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Short communication

Determination of famotidine in human plasma and urine by high-performance liquid chromatography

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Abstract

An improved, rapid and specific high-performance liquid chromatographic assay was developed for the determination of famotidine in human plasma and urine. Plasma samples were alkalinized and the analyte and internal standard (cimetidine) extracted with water-saturated ethyl acetate. The extracts were reconstituted in mobile phase, and injected onto a C_{18} reversed-phase column; UV detection was set at 267 nm. Urine samples were diluted with nine volumes of a mobile phase-internal standard mixture prior to injection. The lower limits of quantification in plasma and urine were 75 ng/ml and 1.0 μg/ml, respectively; intra- and inter-day coefficients of variation were ≤10.5%. This method is currently being used to support renal function studies assessing the use of intravenously administered famotidine to characterize cationic tubular secretion in man.

Keywords: Famotidine

1. Introduction

Famotidine [3-(((2-((aminoiminomethyl)amino)-4-thiazolyl) - methyl)thio) - *N′* - (aminosulfonyl)propanimidamide] (Fig. 1) is a histamine H_2-receptor antagonist that potently inhibits gastric acid secretion and is commonly used in the treatment and prevention of gastric and duodenal ulcers [1]. The major route of elimination of famotidine occurs by renal mechanisms with 70% of a dose being excreted unchanged in urine. Famotidine is filtered at the glomerulus and also extensively secreted within the proximal tubule by the organic cationic secretory pathway resulting in renal clearance values approximately four-fold greater than GFR [2–4]. Famotidine has been used as a probe of cationic transport in in vitro models to elucidate the transport mechanisms of other cationic substrates [5,6] and in animals [7] to study cationic tubular secretion. Capacity-limited tubular secretion of famotidine has been demonstrated in rats and dogs at high plasma concentrations (>10 mg/ml) [8,9]. These data suggest that famotidine may be useful as an in vivo probe of the cationic secretory pathway in humans.

Several HPLC methods for the determination of famotidine in human plasma and urine have been reported. Most methods utilized either solid-phase extraction [10,11], or liquid-phase extraction techniques [12,13]. Limitations of these methods include the requirement to extract large volumes of plasma

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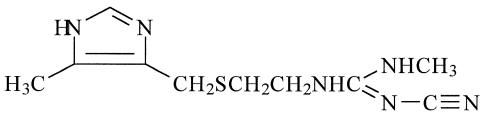
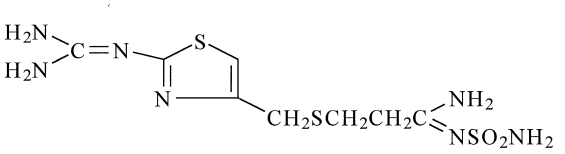


Cimetidine



Famotidine

Fig. 1. Structures of cimetidine and famotidine.

(1–1.5 ml) or urine (1 ml), lack of an internal standard [10], use of an internal standard that is not commercially available [11,13] or lack of data concerning the analysis of famotidine in urine [12]. All previously published methods have used solid-phase or liquid–liquid extraction methods for detection of famotidine in urine [10–13].

This report describes a HPLC method for determining famotidine concentrations in plasma, as well as, a simplified procedure for urine sample analysis that does not require extraction. This method utilizes 250 μl of plasma with liquid-phase extraction using cimetidine as the internal standard. The simplified urine sample preparation requires only 50 μl of urine, which is diluted with mobile phase prior to analysis.

2. Experimental methods

2.1. Reagents and chemicals

Famotidine and the internal standard cimetidine (Fig. 1) were purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile, heptanesulfonic acid, ethyl acetate, sodium hydroxide, sodium carbonate, and sodium acetate were purchased from Fisher Scientific (Pittsburgh, PA, USA). Deionized, distilled water was obtained from a Barnstead Nanopure purification system (Barnstead, Boston, MA, USA). Drug free human plasma was obtained from the Blood Bank. Drug free human urine was obtained from laboratory personnel.

2.2. Equipment/instrumentation

The HPLC system consisted of a Waters model 501 HPLC pump, a model 712 WISP autoinjector and a model 481 tunable absorbance detector set at 267 nm (Waters Assoc., Milford, MA, USA). The chromatographic data was collected and analyzed using Millenium Chromatography Manager (Waters, version 2.15). Separation was achieved at ambient temperature with a Phenomenex (Torrance, CA, USA) Prodigy ODS (3) (5 μm particle size, 25 cm×3.9 mm I.D.) reversed-phase HPLC column preceded by an Alltech guard column packed with C_{18} Bondapak/Corasil 37–50 μm column packing. The mobile phase used for analysis consisted of acetonitrile and heptanesulfonic acid (2.5 g/l) in 20 m*M* sodium acetate buffer (23:77). The mobile phase was adjusted to pH 4.7 with 12 *M* HCl followed by filtration through a 0.45 μm filter. The mobile phase was delivered at a rate of 1.0 ml/min with a pump pressure of approximately 100 bar. The total analysis time for plasma and urine samples was 10 min. However, this time must be extended to 16 min in subjects simultaneously receiving iothalamate to avoid chromatographic interference. Iothalamate is a radiocontrast agent commonly used to estimate glomerular filtration rate [14,15].

