Research Article

Utility of N-Bromosuccinimide for the Titrimetric and Spectrophotometric Determination of Famotidine in Pharmaceutical Formulations

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Two titrimetric and two spectrophotometric methods are described for the assay of famotidine (FMT) in tablets using N-bromosuccinimide (NBS). The first titrimetric method is direct in which FMT is titrated directly with NBS in HCl medium using methyl orange as indicator (method A). The remaining three methods are indirect in which the unreacted NBS is determined after the complete reaction between FMT and NBS by iodometric back titration (method B) or by reacting with a fixed amount of either indigo carmine (method C) or neutral red (method D). The method A and method B are applicable over the range of 2–9 mg and 1–7 mg, respectively. In spectrophotometric methods, Beer’s law is obeyed over the concentration ranges of 0.75–6.0 μg mL⁻¹ (method C) and 0.3–3.0 μg mL⁻¹ (method D). The applicability of the developed methods was demonstrated by the determination of FMT in pure drug as well as in tablets.

1. Introduction

Famotidine (FMT), 3-[2-(diaminomethyleneamino)thiazol-4-ylmethylthio]-N-sulfamoylpropionamidine (Figure 1), is a histamine H₂-receptor antagonist (H₂-RA). It is widely used for the treatment of duodenal ulcers, benign gastric ulcer, reflux oesophagitis, and hyperacid secretory conditions. FMT is official in both the British Pharmacopoeia (BP) [1] and the United States Pharmacopoeia (USP) [2]. The BP [1] recommends thin-layer chromatography using a silica gel F₂₅₄ precoated plate (Fischer Silica Gel GF plates are suitable) and a mixture of 2 volumes of 13.5 M ammonia, 20 volumes of toluene, 25 volumes of methanol, and 40 volumes of ethyl acetate as the mobile phase. The USP [2] recommends a potentiometric nonaqueous method for the determination of FMT using perchloric acid as the titrant and a HPLC method using a mixture of acetic acid buffer of pH 6: acetonitrile (93:7) as a mobile phase with UV detection at 275 nm.

Several procedures have been reported in the literature for the analysis of FMT. The reported methods include HPLC [3–5], HPTLC [6, 7], capillary electrophoresis [8], potentiometry [9], differential pulse voltammetry [10], spectrofluorimetry [11, 12], polarography [13], and UV-spectrophotometry [14]. Some of these methods involve several manipulation steps, which are not simple for routine analysis of pharmaceutical formulations and need sophisticated instruments.

Titrimetry and visible spectrophotometry may serve as useful alternatives to many of the aforesaid sophisticated techniques because of their cost effectiveness, ease of operation, sensitivity, remarkable accuracy and precision, and wide applicability. From the literature survey, it is revealed that two titrimetric methods have been reported using chloramine-T [15] and potassium iodate [16] as oxidimetric reagents. Visible spectrophotometric methods based on diverse reaction chemistry have been proposed for the assay of FMT in pharmaceuticals. Extractive spectrophotometric procedures [17] based on ion pair complexation reaction with bromocresol green (BCG) and bromothymol blue (BTB) have been used for the assay of FMT. Based on the formation of charge-transfer complex, some π
acceptors such as chloranil, dichlorodicyanobenzoquinone and dichloronitrophenol [18], tetracyanoquinodimethane [19], and p-chloranilic acid [20, 21] have been employed for its determination in commercial tablet formulations. Other visible spectrophotometric methods based on different reactions such as complex formation reaction with copper (II) chloride in methanolic medium [22], cupric acetate [23], palladium (II) chloride in Britton Robinson buffer solution in the pH range 2.23–8.5 [24], condensation of amino group in FMT with ninhydrin in DMF medium [25], formation of orange-colored product with sodium nitroprusside in alkaline medium [26], bromination of FMT with brominating mixture [27] reduction of Folin-Ciocalteau reagent [28], and oxidation of FMT by Fe (III) [29] or NBS [30] were reported for the assay of FMT. Most of the above visible spectrophotometric methods suffer from one or another disadvantage such as the use of organic solvents [17–22, 25], poor sensitivity [18, 20–23, 26, 27], less selective [28], use of expensive reagents [19], use of heating step [25, 29], narrow linear range [25, 30], and close pH control [17, 24], as indicated in Table 1. The present investigation aims to develop simple, sensitive, and cost-effective methods for the determination of FMT in pure form and in dosage forms using titrimetric and spectrophotometric techniques. The methods utilized NBS, indigo carmine, and neutral red as reagents. The developed methods offer the advantages of simplicity, speed, accuracy, and precision without the need for costly equipment/chemicals.

2. Experimental

2.1. Apparatus. All absorbance measurements were made on a Systronics model 106 digital spectrophotometer (Ahmedabad, India) provided with 1 cm matched quartz cells.

2.2. Materials and Reagents. All chemicals and reagents used were of analytical or pharmaceutical grade.

2.2.1. N-Bromosuccinimide (NBS). 0.05 M NBS solution was prepared by dissolving 9.93 g of chemical (S. D. Fine Chem. Ltd., Mumbai, India) in one liter of water for use in titrimetric method B.

2.2.2. Sodium Thiosulphate (0.04 M). Prepared by dissolving 9.93 g of chemical (S. D. Fine Chem. Ltd., Mumbai, India) in one liter of water for use in titrimetric method B.

2.2.3. Sulphuric Acid and HCl. Concentrated sulphuric acid (S. D. Fine Chem, Mumbai, India, sp. gr. 1.84) and concentrated HCl (S. D. Fine Chem, Mumbai, India, sp. gr. 1.18) was appropriately diluted with water to get the required concentrations.

