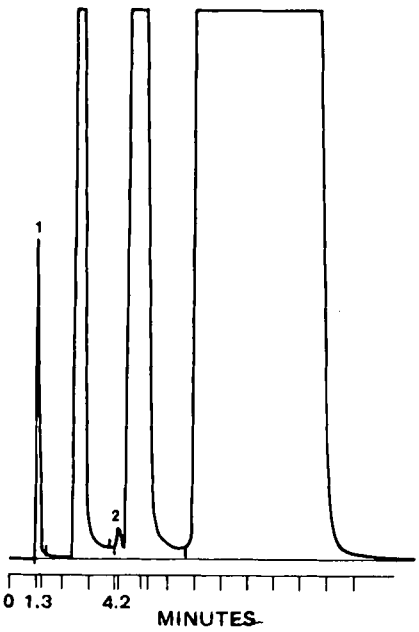

Figure 4—*Chromatogram of freshly coated tablets before drying, showing both methanol and methylene chloride after 15 min of coating. Key: (1) methanol; (2) methylene chloride.*

Statistical limits of detection were established for methanol at 9.5 ± 1.5 ppm per average tablet weight and for methylene chloride at 10.1 ± 2.3 ppm per tablet. Below these limits, sensitivity of the instrument was unreliable. All absolute values were approximate due to two factors. First, precision of measurement is decreased as concentrations used in the calibration curve approach the detection limit. As an alternative, a calibration curve could have been drawn at higher concentrations and extrapolated to the limit of detection (3). Second, this detection limit is also dependent on where the confidence lines are drawn about the regression line and is, at best, an estimate for a limit of guaranteed purity (2). Therefore, the values reported here represented a range within a specified precision for the standards, where the analytical method and the instrumentation were reliable for low-level determinations (6). The levels of solvent residues, ranging from 30 to 300 ppm, obtained during the film-coating process were statistically significant. However, after 24 h of drying, no peaks for either solvent were observed in the chromatograms.

This procedure is easy and reliable for monitoring organic residues on film-coated tablets. Determining lower limits of detection should be an integral part of any statistical package developed for a new analytical method.

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High-Performance Liquid Chromatographic Method for the Determination of Trace Amounts of Acetaminophen in Plasma

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Received July 15, 1982, from the *Department of Drug Metabolism and Pharmacokinetics, Schering-Plough Corporation, Bloomfield, NJ 07003.* Accepted for publication November 5, 1982.

Abstract □ A method is described for the rapid, sensitive, and precise quantitation of acetaminophen in human plasma. The assay involved a single acetonitrile extraction and high-performance liquid chromatographic analysis using a reverse-phase column, with a mobile phase of methanol and water. The limit of quantitation of acetaminophen by this method was 8 ng/mL; only 0.1 mL of the plasma sample was required for the determination. *N*-Propionyl-*p*-aminophenol was used as the internal standard.

Keyphrases □ Acetaminophen—high-performance liquid chromatography determination of trace amounts in plasma □ High-performance liquid chromatography—method for the determination of trace amounts of acetaminophen in plasma

Although acetaminophen is currently one of the most commonly used analgesics and antipyretics, hepatotoxicity has occurred in cases of acetaminophen overdose. Thus, a simple and rapid method for determining acetaminophen concentrations in plasma would be useful.

At present the most sensitive methods for plasma acetaminophen determination are liquid chromatography with electrochemical detection (1) or UV detection (2–6) and GC (7, 8). The quantitation limit for each of these methods is ~100 ng/mL with a 1-mL sample of plasma or serum. These methods, therefore, may be inadequate for microliter samples such as those obtained from newborn infants. Because of time requirements they may not be rapid enough for the determination of acetaminophen in overdose cases. A rapid, specific, high-performance liquid chromatographic (HPLC) method for the determination of acetaminophen in plasma using a 100-μL sample is described in this paper.

EXPERIMENTAL

Reagents and Materials—Acetaminophen[1] standard solutions were prepared in ethanol (1.0 mg/mL, stored at 4°C) and subsequent dilutions

[1] U.S. Pharmacopeial Convention, Inc., Rockville, Md.

