Table I—Predicted and Observed First-Pass Metabolism of Imipramine in Four Subjects

Subject	Sex	Age	Dose, mg	Body Weight, kg	$\int_0^\infty C_0\,dt$, mg min/liter	First Pass Predicted, %	First Pass Observed[a], %
A.B.	f	28	35	52	11.97	66	71
G.A.	f	28	40	55	44.24	37	23
U.F.	m	25	50	68	15.13	68	52
P.L.B.	m	59	50	71	19.84	62	65
Mean ± *SD*						58.25 ± 14.38	52.75 ± 21.36

[a] Reference 1.

Another significant aspect of the correlations presented here is that the area under the plasma concentration–time curve described the first-pass metabolism adequately, signifying that red blood cell transport is not significant in the first-pass metabolism of imipramine. This result may be due either to almost equal partitioning between blood cells and plasma or to quick equilibrations between red blood cells and plasma.

REFERENCES

(1) L. F. Gram and J. Christiansen, *Clin. Pharmacol. Ther.*, **17**, 555(1975).

(2) M. Gibaldi, *J. Pharm. Sci.*, **64**, 1036(1975).

(3) M. Gibaldi, R. N. Boyes, and S. Feldman, *ibid.*, **60**, 1338(1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 25, 1975, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication September 4, 1975.

High-Pressure Liquid Chromatographic Determination of Acetaminophen in Biological Fluids

LAWRENCE T. WONG ˣ, GNANAPRAKASAM SOLOMONRAJ, and BARRY H. THOMAS

Abstract □ A method for the rapid estimation of free acetaminophen in biological fluids is described. The assay involves ether extraction and high-pressure liquid chromatographic analysis on a 10-μm particle-size silica gel column, using a mobile phase of 10% chloroform in tetrahydrofuran. The procedure was used to determine acetaminophen levels in urine from two healthy volunteers who ingested 650 mg of ^{14}C-acetaminophen (20 μCi), and the accuracy of the method was compared with the carbon-14 determination. The limit of detectability for acetaminophen is 1 μg/ml.

Keyphrases □ Acetaminophen—high-pressure liquid chromatographic analysis, biological fluids, compared to radioisotope method □ High-pressure liquid chromatography—analysis, acetaminophen, biological fluids, compared to radioisotope method □ Analgesics—acetaminophen, high-pressure liquid chromatographic analysis, biological fluids

For the determination of free acetaminophen [*N*-(4-hydroxyphenyl)acetamide] in biological fluids, a GLC procedure (1) has been used frequently (2–4). The method requires a silylation step to convert acetaminophen into a volatile disilyl derivative before it is suitable for GLC determination. Although GLC analysis of phenolic drugs (*e.g.*, acetaminophen) is possible by this technique, the silylation step often lengthens the analysis time and also introduces another variable. Liquid chromatography appears to be more suitable for the quantitation of these drugs. It usually requires no derivatization of samples, and nonvolatile compounds can be analyzed just as easily as volatile compounds. The main requirement is that the sample be soluble in the mobile solvent.

This paper describes a simple high-pressure liquid chromatography (HPLC) method for the rapid estimation of acetaminophen in biological fluids using a UV absorption detector. A high-performance liquid chromatographic method for the quantitation of acetaminophen was reported recently (5). The technique, which employs a less common but highly sensitive electrochemical detector, does not involve high pressure in its operation. Determination of acetaminophen in the picogram level using this technique has been reported. However, its sensitivity is limited when applied to the analysis of the drug in biological samples due to the interference of endogenous, electrochemically reactive materials.

EXPERIMENTAL

Reagents and Materials—Acetaminophen was obtained commercially[1]. ^{14}C-Acetaminophen (uniformly labeled), with a specific activity of 17.24 μCi/mg, was custom synthesized[2]. Chloroform[3], methanol[3], and acetic acid[2] were all reagent grade and were used as received. Tetrahydrofuran[4] was freshly distilled prior to use.

