dialuric acid with an (assumed) area of 0.01 m^2/g, a monolayer of water molecules on a 0.100-g sample would weigh 2.14×10^{-7} g. Thus, a water layer 1000 molecules thick would barely be detectable in the absence of the complicating oxidation reaction.

These results indicate that factors other than the apparent permeability of oxygen into the crystal can govern solid-state oxidation reactions. Desolvated crystals of dialuric acid, which are expected to contain voids, are unreactive. Thus, desolvation does not always increase the reactivity of a crystal toward oxygen. Instead, solvated crystals exposed to high humidities react very rapidly, showing that high humidities can accelerate solid oxygen reactions. Also, reactions of the type: solid + gas → solid can be accelerated in high humidities presumably *via* reaction in an invisible moisture layer.

In contrast to other solids, these results indicate that stabilization of pharmaceuticals with solid-state behavior related to dialuric acid would best be accomplished, not by avoiding desolvation as indicated by some published results (1–3, 7, 8), but rather by preventing exposure to high humidities.

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A Rapid 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 nonconjugated acetaminophen present in the plasma following drug administration is determined after plasma protein precipitation by high-pressure liquid chromatography (HPLC) at a wavelength of 240 nm. Acetaminophen (I) is detectable at levels as low as 0.1 μg/ml. Mean recoveries of 94% with a coefficient of variation of 3% were obtained for plasma standards whose concentrations ranged from 0 to 32 μg/ml. Interassay variability of the slope of the standard curve had a coefficient of variation of 2.7%. Application and verification of this method by comparison with another procedure run simultaneously during several human bioavailability studies are described.

Keyphrases □ Acetaminophen—high-performance liquid chromatographic analysis in human plasma □ High-performance liquid chromatography—analysis, acetaminophen in human plasma and blood □ Analgesics—acetaminophen, high-performance liquid chromatographic analysis in human plasma

The widespread use of acetaminophen (I) as an analgesic and antipyretic has stimulated an interest in the development of a simple and rapid free acetaminophen plasma determination suitable for analyzing multiple samples. The pharmacology of acetaminophen is such that ~80% of a dose is conjugated predominately with glucuronic acid and to a lesser extent with sulfuric acid. These conjugated metabolites lack efficacious biological activity (1). Most of the published methods determine total (*i.e.*, free plus conjugated) acetaminophen in plasma (2–4), while those that determine free acetaminophen by a variety of techniques (5–14), including HPLC (7–14), involve time-consuming and labor intensive organic extractions, solvent evaporations (7–10), deal with toxic levels rather than the lower therapeutic levels (11, 12), or are unsuitable for routine multiple therapeutic level samples because of lack of sensitivity, insufficient sample cleanup (11–14), or long analysis time (14).

The present method involves a single plasma protein precipitation step followed by liquid chromatographic determination of acetaminophen in the clear supernatant. The plasma proteins are denatured and precipitated using 0.3 *N* $Ba(OH)_2$ and 5% $ZnSO_4$ solutions described previously (15). The method is capable of detecting <0.1 μg/ml, and the reproducibility eliminates the need for an internal standard. Furthermore, the ease and rapidity of sample workup make this method ideal for multiple sample analysis.

This method has been used routinely for over a year with good results and has been verified by a comparison with an extraction–HPLC acetaminophen method[1] and a colorimetric procedure (16).

EXPERIMENTAL

Reagents—Standard solutions were prepared in distilled water. The 0.3 *N* $Ba(OH)_2$ and 5% $ZnSO_4$ solutions were obtained commercially[2]. The combination of these two salt solutions has been known for years as an effective plasma protein precipitant.

Instrumentation and Operating Conditions—The analysis was performed using a high-performance liquid chromatograph[3] with a variable wavelength UV detector[4] set at 240 nm, an automated injection system[5] fitted with a 75-μl loop, and a 30 cm × 4-mm i.d. reversed-phase,

[1] N. Kalish and S. O'Connell, Bristol-Myers Products, Analytical Chemistry Department, unpublished data, 1977.
[2] Fisher Scientific Co.
[3] Model 6000, Waters Associates, Milford, Mass.
[4] Model 450, Waters Associates, Milford, Mass.
[5] Model 834, Dupont Instrument Co., Wilmington, Del.

