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Validation of a method for the assay of related compounds in famotidine raw materials and formulations*

N. BEAULIEU,† S. J. GRAHAM, R. W. SEARS and E. G. LOVERING

Bureau of Drug Research, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Abstract: A high-performance liquid chromatographic (HPLC) method has been developed for the determination of famotidine and related compounds in drug raw materials and formulations. The minimum detectable amount of the available related compounds is less than 0.02% and the minimum quantifiable amount is less than 0.1%. Famotidine impurity levels were between 0.5 and 2.5% in raw materials, 0.44% in one tablet sample and about 3% in an IV solution, allowing for stabilizers.

Keywords: *Famotidine; H2-receptor antagonists; related compounds; HPLC.*

Introduction

A recently developed HPLC method for ranitidine and related compounds [1] has been modified for determination of synthetic precursors and degradation products in famotidine drug raw materials and formulations (Table 1 and Fig. 1).

Famotidine (**VIII**) is not official in the European, British or United States Pharmacopoeias. A reversed-phase HPLC method for determination of famotidine in formulations provided no data to show that related compounds are resolved from the drug [2], nor did a study of the stability of famotidine in infusion fluids [3]. Methods for the determination of famotidine in biological fluids continue to appear [4, 5].

Experimental

Chemicals

Acetonitrile, phosphoric acid and ammonium hydroxide (Baker Co., Phillipsburg, NJ) and ammonium phosphate (Fisher Scientific, Fairlawn, NJ) were HPLC grade. Deionized water was used. Drug raw materials and related compounds were obtained from Torcan Chemicals Ltd, Toronto (**I–VIII**), Uquifa, Barcelona (**VI, VII** and **VIII**) and Yamanouchi Pharmaceutical Co., Tokyo (**III, IV, V, VIII, IX, X** and **XI**).

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†To whom correspondence should be addressed.

Table 1
Famotidine related compounds

I	3-[[2-[(Diaminomethylene)amino]-4-thiazolyl]methyl]sulphinyl-N^2-sulphamoylpropionamide maleate
II	3-[[[2-[(Diaminomethylene)amino]-4-thiazolyl]methyl]thio]propionic acid
III	3-[[[2-[Diaminomethylene)amino]-4-thiazolyl]methyl]thio]propionamidine hydrochloride
IV	Bis-[2-[(diaminomethylene)amino]-4-thiazolyl]methyl disulphide (mol. wt 374)
V	3,5-Bis-[2-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]-thio]ethyl]-4H-1,2,4,6-thiatriazin-1,1-dioxide dimaleate
VI	3-[(2-Guanidinithiazole-4-yl)methylthio]propionyl sulphamide
VII	Methyl 3-[(2-guanidinothiazol-4-yl)methylthio]propionate
VIII	Famotidine: 3{[([2-(Diaminomethylene)amino]-4-thiazolyl)methyl]thio}-N-sulphamoyl-propionamide
IX	3-[[[2-[(Aminoiminomethyl)amino]-4-thiazolyl]methyl]thio]propanimidic acid methyl ester
X	3-[[[2-[(Diaminomethylene)amino]-4-methyl]thio]propionitrile
XI	3-[[[2-[(Diaminomethylene)amino]-4-thiazolyl]methyl]thio]propionamide

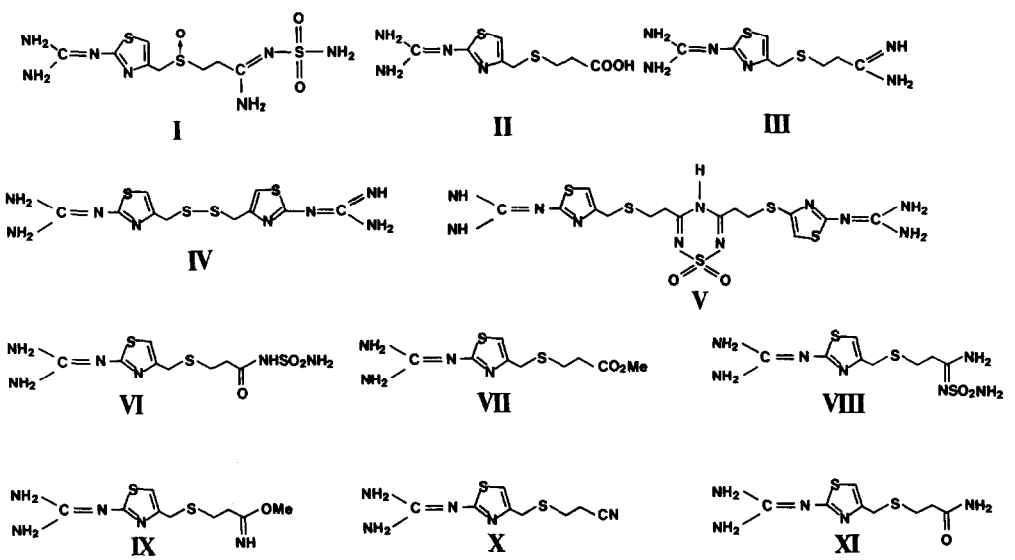

Figure 1
Chemical structures of famotidine and the available related compounds.

