(6) S. Görög and G. Szepesi, *Anal. Chem.*, **44**, 1079 (1972).
(7) R. B. Dean and W. B. Dixon, *ibid.*, **23**, 636 (1951).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 11, 1976, from the *Pharmaceutical Research and Development Laboratories, Warner-Lambert Research Institute, Morris Plains, NJ 07950.*

Accepted for publication September 22, 1976.

The authors thank Mr. T.-M. Chem, Dr. R. E. Daly, and Mrs. C. Moran for technical assistance.

[x] To whom inquiries should be directed.

High-Pressure Liquid Chromatographic Analysis of Cimetidine, a Histamine H_2-Receptor Antagonist, in Blood and Urine

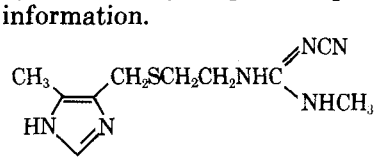
W. C. RANDOLPH [x], V. L. OSBORNE, S. S. WALKENSTEIN, and A. P. INTOCCIA

Abstract □ A method is described for extraction of cimetidine, a histamine H_2-receptor antagonist, from whole blood and urine with subsequent analysis by high-pressure liquid chromatography (HPLC). The drug is extracted from biological fluids with 1-octanol and back-extracted into dilute acid and then into a small volume of ethanol by saturation with potassium carbonate. HPLC analysis is performed on a column of 5-μm silica with a mixed mobile phase consisting primarily of acetonitrile. The method measures concentrations of cimetidine as low as 0.05 μg/ml and is reproducible. Blood levels and urinary excretion data obtained with the analytical procedure are given for a group of human subjects who received 200-mg oral doses of cimetidine.

Keyphrases □ Cimetidine—high-pressure liquid chromatographic analysis, blood and urine □ High-pressure liquid chromatography—analysis, cimetidine, blood and urine □ Histamine H_2-receptor antagonists—cimetidine, high-pressure liquid chromatographic analysis, blood and urine

The two types of pharmacological receptors in the mediation of histamine-induced responses are referred to as H_1 and H_2 (1, 2). The classical antihistaminic compounds are effective through antagonism of the action of histamine H_1-receptor sites. Several new compounds, defined as H_2-receptor antagonists, have been effective in reducing histamine-induced gastric acid secretion. Cimetidine[1], *N″*-cyano-*N*-methyl-*N′*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine (I), is currently undergoing trials to assess its effectiveness in the treatment of gastric and duodenal ulcer patients. The chemistry and pharmacology of this drug were discussed previously (3).

This paper describes methodology for extraction and concentration of cimetidine from whole blood and urine with subsequent analysis by high-pressure liquid chromatography (HPLC). Numerous samples from various clinical studies were analyzed by this method, which proved highly satisfactory in providing necessary bioavailability information.



I

[1] Tagamet, Smith Kline & French Laboratories, Philadelphia, Pa.

EXPERIMENTAL

Instrumentation—A constant flow liquid chromatograph[2] was used in conjunction with a variable wavelength UV detector[3] set at 228 nm. The stainless steel column, 3.2 mm i.d. × 25 cm long, was obtained prepacked with 5-μm silica[4]. A rotary injection valve[5] was used for introducing samples at the column inlet.

Reagents—Certified grade 1-octanol[6] was used for extraction of cimetidine from biological fluids. The HPLC mobile phase consisted of UV grade acetonitrile[7], glass-distilled methanol[7], glass-distilled water, and concentrated ammonium hydroxide (1000:60:20:5).

Standard Solutions (Blood)—Dissolve 10 mg of cimetidine in 10 ml of methanol. Add 0.05 ml of this drug solution to 25 ml of heparinized whole human blood and mix thoroughly. From this blood stock, containing 2.0 μg of cimetidine/ml, make appropriate dilutions with heparinized whole blood to provide a concentration range of 0.1–2.0 μg/ml. Weigh 30 mg of metiamide, *N*-methyl-*N′*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]thiourea (internal standard), into a 100-ml volumetric flask. Dissolve and bring the contents to volume with methanol.

