Table II—Estimated Pharmacokinetic Parameters of Primaquine

	k_a, h^{-1}	Absorption Half-Life, h	k_e, h^{-1}	Elimination Half-Life, h	Lag Time, h	Ratio[a]	r^2
Subject 1[b]	0.81	0.85	0.257	2.7	0.18	7.3	0.946
Subject 2[c]	1.47	0.47	0.287	2.41	0.87	3.5	0.976

[a] Ratio of bioavailability/Vd. [b] After oral administration of 90 mg of primaquine diphosphate. [c] After oral administration of 60 mg of primaquine diphosphate.

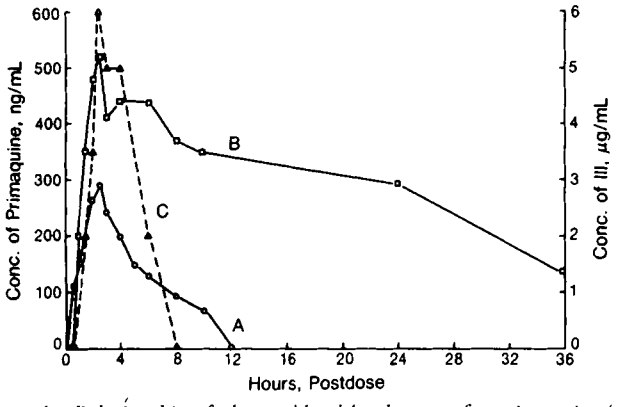

Figure 4—*Relationship of plasma blood level curves for primaquine (A), primaquine metabolite (B), and the more polar metabolite (C), reported as peak height in centimeters.*

tabolites, Fig. 2C. Both of these presumed urinary metabolite compounds are more polar than primaquine in our HPLC system. Neither urinary metabolite appeared to be II or III, but one appears to have a retention time which was the same as that of the more polar plasma metabolite.

The present method utilizing HPLC with UV detection for the simultaneous determination of primaquine and its carboxy metabolite (III), is rapid and precise. The use of this method for plasma and urine samples of volunteers after oral administration of primaquine diphosphate indicates that absorption and metabolism is rapid, that there appear to be at least two plasma metabolites (one identified as an oxidative-deamination product of primaquine, III), and that there appear to be two as yet unidentified urinary metabolites of primaquine.

REFERENCES

(1) P. E. Carson, C. L. Flanagan, C. E. Ickes, and A. S. Alving, *Science*, **124**, 484 (1952).

(2) A. R. Tarlov, G. I. Brewer, P. E. Carson, and A. S. Alving, *Arch. Int. Med.*, **109**, 209 (1962).

(3) "Handbook of Experimental Pharmacology: Antimalarial Drugs," W. Peters and W. H. G. Richards, Eds., Springer-Verlag, New York, NY, 1984, chap. 3.

(4) J. D. Baty, D. A. Price-Evans, and P. A. Robins, *Biomed. Mass Spectrom.*, **2**, 304 (1975).

(5) J. K. Baker, J. D. McChesney, C. D. Hufford, and A. M. Clark, *J. Chromatogr.*, **230**, 69 (1982).

(6) T. G. Rajagopalan, B. Anjaneyulu, V. D. Shanbag, and R. S. Grewal, *J. Chromatogr.*, **224**, 265 (1981).

(7) J. Blanchard, *J. Chromatogr.*, **226**, 455 (1981).

(8) P. E. Carson, R. Hohl, M. V. Nora, G. W. Parkhurst, T. Ahmad, S. Scanlan, and H. Frischer, *Bull WHO*, **59** 427 (1981).

(9) M. Gibaldi and D. Perrier, "Pharmacokinetics," Marcel Dekker, Inc., New York, N.Y., 1975, p. 36.

(10) C. M. Metzler, G. L. Elfring, and A. J. McEwen, *Biometrics*, **30**, 3 (1974).

