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BIOANALYSIS OF CIMETIDINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Cimetidine, N″-cyano-N-methyl-N′-[2[[(5-methyl-1*H*-imidazol-4-yl)methyl]-thio]ethyl]guanidine, is a specific histamine H_2-receptor antagonist drug that is widely used in medicine to treat gastric ulcer disease and other pathological hypersecretory states. To study the bioavailability of cimetidine, it was necessary to develop a rapid and reliable high-performance liquid chromatography procedure for quantitating the drug in body fluids. In this new procedure, cimetidine is adsorbed directly from urine or plasma, without prior clean-up, on to a mini-column prepacked with C_{18} material (Sep-Pak C_{18} cartridge). Acetonitrile is used to elute the drug, and the eluate is analyzed by high-performance reversed-phase liquid chromatography on a Partisil 10 ODS column, with an aqueous phosphate–methanol mixture as the mobile phase and UV detection at 228 nm. This method for analyzing cimetidine in body fluids is rapid, accurate and precise and differs from previously reported methods in that it eliminates the need for performing bothersome single or multiple, dual-phase solvent extractions. Moreover, slight modifications in the composition of the mobile phase permit the simultaneous determination of cimetidine metabolites.

INTRODUCTION

Cimetidine (Tagamet®, Smith Kline and French Labs., Philadelphia, PA, U.S.A.) is a specific histamine H_2-receptor antagonist drug that is widely used to treat gastric ulcer disease and other pathological hypersecretory states. Its pharmacology and chemistry have been extensively reviewed[1,2].

Most of the published procedures for the estimation of cimetidine[3–6] or its major metabolite, cimetidine sulfoxide[7], in body fluids are modifications of the original, adsorption (silica column) high-performance liquid chromatography (HPLC) method[8]. These procedures involve laborious and time-consuming multiple solvent extractions and concentrations, as well as the use of expensive and hazardous organic solvents. Two published procedures for the analysis of cimetidine using reversed-phase (C_{18} column) HPLC[9,10] involve either undesirable multiple solvent extractions and/or the use of hazardous solvents.

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This paper describes a simplified assay procedure using mini-columns pre-packed with C_{18} material [Sep-Pak C_{18} Cartridge (Waters Associates, Milford, MA, U.S.A.)] to extract cimetidine directly from body fluids and a reversed-phase HPLC method in which primarily aqueous solvents are used.

EXPERIMENTAL

Reagents

Cimetidine, N″-cyano-N-methyl-N′-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]-thio]ethyl]guanidine, and metiamide, N-methyl-N′-[2[[(5-methyl-1-*H*-imidazol-4-yl)-methyl]thio]ethyl]thiourea, were supplied by Smith Kline and French Labs. (Philadelphia, PA, U.S.A.). Outdated human plasma (human plasma salvage) used in the preparation of the standard curve was obtained from New Jersey Blood Services (New Brunswick, NJ, U.S.A.). The chromatography solvents (water, acetonitrile and methanol) were HPLC grade (distilled in glass) from J. T. Baker (Phillipsburg, NJ, U.S.A.). The monobasic potassium phosphate, dibasic sodium phosphate and sodium carbonate were of reagent grade and were purchased locally. The Sep-Pak C_{18} cartridges were supplied by Waters Assoc.

Chromatography procedure

The chromatography system consisted of a Waters Model 6000A pump and a Waters Model U6K injector and a Partisil 10 ODS column, 6.6 mm I.D. × 250 mm (Whatman, Clifton, NJ, U.S.A.). A stainless-steel pre-column, 4.6 mm I.D. × 250 mm, packed with Bondapak C_{18}/Porasil B (Waters Assoc.), was positioned between the pump and the injector to protect the Partisil column by pre-saturating the mobile phase with dissolved packing material. The column effluent was monitored with a Perkin-Elmer (Norwalk, CT, U.S.A.) Model LC-55 variable-wavelength spectrophotometer set at 228 nm. The data obtained were recorded on an Omniscribe strip-chart recorder (Houston Instruments, Austin, TX, U.S.A.). The mobile phase consisted of 1/15 *M* monobasic potassium phosphate, 1/15 *M* dibasic sodium phosphate and methanol (815:100:185). Prior to use, the mobile phase was filtered through a 0.45-μm filter, HAWP04700 (Millipore, Bedford, MA, U.S.A.) using a solvent-clarification apparatus (Waters Assoc.), then de-aerated under water-aspirator vacuum in an ultrasonic bath (Model SC-101, Sonicor, Farmingdale, NY, U.S.A.). The mobile phase was maintained under a helium sparge during use. The analyses were performed at a flow-rate of 2.5 ml/min and at a pressure of 3000 p.s.i. Samples were injected with a 100-μl Hamilton (Reno, NV, U.S.A.) syringe.

