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Original Paper

Stability indicating method for famotidine in pharmaceuticals using porous graphitic carbon column

A simple, sensitive and rapid HPLC method was developed and validated for the simultaneous determination of famotidine (FMT) and related impurities in pharmaceuticals. Chromatographic separation was accomplished within 10 min on a porous graphitic carbon (PGC) column using 50:50 v/v ACN – water containing 0.5% pentane sulphonic acid (PSA) as the mobile phase. Separation was achieved with a flow rate of 1 mL/min and a detection wavelength of 265 nm. The calibration curves were linear over a concentration range of 1.5 – 100 μg/mL. The intra- and interday RSDs ($n = 5$) for the retention times and peak area were all less than 2%. The method was sensitive with an LOD (S/N = 3) of 0.1 μg/mL for FMT, imp. C and 0.05 μg/mL for imp. 2, A and D. All recoveries were greater than 98%. The method was demonstrated to be precise, accurate and specific with no interference from the tablet ingredients and separation of the drug peak from the peaks of the degradation products (oxidative degradation and acid and base degradation). The results indicated that the proposed method could be used for the determination of FMT in commercial dosage forms and as a stability-indicating assay.

Keywords: Famotidine / HPLC / Porous graphitic carbon column / Related impurities / Tablets

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1 Introduction

Famotidine (FMT) belongs to a class of drugs known as histamine H_2 receptor antagonist used in the treatment of gastric and duodenal ulcers. It inhibits gastric acid secretion by blocking the H_2 receptors located on parietal cells [1]. Although the H_2 blockers group have generally very limited side effects, several synthetic and degradative impurities of FMT have been identified and are semi-quantitatively determined by using TLC [2, 3]. These impurities often possess unwanted pharmacological or toxicological effects by which any benefit from their administration may be outweighed. Therefore, it is quite obvious that the products intended for human consumption must be characterised as completely as possible. The quality and safety of a drug is generally assured by monitoring and controlling the impurities effectively. Thus, the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis. Several analytical techniques such as spectrophotometry [4 – 8], potentiometry and electrogenerated chemiluminescence [9, 10], flow injection analysis [11, 12], CE [13 – 15] and more extensively HPLC [16 – 23] have been reported for the individual and simultaneous determination of FMT and other antihistaminic analogues. However, among the most extensively used HPLC methods, only few of them describe the simultaneous determination of FMT and related compounds [24 – 32]. These methods, though able to determine FMT impurities, require mobile phase with high proportions of organic solvents and elution gradient and are relatively time consuming.

Porous graphitic carbon (PGC) consists of a robust homogeneous surface that has fewer chromatographic active sites and is good for chromatography of basic and closely related compounds [33]. It has the advantage of extreme pH stability [34]. It can be classified as an adsorbent where the carbon surface acts as a Lewis base towards polar solutes and is involved in π – π interactions and dispersive interactions with aromatic solutes [35 – 37]. The retention factor was found to increase with increase in the number of polar substituents, and was shown to depend on both the fields and mutual resonance effects of the stereo different substituents on the aromatic ring

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Abbreviations: FMT, famotidine; **PGC,** porous graphitic carbon; **PSA,** pentane sulphonic acid