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Quantitation of Metronidazole in Pharmaceutical Dosage Forms Using High-Performance Liquid Chromatography

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Received June 6, 1983, from the *Department of Pharmaceutics, University of Houston, Houston, TX 77030.* Accepted for publication September 26, 1983.

Abstract □ A high-performance liquid chromatographic (HPLC) method for the quantitation of metronidazole in pharmaceutical dosage forms has been developed. The method is accurate and precise with an *RSD* of 0.68%, based on six readings. The excipients present in various dosage forms did not interfere with the assay procedure. A solution of metronidazole decomposed using heat showed 0% potency.

Keyphrases □ Metronidazole—HPLC, pharmaceutical dosage forms □ HPLC—pharmaceutical dosage forms of metronidazole

Metronidazole is extensively used in medicine as an antiprotozoal agent. The USP-NF method (1) for the quantitation of metronidazole in tablets is based on titration with perchloric acid, which requires a tedious extraction-purification procedure; moreover, other weak bases, impurities, and products of decomposition usually interfere with this type of volumetric titration. The quantitation of metronidazole in biological fluids was reviewed by Wood (2). The purpose of these investigations was to develop a stability-indicating assay procedure for the quantitation of metronidazole in pharmaceutical dosage forms based on HPLC.

EXPERIMENTAL SECTION

Chemicals and Reagents—All the chemicals and reagents were USP, NF, or ACS quality and were used without further purification. The USP quality powder of metronidazole[1] (2-methyl-5-nitroimidazole-1-ethanol; I) was used as received.

Apparatus—The high-performance liquid chromatograph[2] (HPLC) was equipped with a multiple-wavelength detector[3] and a recorder[4]. A semipolar column[5] (30 cm × 4 mm i.d.) was used. The average size of the particles was 10 μm.

[1] G. D. Searle & Co., Chicago, Ill.

[2] ALC202 equipped with U6K universal injector; Waters Associates, Milford, Mass.

[3] Spectroflow monitor SF770; Schoeffel Instruments Corp., Ramsey, N.J.

[4] Omniscribe 5213-12; Houston Instruments, Austin, Tex.

[5] μ-Bondapak phenyl; Waters Associates, Milford, Mass.

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Table I—Assay Results

Dosage Form	Metronidazole, mg/mL or mg/tablet	Other Ingredients, (mg/mL)	Percent of Label Claim Found: Using USP-NF Method (*RSD*, %)	Percent of Label Claim Found: Using HPLC Method (*RSD*, %)
		Recovery Data from Commercial Formulations		
Commercial solution 1[d]	5.0	Sodium Phosphate 4.8, Citric Acid 2.3, and Sodium Chloride 7.9	—[a]	99.2 (0.68)
Commercial solution 2[e]	5.0	Sodium Phosphate 4.8, Citric Acid 2.3, and Sodium Chloride 7.9	—[a]	99.8 (0.66)
Commercial solution 3[f]	5.0	Sodium Phosphate 4.8, Citric Acid, 2.3, and Sodium Chloride 7.9	—[a]	99.8 (0.67)
Commercial vial Powder 1[g]	500 mg/vial of metronidazole as HCl salt	Mannitol, 415	—[a]	104.9 (0.69)
Commercial vial Powder 2[h]	500 mg/vial of metronidazole as HCl salt	Mannitol, 415	—[a]	102.8 (0.69)
Tablets, lot 1[i]	250	Blue Coloring Matter and Excipients	99.5 (0.76)	99.2 (0.70)
Tablets, lot 2[j]	250	Blue Coloring Matter and Excipients	99.3 (0.78)	99.6 (0.68)
Tablet 1[k]	250	Blue Coloring Matter and Excipients	—[b]	100.5 (—[c])
Tablet 2[k]	250	Blue Coloring Matter and Excipients	—[b]	98.3 (—[c])
Tablet 3[k]	250	Blue Coloring Matter and Excipients	—[b]	99.2 (—[c])
Tablet 4[k]	250	Blue Coloring Matter and Excipients	—[b]	101.5 (—[c])
Tablet 5[k]	250	Blue Coloring Matter and Excipients	—[b]	97.8 (—[c])
Tablet 6[k]	250	Blue Coloring Matter and Excipients	98.2(—[c])	98.2 (—[c])
Tablet 7[k]	250	Blue Coloring Matter and Excipients	100.3 (—[c])	100.1 (—[c])
		Recovery Data From Synthetic Mixtures		
Synthetic powder 1	160	Mannitol, 160		99.9 (0.68)
Synthetic powder 2	160	Mannitol, 160		100.4 (0.69)
Synthetic solution 1	2.5	Sodium Phosphate 2.5, Citric Acid 1.5, and Sodium Chloride 4.0	—[a]	100.0 (0.69)
Synthetic solution 2	5.0	Sodium Phosphate 5, Citric Acid 2.5, and Sodium Chloride 8	—[a]	100.2 (0.68)
Synthetic solution 3	2.5	Mannitol 2.5	—[a]	100.2 (0.67)

