BIOMEDICAL CHROMATOGRAPHY, VOL. 6, 222–223 (1992)

A Simple Method for the Quantification of Famotidine in Human Plasma and Urine by Paired-ion High Performance Liquid Chromatography

Yasuhiko Imai* and Shin-ichiro Kobayashi

Drug Metabolism Department, Applied Pharmacology & Development Laboratories, Yamanouchi Pharmaceutical Co. Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, Japan

A new method for the quantification of famotidine consists of a simple extraction procedure and paired-ion high performance liquid chromatography with ultraviolet detection. The method has good accuracy and precision and should be suitable for the routine measurement of plasma and urine samples for clinical studies.

INTRODUCTION

Famotidine, one of the histamine H_2-receptor antagonists, is a higher potent inhibitor than cimetidine and ranitidine to gastric acid secretion in man and laboratory animals (Takagi *et al.*, 1982; Pendleton *et al.*, 1983). The drug is widely used for the treatment of gastric ulcers, duodenal ulcers, Zollinger–Ellison syndrome and hypersecretion, and its clinical pharmacokinetic properties have been evaluated after intravenous and oral administration (Campoli-Richard and Clissold, 1986; Kroemer and Klotz, 1987). For the quantification of famotidine in plasma and urine, several methods using high performance liquid chromatography (HPLC) have been reported. In an HPLC–fluorescence detection method (Kawai *et al.*, 1984), reproducible formation of a fluorescent product from famotidine was not obtained because of the instability of the drug under pre-column reaction conditions. Therefore, ultraviolet(UV) absorption of famotidine was used for the HPLC detection system (Vincek *et al.*, 1985; Carlucci *et al.*, 1988; Rahman and Hoffman, 1988). However, in these methods, no internal standard (I.S.) was used. Famotidine is not easily extracted from biological samples and so the measurement of the samples without an I.S. should produce a large variation of data. As described above, previous methods are not consistently reproducible, and therefore it has been necessary to develop a more reliable method for the determination of famotidine in biological samples. In this paper, a simple and reliable method using a suitable I.S. and paired-ion HPLC with UV detection is described.

EXPERIMENTAL

Reagent and chemicals. Famotidine and YAS-423 (3-[[[2-(diaminomethylene)-amino]4-thiazolyl]-methyl]thio)-N^2-methylsulphonylpropionamidine, I.S.) were synthesized in our Central Research Laboratories (Tamazawa and Arima, 1983). Sodium dodecyl sulphate (SDS), sodium carbonate and potassium hydroxide were purchased from Wako Pure Chemical Industries (Tokyo, Japan). Sodium hydrogen carbonate, 2 N hydrochloric acid, 1 N phosphoric acid and organic solvents were obtained commercially from Kanto Chemical (Tokyo, Japan). All reagents were guaranteed grade and used without further purification. A stock solution (1 mg/mL) of famotidine was prepared in methanol and stored at 4 °C. Standard solutions with various concentrations of famotidine were prepared in distilled water from the stock solution for each assay run.

Assay procedure. To 1 mL of plasma sample or 0.1 mL of urine sample in a 10 mL centrifuging tube equipped with a stopper, 1 mL of 0.2 M carbonate buffer (pH 10) containing 500 ng of I.S. and 6 mL of ethyl acetate were added. The tube was shaken for 10 min and centrifuged at 500 × g for 5 min; the organic layer was then transferred to another 10 mL centrifuging tube. To the organic layer, 0.2 mL of 0.1 M phosphoric acid was added. After shaking for 10 min and centrifuging at 500 × g for 5 min, the organic solvent was aspirated. A part of the aqueous layer was introduced into a HPLC system. HPLC was carried out using a LC-6A pump (Shimadzu, Tokyo, Japan), a SIL-6B autosample injector (Shimadzu) and a column (25 cm × 4.6 mm i.d.) packed with TSK-gel ODS-80-Tm (TOSOH, Tokyo, Japan). The column was maintained at 50 °C in a CTO-6A column oven (Shimadzu). A mixture of 100 mM phosphoric acid containing 10 mM SDS and acetonitrile (65:35, v/v) was used as a mobile phase at a flow rate of 1.0 mL/min. Effluent was monitored using a SPD-6A UV detector (Shimadzu) at a wavelength of 266 nm.

RESULTS

In the present method, ethyl acetate extract from the biological sample was back-extracted to the aqueous layer in order to purify the injection sample for HPLC analysis. After such extraction procedures, net recovery of famotidine was 37% for plasma, and 36% for urine. On HPLC chromatograms of human plasma and

* Author to whom correspondence should be addressed.

