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Determination of famotidine in human plasma by high performance liquid chromatography with column switching[1]

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Abstract

A rapid, sensitive and robust reverse-phase high performance liquid chromatographic (HPLC) method with column switching and an internal standard for the quantitative determination of famotidine in human plasma is described. Famotidine and the internal standard were isolated from plasma samples by cation exchange solid phase extraction with SCX cartridges. The chromatographic separation was accomplished by an Inertsil C4 column with a mobile phase of acetonitrile/phosphate aqueous solution, connected by a switching valve to a BDS Hypersil C8 column with a mobile phase of acetonitrile/sodium dodecyl sulfate and phosphate aqueous solution. UV detection was set at 267 nm. The standard curve was linear in the concentration range of 1–100 ng ml^{-1}. The intraday coefficients of variation at all concentration levels were less than 10%. The interday consistency was assessed by running QC samples during each daily run. The limit of quantification for famotidine in human plasma was 1 ng ml^{-1}. The method has been utilized to support clinical pharmacokinetic studies in healthy volunteers who received famotidine 10 mg orally.

Keywords: Famotidine; HPLC; Internal standard; Column switching

1. Introduction

Famotidine, 3-(((2-((aminoiminomethyl)amino)-4-thiazolyl)methyl)thio)-*N′*-(aminosulfonyl) propanimidamide (compound I, Fig. 1), is a potent, competitive and reversible inhibitor of histamine action at the H_2 receptor. Famotidine has been approved in many countries as a prescription agent for the treatment of active duodenal ulcer and gastric ulcer, maintenance therapy of duodenal ulcer disease for up to one year, and the long-term management of Zollinger-Ellison syndrome. The low-dose famotidine OTC tablet is currently approved in the US for the nonprescription, short term, treatment of heartburn, acid indigestion and sour stomach. It is also indicated for the prevention of these symptoms brought on by consuming food and beverages which are known to cause these symptoms.

A number of assay methods for famotidine in biological fluids have been reported [1–8]. For the

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isolation of famotidine from human plasma, liquid-liquid extraction was one of the techniques used in the sample preparation [1,2]. However, the hydrophilic structure of famotidine leads to the low solubility of the compound in many organic solvents [3], therefore, this approach was not efficient. Solid phase extraction of famotidine from plasma on different cartridges, such as silica [3,6,7] and C18 [8], was also less than adequate, yielding either relatively low recovery or poor separation from the plasma endogenous interference. For the purpose of minimizing the variability caused by sample pretreatment, an internal standard was used in some of the reported methods [1,2,6]. In one report [5], an HPLC column switching technique was applied for on-line sample cleanup with an LOQ of 12 ng ml^{-1}. The limit of quantification was 5 ng ml^{-1} or higher in all of the reported cases [1,3–7].

The present paper describes a rapid and sensitive analytical method to determine famotidine concentrations in plasma. The method was based on HPLC separation with column switching. It was developed in order to support human pharmacokinetic studies following the administration of low doses of famotidine.

2. Experimental

2.1. Chemicals and reagents

Famotidine (Compound I) and the internal standard, (4-(((2-(5-amino-4H-1,2,4,6-thiatriazin-3-yl) ethyl) thio) methyl)-2-thiazolyl) guanidine *S,S*(thiatriazine)-dioxide (Compound II), shown in Fig. 1, were synthesized and purified at Merck and Co. Inc.

HPLC grade acetonitrile and methanol were supplied by EM Science (Gibbstown, NJ). Sodium phosphate monobasic, sodium hydroxide, ammonium hydroxide, and HPLC grade *o*-phosphoric acid 85%, were purchased from Fisher (Pittsburgh, PA). Sodium dodecyl sulfate was obtained from Sigma (St. Louis, MO), and citric acid monohydrate was obtained from J.T. Baker (Phillipsburg, NJ). Deionized water was HPLC filtered (>15 Mohm cm^{-1} resistivity) using Milli-Q ultra pure water system (Bedford, MA). All other reagents were of analytical grade. Control human plasma was purchased from Sera-Tech Biologicals (New Brunswick, NJ), and Biological Specialty Corporation (Lansdale, PA).

2.2. Instrumentation

The HPLC system (Fig. 2) consisted of a Varian 9010 solvent delivery system (Varian, Sugarland, TX), a Perkin-Elmer series 10 pump (Perkin-Elmer, Norwalk, CT), a Valco E60 6-port switching valve (VICI, Houston, TX), and a Perkin-Elmer ISS200 autosampler (Perkin-Elmer, Norwalk, CT) equipped with a 200 μl sample loop (Alltech, Deerfield, IL). An ABI 785A programmable absorbance detector (ABI/Perkin-Elmer, Norwalk, CT) was set at 267 nm and 0.005 AUFS. The chromatographic output was collected by an AccessChrom data acquisition system (PE Nelson, Cupertino, CA) via a PE-Nelson 941 analog-to-digital interface.