2.2.4. Potassium Iodide. A 10% solution was prepared by dissolving 10 g of the chemical in 100 mL of water and used in titrimetric method B.

2.2.5. Starch Indicator. One g of the reagent (Merck, Mumbai, India) was made into a paste and poured into 100 mL of boiling water, boiled for 1 min and cooled, and used for titrimetric method B.

2.2.6. Indigo Carmine (200 μg mL\(^{-1}\)) and Neutral Red (240 μg mL\(^{-1}\)). The dye solutions were prepared by dissolving calculated quantity of the chemicals, indigo carmine (S. D. Fine Chem, Mumbai, India, dye content 90%) and neutral red (S. D. Fine Chem, Mumbai, India, dye content 90%) in water and filtered.

2.2.7. Standard FMT Solution. Pharmaceutical grade FMT certified to be 99.98% pure was received as a gift from Cipla India Ltd, Mumbai, India, and used as received. Standard FMT solution (1 mg mL\(^{-1}\), 15 μg mL\(^{-1}\), and 6 μg mL\(^{-1}\)) was prepared by dissolving calculated quantity of pure drug in 0.1 M HCl (method A, method C, and method D) and in 0.5 M H\(_2\)SO\(_4\) (method B).

2.2.8. Pharmaceutical Preparations. 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.

2.3. Materials and Reagents Used in Reference Method

2.3.1. Apparatus. An Elico 120 digital pH meter provided with a combined glass-SCE electrode system (Equip-Tronics, Mumbai, India) was used for potentiometric titration.

2.3.2. Preparation of Modified Glass-Saturated Calomel Electrode. Aqueous potassium chloride solution contained in the saturated calomel electrode was completely removed, and the electrode was rendered anhydrous and filled with 0.1 N lithium perchlorate in acetic anhydride.
### 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⁻¹) and molar absorptivity (l mol⁻¹ cm⁻¹) | Remarks                                                                                           | Reference |
|---------|---------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------|---------------------------------------------------------------------------------------------------|-----------|
| (1)     | Chloramine-T and metol                | Red-colored product was measured at 520 nm                                 | 0.0–40 (2.78 × 10³)                                             | Less sensitive, metol solution is not stable, and even the blank absorbance is high               | [15]      |
| (2)     | Potassium iodate                      | The absorbance of the red-colored solutions was measured at 520 nm         | (1) 2.5–25 (8.40 × 10³) (2) 200–1400 (1.16 × 10⁵)              | Require strict pH control, and the sensitivity of the method which involves extraction was very less | [16]      |
| (3)     | (a) Bromocresol green (b) Bromothymol blue | Ion-pair complex measured at 420 nm                                      | 2.0–23 (ε = 5.0 × 10³) 0.7–8.1 (ε = 1.2 × 10⁴)                | Sensitive but required close pH control and extraction use of organic solvent                    | [17]      |
| (4)     | (a) Chloranil (b) DDQ (c) DCNP        | The increase in the absorbance is measured at 458, 460, and 425 nm, respectively | 50–500                                                      | Poor sensitivity and use of organic solvent                                                      | [18]      |
| (5)     | Tetracyanoquinodimethane (a) P-chloranilic acid in methanol (b) P-chloranilic acid in acetonitrile | Green radical anion measured at 840 nm                                      | 1.0–7.0                                                      | Uses expensive reagents and organic solvents                                                     | [19]      |
| (6)     | Copper (II) chloride in methanolic medium | Blue-colored complex measured at 660 nm                                     | 200–1200 (ε = 1.11 × 10⁵)                                     | Less sensitive, narrow linear range, and use of organic solvent                                  | [20, 21] |
| (7)     | Cupric acetate                        | Complex measured at 630 nm                                                 | 50–1250                                                      | Poor sensitivity                                                                                | [22]      |
| (8)     | Palladium (II)                        | Yellow-colored complex measured at 345 nm in the pH range 2.23–8.50         | 17–200                                                      | Sensitive but required close pH control                                                        | [23]      |
| (9)     | Ninhydrin in methanolic medium        | Blue-colored product measured at 590 nm.                                   | 5.0–30.0                                                     | Requires heating in a boiling water bath and use of organic solvent                              | [24]      |
| (10)    | Sodium nitroprusside                  | Orange species formed in alkaline medium measured at 498 nm                 | 50–500                                                      | Poor sensitivity                                                                                | [25]      |
| (11)    | Brominating mixture                   | Yellow-colored developed measured at 350 nm                                 | 40–200                                                      | Less sensitive and measured at shorter wavelength                                              | [26]      |
| (12)    | F-C reagent                           | Blue-colored product measured at 650 nm                                    | 16–48                                                        | Less selective                                                                                 | [27]      |
| (13)    | Fe (III) with (a) 1,10-phenanthroline (b) 2, 2-bipyridyl | The decrease in the absorption intensity of the colored product measured at 520 nm | (a) 2–12 (ε = 2.9 × 10⁴) (b) 8–16 (ε = 1.6 × 10⁴) | Requires heating and longer reaction time                                                       | [28]      |
| (14)    | NBS with p-aminophenol                | Resulting colored products peaking at 610 nm and 530 nm                      | 0.8–6.0 (ε = 4.199 × 10³) 0.3–3.0 (ε = 1.089 × 10³)     | Highly sensitive, no heating or extraction step, inexpensive instrumental setup, use of ecofriendly chemicals, and aqueous system | Present methods |
| (15)    | NBS (a) indigo carmine (b) neutral red | Resulting colored products peaking at 610 nm and 530 nm                      | 0.8–6.0 (ε = 4.199 × 10³) 0.3–3.0 (ε = 1.089 × 10³)     | Highly sensitive, no heating or extraction step, inexpensive instrumental setup, use of ecofriendly chemicals, and aqueous system | Present methods |

NBS: N-bromosuccinimide; FC: Folin-Ciocalteu; DDQ: dichloro dicyano benzoquinone; DCNP: dichloronitrophenol.
2.3.3. Perchloric Acid (0.1 M). The commercially available 0.1 M perchloric acid (Merck, Mumbai, India) was standardized against 0.1 M potassium dihydrogen phthalate [32].