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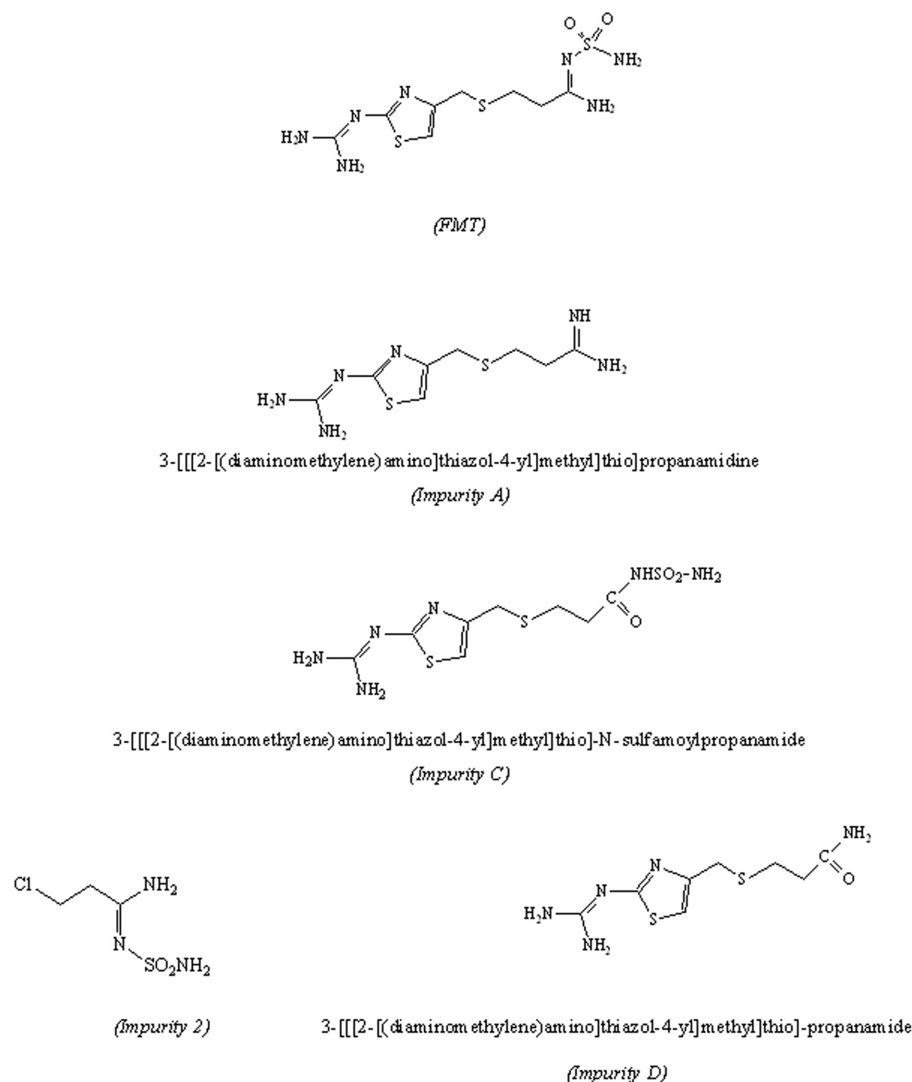

Figure 1. Chemical structures of FMT and related impurities.

[38, 39]. The aim of this work is to investigate the potential of PGC stationary phase for the rapid separation of FMT and its related impurities. The effects of several chromatographic parameters such as the mobile phase nature, buffer pH and ionic strength were evaluated. The performances of the method were evaluated and its potential for the determination of FMT in tablet formulations was investigated.

2 Materials and methods

2.1 Instrumentation and chromatographic conditions

All HPLC experiments were performed on a Beckman system (Beckman Instruments, USA) consisting of a binary pump model 125, a Rheodyne 7725 injector with a 10 μL loop and a spectrophotometer detector (model 166) set at 265 nm. The chromatographic data were collected and analysed using Gold Nouveau software. Separation was achieved with a carbon column (100 × 4.6 mm id, 5 μm particle size) packed with PGC (Thermo Fisher Scientific, France). The mobile phase consisted of 50:50 v/v ACN/phosphate buffer pH 2.0 and 0.5% pentane sulphonic acid (PSA) sodium salt. Analysis was performed at a flow rate of 1 mL/min. Prior to any analysis, mobile phase was degassed and filtered using 0.45 μm filter. The system was equilibrated with the mobile phase for 20 min before injection.

