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Short communication

Development of a rapid HPLC method for determination of famotidine in human plasma using a monolithic column

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Abstract

A rapid and sensitive HPLC method using a monolithic column has been developed for quantification of famotidine in plasma. The assay enables the measurement of famotidine for therapeutic drug monitoring with a minimum detectable limit of 5 ng ml^{-1}. The method involves simple, one-step extraction procedure and analytical recovery was complete. The separation was carried out in reversed-phase conditions using a Chromolith Performance (RP-18e, 100 mm × 4.6 mm) column with an isocratic mobile phase consisting of 0.03 M disodium hydrogen phosphate buffer–acetonitrile (93:7, v/v) adjusted to pH 6.5. The wavelength was set at 267 nm. The calibration curve was linear over the concentration range 20–400 ng ml^{-1}. The coefficients of variation for inter-day and intra-day assay were found to be less than 8%.
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1. Introduction

Famotidine is a histamine H_2-receptor antagonist, which has been widely used for the treatment of peptic ulcers. Famotidine is readily but incompletely absorbed from the gastrointestinal tact with peak concentrations in plasma occurring about 2 h after administration by mouth. A small proportion of famotidine is metabolized in the liver to famotidine S-oxide, but most of it is excreted unchanged in the urine [1]. As therapeutic doses of famotidine recommended to patients are low (40 mg daily) and these doses produce very low therapeutic concentrations in plasma (20–150 ng ml^{-1}) after a 40 mg oral dose [1], a sensitive method is required to determine plasma famotidine concentrations in clinical studies. Several high-performance liquid chromatography methods have been reported for the determination of famotidine in biological fluids [2–9]. HPLC methods differ with respect to the mode of HPLC (normal or reversed-phase) and sample preparation.

Some of these methods employed solid-phase extraction (SPE) procedures for elimination of endogenous plasma interferences and preconcentration in biological fluids by a reversed-phase HPLC analysis [2,4,5]. The other HPLC methods [3,7] involved an extraction of plasma samples with diethyl ether followed by extraction with ethyl acetate before chromatographic separation using C_{18} reversed-phase column. However, these reported methods required tedious liquid–liquid or solid-phase extraction procedures and therefore, sample preparation is time-consuming, complex or both. Moreover, most of the aforementioned methods need long chromatographic elution time for analysis of famotidine in plasma and were not suitable in all conditions. Two HPLC procedures have also been presented by Campanero et al. [8] and Zhong et al. [9] for the analyses of famotidine based on

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MS–MS detection by using tandem mass spectrometry. Both of the methods are very sensitive, having low quatitation limits. However, these methods are not available for most laboratories because of their specialty requirement and financial reasons. Moreover, some purification steps have been used before the samples are injected to chromatographic system as liquid–liquid extraction, solid-phase extraction, etc. In a previous study [6], we reported a simple reversed-phase HPLC procedure using a modification of the solid phase extraction method for determination of famotidine in plasma and urine. Although this method was sensitive, the run time was long and large plasma sample volume (1 ml) was required for drug analysis. Recently, monolithic stationary phases have attracted considerable attention in liquid chromatography due to their simple preparation procedure, unique properties and excellent performance, especially for separation of drugs in biological samples. As opposed to individual particles packed into chromatographic columns, monolithic supports are cast as continuous homogenous phases. They represent an approach that provides high rates of mass transfer at lower pressure drops as well as high efficiencies even at elevated flow rates. Therefore, much faster separations are possible and the productivity of chromatographic processes can be increased by at least one order of magnitude as compared to traditional chromatographic columns packed with porous particles. This enhances the speed of the separation process and reduces backpressure and unspecific binding without sacrificing resolution [10]. The present study describes a rapid and sensitive HPLC method using a monolithic column with UV detection, which enables the determination of famotidine with good accuracy at low drug concentrations in plasma using single-step extraction procedure. Separation was performed on a reversed-phase monolithic column, which has lower separation impedance compared to the particulate packings, and therefore, it allows easy optimizing chromatographic conditions to obtain desirable resolution in a short time. The sample preparation only involves protein precipitation and no evaporation step is required. Also, the use of a smaller sample volume provides an advantage as compared with some previous methods [2–6] that require large sample volume (1–2 ml) for analysis of famotidine. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.