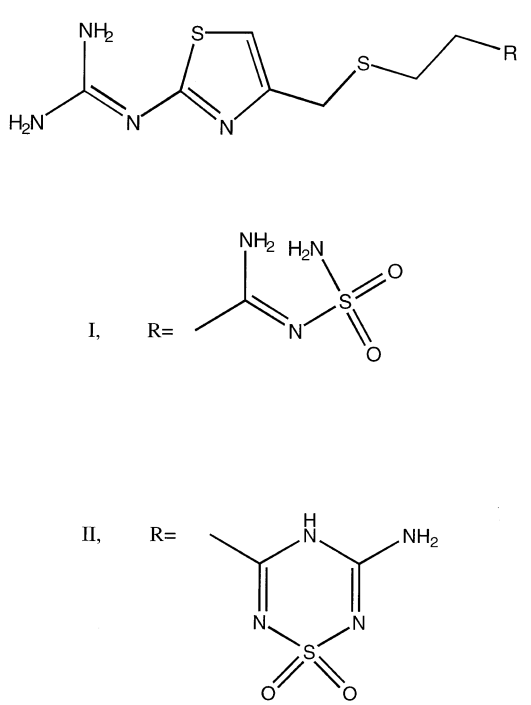

Fig. 1. Chemical structure of famotidine (I) and internal standard (II).

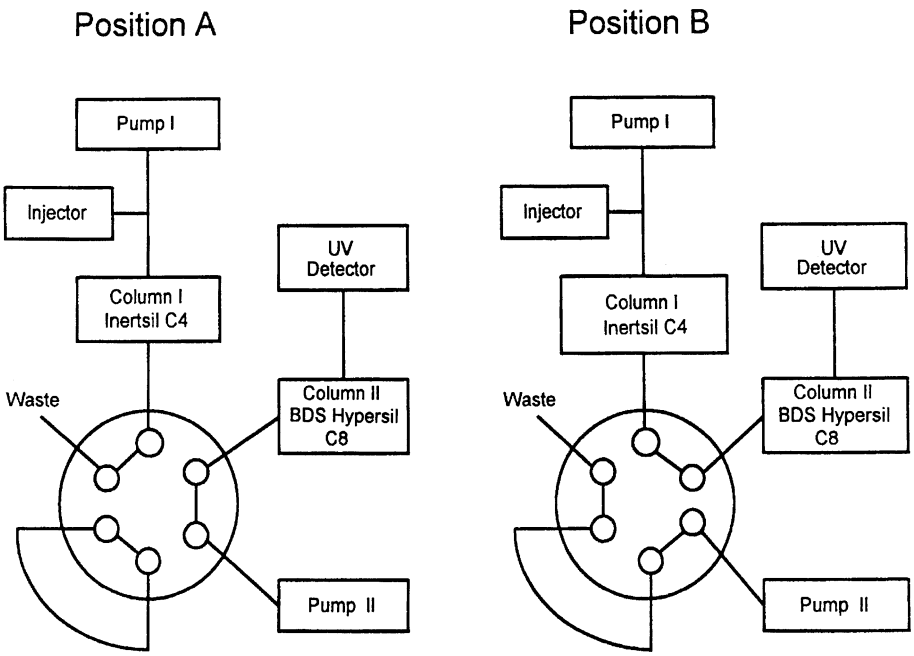

Fig. 2. Block diagram of HPLC system with column switching.

2.3. Chromatographic conditions

HPLC column I was an Inertsil C4 column (5 μm, 50 × 4.6 mm). Column II was a BDS Hypersil C8 column (5 μm, 150 × 4.6 mm). Both were obtained from Keystone Scientific (Bellefont, PA). For Column I, two mobile phase solvent systems were used: solvent A (5% acetonitrile in 15 mM phosphate buffer, pH 6.8) and solvent B (acetonitrile). The flow rate was set at 1 ml min^{-1}. For the first 8 min, the mobile phase consisted of 100% solvent A to assure the passage of famotidine and internal standard through the column. The pump was then programmed for gradient elution to 20% solvent A and 80% solvent B in a period of 8 min for the purpose of removing late-eluting interferences out of the column. The column was then reequilibrated with solvent A isocratically for 7 min. The mobile phase used for Column II was 33% acetonitrile with 15 mM phosphate buffer and 20 mM SDS, pH 3.5. The flow rate was set at 1 ml min^{-1} isocratically. Both columns were heated to 30°C to reduce the variation in retention times. The switching time from column I to column II was controlled by the AccessChrom data acquisition system, and was programmed to switch from Position A to Position B 30 s prior to the elution of famotidine and the internal standard from column I (Fig. 2). The valve was switched back to Position A 30 s after the completion of elution. UV detection was at 267 nm.