Standard Solutions (Urine)—Dissolve 50 mg of cimetidine in 10 ml of methanol. Pipet 1 ml of the drug solution into a 25-ml volumetric flask, and bring to volume with control human urine. Make dilutions of the urine stock (containing 200 μg of cimetidine/ml) with control urine to provide a concentration range of 10–150 μg/ml. Dissolve 30 mg of metiamide in 10 ml of methanol for use as the internal standard.

Extraction (Blood)—Heparinize and freeze whole blood samples as quickly as possible after collection. Prior to extraction, thaw the samples and pipet 5-ml aliquots of the lysed blood into 40-ml centrifuge tubes. Add 10 μl of the internal standard solution (containing 3 μg of metiamide) to each sample and adjust to pH 9 with 1 *N* NaOH. Add 10 ml of 1-octanol to each tube, place horizontally on a reciprocating shaker, and shake 15 min at low speed (about 60 oscillations/min).

After shaking, centrifuge the tubes briefly to obtain a sharp separation of layers. With a pipet, remove 9.5 ml of the octanol layer to a second 40-ml centrifuge tube, add 5 ml of 0.03 *N* HCl, and shake horizontally on a reciprocating shaker at high speed (about 120 oscillations/min). Again, centrifuge to achieve phase separation and aspirate and discard the octanol layer. Transfer 4.5 ml of the aqueous phase to a 12-ml centrifuge tube, add 0.3 ml of absolute ethanol, and vortex briefly to mix. Add sufficient granular potassium carbonate to saturate the solution (about 5.5 g), and vortex vigorously for 30 sec. Centrifuge the tubes at 1300×*g* for 5 min, thereby separating the ethanol as a layer over the aqueous phase. Remove 0.2 ml of the ethanol fraction to a small vial for HPLC analysis.

[2] Perkin-Elmer 1220.
[3] Perkin-Elmer LC-55.
[4] Altex Scientific Inc., Berkeley, Calif.
[5] Valco Instruments Co., Houston, Tex.
[6] Fisher Scientific Co.
[7] Burdick and Jackson.

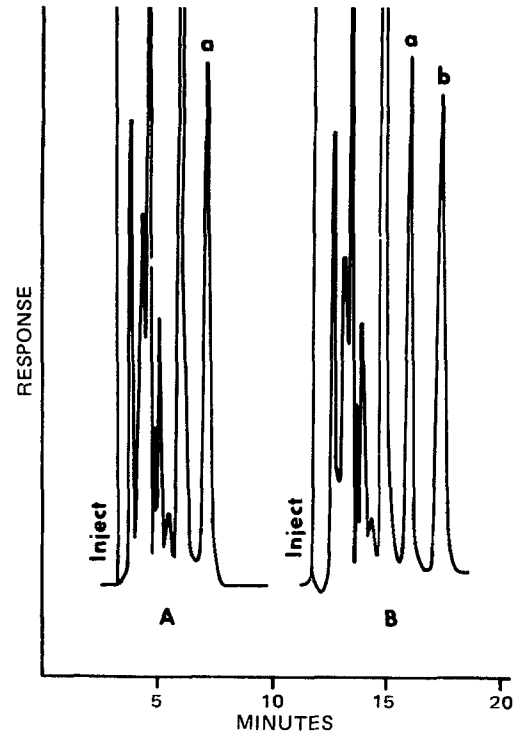


Figure 1—*Chromatograms of extracts from control human blood (A) and blood containing 0.5 μg of cimetidine/ml (B). Key: a, metiamide (internal standard); and b, cimetidine.*

Extraction (Urine)—Dilute 1-ml aliquots of each urine sample with 4 ml of distilled water in 40-ml centrifuge tubes. Add 10 μl of the appropriate internal standard solution (containing 30 μg of metiamide), and adjust the tube contents to pH 9 with 0.1 *N* NaOH. Proceed with the extraction as described for blood.