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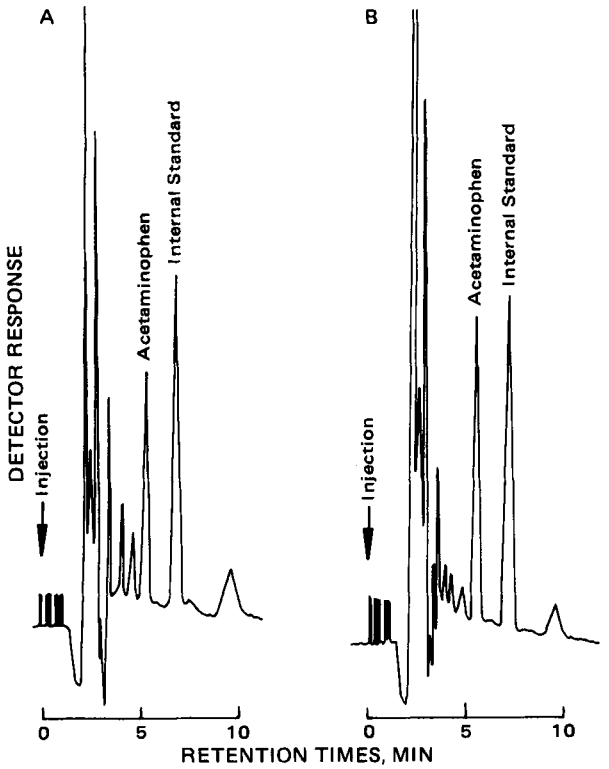

Figure 1—*High-performance liquid chromatogram of human plasma extract from: (A) drug-free plasma to which acetaminophen (1 μg/mL) was added and (B) plasma obtained from a human subject 9 h after 325.0 mg of acetaminophen was given at 0 and 6 hr. The column conditions are given in the text.*

were made with water. The internal standard (*N*-propionyl-*p*-aminophenol)[2] was also prepared in ethanol (1.0 mg/mL, stored at 4°C), with subsequent dilutions with water. Ethanol[3] and acetonitrile[4] (glass distilled) were used in the assay procedure.

Instrumentation—The chromatograph consisted of an automatic injector[5], a high-pressure pump[6], and a UV-absorbance detector[7] with a fixed wavelength of 254 nm. Separation was accomplished on a Spherisorb ODS (5-μm) reverse-phase column (0.46 cm × 25 cm)[8].

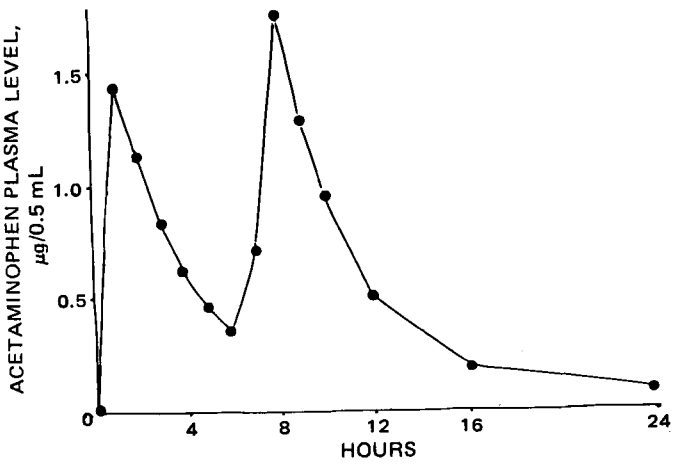

Figure 2—*Mean plasma levels of acetaminophen from nine human volunteers following oral 325.0-mg dose of acetaminophen at 0 and 6 h.*

[2] Schering Corp. (Batch βF504731), Bloomfield, N.J.
[3] Publicker Industries Inc., Philadelphia, Pa.
[4] MCB Manufacturing Chemist Inc., Cincinnati, Ohio.
[5] WISP 710A, Waters Associates Inc., Milford, Mass.
[6] Model 6000A, Waters Associates Inc., Milford, Mass.
[7] Model 440, Waters Associates Inc., Millford, Mass.
[8] Supelco Inc., Bellefonte, Pa.