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Table I—Accuracy of HPLC Assay For Acetaminophen in Plasma

Amount Added, μg	Experiment 1 Amount Found, μg	Experiment 1 Percent Error	Experiment 2 Amount Found, μg	Experiment 2 Percent Error	Experiment 3 Amount Found, μg	Experiment 3 Percent Error	Experiment 4 Amount Found, μg	Experiment 4 Percent Error	Experiment 5 Amount Found, μg	Experiment 5 Percent Error
1.0	1.05	5.0	1.03	3.0	1.04	4.0	1.07	7.0	1.07	7.0
1.0	1.05	5.0	1.07	7.0	1.00	0.0	0.98	2.0	0.99	1.0
2.0	2.10	5.0	2.05	2.5	2.09	4.5	2.14	7.0	2.12	6.0
2.0	2.05	2.5	2.09	4.5	2.09	4.5	2.04	2.0	2.12	6.0
4.0	4.10	2.5	4.00	0.0	4.00	0.0	4.07	1.8	4.15	3.8
4.0	4.02	0.5	4.08	2.0	4.04	1.0	3.95	1.3	3.97	0.8
8.0	7.99	0.1	7.80	2.5	7.99	0.1	7.84	2.0	8.16	2.0
8.0	8.03	0.4	7.93	0.9	7.91	1.1	7.93	0.9	7.67	4.1
16.0	16.19	1.2	15.81	1.2	16.11	0.7	15.97	0.2	16.28	1.8
16.0	16.23	1.4	16.51	3.2	16.03	0.2	15.85	0.9	15.47	3.3
24.0	24.04	0.2	23.68	1.3	24.06	0.3	24.28	1.2	24.45	1.9
24.0	22.74	5.2	24.17	0.7	23.67	1.3	23.90	0.4	23.73	1.1
32.0	32.29	0.9	—	—	32.14	0.4	—	—	—	—
32.0	32.38	1.2	—	—	32.00	0.0	—	—	—	—

high efficiency C_{18} column[6]. The mobile phase was methanol–water (15:85), which was filtered through a 0.45-μm pore filter[7] and degassed before use. The flow rate was 1.0 ml/min. The column was fitted with a precolumn packed with C_{18} Corasil 37–50 μm[8]. This precolumn was repacked once a week during panel use since particulate matter accumulated on the precolumn frit and would cause an increase in column pressure after the weekly load of 300 to 400 injections. The detector was set at a sensitivity of 0.2 aufs.

Plasma Acetaminophen Study in Humans—Patients were fasted overnight and were drug free for 72 hr prior to dosing. Each subject received a 650-mg oral dose of acetaminophen consisting of two 325-mg tablets taken with 100 ml of water. Venous blood specimens were withdrawn by syringe and discharged into centrifuge tubes containing heparin as an anticoagulant. Specimens were taken prior to and at specified times after drug administration through 1 hr. Collected specimens were centrifuged immediately for 15 min at 3500 rpm using a bench-top centrifuge. Plasma was separated and analyzed for free acetaminophen by the proposed method.

Analysis—One milliliter of freshly drawn plasma was added to a 16 × 100-mm screw top culture tube. To each tube was added 1 ml of the saturated 0.3 *N* $Ba(OH)_2$ solution. The tubes were vortexed at sufficient speed to ensure thorough mixing for 2 min on a multiple tube vortex mixer[9]. One milliliter of the 5% $ZnSO_4$ solution was then added to each sample which should have a milky opaque appearance. Each tube was capped and vortexed at high speed for 1 min. The tubes were then centrifuged in a bench-top centrifuge at high speed for 10 min. The resulting clear water-like supernatant was separated from the precipitate and filtered directly into an auto injector vial using a Pasteur pipet plugged

