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Short communication

High-performance liquid chromatographic separation and determination of small amounts of process impurities of famotidine in bulk drugs and formulations[1]

Sajid Husain*, Sara Khalid, V. Nagaraju, R. Nageswara Rao

Analytical Chemistry Division, Indian Institute of Chemical Technology, Hyderabad-500 007, India

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Abstract

A simple and rapid high-performance liquid chromatographic method was developed for the separation and determination of small amounts of process impurities viz., thiourea, chloro-N-(aminosulphonyl)propanimidoamine, diaminomethyleneamino(chloromethyl)thiazole and diaminomethyleneamino(1-amino-1′-iminomethlene)thiomethylthiazole in famotidine. The separation was achieved on a reversed-phase C_8 column using acetonitrile–0.01 *M* aqueous potassium dihydrogenphosphate (25:75, v/v; pH 3.15) as eluent. The method was used not only for quality assurance but also for monitoring the reactions involved in process development of famotidine. The mean recovery of famotidine from authentic samples was 99.48±1.87% and the limit of detection was $5 \cdot 10^{-9}$ g.

Keywords: Pharmaceutical analysis; Famotidine

1. Introduction

Famotidine (FAMT), 3-[({2-[(aminoiminomethyl)amino]4-thiazolyl}methyl)thio]-N-(aminosulfonyl)propanimidoamide is a new H_2-receptor antagonist used in the treatment of gastric and duodenal ulcers [1,2]. It suppresses [3] the secretion of gastric acids induced by histamine and food quite effectively. It is an alternative to cimetidine and ranitidine for healing duodenal ulcers. It is produced synthetically [4,5] by a four-step convergent reactions scheme (Fig. 1). In step 1, diaminomethyleneamino(chloromethyl)thiazole hydrochloride (DACT) is prepared by cyclo addition of 1,3-dichloroacetone (DCA) and guanylthiourea (GTU) initially. Later, it is reacted with thiourea (THU) to obtain diaminomethyleneamino(1-amino-1′-iminomethlene) thiomethylthiazole hydrochloride (DAIT) (step 2). In step 3, chloropropionitrile (CPN) and sulfamide (SFA) are reacted to get 3-chloro-N-(aminosulfonyl)propanimidoamine hydrochloride (CAPA) separately. Finally DAIT and CAPA are condensed together and FAMT is produced (step 4). It is likely that the unreacted intermediates and their precursors during this process may be present in small quantities as impurities in FAMT and reduce its quality. Therefore, separation and determination of FAMT and its process components is quite important not only for quality assurance but also for monitoring the reactions involved in process development.

*Corresponding author.

