and has adequate sensitivity to determine therapeutic plasma ethosuximide concentration in small samples. The present method forms part of our supraregional anticonvulsant service and is ideally suited to the rapid throughput of large batches of samples.

EXPERIMENTAL

Reagents

The following reagents were used: chloroform (AnalaR; Hopkin & Williams, Chadwell Heath, Great Britain); phosphate buffer consisting of 4 *M* sodium dihydrogen orthophosphate in distilled water; internal standard solution consisting of 30 mg/l dimethylphthalate (DMP) (AnalaR; Hopkin & Williams) in chloroform.

Gas chromatography

A Pye 104 model 24 dual-column gas chromatograph equipped with flame ionisation detectors was used throughout, in conjunction with a Hitachi 56 recorder (1 mV f.s.d.). The column was a 1.5 m $\times$ 4 mm I.D. coiled glass tube, which had been silanised with a 5% solution of dimethyldichlorosilane in toluene for 24 h. Glass wool was silanised with the same solution. After emptying and rinsing with methanol, the columns were dried in an oven at 100° and then packed with 4% OV-225 on HP Chromosorb W which was prepared according to the method described previously[13].

The instrument settings were as follows: column oven, 155°; sensitivity, 5×10^{-10} A; carrier flow-rate (nitrogen), 60 ml/min; hydrogen flow-rate, 45 ml/min; air flow-rate, 500 ml/min.

Extraction procedure

Dreyer tubes (Scientific Supplies, Vine Hill, London, Great Britain) were used as the extraction vessel. To each tube 10 μl of phosphate buffer, 100 μl plasma sample and finally 100 μl internal standard solution were added. An Eppendorff pipette was used to dispense buffer and plasma. The internal standard was added using a repeating Hamilton syringe. The contents of the tube were agitated on a vortex mixer for 30 sec before centrifuging for 2–3 min.

After centrifuging, 5 μl of the lower phase was injected into the gas chromatographic column, being careful to follow the procedure described by Flanagan and Berry[10]. Samples were analysed in duplicate and the mean result reported. If the difference between duplicates was greater than 10%, the samples were re-extracted.

Measurement

A range of standard solutions each containing 30 mg/l dimethylphthalate as internal standard and 20–100 mg/l ethosuximide was made up in chloroform. Stock solutions of DMP and ethosuximide were prepared and aliquots of each mixed and diluted to 100 ml with chloroform as indicated in Table I.

TABLE I

COMPOSITION OF STANDARD SOLUTIONS OBTAINED BY DILUTING STOCK SOLUTIONS OF DMP (300 mg/l) AND ETHOSUXIMIDE (1 g/l) TO 100 ml WITH CHLOROFORM

Ethosuximide stock soln. (ml)	*DMP stock soln. (ml)*	*Ethosuximide conc. (mg/l)*
2	10	20
4	10	40
6	10	60
8	10	80
10	10	100

These standard solutions were stable for at least one year when stored in a dark cupboard at room temperature.

A calibration curve was prepared daily by injecting 2–5-μl aliquots of the standards and measuring the peak heights from an extrapolated baseline and plotting a graph of the ratio of peak height of drug to peak height of internal standard. The

calibration curve was rectilinear up to 100 mg/l (Fig. 1). To calculate the ethosuximide concentration in a sample, its peak height ratio was compared directly with the standard calibration graph. Correction for extraction losses was achieved by applying a recovery factor (1.12) which was determined by the following procedure.

