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Impurity profiling of Famotidine in bulk drugs and pharmaceutical formulations by RP-HPLC method using ion pairing agent

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Abstract

An efficient reversed phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for quantitation of famotidine and its process impurities which may coexist in bulk drugs and in solid pharmaceutical dosage forms. The separation was achieved on a C_{18} column (250 mm x 4.6 mm) using a mobile phase of acetonitrile, methanol and 1-Hexane sodium sulfonate. Flow rate was 1.5 ml/min. The photo diode array detector was operated at 266 nm. The method was validated for specificity, linearity, precision, accuracy and limit of quantification. The degree of linearity of the calibration curves, the percent recoveries of famotidine and impurities, the limit of detection and quantitation, for the HPLC method were determined. The method was found to be simple, specific, precise, accurate and reproducible. The method was applied for the quality control of commercial famotidine tablets to quantify the drug and its related substances and to check the formulation content uniformity.

Key words: Famotidine; Impurity profiling; Ion pairing agent; Reversed-phase HPLC.

INTRODUCTION

Famotidine, 3-[({2-[(aminoimino-methyl) 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 acid induced by histamine and food effectively. It is an alternative to cimetidine and ranitidine for healing duodenal ulcers. The active ingredient famotidine has eight potential process impurities (Figure.1) namely 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]propanoic acid(Carboxylic acid impurity), 3-[[[2-[(diaminomethylene)amino]thiazol -4-yl] methyl]sulphanyl]propionamide (Propionamide impurity), 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]isothiourea

dihydrochloride monohydrate(Isothiourea impurity), 3-[[[2-[(diaminomethylene)amino]thiazol-yl]methyl]sulphanyl]-N-sulphamoyl propanamide (Sulfamide impurity), 3-[[[2-[(aminoiminomethyl)amino]-4-thiazolyl]methyl]thio]propanimidic acid methyl ester (Methyl ester impurity), 3-5-bis-[2-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]-ethyl]4H-1,2,4,6-thiatriazine 1,1-dioxide (Bis-Famo impurity), methyl, 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]thio]propionate (propionate impurity) and 3-[[[2-[(diaminomethylene) amino] thiazol-4-yl] methyl] sulfinyl]-N-2-sulfamoyl propionamide (sulfoxide impurity). These impurities may be present in small quantities and reduce the quality of famotidine. Therefore separation and quantitation of famotidine and its process impurities quite important not only for quality assurance but also for monitoring the reactions involved in process development. A literature survey revealed that different high-performance liquid chromatographic (HPLC) methods for determination of famotidine in biological fluids have been reported [4-11]. However two literature assays by N.Beaulieu [12] and Sajid Husain [13] using HPLC were shown to be selective and sensitive enough to monitor impurities. The main aim of the present work was to develop selective and sensitive HPLC method for separation and determination of famotidine and its impurities with better resolution and shortest possible retention time.

The proposed method has also been validated according to the ICH guidelines [14] and applied for controlling of commercially available famotidine tablets containing 20 mg of active substance.

MATERIALS AND METHODS

Experimental

Chemicals and reagents:

All reagents used were of an analytical grade. HPLC grade 1-Hexane sodium sulfonate, acetonitrile and methanol were obtained from E.Merck (India) LTD, Mumbai. Ultra purified water, purified using an ELGA water purification unit (Bucks, UK), was used to prepare the sample and standard solutions.

Chromatographic equipment and conditions:

A high-performance liquid chromatograph (Waters, Milford, MA, USA) consisting of a 600E pump, a 996 photo diode array (PDA) detector, a 717-autoinjector and a degasser module was used. Data was acquired and processed using millennium software ver 2.1.

The mobile phase was made by first preparing a buffer solution of 1-Hexane sodium sulfonate (1.8 g of 1-Hexane sodium sulfonate in 1000 ml of water, pH adjusted to 3.5 with glacial acetic acid). 900 volumes of this buffer solution was then mixed with 94 volumes of acetonitrile and 6 volumes of methanol to yield mobile phase A. Mobile phase B was prepared by mixing 100 volumes of pH 3.5 buffer and 900 volumes of acetonitrile.

