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Note

Determination of antipyrine in plasma by reversed-phase high-performance liquid chromatography

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The kinetics of the elimination of antipyrine (phenazone) from plasma has been used as an index of hepatic microsomal drug metabolism [1—4]. Interest in the detection of antipyrine in biologic fluids has lead to the description of several new methods for the quantitative measurement of this drug [5—7]. Recently Eichelbaum and Spannbrucker [8] have described a rapid and sensitive method for the measurement of antipyrine by high-performance liquid chromatography (HPLC). Their method requires extraction of 0.5-ml samples of plasma or saliva with dichloromethane at alkaline pH prior to chromatography over silica gel.

We report an alternative method for the measurement of antipyrine using reversed-phase HPLC. This method uses smaller aliquots of plasma (0.05—0.20 ml) and does not require extraction into organic solvents. Chromatography is performed at acid pH over bonded octadecylsilane with benzoic acid as an internal standard. The precision, accuracy, and sensitivity of this method is comparable to that reported by Eichelbaum and Spannbrucker.

EXPERIMENTAL

Chemicals

Antipyrine, N.F. was purchased from Mallinckrodt (St. Louis, Mo., U.S.A.). Benzoic acid and acetonitrile were purchased from J.T. Baker (Phillipsburg, N.J., U.S.A.) as "Baker Analyzed Grade". Methanol was "Spectroanalyzed Grade" from Fisher Scientific (Fairlawn, N.J., U.S.A.).

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Apparatus

The chromatographic system consists of a Milton Roy pump (Glass Engineering, Houston, Texas, U.S.A.); a Model U6K Universal Injector and Model 440 Ultraviolet Detector (Waters Assoc., Milford, Mass., U.S.A.). Ultraviolet absorption is measured at 254 nm. Samples are chromatographed at ambient temperature (21—24°) over a 300 × 4 mm I.D. stainless-steel column packed with μBondapak C_{18} (10 μm) (Waters Assoc.). The mobile phase is acetonitrile—acetic acid (1% in water) (35:65) at a flow-rate of 1 ml/min (pressure, 68 bar).

Procedure

1.6 ml of methanol was added to a glass tube containing 0.05—0.20 ml of plasma (0.5 U heparin sodium, USP, per ml) and 10 ng of benzoic acid (in 50 μl of water). The volume was adjusted to 2.0 ml with water. After mixing on a vortex mixer for 15 sec protein was removed by centrifugation at 3,000 *g* for 10 min. The supernatant was filtered through a 0.6-μm paper filter (Millipore, Bedford, Mass., U.S.A.), and 25 μl of the filtrate were injected into the chromatographic system.

RESULTS AND DISCUSSION

With the above procedure, the retention time of antipyrine was 5.5 min and of benzoic acid 7.7 min (Fig. 1). Column efficiency (HETP) for antipyrine was 0.4 mm and for benzoic acid, 0.1 mm. (Antipyrine was as easily measured at lower ratios of acetonitrile to acetic acid but the retention times were increased.)

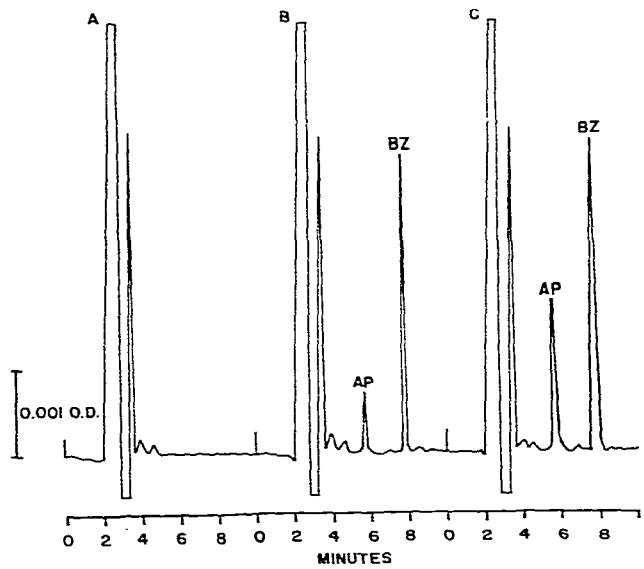

Fig. 1. Chromatograms of plasma containing antipyrine (AP) and benzoic acid (BZ). 50 μl of plasma containing (A) 0, (B) 8 and (C) 20 μg/ml antipyrine (AP) were treated as noted in the text. Benzoic acid was added to (B) and (C) as internal standard. 25 μl of the diluted and deproteinized plasma were injected. Absorbance at full scale deflection was 0.005 O.D.

Optical density was linear with concentration between 0.5 and 80 μg antipyrine per ml of plasma. Using the peak-height ratio method of quantification, the coefficient of variation for 10 replicate samples of plasma was 2.3% at 1 μg/ml and 2.1% at 8 μg/ml with 0.05-ml plasma samples; and 0.1% at 8 μg/ml and 0.1% at 20 μg/ml with 0.20-ml samples. Precision over a 45-day period was ± 5.5% (n = 16) using 0.05-ml aliquots of plasma (19 μg/ml) obtained from a subject 4 h after receiving 18 mg antipyrine per kg body weight by mouth.

Recovery of antipyrine added to plasma was linear in the range 1—8 μg/ml (0.05-ml samples) and 8—80 μg/ml (0.20-ml samples). The correlation coefficient for each group was greater than 0.99. The slope of the regression line was 1.02 ± 0.01 for the 0.20-ml samples and 1.08 ± 0.06 for the 0.05-ml samples.

We have performed over 200 determinations of antipyrine in human plasma with this method. No interfering peaks were noted in plasma from patients receiving a variety of drugs including salicylates, hydrochlorothiazide, prednisone, or insulin. 4-Aminoantipyrine is detected by this method with a retention time of 5.9 min. However, its molar extinction coefficient at this pH is less than 3% of that of antipyrine. The more polar antipyrine metabolites do not interfere with the antipyrine or benzoic acid peaks. In two separate patient studies, we compared the rate of elimination of antipyrine from plasma derived from assays using 0.05-ml and 0.20-ml aliquots. The apparent first-order rate constants for the elimination of antipyrine from plasma were 0.0422 and 0.0491 h^{-1} with 0.20-ml specimens and 0.0452 and 0.0475 h^{-1} with 0.05-ml specimens, respectively.

We believe that this method for quantification of antipyrine offers a useful alternative to that of Eichelbaum and Spannbrucker [8]. Our method requires smaller sample volumes and may be more suitable for studies in laboratory animals or pediatric patients. This method also eliminates the requirement for quantitative extraction into organic solvents and may prove useful for simultaneous determination of antipyrine and other compounds.

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