Apparatus

The HPLC system (Varian 5060) was fitted with a 5 μl loop injector (Spectra-Physics Model SP8780 XR), a data station (Varian Vista model 402) and a variable wavelength detector (Varian Model UV–100) set at 269 nm. Spherisorb 3 μm cyano bonded phase columns (150 × 4.6 mm, Chromatography Sciences Co.), were used at ambient temperature with mobile phase flow rates of 1.0 ml min^{-1}. Other equipment used was as follows: centrifuge — International Equipment Co. Model K; UV–vis spectrophotometer — Varian DMS 90 connected to a HP 85 computer with plotter and disk drive; wrist action shaker — Burrell Model 75; yellow light — Sylvania "Gold" fluorescent tubes.

Mobile phase

Buffer–acetonitrile (85:15, v/v). The buffer was prepared by adding 0.05 M phosphoric acid to 0.05 M dibasic ammonium hydrogen phosphate to obtain a final pH of 7.0.

Solutions

The drug substance was dried under vacuum at 80°C for 5 h. The following solutions were made with acetonitrile–deionized water (50:50, v/v): resolution solution (0.04 mg ml^{-1} famotidine standard and 0.04 mg ml^{-1} compound **X**); related compounds standard solution (0.004 mg ml^{-1} famotidine standard); related compounds test solution (2.0 mg ml^{-1} famotidine). The test solution for tablets was prepared by grinding 20 tablets, shaking a portion equivalent to 20 mg of drug in 10 ml of acetonitrile–water (50:50, v/v) for 20 min and filtering. Liquid formulations were diluted quantitatively with the same solvent to a final concentration of 2 mg ml^{-1}.

System suitability

Five 5-μl aliquots of the resolution solution were injected into the chromatograph. The system was deemed to be suitable for use if the resolution between famotidine and **X** was greater than four, the efficiency of the column, calculated using the drug peak, was not less than 25,000 plates/meter, the tailing factor was 1.5 or less and the relative standard deviation of the five famotidine peak responses was no more than 5%. The retention times of famotidine and **X** were typically 3.1 and 4.5 min. In some cases, column conditioning for 24 h was helpful.

Procedure

Five 5-μl aliquots of the related compounds standard solution and the related compounds' test solution were injected into the chromatograph and the chromatogram recorded for 30 min. The percentage of each impurity in the test sample was calculated from $[100(A_i/A_r)(C_r/C_u)]$ where A_i is the peak area response of each impurity peak, A_r is the peak area response of the drug peak in the standard solution, and C_u and C_r are the concentrations of drug in the test and standard solutions, respectively.

UV spectra

The UV spectra of famotidine and its related compounds were measured in acetonitrile–water (50:50, v/v).

IR spectra

The IR spectra (0.3% KBr) of the famotidine samples were virtually identical (1638, 1534, 1459, 1428, 1332, 1320, 1172, 1161, 1150, 1148 cm^{-1}), except for a sample of another polymorph of the drug, referred to as B (Torcan) [1672, 1645, 1549 (broad), 1459, 1329, 1140 (broad) cm^{-1}].

Results

Figure 2 shows the resolution of famotidine (**VIII**) from a number of related compounds. Compound **XI**, eluting shortly after the drug is resolved sufficiently at the 0.1% level to be integrated, although there may be some loss of resolution after several weeks of column use. The UV maxima and relative absorbance, and the HPLC relative responses and retention times for the available related compounds are listed in Table 2. The response of the HPLC system was linear over the range 0.0056–2.01 μg famotidine injected onto column (1.12–4.05 μg ml^{-1}) and from 0.0035 to 0.11 μg injected onto column (0.7–22 μg ml^{-1}) for the available related compounds. The relative standard deviation (precision) of the system, determined by making six replicate injections of a 0.1 mg ml^{-1} solution of famotidine, was less than 1%.