Chromatography—Condition new columns by flushing for 8 hr with methanol–water (98:2) at 0.5 ml/min followed by the analytical mobile phase, 1 ml/min for 2 hr. Perform all analyses at a flow rate of 1 ml/min with the pressure variable between 1000 and 2000 psi, depending on column characteristics. (If pressures rise above 2000 psi, replacement of both inlet and outlet frits will usually reduce the back-pressure significantly.)

Inject 15-μl aliquots of the ethanol extracts. Monitor the column effluent with a variable wavelength UV detector at 228 nm, and record detector response with a strip-chart recorder. For blood extracts, use attenuations of 0.02 and 0.04 absorbance unit full scale (aufs). For urine samples, use attenuations from 0.10 to 1.00 aufs. Retention times with this system are about 5.5 min for cimetidine and 4 min for metiamide.

Quantitation—Calculate the ratio of cimetidine peak height to metiamide peak height for standards, and plot ratio *versus* concentration of cimetidine. Use the resulting curve to convert peak height ratios of unknown samples to cimetidine concentration.

Clinical Study—A clinical study was performed in which 12 normal human subjects each received two identical oral doses of 200 mg of cimetidine in aqueous solution; 7 days separated the doses. Whole blood samples were collected by venipuncture at designated time intervals, immediately heparinized, and frozen for analysis. Total urine output was collected in the intervals of 0–3, 3–12, and 12–24 hr. Urine volumes were measured, and aliquots were kept frozen prior to analysis.

RESULTS AND DISCUSSION

The development of an analytical method for measuring cimetidine in biological fluids was complicated by the difficulty in extracting the drug from aqueous media. Of various solvents tested, 1-octanol provided the most efficient extraction. The low volatility of octanol, combined with characteristics of the drug itself, made GLC analysis impractical. Certain other analytical techniques, such as spectrophotometry, were ruled out by the small amount of drug present in blood and the large number of interfering substances extracted from biological fluids by octanol. Two relatively new advances in HPLC, variable wavelength UV detectors and microparticulate column packings, made possible the HPLC analysis of cimetidine following extraction from blood and urine.

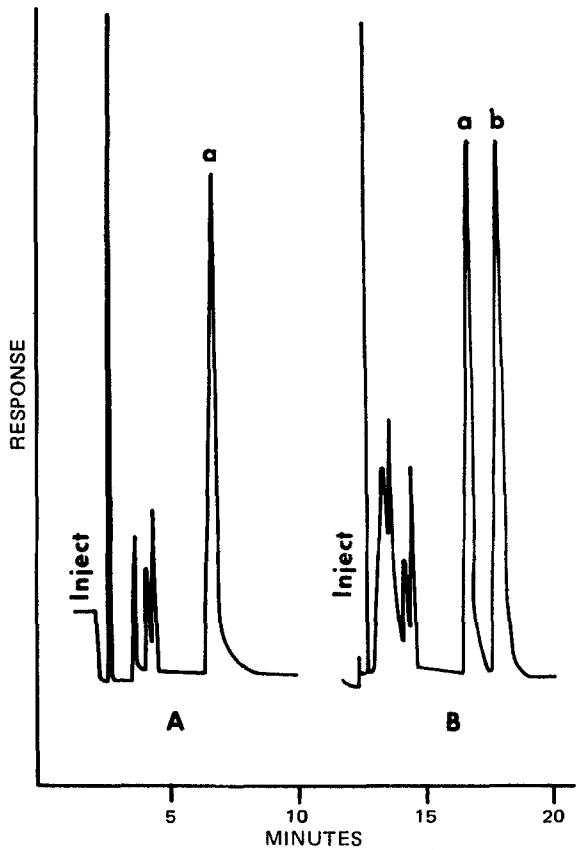


Figure 2—*Chromatograms of extracts from control human urine (A) and urine containing 25 μg of cimetidine/ml (B). Key: a, metiamide (internal standard); and b, cimetidine.*

The recovery of cimetidine from whole blood was determined by addition of the drug, labeled with carbon-14, to control blood and extraction as described under *Experimental*. The radioactive content of the final 0.3-ml ethanol fraction, measured by liquid scintillation counting, contained 63% of the initial blood radioactivity (absolute recovery).

