Direct Determination of Acetaminophen in Plasma by Differential Pulse Voltammetry

JAMES W. MUNSON ˣ **and HASSAN ABDINE**

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Abstract □ A rapid, simple method for the direct determination of acetaminophen in plasma at toxic levels was developed. The method is based on the oxidation of the phenolic moiety of acetaminophen at a carbon paste electrode in a differential pulse voltammetric mode. Linear calibration curves are obtained from 20 to 400 μg/ml. No sample cleanup is required.

Keyphrases □ Acetaminophen—differential pulse voltammetric analysis in plasma □ Differential pulse voltammetry—analysis, acetaminophen in plasma □ Analgesics—acetaminophen, differential pulse voltammetric analysis in plasma

The increasing use of acetaminophen as a nonprescription analgesic has led to an increase in the occurrence of accidental and intentional ingestion of toxic amounts of this material. In the United Kingdom, deaths from acetaminophen ingestion have increased steadily since 1966 (1). Death from acetaminophen toxicity is usually attributable to liver failure. In all cases of acetaminophen overdose, some degree of liver damage is present.

BACKGROUND

Since plasma acetaminophen levels are indicative of the severity of probable liver damage, the determination of plasma levels is necessary for the selection of the appropriate treatment procedures.

An assay for plasma acetaminophen levels should be rapid and simple. Since overdose patients may be admitted at any time, the assay must be of sufficient simplicity to be performed in the emergency room. A method of estimation frequently employed is the differential absorbance procedure of Routh *et al.* (2), but this method has been criticized because of interferences by other drugs (3). Several methods are based on the nitration procedure of Chafetz *et al.* (4). This reaction was used to determine acetaminophen in plasma following the precipitation of plasma proteins by trichloroacetic acid (5, 6).

The described method is based on oxidation of acetaminophen at a carbon paste electrode (graphite–mineral oil) using differential pulse voltammetry. This method is similar to differential pulse polarography, with the principal difference being in the construction of the working electrode. The current measured in the oxidation of acetaminophen is proportional to the amplitude of the applied potential pulse, the surface area of the working electrode, and the concentration of the electroactive species.

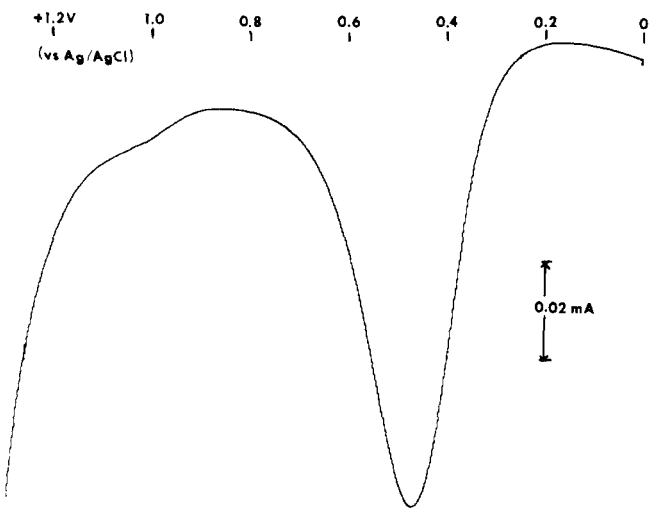

Figure 1—*Differential pulse voltammogram for acetaminophen in plasma. The concentration was 200 μg/ml. The plasma sample was diluted with an equal volume of pH 8 phosphate buffer.*

Differential pulse polarography was utilized in assays for various drugs including chlordiazepoxide (7), diazepam (8), and nitrazepam (9). The differential pulse mode also was applied to the *in vivo* detection of catecholamines with a surface-modified platinum electrode (10). Recently, differential pulse voltammetry at the carbon paste electrode was used to measure trace levels of phenols and aromatic amines (11). This configuration also was used for the determination of theophylline in plasma (12).

Acetaminophen was analyzed in a variety of dosage forms by a method based on linear dc voltammetry at the glassy carbon electrode (13).

This paper describes a method for determining acetaminophen directly in plasma at concentrations of toxicological interest.

EXPERIMENTAL

Reagents—All water was double distilled. Inorganic chemicals were analytical reagent grade and were used without further purification. Acetaminophen[1] was used as received. Individual plasma was obtained from the local blood bank. Phenobarbital, salicylic acid, theophylline, and aspirin were USP grade and were used directly. Theobromine[2] also was used without purification.