Standard solutions

Plasma standards. Stock solutions of cimetidine (10 mg/100 ml) and metiamide, the internal standard, (30 mg/100 ml) were prepared in methanol. Appropriate dilutions of the cimetidine stock solution were prepared in drug-free human plasma salvage to provide a series of plasma standards containing from 0.25 to 2.0 μg of cimetidine per ml.

Urine standards. Stock solutions of cimetidine (50 mg/10 ml) and metiamide (300 mg/10 ml) were prepared in methanol. A 1-ml portion of the cimetidine stock solution was diluted to 25 ml with drug-free control human urine; this solution

contained 200 μg of cimetidine per ml and was appropriately diluted with control human urine to provide a series of urine standards containing from 10 to 200 μg of cimetidine per ml.

Extraction procedure

Plasma. The inlet of a Sep-Pak cartridge was slipped over the Luer adapter of a 10-ml glass syringe barrel. For convenience, the syringe barrel (with the cartridge at the bottom) was clamped in the vertical position to a ring stand. Various solvents and plasma and urine samples were pipetted into the syringe barrel atop the cartridge and then were slowly injected into the cartridge using the syringe plunger. The Sep-Pak cartridge was prepared by treatment with successive solvent washes of 4 ml of methanol, 4 ml of water, 4 ml of methanol and 4 ml of water, after which it was ready for use. Then a 3-ml sample of plasma to which had been added 0.01 ml of internal standard solution (containing 3 μg of metiamide) was loaded on to the Sep-Pak cartridge. The cartridge was then washed first with 5 ml of 1/15 *M* monobasic potassium phosphate, followed by 5 ml of 0.1 *M* sodium carbonate, to remove the more polar plasma components while retaining the less polar cimetidine and metiamide. Finally, the cimetidine and metiamide were eluted from the cartridge with 3 ml of acetonitrile, and the eluate was collected in a 5-ml tapered-bottom vial (Micro-Reaction Vessel, Supelco, Bellefonte, PA, U.S.A.). The acetonitrile eluate was evaporated to dryness under a stream of nitrogen gas at 37°C, the residue was reconstituted in 0.1 ml of the mobile phase, and this sample was then centrifuged at 250 *g* for 5 min. Finally, a 40-μl aliquot of the clear supernate was injected on to the chromatography column. Although the Sep-Pak cartridge is intended as a single-use, disposable item, it was found that careful washing of the used cartridge with methanol and water as described above would permit the processing of about 20–25 plasma samples using a single cartridge.

Urine. A 3-ml sample of urine to which had been added 0.01 ml of the internal standard solution (containing 300 μg of metiamide) was loaded on to a washed Sep-Pak cartridge and extracted as described for the plasma sample. However, because of the high concentrations of cimetidine normally present in urine (*i.e.*, *ca.* 100–200 μg/ml), it was not necessary to concentrate the acetonitrile eluate by evaporation as for the plasma samples. Hence, 40 μl of the acetonitrile eluate was injected directly on to the chromatography column. Also, as noted for the plasma samples, the cartridges could be re-used after washing between samples.

TABLE I

ACCURACY AND PRECISION OF ASSAY FOR CIMETIDINE IN PLASMA

Each result is the mean of four determinations.

Cimetidine added (μg/ml)	*Mean cimetidine determined (μg/ml)*	*Recovery (%)*	*Relative standard deviation (%)*
0.25	0.30	121.73	±7.58
0.50	0.47	94.25	±3.63
1.00	0.97	97.30	±5.69
1.50	1.45	96.46	±7.15
2.00	2.05	102.65	±6.87

Calculations

With use of the standard solutions prepared above, calibration curves were constructed for both plasma and urine samples by plotting the ratios of the peak areas for cimetidine and metiamide against the known cimetidine concentration in μg/ml. Peak areas were determined by multiplying the height of each peak by the full width of the peaks at one-half of the peak height. Each point on the curves represented the mean value of four separate assays.