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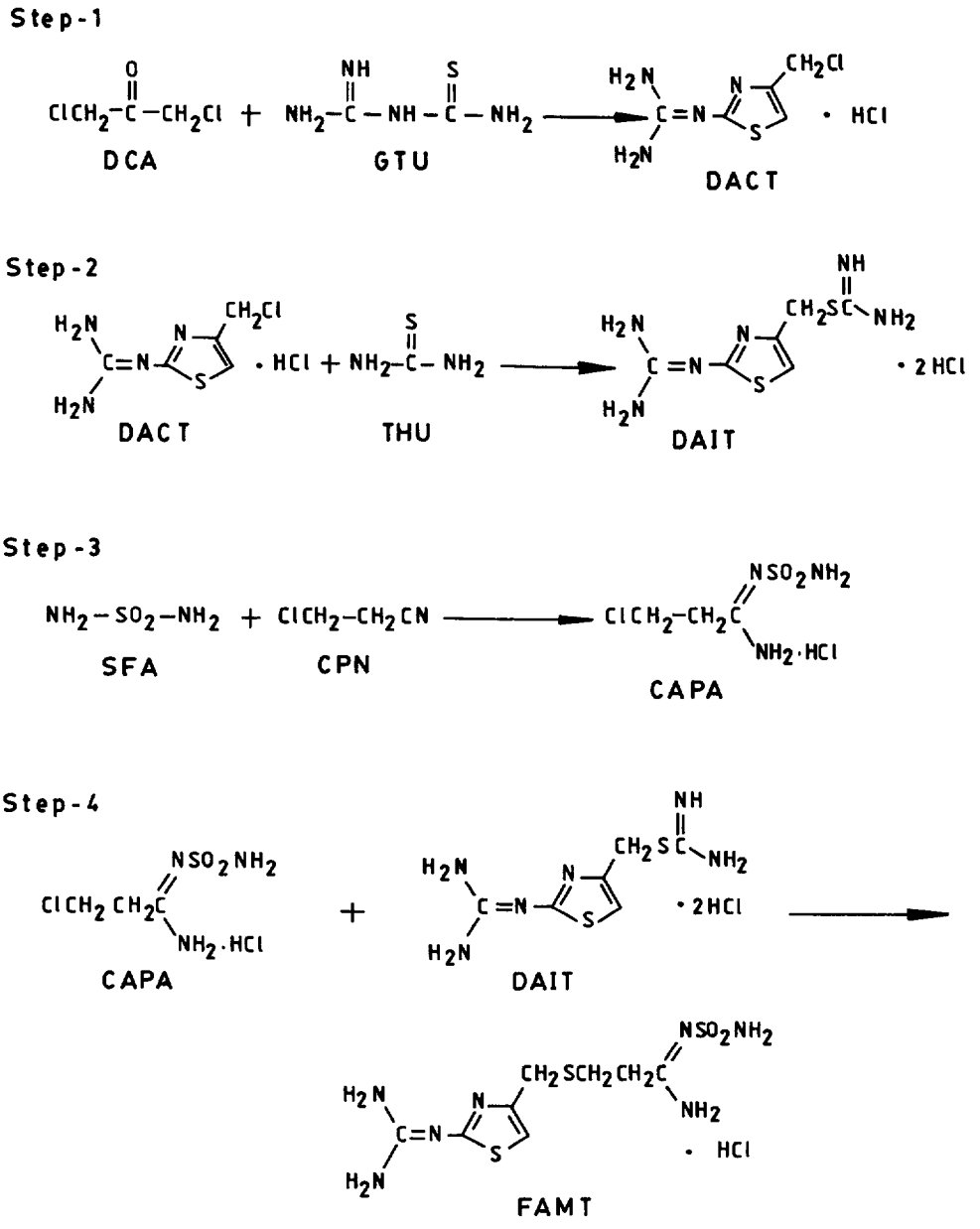

Fig. 1. Reactions involved in the preparation of famotidine hydrochloride.

Different high-performance liquid chromatographic (HPLC) methods for determination of FAMT and its metabolites in biological fluids have been reported [5–8]. Nevertheless, these are not suitable for separation of process impurities in bulk drugs and formulations. Reversed-phase HPLC methods [9,10] for separation of FAMT and its degradation products in pharmaceutical formulations have been tried but are not reproducible. Biffar and Mazzo [11] have improved the reproducibility of these methods by, unconventionally, using silica as a stationary phase. Differential pulse polarographic behaviour of FAMT has been studied [12], but its adsorption on the surface of the electrodes affects the accuracy and

precision. Spectrophotometric methods [13,14] involve tedious procedures for removing excipients from tablet matrices, thus lacking simplicity and specificity. A thorough search of the literature has revealed that no single method for separation and determination of process impurities in FAMT has been reported. In this paper, we describe a simple and rapid HPLC method for separation and determination of process components of FAMT in bulk drugs and formulations using a reversed-phase C_8 column and 0.01 *M* potassium dihydrogenphosphate–acetonitrile (75:25, v/v; pH 3.15) as eluent at ambient temperature.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Glass-distilled water, HPLC-grade acetonitrile (Spectrochem, Bombay, India) and potassium dihydrogenphosphate (Qualigens, Bombay, India) were used. Samples of FAMT and its intermediates prepared by known methods were used.

2.2. Apparatus

A high-performance liquid chromatograph (Waters, Milford, MA, USA) with a 20-μl loop injector was used. A Waters 440 absorption detector was connected after the column. A reversed-phase C_8 (Spherisorb, Leonberg, Germany) column (300×3.5 mm I.D.; particle size 10 μm) was used for separation. The chromatographic and the integrated data were recorded with a Omniscribe D 5000 recorder and Chromatopac E1A integrator respectively.

