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QUANTITATIVE DETERMINATION OF ETHOTOIN IN SERUM BY GAS CHROMATOGRAPHY

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SUMMARY

A gas chromatographic method for the estimation of ethotoin (Peganone®) in serum has been developed. The method involves the use of a flame ionization detector and allows for the determination of the drug down to 1 mg/l. This is sufficient for analysing ethotoin in serum after therapeutic doses. The specificity of the method has been confirmed by mass fragmentography.

INTRODUCTION

Few other drugs have been investigated so intensively to obtain reliable kinetic information as the anti-epileptic drugs. The determination of serum concentrations has been successfully used in the treatment of epileptic patients with, *e.g.*, phenytoin, carbamazepine and phenobarbital. Several procedures[1–8], some of them gas chromatographic, are available for the determination of these drugs. Ethotoin (3-ethyl-5-phenylhydantoin) has been used as an anti-epileptic drug for several years; however, in spite of the fact that the molecular structure of ethotoin indicates a chemical relationship between this drug and phenytoin (5,5-diphenylhydantoin), to our knowledge no analytical method for the determination of this drug has been published.

This paper describes a procedure for the determination of ethotoin in serum. The concentration range after therapeutic doses seems to lie between 15 and 50 mg/l, estimated from preliminary patient observations.

EXPERIMENTAL

Apparatus

A Pye Series 104, Model 64 gas chromatograph was used. The pre-heater temperature was 235°, the column temperature 230°, and the detector temperature 340°. A glass column, 1.5 m × 4 mm I.D., filled with 3% (w/w) OV-17 on Celite JJ CQ, 100–120 mesh BS, was applied. The amount of column material was 12 g.

The column was conditioned at 350° for 48 h. The carrier gas (nitrogen) flow-rate was 60 ml/min, the hydrogen flow-rate 40 ml/min, and the air flow-rate 350 ml/min.

Reagents

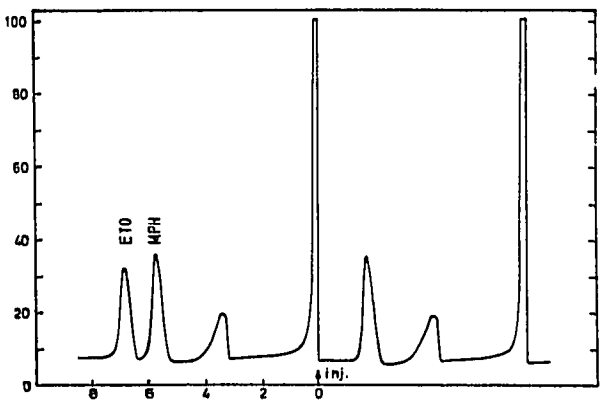
The following reagents were used: Dichloromethane, analytical grade (Merck, Darmstadt, G.F.R.); phosphate buffer, 1 *M*, pH 7.4 (Merck), ethanol, 95%; ethotoin, ethanolic solution (1 g/l); mephenetoin, ethanolic solution (1 g/l).

Extraction procedure

A quantity of 4.0 ml dichloromethane (DCM) is added to 1.0 ml serum containing 30 μg mephenetoin (MPH) as internal standard and carefully mixed for 3 min in a rotary mixer (20–30 rpm). After centrifugation for 3 min at 1000 × *g* the organic phase is transferred to another centrifuge tube containing 4.0 ml phosphate buffer. This mixture is held in a rotary mixer for 3 min (20–30 rpm). After centrifugation the organic phase is transferred to a tapered tube and evaporated to dryness under a stream of nitrogen at room temperature. The residue is dissolved in 25 μl ethanol and a 5-μl quantity is injected into the gas chromatograph.

Calculations

The serum concentrations are read from standard curves constructed from chromatograms of serum samples containing varying amounts of ethotoin (Fig. 1). The peak height ratio between ethotoin (ETO) and MPH is plotted against the concentration in mg/l (Fig. 2).



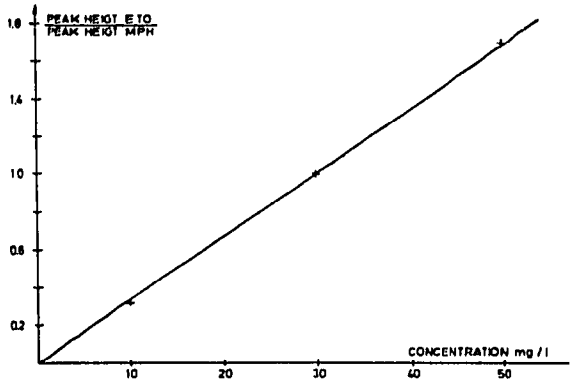


Fig. 1. Read from right to left a demonstration of chromatograms of two serum samples, respectively, a serum blank and a serum sample containing ETO. The internal standard (MPH) appears after about 6 min, the ETO after about 7 min.

Fig. 2. Standard curve constructed from analyses of serum samples with varying but known amounts of ETO.