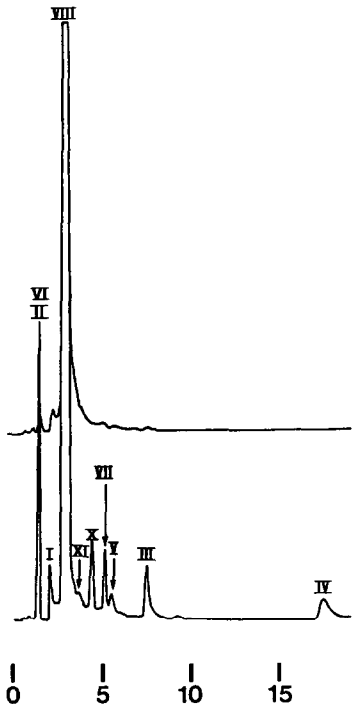

Figure 2
Famotidine (upper chromatogram) and a mixture of famotidine and available related compounds (lower chromatogram). The amount on the column of each compound was: **I** (0.013 μg); **II** (0.018 μg); **III** (0.020 μg); **IV** (0.018 μg); **V** (0.020 μg); **VI** (0.016 μg); **VII** (0.019 μg); **VIII** (famotidine) (10 μg); **X** (0.016 μg) and **XI** (0.016 μg).

Table 2
UV and HPLC characteristics of famotidine related compounds

	UV absorbance			HPLC characteristics	
Compound	Conc.* (μg ml^{-1})	Maximum (nm)	Relative absorptivity†	Relative response‡	Relative retention§
I	6.81	269	1.14	0.45	0.78
II	10.92	265	1.57	1.20	0.60
III	8.45	283	1.19	0.88	3.35
IV	9.08	287	1.84	1.33	4.91
V	7.63	277	1.68	0.69	1.69
VI	9.96	268	1.06	0.84	0.60
VII	8.54	265	1.37	1.11	1.59
VIII	11.15	287	1.00	1.00	1.00
IX	11.0	284	1.39	0.79	1.1
X	19.2	285	1.28	1.16	1.5
XI	13.0	285	1.25	1.20	1.1

*The solvent was acetonitrile–water (50:50,v/v), except for **IX**, **X** and **XI** which were determined in acetonitrile–water (30:70, v/v).

†Absorbance at 269 nm relative to famotidine absorbance of 0.278.

‡Relative to a response of 330 area counts per nanogram for famotidine. The correlation coefficients for the concentration response curves were 0.99 or greater.

§Retention time relative to famotidine at 3.1 min.

Table 3
Relative abundance of related compounds in famotidine and formulations %, (quantitate against an external formation standard)*

RRT†	A	B	C	D	E	F‡	G
0.58	0.24	0.23	0.15	0.45	0.16	2.4	0.14
0.69							0.10
0.73	0.11	0.13	0.17	0.19	0.16		
0.76							0.07
0.83	0.06	0.07	0.09	0.14	0.08	1.23	0.03
0.89				0.08			
1.07						0.25	
1.52				0.15		0.03	
1.73				0.75			
1.84		0.02			0.02	0.05	0.05
1.95	0.02		0.02	0.04	0.07		
2.2				0.22		0.04	
2.5				0.02		0.08	
5.5	0.11	0.18	0.17	0.44	0.25	0.07	0.04
Total	0.54	0.63	0.60	2.48	0.74	2.9	0.43

*Samples A–E are drug substances, F is an injectable solution, 10 mg ml^{-1}, and G is 20 mg tablets.
†Relative to famotidine at a retention time of 3.1 min. Relative retention times of the related compounds are given in Table 2.
‡This sample is labelled to contain 1-aspartic acid, mannitol and benzyl alcohol, in addition to famotidine. The former elutes at RRT 0.45 and the latter at 0.83. Mannitol does not absorb significantly. The total excludes the compound eluting at 0.83.

Ruggedness of the method

No apparent change in the concentration of related compounds was observed when 4 mg ml^{-1} solutions of famotidine in acetonitrile–water (50:50,v/v) were exposed to yellow light for 10 h at room temperature.

The famotidine method was developed, and the results checked, on two columns. An increase in the pH from 7.0 to 7.5, or a decrease in the proportion of acetonitrile in the mobile phase, lead to an increase in the relative retention time (RRT) of **IV**. A decrease in pH to 6.5, or an increase in the proportion of acetonitrile, resulted in a decrease in the RRT of **III** and **VII**. Other peaks were little affected by these changes.

Analysis of available products

Five samples of famotidine raw material, one of tablets and one injectable were analysed for related compounds (Table 3).

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