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SPECTROPHOTOMETRIC DETERMINATION OF FAMOTIDINE USING SULPHONPHTHALEIN DYES

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Four new extraction-free spectrophotometric methods have been established for the quantitation of famotidine (FMT). The methods are based on the formation of yellow ion-pair complexes between FMT and four sulphonphthalein dyes *viz.,* bromothymol blue (method A), bromophenol blue (method B), bromocresol purple (method C) and bromocresol green (method D) in dioxane or acetone medium. The experimental variables such as reagent concentration, solvent medium and reaction time have been carefully optimized to achieve the highest sensitivity. The proposed methods were applied successfully to the determination of famotidine in tablets with good accuracy and precision and without interferences from common excipients. The results obtained by the proposed methods were compared favorably with those of the reference method.

Keywords: famotidine assay; spectrophotometry; tablets.

INTRODUCTION

Famotidine(FMT),3-[2-(diaminomethyleneamino)thiazol-4-ylmethylthio]-*N*-sulfamoylpropionamidine (Figure 1), is a histamine H_2-receptor antagonist (H_2-RA) which competitively inhibits the action of histamine on the H_2-receptors of parietal cells and thereby reduces the gastric acid secretion under daytime and nocturnal basal conditions. It is widely used in the management of gastrointestinal disorders, such as aspiration syndrome, dyspepsia, gastro-esophageal reflux disease, peptic ulcer and Zollinger-Ellison syndrome.[1] FMT is official in both the British Pharmacopoeia (BP)[2] and the United States Pharmacopoeia (USP).[3] The BP[2] recommends a potentiometric non aqueous method for the determination of FMT using perchloric acid as the titrant, while the USP[3] recommends a similar approach for the determination of FMT in its bulk form, and an HPLC method using a mixture of acetate buffer of pH 6: acetonitrile (93:7) as a mobile phase with UV detection at 275 nm.

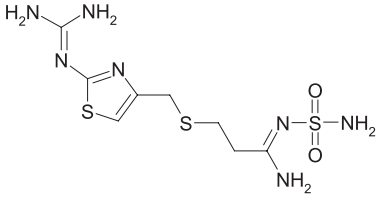

***Figure 1**. Structure of famotidine*

A survey of the literature revealed that FMT has been estimated in pharmaceuticals by UV-spectrophotometry,[4] HPLC,[5,6] HPTLC,[7,8] capillary electrophoresis [9] and electrochemical methods.[10-13] Some of these methods have enough sensitivity to determine lower concentration of the drug, however, it is always required to develop simple, fast, inexpensive analytical methods that can be readily adopted for routine analysis at relatively low-cost to the different requirements of analytical problems.

Visible spectrophotometry is still considered to be a very convenient and economical technique because of its simplicity and speed, the inexpensive equipment needed and accuracy of results. Visible spectrophotometric methods based on diverse reaction chemistries have been proposed for the assay of FMT in pharmaceuticals. Methods based on charge-transfer complexation reactions between famotidine and π acceptors such as chloranil, dichlorodicyanobenzoquinone and dichloronitrophenol,[14] tetracyanoquinodimethane[15] and *p*-chloranilic acid[16,17] have been used for its determination in commercial tablets. Many other methods using copper (II) chloride in methanolic medium,[18] cupric acetate,[19] palladium (II) chloride,[20] ninhydrin in DMF medium,[21] sodium nitroprusside in alkaline medium,[22] NBS with *p*-aminophenol,[23] Folin-Ciocalteau reagent[24] and bromate-bromide[25] have also been reported. Most of the above visible spectrophotometric methods suffer from one or other disadvantage such as poor sensitivity,[14,16-19,22,25] poor selectivity,[24] use of expensive reagent[15] or heating step,[21] narrow linear range,[21,23] strict pH control[20]etc., as indicated in Table 1. Ali *et al.*[26] have earlier employed bromocresol green (BCG) and bromothymol blue (BTB) as chromogenic agents in which the ion-pairs formed at specific pH values were extracted into chloroform before measurement. These procedures involve strict pH control besides the cumbersome extraction step. Many a time, incomplete separation may lead to erratic results.

In the present article, we report the development and validation of four simple, highly accurate and precise spectrophotometric methods using the four dyes based on ion-pair complex formation between the drug and dyes. The hall marks of these methods are that they are free from pH control and extraction step. The methods were demonstrated to be more sensitive than the extraction methods using the same dyes.[26] The proposed methods were applied to the determination of FMT in tablets, and no interference from common excipients in levels found in tablets was observed in the assay.