2.2 Reagents and chemicals

Analytical and technical grade FMT was obtained from the pharmaceutical company Ibn Al Baytar Pharma (Tunis, Carthage, Tunisia). FMT-related impurities (Fig. 1) were obtained from Ercros Industrial, S.A., FYSE (Madrid,

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Spain). Ultra pure water was drawn from a Milli-Q water purification system (Millipore, USA). ACN and methanol (HPLC grade), potassium phosphate, orthophosphoric acid, TCA and sodium pentane sulphonate (analytical grade) were purchased form Prolabo (Paris, France).

2.3 Preparation of solutions

Stock solutions of FMT and its related impurities (500 μg/mL each) were prepared in the mobile phase. The working standard solutions (1–100 μg/mL) were prepared by serial dilutions of the stock solution with the mobile phase.

FMT-containing tablets were prepared by crushing more than 20 tablets and an accurately weighed portion of the mixed powder equivalent to FMT content of one tablet was transferred to 25 mL volumetric flask and dissolved by sonication. The sample was filtered and diluted to make final FMT concentrations ranging from 5 to 10 μg/mL.

In an attempt to study the method selectivity, 50 mg of FMT was hydrolysed in 100 mL of 0.1 M HCl or NaOH, the solution was then refluxed for 30 min. A portion of 1 mL of the hydrolysed solutions was filtered through a 0.45 μm filter before injecting into the column.

3 Results and discussion

3.1 Optimisation of the chromatographic variables

Optimum conditions, which are necessary for the quantitative analysis of the drug and impurities with maximum sensitivity, were established by a number of preliminary experiments. Optimum conditions were fixed by varying one parameter at a time while fixing other parameters constant and observing its effect on the peak resolution and also on the response.

As FMT and related impurities are basic compounds, they are highly retained at high pH value, which indicates their presence in the neutral form; therefore, their analysis is investigated in acidic medium. Initially, results obtained with a mobile phase containing different proportions of methanol as the organic modifier with phosphate buffer at pH 2.0 showed very large peaks accompanied with a long analysis time. Methanol was therefore replaced with ACN in an attempt to improve the separation, peak shape and to reduce the retention time of the last eluting solutes. As expected, retention times were reduced with no remarkable improvement in resolution especially at high proportions of ACN, because retention of last eluting solutes (C and FMT) decreased as the amount of ACN was increased. Therefore, a poor separation appears with a mobile phase that contains 50:50 ACN/buffer pH 2.0 (Fig. 2).

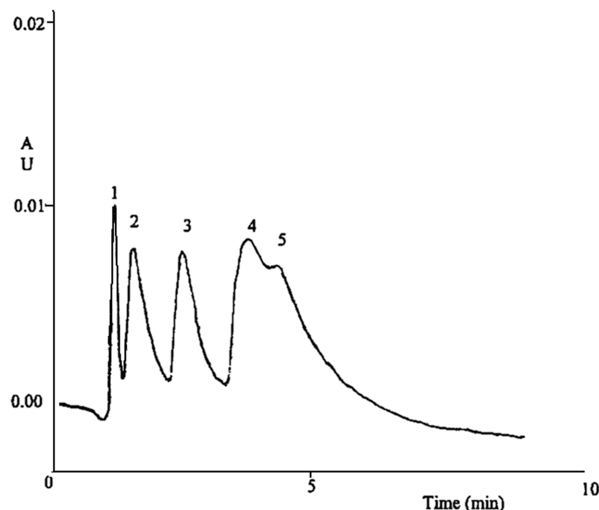

Figure. 2. Typical chromatogram of FMT and its potential impurities separated on PGC column with a mobile phase containing 50:50 v/v ACN/0.05 M phosphate pH 2.0 Peaks: 1 = impurity 2; 2 = impurity A; 3 = impurity D; 4 = impurity C; 5 = FMT.