2.3. Preparation of stock solutions and spiked standards

Stock solutions of 0.1 and 1 mg/ml for famotidine and 1.0 mg/ml for cimetidine were made in methanol and stored at 4°C. These solutions were stable for at least 8 months when stored at 4°C and were used to spike plasma and urine samples in the preparation of standards. Standards and quality control samples were made by addition of the determined quantity of stock solution to drug free plasma and urine and stored at −20°C in aliquots.

2.4. Preparation of plasma samples

Plasma (250 μl) was combined with 30 μl of 2 *M* NaOH, 250 μl of saturated sodium carbonate solution, and 30 μl of internal standard stock solution (cimetidine 1 mg/ml) in an 8 ml round bottom 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 (85 cycles/min) for 10 min and then centrifuged for 10 min at 2000 *g*. The upper organic layer was transferred to a clean 4.5 ml conical polypropylene tube and the mixture was again extracted with 3 ml ethyl acetate followed by vortex mixing and centrifugation as described above. The upper organic layer was combined with that from the first extraction 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 injected into the HPLC system.

2.5. Preparation of urine samples

Urine samples were prepared by adding 400 μ1 of mobile phase, 50 μ1 of internal standard (1 mg/ml) and 50 μl of urine (standard, quality control or patient samples) to a clean 1.5 ml microcentrifuge tube. 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 injected into the HPLC system.

2.6. Calibration and linearity

Calibration curves were constructed using six standard concentrations in plasma or urine that were run in duplicate. Curves were obtained daily for three days by plotting the peak-height ratios of famotidine to the internal standard against the corresponding concentration of famotidine. Linear calibration curves were generated by weighted ($1/y^2$) linear regression analysis and obtained over the respective standard concentration range. The lower limit of quantitation (LOQ) for famotidine in each matrix was selected as the concentration at which the assay precision was within 20% and the signal to noise ratio exceeded 3:1. In plasma and urine, the standard concentrations ranged from 75.0 to 1500 ng/ml and 1 to 20 μg/ml, respectively. 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

The precision and accuracy of the assay was ascertained based on analysis of plasma and urine QC samples. Plasma QC sample concentrations for famotidine were 100, 750 and 1250 ng/ml and urine QC sample concentrations were 1.5, 7.5, 17.5 μg/ml. Six replicate QC samples at each concentration were analyzed on two consecutive days and twelve replicate samples were analyzed on a third day, after which inter- and intra-day means, standard deviations, and coefficients of variation (C.V.) were calculated by standard methods.

3. Results and discussion

Representative chromatograms of a blank sample, spiked standard, and patient sample for both plasma and urine are shown in Fig. 2. Retention times for cimetidine and famotidine were approximately 6.8 and 8.4 min, respectively. Calibration curves generated using weighted ($1/y^2$) least squares regression were linear over the concentration range in each matrix with correlation coefficients (r)>0.995. The intra- and inter-day precision and %C.V. for famotidine in plasma (Table 1) and urine (Table 2) were 10.5% or less. At the LOQ in plasma and urine, the signal to noise ratio was greater than 5:1 and inter- and intra-day C.V. was less than 6%.

Although the plasma LOQ (75 ng/ml) is higher than that previously reported (5–20 ng/ml) [10–13], it was our intention to use this method to analyze plasma and urine samples obtained during an intravenous infusion of famotidine (7.5 to 120 mg/h) where the plasma concentrations achieved are typically greater than 100 ng/ml. Enhanced sensitivity can be easily achieved by increasing the sample volume and amount injected on column. In addition to incorporating a readily available internal standard, the primary advantage of this assay over previously

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

	Concentration (ng/ml)		C.V. (%)	% Deviation (found vs. added)
	Added	Found (mean±SD)		
Intra-assay reproducibility[a]				
Quality controls	100	95.1±5.4	2.6	0.1
	750	823.3±40.0	4.9	9.8
	1250	1261.4±125.3	9.9	0.9
Inter-assay reproducibility[b]				
Quality controls	100	99.9±8.8	8.8	−0.1
	750	791.2±83.3	10.5	5.5
	1250	1272.2±113.9	9.0	1.8
Standards	75	75.0±3.8	5.1	0.0
	150	151.0±11.4	7.5	0.6
	300	302.3±14.5	4.8	0.8
	500	475.0±40.7	8.6	−5.0
	1000	991.3±55.7	5.6	−0.9
	1500	1552.5±63.2	4.1	3.5

[a] Six to twelve quality control samples per concentration.
[b] Six to twelve quality control samples or two standards per day per concentration for three days.

published assays is simplified urine sample processing; dilution of urine with a mobile phase–internal standard mixture is cheaper and more time efficient than the liquid or solid-phase extraction methods employed previously.