Typical chromatograms obtained from control human blood and blood containing 0.5 μg of cimetidine/ml are shown in Fig. 1. Of the several hundred control blood samples analyzed, none contained any material that interfered with the analysis of cimetidine. The major metabolite of cimetidine, the sulfoxide, was tested in the chromatographic system and eluted much later than the parent compound. The internal standard peak (metiamide) was completely resolved from the drug peak and eluted prior to cimetidine, thus not adding to analysis time.

The efficiency of the column used to obtain the chromatogram in Fig. 1 was about 7000 plates/25 cm. Efficiency varies somewhat between columns but may often be improved by reconditioning and paying particular attention to elimination of all possible deadspace. Column life has been very good, especially when considering the alkaline nature of the mobile phase. Approximately 1000 samples were analyzed on one column before a significant loss of efficiency was noted.

The relationship between peak height ratios and concentration of cimetidine in whole blood from 0.05 to 2.00 μg/ml was linear. Two groups of replicate blood samples, eight each containing 0.50 and 1.00 μg of drug/ml, were prepared to test the accuracy of the method. The groups were divided into halves, and one-half was extracted and analyzed on each successive day. Of all 16 samples, an average of 102 ± 4% of the actual cimetidine content was measured by HPLC analysis. Although peaks were obtained at lower concentrations, 0.05 μg/ml represented the lowest level of cimetidine that could be measured accurately in blood.

Figure 2 shows typical chromatograms obtained with control human urine and urine containing 25 μg of cimetidine/ml. The relationship between drug concentration in urine and peak height ratio was linear with drug concentrations between 10 and 150 μg/ml. The accuracy of the urinary analysis was tested by adding known amounts of drug to eight urine samples at two concentrations, 25 and 100 μg/ml. One-half of each group was analyzed on each successive day. The average recovery of added

Table I—Urinary Excretion and Whole Blood Concentrations of Cimetidine with Related Parameters in 12 Human Subjects following Administration of Two Identical Single Oral Doses of 200 mg of Cimetidine

Parameter	Dose I	Dose II	Δ Dose II[a]
Average whole blood concentration and average intrasubject change, Dose I → Dose II, μg/ml, at:			
0.5 hr	0.65	0.71	0.19
1.0 hr	1.02	0.98	0.16
1.5 hr	0.86	0.85	0.12
2.0 hr	0.72	0.71	0.13
3.0 hr	0.59	0.58	0.15
4.0 hr	0.37	0.40	0.09
6.0 hr	0.16	0.17	0.04
8.0 hr	0.09	0.08	0.03
Average of individual peak whole blood concentrations, μg/ml	1.07	1.01	
Average peak time, hr	1.13	1.25	
Average area under whole blood concentration curves, 0 → 8 hr, μg/ml × hr	3.52	3.59	
Mean elimination half-life, hr	1.90	1.72	
Mean urinary excretion of cimetidine and mean intrasubject change, Dose I → Dose II, as % of dose, in intervals of:			
0–3 hr	32.4	33.0	5.6
3–12 hr	23.4	23.2	7.7
12–24 hr	1.9	1.6	1.1

[a] Δ Dose II is the absolute difference, regardless of the direction of change.

drug from the 16 spiked samples was 99.2 ± 1.1%. None of the control urines tested contained material that interfered with the analysis of cimetidine.

Mean blood levels and urinary excretion of drug for the 12 human subjects in the clinical study are shown in Table I. Also presented are parameters related to blood concentration data. Elimination half-lives of the drug in blood were obtained by fitting concentration data from the 4-, 6-, and 8-hr blood samples, using the method of least squares. Areas under blood level curves were computed with a trapezoidal program. No statistically significant differences were found in mean blood levels or urinary excretion of cimetidine, or in any related parameters, when Doses I and II were compared.