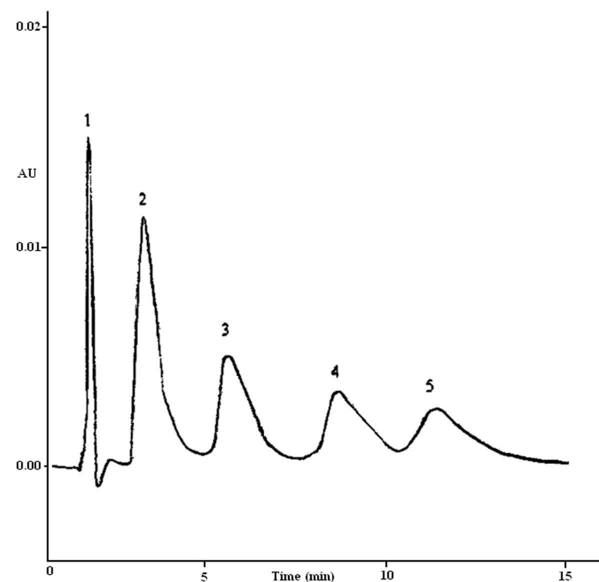

Figure 3. Typical chromatogram of FMT and its potential impurities separated on PGC column with a mobile phase containing 40:60 v/v ACN/0.05 M phosphate buffer and 0.4% TCA acid. Peaks: 1 = impurity 2; 2 = impurity A; 3 = impurity D; 4 = impurity C; 5 = FMT.

The variation of mobile phase nature or ratio was not sufficient to improve resolution, so it was necessary to include modifiers in the eluent. Therefore, to enhance separation, TCA was added to the mobile phase as an ion pair reagent. It was found that increasing TCA concentration from 0.1 to 0.4% increases the retention time of all solutes, however, these changes do not improve peak shape. During this stage, the best results (Fig. 3) were

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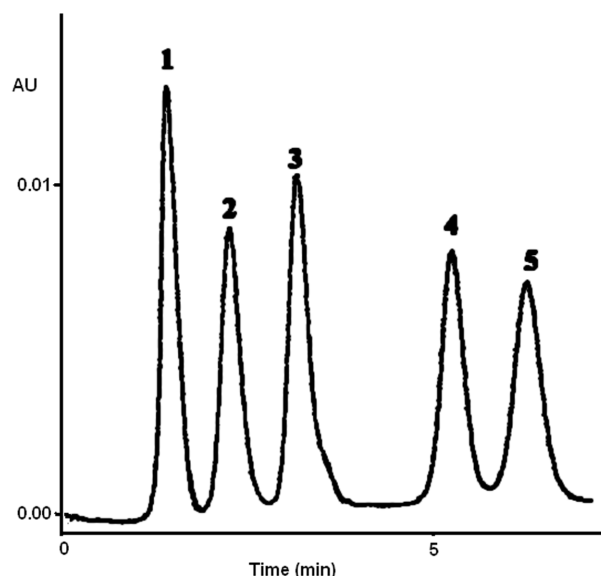

Figure 4. Typical chromatogram of FMT and its potential impurities separated on PGC column with a mobile phase containing 50:50 v/v ACN/0.05 M phosphate buffer and 0.5% PSA Peaks: 1 = impurity 2; 2 = impurity A; 3 = impurity D; 4 = impurity C; 5 = FMT.

obtained with 0.4% TCA in a mobile phase containing 40:60 ACN/buffer pH 2.0. The separation achieved with a mobile phase containing 40:60 ACN/phosphate buffer at pH 2.0 with TCA was not sufficient for their quantitative analysis in real samples. Therefore, the modifier TCA was replaced with a moderately hydrophobic reagent, PSA. The effect of PSA added to the mobile phase was tested over the range of 0.1–0.5%. It was found that the retention time for all solutes increased as the concentration of PSA in the mobile phase increased. The concentration of PSA selected was therefore 0.5%, because this resulted in the best resolution between all peaks with a separation time within 6 min (Fig. 4). Therefore, a mobile phase of 50:50 v/v ACN/0.05 M phosphate buffer containing 0.5% PSA was selected for method validation.