0269–3879/92/050222–02 $06.00

Received 2 November 1991
Accepted 30 April 1992

Table 1. Accuracy and reproducibility of the method for the determination of famotidine in human plasma and urine samples

Prepared concentration (ng/mL)	Measured concentration (ng/mL)[a]	*n*	CV (%)
Plasma			
5	5.4 ± 1.0	4	18.5
50	48.9 ± 0.8	4	1.6
500	530 ± 6	4	1.2
Urine			
50	55 ± 2	4	3.6
500	518 ± 6	4	1.2
5000	5094 ± 36	4	0.7

[a] Values are expressed as mean ± standard deviation.

urine samples, the peaks of famotidine and I.S. resolved well from numerous peaks of endogenous components. A calibration curve constructed by plotting peak height ratios of famotidine to I.S. versus famotidine concentrations was linear in the concentration range of 5–500 ng/mL in plasma with a correlation coefficient (r) of 0.999, and 50–5000 ng/mL in urine with r of 0.999. The detection limit of famotidine (S/N ratio = 3) was 5 ng/mL for plasma and 50 ng/mL for urine. Intra-assay deviation of the method was examined by replicate measurements of plasma and urine samples prepared at several concentrations. The results shown in Table 1 indicate that the method has sufficient accuracy and precision for both plasma and urine samples, except for the coefficient of variation at the concentration of 5 ng/mL in plasma samples.

DISCUSSION

Famotidine is unstable in strong alkaline solution, possibly due to the hydrolysis of a sulphonamide group in the molecule. In the HPLC–fluorescence detection method described by Kawai *et al.* (1984), strongly basic conditions have been adopted for the precolumn reaction of famotidine with 1,4-naphthoquinone, resulting in uncertain formation of a fluorescent product. Famotidine was stable for about 6 h in the 0.2 M bicarbonate buffer (pH 10) used for the extraction process of the present method. The solubility of famotidine in organic solvents is very low because of the high hydrophilicity of a guanidino group. Vincek *et al.* (1985) reported that the use of ethyl acetate for the extraction of famotidine from plasma gave highly variable recovery, and therefore adopted a solid phase extraction. However, the recovery of famotidine from plasma and urine after the sample preparation process in our method was relatively constant over the wide range of concentrations. In addition, the variability of measurements could be depressed by using a suitable I.S., YAS423, which corrected the variation of the famotidine extraction rate. The detection limit for plasma in the present method was 5 ng/mL. The sensitivity is identical to those of previous methods reported by Kawai *et al.* (1984) and Vincek *et al.* (1985) and is higher than that of the method by Carlucci *et al.* (1988) with a detection limit of 10 ng/mL. In their methods, famotidine was determined without an I.S., causing a large variation in the measured values at the concentrations around the detection limit. On the other hand, the method in this report has sufficient sensitivity and reproducibility to permit the pharmacokinetic analysis of plasma concentrations of famotidine administered at the minimum oral effective dose of 20 mg. The detection limit of 50 ng/mL for urine in this method is lower than those of other methods (Vincek *et al.*, 1985; Carlucci *et al.*, 1988, Rahman and Hoffman 1988) with detection limits of 500 ng/mL, 100 ng/mL and 70 ng/mL, respectively. The sensitivity is lower than that for plasma but sufficient for the determination of clinical urine samples, because famotidine is hardly metabolized in humans and most of the drug is excreted into the urine in an unchanged form.

REFERENCES

Campoli-Richard, D. M. and Clissold, S. P. (1986). *Drugs* **32**, 197.

Carlucci, G., Bioldi, L., Napolitano, T. and Bologna, M. (1988). *J. Pharmaceutical and Biomedical Analysis* **6**(5), 515.

Kawai, R., Yamada, S., Kawamura, S., Miwa, T. and Miwa, M. (1984). *Pharmacometrics* **27**(1), 73.

Kroemer, H. and Klotz, U. (1987). *Therapy and Toxicology* **25**, 458.

Pendleton, R. G., Torchiana, M. L., Chung, C. Cook, P., Wiese, S. and Clineschmidt, B. V. (1983). *Arch. Int. Pharmacodyn.* **256**, 4

Rahman, A. and Hoffman, N. E. (1988). *J. Chromatogr.* **428**, 305.

Takagi, T., Takeda, M. and Maeno, H. (1982). *Arch. Int. Pharmacodyn.* **256**, 49.

Tamazawa, K. and Arima, H. (1983). *J. Labelled Compound Radiopharm.* **20**(10), 1193.

Vincek, W. C., Constanzer, M. L., Hessey, II G. A. and Bayne, W. F. (1985). *J. Chromatogr.* **374**, 209.