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Note

Convenient and sensitive high-performance liquid chromatography assay for cimetidine in plasma or urine

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Cimetidine (Fig. 1) is a competitive histamine H_2-receptor antagonist which inhibits gastric acid secretion and is used for the treatment of peptic ulcer. For a review of the chemistry and pharmacology of cimetidine see Brogden et al. [1]. Previously reported high-performance liquid chromatographic (HPLC) methods of measuring cimetidine and its major metabolite cimetidine sulfoxide in blood and plasma require a triple extraction sample preparation utilizing either normal-phase or reversed-phase chromatography [2, 3]. This paper reports an HPLC cimetidine assay for plasma and urine that benefits from a simplified sample work-up and improved reversed-phase chromatography resulting in high accuracy, reproducibility and sensitivity.

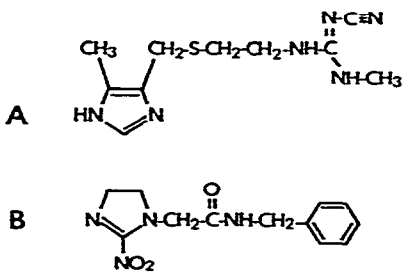

Fig. 1. Structures of (A) cimetidine and (B) internal standard R07-1051.

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MATERIALS AND METHODS

Reagents

Acetonitrile, methanol and ethyl acetate were HPLC grade from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Water was deionized and glass-distilled and reagents were of analytical grade. Cimetidine and cimetidine sulfoxide were obtained from Smith, Kline and French (Philadelphia, PA, U.S.A.) and the internal standard, R07-1051 (Fig. 1), from Hoffmann-La Roche (Nutley, NJ, U.S.A.). Stock solutions of cimetidine (2.5, 25, 250 μg/ml) used to construct standard curves were made with 1 m*M* hydrochloric acid and the internal standard stock solution (25 μg/ml) was made with water; both were stored at 4°C. The HPLC eluent was filtered through a 0.45-μm membrane filter (Type HA, Millipore, Bedford, MA, U.S.A.) immediately prior to use.

Chromatography

A dry-packed 150 mm × 4.6 mm pre-pre-column (Perisorb RP-2, 30—40 μm, Merck, Darmstadt, G.F.R.) was placed between the pump and injector. A slurry-packed 40 mm × 2.1 mm pre-column (LiChrosorb RP-2, 10 μm, Merck) was used to protect the analytical column (Ultrasphere ODS, 150 mm × 4.6 mm, 5 μm, Altex, Berkeley, CA, U.S.A.) from irreversible sample absorption. The HPLC eluent was composed of 10 m*M* phosphate buffer, pH 8.0 with 20% methanol. An Altex 100A pump was used to deliver eluent at 1.7 ml/min at a pressure of about 3500 p.s.i. An Altex-Hitachi 155-30 Vis—UV detector monitored the column eluent at 220 nm. A WISP 710A auto sampler (Waters Assoc.) initiated a Spectra Physics 4100 integrator which was used for data reduction. The integrator was operated in the peak height mode and was supplemented with customized multilevel calibration programs.

Plasma sample preparation

To a culture tube were added 250 μl of the plasma sample, 75 μl water, 75 μl of internal standard stock solution and 2.0 ml of acetonitrile. The mixture was vortexed for 30 sec and centrifuged for 5 min at 2000 *g* to precipitate protein. The liquid was decanted and evaporated to dryness in a vortex evaporator (Buchler, Fort Lee, NJ, U.S.A.) at 45°C with aspirator vacuum. Alternatively, a stream of nitrogen may be used for evaporation at 40°C. The dried samples were reconstituted in 275 μl of HPLC eluent, vortexed for 20 sec and transferred to polyethylene micro centrifuge tubes (250 μl, Brinkmann, Westbury, NY, U.S.A.). The samples were centrifuged in a Brinkmann centrifuge at 12,000 *g* for 2 min to pellet undissolved debris. The centrifuge tube caps were removed and the tubes used directly as disposable limited-volume sample holders in an automated WISP HPLC injector, facilitating injection of a large proportion of the sample. The injection volume is 150 μl. For calibration standards part of the 75 μl of water in samples was replaced by the appropriate volume of cimetidine stock solution and added to drug-free plasma.