[a] USP-NF method not specified for solutions and not applicable to metronidazole hydrochloride powder. [b] These single tablets were not assayed using the USP-NF method. [c] Not determined for single-tablet assay. [d] All commercial solutions were obtained from G. D. Searle & Co.; solution no. 1 from lot 52V054N4. [e] Lot ZH 104. [f] Lot 3L 011. [g] Lot 1181-166. [h] Lot 483-174. [i] Lot 782-008; average of 10 tablets, one determination. [j] Lot 683-048; average of four tablets, one determination. [k] Lot 782-008.

Chromatographic Conditions—The mobile phase was an aqueous solution of 0.02 M KH_2PO_4 (pH ~4.2). The flow rate was 2.5 mL/min, and the temperature was ambient. The sensitivity was set at 0.04 (254 nm) and the chart speed was 30.5 cm/h.

Solution Preparation—The stock solutions of metronidazole (1.60 mg/mL) and phenylpropanolamine hydrochloride (10.0 mg/mL) in water were prepared fresh daily. Metronidazole was dissolved in water with the aid of gentle heat (≤60°C) to hasten dissolution. Preliminary investigations indicated that there was no decomposition with gentle heat. A stock solution of acetaminophen (0.40 mg/mL) was prepared by dissolving 40.0 mg of acetaminophen in 1 mL of methanol and then bringing it to volume (100.0 mL) with water. The standard solutions were prepared as needed by diluting the stock solutions with water. For assays, the concentrations (in μg/mL) of metronidazole, phenylpropanolamine hydrochloride, and acetaminophen were 200, 1400 and 40, respectively.

The assay solutions were prepared by mixing a solution (from ready-to-use bags or vials) representing 20.0 mg of metronidazole with 14.0 mL of the stock solution of phenylpropanolamine hydrochloride (the internal standard) and then bringing it to volume (100.0 mL) with water. To assay the contents of the vials containing powder (metronidazole hydrochloride ≅500 mg of metronidazole), the contents were dissolved in water and brought to volume (100.0 mL) with water. Four milliliters of the mixture were combined with 14.0 mL of the stock solution of phenylpropanolamine hydrochloride and brought to volume (100.0 mL) with water.

Extraction Procedure for Tablets—Ten tablets were accurately weighed and ground to a fine powder. (When content uniformity was to be determined, a single tablet was ground to a fine powder.) Powder representing 80.0 mg of metronidazole was added to ~90 mL of water in a 150-mL beaker. The solution was gently heated (with stirring) to 60°C on a hot plate and then allowed to cool to room temperature. The solution was transferred to a 100-mL volumetric flask, brought to volume with water, and filtered[6]. The first 20 mL of the filtrate was rejected; the rest was further diluted. Then, 12.5 mL of the solution was mixed with 7.0 mL of the stock solution of phenylpropanolamine hydrochloride and brought to volume (50.0 mL) with water.

