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Note

Analytical method for the quantification of famotidine, an H_2-receptor blocker, in plasma and urine

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The new H_2-receptor blocker famotidine (Fig. 1) is a potent inhibitor of gastric acid secretion in man [1,2]. In anticipation of the analysis of biological samples from clinical studies, a selective and sensitive analytical method was developed for the quantification of famotidine both in plasma and urine samples. A straightforward high-recovery sample preparation procedure was effected by using silica-gel cartridges to adsorb the hydrophilic drug from the biological matrices. The described sample preparation scheme permitted the quantification of famotidine in plasma (1 ml) by UV detection (λ_{max} = 267 nm, $\epsilon = 1.58 \cdot 10^4$) alone. Resolution of famotidine from endogenous interferences was accomplished on an RP-8 column with a 0.019 *M* phosphoric acid—acetonitrile (90:10) mobile phase.

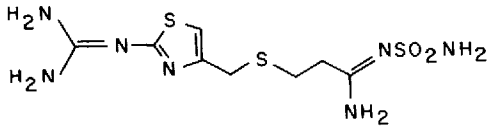

Fig. 1. Chemical structure of famotidine.

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EXPERIMENTAL

Materials

All extractions were performed on a Baker-10 extraction system using Bond-Elut 2.8-ml silica columns (Analytichem International, No. 601303). The water was of Milli-Q quality (18 M Ω cm resistivity), cartridge sequence: Super-C, Ion-Ex, Ion-Ex, Organex-Q. Glacial acetic acid and phosphoric acid (HPLC grade) were purchased from Fisher. Methanol, acetonitrile, and N,N-dimethylformamide (DMF), all HPLC grade, were obained from Burdick & Jackson Labs. Frozen heparinized control plasma was purchased from Sera-Tec Biologicals (New Brunswick, NJ, U.S.A.). Control human urine consisted of pooled urine collections from Merck laboratory personnel and placebo treatment groups from clinical studies. A famotidine analytical standard was supplied by the Chemical Data Section of Merck Sharp & Dohme (Rahway, NJ, U.S.A.). The prepared samples were evaporated in a Organomation analytical evaporator.

Glassware

All glassware was washed in a laboratory dishwasher with detergent at pH 12, rinsed with distilled water and dried at 60° C.

Instrumentation and accessories

The high-performance liquid chromatographic (HPLC) instrumentation consisted of Waters Assoc. 703 HPLC system equipped with a 730 data module, a 720 system controller, a WISP 710B automatic injector (limited-volume inserts), a Model 441 UV detector (254 nm and 0.01 a.u.f.s. for urine) or a Model 480 UV detector (267 nm and 0.002 a.u.f.s. for plasma). The analytical column was an Altex RP-8, particle size 5 μm (25 cm × 4.6 mm). The pre-column was purchased from Brownlee Labs: RP-8, 10 μm, 4 cm Spherisorb.

Mobile phase preparation

The analyses were performed at room temperature (22—25° C). The mobile phase consisted of a mixture of 0.019 *M* phosphoric acid—acetonitrile (90:10, v/v). The mobile phase components were filtered through a 0.45-μm Nylon 66 membrane separately. After sparging the mobile phase with helium or argon for 5 min, the mobile phase containing bottle was covered. Long-term sparging resulted in evaporation of acetonitrile from the mobile phase.

Stability in plasma

Famotidine was stable in plasma for at least five weeks at −15° C. When stored at −15° C for a period of one year, the amount of famotidine remaining in the sample was approximately 80% of the original concentration.

Stability in urine

Drug was unstable in urine at room temperature (22—25° C); there was a 10—15% loss of drug when stored at room temperature for a period of 4 h. Famotidine was stable in urine for at least five weeks at −15° C and for at

least two days at 5° C. The amount of famotidine remaining in the sample after storage at −15° C for one year was between 84% and 94%.

Famotidine was stable in methanol for at least one week at 5° C. All stock standards were prepared weekly and all working standards were prepared daily from the appropriate stock standard.

Preparation of urine sample

Frozen (−15° C) specimens were thawed to ⩽ 5° C, shaken and sampled. To a 13-ml centrifuge tube were added 1.0 ml of urine and 0.1 ml of methanol and the sample was mixed by vortex. To a 2.8-ml silica cartridge, attached to a Baker-10 extraction system under vacuum, were added 1.0 ml methanol, to activate the cartridge, 1.0 ml water, 0.5 ml of the urine—methanol mixture prepared above, followed by 3.0 ml water. The vacuum was relieved and 2-ml collection tubes were positioned under the cartridges. The system was evacuated and 2.0 ml DMF—water (50:50) mixture were added to elute famotidine. The eluate was placed in WISP limited-volume insert and 150 µl were injected in the HPLC system. The pre-column was changed every 100—125 injections.