Urine sample preparation

To 100 μl of urine sample in a screw-capped culture tube were added 250 μl

of internal standard stock solution, 300 μl water, 50 μl of freshly prepared 1 *M* sodium hydroxide solution, and 10.5 ml ethyl acetate. The extraction mixture was vortexed rapidly in a Buchler vortex evaporator for 5 min at room temperature and centrifuged at 2000 *g* for 3 min. The organic layer was transferred to a fresh culture tube, evaporated at 40°C with a stream of nitrogen and reconstituted with 275 μl of HPLC eluent. The injection volume was 50 μl. For calibration standards part of the 300 μl water in samples was replaced with the appropriate volume of cimetidine stock solution and added to drug-free urine.

RESULTS

Chromatography

An eluent pH of 8.0 yielded excellent peak shape of both cimetidine and internal standard (Fig. 2) by reducing peak tailing of these basic compounds.

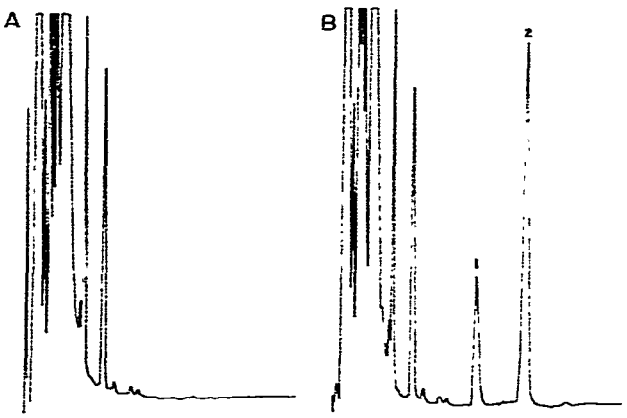

Fig. 2. Chromatograms of extracts of human plasma (A) before and (B) after spiking with 0.5 mg/ml cimetidine (1) and internal standard (2).

Column life was extended by the use of a pre-pre-column connected between the pump and injector. The purpose of such a column was to increase the concentration of dissolved silica in the eluent, thus reducing hydrolysis of silica on the analytical column [4]. Over 600 plasma and urine samples were analyzed on one Ultrasphere ODS column, indicating good stability under conditions of high pH and high pressure. Cimetidine and internal standard peaks fell in a clear area of the chromatogram (Fig. 2) with a few samples containing very small peaks occurring to either side of cimetidine. There was no interference from cimetidine sulfoxide, a primary metabolite [5] and several drugs tested: caffeine, theophylline, naproxen, ketoprofen and acebutolol.

Plasma

Extraction of cimetidine from plasma was found to suffer from poor reproducibility at low concentrations. In contrast, the procedure described (protein precipitation and direct injection of the reconstituted sample), gave

TABLE I

SPECIFICATIONS OF CIMETIDINE ASSAY IN PLASMA

	Concn. of cimetidine (μg/ml)				
	0.1	0.5	2.5	5.0	10
Precision					
C.V.%					
Intraday (6 samples)	3.7	1.2	1.7		2.2
Interday (16 days)		5.9		2.6	
Bias					
Deviation of mean from amount spiked (%)					
Intraday (6 samples)	−2.6	−1.8	−3.4		+3.4
Interday (16 days)		+3.2		−2.0	

Linearity	at 0.1—1.0	1.0—25 μg/ml
Average C.V. of concentration-normalized peak height ratios (%)		
1 per day, 25 days; 5 points per range	4.3 ± 1.5	3.3 ± 1.7

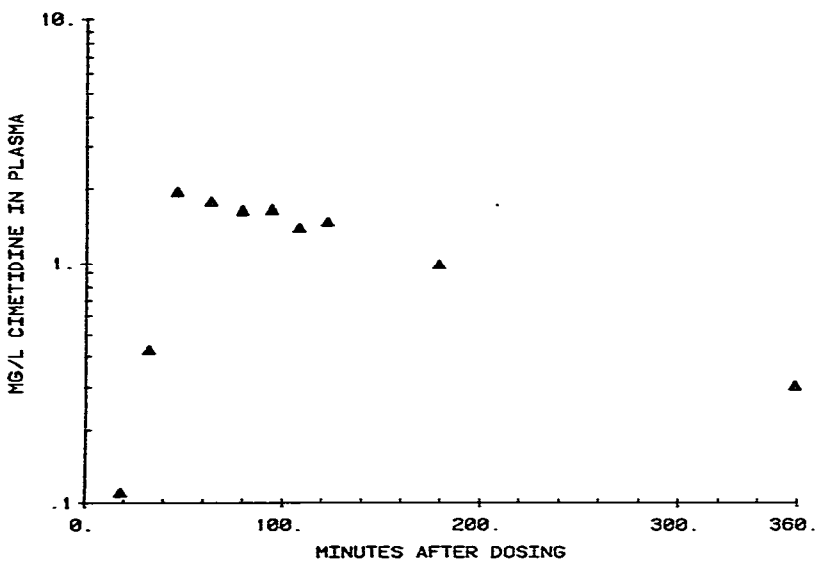

Fig. 3. Plasma concentration—time profile after a 500-mg dose of cimetidine.