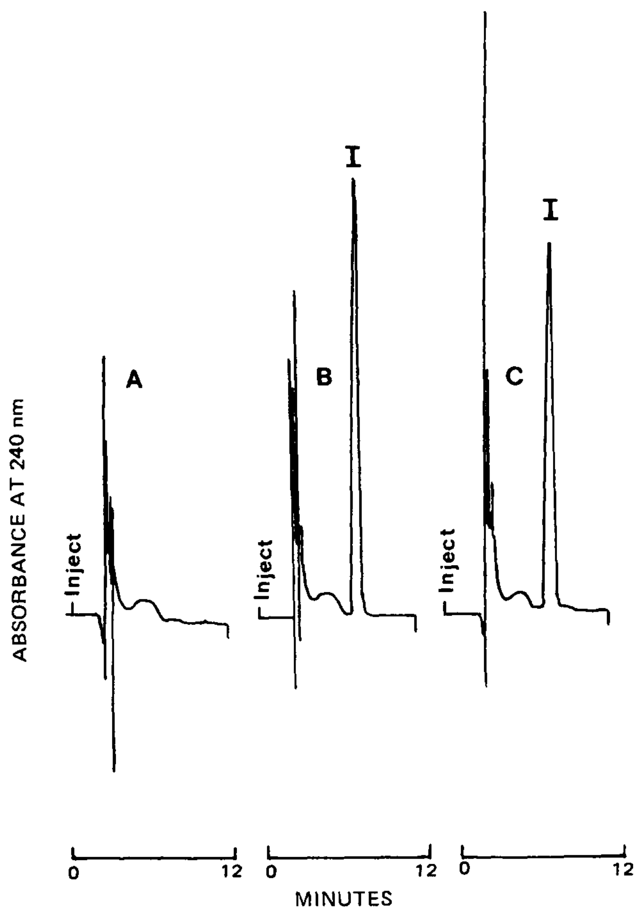

Figure 1—*Chromatograms for a typical subject. Key: (A) taken prior to acetaminophen (I) administration; (B) taken 20 min after oral administration of two 325-mg tablets; (C) taken 40-min postdosing.*

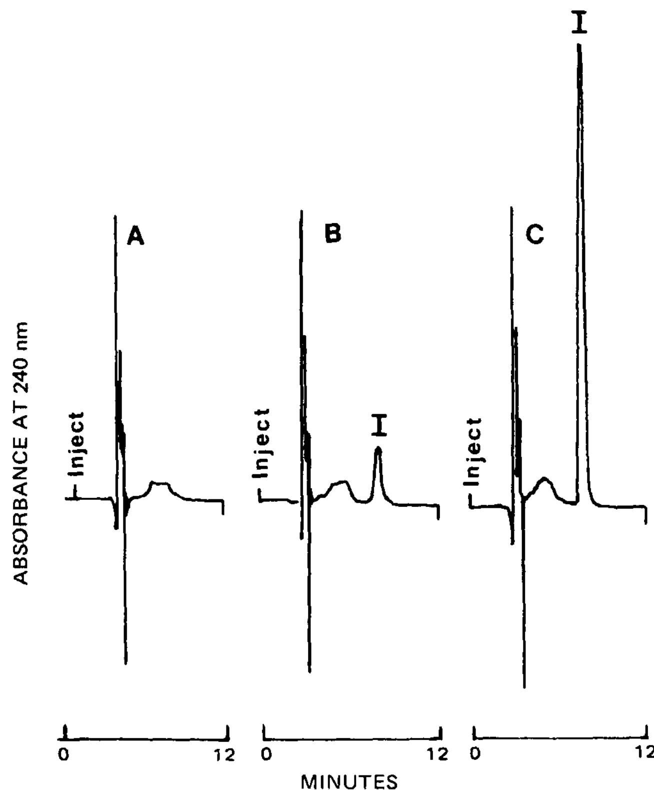

Figure 2—*Chromatograms of 1-ml human plasma sample processed by the described precipitation method. Key: (A) blank; (B) spiked with 1 μg of acetaminophen (I); (C) spiked with 8 μg of I. The mobile phase was 15% (v/v) methanol at a flow rate of 1.0 ml/min, and the chart speed was 15 cm/hr.*

[6] μ-Bondapak C_{18}, Waters Associates, Milford, Mass.
[7] Millipore Corp., Bedford, Mass.
[8] Waters Associates, Milford, Mass.

[9] Kraft Apparatus, Inc., Mineola, N.Y.

Table II—Comparison of Acetaminophen Levels in Plasma Analyzed Simultaneously by Plasma Protein Precipitation HPLC Assay and a Second Extraction Method