RESULTS AND DISCUSSION

Plasma assay

The relationship between the peak area ratios and the concentration of cimetidine in plasma was linear within the range of 0.25 to 2.0 μg/ml ($y = 1.52x - 0.21$; $r = 0.998$). Table I illustrates the accuracy and precision of the method. The limit of detection (see ref. 11) was 0.1 μg/ml. Figs. 1 and 2 show typical chromatograms for a blank plasma extract and a plasma sample spiked with cimetidine and metiamide; no significant interfering peaks are observed.

The extraction efficiency for cimetidine from plasma (and urine) using the Sep-Pak C_{18} cartridges was measured by comparing the peak areas with the peak areas of

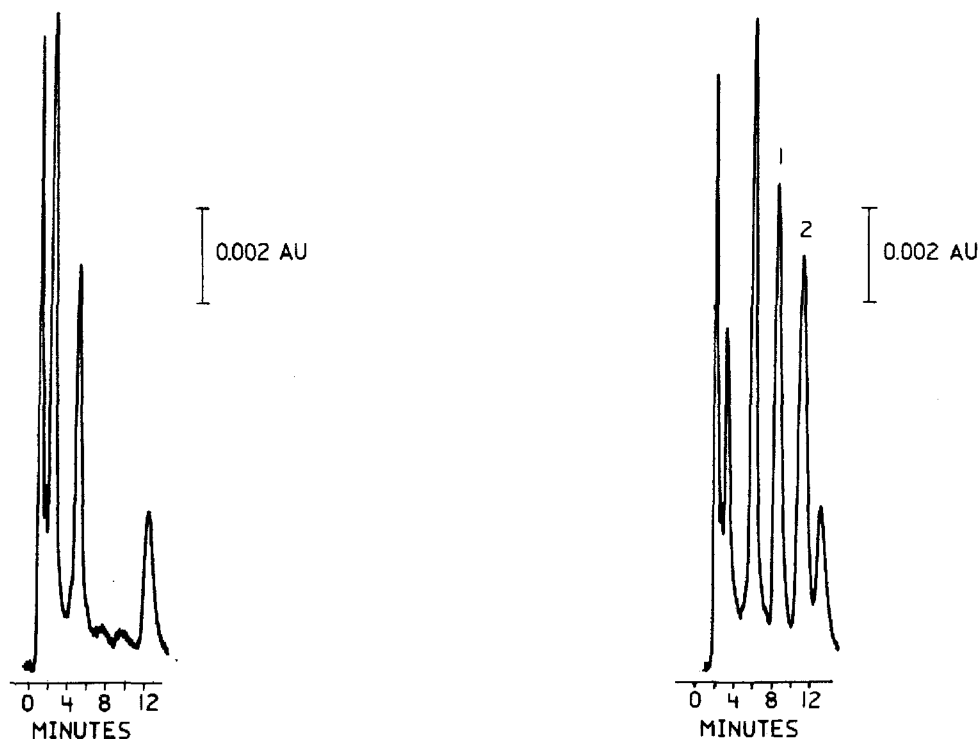

Fig. 1. Chromatogram of control human plasma extract.

Fig. 2. Chromatogram of human plasma extract. Peaks: 1, metiamide, the internal standard (1 μg/ml); 2, cimetidine (1 μg/ml).

TABLE II

ACCURACY AND PRECISION OF ASSAY FOR CIMETIDINE IN URINE

Each result is the mean of four determinations.

Cimetidine added (μg/ml)	*Mean cimetidine determined (μg/ml)*	*Recovery (%)*	*Relative standard deviation (%)*
10	9.74	97.40	±7.28
50	50.84	101.68	±2.61
100	99.31	99.31	±2.50
150	149.88	99.92	±3.00
200	200.24	100.12	±2.69

known amounts of cimetidine in methanol, which were injected directly on to the column. The absolute recovery averaged 59%, which compared favorably with the absolute recoveries reported by other workers[4,8–10].