Apparatus—A polarographic analyzer system[3] was used for all measurements. A three-electrode configuration with a carbon paste electrode (14) as the working electrode, a silver–silver chloride reference electrode, and a platinum wire auxiliary electrode was used. The surface area (circular) of the carbon paste was 33 cm^2. All scans were taken at a rate of 10 mv/sec with a modulation amplitude of 50 mv and a drop time of 0.5 sec. All measurements were made in the differential pulse mode at ambient temperatures (21–24°).

The working electrode was prepared from a polytef plug (20 mm long × 15-mm diameter). A well (6-mm diameter, 4-mm depth) was drilled in one end of the polytef plug, and a 1-mm hole was drilled through the bottom of the well. A platinum wire (20 gauge) was glued (epoxy resin) into this hole so that 2 mm of the wire protruded into the well. The other end of the wire was soldered to a copper wire, and the polytef plug was glued (epoxy resin) into an open glass tube.

The carbon paste was prepared by levigating 3 g of powdered graphite[4] with 2 g of mineral oil. The paste was packed into the well to exclude any air bubbles, and the surface of the paste was smoothed with a stiff paper card (14).

Procedure—A spiked plasma sample (5.0 ml) was diluted with 5.0 ml of pH 8 phosphate buffer (0.5 *M*). The electrodes were inserted into this mixture, and 5 min was allowed to ensure a quiet solution. Then a differential pulse voltammogram was obtained from +0.2 to 0.8 v. The diffusion current was determined from the peak height and the appropriate scaling factor (current range) of the instrument.

RESULTS AND DISCUSSION

Figure 1 shows a typical differential pulse voltammogram for acetaminophen in plasma with the peak potential of +0.51 v *versus* the silver–silver chloride reference electrode. A typical calibration curve for

[1] McNeil Laboratories, Ft. Washington, Pa.
[2] Pfaltz and Bauer.
[3] Princeton Applied Research Co., model 174.
[4] Ultra Carbon, UCP-1-325, Ultra Carbon Corp., Bay City, MI 48706.

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Table I—Precision Study on Spiked Plasma Samples [a]

Acetaminophen, μg/ml	Current, μamp	CV, %
41	22.7, 21.5, 19.3, 19.5, 17.9 16.9, 18.3, 19.3, 19.3[b]	9.23
101	50.3, 49.1, 51.5, 47.6, 49.9 53.1, 51.1, 53.8, 55.0, 55.4	4.99
202	118.1, 118.1, 114.1, 106.3, 107.0 105.1, 103.1, 101.5, 99.9, 96.8	6.84
403	191.1, 195.0, 191.1, 193.1, 200.9 199.0, 199.0, 195.0[c]	1.97

[a] The data in Table I can be fitted by the equation: current (microamperes) = [acetaminophen (μg/ml)] + 2.88, with $r = 0.9960$ and $S_{y \cdot x} = 5.93$. [b] One determination was rejected because of an irregular peak shape. [c] Two determinations were rejected on a statistical basis according to the method of Dean and Dixon (16).

the range of 20–400 μg/ml can be described by the equation: current (μamp) = 0.438 concentration (μg/ml) + 12.9, with a correlation coefficient of 0.991 and a standard error of the estimate ($S_{y \cdot x}$) of 7.76. Determination of multiple spiked samples gave the results presented in Table I.

Daily minor variations in the slopes of standard curves were attributed to aging of the electrode surface. Consequently, it was necessary to prepare standard curves daily. Plasma samples containing phenobarbital, salicylic acid, theobromine, theophylline, or aspirin at levels of 100 μg/ml gave voltammograms (+0.2–0.8 v) identical to those of blank plasma at a sensitivity setting that produced a half-scale response for acetaminophen at 100 μg/ml.

This method is sufficiently simple and sensitive for the rapid determination of acetaminophen in plasma at levels likely to be encountered in cases of toxic overdosage. Since no sample preparation is required and the instrumentation requires virtually no startup time, this method represents an extremely rapid method for acetaminophen. Since the measurements are made at potentials positive with respect to the reference electrode, dissolved oxygen does not interefere. Hence, sample degassing is not required.