Separations were performed on a Hypersil BDS C_{18} column (250 x 4.6 mm, 5 µm particle size). The mobile phase flow rate was 1.5 ml/min with ambient column temperature, 20 µl injection volume and UV detection at 266 nm. Two step gradient profiles begin with a 20 min linear gradient from 100% mobile phase A to mobile phase A-mobile phase B (90: 10 v/v). This is

followed by a linear ramp over the next 15 min to mobile phase A-mobile phase B (80:20 v/v). Finally, the composition is returned to 100% mobile phase A over 2 min followed by a 8 min equilibration for a total run time of 45 min.

Standard preparation:
Standard solutions were prepared by dissolving famotidine and all the impurities in the mobile phase A to obtain concentration of 0.5 mg/ml for famotidine and 2.5 µg/ml for all the impurities.

Sample preparation:
Twenty tablets were weighed and ground to a homogenous powder. A portion of the powder equivalent to 25 mg of famotidine was transferred to a 25 ml volumetric flask. 20 ml of the mobile phase A was added and the flask was placed in an ultrasonic bath for 30min. The volumetric flask was filled with mobile phase A to the mark and the solution was filtered. From this stock solution, 0.15 mg/ml of famotidine solution was prepared and injected in to the column.

RESULTS AND DISCUSSION

The method described herein was developed for the simultaneous determination of the famotidine and its potential impurities present in bulk drugs and in pharmaceutical formulations. The chromatogram was shown in figure 2. The peaks were identified by injecting the individual compounds. Individual chromatograms were shown in figure 3.

In the reversed- phase chromatography of nitrogen containing bases on chemically bonded ODS-silica; peak tailing, poor separation and reproducibility are often considerable problems. These problems are probably due to the presence of residual silanol groups on the surface of the column material. To solve these problems 1-Hexane sodium sulfonate (anionic ion pairing agent) was added to the mobile phase.

The ion-pair reagent is attracted to the stationary phase because of its hydrophobic alkyl group, and the charge carried by the reagent (C_6-So^-_3) there by attaches to the stationary phase. This negative charge on the stationary phase is balanced by positive ions (Na^+) from the reagent. A positively charged sample ion (protonated base) can now exchange with Na^+ ion, resulting in the retention of the sample ion by an ion exchange process. By this way hexane sulfonate adjusts the retention and improves the resolution.

The optimum resolution between the compounds of interest and better peak shapes were obtained using the Hypersil BDS C_{18} column with proposed gradient method. The UV detector was set at a wavelength of 266 nm and used both for detection and quantification. Figure 4 shows the UV spectra of famotidine impurities. A good linearity was found between the mass and integral response of each compound under investigation. The data showing the linearity between mass and integral response for different concentrations of impurities is recorded in table 1.

For the evaluation of method precision (Table 2) and accuracy (Table 3) the standard deviation (SD), coefficient of variation (CV) and recoveries were calculated. Standard deviation and coefficient of variation have the required small values.

For quantitative analysis it was important to define the values of limit of detection (LOD) and limit of quantification (LOQ) (Table 4). The signal-to-noise ratio of 3:1 and 10:1 were taken as LOD and LOQ respectively.

After evaluation, the proposed method was applied for the determination of the content of famotidine in commercially available tablets. The found content of famotidine ranged from 98.0 to 99.5 % (Table 5).

In conclusion the proposed method for separation and quantitation of famotidine and its impurities has been shown to be simple, specific, accurate and precise for routine quality control analysis of famotidine in bulk drugs and in pharmaceutical formulations.

Table 1: Linearity Data

Substance	Concentration range (µg/ml)	b	a	r
Famotidine	0.005-200	7364.2	90.72	0.9998
Sulfoxide impurity	0.04-90.0	3987.5	0.7959	0.9999
Propionamide impurity	0.06-90.0	13489.7	41.87	0.9998
Carboxylic acid impurity	0.07-80.0	14802.9	45.32	0.9999
Propionate impurity	0.05-120	12374.4	30.56	0.9999
Sulfamide impurity	0.06-80.0	10718.5	22.59	0.9999
Iso thiourea impurity	0.07-80.0	12249.02	-41.12	0.9998
Methyl ester impurity	0.06-100.0	10215.6	-5.32	0.9999
Bis famo impurity	0.03-140.0	7122.6	5.16	0.9999

$y = bx + a$, y is peak area of the substance, x is concentration of the substance, b is slope and a is intercept