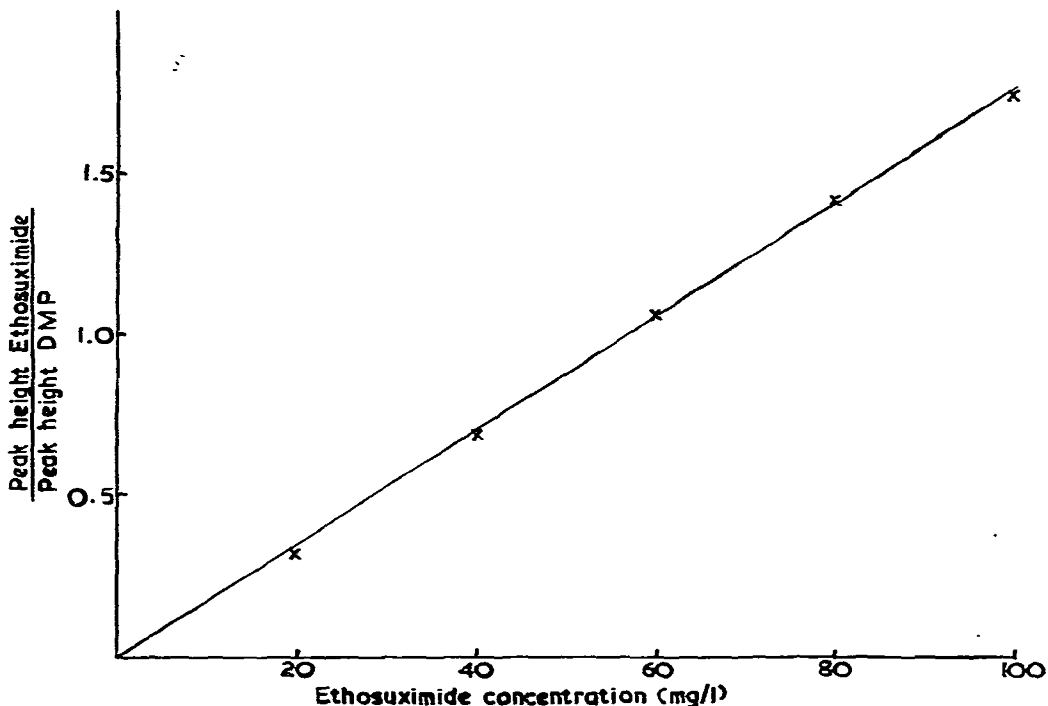

Fig. 1. Standard calibration graph relating the ratio of the peak heights of ethosuximide and dimethylphthalate to the concentration of ethosuximide in the extract.

RESULTS AND DISCUSSION

Recovery studies

Recovery experiments were performed by adding ethosuximide to human plasma over the range 20–80 mg/l and determining the drug concentration using the described method. These additions were made by preparing a solution of ethosuximide in ethanol (4 mg/ml) and using a repeating Hamilton dispenser to add 50–200-μl aliquots to 10 ml plasma. After mixing thoroughly the plasma drug concentration was determined. Over the range studied, drug recoveries were 84 $\pm$ 5%.

Quality control

A quality control sample was prepared as described above at a concentration of 60 mg/l. This was divided and frozen in 1.0-ml aliquots until required. The quality control sample was analysed in duplicate with each batch of samples.

Reproducibility

The reproducibility of the method was determined by analysing a plasma sample twenty times. Results were: mean concentration, 42.6 mg/l; standard deviation, 0.94 mg/l; coefficient of variation, 2%.

Chromatography

The 4% OV-225 has proved to be an excellent column for ethosuximide analysis. Both drug and standard give sharp, symmetrical peaks (Fig. 2) with baseline separation nicely separated from the solvent front. Sensitivity is high with no evidence of irreversible absorption by the columns.