The previously described HPLC methods and that of the USP assay for FMT and related impurities list RP ODS columns with a total run time of about 20 min and an elution order as following: imp. A, FMT, imp. D and imp. C. The elution order obtained with these columns was different from that obtained with PGC column. The difference in the elution order between PGC and ODS columns suggests that molecular interactions determining solute retention are different for the two packing materials.

3.2 Method performances

3.2.1 Selectivity and stability study

The selectivity of the optimised method was determined by injecting a mixture of FMT and its four impurities. As shown in Fig. 4, there was an adequate resolution of all compounds. The selectivity of the method was further assessed by the analysis of FMT solutions that had been hydrolysed under reflux with HCl and NaOH for 20 min. Figure 5 (a, b and c) shows the appearance of other peaks in addition to FMT peak. The area of FMT peak decreases as a function of time as the area of other peaks increases. However, the peaks were separated and hence the proposed method is applicable to the selective determination of FMT in the presence of related impurities.

3.2.2 Linearity

Linearity and range of the method were investigated by analysing different concentrations of the mixed standard solutions containing 1.5–100 μg/mL of FMT and its related impurities under the chromatographic conditions mentioned above. Calibration curves (peak area *vs.* concentration of the standard solutions) were constructed with nine different concentrations. Each point of the calibration curve corresponded to the mean value obtained from five independent measurements. Calibration curves were found to be linear with correlation coefficients greater than 0.999. The data were analysed by linear regression least squares fit method. The calibration curve shows a calibration equation $y = a + bx$, where y is the peak area, 'b' the slope, 'a' the intercept and 'x' is the concentration of the analyte expressed in μg/mL. Linear regression least squares fit data are given in Table 1 which shows that the variability (RSD) of the slope and intercept varied between 0.88 and 3.0%.

To calculate the LOQ and LOD a S/N of approximately 3:1 is generally considered to be acceptable for estimating the LOD, which is the lowest concentration that can be detected. The LOD was estimated at: 0.1 μg/mL for FMT, imp. C and 0.05 μg/mL for imp. 2, A and D.

The LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy and could be estimated as S/N of approximately 10:1. The LOQ of FMT, imp. C, imp. 2, imp. A and imp. D was varied between 0.2 and 0.4 μg/mL.

3.2.3 Precision

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. It was evaluated by measuring intraday and interday repeatability of retention times, peak areas, concentration of FMT and related impurities. In order to measure the repeatability of the system, with respect to retention times and peak areas, five successive injections of the same mixture of the five analytes were performed. The precision of the analysis was determined by calculating the RSD%. The intraday RSD values obtained for retention times were less than 2% and for peak areas were varied between 1.07 and 1.64%. The interday RSD values obtained for retention