2.3. Chromatographic conditions

The mobile phase was acetonitrile–0.01 *M* aqueous potassium dihydrogenphosphate (25:75, v/v) adjusted to pH 3.15 with phosphoric acid. Samples were dissolved in the mobile phase. The analysis was carried out under isocratic conditions using a flow-rate of 1.2 ml/min, at room temperature (27°C). Chromatograms were recorded at 254 nm using the UV detector.

2.4. Analytical procedure

Samples (10 mg) were dissolved in the mobile phase (100 ml) and a 20-μl volume of each sample was injected and chromatographed under the above conditions. Synthetic mixtures, bulk drug and formulations were analysed under identical conditions. The quantities of impurities were calculated from their respective peak areas.

3. Results and discussion

Fig. 1 shows the molecular structure of potential impurities of FAMT produced industrially. The

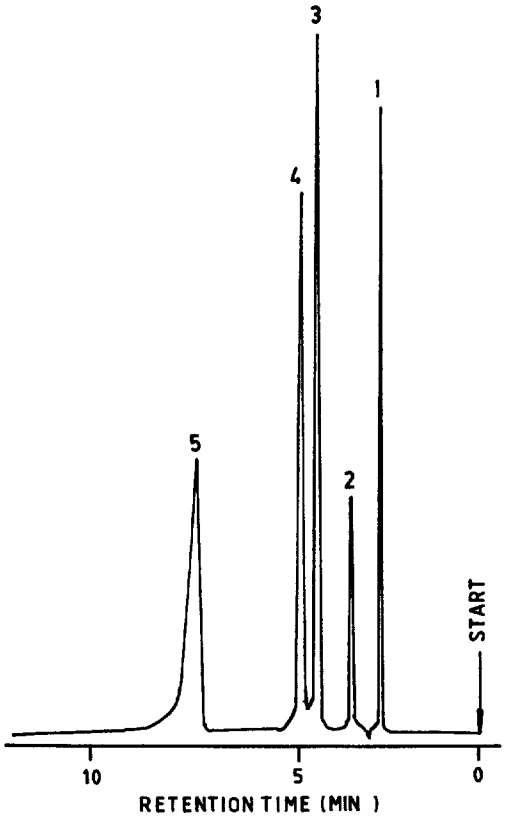

Fig. 2. Typical chromatogram of a mixture containing (1) THU (0.2 μg), (2) CAPA (20.0 μg), (3) DACT (0.2 μg), (4) FAMT (0.2 μg) and (5) DAIT (0.2 μg).

Table 1
Retention data

Compound	Abbreviation	t_R (min)	k'	R_S	λ_{max} (nm)	ASF
Thiourea	THU	2.40	0.14		250	1.25
				3.0		
Chloro-N(amino-sulphonyl)propanimidoamine	CAPA	3.96	0.89		215	1.40
				2.36		
Diaminomethylene amino(chloromethyl)thiazole	DACT	4.43	1.11		2.60	1.32
				2.86		
Famotidine	FAMT	5.08	1.42		265	1.14
				3.58		
Diamino methyleneamino(1-amino-1-iminomethlene) thiomethyl)thiazole	DAIT	7.07	2.37		280	0.71

impurities and FAMT were subjected to separation by HPLC. The chromatogram is shown in Fig. 2. The peaks were identified by injecting the individual compounds. The values of resolution (R_s) are given in Table 1.