EXPERIMENTAL

Apparatus

All absorbance measurements were made on a Systronics model

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Table 1. Comparison of the performance characteristic of the existing spectrophotometric methods with the proposed methods

Sl. No.	Reagent/s used	Methodology	Linear range, μg/mL and molar absorptivity(L/mol cm)	Remarks	Ref.
1.	a)Chloranil, b) DDQ and c) DCNP	Charge-transfer complex measured at 458, 460 and 425 nm, respectively.	50-500	Poor sensitivity	14
2.	Tetracyanoquinodimethane	Green radical anion measured at 840 nm.	1.0-7.0	Uses expensive reagents	15
3.	a)*p*-chloranilic acid in methanol b) *p*-chloranilic acid in acetonitrile	C-T complex measured at 525 nm	a) 16000-110000 b) 25-240	Less sensitive, uses acetonitrile which is very expensive	16 17
4.	Copper (II) chloride in methanolic medium	Blue color complex measured at 660 nm.	200-1.200 ($\varepsilon = 1.11 \times 10^2$)	Less sensitive	18
5.	Cupric acetate	Complex measured at 630 nm.	50-1250	Poor sensitivity	19
6.	Palladium (II)	Yellow color complex measured at 345 nm in the p^H range 2.23-8.50.	17-200	Less sensitive ant requires close pH control	20
7.	Ninhydrin in methanolic medium	Blue colored product measured at 590 nm.	5.0-30.0	Requires heating in a boiling water bath and narrow linear range	21
8.	Sodium nitroprusside	Orange species fomed in alkaline medium measured at 498 nm.	50-500	Poor sensitivity	22
9.	NBS with *p*-aminophenol	The decrease in the absorbance of the colored product measured at 552 nm.	6.0-22.0	Two step reaction, longer reaction time and narrow linear range	23
10.	F-C reagent	Blue color product measured at 650 nm	16-48	Less selective, less sensitive and narrow linear range	24
11.	Brominating mixture	Yellow color developed measured at 350	40-200	Less sensitive and measured at shorter wavelength	25
12.	a) Bromocresol green b) Bromothymol blue	Ion-pair complex measured at 420 nm.	2.0-23 ($\varepsilon = 5.0 \times 10^3$) 0.7-8.1 ($\varepsilon = 1.2 \times 10^4$)	Require close pH control and extraction	26
13.	a) BTB b) BPB c) BCP d) BCG	In all the methods, resulting yellow colored ion-pair complexes were measured at 410nm.	a) 1.0-12 ($\varepsilon = 2.07 \times 10^5$) b) 1.0-12 ($\varepsilon = 2.28 \times 10^5$) c) 0.75-7.5 ($\varepsilon = 2.91 \times 10^5$) d) 0.75-9.0 ($\varepsilon = 1.66 \times 10^5$)	Highly sensitive with wide linear dynamic ranges, no heating or extraction step, no pH-adjustment, single step reaction and inexpensive instrumental setup.	Present methods

DDQ: dichloro dicyano benzoquinone; DCNP: dichloronitrophenol; NBS: N-bromosuccinimide; FC: Folin-Ciocalteu; BTB: bromohtymol blue; BPB: bromophenol blue; BCP: bromocresol purple; BCG: bromocresol green.

106 digital spectrophotometer (Ahmedabad, India) equipped with 1-cm matched quartz cells.

Materials and reagents

All chemicals and reagents used were of analytical-reagent grade. The solvents used were of HPLC-grade.

Two brands of tablets containing FMT, Topcid-20 (Torrent Pharmaceuticals Ltd., H. P, India) and Famocid-20 (Sun Pharmaceuticals Industries, Jammu, India), used in the investigation were purchased from local commercial sources.

0.03% solution of bromothymol blue (BTB, Loba Chemie, Mumbai, India) and 0.1% solution of bromophenol blue (BPB, Loba Chemie, Mumbai, India) were prepared in dioxane (Merck, Mumbai, India).

0.01% solution each of bromocresol purple (BCP, Loba Chemie, Mumbai, India) and bromocresol green (BCG, Qualigens Fine Chemicals, Mumbai, India) were prepared in acetone (Merck, Mumbai, India).

Standard solution preparation

Pharmaceutical grade FMT, certified to be 99.98 % pure was procured as gift from Cipla India Ltd., Mumbai, India, and was used as received. A 200 μg/mL stock standard solution was prepared by dissolving the calculated amount of pure FMT either in dioxane (Merck, Mumbai, India) (method A and method B) or in acetone (Merck, Mumbai, India) (method C and method D) and diluted to a definite volume with the respective solvents. The stock solutions were diluted to get working concentrations of 20 μg/mL for method A and method B, and 15 μg/mL for method C and method D with the respective solvents.