	Acetaminophen by Extraction				Acetaminophen by Precipitation–HPLC			
				Tablet A				
Time, min / Subject	10	20	40	60	10	20	40	60
1	3.2	12.9	8.2	6.7	3.4	13.8	8.8	6.9
2	0.7	4.3	5.0	4.7	0.8	4.2	5.0	4.7
3	0	1.1	3.4	3.1	0	1.1	3.3	3.2
4	0.8	2.8	5.5	5.9	0.8	2.9	5.7	6.0
5	1.3	8.5	7.0	6.6	1.2	8.7	7.0	6.0
6	0	1.2	7.4	6.1	0	1.2	7.2	6.5
7	0	2.2	6.8	7.4	0	2.8	6.9	7.4
8	0	6.8	7.6	6.4	0	7.0	7.7	6.1
9	0.9	3.2	5.3	5.2	1.2	3.5	5.9	5.7
10	0	0.7	9.3	7.6	0	1.1	9.8	8.0
11	0.7	4.5	9.1	7.6	1.0	5.1	8.8	7.2
12	11.3	15.1	14.0	12.3	11.2	14.7	13.3	12.1
13	0	5.7	11.3	9.6	0	5.4	11.2	10.5
14	0	0.9	3.0	3.9	0	1.0	3.1	4.1
15	0	1.0	2.5	4.1	0	0.6	2.9	4.3
16	0	1.2	5.4	7.3	0	1.0	4.8	7.1
				Tablet B				
Time, min / Subject	10	20	40	60	10	20	40	60
1	1.5	5.5	6.5	8.0	1.6	5.8	6.7	7.9
2	0	0.7	2.6	4.9	0	0.5	2.6	4.8
3	0	8.1	7.5	5.7	0	7.7	7.4	5.6
4	0	0	0.8	1.0	0	0	0.8	1.0
5	2.8	6.5	6.4	5.3	2.7	7.0	6.9	5.1
6	2.1	9.7	7.1	5.9	2.1	9.8	7.3	6.0
7	0	2.6	11.5	8.4	0	2.6	10.9	8.4
8	0	6.5	6.9	5.9	0	6.4	6.7	5.8
9	0	0.6	2.8	3.4	0	0.9	3.1	3.7
10	5.4	4.2	3.8	3.4	5.1	4.7	4.3	3.8
11	4.2	12.8	7.9	6.5	4.5	13.2	8.2	7.0
12	11.7	14.3	13.3	12.1	11.9	14.3	13.3	12.0
13	0.7	1.6	5.0	8.9	0.8	1.8	5.3	8.9
14	0	1.4	4.0	5.8	0	1.5	4.0	6.1
15	0	0	1.3	3.6	0	0	1.7	4.0
16	0	1.8	6.2	7.4	0	2.0	6.2	7.4
				Tablet C				
Time, min / Subject	10	20	40	60	10	20	40	60
1	0.7	3.3	8.4	7.0	0.8	3.6	8.6	7.4
2	0	2.5	12.2	8.7	0	2.5	12.3	8.9
3	0	1.6	4.7	7.0	0	1.5	4.7	6.3
4	5.3	5.6	7.2	6.0	5.2	5.5	7.0	6.1
5	1.1	2.2	4.1	4.6	1.1	2.2	4.2	4.8
6	0	6.0	7.3	5.9	0	6.4	7.4	6.1
7	4.9	12.9	9.2	7.3	5.4	13.1	9.5	7.5
8	0	2.0	7.7	6.9	0	2.0	7.3	7.2
9	0	0	1.6	3.5	0	0	1.8	3.6
10	0	1.0	3.0	4.1	0	1.2	3.3	4.3
11	1.4	5.6	8.0	7.5	1.6	5.8	8.1	7.6
12	2.6	12.6	14.1	13.3	2.3	12.3	14.1	13.1
13	0.5	17.0	11.7	9.3	0.6	17.3	11.9	9.5
14	0	0.9	2.5	4.2	0	1.0	2.7	4.1
15	0	0	2.2	3.3	0	0	2.3	3.4
16	1.6	5.7	9.4	7.6	1.8	5.6	9.3	7.7

with glass wool. This ensured a minimal volume loss and a particulate-free sample.

Standard Curves—Calculations were carried out using standard curves constructed by analyzing 1-ml samples of the pooled predose plasma spiked with 1–32 μg of acetaminophen and plotting the area counts *versus* the corresponding concentration. The standard curve data were subjected to least-squares linear regression analysis, and the resulting equation was utilized for the calculation of the drug concentration in the unknown samples.

RESULTS AND DISCUSSION

Typical chromatograms of a predose plasma sample (Fig. 1A) and the 20- and 40-min postdose plasma samples (Fig. 1B and C) obtained for a typical subject, demonstrate the specificity of this method by the absence of interfering peaks. With 15% methanol in water as the eluent at a flow rate of 1.0 ml/min, the retention time for acetaminophen was 7 min. Additional peaks are observed eluting close to the solvent front. They are ascribed to the more polar metabolites of acetaminophen. Earlier elution of the acetaminophen is possible by increasing the methanol content of the mobile phase, but an occasional interfering peak was observed eluting close to the peak of interest.

Figure 2 represents the chromatograms of a 1-ml blank plasma sample, a 1-ml plasma sample spiked with 1 μg of acetaminophen, and a 1-ml plasma sample spiked with 8 μg of acetaminophen. The standards and samples are chromatographically clean to the extent that acetaminophen concentrations <1 μg/ml can be detected with this procedure. Since therapeutic acetaminophen levels normally encountered in blood plasma monitoring require detection of ≥1 μg/ml, the sensitivity of this method is more than adequate for use in blood level studies.

