Improved Ultraviolet Spectrophotometry of Serum Theophylline

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We present an improved method for ultraviolet spectrophotometry of theophylline in serum. We studied various extraction techniques aimed at eliminating interferences from co-extractable serum constituents. In the resulting modified procedure, 1 ml of serum is required and a salt-solvent pair of ammonium sulfate and chloroform/hexane is used for extraction. The solvent forms the top phase after extraction, the lower phase after back-extraction, thereby permitting easy removal of the appropriate phase from culture tubes. The use of ammonium sulfate coupled with the added specificity of the extraction solvent results in an extract with low background absorption and a well-defined spectrum for the extracted theophylline.

In laboratories with no gas-liquid or high-performance liquid-chromatographic equipment, theophylline is usually determined by ultraviolet spectrophotometry. Such methods generally require 2–3 ml of serum, relatively large volumes of extracting solvent, and filtration of the solvent before the drug is back-extracted into an aqueous medium (1, 2). Here we describe a procedure for the determination of theophylline in 1 ml of serum with use of 16 x 150 mm culture tubes, and validate the use of a salting-out technique and a selective extraction solvent for the analysis.

Materials and Methods

Apparatus

We used an “Acta III” double-beam recording spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif. 92630) for these studies.

Reagents

Theophylline, caffeine, and theobromine, anhydrous crystals, were obtained from Sigma Chemical Co., St. Louis, Mo. 63178.

Ammonium sulfate, granular, was from Mallinckrodt Inc., St. Louis, Mo. 63160.

Theophylline stock standards. Aqueous theophylline standards were prepared in concentrations of 100, 200, and 300 mg/liter. The standards, stored at 4°C, were stable for at least six months.

Theophylline standards in plasma. For assessment of analytical recoveries, standard curves, and other studies we used outdated blood-bank plasma. For the standard curves, add 1 ml of plasma to each of a series of 16 x 150 mm (or 20 x 125 mm) culture tubes, add 100 µl of each theophylline stock standard, and mix on a vortex-type mixer for 15 s.

Procedure

Add a constant amount of granular ammonium sulfate (about 0.8 g in the spoon end of a porcelain spatula, Coors, 19-K) in sequence to the culture tubes containing 1 ml of patient’s serum or plasma-based standards. Mix the contents of each tube on a vortex-type mixer for 15 s. Add 15 ml of chloroform/hexane (7/3 by vol) to each tube, cap the tubes with Teflon-lined caps, and hand-shake for 10 s to ensure that the caps do not leak and to facilitate uniform dispersion of serum. Then extract for 15 min on either an Eberbach shaker or a rotating-type mixer. Centrifuge (2000 x g, 5 min) to separate the phases and transfer 14 ml of the organic phase to a 16 x 150 mm culture tube. Add 3 ml of bicarbonate-carbonate buffer (pH 9.0, 0.1 mol/liter), prepared as described in reference 2, to each tube and back-extract the theophylline by mechanically shaking it for 10 min. After a 1-min centrifugation, transfer the aqueous (top) phase to a 1-cm quartz cuvet and scan in the wavelength range from 330 to 240 nm.
to ensure that the maximum absorbance is at 275 nm, the wavelength used in calculations.

Results and Discussion

We evaluated several solvent combinations. An amount of ammonium sulfate that completely saturates the aqueous phase improves the extraction with chloroform/hexane from 30 to 88% and with chloroform from 44 to 78%, and also effectively precipitates serum proteins and decreases the amount of co-extractable material in serum extracts (3). The relatively nonpolar chloroform/hexane mixture has distinct advantages over either chloroform or chloroform/isopropanol (95/5 by vol). Chloroform/isopropanol extracts of patients' sera exhibited higher baselines and a higher absorbance ratio at 250/275 nm than did extracts with chloroform/hexane. Use of chloroform/hexane also resulted in higher recoveries than was true for chloroform. Unlike chloroform or chloroform/isopropanol, chloroform/hexane is the upper phase after extraction of serum, but is the lower phase after subsequent back-extraction into the buffer. This permits easy transfer both of the organic phase, for back-extraction, and the bicarbonate/carbonate phase, for spectrophotometric scanning.

Assays of sera from patients not receiving theophylline resulted in a baseline absorbance at 275 nm equivalent to 1.0 ± 0.4 mg/liter with the proposed procedure, whereas values with the chloroform/isopropanol method (1) were 2.5 ± 1.0 mg/liter, with correction for absorbance at 300 nm (n = 10). With samples from patients on theophylline therapy, the chloroform/isopropanol method produced values that were 9% higher than those obtained with chloroform/hexane (n = 20). The high blank values and higher absorbance at 250 nm appear largely to result from the use of the more polar isopropanol for extraction and the more basic sodium hydroxide for back extraction. Interferences due to endogenous color and turbidity have been mentioned previously as a problem in analyzing theophylline (1, 4).

The procedure of Hicks (2) in which chloroform and a bicarbonate/carbonate buffer are used results in theophylline values that are uniformly 20% lower than those achieved with chloroform/hexane and 25% lower than those obtained with an electron-capture gas-chromatographic method (n > 50). With the proposed method, theophylline values were 3.4% lower than those determined by gas-chromatography.

The procedure results in analytical recoveries of 88% and CV's of 3.1 and 5.0% for within-day and day-to-day analyses, respectively; linearity exceeds 30 mg/liter. A least-squares comparison to an electron-capture gas-chromatographic method (x-axis) yielded values for slope, intercept, and correlation coefficient of 1.002, 0.971, and 0.986, respectively. Serious interference by barbiturates, phenytoin, acetaminophen, allopurinol, salicylates, and phenylbutazone (1) with the procedure of Schack and Waxler has been described recently (5). We have found no interference from these drugs, or from diphenhydramine, ephedrine, or diazepam at concentrations of 20 mg/liter. Acetaminophen, however, at 20 mg/liter would increase values for apparent theophylline by 4.0 mg/liter. Dyphylline interferes with both analytical methods. Caffeine does not interfere with the described method, and 3-methylxanthine would contribute a value of less than 0.6 mg/liter serum based on its known concentration and its low recoveries from serum (20%) with this extraction system. As verified by gas chromatography, we have found interference from unknown drugs or metabolites in 1 to 2% of the patients' samples assayed with the spectrophotometric procedure.

References