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Short communication

Validation of a simplified method for determination of cimetidine in human plasma and urine by liquid chromatography with ultraviolet detection

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Abstract

A HPLC method was developed for determination of cimetidine in human plasma and urine. Plasma samples were alkalinized followed by liquid extraction with water-saturated ethyl acetate then evaporated under nitrogen. The extracts were reconstituted in mobile phase and injected onto a C_{18} reversed-phase column; UV detection was set at 228 nm. Urine samples were diluted with an internal standard/mobile phase mixture (1:9) prior to injection. The lower limit of quantification in plasma and urine were 100 ng/ml and 10 μg/ml, respectively; intra- and inter-day coefficients of variation were ≤4.2%. Advantages of this validated assay include a readily available internal standard, simplified plasma extraction and urine dilution methods, and applicability to clinical studies investigating the renal handling of cimetidine.

Keyword: Cimetidine

1. Introduction

Cimetidine (*N*″-cyano-*N*-methyl-*N*′-[2-[[(5-methyl-1*H*-imidazol-4-yl) methyl]thio]ethyl]-guanidine) is a histamine H(2)-receptor antagonists that is used widely to treat gastric and duodenal ulcers [1,2]. Cimetidine is excreted predominantly unchanged by the kidneys (approximately 70%) and undergoes extensive tubular secretion with renal clearance values approximately four-fold greater than creatinine clearance [3,4]. Cimetidine has also been identified as a substrate for P-glycoprotein (P-GP), an MDR-encoded membrane transporter that is expressed in normal tissues including kidney proximal tubules [5–7]. Thus, evaluation of renal P-GP probe compounds, such as cimetidine, is critical in order to identify potential renal drug interactions, prevent drug toxicity, and optimize drug therapy in patients.

Several HPLC methods for the determination of cimetidine in human plasma and urine have been reported. Most methods utilized either solid-phase extraction or liquid-phase extraction techniques. Limitations of these methods include the requirement to extract large volumes of plasma (0.5–1.0 ml) [8–10], low or inconsistent recovery in plasma [11,12], use of an internal standard that is either not commercially available [10,13] requires an additional protonation step using hydrochloric acid [14], lack of urine analysis capabilities [12,14,15] or requirement of solid-phase or liquid-liquid extraction methods for urine analysis [1,8,16,17]. This report describes a validated HPLC method for determining cimetidine concentrations in plasma that incorporates a simplified procedure for urine sample analysis.

2. Experimental methods

2.1. Reagents and chemicals

Cimetidine, famotidine, heptanesulfonic acid, sodium acetate and sodium carbonate were purchased from Sigma (St. Louis, MO, USA). The purity of cimetidine and famotidine standards was ≥99.0%. HPLC grade acetonitrile,

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ethyl acetate and sodium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA, USA). Deionized distilled water was obtained from a Picopure (Hydro-Picosystem Plus, Rockville, MD). Drug free human plasma was obtained from the University of Maryland Medical System Blood Bank. Drug free urine was obtained from laboratory personnel.

2.2. Equipment/instrumentation

The HPLC system consisted of a Waters 2690 separation module (Waters Millipore, Millford, MA), and a model 2487 dual wavelength absorbance detector set at 228 nm. The chromatographic data was collected and analyzed using Millenium Chromatography Manager (version 3.2). Separation was achieved at ambient temperature with a Phenomenex (Torrance, CA) C18 (4.6 mm × 250 mm), Prodigy 5 μm ODS (3), 100 A reversed-phase HPLC column preceded by a guard column (Alltech, Deerfield, IL) packed with C18 Bondapak/Corasil 37–50 μm. The mobile phase consisted of acetonitrile and heptanesulfonic acid (2.5 g/l) in an aqueous 20 mM sodium acetate buffer (23:77). The mobile phase was adjusted to pH 4.7 with 12 M HCl, degassed and passed through a 0.45 μm filter. The mobile phase was delivered at an isocratic rate of 1.0 ml/min with a pump pressure of approximately 1900 psi. Total run time for plasma and urine samples was 10 min.