Recovery—The recovery of free acetaminophen from plasma relative to its recovery from water as measured by the ratio of the slopes of the

standard curves in plasma and water, respectively, was 0.94 ± 0.03 with a coefficient of variation of 3%. Interassay variability of the slope and y-intercept of the five standard curves generated during the bioavailability study had coefficients of variation of 2.7 and 8%, respectively. A typical calibration regression line is $y = 0.229x + 0.0296$ and is linear over a 1–32-μg/ml concentration range. The good linearity between the peak area and acetaminophen concentration in plasma is indicated by the correlation coefficient of $r > 0.999$.

Accuracy—Table I shows the actual amounts with which 1-ml blank plasma samples were spiked and the amounts found when the plasma was analyzed according to the described method. The experiment was performed in duplicate on each of 5 days. The percent error for each unknown sample was calculated according to (17):

$$\text{percent error} = \frac{\text{amount added} - \text{amount found}}{\text{amount added}} \times 100 \quad \text{(Eq.1)}$$

The average percent error was 2.2%. In no case did it exceed 7.0%.

Reliability—A comparison was made of acetaminophen levels determined by the described method with levels determined simultaneously by a previously validated extraction procedure. Table II shows the acetaminophen levels found with both methods for 16 subjects who had been studied on a three-way crossover with samples taken at 10, 20, 40, and 60 min postdosing. The comparison gave a correlation coefficient of 0.993. In addition, the application of a paired Student's t test and the Wilcoxon-Mann-Whitney rank-sum test to the two sets of data indicated at the 95% confidence level that there was no significant difference between results obtained by the two methods.

Summary—The proposed method is simple and sensitive for the rapid determination of nonconjugated acetaminophen in plasma or blood at levels likely to be encountererd after a usual 650- or 1000-mg total dose. Plasma proteins are denatured by the addition of a saturated $Ba(OH)_2$ solution and then precipitated with 5% $ZnSO_4$ solution. The resulting clear supernatant is analyzed by HPLC. The reproducibility, sensitivity, and selectivity of the method make the use of an internal standard unnecessary.

Since the major biotransformation of acetaminophen in humans is direct conjugation with sulfate and glucuronic acid to form the sulfate and glucuronic metabolites, these polar metabolites do not interfere in the assay as they are eluted along with the solvent. The accuracy and reliability of this method have been proven by comparison of results obtained with an established extraction procedure in over 400 comparative determinations.

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Pharmacokinetics of Bretylium in Dogs and the Effect of Hemoperfusion on Elimination

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Abstract □ The pharmacokinetics of bretylium in dogs and the efficacy of hemoperfusion with a resin column in its removal from the body following intravenous administration of bretylium tosylate were investigated. Five mongrel dogs weighing 18–26 kg were given a bolus dose of 15 mg/kg. Serial blood samples were taken for 24 hr. Hemoperfusion, through a resin column, was then initiated and continued for 4 hr under pentobarbital anesthesia. During hemoperfusion, arterial and venous blood samples were collected several times; venous blood samples were then withdrawn for an additional 8 hr. Urine was collected from each dog in three portions for up to 48–54 hr. Pharmacokinetics of bretylium in dogs could be characterized by a two-compartment open model with a distribution half-life of 7 min and biological half-life of 15.9 ± 1.9 hr. Plasma levels declined rapidly from ~20 μg/ml at 6 min to <2 μg/ml within 1 hr. The ratio of intercompartmental rate constants, k_{12}/k_{21}, was 16.7, and the volume of the central compartment and apparent volume of distribution were 0.245 and 5.22 liter/kg, respectively, indicating a wide distribution of bretylium into the tissues. Plasma dialysis clearance averaged 29.7 ml/min, which is 30% of the total body clearance (98.8 ml/min). These data suggest that resin hemoperfusion may not be useful in the treatment of bretylium intoxication.

Keyphrases □ Pharmacokinetics—bretylium and the effect of hemoperfusion on elimination, distribution, dogs □ Bretylium—pharmacokinetics and the effect of hemoperfusion on elimination, distribution, dogs □ Distribution—pharmacokinetics of bretylium and the effect of hemoperfusion on elimination, dogs

Bretylium tosylate is a quaternary ammonium compound used in the treatment of ventricular tachycardia or ventricular fibrillation. It has been demonstrated that bretylium increases the action potential duration along the entire left ventricular conducting system (1).

In humans and rats, bretylium is primarily eliminated unchanged *via* the kidneys. No metabolites have been identified following administration of bretylium in humans (2–5). A half-life of 9.75 ± 4.19 (*SD*) hr in eight patients aged 48.4 ± 10.8 years has been reported (6). The longest

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