Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, No. 2, pp. 207–210, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00
Pergamon Press plc

Short Communication

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

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Keywords: *Famotidine; plasma; urine; solid-phase extraction; HPLC; internal standard.*

Introduction

Famotidine is a new histamine H_2-receptor antagonist which has been proven to be highly effective and useful in the treatment of peptic ulcers [1, 2]. Absorption is very fast, reaching peak plasma concentrations approximately 1–3.5 h after oral administration [2, 3]. Famotidine is mainly excreted into urine in unchanged form (22–31% of orally administered doses) [4, 5]. Therefore, the determination of famotidine concentrations in biological fluids is of particular interest in pharmacokinetic studies.

Plasma famotidine levels are in the concentration range 20–150 ng ml^{-1} after single therapy with a 40 mg oral dose. For determination of famotidine, the HPLC method with UV detection has been previously reported using a solid-phase extraction procedure for elimination of endogenous plasma and urine interferences and preconcentration of famotidine in biological fluids [6, 7].

In our study, a modification of this method has been used. Since the pretreatment of the sample causes variation in famotidine recovery, the internal standard method was developed.

The present paper describes a rapid and sensitive analytical method for the determination of famotidine in human plasma and urine involving internal standard quantification, suitable for drug level monitoring in a clinical pharmacokinetic study.

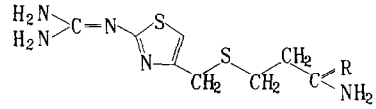
Experimental

Chemicals and reagents

Famotidine and the internal standard, i.e. 3-[({2-[(aminoiminomethyl)amino]-4-thiazolyl}-methyl)thio]-propanamide (Fig. 1), were synthesized at the Chemical Department (KRKA-Pharmaceuticals, Novo mesto, Yugoslavia).

HPLC-grade acetonitrile and ethanol were supplied by Merck (Darmstadt, FRG). Acetic acid and phosphoric acid (analytical grade) were purchased from Kemika (Zagreb, Yugoslavia).

Water was obtained by double distillation and purified additionally with a Milli-Q system.



R : $N-SO_2NH_2$ Famotidine
R : O Internal Standard

Figure 1
Structural formulae of famotidine and the internal standard.

Instrument and chromatographic conditions

HPLC was performed using a Shimadzu liquid chromatography system (Shimadzu Corporation, Tokyo, Japan) consisting of a LC-4A solvent delivery system with a Rheodyne 7125 injection valve, 100 μl loop (Rheodyne, Cotati, CA, USA), a SPD-2AS UV

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detector set at 267 nm and a Chromatopac C-R3A data processor.

A Supelco solid-phase extraction manifold with a Drying attachment kit (Supelco, Gland, Switzerland) using Supelclean silica columns (3 ml capacity) was used for plasma and urine sample preparation. Separation was performed on the analytical column (25 cm × 4.6 mm) using LiChrosorb RP-8, 10 μm stationary phase (Merck, Darmstadt, FRG), protected by a guard column (7.5 cm × 2.1 mm) with a pelicular reversed phase (Chrompack, Middelburg, The Netherlands). The mobile phase consisted of acetonitrile–aqueous 0.02 M phosphoric acid (5:95, v/v) and was delivered to the system at a flow rate of 1.2 ml min^{-1}.

Stock solutions

Stock solutions of famotidine and the internal standard were prepared monthly in methanol and stored at +4°C. No change in stability over the period of 1 month was observed.

Plasma standard curve

Blank human plasma was prepared from heparinized whole blood samples collected from healthy volunteers and stored at −20°C. After thawing, plasma was spiked daily with stock solutions to yield concentrations ranging from 20 to 200 ng ml^{-1} of famotidine and 100 ng ml^{-1} of internal standard.

Urine standard curve

Blank human urine was collected from healthy volunteers and stored frozen at −20°C until used. From the stock solution appropriate dilutions were prepared daily to achieve famotidine levels between 0.5–10 μg ml^{-1}, with an additional 2.5 μg ml^{-1} of internal standard.

Biological samples

Famotidine was administered in a single oral dose of 40 mg to healthy volunteers after overnight fasting. Plasma and urine samples were collected at several intervals after dosing and frozen immediately at −20°C until assayed.