It can be seen that FAMT is well separated from the reactants and intermediates. Due care was given to the pH of the mobile phase while standardizing the HPLC conditions. The resolution obtained was found to be pH dependent. pH values between 3.0–

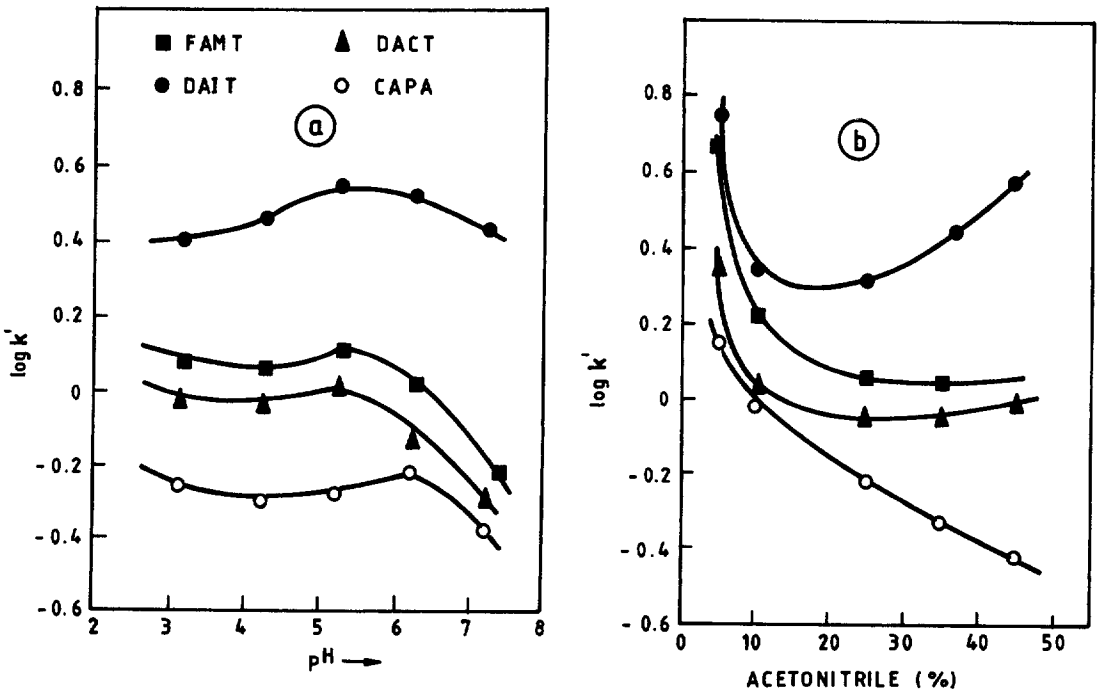

Fig. 3. Effect of (a) pH and (b) concentration of acetonitrile on retention of famotidine and its process impurities.

Table 2
Linearity data

S. No.	Compound	Mass range $\times 10^{-9}$ g	Linear regression ($y=ax+b$)[a]	r[b]
1	THU	0.4–1.5	$y_b=331x_b-12$	0.980
2	CAPA	20.5–35.0	$y_b=0.756x_b+1.8$	0.968
3	DACT	0.3–61.5	$y_b=379x_b-15$	0.987
4	FAMT	90.5–99.9	$y_a=279x_a+357$	0.995
5	DAIT	0.5–1.5	$y_b=317x_b-14$	0.979

[a] y_a=Integral area of FAMT; x_a=mass of FAMT. y_b=Integral area of impurity; x_b=mass of impurity; [b] r=correlation coefficient.

5.0 were ideal for separation. Peaks were overlapped at other values of pH. Acetonitrile was used as an organic solvent modifier to improve the separation. The effect of concentration of acetonitrile on resolution was also studied. The chromatographic separation was found to be dependent on the concentration of acetonitrile. The retention of CAPA, DACT, FAMT and DAIT as a function of pH and concentration of acetonitrile is shown in Fig. 3. It may be noted that the retention behaviour of these compounds on Spherisorb octylsilica is quite unusual. It occurs because of the fact that these compounds are highly basic in nature and probably retain on to the silanols via ion exchange. The optimum resolution between the compounds of interest was obtained using the Spherisorb C_8 with aqueous 0.01 *M* potassium dihydrogenphosphate–acetonitrile (75:25, v/v) as eluent. The peak shapes obtained using a Spherisorb column packed with 5 μm C_8 were reproducible. The peak asymmetry functions (ASF) were calculated for concentrations less than 1.0% of impurities in FAMT and are given in Table 1. From these values, it is clear that the shapes of these peaks are undistorted. Therefore, the Spherisorb C_8 column was preferred over other columns viz., Spherisorb C_{18} and CN, because it provided better resolution of the peaks with symmetry and reproducibility. The retention times (t_R), capacity factors (k') and wavelengths of maximum absorption (λ_{max}) were determined and recorded in Table 1. The UV detector was set at a wavelength of 254 nm and used both for detection and quantification. A good linearity was found between the mass and integral response of each compound under examination. The data showing the linearity between mass and integral response for different concentrations of impurities is recorded in Table 2. When the detector was at 0.001 AUFS the limit of detection for FAMT was $5.0 \cdot 10^{-9}$ g, with a signal-to-noise ratio of 4.0. The response factors for all the compounds are given in Table 3. It may be seen from Table 3 that the precision of the detector response was quite good for all the compounds.

