Quantitative Determination of Acetaminophen in Plasma

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Abstract □ A simple method is described for the rapid quantitative analysis of acetaminophen in plasma. The acetaminophen and its conjugates present in the plasma following drug administration are hydrolyzed with 4 *N* HCl to *p*-aminophenol. This compound is coupled with 5% vanillin reagent to form a stable yellow color whose concentration is determined spectrophotometrically at 395 nm. Application of this method to a study of three dogs treated with 650 mg of acetaminophen is described.

Keyphrases □ Acetaminophen—spectrophotometric analysis in plasma □ Spectrophotometry—analysis, acetaminophen in plasma □ Analgesics—acetaminophen, spectrophotometric analysis in plasma

The widespread use of acetaminophen as an antipyretic and mild analgesic has stimulated an interest in the development of a simple and rapid plasma determination. Several methods have been published for acetaminophen and its metabolites in blood and urine (1–8). A commonly used method (1, 2) involves acid hydrolysis of conjugated acetaminophen following extraction of the resulting *p*-aminophenol into ether and then its extraction from the organic solvent into dilute acid. The *p*-aminophenol is reacted with phenol in the presence of sodium hypobromite to form an indophenol dye whose concentration is determined spectrophotometrically. However, the sample must be allowed to stand for 40 min for maximum development of the blue color. In previously published papers (3, 4), the determination of acetaminophen in capsules by utilizing vanillin reagent was reported.

According to the method presented here for acetaminophen determination in plasma, the *p*-aminophenol is extracted from the ether extract by 1 *N* HCl. The acid extract, upon the addition of 5% vanillin reagent, immediately produces an intense yellow color, which can be measured at 395 nm.

EXPERIMENTAL

Reagents—All chemicals and reagents (analytical grade) were used without further purification, unless otherwise indicated.

Protocol—Three healthy adult beagle dogs were fasted overnight. Each received a 650-mg oral dose of acetaminophen consisting of two 325-mg capsules[1]. Venous blood specimens were withdrawn with evacuated tubes[2] containing ammonium heparinate as an anticoagulant. Specimens were taken prior to and at specified times after drug administration for 10 hr. Collected specimens were centrifuged immediately at 15,000 rpm and 4° using a refrigerated centrifuge[3] for 10 min. Plasma was separated and analyzed for total acetaminophen (1, 2, 5–7) by a modification of the method of Plakogiannis and Saad (4).

Analysis—To 2 ml of plasma in a polytef-lined screw-capped centrifuge tube (20 ml), 4 ml of 4 *N* HCl was added; the sample was then diluted with distilled water to 10 ml. The tube was centrifuged at 5000 rpm for 1 hr, and the clear supernate was placed in a boiling water bath for 1 hr. Each sample was treated two or three times with 4 ml of ether, and the *p*-aminophenol was then extracted from ether with 10 ml of 1 *N* HCl. To 2 ml of the acid extract, 5 ml of 5% vanillin in 2-propanol was added; the yellow color produced was measured[4] at 395 nm. Concentrations were determined from a previously constructed standard curve.

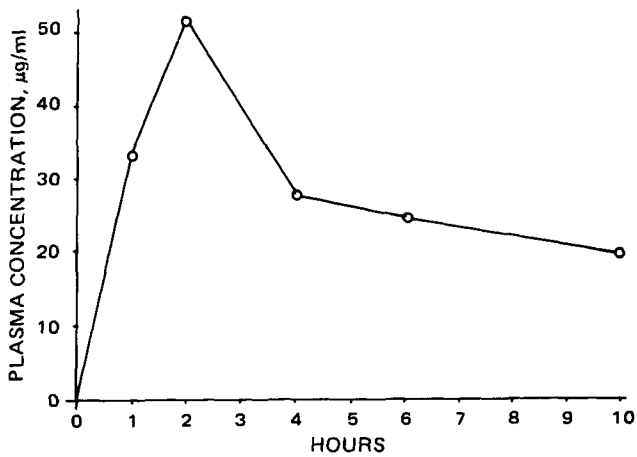

Figure 1—*Plot of plasma acetaminophen concentration* versus *time.*

RESULTS AND DISCUSSION

The biological disposition of acetaminophen in humans and animals has been extensively examined. It has been shown (9, 10) that 80% of the total dose is excreted in a conjugated form, which, upon acid hydrolysis, gives rise to *p*-aminophenol. In the present study, the plasma samples were analyzed for total, *i.e.*, free plus conjugated, acetaminophen. Plakogiannis and Saad (4, 11) showed that 4 *N* HCl and 1 hr of boiling are needed to obtain as complete hydrolysis as possible. The *p*-aminophenol then is reacted with 5% vanillin reagent in 2-propanol to produce immediately an intense yellow color, which is stable up to 24 hr.

The precision and accuracy of the method were determined by analysis of plasma samples spiked with 10, 20, 30, 40, and 50 µg of acetaminophen/ml. The recovered concentrations were equal to 98 ± 5% (mean ± *SD*).

Mean plasma concentrations observed at various sampling times from the three dogs are shown in Fig. 1. Maximum plasma concentrations of acetaminophen were observed within 2 hr, and easily measurable amounts were present at the end of the study (10 hr).

The present method appears to be accurate and precise and has the advantages over the procedure of Brodie and Axelrod (1, 2) of being less time consuming and more dependable due to color stability.

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[2] Vacutainer, Becton-Dickinson & Co.
[3] Sorvall, DuPont Co.
[4] Spectronic 200, Bausch & Lomb.