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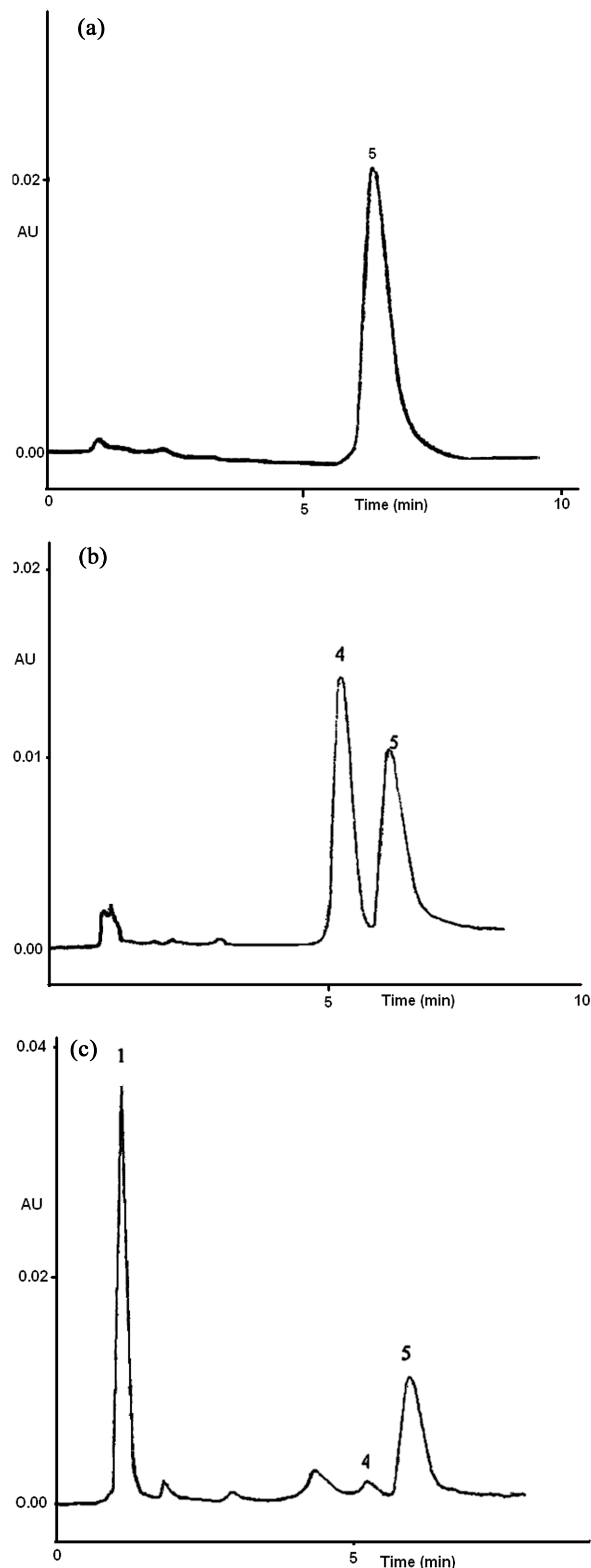

Figure 5. (a) Chromatogram of FMT in aqueous solution, (b) hydrolysed in 0.1 M HCl, (c) hydrolysed in 0.1 M NaOH. Chromatographic condition and peaks identification as in Fig. 4.

times were 0.72–1.87% and for peak areas were varied from 1.12 to 1.94%.

In order to investigate further the precision with respect to the concentration of FMT and related impurities, three independent series (2.5, 50 and 80 μg/mL) in the linear range were analysed in the same day and in three consecutive days. Within each series every sample was injected three times giving a total of nine determinations (three concentrations/three replicates each). The RSD values of intraday and interday studies varied from 0.46 to 2.0%, which showed that the precision of the method was satisfactory (Table 2). The precision around the mean value should not exceed 3% of the RSD%.

3.2.4 Accuracy and recovery

The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value. It is determined by calculating the percentage relative error between the measured mean concentrations and added concentrations of FMT. Table 2 shows the results obtained for intra- and interday accuracy.

Recovery studies for the accuracy of the method were performed by spiking FMT samples (Famodine tablets) and FMT analytical placebos (a mixture of excipients that added to FMT as in pharmaceutical formulation) with known amounts of FMT standard (5, 10 and 20 μg/mL). The mean percentage recoveries for FMT were varied between 98 and 102% (Table 3). The higher values of recoveries and the lower values of the RSD of the assay indicate that the method is precise and accurate. Also, the results depicted that the present method is useful for bulk drug analysis as well as commercial pharmaceutical formulations.

3.2.5 Robustness and ruggedness

Chromatographic parameters were not significantly affected with the slight changes in the chromatographic conditions like the composition of the mobile phase and flow rate (1 ± 0.1 mL/min). Analysis was carried out in triplicate and only one parameter was changed in the experiments at a time. The retention time and peak areas under the various conditions were not significantly different compared to the optimum conditions and the proposed method could be considered robust.