2.3. Preparation of stock solutions and spiked standards

Stock solutions (in methanol) were prepared at 0.1, 1.0 and 10 mg/ml for cimetidine and 50 μg/ml and 1 mg/ml for famotidine (internal standard). Solutions were stored at 4 °C and were used to spike plasma and urine samples. Standards and quality control (QC) samples were made by addition of the determined quantity of stock solution to drug free plasma and urine stored at −20 °C in aliquots. Plasma QC sample concentrations were 300, 800 and 2100 ng/ml and urine QC sample concentrations were 20, 80 and 175 μg/ml.

2.4. Preparation of plasma samples

Plasma (250 μl) was combined with 30 μl of 2 M NaOH, 250 μl saturated sodium carbonate solution, and 30 μl of internal standard (famotidine, 50 μg/ml) in a 4.5 ml polypropylene tube. The sample was vortexed briefly, followed by addition of 3 ml water-saturated ethyl acetate. The tubes were capped and shaken at low speed (120 cycles/min) for 10 min and then centrifuged for 10 min at 2000 × *g*. The upper organic layer was transferred to a clean glass tube and evaporated to dryness at 37 °C under a steady stream of nitrogen. Following reconstitution of the residue in 100 μl of mobile phase, the mixture was transferred to a WISP microinsert and 50 μl was then injected onto the column.

2.5. Preparation of urine samples

In a 2.0 ml microcentrifuge tube, urine samples were prepared by adding 50 μl of urine (standard or QC), 50 μl of internal standard (50 μg/ml) and 400 μl of mobile phase. After capping, each tube was vortex-mixed briefly. An aliquot of 100 μl of each sample was transferred to a WISP microinsert and 50 μl was then injected onto the column.

2.6. Calibration and linearity

Assay performance was determined in accordance with the FDA Guidance for Bioanalytical Methods Validation for Human Studies [18]. Standard curves were constructed using six standard concentrations in plasma and urine

Table 1
Intra- and inter-day precision and accuracy for cimetidine in plasma

	Concentration (ng/ml)		CV (%)	Percentage of deviation (found vs. added)
	Added	Found (mean ± S.D.)		
Intra-assay reproducibility[a]				
Quality controls	300	306.6 ± 8.0	2.6	2.2
	800	796.8 ± 13.7	1.7	−0.4
	2100	2073.0 ± 55.1	2.7	−1.3
Inter-assay reproducibility[b]				
Quality controls	300	308.1 ± 3.0	1.0	2.7
	800	807.0 ± 6.6	0.8	0.9
	2100	2147.6 ± 9.9	0.5	2.3
Standards	100	98.9 ± 2.1	2.2	−1.2
	250	262.5 ± 9.3	3.6	5.0
	500	489.9 ± 20.4	4.2	−2.0
	1000	995.3 ± 28.0	2.8	−0.5
	2000	2002.0 ± 35.3	1.8	0.1
	4000	3991.3 ± 87.6	2.2	−0.2

[a] Six quality control samples per concentration.
[b] Eighteen quality control samples or two standards per day per concentration for 3 days.

Table 2
Intra- and inter-day precision and accuracy for cimetidine in urine

	Concentration (μg/ml)		CV (%)	Percentage of deviation (found vs. added)
	Added	Found (mean ± S.D.)		
Intra-assay reproducibility[a]				
Quality controls	20	20.8 ± 0.22	1.1	4.1
	80	82.6 ± 0.49	0.6	3.3
	175	175.8 ± 1.59	0.9	0.4
Inter-assay reproducibility[b]				
Quality controls	20	20.9 ± 0.06	0.3	4.7
	80	83.5 ± 0.25	0.3	4.4
	175	177.0 ± 0.37	0.2	1.2
Standards	10	10.1 ± 0.90	0.9	1.0
	25	24.7 ± 0.29	1.2	−1.2
	50	48.1 ± 0.42	0.9	−3.9
	100	100.6 ± 0.83	0.8	0.6
	150	153.3 ± 1.57	1.0	2.2
	250	254.6 ± 1.99	0.8	1.6