2. Experimental

2.1. Chemicals

Famotidine and ranitidine were supplied by Kimidarou Pharmaceuticals (Tehran, Iran). Famotidine is available as an oral tablet containing 40 mg of famotidine and other inactive ingredients. HPLC-grade acetonitrile and all other chemicals were obtained from Merck (Darmstadt, Germany). Water was obtained by double distillation and purified additionally with a Milli-Q system.

2.2. Instruments and chromatographic conditions

The chromatographic apparatus consisted of a model Wellchrom K-1001 pump, a model Rheodyne 7125 injector and a model K 2501 UV detector connected to a model Eurochrom 2000 integrator, all from Knauer (Berlin, Germany).

The separation was performed on Chromolith Performance (RP-18e, 100 mm × 4.6 mm) column from Merck (Darmstadt, Germany). The wavelength was set at 267 nm. The mobile phase was a mixture of 0.03 M disodium hydrogen phosphate buffer–acetonitrile (93:7, v/v) adjusted to pH 6.5 at a flow rate of 1.5 ml/min. The mobile phase was prepared daily and degassed by ultrasonication before use. The mobile phase was not allowed to recirculate during the analysis.

2.3. Standard solutions

Stock solutions (4 mg ml^{-1}) of famotidine were prepared in methanol and 40 μg ml^{-1} and 4000 ng ml^{-1} solutions were made by dilution in water. Then, 20, 80, 160, 240, 320 and 400 ng ml^{-1} working standards were prepared in plasma from the 4000 ng ml^{-1} solution and stored at +4 °C.

2.4. Sample preparation

To 500 μl of plasma in a glass-stoppered 15 ml centrifuge tube were added 50 μl of ranitidine as internal standard (6 μg ml^{-1}) and 50 μl of 48% perchloric acid aqueous solution. After mixing (30 s), the mixture centrifuged for 10 min at 8000 rpm. Then, 30 μl of supernatant was injected into liquid chromatograph.

2.5. Biological samples

Twelve healthy male volunteers were included in this study. The study protocol was approved by the Ethics Committee of Shaheed Beheshti University of Medical Sciences and written informed consent was obtained from the volunteers. Famotidine was administered in a single dose of 40 mg to the volunteers after over night fasting. Plasma samples were collected at several intervals after dosing and then frozen immediately at −20 °C until assayed.

2.6. Stability

The stability of famotidine was assessed for spiked plasma samples stored at −20 °C for up to 2 months and at ambient temperature for at least 12 h. The stability of stock solutions stored at −20 °C was determined for up to 1 month by injecting appropriate dilutions of stocks in distilled water on day 1, 15 and 30 and comparing their peak areas with fresh stock prepared on the day of analysis. Samples were considered to be stable if the assay values were within the acceptable limits of accuracy and precision.

2.7. Plasma standard curve

Blank plasma was prepared from heparinized whole-blood samples collected from healthy volunteers and stored at −20 °C. After thawing, stock solution of famotidine was added to yield final concentrations ranging from 20 to 400 ng ml^{-1}. Internal standard solution was added to each of these samples to yield a concentration of 500 ng ml^{-1}. The samples were then prepared for analysis as described above.

2.8. Selectivity and specificity

Control human plasma, obtained from 12 healthy volunteers, was assessed by the procedure as described above and compared with respective plasma samples to evaluate selectivity of the method. Omeperazole and some histamine H_2-receptor antagonists like cimetidine and nizatidine were also tested for potential interferences.

2.9. Precision and accuracy

The precision and accuracy of the method were examined by adding known amounts of famotidine to pool plasma (quality control samples). For intra-day precision and accuracy, five replicate quality control samples at each concentration were assayed on the same day. The inter-day precision and accuracy were evaluated on three different days.