Preparation of plasma sample

The frozen plasma samples were thawed by placing them at approximately 5° C overnight. The next day the specimens were mixed by vortex and centrifuged. To a 2.8-ml silica cartridge, attached to a Baker-10 extraction system under vacuum, were added 1.0 ml methanol, to activate the cartridge, 1.0 ml water, 1.0 ml plasma, and 5.0 ml water. The vacuum was relieved and 2-ml collection tubes were positioned under the cartridges. The system was evacuated and 2.0 ml of acetonitrile were added to elute famotidine. The eluate was evaporated to dryness under a stream of dry nitrogen at 40° C. The residue was reconstituted in 0.2 ml of 0.017 *M* glacial acetic acid. The acid was transferred to a WISP limited-volume insert and 150 µl were injected in the HPLC system.

Quantification

A standard curve was prepared and assayed daily with the unknown samples. The standards were prepared by mixing 1.0 ml of control fluid with a famotidine standard prepared in methanol. At the start of the clinical study, known concentrations of famotidine in biological fluid were prepared and frozen at −15° C. With each days' analyses, these quality control standards were assayed with the unknown samples. Unknown sample concentrations were calculated from an unweighted (weight, $W = 1$) linear regression analysis of the standard curve data (urine = peak area; plasma = peak height).

Selectivity

Famotidine, itself, was well resolved from endogenous interferences, as shown in Figs. 2 and 3. However, the metabolism of famotidine results in the formation of small amounts of an S-oxide metabolite. The famitodine S-oxide was not retained on the analytical column described in this method. The metabolite can be retained and adequately resolved from chromatographic

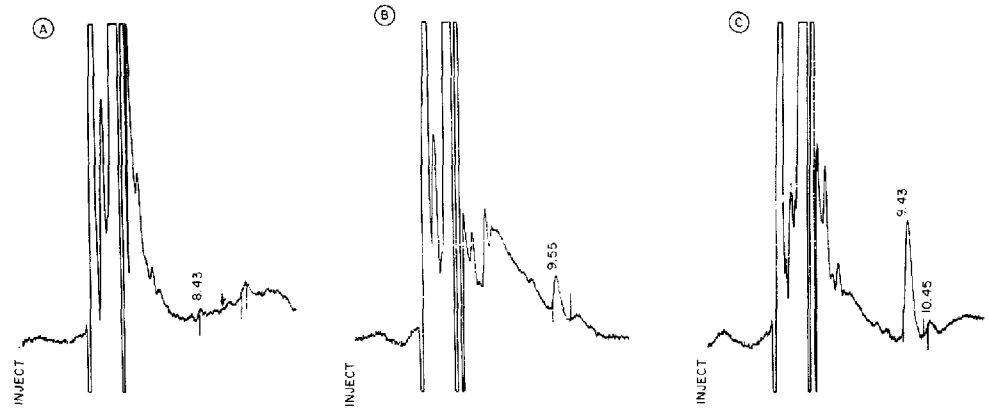

Fig. 2. Chromatograms of famotidine in plasma. (A) Plasma blank; (B) 5 ng/ml standard of famotidine in plasma; (C) normal subject dosed with 10 mg famotidine, 7 h post-dose, 13.6 ng/ml.

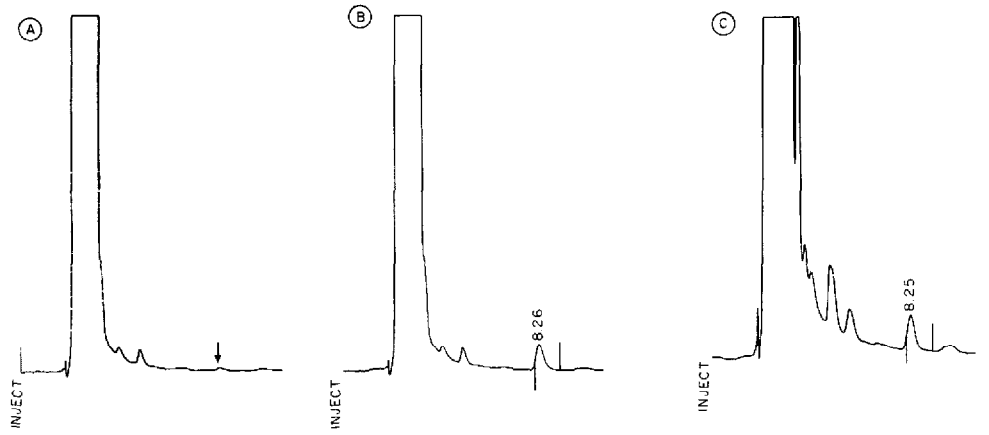

Fig. 3. Chromatograms of famotidine in urine. (A) Urine blank; (B) 500 ng/ml standard of famotidine in urine; (C) normal subject dosed with 20 mg famotidine, 12—24 h collection post-dose, 0.68 μg/ml.