Lithium Perchlorate (0.1 N). The solution was prepared by dissolving calculated quantity of lithium perchlorate (Himedia Lab. Pvt. Limited, Mumbai, India) in acetic anhydride (Merck, Mumbai, India).

2.4. General Procedure

2.4.1. Titrimetry

**Method A.** Different volumes (2–9 mL) of standard solution containing 1 mg mL\(^{-1}\) FMT were accurately measured and transferred into a 100 mL titration flask, and the volume was made up to 10 mL with 0.1 M HCl. Five mL of 2 M HCl and one spatula of KBr were added. The resulting solution was titrated against 0.01 M NBS using 2 drops of methyl orange as indicator. The titration was carried by dropwise addition of NBS with continuous shaking and the end point is the disappearance of pink color.

**Method B.** Different volumes (1–7 mL) of standard solution containing 1 mg mL\(^{-1}\) FMT were taken in a 100 mL titration flask, and the volume was made up to 10 mL with 0.5 M H\(_2\)SO\(_4\). Two mL of 5 M H\(_2\)SO\(_4\) and one spatula of KBr were added into the flask. Ten mL of NBS (0.02 M) was pipetted into the flask; the content was mixed and kept aside for 5 min, then 5 mL of 10% potassium iodide solution was added, and the liberated iodine was titrated against sodium thiosulphate (0.04 M) using starch indicator. A blank titration was performed under identical conditions.
2.4.2. Spectrophotometry

Method C. Different aliquots of 0.25, 0.5, 1.0, 2.0, 3.0, and 4.0 mL of standard FMT solution (15 μg mL⁻¹) were transferred into a series of 10 mL standard volumetric flasks, and the total volume in each flask was adjusted to 4 mL with 0.1 M HCl. To each flask, 1 mL of 0.1 M HCl followed by 1 mL of 150 μg mL⁻¹ NBS was added. The contents of each flask were mixed well and kept aside for 15 minutes with occasional shaking. One mL of 200 μg mL⁻¹ indigo carmine was added, and the volume was made up to the mark with distilled water after 5 min, and the absorbance was measured at 610 nm versus reagent blank prepared in a similar manner.

Method D. Different aliquots (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0) mL of standard 6 μg mL⁻¹ FMT solution were accurately measured and transferred into a series of 10 mL calibrated flasks by means of a microburette, and the total volume was adjusted to 5 mL with 0.1 M HCl. To each flask, 1 mL of 200 μg mL⁻¹ NBS was added using microburette. And the contents of each flask were mixed well and kept aside for 15 minutes. One mL of 240 μg mL⁻¹ neutral red was added to each flask and mixed well. The volume was made up to the mark with distilled water after 5 min, and the absorbance was measured at 530 nm versus reagent blank prepared in a similar manner.

Calibration graphs were prepared by plotting the increasing absorbance values versus concentrations of FMT. The concentration of the unknown was read from the respective calibration graph or deduced from the regression equation derived using the Beer’s law data.

2.4.3. Procedure for the Assay of FMT in Tablets. Twenty tablets were weighed accurately and ground into a fine powder. A quantity of the powder containing 100 mg of FMT was accurately weighed into a 100 mL calibrated flask, and 60 mL of 0.1 M HCl (titrimetric method A) or 0.5 M H₂SO₄ (titrimetric method B) was added. The content was shaken for about 20 min; the volume was diluted to the mark with the respective solvent mixed, and filtered using a Whatman no. 42 filter paper. First, 10 mL portion of the filtrate was discarded, and a convenient aliquot was taken, and the assay was completed according to the titrimetric procedures described earlier. The tablet extract containing FMT at a concentration of 1 mg mL⁻¹ was then diluted stepwise with 0.1 M HCl to obtain working concentrations of 15 μg mL⁻¹ and 6 μg mL⁻¹ in FMT for spectrophotometric method C and method D, respectively. A convenient aliquot was then subjected to analysis by spectrophotometric procedures described above.

2.4.4. Placebo Blank Analysis. A placebo blank of the composition: talc (10 mg), starch (5 mg), acacia (5 mg), methyl cellulose (10 mg), sodium citrate (5 mg), magnesium stearate (10 mg), and sodium alginate (5 mg) was made, and its solution was prepared as described under “Procedure for the assay of famotidine in pharmaceutical preparations” and then subjected to analysis using the procedures described above.

2.4.5. Procedure for the Determination of FMT in Synthetic Mixture. To the placebo blank of the composition described above, 100 mg of FMT was added homogenized, and transferred to a 100 mL calibration flask, and solution was prepared as described under tablets. The solution was mixed well and filtered using a Whatman no. 42 filter paper. The resulting solution was assayed (n = 5) by titrimetry according to the procedures described above. The synthetic mixture solution (1 mg mL⁻¹ in FMT) was then diluted stepwise with 0.1 M HCl to obtain working concentrations of 15 μg mL⁻¹ and 6 μg mL⁻¹ in FMT for spectrophotometric method C and method D, respectively. A convenient aliquot was then subjected to analysis by using the procedures described above.