Table 2: Precision of the proposed HPLC method

Compound	Amount injected	Amount found (n=10)	Recovery (%)	CV (%)
Famotidine(µg/ml)	0.10	0.102±0.002	102.0	2.0
	1.50	1.503±0.03	100.2	1.9
	10.0	10.01±0.25	100.1	2.4
Sulfoxide impurity(µg/ml)	0.15	0.149±0.002	99.3	1.3
	0.80	0.803±0.02	100.4	2.5
	50.0	50.02±0.3	100.04	0.6
Propionamide impurity(µg/ml)	0.12	0.122±0.004	101.6	3.2
	0.60	0.605±0.02	100.8	3.3
	40.0	40.01±0.4	100.03	1.0
Carboxylic acid impurity(µg/ml)	0.20	0.204±0.004	102.0	1.9
	5.0	5.02±0.03	100.4	0.6
	40.0	40.05±0.6	100.1	1.5
Propionate impurity(µg/ml)	0.9	0.91±0.03	101.1	3.2
	6.0	6.07±0.06	101.2	0.9
	50.0	50.03±0.8	100.06	1.6
Sulfamide impurity(µg/ml)	0.15	0.151±0.003	100.7	2.0
	1.5	1.52±0.04	101.3	2.6
	30.0	30.04±0.3	100.1	1.0
Iso thiourea impurity(µg/ml)	0.15	0.152±0.004	101.3	2.6
	3.0	2.98±0.06	99.3	2.0
	60.0	60.03±0.5	100.05	0.8
Methyl ester Impurity(µg/ml)	0.60	0.603±0.006	100.5	1.0
	6.0	6.04±0.08	100.6	1.3
	60.0	60.03±0.4	100.05	0.6
Bis famo impurity (µg/ml)	0.7	0.71±0.02	101.4	2.8
	7.0	7.03±0.08	100.4	1.1
	70.0	70.05±0.5	100.07	0.7

Table 3: Accuracy of the HPLC method for the determination of famotidine and its impurities in standard or placebo solutions

Compound	Amount added (µg/ml)	Standard solutions (n=10)		Placebo solutions (n=10)	
		Recovery (%)	CV (%)	Recovery (%)	CV (%)
Famotidine	0.15	101.2	1.7	100.05	1.9
	5.0	99.40	1.3	101.2	2.2
	20.0	100.5	2.4	99.50	1.6
Sulfoxide impurity	0.10	100.3	2.1	99.86	1.4
	0.60	99.95	0.9	101.1	3.3
	30.0	99.93	1.9	101.4	2.1
Propionamide impurity	0.25	101.4	2.3	99.60	1.5
	5.0	101.2	3.1	100.01	2.1
	35.0	99.60	1.7	101.3	2.6
Carboxylic acid impurity	1.0	99.98	3.3	100.1	2.3
	20.0	100.05	2.7	99.99	2.6
	50.0	101.4	1.6	100.06	2.1
Propionate impurity	1.5	99.60	1.7	98.80	2.2
	10.0	100.1	2.1	99.98	1.5
	60.0	101.0	2.3	100.03	3.1
Sulfamide impurity	0.30	100.03	1.3	101.0	1.7
	3.0	99.86	1.9	99.92	2.4
	25.0	99.30	2.2	100.05	3.1
Isothiourea impurity	0.08	103.2	2.7	99.82	2.7
	0.35	101.2	2.0	102.0	2.5
	40.0	100.03	2.3	100.1	2.1
Methyl ester impurity	0.40	100.1	1.6	99.80	2.6
	8.0	100.02	2.1	100.05	2.3
	40.0	99.97	2.4	100.01	2.8
Bis famo impurity	0.05	102.0	1.9	102.3	3.2
	0.20	101.5	2.5	100.5	2.6
	70.0	102.4	2.7	102.2	2.8

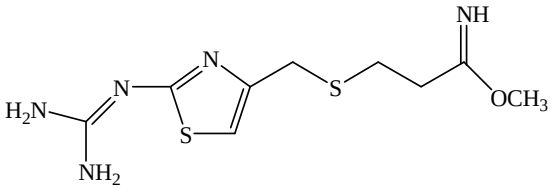
Table 4. Limit of detection (LOD) and limit quantification (LOQ)

Compound	LOD(ng/ml)	LOQ(ng/ml)
Famotidine	1.5	5
Sulfoxide impurity	12	37.5
Propionamide impurity	16.5	57
Carboxylic acid impurity	27	67.5
Propionate impurity	15	48
Sulfamide impurity	18	52.5
Iso thiourea impurity	22.5	63
Methyl ester impurity	22.5	60
Bis famo impurity	7.5	30