RESULTS AND DISCUSSION

Reproducibility tests for ETO present in varying concentrations in serum samples were performed on twelve serum samples in the lower as well as in the upper part of the presumed concentration range. The recovery of ETO from serum is found to lie between 97–102% through the whole range. Details are shown in Table I. The lower limit for reliable quantitation is 1 mg/l.

TABLE I

RECOVERY OF ETHOTOIN FROM SERUM

The serum volume extracted was in all cases 1 ml.

Concentration of ethotoin in serum (mg/l)	*Apparent concentration (mg/l)*	*% Recovery*
5.0	4.9	98
5.0	5.1	102
15.0	14.9	99
15.0	15.0	100
25.0	25.2	100
25.0	25.1	100
35.0	34.7	99
35.0	34.0	97
45.0	45.0	100
45.0	45.6	102
55.0	55.7	102
55.0	54.3	99

TABLE II

SELECTIVITY OF THE METHOD DEMONSTRATED WITH A MIXTURE OF DRUGS IN SERUM SAMPLES

Concentrations (mg/l)							
Ethotoin		*Phenytoin*		*Carbamazepine*		*Phenobarbital*	
Added	*Recovered*	*Added*	*Recovered*	*Added*	*Recovered*	*Added*	*Recovered*
15.0	14.8	5.0	–	–	–	–	–
15.0	14.9	25.0	–	–	–	–	–
15.0	15.1	–	–	5.0	–	–	–
15.0	14.7	–	–	25.0	–	–	–
15.0	15.2	–	–	–	–	5.0	-
15.0	15.0	–	–	–	–	25.0	–
5.0	4.9	15.0	–	15.0	–	15.0	–
15.0	14.8	15.0	–	15.0	–	15.0	–
25.0	25.2	15.0	–	15.0	–	15.0	–
35.0	35.1	15.0	–	15.0	–	15.0	–
45.0	44.7	15.0	–	15.0	–	15.0	–
55.0	55.7	15.0	–	15.0	–	15.0	–

As simultaneous medication with analogous drugs is common, the method has been tested in the presence of other anti-epileptic drugs, in particular phenytoin, carbamazepine and phenobarbital. The results presented in Table II demonstrate a satisfactory selectivity in the concentration range 5–55 mg ETO/l serum. Furthermore, the selectivity was proved by mass fragmentography. The instrument used was a combined gas chromatograph–mass spectrometer LKB 9000. The mass spectra of ETO and MPH are presented in Fig. 3, all ion fragments with an intensity below 5% being excluded. With the mass spectrometer used as an ion-specific detector for the gas chromatograph a mass fragmentographic determination was carried out with ETO focussed on the molecular ion *m/e* 204 and MPH focussed on the ion fragment 189. The mass fragmentogram is demonstrated in Fig. 4. The same serum samples run on the gas chromatograph were determined by mass fragmentography. The results presented in Table III indicate that the two methods give the same results.

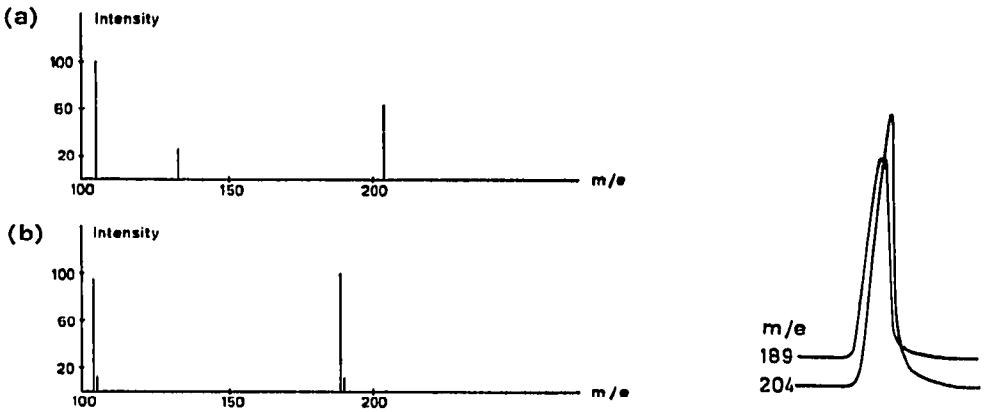


Fig. 3. Mass spectra of (a) ETO and (b) MPH. All intensities below 5% are excluded.

Fig. 4. Mass fragmentogram of a serum sample containing ETO and internal standard, separately registered on two different channels. The instrument settings were as follows: column temperature, 200°; electron energy, 70 eV; trap current, 60 μA. Channel settings: 204 (M^+ for ETO) and 189 (M^+ for MPH).

TABLE III

COMPARISON BETWEEN THE GAS CHROMATOGRAPHIC (GC) AND THE MASS FRAGMENTOGRAPHIC (MF) METHODS

Added concentration of ETO (mg/l)	*Apparent concentration of ETO (mg/l)*	
	GC method	*MF method*
10.0	9.9	10.1
20.0	20.2	19.8
30.0	29.9	30.2
40.0	39.6	40.0
45.0	45.4	45.2
50.0	50.1	50.0

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