Standard mixtures containing known amounts of

Table 3
Precision data

S. No.	Compound	Amount $\times 10^{-9}$ g	Area	Mean±S.D.[a]	Relative response factor (RRF)
1.	THU	151.8	49 025 47 674 49 404	48 701±322	1.139
2	CAPA	20 160	15 354 18 605 17 266	17 075±578	0.003
3	DACT	154.2	58 049 53 726 55 373	55 716±766	1.285
4	FAMT	150.6	41 857 40 693 45 022	42 524±792	1.000
5	DAIT	151.8	47 905 44 284 47 554	46 581±706	1.089

[a] $n=3$.

THU, CAPA, DACT, FAMT and DAIT were prepared and analysed by HPLC. The accuracy of the method was checked by standard addition technique. Small quantities of impurities were added to the sample and chromatographed. It was found that these additions were accurately reflected in their peak areas. All estimations were repeated thrice and standard deviations (S.D.) were calculated (Table 4). The measured amounts have agreed well with actual values and the mean recovery of FAMT from authentic samples was found to be 99.48±1.87% (S.D.). Further experiments for determination of the minimum detectable limits of DACT in the presence of FAMT were conducted. Synthetic mixtures containing FAMT and trace amounts of DACT were prepared and chromatographed. The chromatograms are shown in Fig. 4. The integral areas obtained for different concentrations of DACT are recorded in Table 5. When the detector was set at 0.001 AUFS, the limit of detection for DACT in the presence of 90%+ FAMT was found to be $3.7 \cdot 10^{-9}$ g with a signal-to-noise ratio of 4.0.

Reactants and intermediates involved in steps 1 and 3 of the process were chromatographed separately. The retention times of DCA, SFA and CPN were determined by injecting authentic samples and found to be 3.31, 2.85 and 11.79 min, respectively. The reaction mixtures collected at different intervals of time were analysed by HPLC and quality of the intermediates was checked. Apparently, the detector response for SFA and CPN was found to be too low for their quantitation in FAMT. Therefore, the separation of these two compounds was studied in reaction mixtures of step 3 but not in bulk drug of FAMT.

The quality of FAMT in bulk drugs and different formulations was checked thoroughly. A typical chromatogram of a commercial formulation of FAMT is shown in Fig. 5. The amounts of various impurities were determined and the purity of FAMT was calculated. The results are recorded in Table 6.