excellent intra- and interday reproducibility, even at the limit of assay sensitivity (0.1 μg/ml, Table I). In order to improve reproducibility and accuracy the disposable pipet tips (Finntip, Finnpipette) were rinsed several times prior to use with the cimetidine stock solutions to be pipetted, as random absorption of cimetidine was found to be significant. Perhaps for the same reason a slightly convex standard curve of cimetidine peak height ratios was observed. The following calibrators were processed, covering a 250-fold range of concentrations: 0, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0, 7.5, 10, 25 μg/ml. To avoid excessive weight being given to the more concentrated calibrators, the standard curve was

divided into two ranges (0—1.0 μg/ml and 1.0—25 μg/ml). An unweighted least squares regression was independently fitted to each range with excellent linearity (Table I). A drug study in which eight individuals took 500 mg of cimetidine twice a day resulted in peak concentrations as high as 12.9 μg/ml falling to 0.25 μg/ml in 7 h. A typical plasma concentration—time profile obtained with this assay is shown in Fig. 3.

Urine

Because of interfering peaks in the chromatogram an extraction of cimetidine and internal standard was necessary for urine. A single basic extraction with ethyl acetate was effective in reducing interfering peaks to an insignificant level. Extraction efficiency was 65% for cimetidine and 97% for internal standard. Reproducibility above 100 μg/ml was excellent (Table II) but

TABLE II

SPECIFICATIONS OF CIMETIDINE ASSAY IN URINE

	Concn. of cimetidine (μg/ml)		
	150	300	500
Precision			
C.V.%			
Intraday (6 samples)	3.5	2.7	4.0
Interday (2 days)	8.4	2.3	4.9
Bias			
Deviation of mean from amount spiked (%)			
Intraday (6 samples)	+8.7	+1.9	—0.6
Interday (2 days)	+2.8	+3.5	—0.2
Linearity		at	100—750 μg/ml
Average C.V. of concentration-normalized peak height ratios (%)			
1 per day, 3 days; 11 points per range			7.9 ± 1.9

deteriorated below this concentration. In the drug study previously mentioned, no urine concentrations below this assay sensitivity were encountered. Samples having cimetidine concentrations greater than 750 μg/ml were diluted with drug-free urine prior to extraction. Standard curves calculated with an unweighted least squares regression yielded the linearity figures in Table II.

DISCUSSION

The cimetidine HPLC plasma assay described is a significant improvement over those previously reported [2, 3] in terms of range, sensitivity and ease of sample preparation. Larsen et al. [3] reported an assay range of 0.5—4.0 μg/ml for 750 μl plasma with a potential sensitivity limit of 0.1 μg/ml with 10% reproducibility. The range of the assay described in this paper is 0.1—25 μg/ml

for 250 μl plasma with a sensitivity of 0.1 μg/ml with 4% reproducibility. The requirement of only 250 μl of plasma may be clinically advantageous as little more than a pin prick may be used for cimetidine quantitation in the therapeutic range. Alternatively, the use of 1.0 ml of plasma will increase assay sensitivity to 25 ng/ml with no loss in reproducibility or accuracy. Previous cimetidine assays require triple extractions as the sample purification procedure prior to chromatography. In contrast the assay described herein involves a single protein precipitation step thus greatly reducing sample preparation time and manipulative errors. With the aid of the automated sample injector as many as 70 plasma samples a day could easily be processed and chromatographed by a single technician. The high degree of chromatographic resolution of cimetidine from endogenous peaks is responsible for the abbreviated sample work-up as prechromatographic extractions of plasma are not necessary. In addition, the excellent peak resolution aids in identification and quantification of low concentrations. The urine assay also benefited from the good chromatographic resolution but required a single alkaline extraction to remove acidic urine compounds. The excellent reproducibility of the urine extractions provided good assay accuracy despite a relatively low extraction efficiency of cimetidine.

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