Sample preparation

Tablets

Ten tablets were accurately weighed and powdered. A portion equivalent to 5 mg FMT was accurately weighed and transferred into two separate 50 mL volumetric flasks, 30 mL of dioxane or acetone was added to the flasks and the content was shaken thoroughly for 15-20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with either dioxane or acetone (50 mL flask), mixed well and filtered using a Whatman No. 42 filter paper. An aliquot of the filtrate (100 μg/mL in FMT) was diluted to get required concentrations with the respective solvents.

Placebo blank

A placebo blank of the composition: talc (10 mg), starch (5 mg), acacia (5 mg), methyl cellulose (10 mg), sodium citrate (5 mg), mag-

nesium stearate (10 mg) and sodium alginate (5 mg) was made and its solution was prepared as described under 'Procedure for tablets'.

Synthetic mixture

To the placebo blank of the composition described above, 5 mg of FMT was added and homogenized, transferred to 50 mL volumetric flask and the solution was prepared as described under "Procedure for tablets".

Standard curves construction

Method A and method B

Aliquots of 0.25, 0.5…….3.0 mL FMT standard solution in dioxane (20 µg/mL) were measured accurately and transferred into a series of 5 mL volumetric flasks. To each flask, 1 mL of 0.03% BTB solution in method A or 1 mL of 0.1% BPB in method B, was added, diluted to the mark with dioxane and mixed well. The absorbance of the resulting yellow color chromogen was measured at 410 nm against the respective reagent blank.

Method C and method D

Varying aliquots, 0.25, 0.5…….. 2.5 mL of 15 µg/mL standard FMT solution in acetone (method C) or 0.25, 0.5…….. 3.0 mL of 15 µg/mL standard FMT solution in acetone (method D), were measured accurately and transferred into a series of 5 mL volumetric flasks. To each flask was added 1 mL of 0.01 % BCP in method C or 1 mL of 0.01 % BCG in method D. The content was mixed well and diluted to the mark with acetone. The absorbance of each solution was measured at 410 nm against respective reagent blank.

In all the methods, a standard curve was prepared by plotting the increasing absorbance values *versus* concentrations of FMT. A linear equation for the standard curve was calculated by linear regression.

Statistical analysis

The tablet solutions were analyzed for FMT by following the recommended procedures. The same batch tablets were analyzed by the B P method[2] which consisted of potentiometric titration of acetous FMT solution with acetous perchloric acid and the percent recoveries and standard deviation were computed for the results obtained from which the Student's *t*-value and *F*-value were calculated using the formulae:[27,28]

$$t = \frac{\left|\bar{x}-\bar{y}\right|}{S_p}\sqrt{\frac{n_x n_y}{n_x+n_y}}, \quad S_p = \sqrt{\frac{(n_x-1)S_x^{\,2}+(n_y-1)S_y^{\,2}}{(n_x-1)+(n_y-1)}}$$

where $\bar{x}$ and $\bar{y}$ are the mean recoveries by the two methods, n_x and n_y are the number of individual results obtained by the two methods, S_x and S_y are the standard deviations of the two methods; and S_p is the pooled standard deviation, and

$$F = \frac{S_x^{\,2}}{S_y^{\,2}}, \text{ where } S_x^{\,2} > S_y^{\,2}$$

Procedure for stoichiometric ratio

Job's method of continuous variations of equimolar solutions was employed: 5.927 x 10^{-5} M solution each of FMT and BTB (method A) or BPB (method B) in dioxane; and 4.445 x 10^{-4} M solution each of the FMT and BCP (method C) or BCG (method D) in acetone were prepared separately. A series of solutions was prepared in which the total volume of FMT and reagent was kept at 5 mL. The drug and reagent were mixed in various complementary proportions (0:5, 1:4, 2:3,……..5:0, inclusive) and completed as directed under the recommended procedures. The absorbance of the resultant ion-pair complex in each case was measured at 410 nm.

Method validation

The proposed methods were validated for linearity, sensitivity, precision, accuracy, robustness, ruggedness, specificity, according to the International Conference on Harmonization (ICH)[29] guidelines.

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Linear correlations were obtained from the standard curves between the absorbance and concentration of FMT. To prepare the standard curves, solutions for each FMT concentration were prepared in triplicate in all the four methods. The standard curve is described by the equation: Y = a + bX, (where Y = absorbance, a = intercept, b = slope and X = concentration in µg/mL) obtained by the method of least squares. The limit of detection (LOD) and limit of quantification (LOQ) were computed using the formulae: LOD=3.3σ/s and LOQ=10σ/s, where σ is the standard deviation of replicate reagent blank absorbance readings and s is the slope of the standard curve.