2.4. Preparation of stock solution and working standard solution

A stock solution of famotidine (100 ng μl^{-1}) was prepared by dissolving 1 mg famotidine in 10 ml methanol. The working standard solutions chosen for the standard curve were at seven concentration levels: 1, 2, 5, 10, 20, 50, and 100 ng/100 μl, which were made by further dilution of the stock solution with methanol. These solutions were stable at −20°C for at least 1 month.

A stock solution of the internal standard (50 ng μl^{-1}) was prepared by dissolving 0.5 mg of the

material in 10 ml methanol, from which a working standard solution of 25 ng/100 μl in methanol was made. The solutions were stored at −20°C until required. No degradation was observed under this condition in a period of 8 months.

2.5. Preparation of plasma standard curve

A daily standard curve was prepared by aliquoting 1 ml control plasma into 16 × 150 mm glass culture tubes, followed by the addition of 100 μl of each of the famotidine working standard solutions, and 100 μl internal standard solution. This yielded the standard curve concentration of famotidine in the range of 1–100 ng ml^{-1}. The concentration of the internal standard was 25 ng ml^{-1}. These standard plasma samples were subject to sample preparation as described in the next section. The plasma standard curve was constructed by plotting peak height ratio of famotidine over internal standard (ordinate) vs. famotidine concentration in plasma (abscissa). Peak height ratios of famotidine over the internal standard were calculated using the Access Chrom data acquisition system. Famotidine concentrations in clinical samples were calculated from the equation ($Y = MX + B$) as determined by the $1/X$ weighted linear regression analysis of the standard curve.

2.6. Sample preparation

A vacuum manifold (J.T. Baker) was used in the sample preparation procedure. A single solid phase extraction with SCX solid phase cartridge was used to isolate the drug and internal standard (25 ng ml^{-1}) from 1 ml^{-1} plasma sample buffered with 1 ml^{-1} 50 mM citric acid solution (pH 2.5). The cartridge was conditioned sequentially by elution with 1 ml methanol, 2 ml water, and followed by 1 ml of 50 mM citric acid solution. The buffered plasma sample with the internal standard was transferred from the culture tube into the cartridge under vacuum at 4 psi. Water (1 ml) was used to rinse the culture tube, and the rinse was transferred onto the cartridge. The clean-up was accomplished by elution with an additional 2 ml of water, and 1 ml of methanol through the cartridge. The cartridge was then placed in a clean culture tube. The analytes were eluted with 2 ml of NH_4OH/MeOH (v/v: 1/50) solution under centrifugation at 1000 rpm. The eluate was evaporated to dryness under N_2 for 20 min at 40°C, and reconstituted into 200 μl of the mobile phase. The entire volume was injected into the HPLC system.