2.4.6. Procedure for Reference Method. Two hundred and fifty mg of FMT was accurately weighed and dissolved in 80 mL of glacial acetic acid and titrated with 0.1 M perchloric acid, using modified glass-saturated calomel electrode which was described above. Near the equivalence point, the titrant was added in 0.05 mL increments, and after each addition of titrant, the solution was stirred for 30 s, and the steady potential was recorded. The titration was continued until there was no significant change in the potential on further addition of the titrant. A blank determination was performed to make necessary correction.

3. Results and Discussion

NBS is a brominating and oxidizing agent that is perhaps the most important positive bromine containing organic compound, and it is used for the specific purpose of brominating
alkenes at the allylic position [33]. NBS has earlier been used for the assay of FMT using p-aminophenol as reagent, and the present work extended the utility of NBS as an oxidimetric as well as brominating agent. Two titrimetric and two spectrophotometric methods to the determination of FMT were developed and validated as per the current ICH guidelines [34].

The titrimetric method A is direct where the FMT is directly titrated against NBS in HCl medium using methyl orange as indicator, whereas in back titration (method B), the reactants were allowed to stand for some time in H2SO4 medium, and the unreacted NBS is determined iodimetrically (Figure 2). The proposed spectrophotometric methods involve two steps: oxidation/bromination of FMT by NBS (first step) and estimation of unconsumed NBS with indigo carmine (method C) which give maximum absorption at 610 nm (Figure 3) or neutral red (method D) which give maximum absorption at 530 nm (Figure 3) (second step). The tentative reaction scheme is shown in Figure 2.

3.1. Optimization of Reaction Conditions

3.1.1. Titrimetry. The reaction stoichiometry was found to be 1 : 2 (FMT : NBS) in method A and 1 : 5 (FMT : NBS) in method B. In method A, titration was carried out instantaneously, and in method B, the reaction between FMT and NBS was kept for 5 min. The difference in the reaction stoichiometry in method A and B is perhaps due to the difference in reaction time and possibility of getting two different reaction products. The structure of FMT features aliphatic sulphur which can easily undergo oxidation to form sulphone and sulphone [35, 36] and also bromination at allylic position [33]. On addition to these reactions, the bromination at C5 position of thiazole ring in FMT and oxidation of sulphur in thiazole to sulphoxide [37] are also possible routes. Electrophilic aromatic substitution at C5 of thiazole requires activating groups [38]. The tertiary-amino group present in FMT is a strong activating group, so the bromination of thiazole in FMT is possible. Based on these observations, two possible reaction products are proposed and shown in Figure 2. In both the methods, constant reaction stoichiometry was obtained only in the presence of KBr. One spatula of KBr was found to be optimum to accelerate the oxidation/bromination process. In direct titration, quantitative results were obtained in HCl medium, and the reaction stoichiometry was unaffected in the concentration range of 0.53–0.8 M HCl. Hence, 5 mL of 2 M HCl in a total volume of 15 mL was fixed. In indirect titration, 2 mL volume of 5 M H2SO4 in a total volume of 26 mL was found adequate although 1–3 mL resulted in the same value of “n.” The oxidation/bromination reaction was found to be complete and quantitative in 5 min, and contact time upto 20 min had no effect on the stoichiometry or the results. Hence, it is necessary to terminate the oxidation/bromination step at the end of 5 min to obtain accurate and precise results. A 10 mL aliquot of 0.02 M NBS solution was found adequate for quantitative oxidation/bromination of FMT in the range determined to be 1–7 mg.

3.1.2. Spectrophotometry. Preliminary experiments were performed to determine the maximum concentration of indigo carmine or neutral red in the acid medium employed, and this was found to be 20 μg mL−1 and 24 μg mL−1 for indigo carmine and neutral red, respectively. NBS concentration of 15 μg mL−1 was found optimum to bleach the blue color due to 20 μg mL−1 indigo carmine, whereas in the case of neutral red, 20 μg mL−1 NBS was sufficient to destroy the pink color of 24 μg mL−1 neutral red. Hence, different amounts of FMT reacted with 15 μg mL−1 NBS in method C and 20 μg mL−1 NBS in method D.

Hydrochloric acid was the ideal medium for the oxidation/bromination of FMT by NBS as well as the latter’s determination employing either dye. In method C, the reaction between FMT and NBS was unaffected when 0.5–2.0 mL of 1 M HCl in a total volume of 10 mL was used. Hence, 1 mL of 1 M HCl was used for both steps in method C, whereas in method D, the reaction was found to be faster in lower acid concentration. Hence, 0.1 M HCl which was used to dilute the series of drug solution upto 5 mL was found sufficient for both steps. For a quantitative reaction between FMT and NBS, a contact time of 15 min was found necessary in both the methods C and D; and constant absorbance readings were obtained when contact times were extended.
Table 2: Sensitivity and regression parameters.