Precision

Intra-day precision (repeatability) of the proposed methods was evaluated by carrying out the determination of seven replicates (n=7) of pure drug at 3 different concentration levels on the same day. Inter-day precision (intermediate precision) was determined by assaying the pure drug solution in 7 replicates (n=7) at the same concentration levels on 5 consecutive days. The relative standard deviation for replicate analysis of intra-day precision (repeatability) and inter-day precision (intermediate precision) were calculated.

Accuracy

Intra-day accuracy (on the same day) and inter-day accuracy (on 5 consecutive days) were evaluated in terms of percent relative error (% RE) by assaying the pure drug solution in 7 replicates (n=7) at three different concentration levels. The % RE was calculated by using the formula: $\%R.E = \left[\frac{found-taken}{taken}\right]\times 100$. The accuracy of the proposed methods was further ascertained by performing recovery studies *via* standard-addition procedure. Pre-analysed tablet powder was spiked with pure FMT at 3 concentration levels (50, 100 and 150% of that in tablet powder) and the total was found by the proposed methods. Each determination was repeated 3 times. The recovery (%) of the pure drug was calculated as $[(C_1-C_2)/C_3] \times 100$, where C_1 is the total drug concentration found, C_2 is the drug concentration in the tablet taken and C_3 is the pure drug concentration added to the formulation.

Robustness and ruggedness

Method robustness was evaluated by the analysis of tablet and standard solutions at different concentrations of dye (n=3) in ± 0.2 units and by maintaining the solutions at room temperature (25 ± 2 °C). In order to demonstrate the ruggedness of the method, determinations at 3 different concentrations of the pure drug and tablet solutions were carried out by 4 different analysts, and also with 3 different instruments by a single analyst.

Specificity

The specificity of the proposed methods was evaluated through the analysis of placebo blank and synthetic mixture solutions. A con-

venient aliquot of the placebo blank solutiotn and synthetic mixture solution was subjected to analysis according to the recommended procedures. For all the methods, the interference from the formulation matrix was evaluated.

RESULTS AND DISCUSSION

Ion-pair extraction-free spectrophotometry has received considerable attention in the recent years for the quantitative determination of many pharmaceuticals.[30-33] This technique depends on the reaction of a drug that has basic cationic nitrogen and an anionic dye, where a highly colored ion-pair complex is formed. The structural formula of FMT features amino groups; therefore, attempts were made to determine FMT based on the formation of ion-pair complex with anionic dyes.

Absorption spectra

FMT either in dioxane or acetone does not absorb in the visible region, but upon addition of any of four dyes (BTB, BPB, BCP and BCG), yellow color is immediately produced with the absorption maximum at 410 nm (Figure 2). The developed yellow color is due to the conversion of the dye into an open quinoidal anionic derivative,[34,35] which subsequently forms an ion pair with FMT as shown in Scheme 1.

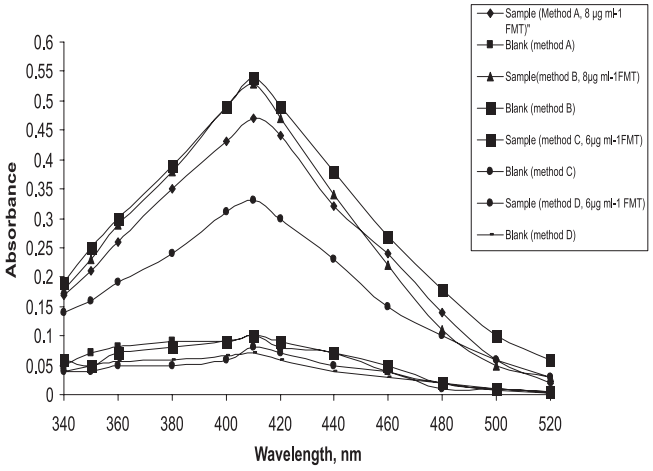


Figure 2. *Absorption spectra of ion-pair complexes (FMT: BTB; FMT: BPB; FMT: BCP; FMT: BCG) against respective reagent blank*

Optimization of reaction conditions

Optimum reaction conditions for quantitative determination of ion-pair complexes were established *via* various preliminary experiments such as choice of organic solvent, concentration of the dye and reaction time.

Choice of organic solvent

Since FMT is insoluble in most of the organic solvents, few solvents such as methanol, acetone and dioxane were tested. FMT is easily soluble in methanol but the dye alone in methanol (blank) gave an intense yellow color. Hence, acetone and dioxane were preferred to carry out the experiments. In the case of BTB and BPB, better sensitivity was achieved in dioxane medium whereas acetone was selected as the suitable solvent for BCP and BCG, yielding maximum absorbance. The blanks yielded least absorption in these solvents.

