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Note

High-performance liquid chromatographic determination of famotidine in urine

ASIRUR RAHMAN and NORMAN E. HOFFMAN*

Todd Wehr Chemistry Building, Marquette University, Milwaukee, WI 53233 (U S A.)

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Famotidine is a relatively new histamine H_2-receptor antagonist. It has been found to be effective in inhibiting gastric acid secretion induced by histamine and food [1,2]. The chemical structure of famotidine is shown in Fig. 1. Only one high-performance liquid chromatographic (HPLC) technique has been reported for the quantitation of famotidine in plasma and urine [3]. In a second report an HPLC technique was used for the determination of famotidine in pharmaceutical formulations [4]. This paper reports a rapid and sensitive HPLC method for the determination of famotidine in urine.

EXPERIMENTAL

Equipment

The chromatographic system consisted of a Waters M-6000A solvent delivery pump (Waters Assoc., Milford, MA, U.S.A.). A Rheodyne Model 7125 injector (Cotati, CA, U.S.A.) with a 200-μl sample loop was used as the injection port. A Kratos Spectroflow 773 variable-wavelength UV–Vis detector (Ramsey, NJ, U.S.A.) was used. A Houston Instrument Model 4511 strip chart recorder (Austin, TX, U.S.A.) was used. An Econosphere 150 mm × 4.6 mm, 5-μm CN column was purchased from Alltech (Deerfield, IL, U.S.A.). A Sybron-Barnstead D2794 water purification system (Boston, MA, U.S.A.) was used to prepare the deion-

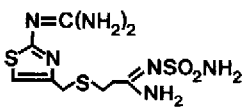

Fig. 1. Structure of famotidine.

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ized water. A Sorvall GLC-1 centrifuge from Ivan Sorvall (Norwalk, CT, U.S.A.) was also used. An ultrasonic water bath from L&R Manufacturing Industry (Kearny, NJ, U.S.A.) was used to degas the mobile phase.

Materials

Famotidine was obtained from Merck Sharp & Dohme (Rahway, NJ, U.S.A.). Acetonitrile obtained from Curtin Matheson Scientific (Houston, TX, U.S.A.) was HPLC grade. Anhydrous potassium carbonate and chloride were obtained from Alfa Products (Danvers, MA, U.S.A.). Anhydrous sodium carbonate, potassium dihydrogenphosphate, dipotassium hydrogenphosphate and sodium dihydrogenphosphate were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). The urine samples were collected from ten volunteers. When not in use the urine was kept at $-10°C$.

Procedure

Preparation of the stock solution. A stock solution of famotidine with a concentration of 50.0 μg/ml was made by dissolving 12.5 mg of famotidine in 250 ml of acetonitrile. The solution was kept at $-10°C$ and brought to room temperature when needed.

Extraction of standard solutions of famotidine in urine. Standard solutions of famotidine were prepared in 1.0 ml of thawed urine. The required volume of famotidine stock solution was added to the urine and the mixture was vortex-mixed for 10 s. The standard solutions had famotidine concentrations of 0.4, 1.0, 2.5, 5.0, 10.0, 17.5 and 25.0 μg/ml of urine. In a typical extraction, the necessary volume of acetonitrile was added to a standard solution to make the total volume of acetonitrile equal to 500 μl. The mixture was vortex-mixed for 10 s and an excess of potassium carbonate was added together with vortex-mixing for 30 s. The mixture was centrifuged at 1200 *g* for 2 min. At this point two liquid phases were obtained. A 300-μl portion of the organic layer was transferred to a test tube and the remaining was discarded. To this 300 μl of acetonitrile solution were added 300 μl of 3.0 m*M* potassium carbonate solution and the mixture was vortex-mixed for 10 s. Finally, 40 μl of this solution of famotidine were injected into the chromatograph. The above procedure was duplicated for water as the sample medium.

Preparation of standard solutions of famotidine without extraction. Standard solutions of famotidine were made in 50:50 (v:v) acetonitrile–3 m*M* potassium carbonate by adding volumes of famotidine stock solution to seven different volumes of acetonitrile to give a total volume of 500 μl each and concentrations of 0.8, 2.0, 5.0, 10.0, 20.0, 35.0 and 50.0 μg/ml, respectively. The solutions were vortex-mixed separately and 300 μl of each of those were mixed with equal volumes of 3 m*M* potassium carbonate. The seven standard solutions had concentrations equal to the urine concentrations.

