Journal of Chromatography, 145 (1978) 328—331
Biomedical Applications

CHROMBIO. 122

Note

Determination of acetaminophen in plasma by high-performance liquid chromatography with electrochemical detection

JAMES W. MUNSON, RICHARD WEIERSTALL* and HARRY B. KOSTENBAUDER

College of Pharmacy, University of Kentucky, Lexington, Ky. 40506 (U.S.A.)

(Received August 2nd, 1977)

The lack of extreme sensitivity in detectors for high-performance liquid chromatography (HPLC) often limits its application in the determination of drugs in blood and plasma. The development of an electrochemical detector by Kissinger et al. [1] provides for very sensitive detection of compounds that can be oxidized at the carbon-paste electrode. By using this electrochemical detector, these authors were able to detect picogram quantities of norepinephrine and dopamine. Subsequently this detector has been used in the analysis of epinephrine [2], isoproterenal [2], L-dopa [2], α-methyldopa [2], phenylephrine [2], norepinephrine [2—4], dopamine [3, 4], ascorbic acid [5] and homogentisic acid [6]. Recently, this detector was used in the analysis of acetaminophen (N-acetyl-*p*-aminophenol) in commercial dosage forms, urine and plasma [7]. While these authors reported the detection of acetaminophen in plasma they did not demonstrate the application of this method in clinical studies.

This paper reports the application of HPLC with electrochemical detection to a study on acetaminophen bioavailability which required an analytical method capable of quantitating acetaminophen in plasma at a concentration of 0.2 μg/ml.

MATERIALS AND METHODS

Materials

Reagent grade potassium dihydrogen phosphate, disodium hydrogen phosphate, ethyl acetate and methanol were used directly. Acetaminophen (Chem. Service Co., Westchester, Pa., U.S.A.) and *p*-butoxyphenol (McNeil Labs.,

*Present address: Banner Gelatin Products Corp., Box 157, Chatsworth, Calif. 91311, U.S.A.

Ft. Washington, Pa., U.S.A.) were also used directly. All water was double distilled from glass.

Extraction procedure

3 ml of pH 7.4 phosphate buffer, prepared by mixing 19.6 ml of solution of KH_2PO_4 (9.08 g/l) and 80.4 ml of a solution of $Na_2HPO_4 \cdot 2H_2O$ (11.8 g/l), were added to 1 ml of plasma in a round-bottom centrifuge tube. 1 ml of internal standard solution (*p-n*-butoxyphenol, 5.0 μg/ml) and 8.0 ml of ethyl acetate were added to the tube. The tube was rocked for 10 min followed by centrifuging for 5 min at 300 *g*. The ethyl acetate layer was transferred to a conical centrifuge tube and evaporated to dryness under reduced pressure at 40°. The residue was redissolved in 70 μl of methanol with 2—5 μl of the resulting solution being used for HPLC.

Instrumentation

All samples were analyzed by HPLC with electrochemical detection as previously described by Kissinger et al. [1]. Stainless-steel columns (0.5 m × 2.6 mm I.D.) packed with Pellidon (Pellicular polyamide, 37 μm; Reeve Angel, Clifton, N.J., U.S.A.) were used with an aqueous mobile phase consisting of 0.04 *M* NaH_2PO_4 adjusted to pH 7.4 and 5% methanol (v/v). The flow-rate was 60 ml/h. Solvent pumping was accomplished with either a Varian Model 4100 syringe pump or a Milton Roy reciprocating piston Minipump®.

A three-electrode system consisting of a carbon-paste electrode (working electrode), platinum electrode (auxiliary electrode), and saturated calomel electrode (reference electrode) was utilized to measure the current arising from the oxidation of the phenol group at a potential of +0.7 V (vs. SCE).

Analysis of acetaminophen

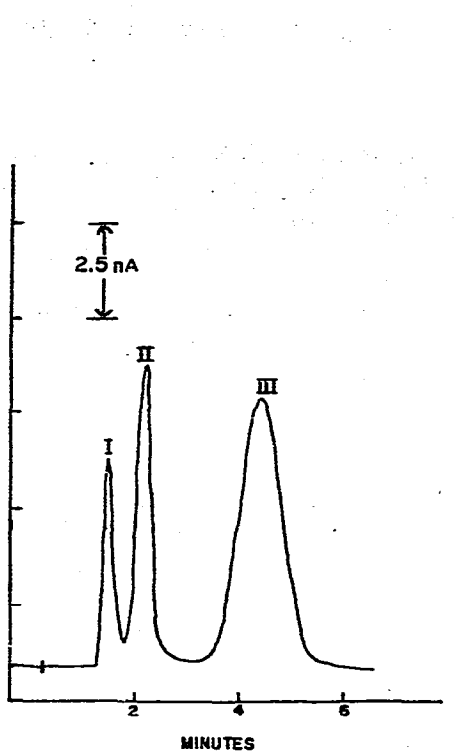
Acetaminophen concentrations in plasma were determined from a standard curve obtained by plotting drug/internal standard peak height ratio versus acetaminophen concentration. The standard curve was obtained by carrying through the assay procedures, samples prepared by adding known amounts of acetaminophen to pooled human plasma.