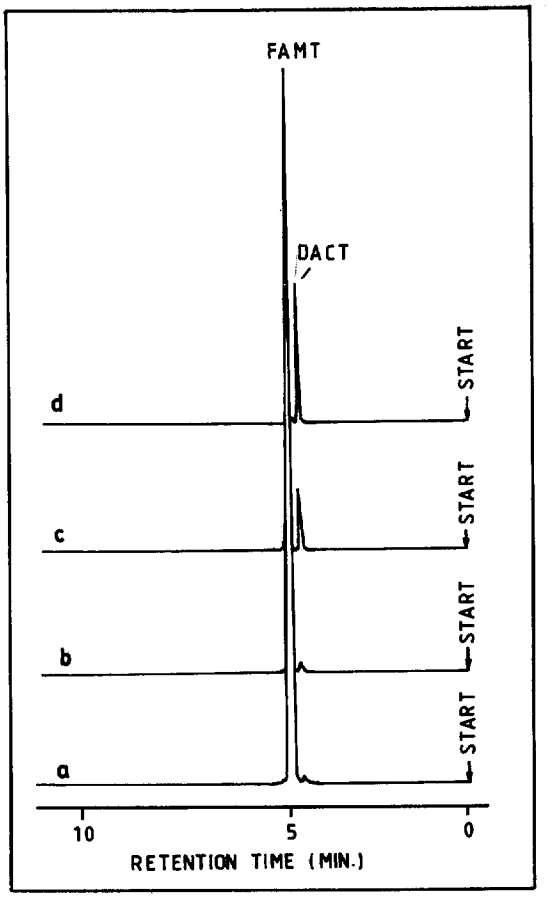

Fig. 4. Chromatogram showing the separation of trace amounts (a) 0.1%, (b) 0.2%, (c) 0.5% and (d) 1.0% DACT in presence of 90%+ FAMT.

Table 4
Analytical data for mixtures

Compound	Taken (%)	Found mean±S.D.[a]	Error (%)
THU	0.94	0.97±0.04	3.19
CAPA	1.36	1.40±0.03	2.94
DACT	0.95	0.99±0.05	4.20
FAMT	95.83	95.29±1.79	0.56
DAIT	0.92	0.95±0.04	3.26

[a] $n=3$.

Table 5
Integral data of trace amounts of DACT in the presence of 90%+ FAMT

S. No	Amount of DACT $\times 10^{-6}$ g	Amount of FAMT $\times 10^{-6}$ g	Integral area of DACT±S.D.[a]
1.	0.01	10.09	525±21
2.	0.02	9.99	1144±35
3.	0.05	9.69	2502±89
4.	0.10	9.18	5311±157

[a] $n=3$.

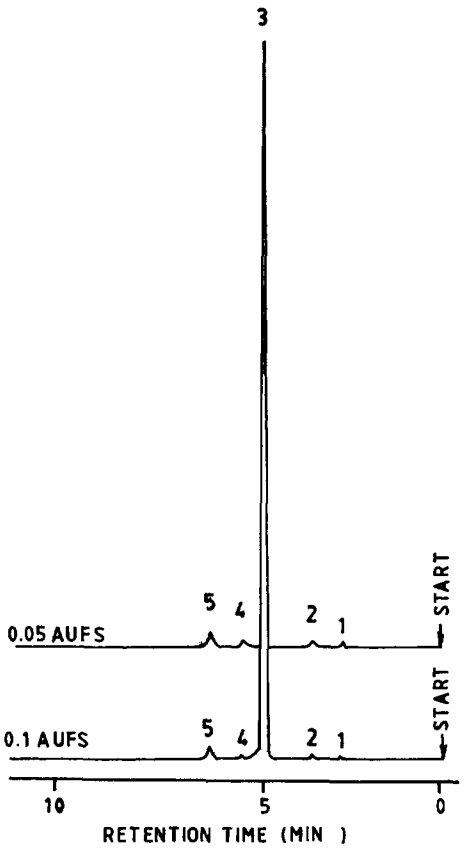

Fig. 5. Chromatogram of a commercial formulation of famotidine. Peaks: (1) THU; (2) CAPA; (3) FAMT; (4) unknown and (5) DAIT.

Table 6
Determination of process impurities in a typical sample of famotidine

S. No.	Compound	Concentration (%)	S.D. (%)
1	THU	0.15	2.18
2	CAPA	0.43	1.89
3	DACT	0.07	2.58
4	DAIT	0.59	1.94
5	Unknown	0.18	2.37

[a] $n=3$.

From these results, it is clear that the method is precise and accurate for the separation and determination of small quantities of some of the process impurities that are present in FAMT generally. In conclusion, the developed HPLC method is suitable not only for separation and determination of process impurities viz., THU, CAPA, DACT and DAIT in FAMT but also for monitoring the reaction of SFA and CPN and the quality of CAPA prepared during the process, Thus the method is suitable not only for process development but also quality assurance of FAMT and related products.

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