Chromatographic conditions. The analytical column used was 150 mm$\times$4.6 mm, packed with 5-μm silica-bonded cyanopropyl stationary phase. The mobile phase was prepared by mixing 8 volume percent of acetonitrile with 92 volume percent of 10 m*M* aqueous sodium dihydrogenphosphate. No pH adjustment was made. The mobile phase was degassed for 15 min by using an ultrasonic water

bath. It was then passed through the analytical column at a flow-rate of 1.0 ml/min. The UV–Vis detector was set at 267 nm with its sensitivity varying between 0.01 and 0.10 a.u.f.s. A 2-cm guard column packed with 8-μm cyanopropyl stationary phase was also provided. The packing of the guard column needed changing after 50 to 60 injections as shown by increased back-pressure.

Study of the effect of injection solvent ionic strength on famotidine peak height. Solutions of 25.0 μg/ml famotidine were prepared by mixing equal volumes of famotidine solution and 0.8–50 m*M* dipotassium hydrogenphosphate, sodium carbonate, potassium carbonate, potassium chloride and potassium dihydrogenphosphate. Of these solutions, 40 μl were then injected into the chromatograph.

RESULTS

A typical chromatogram of urine spiked with famotidine is shown in Fig. 2a. The famotidine peak was found to be well resolved from the endogenous peaks of urine. The chromatogram of control urine is shown in Fig. 2b. No interfering peak was found at the famotidine peak position. Under optimum chromatographic conditions the retention time for the famotidine peak was found to be 13.5 min.

Famotidine concentration was measured by using a calibration curve method. The curve was constructed with peak height versus concentration. The concentration range was kept similar to that previously reported [3]. Two different calibration curves for famotidine were obtained using the extraction process: one

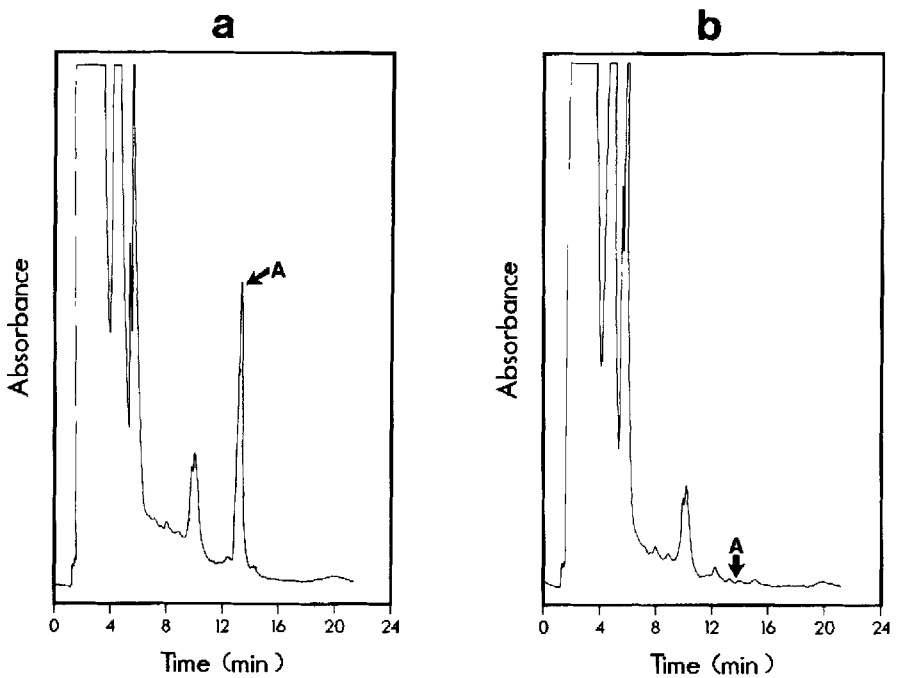

Fig 2. Chromatograms of an extract of (a) urine spiked to give 2.5 μg/ml famotidine and (b) control urine. A = famotidine peak location. Detector, 0.01 a.u.f.s.