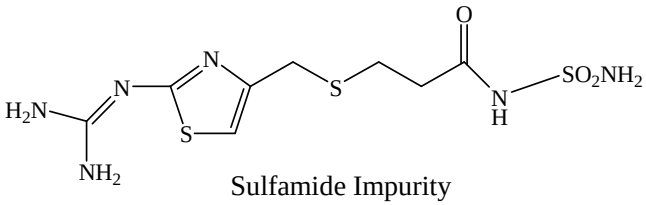
Table 5. Determination of famotidine content in pharmaceutical formulations

Pharmaceuticals	Amount (mg/tablet)	Amount found (mg/tablet)	Recovery (%)	CV (%)
Tablets-1	20	19.6±0.1*	98.0	0.5
Tablets-2	20	19.9±0.2	99.5	1.0
Tablets-3	20	19.9±0.2	99.5	1.0

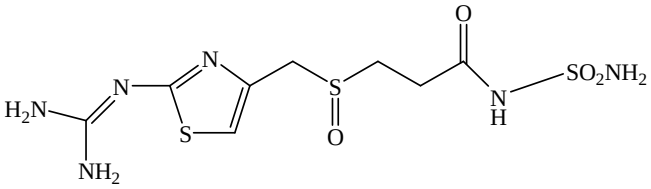