| Parameter                                | Method C                  | Method D                  |
|------------------------------------------|---------------------------|---------------------------|
| \( \text{max} \lambda \text{ nm} \)  | 610                       | 530                       |
| Linear range, \( \mu \text{ gm L}^{-1} \) | 0.75–6.0                  | 0.3–3.0                   |
| Molar absorptivity(\( \varepsilon \)), \( \text{L mol}^{-1} \text{ cm}^{-1} \) | \(4.199 \times 10^4\) | \(1.089 \times 10^5\) |
| Sandell sensitivity\( a \), \( \mu \text{ gm cm}^{-2} \) | 0.008                     | 0.003                     |
| Limit of detection (LOD), \( \mu \text{ gm L}^{-1} \) | 0.05                      | 0.02                      |
| Limit of quantification (LOQ), \( \mu \text{ gm L}^{-1} \) | 0.16                      | 0.06                      |
| Regression equation, \( Y = a + bX \)  |                           |                           |
| Intercept \( (a) \)                      | 0.011                     | 0.02                      |
| Slope \( (b) \)                          | 0.122                     | 0.303                     |
| Standard deviation of \( a \) (\( S_a \)) | 0.048                     | 0.063                     |
| Standard deviation of \( b \) (\( S_b \)) | 0.009                     | 0.023                     |
| Regression coefficient \( (r) \)        | 0.996                     | 0.999                     |

\( a \) Limit of determination as the weight in \( \mu \text{ g} \) 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 \( l = 1 \text{ cm} \).

\( b \) \( Y = a + bX \), where \( Y \) is the absorbance, \( X \) is concentration in \( \mu \text{ gm L}^{-1} \), \( a \) is intercept, and \( b \) is slope.

Table 3: Evaluation of intraday and interday accuracy and precision.

| Method\( a \) | FMT taken | Intraday accuracy and precision | Interday accuracy and precision |
|---------------|-----------|---------------------------------|---------------------------------|
|               | FMT found | %RE    | %RSD  | FMT found | %RE    | %RSD  |
| Titrimetry method A | 3.0       | 3.08   | 2.66  | 1.06      | 3.10   | 3.3   | 1.32 |
|                | 6.0       | 6.1    | 1.67  | 1.01      | 6.13   | 2.17  | 0.97 |
|                | 9.0       | 8.75   | 2.77  | 0.95      | 8.72   | 3.11  | 1.18 |
|                | 1.0       | 1.02   | 2.01  | 1.02      | 0.97   | 3.01  | 1.12 |
| Method B       | 5.0       | 4.94   | 1.22  | 0.98      | 4.89   | 2.22  | 1.34 |
|                | 7.0       | 6.92   | 1.14  | 1.11      | 6.87   | 1.86  | 1.29 |
|                | 3.0       | 3.07   | 2.33  | 0.92      | 3.10   | 3.33  | 1.15 |
| Spectrophotometry method C | 4.5       | 4.59   | 2.00  | 1.14      | 4.61   | 2.44  | 1.28 |
|                | 6.0       | 5.8    | 3.33  | 1.02      | 5.82   | 3.01  | 1.11 |
|                | 0.6       | 0.62   | 3.33  | 1.25      | 0.58   | 3.33  | 1.35 |
| Method D       | 1.8       | 1.85   | 2.78  | 1.21      | 1.76   | 2.22  | 1.29 |
|                | 3.0       | 2.94   | 2.02  | 1.13      | 3.10   | 3.33  | 1.17 |

RE: Relative error and RSD: Relative standard deviation.

\( a \) In titrimetry, FMTs taken/found are in mg and they are \( \mu \text{ gm L}^{-1} \) in spectrophotometry.

upto 60 min. In both the methods, a standing time of 5 min was necessary for the bleaching of the dye color by the residual NBS, and the measured color was found to be stable for several hours in the presence of the reaction product/s.

3.1.3. Methodology of the Reference Method. The method is based on the neutralization reaction of the weak base FMT with perchloric acid as a titrant in glacial acetic acid medium. When a strong acid, such as perchloric acid, is dissolved in a weaker acid, such as acetic acid, the acetic acid is forced to act as a base and accept a proton from the perchloric acid forming an onium ion [39]. The formed onium ion (CH\(_3\)COOH\(^+\)) can very readily give up its proton to react with FMT, so basic properties of the drug are enhanced, and hence titration between FMT and perchloric acid was accurately carried out using acetic acid as solvent.

3.2. Method Validation Procedures. The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity, and recovery.

3.2.1. Linearity and Sensitivity. Over the range investigated (2–9 mg in method A and 1–7 mg in method B), fixed stoichiometry of 1:2 (FMT:NBS) and 1:5 (FMT:NBS) in method A and method B, respectively, was obtained in titrimetry which served as the basis for calculations. In spectrophotometry, under optimum conditions, a linear relation was obtained between absorbance and concentration of FMT in the range of 0.75–6.0 \( \mu \text{ gm L}^{-1} \) (method C) and 0.3–3.0 \( \mu \text{ gm L}^{-1} \) (method D) (Figure 4). The calibration graph is described by the equation

\[ Y = a + bX, \]
Table 4: Recovery of the drug from synthetic mixture.

| Method | FMT in synthetic mixture taken<sup>a</sup> | FMT recovered<sup>b</sup> (percent ± SD) |
|--------|-----------------------------------------|---------------------------------------|
| Method A | 2.0 | 105.4 ± 1.13 |
|         | 5.0 | 99.17 ± 1.21 |
|         | 9.0 | 96.54 ± 1.08 |
| Method B | 2.0 | 96.01 ± 1.14 |
|         | 4.0 | 95.93 ± 1.26 |
|         | 6.0 | 94.78 ± 1.46 |
| Method C | 3.0 | 101.0 ± 1.04 |
|         | 4.5 | 100.8 ± 1.01 |
|         | 6.0 | 96.82 ± 0.98 |
| Method D | 0.6 | 117.3 ± 1.19 |
|         | 1.8 | 122.1 ± 1.05 |
|         | 3.0 | 113.7 ± 1.15 |

<sup>a</sup>mg in titrimetry and μg mL<sup>−1</sup> in spectrophotometry.
<sup>b</sup>Mean value of five determinations.