Furthermore, the ruggedness of the method was evaluated by applying the optimised procedure to the analysis of 50 μg/mL FMT by different analysts using the same instrument. No considerable changes were noticed since there was no difference between results obtained with all analysts (RSD = 0.66%). Thus, the proposed chromatographic procedure could be considered rugged.

With regard to stability, it was observed that analyte concentration in solutions was stable over a period of

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Table 1. Linear regression calibration data for the analysis of FMT and its related impurities

Compounds	Concentration range (μg m/L)	Equation of the line	RSD of the slope (%)	RSD of the intercept (%)	R^2
Impurity 2	1.5–100	$Y = 9834.4x + 13368$	0.96	2.0	0.9994
Impurity A	0.25–62.5	$Y = 5763.9x + 1067.7$	1.82	2.8	0.9993
Impurity D	1.5–100	$Y = 7253.7x + 7899.7$	1.64	2.4	0.9991
Impurity C	1.5–100	$Y = 3463.7x + 2323.1$	2.04	3.0	0.9997
FMT	1.5–100	$Y = 9702.2x + 11063$	0.88	1.8	0.9991

Table 2. Precision and accuracy data for the proposed method

	Intraday				Interday		
Compounds	Added (μg/mL)	Found (μg/mL)	Precision (RSD%)	Accuracy (%)	Found (μg/mL)	Precision (RSD%)	Accuracy (%)
Impurity 2	2.5	2.48	1.42	−0.8	2.47	1.98	−1.20
	50	50.20	0.46	0.40	50.30	0.34	0.60
	80	79.0	0.66	−1.25	78.8	0.94	−1.50
Impurity A	2.50	2.53	1.68	1.20	2.46	2.0	−1.60
	20.0	19.80	0.54	−1.00	19.72	1.22	−1.40
	40.0	39.4	1.24	−1.50	39.3	1.45	−1.75
Impurity D	2.5	2.52	1.20	0.80	2.52	1.68	0.80
	50	49.60	0.98	−0.80	49.20	1.46	−1.60
	80	79.4	1.80	−0.75	79.0	1.88	−1.25
Impurity C	2.5	2.53	1.43	1.20	2.54	1.86	1.60
	50	50.3	0.68	0.60	50.70	1.07	1.40
	80	79.0	0.60	−1.25	78.50	1.56	−1.88
FMT	2.5	2.46	0.94	−1.60	2.45	1.90	−2.00
	50	50.8	0.52	1.60	50.9	1.50	1.80
	80	81	1.20	1.25	81.2	1.20	1.50

Accuracy: ((found − added)/added) × 100.

Table 3. Percentage recovery values of FMT in drug added tablets

Drug	FMT (μg/mL)		Recovery (%)
	Added	Found	
Famodine 40	5	4.96	99.20
	10	10.18	101.88
	20	19.62	98.10

Table 4. FMT contents in drug tablets expressed as % with respect to label amount claim

Drug	Claimed value (mg)	FMT found (mg)		%
		Proposed method	Compared method	
Famodine 20	20	20.3	20.2	102
Famodine 40	40	39.6	39.2	98

3 months in the freeze and also found to be stable during the analysis.

3.2.6 Method application

The applicability of the method for the determination of FMT was examined by analysing commercially available FMT-containing formulations. The amounts of FMT in tablets were calculated using calibration curve method. The obtained results demonstrate that the content of drug corresponds to the drug label (Table 4), which confirms the good accuracy of the proposed method. The method was further evaluated by comparing the results obtained by the proposed method with those obtained with previously published method [31]. Results obtained with the proposed method (98–102% with respect to label) were in close agreement with the published method (99–100% with respect to label) and compare reasonably with the label claimed.

4 Concluding remarks

The method showed acceptable performance with respect to linearity, inter- and intraday precision and specificity with no interference from the tablet ingredients. It is successfully applied for the separation of the drug peak from the peaks of the induced degradation products (acid and base degradation). It is a potential method for the quality control of FMT as drug substance and for

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the determination of FMT in tablet formulation and it could also be used as a stability indicating method.

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