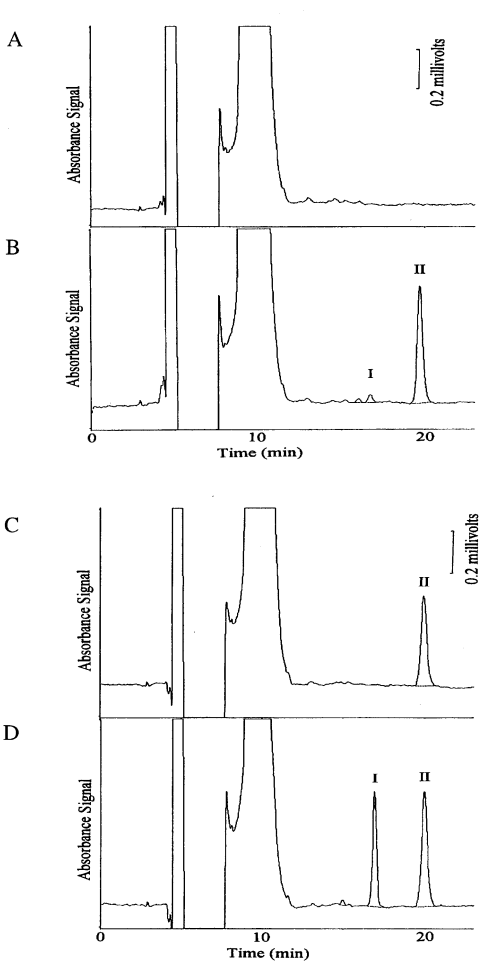

Fig. 3. Representative chromatograms of plasma (I = famotidine; II = internal standard): (A) Control human plasma; (B) plasma spiked with 1 ng ml^{-1} of I and 25 ng ml^{-1} of II; (C) Predose plasma sample of a human subject in the clinical study, spiked with 25 ng ml^{-1} of II; (D) Plasma sample of a human subject, 3 h after the administration of 10 mg famotidine in the clinical study, spiked with 25 ng ml^{-1} of II. The concentration of famotidine in sample D was determined to be 15.30 ng ml^{-1}.

2.7. Recovery

The absolute recovery from the extraction procedure was determined at 1, 10, and 100 ng ml^{-1} for famotidine, and at 25 ng ml^{-1} for the internal standard. The recovery was obtained by comparing the absolute peak height of analyte from the extracted plasma samples after spiking with that from direct spiking of analyte into the eluate of control plasma after extraction.

2.8. Clinical study

The assay method has been applied to support clinical biopharmaceutical studies. Prior to the analysis of clinical samples, quality control (QC) samples in plasma were prepared at the concentration levels of 2, 10, 50 ng ml^{-1}, and stored at -70°C. Two sets of these QC samples were then assayed daily along with the clinical samples.

3. Results and discussion

3.1. Sample preparation

Based on its structure, famotidine is a strong hydrophilic drug, with a pK_a of 6.7. The solubility of famotidine in many organic solvents is low [3]. Common solvents, such as ethyl acetate, chloroform and methyl-*t*-butyl ether have been tried for the purpose of liquid-liquid extraction under basic conditions, including back extraction of the drug from the organic solvent. The result showed poor recovery and insufficient separation from the endogenous interference peaks. The unique structure of famotidine suggests the potential advantage of solid phase extraction. The efficiency of solid phase extraction by different types of cartridges was investigated. Octadecyl, silica, cyanopropyl, aminopropyl, and primary/secondary amine cartridges were initially tried to separate famotidine in basic solutions as the molecule becomes neutral under such conditions. Separations on these cartridges were less than adequate, yielding either low recovery or poor isolation. In acidic solutions below its pK_a, the amine function groups on famotidine are protonated. This suggested the possibility of separation and isolation based on a cation exchange mechanism. Cartridges with weak cation exchange functional groups, such as carboxylic acid (CBA), and with strong cation exchange functional groups, such as benzenesulfonic acid (SCX), were tested. SCX cartridges particularly showed excellent separation and high recovery. The strong cation-anion binding between famotidine cation and benzenesulfonic acid anion allowed the use of various solvents with different polarities, ranging from water to methanol, to wash out the cartridge. The remaining endogenous components were resolved efficiently from famotidine by a column switching HPLC.

3.2. Extraction recovery

The recovery of the SCX solid phase extraction procedure averaged 86.6% for famotidine over the standard curve range. The recovery for the internal standard was 80.0%.

3.3. Chromatographic conditions

In order to enhance the separation of famotidine by HPLC, column switching technique was used to remove the early-eluting endogenous peaks on the chromatogram. It also enabled the application of a stronger mobile phase for the second column to improve the detection limit by peak-compression. When used in combination with a weak mobile phase, column I, with its low carbon loading, allowed the early elution of famotidine and the internal standard. This combination also allowed their initial separation from other endogenous components. The switching window was selected to isolate the famotidine and the internal standard peaks from the eluent of the first column for 'heart-cut' onto the second column. And it was set to be sufficiently narrow for the purpose of eliminating the late-eluting endogenous interferences from being carried into the second column. The mobile phase for the second column was significantly stronger than the first one. Besides having a higher percentage of acetonitrile, it contained sodium dodecyl sulfate which acted as an ion pairing agent. The pH of the mobile phases also played a key role. At

Table 1
Intraday precision and accuracy of the assay as assessed by the replicate ($n = 5$) analysis of plasma spiked with famotidine standards