(\text{where } Y = \text{absorbance, } a = \text{intercept, } b = \text{slope, and } X = \text{concentration in } \mu\text{g mL}^{-1} \text{) obtained by the method of least squares. Correlation coefficient, intercept, and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values and the limits of detection and quantification are calculated as per the current ICH guidelines [34] which are compiled in Table 2 that speaks of the excellent sensitivity of the proposed method. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae

\begin{equation}
\text{LOD} = 3.3\frac{\sigma}{s}, \quad \text{LOQ} = 10\frac{\sigma}{s},
\end{equation}

where \( \sigma \) is the standard deviation of five reagent blank determinations, and \( s \) is the slope of the calibration curve.

3.2.2. Precision and Accuracy. Intraday precision and accuracy of the proposed methods were evaluated by replicate analysis (\( n = 5 \)) of calibration standards at three different concentration levels in the same day. Interday precision and accuracy were determined by assaying the calibration standards at the same concentration levels on five consecutive days. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively (Table 3).

3.2.3. Selectivity. The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. A convenient aliquot of the placebo blank solution prepared as described earlier was subjected to analysis by titrimetry and spectrophotometry according to the recommended procedures. In all the cases, there was no interference by the inactive ingredients.

A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution prepared above yielded percent recoveries which ranged of 94.78–122.1 with standard deviation of 0.98–1.46 in all the cases. The results of this study are presented in Table 4 indicating that the inactive ingredients did not interfere in the assay. These results further demonstrate the accuracy as well as the precision of the proposed methods.

3.2.4. Application to Formulations. In order to evaluate the analytical applicability of the proposed methods to the quantification of FMT in commercial tablets, the results obtained by the proposed methods were compared to those of the reference method [2] by applying Student’s \( t \)-test for accuracy and \( F \)-test for precision. The results (Table 5) show that the Student’s \( t \)- and \( F \)-values at 95% confidence level are less than the theoretical 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.

3.2.5. Recovery Studies. The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Preanalysed tablet powder was spiked with pure FMT at three concentration levels (50, 100, and 150% of that in tablet powder), and the total was found by the proposed methods. In all cases, the added FMT recovery percentage values ranged from 94.78 to 103.9% with standard deviation of 1.13–0.92 (Table 6) indicating that the recovery was good and that the coformulated substance did not interfere in the determination.

4. Conclusion

The methods described in this paper are simple, relatively specific, accurate, and precise for the determination of FMT. In particular, the proposed direct titrimetry is the simplest of all the methods reported so far for famotidine. The chromatographic techniques [3–8], although sensitive, require judicious control of pH of the mobile phase besides requiring expensive and sophisticated instruments. A large volume of solvents is required for these techniques, which are expensive, hazardous to health, and harmful to the environment. The methods based on such techniques as potentiometry [9], voltammetric [10], and polarography [13] require rigid pH control. The reliability and precision of the results by polarography [13] depend on the capillary characteristics which are often not reproducible. Even though the spectrofluorimetric methods [11, 12] are very sensitive, they use organic solvent which is not always desirable. The reported visible spectrophotometric methods based on ion-pair complexation reaction [17] require liquid-liquid extraction step, strict pH control, and large amounts of high purity solvents, which are often hazardous and result in the production of toxic lab waste. Other methods based on charge-transfer complexation reactions [18–21], complexation reaction using reagents like copper (II) chloride [22], cupric acetate [23], palladium (II) [24], condensation reaction [25], and redox reactions [15, 16, 26–30] suffer from disadvantages like poor sensitivity [15, 16, 18–28, 30], use
Table 5: Results of analysis of tablets by the proposed methods.

| Tablet brand name | Label claim, mg/tablet | Method | Titrimetry | Spectrophotometry |
|-------------------|------------------------|--------|------------|------------------|
|                   |                        | Found (percent of label claim ± SD) |            |                  |
|                   |                        | Method A | Method B | Method C | Method D |
| Topcid 20\textsuperscript{b} | 20 | 102.5 ± 0.93 | 101.21 ± 1.19 | 101.24 ± 0.87 | 103.5 ± 0.91 | 103.7 ± 1.07 |
|                   |                        |          | t = 1.92 | F = 1.63 | 101.3 ± 1.07 | 101.1 ± 0.92 | 103.5 ± 0.91 | 103.7 ± 1.07 |
| Famocid 20\textsuperscript{c} | 20 | 101.3 ± 1.02 | 99.56 ± 1.17 | 99.86 ± 1.06 | 102.7 ± 1.09 | 102.5 ± 0.95 |
|                   |                        |          | t = 2.51 | F = 0.76 | 101.24 ± 0.87 | 101.3 ± 0.92 | 103.5 ± 0.91 | 103.7 ± 1.07 |

\textsuperscript{a}Mean value of five determinations. \textsuperscript{b}Torrent Pharmaceuticals Ltd., H. P, India; \textsuperscript{c}Sun Pharmaceuticals Industries, Jammu, India.

The value of $t$ (tabulated) at 95% confidence level and for four degrees of freedom is 2.77.
The value of $F$ (tabulated) at 95% confidence level and for four degrees of freedom is 6.39.