** Standard deviation (n=10)*



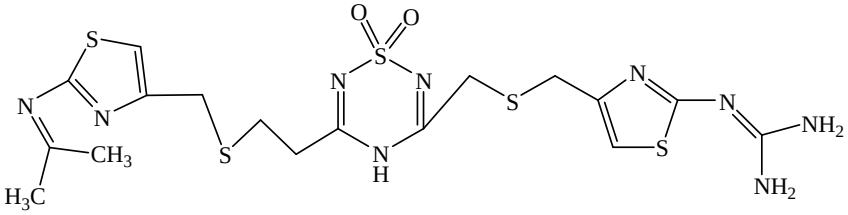
Methyl ester impurity



Sulfamide Impurity



Sulfoxide impurity



Bis-Famo impurity

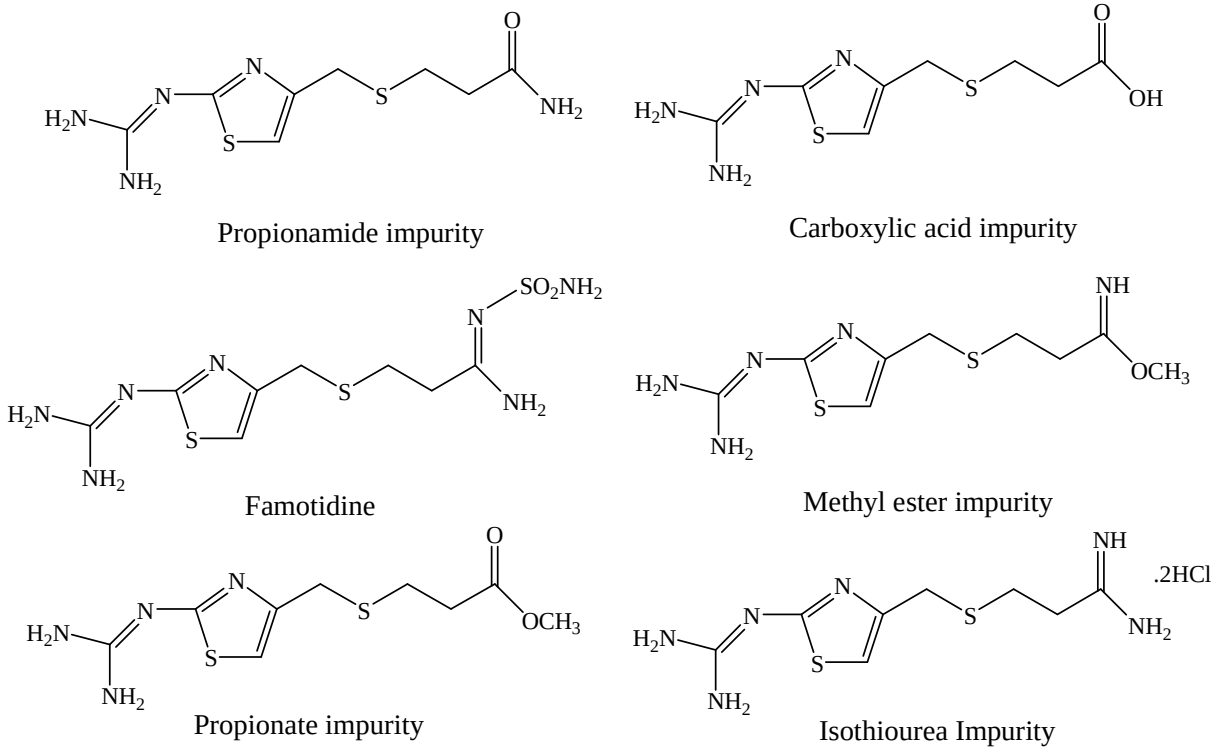


Figure 1. Chemical structures of famotidine and its impurities

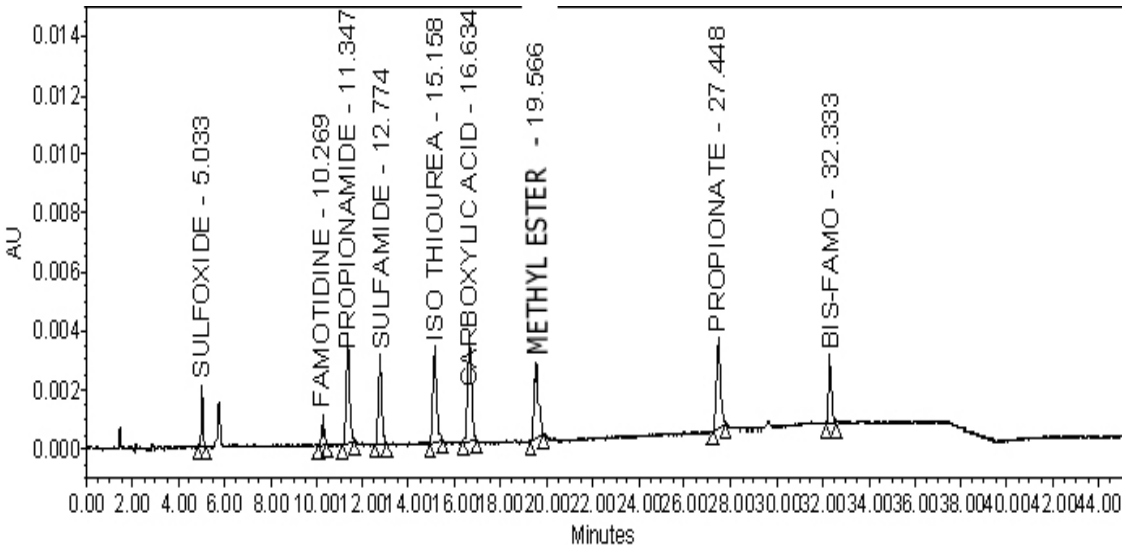
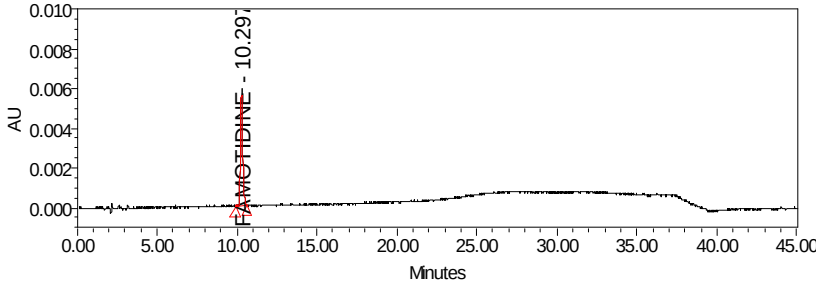
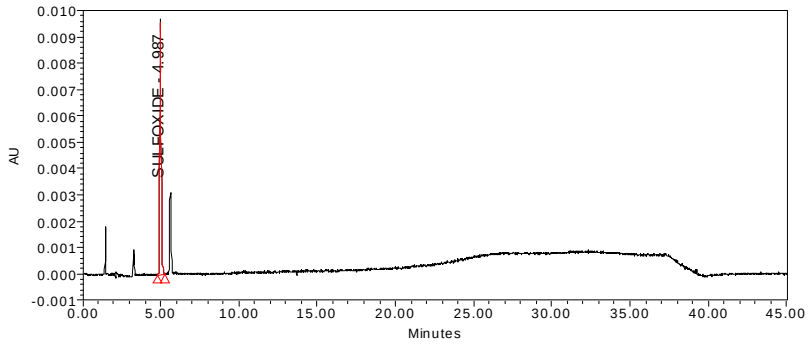