interferences on a silica column. Unfortunately, metabolite concentrations in human urine were too low for reliable quantification.

RESULTS AND DISCUSSION

Anticipating peak plasma famotidine levels in the 5—10 ng/ml range for a 5-mg dose of the drug, initial detection schemes were centered around pre- and post-column derivatization of famotidine with fluorescence or UV tags. No acceptable derivatization method was developed for either the sulfonamide or the guinidine moieties [3—6]. Oxidation of famotidine on either glassy or porous carbon also was unsuitable for routine analysis.

Apparently, famotidine was so highly associated with plasma proteins that any attempt to clean-up plasma by protein precipitation resulted in low drug recovery.

TABLE I

SOLUBILITY OF FAMOTIDINE AT 20° C

Solvent	Solubility (%, w/v)
N,N-Dimethylformamide	80
Glacial acetic acid	50
Methanol	0.3
Water	0.1
Ethanol (100%)	<0.01
Ethyl acetate	<0.01
Chloroform	<0.01

TABLE II

INTRA- AND INTER-DAY VARIABILITY OF FAMOTIDINE

Sample	Intra-day		Inter-day*		
	Mean**	C.V. (%)	Mean***	*n*	C.V. (%)
Urine § *(μg/ml)*					
0.5	0.5	4.8			
1.0	1.0	1.6	0.9 (1.0)	31 (24)	6.0 (12.3)
2.0	2.0	5.9			
5.0	5.0	2.1			
10.0	10.0	4.1	9.0	32	3.8
15.0	15.1	1.0			
20.0	19.8	4.1	17.7 (19.8)	32 (22)	4.0 (4.7)
30.0	29.9	4.7			
Plasma (ng/ml)					
5.0	5.2	8.9			
10.0	9.8	5.7	10.8 (10.4)	9 (38)	9.0 (9.1)
20.0	19.8	5.9			
30.0	29.3	7.2			
40.0	40.0	7.6	40.6 (39.3)	9 (38)	5.3 (6.0)
50.0	51.0	6.7			

*Values determined from an additional study, new quality control preparations, are given between parentheses.
**Mean calculated concentration, $n = 6$.
***Quality control standards.
§ Nominal concentration.

The solubility of famotidine in many organic solvents is low (see Table I) and extraction of the drug ($pK_a = 6.7$) from plasma (pH 4, 6 or 8) with ethyl acetate gave highly variable recoveries (20—60%). In addition, these extracts, when evaporated and reconstituted with mobile phase, contained many endogenous interferences which could not be resolved from famotidine using a variety of HPLC columns. Liquid—solid extraction of drug from plasma or urine on different bonded phase cartridges (Baker) was less than adequate, yielding either low recovery or background interferences. On the other hand, silica cartridges were adequate, since famotidine, containing polar substituents

like other H_2-receptor blockers, adsorbs well to silica [7, 8]. Endogenous chromatographic peaks in the eluate from the silica cartridges were resolved from famotidine using a C_8 analytical column. The use of two different supports, silica and C_8, for the method, in toto, likely contributes to the very clean backgrounds. The mean recoveries of famotidine over the range of the standard curve were 90.3 ± 1.0% and 90.2 ± 3.7% for plasma and urine, respectively. The recovery was based upon the peak height (area) of a direct injection of the corresponding standard.

The limit of reliable quantification was 5.0 ng/ml for plasma and 500 ng/ml for urine. Drug was stable in the injection solvent for 24 h at 20—22°C. Analytical method accuracy and precision data are presented in Table II. The calibration curves were linear within the concentration range of interest (urine 0.5—30.0 μg/ml; plasma 5.0—50.0 ng/ml). The mean regression line (fifteen standard curves) for urine had a correlation coefficient of 0.99992 and for plasma (seven standard curves) 0.99839.

This method affords a simple, sensitive and direct method for the determination of famotidine levels in clinical study specimens.

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