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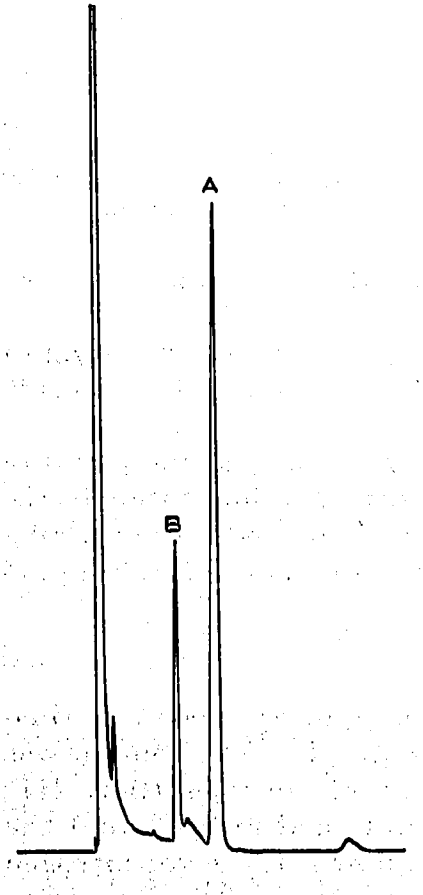
The determination of 5,5-diphenylhydantoin (dilantin) in serum by gas chromatography

A gas chromatographic (GC) method is described for the rapid detection of dilantin in serum. The procedure, in which decyl stearate is used as an internal standard, offers sensitivity, linearity, specificity and can be completed in 30 min.

In recent years there has been an increasing need for determinations of dilantin in serum for monitoring therapeutic levels as well as for the treatment of accidental overdosages. Many methods are available for the determination of dilantin. However, of all the techniques developed, GC appears to be the method of choice as it offers quantitation, sensitivity and specificity that are unattainable by other procedures.

Of the current GC methods available, several require methylation[1-4] of dilantin, which is time-consuming and tedious. Others involve lengthy extraction procedures[3,5], or give chromatograms that contain extraneous peaks and interferences[6,7]. Finally, one method has low sensitivity and cannot measure dilantin quantitatively[8].

The method described here offers the sensitivity that is needed for detecting therapeutic as well as toxic concentrations of dilantin. The chromatograms obtained show sharp peaks without significant tailing, enabling simple quantitation based on



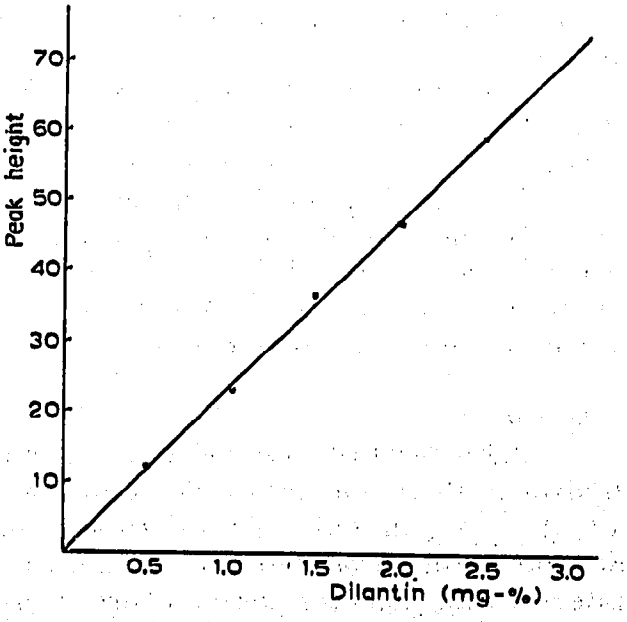


Fig. 1. Recovery of dilantin from serum. A, internal standard (decyl stearate); B, dilantin.
Fig. 2. Recovery of 0.5–2.5 mg % of dilantin from blood.

Fig. 3. Comparison of serum (patient) sample with recovery of 3 mg % dilantin. A, 3 mg % recovery; B, unknown serum sample. Peaks: 1, recovery of 3 mg % dilantin in acetone; 2, decyl stearate (internal standard); 3, phenobarbital; 4, unknown; 5, decyl stearate; 6, dilantin.

peak height ratios using decyl stearate as an internal standard. An example is shown in Fig. 1.

The linearity of the procedure is demonstrated in Fig. 2, where 0.5–2.5 μg of dilantin, representing 0.5–2.5 mg % in blood, is shown. We have found that levels as low as 0.1 mg % can be detected.

In Fig. 3, a serum sample is analysed for dilantin by comparing the ratios of the peak heights of dilantin and decyl stearate with the corresponding ratios in the 3 mg % recovery. The unknown peak shown in Fig. 3 always precedes the internal standard and causes no interferences in the determination of dilantin. Additional work is being carried out to identify this peak.

Apparatus

A Barber-Coleman Selecta-System/5000 gas chromatograph, equipped with dual hydrogen flame ionization detectors, was used for chromatography. The column was a 6 ft. × 1/4 in. O.D. glass U-tube packed with 3% OV-17 on Chromosorb W HP, 80–100 mesh (Analabs). The column temperature was maintained at 250°, and the injector and detector temperatures were held at 265°. The flow-rates were: nitrogen carrier gas, 75 ml/min; hydrogen, 16.7 ml/min; and air, 300 ml/min. The sensitivity setting was at range 10^2 and the attenuation was × 3.

Reagents

The reagents used were anhydrous diethyl ether (Mallinckrodt Co.); acetone, reagent grade (J. T. Baker Co.); a saturated solution of monobasic sodium phosphate; and a 0.5 mg/ml solution of decyl stearate (internal standard) in acetone.

Procedure

To a 15-ml graduated centrifuge tube (Corning 8084) containing 2 ml of serum are added 2 ml of saturated sodium dihydrogen phosphate solution, followed by 10 ml of diethyl ether. The tube is stoppered and shaken vigorously by hand for 30 sec and then centrifuged for 1 min at 2000 r.p.m. Then 7 ml of the clear ethereal extract are transferred into a 15-ml conical tube (Corning 8500), in which the ether is evaporated almost to dryness by heating it in a water bath (40°) with a stream of dry air. The residue is concentrated in the tip of the conical tube by washing down the side with 1 ml of diethyl ether and evaporating it to dryness. The resulting residue is dissolved by adding 100 μl of acetone solution containing 0.5 mg/ml of decyl stearate, and a 5-μl aliquot is injected into the gas chromatograph under the conditions described above.

Discussion

The recovery of dilantin from serum was determined by adding fixed amounts of dilantin to serum and comparing the recovery with a known standard. The results are shown in Table I.

TABLE I

RECOVERY OF DILANTIN ADDED TO BLOOD

Dilantin added (μg/ml)	*Recovered (μg/ml)*	*Recovery (%)*
5	5.1	105
10	10	100
20	21	105
30	29.1	97
40	38	95

The excellent recoveries may be due in part to the use of the saturated solution of monobasic sodium phosphate as a protein precipitant. The protein precipitated by this salting-out effect is unaltered and can be re-dissolved if further water is added. The subsequent ether extraction produces a clear organic phase with no emulsions or turbidity interferences.

Although metabolites of dilantin are not a major problem in serum analyses, further work is being carried out to isolate and identify these substances.

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