Figure 2. The representative chromatogram of famotidine and its impurities


FAMOTIDINE - 10.297
AU
Minutes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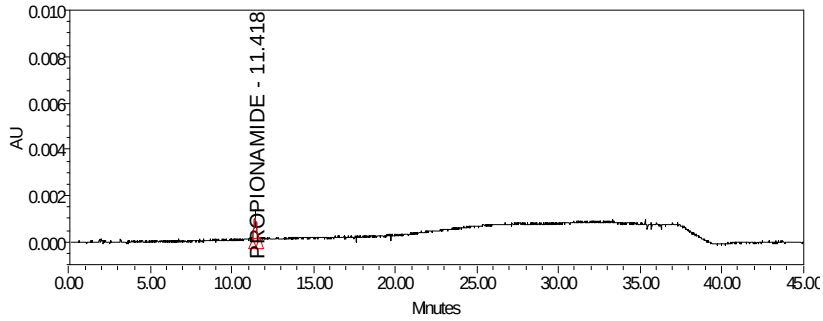
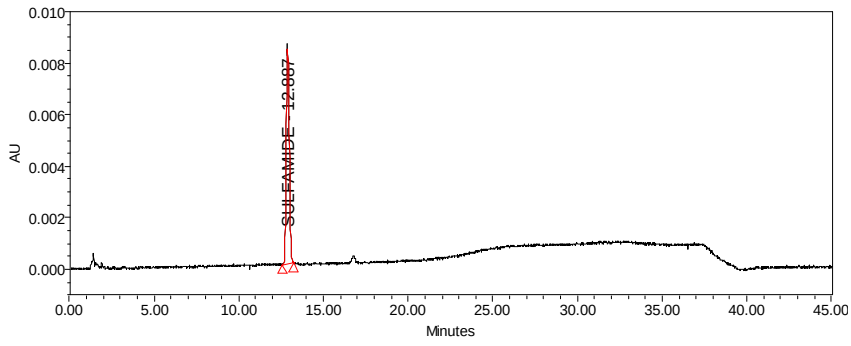