Effect of dye concentration

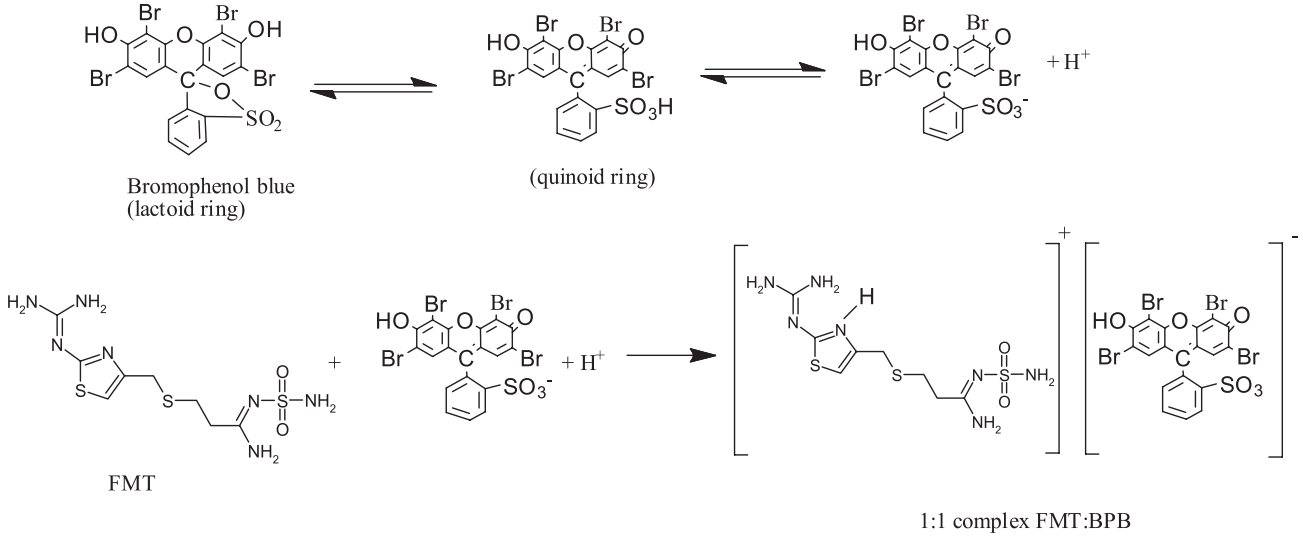
The influence of the concentration of BTB, BPB, BCP and BCG on the intensity of the color developed at the selected wavelength was studied. 1 mL each of 0.03% BTB, 0.1% BPB, 0.01% BCP or BCG was sufficient to produce maximum and reproducible color with minimum blank absorbance.

Effect of reaction time

The optimum reaction time for the development of color at ambient temperature (25 ± 2 °C) was studied and it was found that complete color development was instantaneous in all the methods. The formed color was stable for at least 3 h in all cases.

Stoichiometric ratio

The molar ratio of FMT to dyes in the complex was determined by applying the Job's method of continuous variations. In all the cases, the plot reached a maximum value at a mole fraction of 0.5 which indicated the formation of 1:1 (FMT:Dye) complex (Figure 3). Based on this it was confirmed that only one nitrogen atom in the drug is protonated and through the electrostatic attraction ion-pair complex is formed with the negatively charged dye. In FMT nitrogen atom present in the thiazole ring is the most vulnerable one to protonate