for urine and the other for pure water. In both cases 1.0 ml of urine or water was spiked with famotidine and extracted into acetonitrile. A third calibration curve was obtained with acetonitrile as the sample matrix. No extraction was performed in this case. The statistical data for these three calibration curves are shown in Table I. The curves were found to be linear with correlation coefficients of 0.999. At the 95% confidence interval t-value calculation showed no significant difference among the slopes of these three curves. A regression analysis showed the three intercepts to be negligible. This implies that the curves were identical to each other at the 95% confidence level and any one of the calibration curves can be used for the quantitation of famotidine in urine. The method was found to be reproducible for both within day and between days. The results are shown in Table II. The relative standard deviation (R.S.D.) varied between 1.7 and 6.8% for within-day determination and between 1.2 and 7.9% for between-day determination.

Cimetidine, clindamycin phosphate, dexamethasone, diphenhydramine, halo-

TABLE I

LINEAR REGRESSION ANALYSIS OF CALIBRATION CURVES

$y=mx+b$ where y is the peak height in absorbance units and x is concentration in μg/ml. For all curves $r=0.999$, $n=8$.

Curve	Fluid extracted	Slope (mean ± S.D.) ($\times 10^3$)	Intercept (mean ± S.D.) ($\times 10^3$)	Identity of slopes, t_{calc}*
A	Urine	2.16 ± 0.01	0 0335 ± 0.0560	1.066 (A vs C)
B	Water	2.17 ± 0.01	−0.0163 ± 0.108	1.152 (A vs. B)
C	No extraction	2.17 ± 0.01	0.0989 ± 0.0951	0.1580 (B vs C)

*$t_{table}=2.179$, 95%.

TABLE II

ASSAY REPRODUCIBILITY OF THE METHOD OVER A RANGE OF FAMOTIDINE CONCENTRATIONS

Actual concentration (μg/ml)	Mean concentration ($n=5$)			
	Within day		Between days	
	μg/ml	R.S.D. (%)	μg/ml	R.S.D. (%)
0.4	0.39	6.8	0.38	7.9
1.0	1.04	3.4	0.96	6.0
2.5	2.41	3.2	2.42	3.7
5.0	4.75	3.8	4.82	4.2
10.0	10.1	3.2	9.86	3.3
17.5	17.3	2.3	17.5	1.2
25.0	25.1	1.7	25.5	2.2

peridol, heparin sodium, hydroxyzine, midazolam, nafcillin sodium, phenobarbital, phenytoin sodium, prochlorperazine, ranitidine and tobramycin sulfate showed no interference with famotidine determination. A stability study of famotidine was performed at room temperature. Three different urine samples were spiked with famotidine. The concentration of famotidine in urine was then determined at various time intervals. There was a 12–18% loss of famotidine over a time period of 3–4 h. The result agrees with that reported earlier [3]. Fig. 3 shows the stability curve of famotidine in one of the urine samples. After 54 h the urine sample showed a 23% drug loss.

The extraction efficiency of famotidine from urine was found to be 100 ± 1%. It was calculated from the ratio of the slopes obtained from the calibration curve for urine and the curve from the non-extracted standards. The limit of detection for the method was found to be 70 ng/ml at a signal-to-noise ratio of 2.

DISCUSSION

In the method developed, the famotidine after extraction was dissolved in acetonitrile. Acetonitrile, however, is a much stronger solvent than the mobile phase. As observed by others and us [5,6], injection of eluites in a solvent that is stronger than the mobile phase produces a leading peak and sometimes multiple peaks. In the case of famotidine a leading peak was produced. This peak distortion in turn gave a reduction in peak height. Water was added to acetonitrile to remove peak distortion. The volume added was chosen to make the concentration in the diluted extract equal to that in the urine (100% of the famotidine was extracted). The dilution reduced the peak height but resolution was improved through less band spreading.