PROPIONAMIDE - 11.418
AU
Mnutes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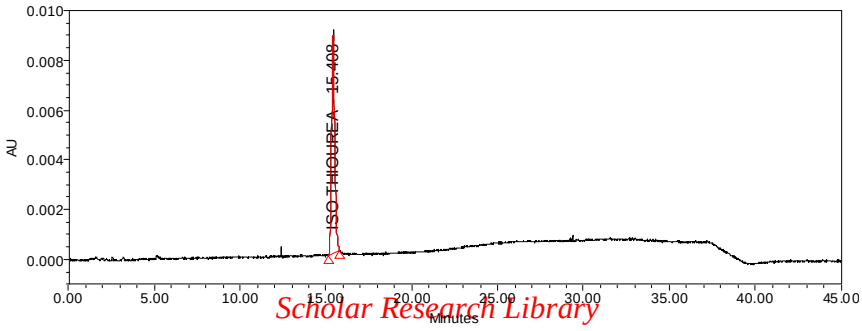
Minutes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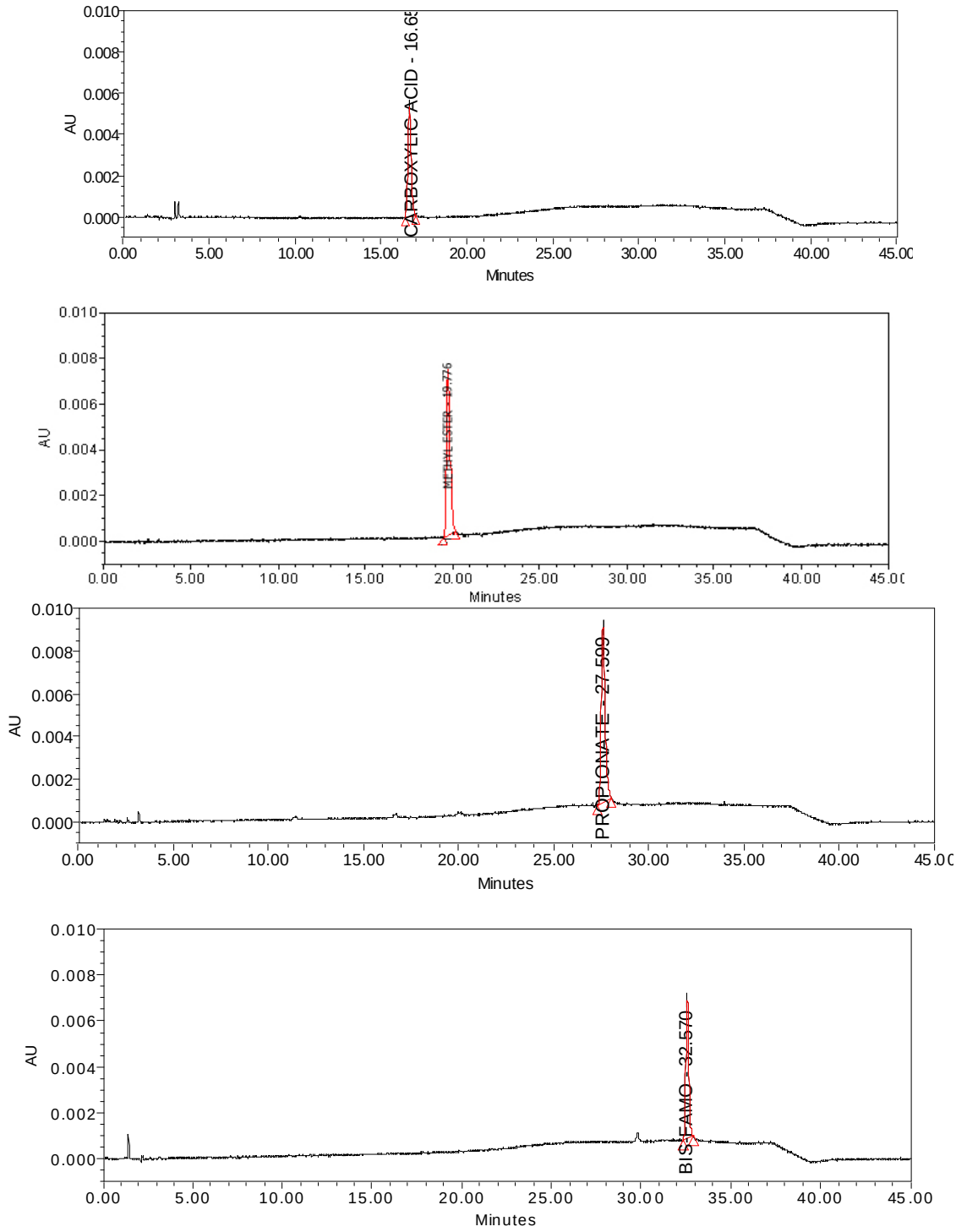

Figure 3. Individual chromatograms of famotidine and its impurities

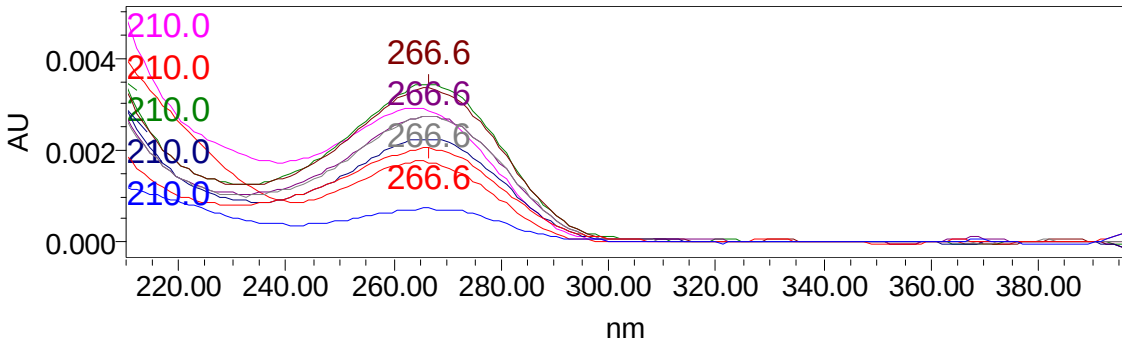

Figure 4. UV spectra of famotidine impurities

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