CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Phenobarbital in Serum

Purpose
Determination of phenobarbital in serum.

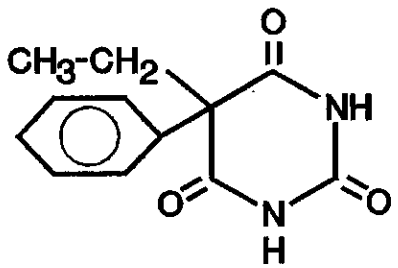

Figure 1. Structure of phenobarbital.

Phenobarbital (F1, 5-ethyl-5-phenyl-2,4,6(1H,3H,5H)-pyrimidinetrione) is a widely used barbiturate classified as an anticonvulsant and sedative-hypnotic. It is used in the treatment of seizures (including those of the newborn) and as a long-acting sedative. Therapeutic doses range from 10-40 µg/mL blood. Side effects associated with the use of phenobarbital may include impaired coordination (ataxia), liver damage, and drowsiness.

Existing Methods
GC coupled with chemical ionization mass spectrometry, enzyme immunoassay and LC. Numerous methods have been published and are available commercially.

Conditions
Detector: BAS UV-108 variable wavelength UV detector (220 nm)
Column: 3 µm, C 18 reverse-phase, 100 x 3.2 mm (PN MF-6213)
Mobile Phase: 80% (v:v) 25 mM Na_2HPO_4, pH 7.0 , 20% acetonitrile. Flow rate was 1 mL/min.
Detection Limit: 175 pg injected standard, 35 ng/mL serum (S/N = 3).
Linear Range: 0.5-100 ng injected standards, 5-40 µg/mL serum.

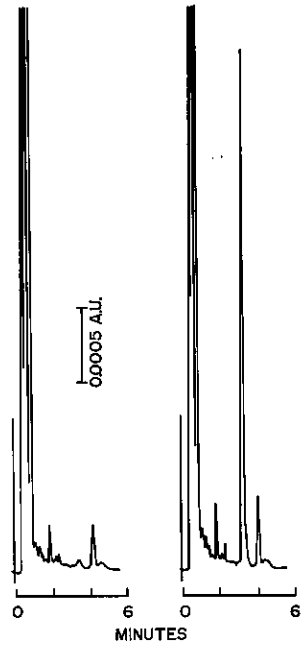

Figure 2. Calibration curve for spiked serum samples.

Sample Preparation
1. Prepare Bond-Elut® C 18 solid-phase extraction columns by washing with 1 mL methanol followed by 1 mL water.

2. Load the following onto each column: 50 µL serum, standards as appropriate, and 0.05 M NaH_2PO_4 (pH 5.0) for a final volume of 1 mL.

3. Wash the column with an additional 1 mL of phosphate buffer followed by two 1-mL washes of water.

4. Elute the samples with 1 mL methanol. Dry each in a stream of nitrogen or in a vacuum evaporator. Redissolve in 0.350 mL mobile phase and inject in 20 µL aliquots.

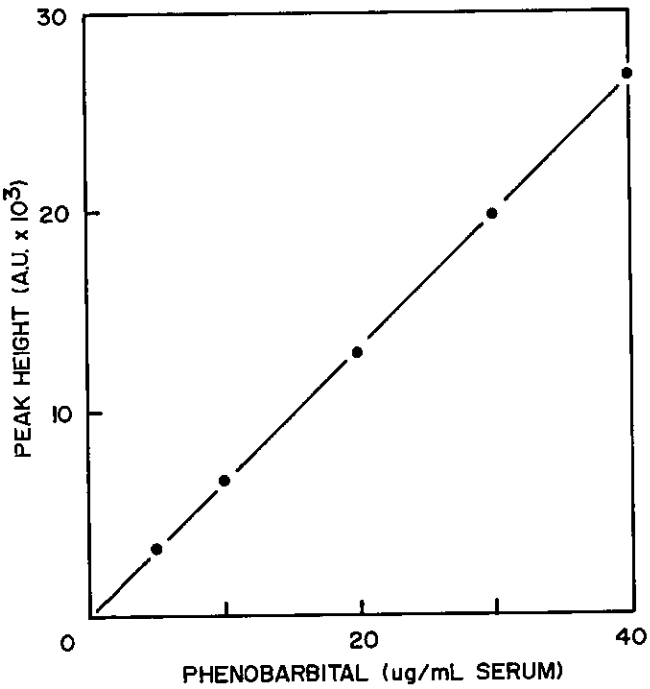

Figure 3. Sample chromatograms of blank (left) and spiked (5 µg phenobarbital/mL) serum samples.

Notes

A calibration curve for spiked serum samples is presented in F2, and sample chromatograms are shown in F3.

Recovery of phenobarbital from spiked serum samples was 98%.

5-methyl-5-phenylhydantoin [1], guaifenesin [2] and 5-(p- tolyl)-5-phenylhydantoin [3] have been used as internal standards.

References

1. Bhargava, V.O., J. Chromatogr. 419 (1987) 421-425.

2. Shabbir, U. and F. Castro, J. Liq. Chromatogr. 9 (1986) 2269-2279.

3. Soto-Otero, R., E. Mendez-Alvarez and G. Sierra-Marcuno, J. Liq. Chromatogr. 8 (1985) 753-763.