[a] Seven quality control samples per concentration.
[b] Twenty-one quality control samples or two standards per day per concentration for 3 days.

that were processed separately and run in duplicate daily for three consecutive days. Drug concentrations were reported as the ratios of peak height for cimetidine to the internal standard (famotidine). In plasma and urine, the standard concentrations ranged from 100 to 4000 ng/ml and 10 to 250 μg/ml, respectively. Calibration curves were generated using weighted least squares regression analysis and obtained over the respective standard concentration range. Individual standard concentrations in plasma and urine are shown in Tables 1 and 2, respectively. All standards and QC samples were stored at −20 °C until analysis.

2.7. Precision and accuracy

Standards and replicate QC samples of plasma and urine at each concentration were analyzed on three consecutive days, after which inter- and intra-day means, standard deviations (S.D.), and coefficients of variation (CV%) were calculated by standard methods [18].

2.8. Stability and recovery

Low and high QC for both plasma and urine were tested in duplicate for stability over three freeze/thaw cycles and

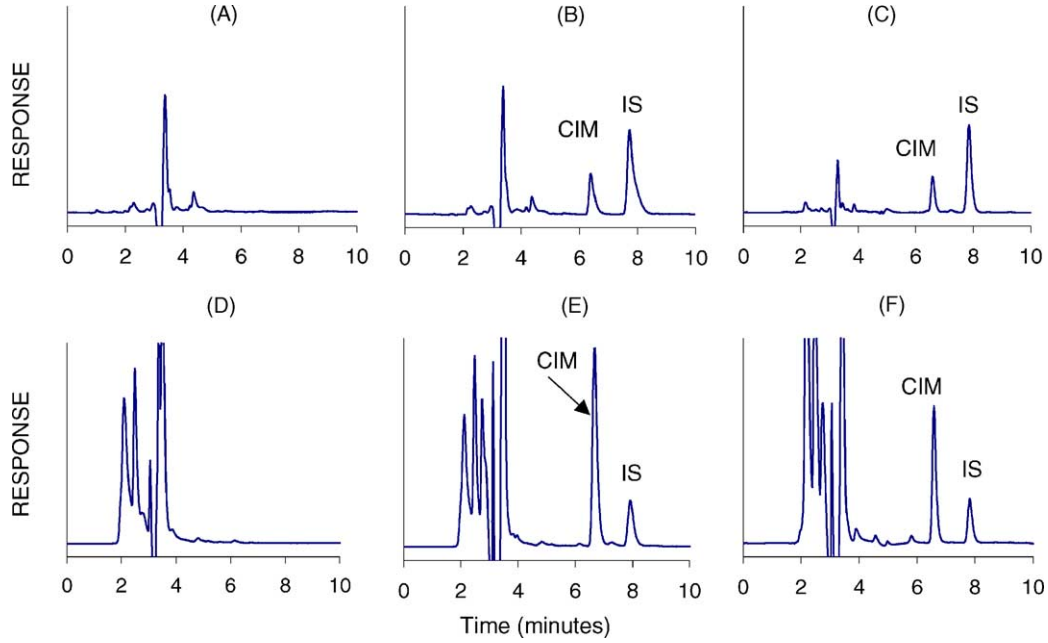

Fig. 1. Representative chromatograms of (A) blank plasma; (B) plasma standard (1000 ng/ml); (C) human volunteer plasma (844.2 ng/ml); (D) blank urine; (E) urine standard (100 μg/ml); and (F) human volunteer urine (72.9 μg/ml). CIM, cimetidine; IS, internal standard.

at room temperature for 24 and 48 h (processed and unprocessed). Recovery was determined for cimetidine and famotidine from plasma low QC (300 ng/ml) samples.