Assay Sample of Decomposed Ready-to-Use Solution (5.0 mg/mL)—Ten milliliters of the solution was mixed with ~4 mL of ~1 M NaOH solution in a 150-mL beaker and then heated to boiling on a hot plate for ~25 min (water was replaced as necessary). The mixture was cooled, the pH adjusted to ~6 using ~0.5 M HCl, and brought to volume (250.0 mL) with water.

Chromatographic Procedure—A 20-μL aliquot of the assay solution was injected into the chromatograph under the conditions described above. For comparison, a 20-μL aliquot of the standard solution containing identical concentrations of metronidazole and phenylpropanolamine hydrochloride was injected after the assay was eluted.

Calculations—Since preliminary investigations indicated that the ratio of the peak heights of metronidazole and phenylpropanolamine hydrochloride were directly related to the concentration of metronidazole (range tested 2–6 μg), the results were calculated using the equation:

$$\frac{R_{pha}}{R_{phs}} \times 100 = \text{percent of label claim found}$$

where R_{pha} is the ratio of the peak heights of drug–internal standard of the

[6] Whatman # 1 filter paper.

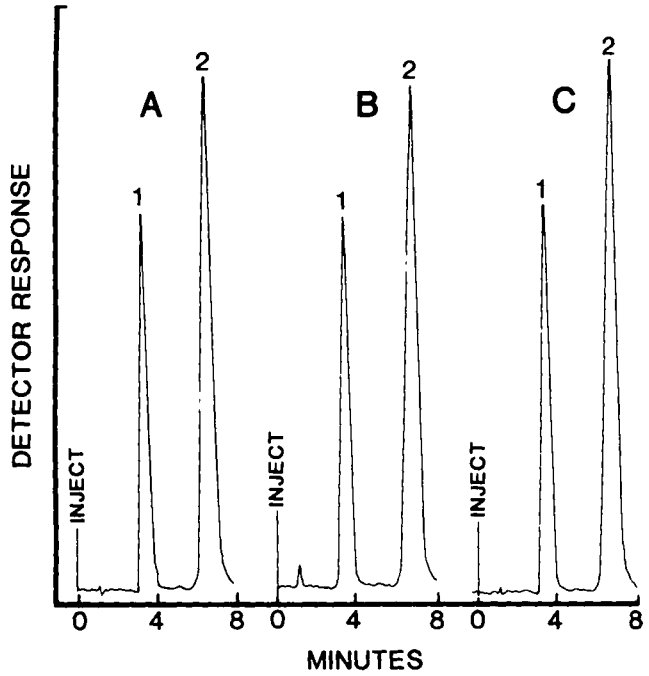

Figure 1—*Typical chromatograms; peaks 1 and 2 are from phenylpropanolamine and metronidazole, respectively. Chromatograms A–C are from a standard solution, a ready-to-use solution (No. 1, Table I), and from tablets, respectively.*

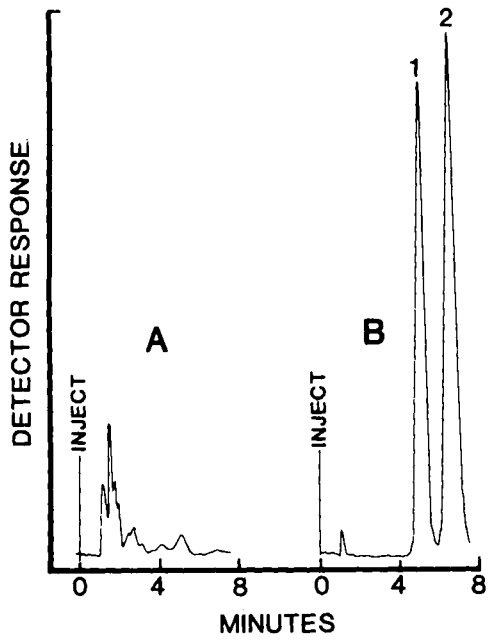

Figure 2—*Typical chromatograms; chromatogram A is from a decomposed solution (see text). All the peaks are from the decomposition products; no internal standard was added to this solution. Chromatogram B is from a standard solution with acetominophen (peak 1) as the internal standard and metronidazole (peak 2).*