2.10. Limit of quantification (LOQ) and recovery

For the concentration to be accepted as LOQ, the percent deviation from the nominal concentration (accuracy) and the relative standard deviation must be ±10% and less than 10%, respectively, considering at least five times the response compared to the blank response. The relative analytical recovery for plasma at three different concentrations of famotidine (50, 150 and 300 ng ml^{-1}) was determined. Known amounts of famotidine were added to drug-free plasma and the internal standard was then added. The relative recovery of famotidine was calculated by comparing the peak areas for extracted famotidine from spiked plasma and a standard solution of famotidine in methanol containing internal standard with the same initial concentration (six samples for each concentration level).

3. Results and discussion

Under the chromatographic conditions described, famotidine and the internal standard peaks were well resolved. Endogenous plasma components did not give any interfering peaks. Fig. 1 shows typical chromatograms of blank plasma in comparison to spiked samples analyzed for a pharmacokinetic study. The average retention times of famotidine and ranitidine were 3.3 and 4.3 min, respectively. None of the drugs mentioned above interfered with analytes peaks as well.

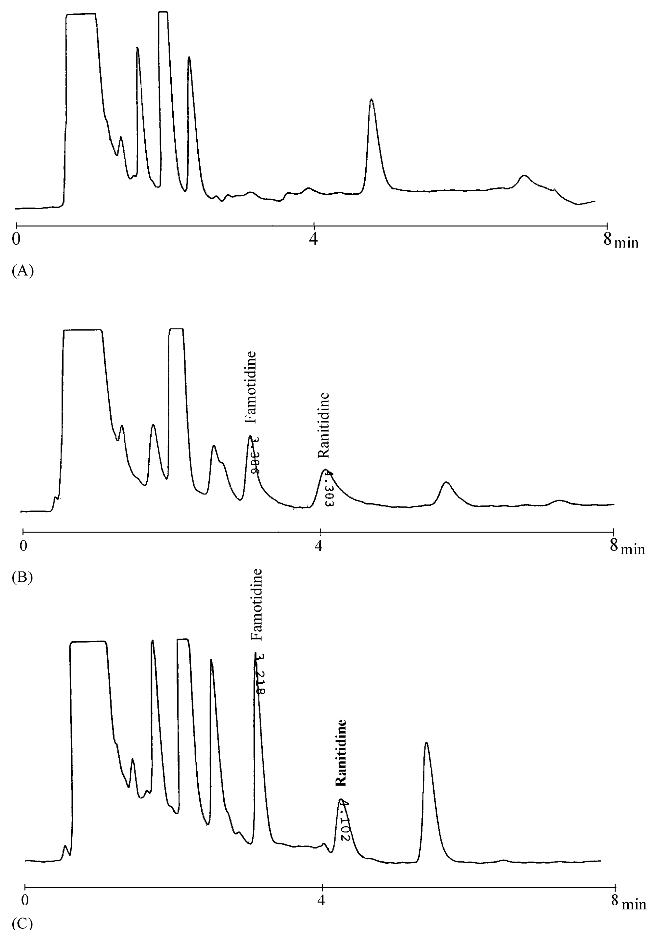

Fig. 1. Chromatograms of: (A) blank plasma; (B) blank plasma spiked with 150 ng ml^{-1} famotidine and 500 ng ml^{-1} ranitidine (internal standard); (C) plasma sample from a healthy volunteer 2 h after oral administration 40 mg of famotidine.

The calibration curve for the determination of famotidine in plasma was linear over the range 20–400 ng ml^{-1}. The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients (r) for calibration curves were equal to or better than 0.999. The relative standard deviation (R.S.D.) values of the slope were equal to or better than 6%. For each point of calibration standards, the concentrations were recalculated from the equation of the linear regression curves (Table 1). The relative analytical recovery for plasma at three different concentrations of famotidine was determined. Known amounts of famotidine were added to drug-free plasma in concentrations ranging from 50 to 300 ng ml^{-1}. The internal standard was added and the relative recovery of famotidine was calculated by comparing the peak areas for extracted famotidine from spiked plasma and a standard solution of famotidine in methanol containing internal standard with the same initial concentration. The average recovery was 98.1 ± 1.7% (n = 6) (Table 2). The limit of quantification, as previously defined, was 15 ng ml^{-1} for famotidine. This is sensitive enough for drug monitoring and