3. Results

Representative chromatograms of plasma and urine samples are shown in Fig. 1. Retention times for cimetidine and famotidine were approximately 6.8 and 7.9 min, respectively. The plasma standard curve for cimetidine was found to be linear in the range of 100–4000 ng/ml with a slope (reported as mean ± S.D.) of $4.9 \times 10^{-4} \pm 1.9 \times 10^{-5}$, a correlation coefficient of 0.998 ± 0.001, and *y*-intercept of $-0.002 \pm 6.5 \times 10^{-4}$. The urine standard curve for cimetidine was found to be linear in the range of 10–250 μg/ml with a slope of 0.042 ± 0.005, a correlation coefficient of $0.999 \pm 1.15 \times 10^{-4}$ and *y*-intercept of -0.024 ± 0.002. The intra- and inter-day precision and CV for cimetidine in plasma (Table 1) and urine (Table 2) were ≤4.2%. At the LOQ in plasma and urine, the signal to noise ratio was greater than 5:1, and inter- and intra-day CV was less than 2.2%. Low and high QC sample (plasma and urine) values were unchanged (average difference <10%) during freeze/thaw and room temperature stability tests. The variability (CV%) between samples during the three freeze/thaw cycles for plasma and urine ranged from 2.6 to 6.3%. The variability between samples during the stability testing for processed and unprocessed plasma and urine samples at 48 h (room temperature) ranged from 3.8 to 6.7%. The recovery of cimetidine and famotidine in plasma was 94.0 ± 2.2 and 62.0 ± 3.4%, respectively. Blank plasma and urine samples obtained from 14 patients with congestive heart failure taking medicines shown in Table 3 were analyzed with no interferences observed at retention times for cimetidine or famotidine.

Table 3
List of medications demonstrating no interference with cimetidine or famotidine in plasma and urine samples

Albuterol	Enoxaparin	Metformin
Allopurinol	Ferrous sulfate	Methadone
Amiodarone	Fluticasone	Metolazone
Aspirin	Furosemide	Metoprolol
Atorvastatin	Glimepiride	Nadolol
Bumetanide	Glyburide	Nitroglycerin
Buspirone	Heparin	Nitroprusside
Celecoxib	Insulin	Pantoprazole
Clonidine	Ipratropium	Potassium chloride
Clopidogrel	Irbesartan	Prednisone
Colchicine	Isosorbide	Salmeterol
Dalteparin	Levothyroxine	Simvastatin
Digoxin	Lisinopril	Spironolactone
Diphenhydramine	Losartan	Vitamin B complex
Docusate	Megestrol	Warfarin
Enalapril	Metaxolone	

4. Discussion

The assay described herein is a modification of a previous assay we developed for famotidine with a lower detection wavelength (228 nm) and a single-step extraction [19]. Additional advantages of this method include small sample volume, excellent extraction recovery from plasma, simplified urine sample processing, and a readily available internal standard. A mobile phase with pH 4.7 was chosen based on previous success and to minimize column degredation,