Table 6: Accuracy assessment by recovery experiments.

| Method | Tablet studied | FMT in tablet$^a$ | Pure FMT added$^a$ | Total found$^a$ | Pure FMT recovered$^b$ percent ± SD |
|--------|----------------|-------------------|-------------------|----------------|-----------------------------------|
| Titrimetry method A | Topcid 20 | 3.0 | 1.5 | 4.52 | 101.3 |
| Method B | Topcid 20 | 3.0 | 3.0 | 5.97 | 99.0 |
| Spectrophotometry method C | Topcid 20 | 2.0 | 4.5 | 7.40 | 97.78 |
| Method D | Topcid 20 | 1.5 | 0.75 | 2.27 | 102.7 |
|                  |              | 1.5 | 1.5 | 3.04 | 103.1 |
|                  |              | 1.5 | 2.25 | 3.81 | 102.7 |
|                  |              | 1.2 | 0.6 | 1.82 | 103.3 |
|                  |              | 1.2 | 1.2 | 2.43 | 102.5 |
|                  |              | 1.2 | 1.8 | 3.07 | 103.9 |

$^a$mg in titrimetry and $\mu$g mL$^{-1}$ in spectrophotometry.
$^b$Mean value of three measurements.

of organic solvents [18–21, 25], strict pH control [16, 24], use of expensive reagent [19], boiling step [25, 29], and besides suffering from narrow linear range of applicability [22].

In contrast to the above-published methods, the present methods are sensitive, simple, and using ecofriendly chemicals, free from unwelcome steps such as heating or extraction and also from critical pH conditions. The present methods have wide linear dynamic ranges and can measure concentrations down to 0.8 and 0.3 $\mu$g mL$^{-1}$ with good precision and accuracy. The proposed methods are most sensitive compared to other reported visible spectrophotometric methods which are confirmed by the molar absorptivity values of $4.20 \times 10^4$ and $1.09 \times 10^3$ L mol$^{-1}$ cm$^{-1}$. The relative cheapness of apparatus and reagents demonstrate their advantageous characteristics. The methods are also useful due to high tolerance limit for common excipients found in drug formulations. These merits coupled with the use of simple and inexpensive instrument make the proposed methods acceptable in quality control laboratories for routine use.

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References

[1] British Pharmacopoeia, The Stationary Office London, 1998.
[2] U. S. Pharmacopoeia 30, National Formulary 35, U. S. Pharmacopoeial Convention, Rockville, Md, USA, 2007.
[3] S. E. Biffar and D. J. Mazzo, "Reversed-phase determination of famotidine, potential degradates, and preservatives in pharmaceutical formulations by high-performance liquid chromatography using silica as a stationary phase," Journal of Chromatography, vol. 363, no. 2, pp. 243–249, 1986.
[4] A. Mutaz, S. Sheikh, A. N. Hanan, and B. A. Adnan, "High pressure liquid chromatographic analysis and dissolution of famotidine in tablet formulation," Analytical Letters, vol. 22, no. 11&12, pp. 2501–2510, 1989.
[5] C. Ho, H. M. Huang, S. Y. Hsu, C. Y. Shaw, and B. L. Chang, "Simultaneous high-performance liquid chromatographic analysis for famotidine, ranitidine HCl, cimetidine, and nizatidine in commercial products," Drug Development and Industrial Pharmacy, vol. 25, no. 3, pp. 379–385, 1999.

[6] J. Novaković, "High-performance thin-layer chromatography for the determination of ranitidine hydrochloride and famotidine in pharmaceuticals," Journal of Chromatography A, vol. 846, no. 1–2, pp. 193–198, 1999.

[7] A. N. Campbell and J. Sherma, "Determination of famotidine in acid reduction tablets by HPTLC and videodensitometry of fluorescence quenched zones," Journal of Liquid Chromatography and Related Technologies, vol. 26, no. 16, pp. 2719–2727, 2003.

[8] S. M. Wu, Y. U. H. Ho, H. L. Wu, S. U. H. Chen, and H. S. Ko, "Simultaneous determination of cimetidine, famotidine, nizatidine, and ranitidine in tablets by capillary zone electrophoresis," Electrophoresis, vol. 22, no. 13, pp. 2758–2762, 2001.

[9] M. M. Ayad, A. Shalaby, H. E. Abdellatef, and H. M. Elsaid, "Potentiometric determination of famotidine in pharmaceutical formulations," Journal of Pharmaceutical and Biomedical Analysis, vol. 29, no. 1–2, pp. 247–254, 2002.

[10] J. A. Squeella, C. Rivera, I. Lemus, and L. J. Nuñez-Vergara, "Differential pulse voltammetric determination of famotidine," Microchimica Acta, vol. 100, no. 5–6, pp. 343–348, 1990.

[11] M. I. Walash, A. El-Brashy, N. El-Enany, and M. E. Kamel, "Spectrofluorimetric determination of famotidine in pharmaceutical preparations and biological fluids. Application to stability studies," Journal of Fluorescence, vol. 19, no. 2, pp. 333–344, 2009.

[12] A. Ael-Bayoumi, A. A. El-Shanawany, M. E. El-Sadek, and A. Abd El-Sattar, "Synchronous spectrofluorimetric determination of famotidine, fluconazole and ketoconazole in bulk powder and in pharmaceutical dosage forms," Spectroscopy Letters, vol. 30, no. 1, pp. 25–46, 1997.

[13] M. I. Walash, M. K. Sharaf-El-Din, M. E. S. Metwally, and M. R. Shabana, "Polarographic determination of famotidine through complexation with Nickel(II) chloride," Journal of the Chinese Chemical Society, vol. 52, no. 5, pp. 927–935, 2005.