Scheme 1. *The possible reaction mechanisms*

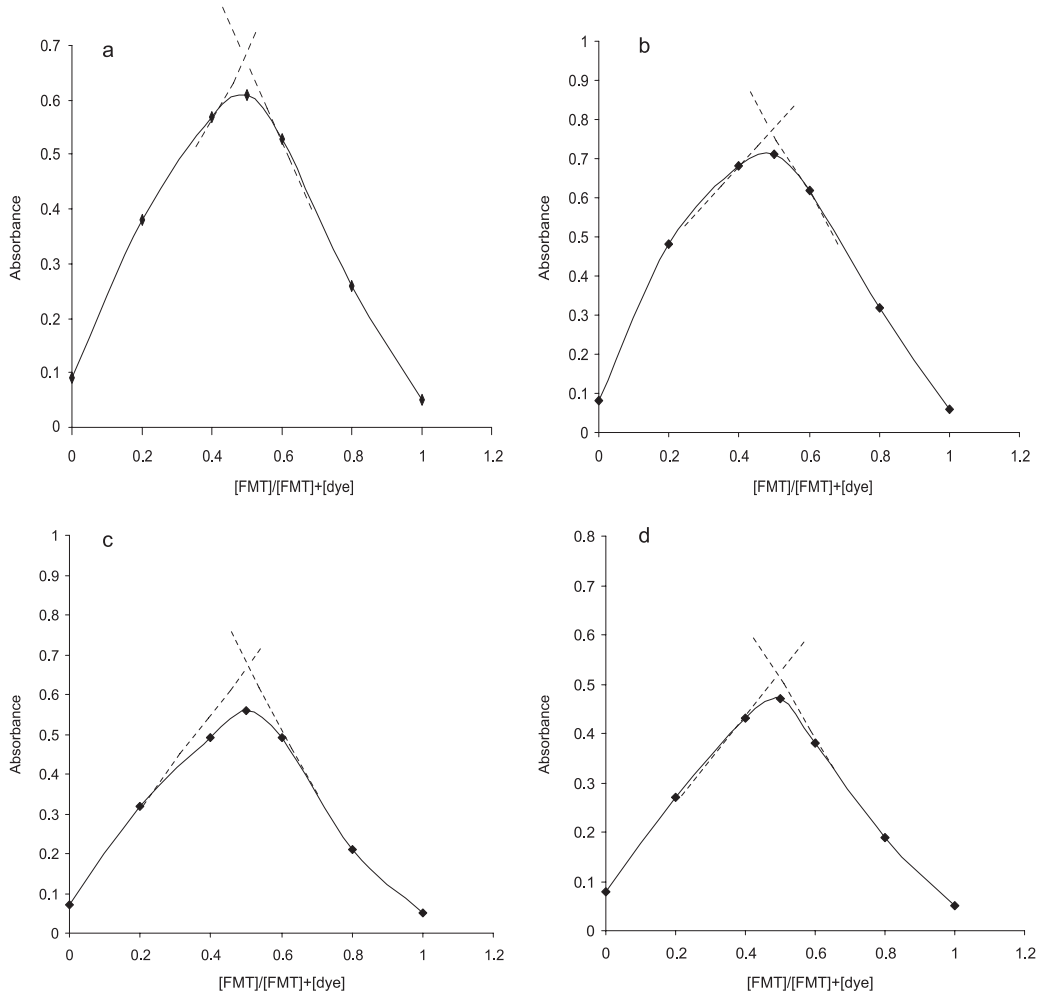


***Figure 3**. Job's Continuous - variations plots (a) FMT + BTB; (b) FMT + BPB; (c) FMT+BCP and (d) FMT+BCG*

when compared to other nitrogen atoms. The reaction pathway is proposed to proceed as shown in Scheme 1.

Conditional stability constants (K_f) of the ion-pair complexes

The conditional stability constants (K_f) of the ion-pair complexes for famotidine were calculated from the continuous variation data using the following equation:[36]

$$K_f = \frac{A/A_m}{[1\text{-}A/A_m]^{n+2}\, C_M(n)^n}$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the molar concentration of drug at the maximum absorbance and n is the stoichiometry with which dye ion associates with drug. The log K_f values for FMT-BTB, FMT-BPB, FMT-BCP and FMT-BCG ion-pair associates were 7.554 ± 0.225, 7.196 ± 0.241, 8.146 ± 0.219 and 7.164 ± 0.326, respectively.

Experimental results

Under optimum experimental conditions, linear correlations were obtained between the absorbance and concentration of FMT in the range of 1.0-12 µg/mL (method A and method B), 0.75-7.5 µg/mL (method C) and 0.75-9.0 µg/mL (method D). In all the methods regression coefficient was 0.999 indicating good linearity. The intercept and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, LOD and LOQ are compiled in the same table, speak of the excellent sensitivity of the proposed methods. The LOD and LOQ values are small in method C, indicates that BCP method is most sensitive among the proposed four methods.

Table 3 summarizes the intra-day and inter-day precision expressed as percentage relative standard deviation (RSD, %) and intra-day and inter-day error data (RE %) for the assay of FMT in pure drug by the proposed methods. The intra-day and inter-day RSD values were found to be < 1.5 %, reflecting the usefulness of the methods in routine use, and RE % data was ≤ 3%.

Method robustness was determined with small incremental change in dyes concentrations (n=3). The % RSD with the altered dyes concentration was < 1.5 % in both pure drug and tablet solutions indicates that the absorbance value remains unaffected by small deliberate variations. Regarding the evaluation of ruggedness of the method, the inter-analysts RSD were < 1.5%, whereas the inter-instrumental variation expressed as RSD were < 2%. These low values of precision demonstrate the robustness and ruggedness of the proposed methods (Table 4).