Further study showed that salts in the water used for acetonitrile dilution have a profound effect on the famotidine peak height. Fig. 4 shows typical results. By

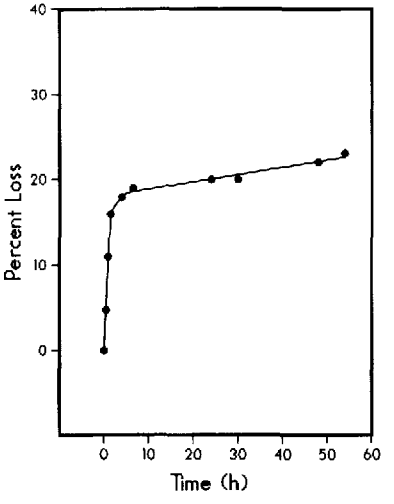

Fig. 3. Percent loss in famotidine in an urine sample at room temperature over a time period of 54 h.

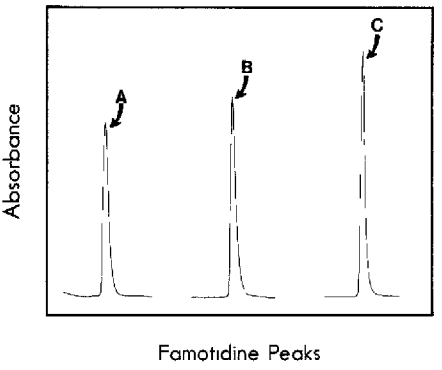

Fig. 4 Famotidine peaks in different injection solvents: (A) in acetonitrile; (B) in acetonitrile–water (50:50); (C) in acetonitrile–3 m*M* potassium carbonate (50:50). For each peak the amount of famotidine injected was 1.0 μg. The injection volume was 40 μl and the detector sensitivity was 0.10 a.u.f.s.

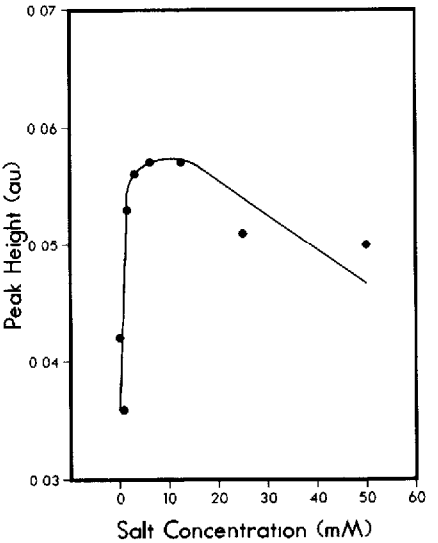

Fig. 5. Peak height of famotidine at different concentrations of potassium carbonate used to dilute the famotidine extract. For all points, injection volume and injection amount were 40 μl and 1.0 μg, respectively.

water dilution of the acetonitrile solution which gave chromatogram A, a taller peak (B) was obtained. Close inspection reveals that this occurred at the expense of the small leading section of the peak in chromatogram A. Making the same dilution with 3 m*M* potassium carbonate gave an even taller peak (C).

A systematic study of the effect on peak height of the salt concentration in the water used to dilute the famotidine extract was made by using potassium chloride, potassium carbonate, dipotassium hydrogenphosphate, potassium dihydrogenphosphate and sodium carbonate. Fig. 5 shows the curve obtained with potassium carbonate. As the salt concentration increased the famotidine peak height in-

creased until a maximum was reached in the concentration range 3–15 m*M*. The peak height, on further potassium carbonate concentration increase, dropped. This curve was typical of those found with the other salts studied, that is, within experimental error there appeared to be little or no relationship between the type of salt and its effect on peak height. However, in the final method potassium carbonate was chosen to be the salt in the injection solvent because it gave as high or higher famotidine peak than the other salts.

CONCLUSION

The HPLC method developed for famotidine was simple and rapid. The typical assay time was less than 20 min. The method described here was more sensitive than the previously reported method for urinary famotidine [3]. The analytical column showed no significant deterioration even after several hundred injections. Urinary famotidine was somewhat unstable. However, because its rate of disappearance was slow and the method developed was rapid, accurate and precise quantitation of the drug in urine was possible. Because some common drugs did not show any chromatographic interference, the method can be used to monitor the famotidine level in patients who would receive those drugs.

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