Journal of Chromatography, 104 (1975) 215–216
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CHROM. 7944

Note

Gas chromatographic microdetermination of acetanilide in blood

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(Received August 22nd, 1974)

The colorimetric method of Brodie and Axelrod[1] has been used for the determination of acetanilide in biological fluids. This method involves hydrolysis to aniline and its subsequent determination by diazotization and coupling. Gas chromatography (GC) has been used extensively for the analysis of related compounds such as acetaminophen[2–4] and phenacetin[3,4]; Prescott[3] reported the GC retention time data of acetanilide on various stationary phases. We report here a simple GC assay which requires only 100 µl of whole blood. The method was used for the determination of the half-life of acetanilide in rat blood.

EXPERIMENTAL

Reagent grade ethyl acetate was used. Acetanilide (zone-refined) was obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Male Holtzman rats of 280 g average weight were dosed with 50 mg/kg acetanilide intraperitoneally. Blood samples (100 µl) were collected from the tail vein in heparinized tubes just before the administration of acetanilide and at various time intervals thereafter. To each sample was added 100 mg of sodium chloride and 100 µl of ethyl acetate. The contents were stirred on a Vortex mixer for 30 sec and centrifuged to separate the layers. Aliquots of the ethyl acetate layer were injected into the gas chromatograph.

The analyses were performed on a Varian Aerograph Model 1200 gas chromatograph equipped with a flame ionization detector. A 6-ft. × 0.07-in.-I.D. stainless-steel column, fitted with a 6-in. × 0.1-in.-I.D. glass injector, was packed with 3% Versamid 900 (Supelco, Bellafonte, Pa., U.S.A.) on Gas-Chrom Q, 80–100 mesh, and operated at 150°. The injector and detector temperatures were 220° and 270°, respectively. Peak heights were used for quantitation.

For recovery studies, acetanilide in the range of 60–400 µg/ml was added to rat blood. A recovery of 88.9 ± 4.3% (mean ± S.E.) was obtained, and the standard curves were linear over this range. Control blood samples showed no interfering peaks.

Semi-logarithmic plots of blood concentrations *vs.* time showed first-order decay. Half-life data were calculated by the method of least squares using computer programs written in our laboratory.

RESULTS AND DISCUSSION

The method described here is very rapid, simple and permits the analyses of

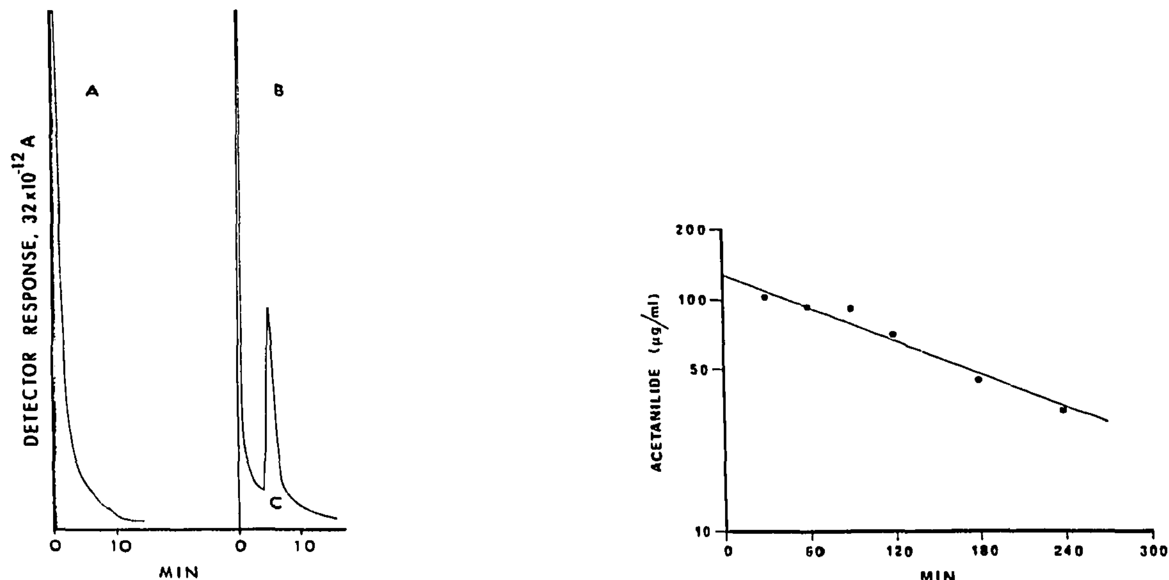

Fig. 1. Gas chromatograms of: (A) control and (B) experimental rat blood containing 100 μg/ml of acetanilide; (C) acetanilide. Column, 6-ft. × 0.07-in.-I.D. stainless-steel tube packed with 3% Versamid 900 on Gas-Chrom Q, 150°; injector, 6-in. × 0.1-in.-I.D. glass tube, 220°; detector temperature, 270°; nitrogen flow-rate, 10 ml/min.

Fig. 2. Half-life of acetanilide in rat blood; dose, 50 mg/kg; $t\frac{1}{2}$, 122.9 min.

many samples a day. Fig. 1 shows chromatograms of control and experimental blood samples. The colorimetric method[1] was much more time-consuming and involved several steps and the minimum amount of acetanilide that could be detected was 4 μg. With the GC method described here the lower limit of detection was 60 ng, but this could be substantially lowered by increasing the sensitivity setting of the chromatograph.

The half-life of acetanilide in rats was determined. Fig. 2 shows a representative experiment. Using four animals, a half-life of 117.2 ± 7.6 min (mean ± S.E.) was obtained. Also, this method has been employed in our laboratory in studying the inhibition of acetanilide metabolism by various compounds in rats and human subjects. Furthermore, the small sample size would permit use in pediatric subjects.

ACKNOWLEDGEMENTS

This work was supported by U.S.P.H.S., N.I.H. Grant No. GM 17699. The authors acknowledge the assistance of Jerry Soechting and Adrian Swanson.

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