Table I—Standard Curve of Acetaminophen [a]

Concentration, ng/mL	Peak Height Acetaminophen/ Peak Height Internal Standard Ratio			
	Mean	*SD*	*CV*, %	*n*
1.0	0.008	0.001	1.68	2
2.5	0.021	0.001	3.28	2
5.0	0.048	0.004	9.49	3
10.0	0.083	0.007	8.27	6
25.0	0.242	0.020	8.50	4
50.0	0.495	0.019	3.95	10
100.0	0.982	0.049	5.08	4
200.0	2.163	0.042	1.95	6
250.0	2.512	0.115	4.60	4
500.0	4.947	0.041	0.83	4
1000.0	10.185	0.021	0.20	2

[a] Various concentrations of acetaminophen were added to drug-free plasma samples prior to extraction as described in the text.

Analyses were performed using a deaerated mobile phase of methanol–water (25:75, v/v) at a flow rate of 1.0 mL/min (2200 psi). The UV detector was set at 0.02–2.0 AUFS. The absorbance detector output was monitored with a 1.0-mV recorder[9]. An automation system[10] was used for the measurements of peak height and retention time. All operations were done at ambient temperature.

Extraction and Analysis Procedure—To 0.1 mL of plasma in a 15-mL glass-stoppered centrifuge tube, 0.2 mL of *N*-propionyl-*p*-aminophenol (internal standard, 2.0 μg/mL) was added, mixed, and extracted with 5 ml of acetonitrile by shaking for 10 min on a shaker[11]. The tubes were then centrifuged for 10 min (1500 × *g*) after which 4.5 mL of the organic layer was transferred to another glass tube and evaporated to dryness under a nitrogen stream at 40°C. The residue was reconstituted with 2.0 mL of distilled water, and aliquots of 0.05 mL were injected into the HPLC using an automatic injector[5]. For calibration of detector response, various concentrations (0.10–20.0 μg/ml) of acetaminophen were added to several drug-free plasma samples, which were subsequently processed with each set of study samples. Calculation of acetaminophen concentration was based on the peak height ratio of acetaminophen relative to that of the internal standard. The linearity of the HPLC response was confirmed daily.

RESULTS AND DISCUSSION

The chromatogram yielded retention times (t_R) of 5.44 min, for acetaminophen and 6.86 min for the internal standard (Fig. 1). The extraction procedure using acetonitrile produced a clean extract. The absolute recoveries of acetaminophen from plasma were virtually complete (0.5 μg/mL gave 101.5% recovery, n = 7, and 1.0 μg/mL gave 99.8% recovery, n = 11); the recovery of the internal standard was 100.0%, n = 6. The linearity of the assay was evaluated by adding the acetaminophen to the drug-free plasma samples (Table I). The limit of acetaminophen quantitation in plasma by this method was 8.0 ng/mL (CV = 3.8%, n = 8). A straight line relationship was obtained between acetaminophen concentration and chromatographic response with a correlation coefficient of 1.000. The reproducibility of the assay was determined in six drug-free samples to which acetaminophen was added (CV of 2.7% at 0.5 μg/mL and CV of 1.8% at 2.0 μg/mL). The stability of acetaminophen was checked by reassaying the frozen and thawed plasma samples containing acetaminophen (over several weeks of storage). The coefficient of variation for the mean of three samples on each occasion and five determinations was 1.89%. None of the other drugs tested (theophylline, β-hydroxytheophylline, methylxanthine, theobromine, and caffeine) interfered with acetominophen detection; only the methylxanthine retention time (5.46 min) was close to that of acetaminophen. Table II shows individual plasma levels, and Fig. 2 illustrates the mean plasma levels of acetaminophen obtained from the nine human volunteers who received oral doses of 325.0 mg of acetaminophen[12] at 0 and 6 h. This simple, rapid, specific, and reproducible HPLC assay procedure developed for determination of acetaminophen in plasma could be used to monitor plasma acetaminophen levels in newborn infants, cases of overdose, and bioavailability studies.

[9] Model 9176, Varian, Palo Alto, Ca.
[10] Lab Automation System, Model 3353, Hewlett-Packard, Paramus, N.J.
[11] Model 6010, Eberbach Corp., Ann Arbor, Mich.
[12] McNEILAB, Inc., Spring House, Pa.