Instrumentation—A liquid chromatograph[5] equipped with a positive displacement pump capable of developing a pressure of 5000 psi, a stop-flow injection port, a variable wavelength UV absorbance detector operated at 247 nm, and an integrator[6] was employed. The

[1] McNeil Laboratories (Canada) Ltd., Don Mills, Ontario, Canada.
[2] Mallinckrodt, St. Louis, MO 63160
[3] Caledon Labs., Georgetown, Ontario, Canada.
[4] BDH (Canada) Ltd., Toronto, Canada.
[5] Varian model 4100, Varian Aerograph, Walnut Creek, Calif.
[6] Model 3370A, Hewlett-Packard, Avondale, PA 19311

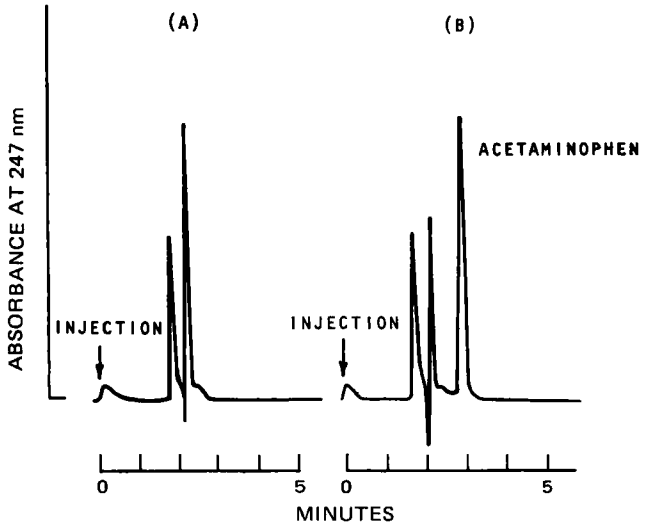

Figure 1—*High-pressure liquid chromatogram of human plasma extract from: (A) blank plasma and (B) plasma spiked with acetaminophen (7 μg/ml).*

column[7] (50 cm × 2.2 mm) was packed with silica gel with an average diameter of 10 μm.

Analyses were performed using a mobile phase of tetrahydrofuran–chloroform–5% acetic acid in methanol (90:10:0.04 v/v) at a flow rate of 40 ml/hr (1000 psi) and ambient temperature. The UV absorbance detector was set at 0.1–0.5 absorbance unit full scale (aufs), depending on the sample, to keep the acetaminophen signal on scale for proper integration of the peak. The mobile phase was degassed by applying vacuum to the solvent reservoir for 1 min before use.

Extraction and Analysis Procedure—Urine or plasma (1 ml) and 1 *M* phosphate buffer, pH 7.4 (1 ml), were saturated with sodium chloride and extracted two times with ether (2 ml each). The ether layer was removed and dried with a current of dry nitrogen. The inner wall of the tube was rinsed with tetrahydrofuran (1 ml), which also was evaporated. The resultant residue was redissolved in 50–250 μl of a chloroform–tetrahydrofuran (2:1 v/v) mixture to give an anticipated acetaminophen concentration of 1–10 μg/μl, and a 2-μl aliquot was injected into the liquid chromatograph.

Urinary Excretion of Free Acetaminophen—Two healthy human volunteers each took a single dose of ^{14}C-acetaminophen (650 mg, 20 μCi). Urine samples were collected at 3, 6, 9, and 12 hr. A working standard curve was prepared by adding known amounts of acetaminophen at five different concentrations to drug-free urine. The urine samples were worked up as already described.

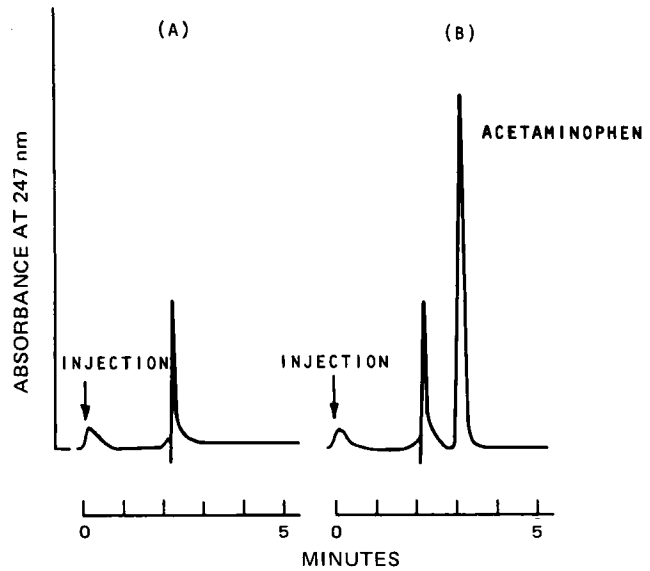

Figure 2—*High-pressure liquid chromatogram of human urine extract from: (A) blank urine and (B) urine spiked with acetaminophen (10 μg/ml).*