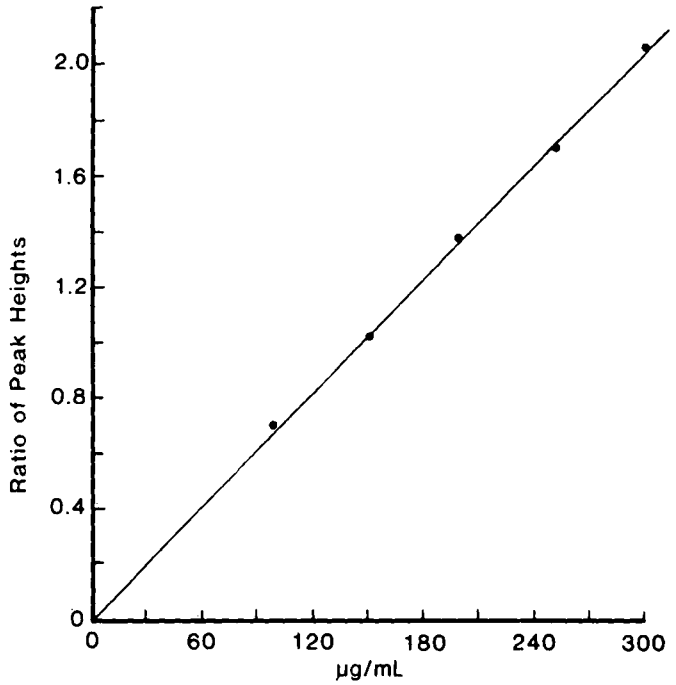

Figure 3—*A calibration curve of the ratio of the peak heights (metronidazole–phenylpropanolamine) and concentration of metronidazole. An average* RSD *based on six injections, was 0.68%.*

assay solution, and R_{phs} is the ratio of the peak heights of drug–standard solution. Alternatively, the results may be calculated using the standard curve.

RESULTS AND DISCUSSION

The assay results (Table I) indicate that the developed HPLC method for the quantitation of metronidazole in pharmaceutical dosage forms can be adopted. It is accurate and precise with an *RSD* of 0.68%, based on six injections. Before developing phenylpropanolamine hydrochloride as the internal standard (Fig. 1), acetaminophen was tried (Fig. 2). Acetaminophen can be used as an internal standard if some active or inactive products of decomposition interfere with the phenylpropanolamine peak. The concentrations were directly related to the ratio of peak heights between a range of 2–6 μg (Fig. 3). No preliminary extraction procedure was required to assay the ready-to-use solutions or the powders, which contained 415 mg of mannitol for each 500 mg of metronidazole. The presence of sodium phosphate, citric acid, and sodium chloride in the ready-to-use solutions, and mannitol in the powders, did not interfere with the assay procedure.

A very simple preliminary extraction procedure was required to assay the tablets. The coloring matter and the excipients present in the tablets did not interfere with the assay procedure. The method is stability indicating since a sample decomposed by using heat (see *Experimental Section*) showed almost 0% potency and a number of new peaks in the chromatogram (Fig. 2A). The developed method can be used to assay metronidazole in various dosage forms and also can be used to test content uniformity of the tablet formulations.

REFERENCES

(1) The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Inc., Rockville, Md., 1980, p. 531.

(2) N. F. Wood, *J. Pharm. Sci.*, **64**, 1048 (1975).