Table II—Plasma Levels of Acetaminophen (μg/0.5 mL) Following Administration of 325.0 mg of Acetaminophen at 0 and 6 h

Subject	Hours After Treatment													
	0	1	2	3	4	5	6	7	8	9	10	12	16	24
1	N.D.[a]	1.421	0.905	0.936	0.671	0.496	0.449	0.711	1.196	1.368	0.911	0.626	0.237	0.078
2	N.D.	1.058	0.923	0.615	0.464	0.294	0.238	0.343	1.688	1.178	0.814	0.454	0.187	0.065
3	N.D.	1.512	0.989	0.702	0.517	0.436	0.333	1.074	1.506	1.010	0.701	0.381	0.119	N.D.
4	N.D.	1.357	1.049	0.744	0.530	0.406	0.292	1.169	1.494	0.982	0.777	0.384	0.109	N.D.
5	N.D.	1.110	0.758	0.584	0.399	0.271	0.210	0.189	0.671	0.711	0.811	0.376	0.143	0.073
6	N.D.	1.239	1.542	0.798	0.617	0.361	0.249	0.651	1.954	1.233	0.861	0.400	0.165	0.078
7	N.D.	2.249	1.557	1.181	0.939	0.801	0.523	0.696	2.703	2.055	1.609	0.752	0.278	0.094
8	N.D.	1.265	1.360	1.110	0.702	0.554	0.441	0.885	2.212	1.354	0.878	0.494	0.172	0.056
9	N.D.	1.696	1.200	0.811	0.551	0.439	0.378	0.279	2.199	1.488	1.117	0.551	0.230	0.089

[a] N.D.—not detectable.

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Determination of a New Antihypertensive Agent (2*R*,4*R*)-2-(2-Hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic Acid in Blood by High-Performance Liquid Chromatography with Electrochemical Detection

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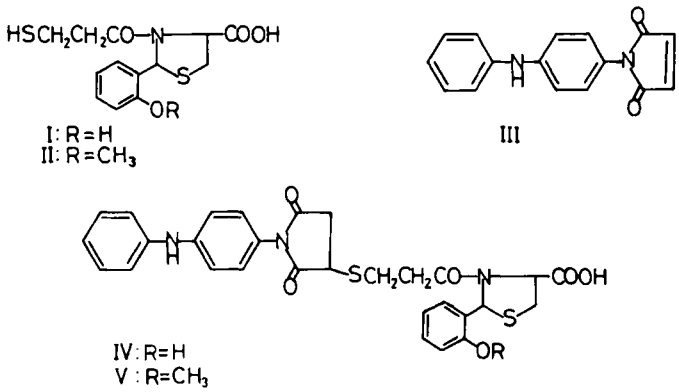
Received August 23, 1982, from the **Pharmaceutical Institute, Tohoku University, and* ‡*The Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai 980, Japan.* Accepted for publication November 22, 1982.

Abstract □ A sensitive method has been developed for the determination of a new antihypertensive agent, (2*R*,4*R*)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (I), in human blood. Because the drug is unstable in biological fluids, it was immediately derivatized by treating the freshly drawn blood specimens with *N*-(4-anilinophenyl)maleimide. The adduct was separated and determined by high-performance liquid chromatography with electrochemical detection on a reverse-phase column. The assay method was satisfactory with respect to the sensitivity and precision, providing a quantitation limit of 2 ng/ml and coefficient of variation of 3%. Preliminary pharmacokinetic data were obtained by orally administering I to two patients with essential hypertension.

Keyphrases □ Antihypertensive agents—(2*R*,4*R*)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid, determination in human blood, high-performance liquid chromatography with electrochemical detection □ High-performance liquid chromatography—determination of (2*R*,4*R*)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid in human blood, electrochemical detection □ (2*R*,4*R*)-2-(2-Hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid—determination in human blood, high-performance liquid chromatography with electrochemical detection

(2*R*,4*R*) - 2 - (2-Hydroxyphenyl) - 3 - (3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (SA 446, I), a newly developed potent and specific inhibitor of angiotensin-converting enzyme, appears to be a promising antihypertensive agent (1, 2). This drug is several times more potent *in vitro* than captopril, a specific and orally active inhibitor of angiotensin-converting enzyme proven useful in patients with hypertension and congestive heart failure (3, 4). The lower therapeutic dose of I requires a more sensitive method for the determination of the drug in biological fluids for pharmacokinetic studies. A sensitive GC method using an electron capture detector has been reported, but this procedure requires a two-step derivatization (5). High-performance liquid chromatography (HPLC) with



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