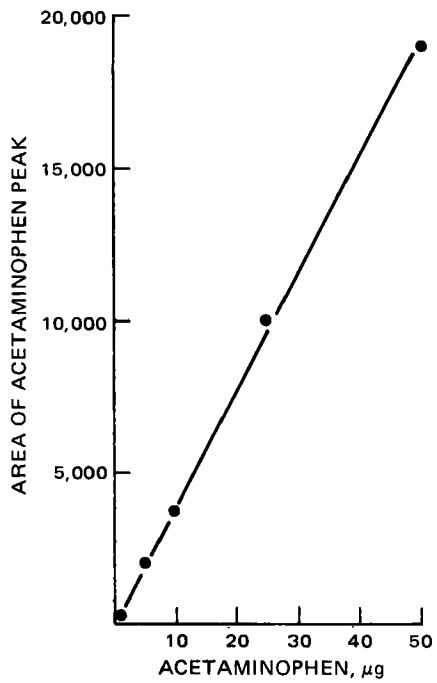

Figure 3—*Standard curve for acetaminophen.*

Determination of ^{14}C-Acetaminophen in Urine by Radioisotope Counts—Urine (25 μl) was applied as a band to strips of chromatography paper[8] and developed using the alkaline paper chromatography system described by Shahidi (6, System C). Radioactivity on the developed chromatograms was detected by serial sectioning into 1-cm strips and counting in a liquid scintillation counter. The peak corresponding to standard ^{14}C-acetaminophen was quantitated.

RESULTS AND DISCUSSION

The mobile phase used in the chromatograph was a simple solvent system of tetrahydrofuran modified with 10% chloroform. A small amount of acetic acid (0.002%) was added to reduce the slight tendency of acetaminophen to tail. The sample residues were redissolved in a chloroform–tetrahydrofuran (2:1) mixture to minimize the diffusion of the compound when injected into the mobile phase.

The extraction procedure using ether gave a cleaner extract with fewer extraneous peaks. The recoveries of processed standards of acetaminophen from plasma and urine were virtually complete, as reported previously (3). The chromatograms (Figs. 1 and 2) yielded an acetaminophen peak with a retention time (t_R) of 3 min. Chromatograms from control plasma (Fig. 1) and control urine (Fig. 2) also yielded an extraneous peak around 2.2 min, but it did not interfere with the acetaminophen peak. The plasma extract had an additional peak at 1.8 min. No other interfering compounds were extracted when ether was used as the solvent.

This method was used to study the urinary excretion of free acetaminophen in two healthy human volunteers. Figure 3 represents the standard curve obtained by plotting peak areas *versus* acetaminophen concentrations obtained from the analysis of spiked urine samples. The plot is a straight line over the concentration range of 1–50 μg/ml. The data obtained from duplicate analyses were reproducible to within 4%.

Figure 4 illustrates the cumulative urinary excretion of free acetaminophen by the two subjects after ingestion of 650 mg. The slopes of the curves tend to level off at about 6 hr. The total acetaminophen excreted at 12 hr was 19.12 mg in one subject and 29.08 mg in the other as determined by HPLC. These results were compared with those obtained from ^{14}C-radioisotope counts (Fig. 4). In the latter deter-

[7] Varian MicroPak Si-10, Varian Aerograph, Walnut Creek, Calif.

[8] Whatman No. 1.

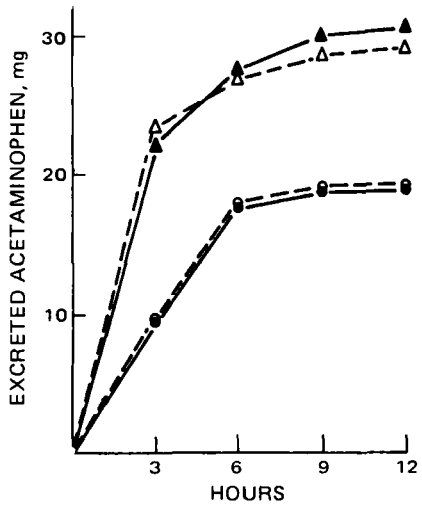

Figure 4—*Cumulative urinary excretion of free acetaminophen in two healthy subjects as determined by HPLC (○, △) and radioisotope (●, ▲) methods.*

mination, the paper chromatography system of Shahidi (6) was used to separate the ^{14}C-acetaminophen from its metabolites. The overall difference between the two methods was less than 6%.