Sample preparation

A Supelco vacuum manifold with Supelclean silica columns (cartridges) was used to shorten the sample preparation procedure. Cartridges were prepared for use by washing with 1 ml of methanol followed by 1 ml of water. One millilitre of the plasma or urine sample was applied to the activated cartridge and washed with 5 ml of water. Analytes retained were then eluted by 2 ml of an acetonitrile–ethanol mixture (1:1). The extract was evaporated to dryness in a stream of dry nitrogen at 40°C. The residue was redissolved in 250 μl of 0.017 M acetic acid, filtered through a Millipore HV4 filter (0.45 μm) and 100 μl of the extract was injected into the HPLC system.

Results and Discussion

Under the chromatographic conditions described, famotidine and the internal standard peaks were well resolved. Endogenous plasma or urine components did not give any interfering peaks. Figure 2 shows typical chromatograms of blank plasma (a) and urine (b) in comparison to spiked samples analysed for a pharmacokinetic study.

The detection limit for famotidine was 5 ng ml^{-1} in plasma and 10 ng ml^{-1} in urine samples. The recovery of the extraction procedure was determined by comparing the peak areas for extracted famotidine from spiked plasma or urine and a standard solution of famotidine in methanol with the same initial concentration. The recovery ranged from 80% in plasma to 84% in urine. The separation efficiency of cartridges for famotidine from biological samples differs from one cartridge to another, since the degree of activation of the silica stationary phase was not the same. To eliminate these differences, the method of internal standardization was used for the quantification of famotidine.

Calibration curves for plasma and urine were obtained by plotting peak area ratios of famotidine and the internal standard against famotidine concentration, and were linear within the concentration range of interest (25–200 ng ml^{-1} in plasma and 0.5–10 μg ml^{-1} in urine). The regression equation was: $Y = 0.00854\ X - 0.0139$ ($r = 0.9979$) for plasma and $Y = 0.7103\ X - 0.0094$ ($r = 0.9999$) for urine. Data on the accuracy and precision of the analytical method are presented in Table 1.

The aim of our study was to develop a simple and fast method for the routine analysis of biological samples in pharmacokinetic famotidine research. Over 500 plasma and urine samples were analysed by this method without problems, thus proving its suitability. A typical