[14] A. Apostu, N. Bibire, and V. Dorneanu, "UV spectrophotometric analysis of famotidine in combination with picrolic acid, picrolinateDetermirearea quantitativa spectrofotometrica in uv a famotidinei sub forma de picrolonat," Revista Medico-Chirurgicală a Societăţii de Medici şi Naturalişti din Iaşi, vol. 109, no. 2, pp. 422–425, 2005.

[15] K. Basavaiah and H. C. Prameela, "Titrimetric and spectrophotometric determination of famotidine using chloramine-T," Bulgarian Chemical Communications, vol. 35, no. 1, pp. 37–42, 2003.

[16] K. Basavaiah and H. C. Prameela, "Four simple methods for the determination of famotidine in bulk form and formulations," Bulgaria Chemical Industry, vol. 74, no. 2, pp. 50–55, 2003.

[17] A. Z. Abu Zuhri, R. M. Shubietah, and G. M. Badah, "Extractional-spectrophotometric determination of famotidine in pharmaceutical formulations," Journal of Pharmaceutical and Biomedical Analysis, vol. 21, no. 2, pp. 459–465, 1999.

[18] B. V. Kamath, K. Shivram, and V. Saroj, "Spectrophotometry determination of famotidine by charge transfer complexation," Analytical Letters, vol. 25, no. 12, pp. 2239–2247, 1992.

[19] S. Al-Ghanam and F. Belal, "Spectrophotometric determination of three anti-ulcer drugs through charge-transfer complexation," Journal of AOAC International, vol. 85, no. 5, pp. 1003–1008, 2002.

[20] B. K. C. Chukwurah and U. Ajali, "Quantitative determination of famotidine through charge-transfer complexation with chloranilic acid," Bollettino Chimico Farmaceutico, vol. 140, no. 5, pp. 354–360, 2001.

[21] H. A. Mohammad, "Spectrophotometric determination of famotidine using p-chloranilic acid," Bulletin of Pharmaceutical Sciences (Assiut University), vol. 23, pp. 157–163, 2000.

[22] K. Basavaiah and H. C. Prameela, "Spectrophotometric determination of famotidine in pharmaceutical preparations," Industrial Pharmacy, vol. 3, no. 20, pp. 59–61, 2004.

[23] B. Guvener and S. Ates, "Method for the assay of famotidine," Acta Pharmaceutica Turcica, vol. 30, pp. 67–68, 1998.

[24] Z. Koricanac, T. Jovanovic, J. Petkovic, and D. Minić, "Spectrophotometric investigation of famotidine-Pd(II) complex and its analytical application in drug analysis," Journal of the Serbian Chemical Society, vol. 69, no. 6, pp. 485–491, 2004.

[25] N. Rahman and M. Kashif, "Application of ninhydrin to spectrophotometric determination of famotidine in drug formulations," IL Farmaco, vol. 58, no. 10, pp. 1045–1050, 2003.

[26] Y. K. Agrawal, K. Shivramchandra, G. N. Singh, and B. E. Rao, "Spectrophotometric determination of famotidine in pharmaceutical preparations," Journal of Pharmaceutical and Biomedical Analysis, vol. 10, no. 7, pp. 521–523, 1992.

[27] N. R. Reddy, K. Prabhavathi, Y. V. B. Reddy, and I. E. Chakravarthy, "A new spectrophotometric determination of famotidine from tablets," Indian Journal of Pharmaceutical Sciences, vol. 68, no. 5, pp. 645–647, 2006.

[28] G. R. Rao, G. Kanjilal, and K. R. Mohan, "Extended application of folin-ciocalteu reagent in the determination of drugs," The Analyst, vol. 103, no. 12, pp. 993–994, 1978.

[29] M. M. Ayad, A. Shalaby, H. E. Abdellatef, and M. M. Hosny, "New colorimetric methods for the determination of trazodone HCl, famotidine, and diltiazem HCl in their pharmaceutical dosage forms," Analytical and Bioanalytical Chemistry, vol. 376, no. 5, pp. 710–714, 2003.

[30] A. Ibrahim, A. Darwish Samaia, M. Hussein Ashraf, and I. Mahmoud Ahmed Hassan, "Sensitive spectrophotometric method for the determination of H2-receptor antagonists by means of N-bromosuccinimide and p-aminophenol," International Journal of Biomedical Science, vol. 3, no. 2, pp. 123–130, 2007.

[31] A. Berka, J. Vlterin, and J. Zyroka, Newer Redox Titrants, Pargamon Press, New York, NY, USA, 1965.

[32] R. N. J. Kucharsky and L. Safarik, Titrations in Non-Aqueous Solvents, Elsevier, Amsterdam, The Netherlands, 1965.

[33] J. D. Morrison and R. N. Boyd, Organic Chemistry, Prentice Hall, New York, NY, USA, 6th edition, 1992.

[34] ICH Steering Commitee, "ICH harmonised tripartite guideline, validation of analytical procedures: text and methodology Q2(R 1)," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, London, UK, November 1996.

[35] J. March, Oxidations and Reductions. In advanced Organic Chemistry- Reactions, Mechanisms, and Structure, John Wiley & Sons, Singapore, 4th edition, 2006.

[36] B. M. Trost and D. P. Curran, "Chemoselective oxidation of sulfides to sulfones with potassium hydrogen persulfate," Tetrahedron Letters, vol. 22, no. 14, pp. 1287–1290, 1981.
[37] http://www.inchem.org/documents/jecfa/jecmono/v50je12.htm.
[38] http://www.answers.com/topic/thiazole.
[39] D. Cairns, *Essentials of Pharmaceutical Chemistry*, Pharmaceutical Press, Cornwall, UK, 3rd edition, 2008.