The results of the selectivity study confirmed that the proposed methods are accurate and precise even in the presence of various excipients. The analysis of synthetic mixture solution yielded percent recoveries which ranged from 96.82-101.0 with standard

Table 2. Sensitivity and regression parameters

Parameter	Method A	Method B	Method C	Method D
λ_{max}, nm	410	410	410	410
Linear range, µg/mL	1.0-12	1.0-12	0.75-7.5	0.75-9.0
Molar absorptivity(ε), L/mol cm	2.07 x 10^5	2.28 x 10^5	2.91 x 10^5	1.66 x 10^5
Sandell sensitivity[a], µg/cm^2	0.016	0.015	0.012	0.022
Limit of detection (LOD), µg/mL	0.11	0.09	0.07	0.12
Limit of quantification (LOQ), µg/mL	0.32	0.27	0.20	0.36
Regression equation, Y[b]				
Intercept (a) ± CL	0.010 ± 0.053	0.002 ± 0.047	0.003 ± 0.049	0.002 ± 0.025
Slope (b) ± CL	0.059 ± 0.0088	0.067± 0.0035	0.086 ± 0.0067	0.049 ± 0.0038
Standard deviation of a (S_a)	0.039	0.019	0.019	0.010
Standard deviation of b (S_b)	0.004	0.002	0.003	0.002
Regression coefficient (R^2)	0.999	0.999	0.999	0.999

[a] Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. [b] Y=a+bX, Where Y is the absorbance, X is concentration in µg/mL, a is intercept, b is slope. CL is confidence limit at 95%.

Table 3. Evaluation of intra-day and inter-day precision and accuracy of the methods

Method	FMT taken µg/mL	Intra-day precision and accuracy (n=7)			Inter-day precision and accuracy (n=7)		
		FMT found µg/mL	%RSD	%RE	FMT found µg/mL	%RSD	%RE
Method A	4.0	4.07	0.92	1.75	4.06	1.02	1.50
	8.0	7.84	1.01	2.00	8.13	0.97	1.63
	12.0	11.86	0.98	1.17	11.80	1.05	1.67
Method B	4.0	4.10	1.02	2.50	4.08	1.11	2.00
	8.0	8.11	0.96	1.38	8.15	1.20	1.88
	12.0	11.76	1.08	2.00	12.23	1.15	1.92
Method C	1.5	1.47	1.12	2.00	1.53	1.15	2.33
	4.5	4.40	1.15	2.22	4.59	1.21	2.00
	7.5	7.31	1.02	2.53	7.67	1.11	2.26
Method D	3.0	3.09	1.17	3.00	3.05	1.25	1.66
	6.0	6.17	1.21	2.83	6.13	1.19	2.17
	9.0	9.14	1.14	1.56	9.21	1.27	2.33

RSD. Relative standard deviation; RE. Relative error.

Table 4. Robustness and ruggedness expressed as intermediate precision (%RSD)

Method	FMT taken µg/mL	Method robustness[a]	Method ruggedness	
		RSD, % (n=3)	Inter-analyst, RSD, % (n=4)	Inter-instruments, RSD, % (n=3)
Method A:BTB (Pure drug)	4.0	1.21	0.83	1.35
	8.0	1.34	0.98	1.21
	12.0	1.27	0.92	1.27
Tablet (Topcid-20)	4.0	1.42	1.11	1.31
	8.0	1.37	1.03	1.35
	12.0	1.32	0.98	1.28
Method B:BPB (Pure drug)	4.0	1.41	0.89	1.52
	8.0	1.32	0.96	1.47
	12.0	1.29	0.83	1.41
Tablet (Topcid-20)	4.0	1.35	0.96	1.31
	8.0	1.14	1.14	1.43
	12.0	1.31	1.07	1.38
Method C:BCP (Pure drug)	1.5	1.13	0.79	1.52
	4.5	1.19	0.85	1.38
	7.5	1.24	0.81	1.45
Tablet (Topcid-20)	1.5	1.28	0.97	1.37
	4.5	1.32	1.17	1.42
	7.5	1.37	1.08	1.29
Method D:BCG (Pure drug)	3.0	1.25	0.87	1.61
	6.0	1.17	0.72	1.50
	9.0	1.32	0.76	1.46
Tablet (Topcid-20)	3.0	1.25	1.09	1.54
	6.0	1.37	1.13	1.36
	9.0	1.29	1.25	1.41

[a] Dyes (BTB, BPB, BCP and BCG) volumes used were 0.8, 1.0 and 1.2 mL.

Table 5. Recovery of the drug from synthetic mixture

Method	FMT in synthetic mixture taken µg/mL	FMT recoverd[a] (Percent ±SD)
Method A	4.0	97.85 ± 1.16
	8.0	97.57 ± 1.25
	12.0	97.42 ± 1.11
Method B	4.0	101.0 ± 1.24
	8.0	100.3 ± 1.26
	12.0	99.78 ± 1.36
Method C	1.5	97.82 ± 1.34
	4.5	97.45 ± 1.21
	7.5	96.82 ± 1.28
Method D	3.0	98.21 ± 1.19
	6.0	97.83 ± 1.25
	9.0	97.72 ± 1.21

[a] Mean value of five determinations

deviation of 1.11-1.36 in all the cases. The results of this study are presented in Table 5 indicating that the inactive ingredients did not interfere in the assay.