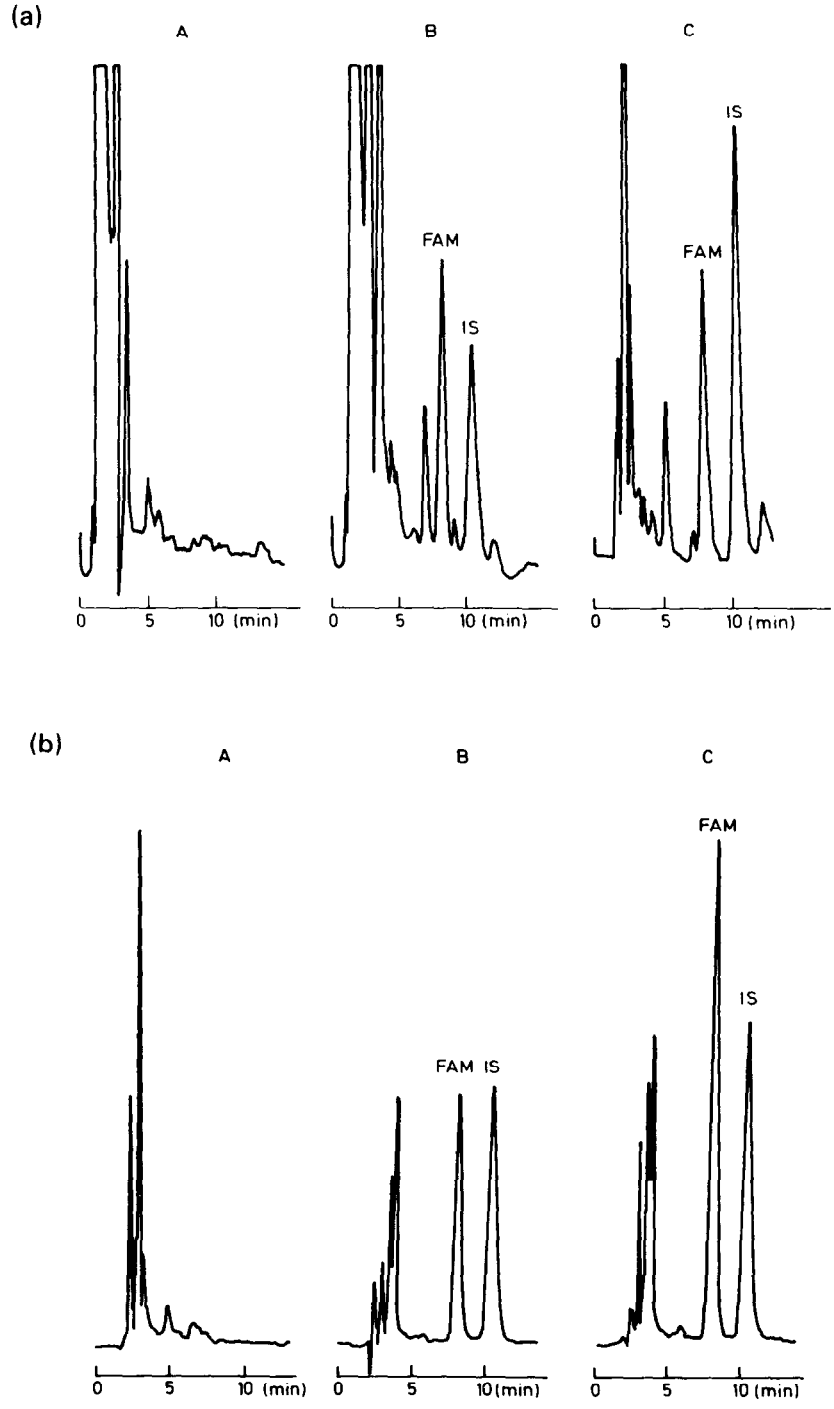

Figure 2
(a) Chromatograms of human plasma extracts: (A) blank plasma; (B) plasma spiked with 100 ng ml^{-1} of famotidine (FAM) and 100 ng ml^{-1} of internal standard (IS); (C) plasma sample taken 2 h after oral administration of 40 mg of famotidine spiked with 100 ng ml^{-1} of internal standard; (b) Chromatograms of human urine extracts: (A) blank urine; (B) urine spiked with 2 μg ml^{-1} of famotidine (FAM) and 2.5 μg ml^{-1} of internal standard (IS); (C) urine sample taken 2 h after oral administration of 40 mg of famotidine spiked with 2.5 μg ml^{-1} of internal standard.

Table 1
Assay accuracy and precision data

	Famotidine-added concentration (ng ml^{-1})	Mean (n = 5)	RSD (%)
Plasma	25	25.6	6.4
Intraday	75	75.3	3.9
Variability	150	151.1	4.3
Plasma	25	25.8	9.4
Interday	75	74.8	4.5
Variability	150	151.0	6.8
Urine	500	504	9.0
Interday	2000	2008	5.9
Variability	5000	5013	6.1

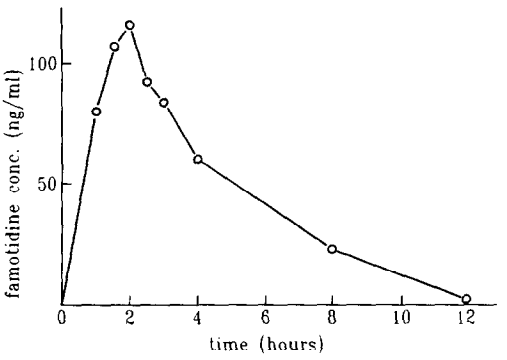

Figure 3
Typical human plasma concentration–time curve after administration of 40 mg famotidine.

result of the pharmacokinetic study is depicted in Fig. 3.

Acknowledgements — Financial support of the Research Community of Slovenia is gratefully acknowledged. The authors thank Professor F. Kozjek for providing the samples, Mr M. Florjanič for synthesis of the internal standard and technicians in the Laboratory of Pharmacokinetics for their technical assistance.

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[*Received for review 16 April 1990*]