The mobile phase is versatile in that it can be easily modified to meet certain

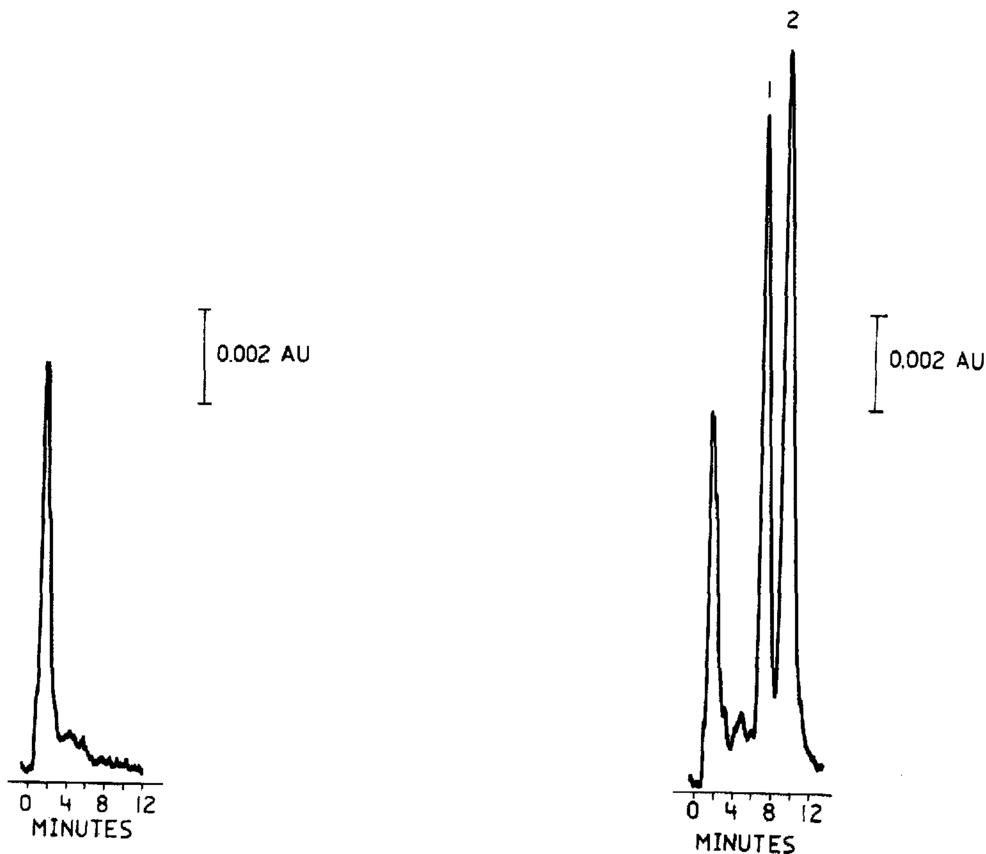

Fig. 3. Chromatogram of control urine extract.

Fig. 4. Chromatogram of urine extract. Peaks: 1, metiamide, the internal standard (100 μg/ml); 2, cimetidine (100 μg/ml).

needs. For example, we have been studying the bioavailability (in dogs) of cimetidine when co-administered orally with certain foods. It was initially found that certain foods led to additional peaks in the plasma chromatograms, which interfered with the analysis of cimetidine. By altering the ratio of the components of the mobile phase from 1/15 *M* monobasic potassium phosphate–1/15 *M* dibasic sodium phosphate–methanol (815:100:185) to 1/15 *M* monobasic potassium phosphate–1/15 *M* dibasic sodium phosphate–methanol (775:95:130), these interfering peaks were completely resolved from the peaks for cimetidine and internal standard, thereby allowing accurate analyses of plasma cimetidine levels to be performed. A single plasma sample could be analyzed for cimetidine in about 30–40 min.

