CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of Acetaminophen In Plasma

Purpose

Determination of acetaminophen in plasma by LC with EC or UV detection.

Acetaminophen (F1, N-acetyl-p-aminophenol) is an accepted aspirin substitute which provides analgesic relief through the elevation of the pain threshold. It also reduces fever by acting on the hypothalmic heat-regulating center. Therapeutic concentrations range from 1 - 10 µg/mL plasma. In cases where toxic levels are suspected, therapeutic monitoring of this drug is important due to the necrotic effects of overdose concentrations on the hepatic systems. This determination can be accomplished using several techniques, including gas chromatography, thin-layer chromatography, spectrophotometry, and LCUV. The plasma concentrations encountered in acetaminophen overdose are enormous and detection limits are rarely an issue. On the other hand, for basic metabolism studies (including pharmacokinetics) there is a far better method. Because of its sensitivity and selectivity, liquid chromatography/electrochemistry provides a method of choice for accurate low level acetaminophen detection. The small volume of serum necessary and the limited amount of time required for sample preparation makes this a fast and simple analysis technique.

Conditions

System: BAS 400 Liquid Chromatograph
Column: BAS 3 µM Phase II ODS (100 x 3.2 mm) (PN MF-6213)
EC Detector: BAS Dual 4B/17A
Electrode: BAS Glassy Carbon
Potential: +0.8 V vs Ag/AgCl
UV Detector: BAS UV-8 fixed wavelength (254 nm).
Mobile Phase: 95% (v:v) 0.2 M sodium perchlorate, 0.005 M sodium citrate, pH 5.0; 5% methanol. Flow rate was 0.8 mL/min.
Detection Limits: EC: 5 pg (injected standard, S/N = 6); 5 ng/mL plasma UV: 50 pg (injected standard, S/N = 3); 50 ng/mL plasma

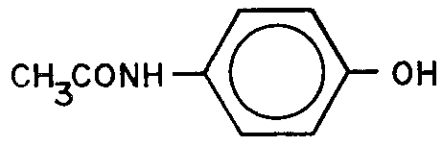

Figure 1. Structure of acetaminophen

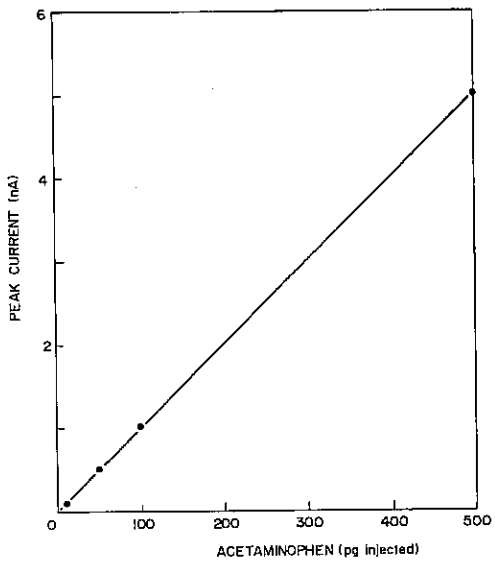

Figure 2. EC calibration curve for acetaminophen standards.

Linear Range: Linear from detection limits to at least 10 ng (injected standard) and 5 µg/mL plasma.

Sample Preparation

1. Prepare working phosphate buffer: combine 173 mg Na_2HPO_4 and 47 mg KH_2PO_4 with 80 mL distilled water. Adjust to pH 7.4 with NaOH,then add water to 100 mL. Saturate with NaCl (about 40 g) and refrigerate. Make a fresh batch weekly.

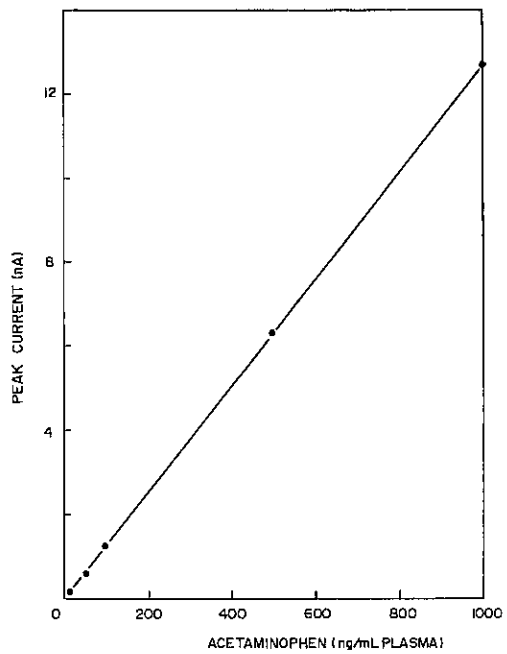

Figure 3. EC calibration curve for spiked plasma samples.

2. In a 3 mL screw-top test tube, add 50 mg NaCl, 100 µL working phosphate buffer, 100 µL plasma, and 2.5 mL ethyl acetate.

3. Cap and shake on a reciprocal shaker for 10 minutes.

4. Centrifuge for 10 minutes.

5. Transfer 2 mL of the organic layer to a 3 mL culture tube and evaporate to dryness under nitrogen at 40°C.

6. Reconstitute in 500 µL of the mobile phase.

7. Filter in a microfilter (PN MF-5500) through an RC-55 membrane (PN MF-5655) and inject 20 µL of this solution.

For calibration purposes both acetaminophen standards (F2) and plasma samples spiked with acetaminophen (F3, F4) were prepared and chromatographed.

Notes

Recovery of acetaminophen from spiked plasma samples was 78%. EC detection was about 10 times more sensitive than was UV detection.

The determination of acetaminophen presented above also can be performed on the BAS 200 Problem Solver.

Liquid Chromatography with electrochemical detection is a highly useful tool for biomedical research. As with any instrumental technique, the precision and accuracy of the measurement depends on the instrumentation, the skill and knowledge of the operator and the integrity of the sample preparation procedure. Use of these techniques for medical diagnosis and accountability for the same rests entirely with the user of this equipment.

References

1. D.J. Miner and P.T. Kissinger, Anal. Chem., 68(1979) 96- 97.

2. L. A. Pachla and K. T. Ng, J. Chromatogr., submitted.

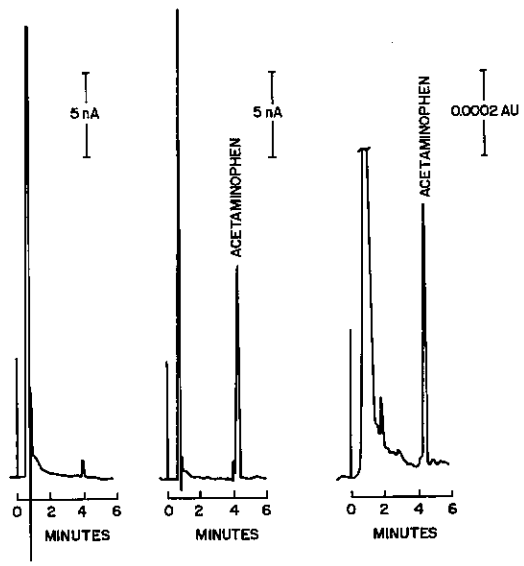

Figure 4. Chromatograms of unspiked (left) and spiked (1 µg acetaminophen/mL) plasma. Detection was by EC(left, middle) and UV (right).