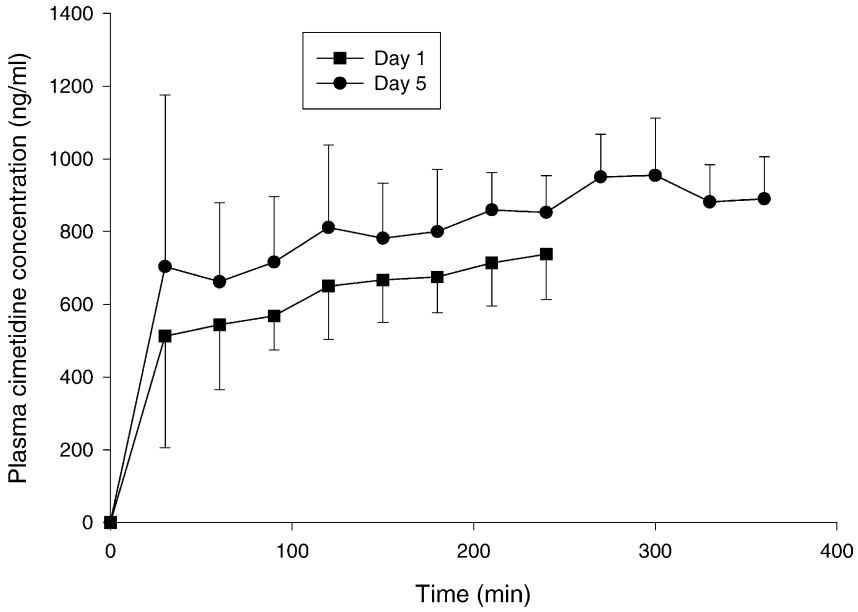

Fig. 2. Plasma cimetidine concentrations (mean ± S.D.) obtained following administration of intravenous bolus (0.2 mg/kg) and 0.6 mg/min continuous infusion in healthy volunteers ($n = 8$) on day 1 (baseline) and day 5 (post-itraconazole dosing).

although a lower pH (4.2) may also be utilized without analytical interferences. Use of heptanesulfonic acid as the ion-pairing agent improved the peak shape and height for both famotidine and cimetidine compared to standard aqueous:organic mobile phases. We chose ethyl acetate as the extraction solvent based on previously observed excellent recovery for both famotidine and cimetidine, with minimal extraction of endogenous compounds. Alkalinizing agents (NaOH and Na_2CO_3) were used for extraction based on previous studies showing that this markedly improves the extraction of cimetidine and minimizes extraction and interference from endogenous compounds [17].

It is known that the two primary metabolites of cimetidine are cimetidine sulfoxide (CSO) and hydroxymethyl cimetidine (COH). Following oral or IV administration of cimetidine in humans, approximately 65% of the dose is excreted unchanged, 8% is excreted as CSO, and 4% is excreted as COH. Previous investigators, using similar HPLC conditions and column as ours, reported that the short retention times for CSO and COH resulted in elution of these compounds earlier than cimetidine or in the solvent front (<3 min) [16,17]. Since these metabolites have higher polarity than the parent compound, interference between CSO or COH with cimetidine is unlikely.

Although lower LOQ have been reported using methods such as liquid chromatography coupled with atmospheric pressure chemical ionization (APCI) and selected reaction monitoring (SRM) mass spectrometry (MS) [13] or ion-pair solid phase extraction [20], these methods are limited by expense and often require technical expertise (i.e., APCI–MS). The small plasma volume (0.25 ml) requirement allows for studies to be conducted in children and renal or hepatic failure patients where sample volume must be minimized. Furthermore, this assay complies with FDA guidelines for accuracy, precision and stability for standards and QC samples in both plasma and urine.

This method can be used to support a wide range of clinical trials where plasma concentrations of cimetidine are typically greater than 100 ng/ml. Steady-state plasma concentrations of ~900 ng/ml have been reported during continuous infusion dosing of 37.5 mg/h [21]. Calculation of renal clearance as part of clinical pharmacokinetics studies requires accurate quantitation of cimetidine concentrations in urine. The method described above was recently used to evaluate the renal handling of cimetidine in healthy volunteers. A representative plasma concentration versus time profile obtained following administration of cimetidine given as an intravenous bolus (0.2 mg/kg) and continuous infusion of 0.6 mg/min is shown in Fig. 2. In this study evaluating a drug interaction with itraconazole (200 mg daily), the renal secretory clearance values ranged from 300 to 568 ml/min, which is consistent with previous pharmacokinetic evaluations [4,17].

In summary, the rapid and reproducible analytical method reported here can be utilized to study the pharmacokinetics of cimetidine, renal drug handling mechanisms and drug interactions in phase I/II clinical pharmacology trials.

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