Table 1
Assay linearity

	Coefficient of the linear regression analysis ($r \pm$ S.D.)	Slope $\pm$ S.D.	Intercept $\pm$ S.D.
Intra-assay ($n = 6$)	$0.9995 \pm 7.65 \times 10^{-4}$, R.S.D. = 0.0765%	0.0059 ± 0.0003, R.S.D. = 5.08%	0.0154 ± 0.0009
Inter-assay ($n = 9$)	$0.9992 \pm 9.12 \times 10^{-4}$, R.S.D. = 0.0913%	0.0064 ± 0.0003, R.S.D. = 4.69%	0.0165 ± 0.0011

Table 2
Relative recovery of famotidine from plasma

Famotinine spiked concentration (ng ml^{-1})	Famotinine concentraion found ($n = 6$)	Recovery% (mean $\pm$ S.D.)
50	48.1	96.1 $\pm$ 1.9
150	148.5	99.0 $\pm$ 1.6
300	297.6	99.2 $\pm$ 1.6

Table 3
Reproducibility of the analysis of famotidine in human plasma ($n = 5$)

Concentration added (ng ml^{-1})	Concentration measured (mean $\pm$ S.E.)	
	Intra-day	Inter-day
50	53.4 $\pm$ 3.8 (7.1)	52.9 $\pm$ 3.4 (6.4)
150	156.6 $\pm$ 6.3 (4.0)	154.3 $\pm$ 6.3 (4.1)
300	322.9 $\pm$ 10.3 (3.2)	320.6 $\pm$ 12.5 (3.9)

Values in parentheses are coefficients of variation (%).

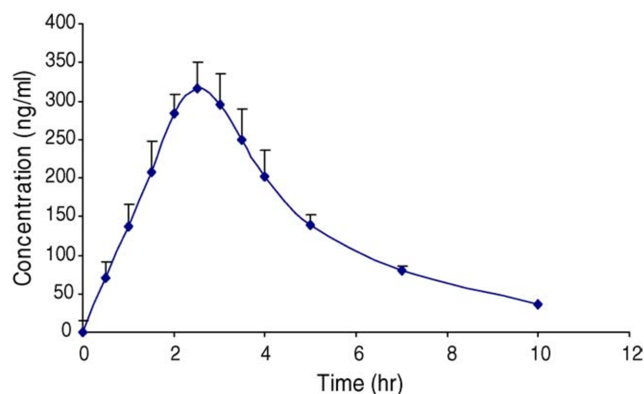

Fig. 2. Mean plasma concentration–time profile of famotidine in healthy volunteers ($n = 12$) after a single 40 mg famotidine.

other purposes such as pharmacokinetic studies. We assessed the precision of the method by repeated analysis of plasma specimens containing known concentrations of famotidine. As shown in Table 3, coefficients of variation were less than 8%, which is acceptable for the routine measurement of famotidine. Stability was determined for spiked plasma samples under the conditions as previously described. The results showed that the samples were stable during the mentioned conditions. The aim of our study was to develop a rapid and sensitive method for the routine analysis of biological samples in pharmacokinetic famotidine research. This method is well suited for routine application in the clinical laboratory because of the speed of analysis and simple extraction procedure. Owing to use of the monolithic column, which has lower separation impedance compared to the particulate packings, much faster separations are possible and the productivity of chromatographic processes can be increased by at least one order of magnitude as compared to traditional chromatographic columns packed with porous particles. Accordingly, the chromatographic elution step is undertaken in a short time (less than 8 min) with high resolution. The sample preparation only involves protein precipitation and no evaporation step is required. Also, the use of a smaller sample volume provides an advantage as compared with some previous methods that require 1–2 ml of plasma for analysis of famotidine. Over 700 plasma samples were analyzed by this method without any significant loss of resolution. No change in the column efficiency and back pressure was also observed over the entire study time, thus proving its suitability. In this study, plasma concentrations were determined in 12 healthy volunteers, who received 40 mg of famotidine each. Fig. 2 shows the mean plasma concentration–time curve of famotidine: plasma concentration reached a maximum 2.33 ± 0.32 h after dosing with a level of 325.65 ± 17.56 ng ml^{-1}. These pharmacokinetic parameters are in good agreement with that found previously [11].

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