Metiamide, also an H_2-receptor antagonist, was employed in earlier studies of the effects of this class of compounds on gastric acid secretion. Obviously, since the method employs metiamide as the internal standard in the analysis of cimetidine, the reverse situation may be applied to analyze for metiamide. This analysis was successful on a number of samples. The only changes were that a detector wavelength of 235 nm was used and that concentrations of cimetidine in internal standard solutions were 0.5 (blood) and 5 (urine) mg/ml.

The described method is satisfactory for analysis of cimetidine and metiamide, both in its reproducibility and sensitivity. By application of the analytical procedure, it was determined that cimetidine is well absorbed following an oral dose, as indicated by the urinary excretion and blood levels of the drug. In clinical trials, the blood levels of cimetidine were measured and correlated with the inhibition of gastric acid secretion (4–6). These correlations, obtained with different doses of cimetidine, have been helpful in assessing the effect of circulating drug on gastric acid secretion.

REFERENCES

(1) A. S. F. Ash and H. O. Schild, *Br. J. Pharmacol. Chemother.*, **49**, 427 (1966).

(2) J. W. Black, W. A. M. Duncan, G. J. Durant, C. R. Ganellin, and M. E. Parsons, *Nature*, **236**, 385 (1972).

(3) R. W. Brimblecombe, W. A. M. Duncan, G. J. Durant, J. C. Emmett, C. R. Ganellin, and M. E. Parsons, *J. Int. Med. Res.*, **3**, 86 (1975).

(4) R. M. Henn, J. I. Isenberg, N. Maxwell, and R. A. L. Sturdevant, *N. Engl. J. Med.*, **293**, 371 (1975).

(5) R. E. Pounder, J. G. Williams, J. J. Misiewicz, and G. J. Milton-Thompson, *Proc. Br. Soc. Gastroenterol.*, **1975**, 32 (Abstract).

(6) G. F. Longstreth, J. R. Malagelada, and V. L. W. Go, *Gastroenterology*, **68**, A-85/942 (Abstract) (1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 18, 1976, from *Smith Kline & French Laboratories, Philadelphia, PA 19101.*

Accepted for publication September 20, 1976.

The authors thank R. Stote, M.D., and the staff of the Clinical Research Group of Presbyterian-University of Pennsylvania Medical Center for conducting the bioavailability study described. The authors also gratefully acknowledge the contributions of the biochemistry staff of The Research Institute, Welwyn Garden City, United Kingdom, and the technical assistance of Mr. Albert Bernstein and Mr. Gerald Joseph.

[x] To whom inquiries should be directed.

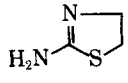
Anthelmintic 2-Arylhydrazino- and 2-Arylazo-2-thiazolines

M. T. WU, F. S. WAKSMUNSKI, D. R. HOFF, M. H. FISHER[x], J. R. EGERTON, and A. A. PATCHETT

Abstract □ Some 2-arylhydrazino- and 2-arylazo-2-thiazolines were synthesized for anthelmintic testing. The most potent compound, 2-(*o*-tolylazo)-2-thiazoline, was orally effective in sheep against a broad range of helminths.

Keyphrases □ Thiazolines, substituted—synthesized, evaluated for anthelmintic activity in sheep □ Anthelmintic activity—evaluated in various substituted thiazolines in sheep □ Structure–activity relationships—various substituted thiazolines evaluated for anthelmintic activity in sheep

In a search for anthelmintic activity in novel structures, the known neuromuscular blocking agent 2-amino-2-thiazoline (I) (1) had a paralytic effect on third-stage larvae of *Haemonchus contortus in vitro*, similar to that of the anthelmintic drugs tetramisole and pyrantel. No activity could be demonstrated in *in vivo* assays. However, with this *in vitro* activity as a starting point, a series of related compounds was synthesized and tested for *in vivo* anthelmintic activity.



I