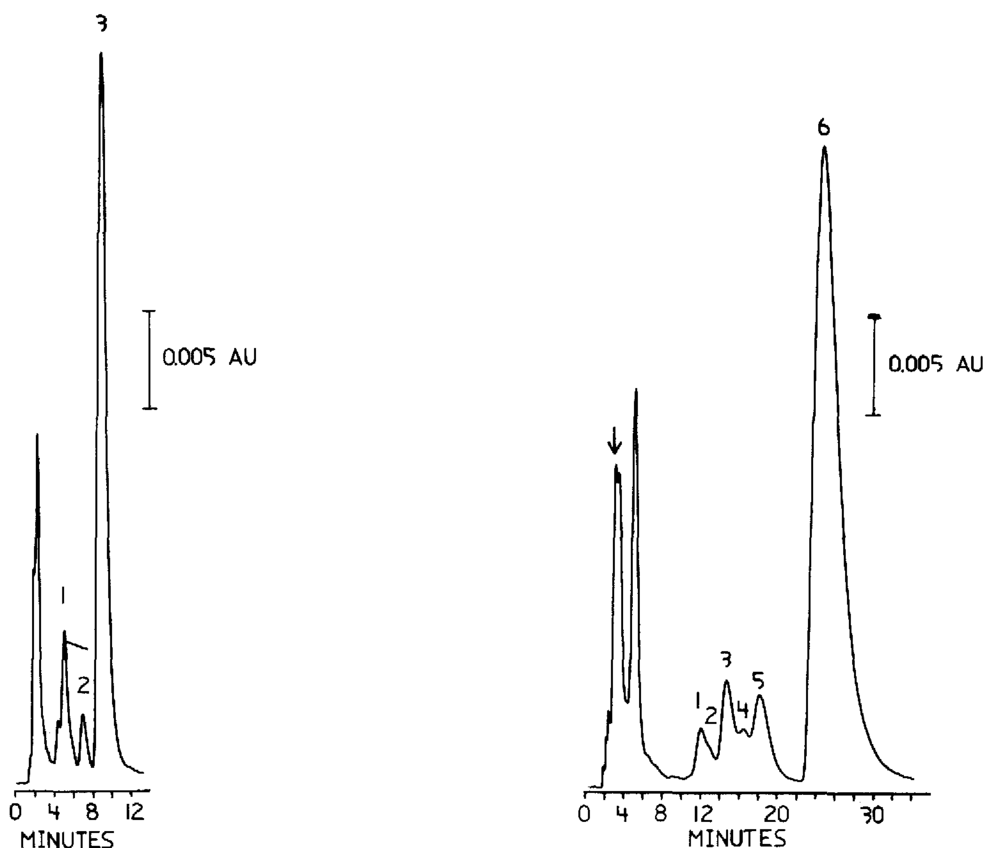

Fig. 5. Chromatogram of urine extract from a human volunteer 3 h after oral administration of a 300-mg cimetidine tablet. Peaks: 1 = possible cimetidine metabolites, including cimetidine sulfoxide; 2 = metiamide, 100 μg/ml; 3 = cimetidine.

Fig. 6. Chromatogram of urine extract shown in Fig. 5, but re-run in mobile phase containing 1/15 *M* KH_2PO_4–1/15 *M* Na_2HPO_4–methanol (715:100:83) to separate possible metabolite peaks. Peaks: 1, 2 and 4 are unidentified, but considered to be possible cimetidine metabolites; 3 = cimetidine sulfoxide; 5 = metiamide; 6 = cimetidine. Creatinine was eluted under the peak identified by the arrow.

Urine assay

The relationship between the peak area ratios and the concentration of cimetidine in urine was linear within the range 10 to 200 μg/ml ($y = 1.00x + 0.001$; $r = 0.999$). Table II illustrates the accuracy and precision of the method. Figs. 3 and 4 show typical chromatograms of a control urine extract and an extract from a urine sample containing 100 μg/ml each of cimetidine and methiamide. The limit of detection (see ref. 11) was 2.0 μg/ml. A single urine sample can be analyzed for cimetidine in about 20 min.

Fig. 5 shows the chromatogram of a urine extract from a human volunteer 3 h after oral administration of a single 300-mg cimetidine tablet. However, the mobile phase used, 1/15 *M* monobasic potassium phosphate–1/15 *M* dibasic sodium phosphate–methanol (815:100:815), was unable to resolve the mixture of cimetidine metabolites under peak 1. Fig. 6 shows the chromatogram of the same extract analyzed in Fig. 5 but re-run in a modified mobile phase containing 1/15 *M* monobasic potassium phosphate–1/15 *M* dibasic sodium phosphate–methanol (715:100:83). In this instance, the cimetidine sulfoxide (peak 3), metiamide (peak 5) and cimetidine (peak 6) were sufficiently resolved to permit accurate determinations of these substances by using appropriate calibration curves.

The assay presented here provides a rapid, sensitive, accurate, precise and inexpensive method for the determination of cimetidine in plasma and urine and of cimetidine sulfoxide in urine. It should prove to be useful for studies that require monitoring of serum and urine levels of cimetidine and its major metabolite cimetidine sulfoxide.

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