Since the major biotransformation of acetaminophen in humans is direct conjugation with sulfate and glucuronic acid to form the sulfate and glucuronate metabolites (15), these metabolites should not interfere in this assay. In both of these metabolites, the phenolic group, which is oxidized at the carbon paste electrode, is conjugated and unavailable for electrochemical oxidation. Although the instrumentation required for this method is probably not available in many clinical laboratories, the recent introduction of a low-priced unit[5] will undoubtedly make differential pulse voltammetry a more common tool.

REFERENCES

(1) B. G. Gazzard, M. Davis, J. B. Spooner, and R. S. Williams, *J. Int. Med. Res., Suppl. 4*, **4**, 25 (1976).

(2) J. I. Routh, N. A. Shane, E. G. Arrendondo, and W. D. Paul, *Clin. Chem.*, **14**, 882 (1968).

(3) K. Wiener, M. G. Longlands, and B. H. A. Tan, *Ann. Clin. Biochem.*, **13**, 452 (1976).

(4) L. Chafetz, R. E. Daly, H. Schriftman, and J. J. Lomner, *J. Pharm. Sci.*, **60**, 463 (1971).

(5) J. P. Glynn and S. E. Kendal, *Lancet*, **1**, 1147 (1975).

(6) K. Wiener, *Ann. Clin. Biochem.*, **14**, 55 (1977).

(7) M. R. Hackman, M. A. Brooks, J. A. F. de Silva, and T. S. Mas, *Anal. Chem.*, **46**, 1075 (1974).

(8) M. A. Brooks, J. A. F. de Silva, and M. R. Hackman, *Am. Lab.*, **5**, 23 (1973).

(9) S. Halvorsen and E. Jacobsen, *Anal. Chim. Acta*, **59**, 127 (1972).

(10) R. F. Lane, A. T. Hubbard, K. Funkunaga, and R. J. Blanchard, *Brain Res.*, **114**, 346 (1976).

(11) W. M. Chey and R. N. Adams, *J. Electroanal. Chem.*, **75**, 731 (1977).

(12) J. W. Munson and H. Abdine, *Talanta*, **25**, 221 (1978).

(13) C. M. Shearer, K. Christenson, A. Mukheji, and G. J. Papariello, *J. Pharm. Sci.*, **61**, 1627 (1972).

(14) P. T. Kissinger, C. Refshauge, R. Dreiling, and R. N. Adams, *Anal. Lett.*, **6**, 465 (1973).

(15) R. S. Andrews, C. C. Bond, J. Bernett, A. Saunders, and K. Watson, *J. Int. Med. Res., Suppl. 4*, **4**, 34 (1976).

(16) R. B. Dean and W. J. Dixon, *Anal. Chem.*, **23**, 636 (1951).

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[5] Model 364, Princeton Applied Research, Princeton, N.J.

COMMUNICATIONS

Simplified Derivation of Chiou–Hsu Equation for Rapid Estimation of Total Body Drug Clearance during Constant-Rate Intravenous Infusion

Keyphrases □ Total body drug clearance—rapid estimation during constant-rate intravenous infusion, equation derived □ Clearance, total body drug—rapid estimation during constant-rate intravenous infusion, equation derived □ Pharmacokinetics—rapid estimation of total body drug clearance during constant-rate intravenous infusion, equation derived

To the Editor:

An equation using two sets of plasma level data to estimate the total body clearance (*TBC*) of a substance during a zero-order input to the body was reported previously by Chiou and Hsu (1). The equation is:

$$TBC = \frac{2K_0}{C_{p_1} + C_{p_2}} + \frac{2V_d(C_{p_1} - C_{p_2})}{(C_{p_1} + C_{p_2})(t_2 - t_1)} \qquad \text{(Eq. 1)}$$

where K_0 is the zero-order input rate constant, V_d is the literature estimated apparent volume of distribution based on the linear one-compartment open model, and C_{p_1} and C_{p_2} are plasma levels of the substance at times t_1 and t_2 during the zero-order input, respectively.

This equation was originally proposed to estimate the total body clearance of endogenous creatinine and renal creatinine clearance in patients after a slight modification (1). It also was proposed recently to estimate rapidly the total body clearance of a drug in patients during a constant-rate intravenous infusion (2). Some precautions in using the equation for dosing individualization were discussed (2).

The derivation of Eq. 1 involved the Laplace transform

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