This HPLC method is rapid and simple for the determination of acetaminophen in biological fluids. It involves no silylation of the compound as is required in GLC determinations. Because the extraction procedure is simple and complete, the use of an internal standard is not necessary; reproducible results to within 4% were achieved. The limit of detectability for acetaminophen by this method is 1 μg/ml. Below this concentration, the reproducibility of acetaminophen estimations is more variable.

This HPLC technique also could be used for the determination of acetaminophen metabolites. The estimation of the glucuronide and sulfate of acetaminophen is usually carried out by analyzing the free acetaminophen generated from enzymatic cleavage of these metabolites (7). Likewise, the cysteine and mercapturate metabolites could be estimated after chemical cleavage with Raney nickel (8).

REFERENCES

(1) L. F. Prescott, *J. Pharm. Pharmacol.*, **23**, 111(1971).

(2) G. Ramachander, F. D. Williams, and J. F. Emele, *J. Pharm. Sci.*, **62**, 1498(1973).

(3) B. H. Thomas and B. B. Coldwell, *J. Pharm. Pharmacol.*, **24**, 243(1972).

(4) R. P. Miller and L. J. Fischer, *J. Pharm. Sci.*, **63**, 969(1974).

(5) R. M. Riggin, A. L. Schmidt, and P. T. Kissinger, *ibid.*, **64**, 680(1975).

(6) N. J. Shahidi, *Ann. N.Y. Acad. Sci.*, **151**, 822(1968).

(7) G. Levy and H. Yamada, *J. Pharm. Sci.*, **60**, 215(1971).

(8) J. R. Mitchell, S. S. Thorgeirsson, W. Z. Potter, D. J. Jollow, and H. Keiser, *Clin. Pharmacol. Ther.*, **16**, 676(1974).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 30, 1975, from the *Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2*

Accepted for publication September 4, 1975.

[x] To whom inquiries should be directed.

Enthalpies of Hydrogen Bonding in Psychotropic Drugs

TIMOTHY B. PATRICK [x] and RANDALL R. BRESEE

Abstract □ The enthalpy of hydrogen bonding of some antipsychotic, antidepressant, anticonvulsant, and antianxiety agents with phenol, as determined from IR and NMR spectroscopic measurements, was shown not to be responsible for differences in activity within the drug classes. These results support a theoretical prediction advanced for anticonvulsant activity.

Keyphrases □ Enthalpy—hydrogen bonding between various psychotropic drugs with phenol, IR and NMR spectral measurement, related to differences in activity □ Hydrogen bonding—various psychotropic drugs with phenol, IR and NMR spectral measurement, related to differences in activity □ Psychotropic drugs—enthalpy of hydrogen bonding with phenol, IR and NMR spectral measurement, related to differences in activity

Hydrogen bonding is often mentioned when the mechanism of drug action is considered. However, little quantitative information concerning this subject has been reported (1, 2). Therefore, the enthalpy of hydrogen bonding of several psychotropic drugs with phenol was investigated to obtain factual information for testing the theoretically based conclusion that hydrogen bond acceptance is not responsible for variations in anticonvulsant activity (3). Furthermore, hydrogen bond strengths of some tranquilizing and anticonvulsant drugs were examined so that information regarding the importance of hydrogen bonding in diazepam (V), which acts as both a tranquilizer and an anticonvulsant (4), could be evaluated.

DISCUSSION

Hydrogen-bonding enthalpies with phenol were obtained using the IR and NMR spectral techniques developed by Drago and coworkers (5, 6). The IR technique relies on a linear relationship between the enthalpy of hydrogen bonding and the hydroxyl frequency difference of free phenol and hydrogen-bonded phenol. The NMR method is based on a linear relationship between hydrogen-bonding enthalpy and the chemical shift of a hydroxyl proton in a hydrogen-bonded phenol–base adduct. The IR procedure is better because measurements are straightforward and small sample concentrations are used; the NMR method requires many sample variations and an estimate of anisotropic corrections (6).

The IR–enthalpy relationship obtained was:

$$-\Delta H \text{ (kcal/mole)} = 0.010\,(\Delta\nu) + 3.67 \pm 0.04 \qquad r = 0.986 \quad \text{(Eq. 1)}$$

The NMR–enthalpy relationship is:

$$-\Delta H \text{ (kcal/mole)} = 1.89\,(\delta \text{ adduct}) - 9.14 \pm 0.52 \qquad r = 0.991 \quad \text{(Eq. 2)}$$

Errors are reported at the 99% confidence level (7).