In summary, the rapid and simple method reported here has recently been utilized in clinical studies investigating the pharmacokinetics and renal handling of famotidine during a sequential intravenous infusion methodology. This approach is currently being used to characterize cationic tubular secretion in man and may be used to assess the effects of renal disease, drug interactions and nephrotoxins on the cationic secretory pathway.

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

	Concentration (μg/ml)		C.V. (%)	% Deviation (found vs. added)
	Added	Found (mean±SD)		
Intra-assay reproducibility[a]				
Quality controls	1.5	1.50±0.04	2.6	0.1
	7.5	7.72±0.19	2.4	2.9
	17.5	17.57±0.22	1.3	0.4
Inter-assay reproducibility[b]				
Quality controls	1.5	1.49±0.01	9.9	−0.7
	7.5	7.88±0.03	4.1	5.1
	17.5	17.42±0.06	3.4	−0.4
Standards	1.0	0.99±0.05	5.4	−0.9
	2.0	2.0±0.14	7.1	2.0
	5.0	5.0±0.08	1.6	−0.5
	10.0	9.9±0.22	2.2	−0.8
	15.0	15.4±0.30	1.9	2.7
	20.0	19.5±0.46	2.4	−2.6

[a] Six to twelve quality control samples per concentration.
[b] Six to twelve quality control samples or two standards per day per concentration for three days.

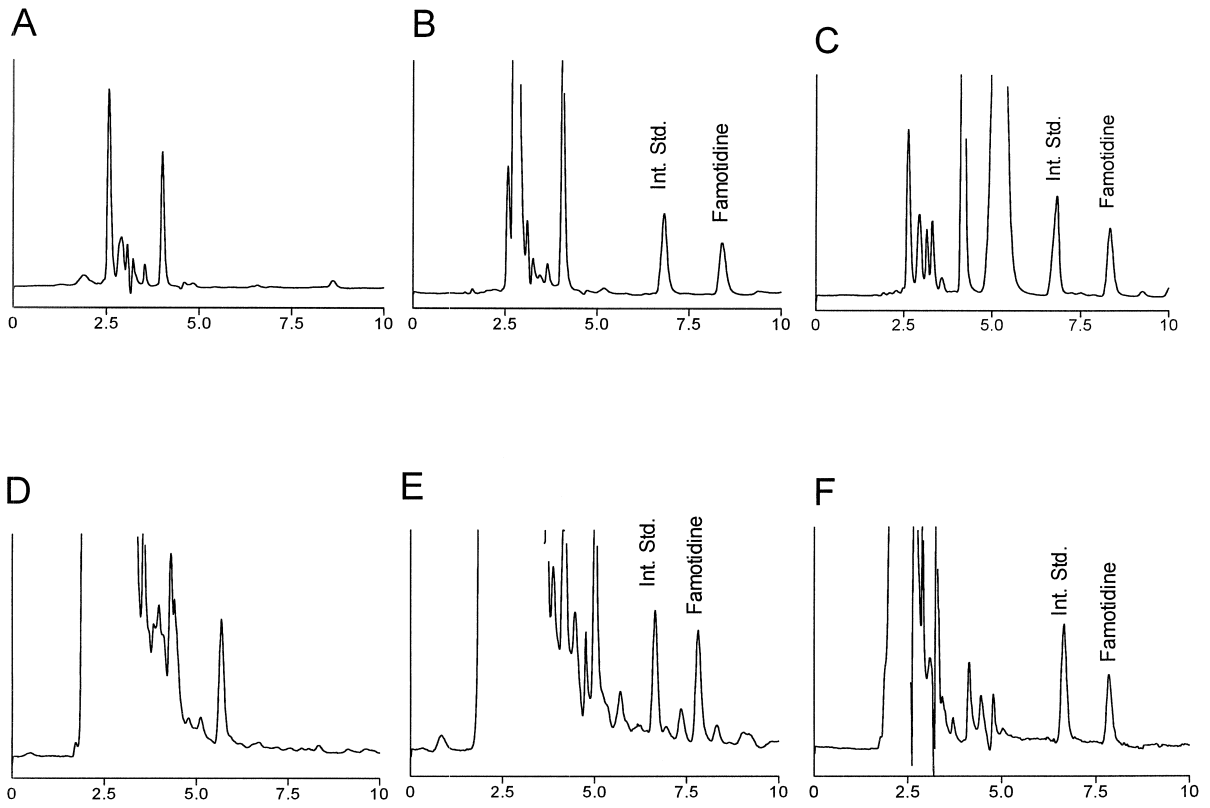


Fig. 2. Representative chromatograms of (A) blank plasma, (B) plasma standard (500 ng/ml), (C) patient sample (442 ng/ml), (D) blank urine, (E) urine standard (5.0 μg/ml), and (F) patient sample (3.2 μg/ml). Patient samples were obtained during continuous intravenous infusion (see text). The peak occurring at 5.0 min in the patient plasma sample is iothalamate eluting from the previous injection; increasing the run time to 16 min prevented any interference.

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