CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Plasma Theophylline

Purpose

Monitoring therapeutic levels of theophylline in plasma.

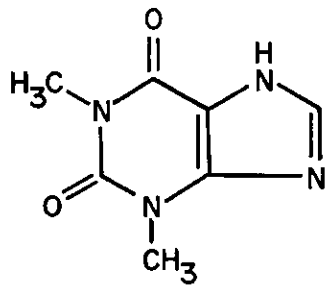

Figure 1. Structure of theophylline.

Theophylline (F1, 1,3-dimethylxanthine) is a potent bronchodilator frequently used for the treatment of respiratory problems, most notably acute bronchial asthma. Therapeutic levels of theophylline in plasma normally range from 5 to 20 µg/mL, while toxic effects may occur at concentrations of 25 µg/mL or greater. Metabolic variations among treated individuals make routine monitoring of drug levels necessary.

The determination of plasma theophylline is easily accomplished using liquid chromatography combined with UV detection (LCUV). The superior resolving abilities of reverse-phase columns eliminates the need for extensive sample clean-up. Simple deproteinization of plasma samples is generally adequate. The procedure outlined below, a composite of several previously published methods, illustrates the suitability of LCUV combined with very simple sample preparation for monitoring plasma theophylline levels.

Conditions

Detector: BAS UV-8 fixed wavelength (254 nm)
System: BAS 400 Liquid Chromatograph

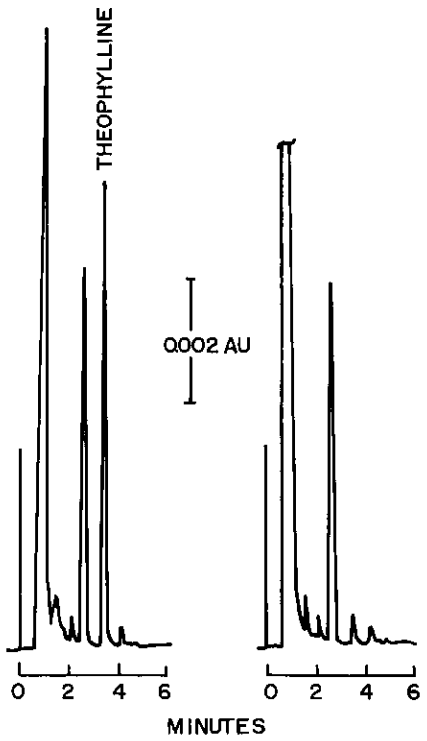

Figure 2. Chromatograms of unspiked plasma (right) and plasma spiked with 10 µg/mL theophylline.

Column: BAS 3 µm Phase-II ODS (100 x 3.2 mm) (PN MF-6213)
Mobile Phase: 95% (v:v) 50 mM sodium acetate adjusted to pH 5.0, 5% acetonitrile. Flow rate was 0.8 mL/min.
Linear Range: 5 - 60 µg/mL plasma

Sample Preparation

1. Add 100 µL aliquots of plasma to centrifuge tubes.
2. Add theophylline standards and/or water for a total volume (including plasma) of 140 µL.

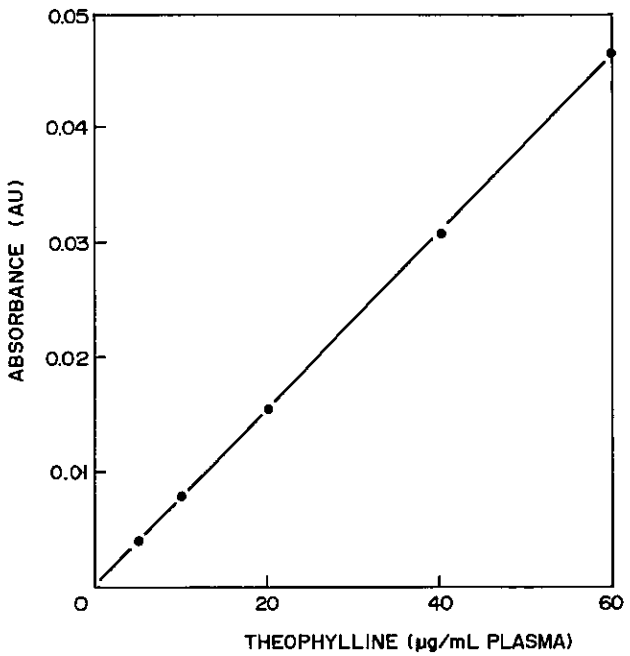

Figure 3. Calibration curve for plasma samples spiked with theophylline and processed as described in text. Each point represents the mean of 2 determinations.

3. Add 10 µL 60% $HClO_4$ and vortex for 10 - 20 seconds.
4. Place samples in freezer for 10 minutes.
5. Add 350 µL water and vortex. Total volume is now 500 µL.
6. Centrifuge 5 minutes at 1600 x g.
7. Transfer supernatant to microfiltration tubes (PN MF-5500) containing 0.45 µm membranes (PN MF-5655) and centrifuge for another 5 minutes.
8. Inject sample. Injection volume was 20 µL throughout this calibration.

Notes

Representative chromatograms from control and theophylline-containing plasmas are shown in F2. The high resolving power of a 3 µm reverse-phase column is evident in this separation, where each sample required less than 6 minutes elution time.

A typical calibration plot is illustrated in F3.

Although only therapeutically relevant theophylline concentrations in plasma were considered here, the system should be sensitive to levels as low as 250 ng/mL plasma.

Theophylline has an absorbance maximum at 271 nm. Additional sensitivity might be achieved at this wavelength.

In the clinical laboratory, where a liquid chromatograph may be dedicated indefinitely to a particular assay due to large sample volume and the need for rapid results, the proposed method meets several requirements. First, sample preparation is simple and no expensive reagents are required. Second, the analysis time on the LC is short, allowing the necessary sample throughput. Finally, the method obviates the use of expensive gradient systems and allows the clinical laboratory the luxury of dedicated systems for specific high volume applications without the associated expense.

This determination of theophylline in plasma can also be performed on the BAS 200 Problem Solver.

References

1. J. Orcutt, P. Kozak, S. Gillman, and L. Cummins, Clin. Chem., 23(1977) 599.