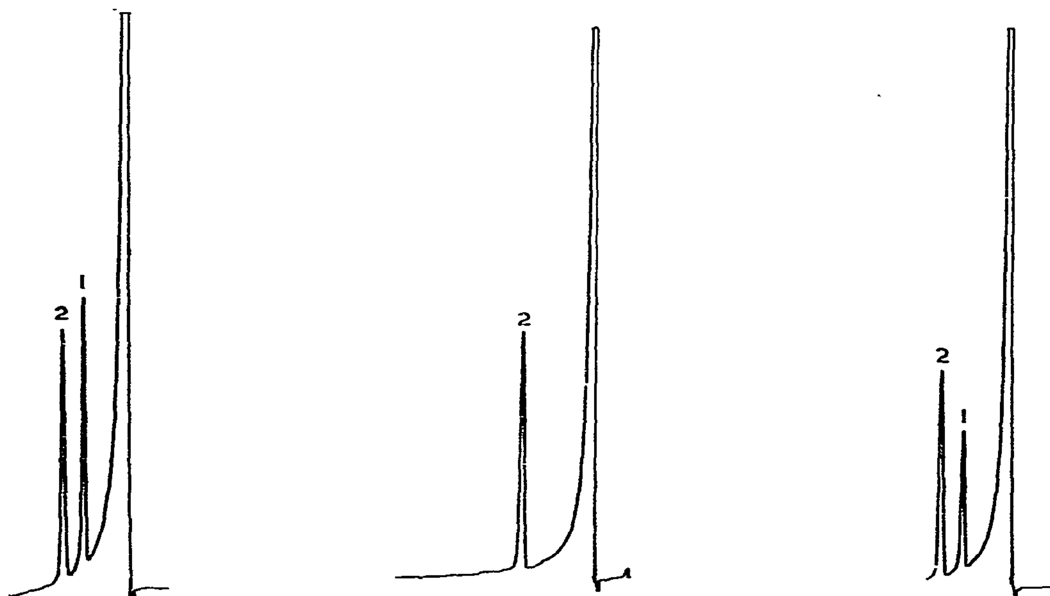

Fig. 2. Separation of ethosuximide and dimethylphthalate under the chromatographic conditions described. 1 = Ethosuximide; 2 = DMP.

Fig. 3. Gas chromatogram of a normal plasma extract with addition of internal standard. 2 = DMP.

Fig. 4. Gas chromatogram of a plasma extract from an individual treated with ethosuximide. 1 = Ethosuximide; 2 = DMP.

Fig. 3 shows that no interfering peaks in the region of either ethosuximide or DMP have been encountered from constituents of normal plasma, and Fig. 4 is a typical trace from a patient sample.

The anticonvulsant drug tridione has a similar retention time to ethosuximide on the OV-225 column; therefore, the procedure cannot be used for a patient who is prescribed both drugs, since plasma concentrations of each drug are of the same order. In Great Britain tridione is rarely prescribed so this is not a serious problem.

The main difficulty we encountered with ethosuximide determination arose because the drug is a volatile and light-sensitive compound. By extracting directly

from plasma into an equal volume of chloroform, solvent concentration is avoided, together with any possible loss caused by volatility. Extractions were always performed away from direct sunlight, thus avoiding problems from this source.

Another factor which was found to affect the efficiency of recovery was extraction pH. When plasma was strongly acidified by the addition of 1 drop of concentrated hydrochloric acid prior to extraction instead of using phosphate buffer, the recovery fell from 84 ± 5% to 67 ± 5%.

In summary, with the method described the concentration of ethosuximide in plasma can be determined accurately and rapidly, within the range that is encountered following normal therapeutic dosing.

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REFERENCES

1 A. K. Sherwin, J. P. Robb and M. Lechter, *Arch. Neurol.*, 28 (1973) 178.
2 J. K. Penry, R. J. Porter and F. E. Dreifuss, in D. M. Woodbury, J. K. Penry and R. P. Schmidt (Editors), *Antiepileptic Drugs*, Raven Press, New York, 1972.
3 S. E. Hansen and L. Felburg, *Dan. Med. Bull.*, 11 (1964) 54.
4 H. M. H. G. Cremers and P. E. Verheesen, *Clin. Chim. Acta*, 48 (1973) 413.
5 J. W. A. Meijer, *Epilepsia*, 12 (1971) 341.
6 A. J. Glazko and W. A. Dill, in D. M. Woodbury, J. K. Penry and R. P. Schmidt (Editors), *Antiepileptic Drugs*, Raven Press, New York, 1972.
7 E. B. Solow and J. B. Green, *Clin. Chim. Acta*, 33 (1971) 87.
8 E. van der Kleijn, P. Collste and B. Norlander, *J. Pharm. Pharmacol.*, 25 (1973) 324.
9 J. Bonitati, *Clin. Chem.*, 22 (1976) 341.
10 R. J. Flanagan and D. J. Berry, *J. Chromatogr.*, 131 (1977) 131.
11 D. M. Rutherford, *J. Chromatogr.*, 137 (1977) 439.
12 D. J. Berry and L. A. Clarke, in preparation.
13 D. J. Berry, *J. Chromatogr.*, 86 (1973) 89.