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Letter to the Editor

Rapid extraction method for ethosuximide and other antiepileptics in serum for determination by high-performance liquid chromatography

Sir,

Our recently published high-performance liquid chromatographic method [1] for the simultaneous determination of eleven antiepileptic compounds showed a rather high day-to-day coefficient of variation (C.V.) of 9.8% for ethosuximide, the other substances having a mean C.V. of 5.4%. The high C.V. for ethosuximide can be partly attributed to the high water-solubility of this drug, which reduces the extraction into an organic solvent. Equal extraction conditions were guaranteed by shaking the standards and tests simultaneously [1]. The low melting point of ethosuximide (64—65°C) and possibly low vapour pressure also contribute to the high C.V., although the evaporation was done at 37°C by means of a direct air stream [1]. The extraction method described has a more pronounced salting out effect than the earlier method [1] and the evaporation step is omitted. The method described by Kabra et al. [2], where the extraction step is also left out, was not selected because we believe that the present extraction results in a cleaner and a more concentrated injection solution.

Our method is as follows: 400 μl of serum, 400 μl of saturated sodium dihydrogen phosphate in water (ca. 850 g of $NaH_2PO_4 \cdot H_2O$ dissolved in 1 l of water, pH 3.4) and 600 μl of acetonitrile containing the internal standard (45 mg of hexobarbital in 1 l of acetonitrile) are placed in a 100 × 15 mm glass tube. The tubes are vortex-mixed for 15 sec and centrifuged for 10 min at 3000 *g*. Due to the high salt content, an acetonitrile extract separates from the water phase and 300 μl of supernatant can be pipetted away from the protein pellet; 8 μl of this extract are injected into a Hewlett-Packard 1084 B high-performance liquid chromatograph. The liquid chromatographic settings are the same as published [1], except for the wavelength which is 204 nm.

A chromatographic separation of a drug standard which was added to drug-free serum and extracted as a patient serum is shown in Fig. 1. Fig. 2 shows a chromatogram of the Seronorm Pharmaca AED control serum (toxic level, diluted 1:1 with water; Nygaard, Norway), which was used for the within-day precision of the described method (Table I). The day-to-day C.V. was 5.6% (425.1 ± 25.3 μmol/l, n = 27) and was determined by analysing pooled serum

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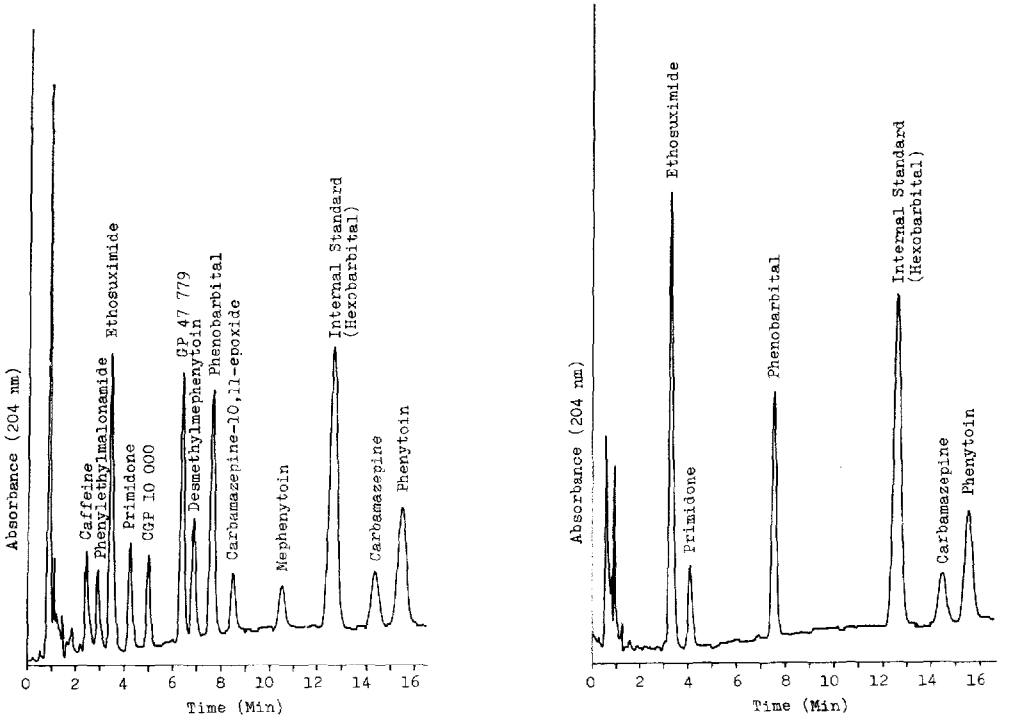

Fig. 1. Chromatographic separation of the drug standard [1] which was added to drug-free serum and extracted as a patient serum. Column: LiChrosorb RP-8, 10 μm, 25 × 0.4 cm. Gradient elution with 17—23% acetonitrile in water for 6 min, remaining isocratic for the rest of the run. Flow-rate: 2.3 ml/min. Temperature: 35°C. Wavelength: 204 nm.

Fig. 2. Chromatographic separation of Seronorm Pharmaca AED control serum. The concentrations are those listed in Table I. Conditions are the same as in Fig. 1.

TABLE I

WITHIN-DAY PRECISION OF THE METHOD

n = 32 in every case.

Drug	Concentration (mean ± S.D.) (μmol/l)	C.V. (%)
Carbamazepine	30.5 ± 0.9	2.9
Ethosuximide	442.3 ± 11.0	2.5
Primidone	30.1 ± 2.5	8.3
Phenobarbital	89.5 ± 1.3	1.5
Phenytoin	45.6 ± 1.6	3.5

containing only ethosuximide. The determination of primidone is not recommended with these liquid chromatographic settings, because in some cases an unidentified peak with the same retention time as primidone can appear with drug-free serum.

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1 N. Wad, J. Chromatogr., 305 (1984) 127—133.
2 P.M. Kabra, D.M. McDonald and L.J. Marton, J. Anal. Toxicol., 2 (1978) 127—133.

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