Nominal standard concentration (ng ml^{-1})	Mean analyzed standard concentration (ng ml^{-1})	Precision (% C.V.)	Accuracy (% Deviation from nominal)
1	1.1	4.9	6.6
2	2.1	5.7	7.1
5	4.9	3.7	−1.2
10	9.4	5.0	−5.6
20	18.7	2.4	−6.6
50	47.9	5.7	−4.2
100	103.8	3.7	3.8

different pHs, analytes with multiple primary and secondary amine groups can be partially or completely protonated. The pH (6.8) of the first mobile phase was very close to the pK_a of famotidine, resulting in partial protonation of the molecules. The famotidine peak and the internal standard peak were incompletely resolved on the first column. The pH of the mobile phase for the second column was lower (pH = 3.5), causing the two analytes to be charged differently because of the different numbers of amine groups in their structures. This resulted in different interactions of these compounds with the ion pairing agent, and contributed to the complete baseline separation of famotidine and the internal standard. Thus, the combined use of the SCX solid phase extraction step together with the HPLC column switching was necessary in the present method to achieve the improved quantification limit. The recovery efficiency of 86.6% for famotidine in the present method was comparable to the highest recovery of 91.2% reported by other workers [7]. However, none of the published assay methods has achieved the LOQ below 5 ng ml^{-1}. The initial separation on the first column was necessary to achieve the benefit of peak compression on the second column. While separation of the two analytes from the endogenous materials might be theoretically possible with a single column without column switching, it would not be adequate to achieve the 1 ng ml^{-1} LOQ using either an isocratic or a gradient technique.

3.4. Assay specificity

Fig. 3 shows the typical chromatograms of control plasma (Fig. 3A), a plasma standard containing 1 ng ml^{-1} famotidine and 25 ng ml^{-1} internal standard (Fig. 3B), and a predose plasma sample from a subject (Fig. 3C) and 3 h after a 10-mg famotidine dose (Fig. 3D). None of the predose or control plasma samples analyzed contained a detectable interference at the retention time range of interest.

3.5. Linearity, precision, accuracy and detection limit

The weighted least square regression standard curve was linear over the concentration range of 1–100 ng ml^{-1}. The coefficient of regression was greater than 0.998 for a typical curve. Replicate standards ($n = 5$) were analyzed to assess the within-day variability of the assay. The precision, expressed as coefficient of variation (%C.V.), as well as accuracy, expressed as a precentage of deviation from the theoretical value, are listed in Table 1.

Limit of quantification for the assay was 1 ng ml^{-1}. The detection limit, based on 4/1 of the signal to noise ratio, was 0.42 ng ml^{-1}.

Plasma quality control samples at low (2 ng ml^{-1}), middle (10 ng ml^{-1}), and high (50 ng ml^{-1}) concentrations were prepared prior to the start of the study and subjected to replicate ($n = 5$) within-day analysis. Two sets of QCs at

Table 2
Interday variability of the assay as assessed by the coefficient of variation (% C.V.) of quality control samples at three concentration levels

Nominal concentration (ng ml^{-1})	Mean ($n = 30$) analyzed concentration (ng ml^{-1})	% C.V.
2	2.03	13.6
10	9.22	10.3
50	47.81	10.3

each of the three concentration levels were analyzed daily over a period of 1 year to assess the interday precision (see Table 2 for results). The overall interday variability of the assay, as measured by the coefficient of variation (% C.V.), is below 14%. These data also indicate that famotidine is stable in plasma stored at -70°C for at least 1 year.

Representative famotidine plasma profiles are shown in Fig. 4.

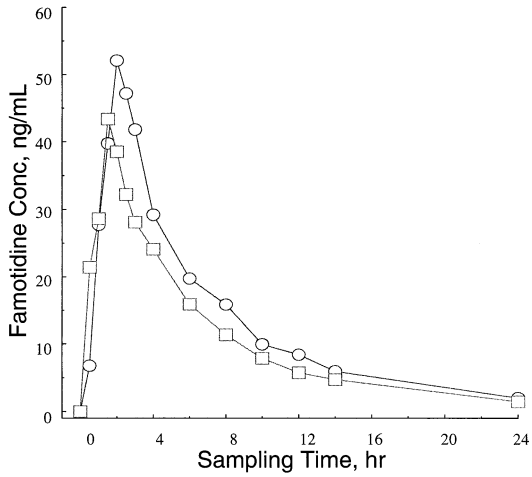

Fig. 4. Plasma concentration profiles of famotidine following administration of two dosage forms of famotidine (Dose = 10 mg), in a healthy volunteer.

4. Conclusion

Solid phase extraction, with SCX cartridge as the sample preparation step, and HPLC column switching technique offers significant advantage in the determination of famotidine in plasma. The method is rapid, robust and sensitive. The limit of quantification is improved to 1 ng ml^{-1}, which is at least 5-fold more sensitive than the published values.

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