For a statistical comparison of the tablet assay results obtained by the proposed methods with those obtained by reference method,[2] Student's *t*-test for accuracy and *F*-test for precision were applied to the experimental results. The results (Table 6) showed that the Student's *t*- and *F*-values at 95% confidence level did not exceed the tabulated values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. In all the cases, the percentage recovery values of pure FMT added was in the range of 96.67-101.5% with standard deviation of 0.98-1.21. The results of this study summarized in Table 7 indicate that neither the accuracy nor the precision of the methods is affected by the co formulated substances.

Comparison of the tablet assay results by the four proposed methods

The results of the four different methods for the assay of FMT in tablets were compared by calculating average percent recovery, average % RSD for precision and robustness. The results shown in Table 8 indicate that method B is more accurate, precise and robust, hence BPB is found to be the best dye for quantitative analysis of FMT from tablets.

CONCLUSIONS

This paper describes for the first time the application of extraction-free spectrophotometric technique for the determination of FMT in pharmaceutical formulations. Compared with the existing methods, the proposed methods are very simple, selective, free from auxiliary reagents, no-pH adjustment unlike other reported methods,[19,26] single

Table 6. Results of analysis of tablets by the proposed method

Tablet brand name	Label claim, mg/tablet	Found[a] (Percent of label claim ±SD)				
		Reference method	Method A	Method B	Method C	Method D
Topcid-20[b]	20	99.94 ± 1.05	98.21 ± 1.19 *t* = 2.44 *F* = 1.28	98.92 ± 1.07 *t* =1.52 *F* = 1.04	98.97 ± 1.12 *t* =1.41 *F* = 1.13	102.3 ± 0.97 *t* = 2.34 *F* = 1.51
Famocid-20[c]	20	99.87 ± 1.03	98.56 ± 1.11 *t* = 1.93 *F* = 1.16	102.1 ± 1.06 *t* =2.13 *F* =1.06	98.31 ± 1.18 *t* =2.20 *F* =1.31	98.59 ± 1.15 *t* =1.86 *F* =1.25

[a] Mean value of five determinations. [b] Torrent Pharmaceuticals Ltd., H. P, India; [c] Sun Pharmaceuticals Industries, Jammu, India. The value of *t* (tabulated) at 95% confidence level is 2.78. The value of *F* (tabulated) at 95% confidence level is 6.39.

Table 7. Accuracy assessment by recovery experiments

Method	Tablet studied	FMT in tablet µg/mL	Pure FMT added µg/mL	Total found µg/mL	Pure FMT recovered[a] Percent ± SD	RSD %
Method A	Topcid-20	3.93	2.0	5.96	101.5 ± 1.21	1.19
		3.93	4.0	7.92	99.75 ± 1.13	1.13
		3.93	6.0	9.87	99.00 ± 1.17	1.18
Method B	Topcid-20	3.96	2.0	5.98	101.0 ± 1.05	1.04
		3.96	4.0	7.95	99.75 ± 1.08	1.08
		3.96	6.0	9.93	99.50 ± 1.02	1.03
Method C	Topcid-20	2.97	1.5	4.46	99.33 ± 1.16	1.17
		2.97	3.0	5.95	99.33 ± 1.07	1.08
		2.97	4.5	7.42	98.89 ± 1.13	1.14
Method D	Topcid-20	3.07	1.5	4.52	96.67 ± 1.07	1.11
		3.07	3.0	6.08	100.3 ± 0.98	0.98
		3.07	4.5	7.64	101.5 ± 1.04	1.08

[a] Mean value of three measurements.

Table 8. Comparison of the tablet assay results obtained from four proposed methods

Methods	% Average recovery (n=3)	Precision (average, % RSD) (n=3)	Robustness (average, % RSD) (n=3)
Method A	100.08	1.17	1.37
Method B	100.08	1.05	1.27
Method C	99.18	1.13	1.32
Method D	99.49	1.06	1.30

step reaction and cost-effective. Besides simplicity and selectivity, the proposed spectrophotometric methods are the most sensitive ever reported for FMT (Table 1). The methods are one order of magnitude more sensitive than the reported methods using the same dyes with extraction procedure. The most attractive feature of the method is its relative freedom from interference by the usual tablet diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The high accuracy and precision of the methods may be attributed to the absence of experimental variables which normally would affect the absorbance values. Hence, recommended methods are well suited for the assay and evaluation of drug in pharmaceutical industrial quality control.

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