Clinical studies

Nine normal, healthy male subjects ranging in age of 22—26 years and weight of 68—92 kg were administered three dosage forms of acetaminophen in a three-way crossover study. The dosage forms used in this study were an experimental suppository dosage form consisting of a gelatin encapsulated water-soluble solution of acetaminophen, a glycol base suppository and an oral elixir. A 300-mg dose of acetaminophen was administered in each case. Following dosing, venous blood samples (15 ml) were withdrawn from the right or left antecubital fossa into heparinized vacutainers. The plasma was harvested immediately and frozen until analysis.

RESULTS AND DISCUSSION

Acetaminophen and the internal standard appeared as well-resolved symmetrical peaks under these conditions with retention times of 2 and 4.25 min,



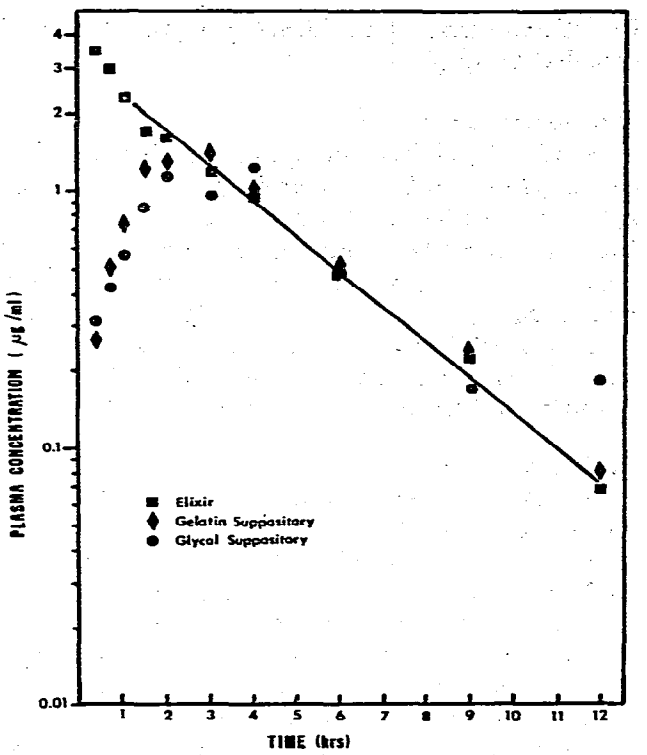


Fig. 1. Typical chromatogram for acetaminophen (II) and internal standard (III). Peak I represents the solvent front.

Fig. 2. Averaged plasma acetaminophen concentrations in nine human subjects following administration of 300 mg of acetaminophen as an oral elixir, glycol base suppository or gelatin encapsulated suppository. Straight line corresponds to an elimination half-life of 2.2 h.

respectively. A typical chromatogram is shown in Fig. 1. The standard curve is described by the following regression equation: peak height ratio = 0.180 X concentration (μg/ml) + 0.005. The correlation coefficient for this line is 0.997 with a standard error of the estimate ($S_{x.y}$) of 0.0099. Analysis of multiple standards gave coefficients of variation (n = 3) of 4.8, 9.6, 4.2 and 4.1% at concentrations of 0.2, 1.0, 2.0, and 4.0 μg/ml, respectively. During the course of the study standard curves were determined on a daily basis. This was necessary since the carbon-paste in the detector lost sensitivity with use and was changed periodically. Fig. 2 shows the average data for all subjects for all three dosage forms. The straight line represents an elimination half-life of 2.2 h for acetaminophen.

HPLC with electrochemical detector has been shown to be useful for measuring plasma concentrations of acetaminophen at low levels. However, it should be noted that the electrochemical detector is quite sensitive to solvent flow variation. Because of this, extensive pulse dampening is necessary when the reciprocating piston type pump is used. Also, the electrochemical detector

is quite sensitive to fluctuations in the line voltage. Consequently, best performance of the detector is achieved when no other electrical devices are used on the same circuit.

Preliminary results obtained in this laboratory also indicate that acetaminophen levels can be assayed in very small volumes of plasma using electrochemical detection. Plasma samples (50 μl) with concentrations ranging from 0.5 to 8 μg/ml have been measured in our laboratory using a variation of this method. Since this type of method would be quite suitable for measurement for acetaminophen in neonates and children, where only small plasma samples are available, further investigations along these lines are in order.

ACKNOWLEDGEMENTS

This work was supported by a grant from Banner Gelatin Products Co., Chatsworth, Calif. The authors thank Mr. Kenneth Pearce and Mr. Michael Swintosky for their technical assistance and Mr. Joseph Graft for his assistance in the construction of the electrochemical detector. Dr. Thomas Foster conducted the clinical portion of this study.

REFERENCES

1 P.T. Kissinger, C. Refshauge, R. Dreiling and R.N. Adams, Anal. Lett., 6 (1973) 465.
2 R.M. Riggin, L. Rau, L. Alcorn and P.T. Kissinger, Anal. Lett., 7 (1974) 791.
3 C. Refshauge, P.T. Kissinger, R. Dreiling, C.L. Blank, R. Freeman and R.N. Adams, Life Sci., 14 (1974) 311.
4 C.L. Blank, J. Chromatogr., 117 (1976) 35.
5 K.V. Thrivikraman, C. Refshauge and R.N. Adams, Life Sci., 15 (1975) 1335.
6 P.H. Zoutendam, C.S. Brunlett and P.T. Kissinger, Anal. Chem., 48 (1976) 2200.
7 R.M. Riggin, A.L. Schmidt and P.T. Kissinger, J. Pharm. Sci., 65 (1976) 680.