Development and Validation of A Visible Spectrophotometric Method for the Assay of Selected Pharmaceutical Formulations

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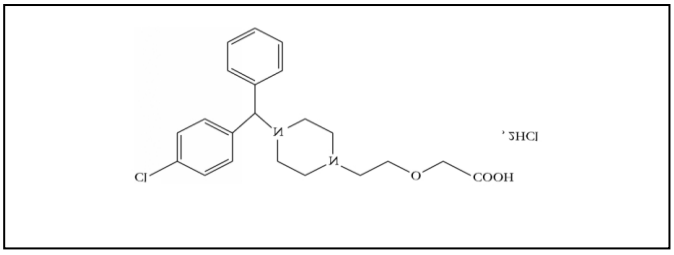
Abstract:
A reliable, sensitive, and precise spectrophotometric method was established for the determination of Cetirizine Dihydrochloride (CTZ), Imatinib Mesylate (ITM), and Voriconazole (VCZ) in bulk materials and tablet formulations. The method relies on the oxidation of the drugs by an excess of N-Bromosuccinimide (NBS) in acidic medium, with unreacted NBS quantified via Methylene Blue. Validation parameters, including linearity, accuracy, precision, %RSD, LOD, LOQ, robustness, and ruggedness, were assessed. The method exhibited excellent recoveries (97–100%) with %RSD values below 2%, confirming high reliability. Comparative statistical evaluation using Student's t-test and F-test showed no significant differences from reference procedures. The developed approach is practical for routine quality control in pharmaceutical laboratories.

Keywords: Spectrophotometry, N-Bromosuccinimide, Methylene Blue, Pharmaceutical Analysis, Method Validation.

INTRODUCTION

1. Cetirizinedihydrochloride (CTZ):



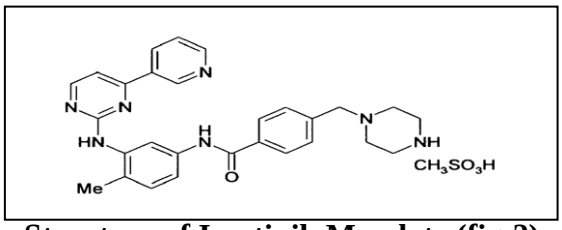
Structure of Cetirizinedihydrochloride (fig:1)

Cetirizine Dihydrochloride (CTZ) is a selective, second-generation antihistamine, chemically designated as **(RS)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid dihydrochloride** (IUPAC) (Fig. 1). Compared to first-generation antihistamines, CTZ exhibits fewer central nervous system side effects due to its limited penetration across the blood–brain barrier. It is commonly used in the management of allergic disorders, including allergic rhinitis and chronic urticaria.

A review of the literature indicates that CTZ has been analyzed in pharmaceutical formulations using a variety of techniques such as spectrophotometry [1], high-performance liquid chromatography (HPLC) [1], spectrofluorimetry [2], titrimetry [3], and liquid chromatography–mass spectrometry (LC–MS) [4]. However,

the current study introduces a novel approach for the determination of CTZ, which has not been previously reported.

2. Imatinib Mesylate:

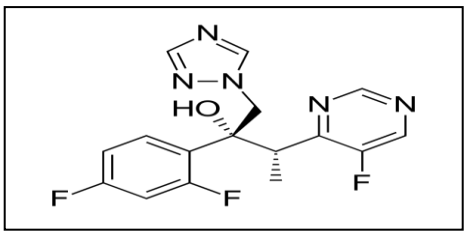


Structure of Imatinib Mesylate (fig:2)

Imatinib Mesylate (ITM) is the mesylate salt form of imatinib, chemically named **4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]benzamide monomethane sulfonate** (IUPAC) (Fig. 2). ITM is the first therapeutic agent developed as part of a novel class of targeted cancer drugs that selectively act on malignant cells while sparing normal, rapidly dividing cells. It is extensively used in the treatment of several cancers, most notably **Philadelphia chromosome–positive (Ph^+) chronic myelogenous leukemia (CML)**, as well as lymphoblastic leukemia and gastrointestinal stromal tumors [5].

According to the literature, ITM has been quantified in biological matrices and pharmaceutical formulations using techniques such as spectrophotometry [6], high-performance liquid chromatography (HPLC) [7], high-performance thin-layer chromatography (HPTLC) [8], and spectrofluorimetry [9]. However, the present study introduces a novel method for the determination of ITM, which has not been previously reported.

3. Voriconazole:



Structure of Voriconazole (fig:3)

Voriconazole (VCZ) is a second-generation, broad-spectrum triazole antifungal agent commonly employed in the management of severe fungal infections, including **invasive aspergillosis** and **esophageal candidiasis**. It is primarily indicated for patients with progressive or potentially life-threatening fungal diseases. The drug exerts its antifungal effect by inhibiting **fungal cytochrome P450–dependent 14α-sterol demethylase**, a key enzyme in the biosynthesis of **ergosterol**, which is essential for fungal cell membrane integrity. Chemically, VCZ is designated as **(αR,βS)-(2,4-difluorophenyl)-5-fluoro-β-methyl-α-(1H-1,2,4-triazol-1-yl-methyl)-4-pyrimidineethanol** (IUPAC) (Fig. 3) [10].

Literature reports indicate that VCZ has been quantified using various analytical techniques, such as spectrophotometry [11], reversed-phase ultra-performance liquid chromatography (RP-UPLC) [12], high-performance thin-layer chromatography (HPTLC) [13], and Colorimetric methods [14].

2. ABOUT THE METHOD

N-Bromosuccinimide (NBS) is widely used in analytical chemistry due to its strong oxidative nature, good stability in solution, and broad applicability arising from the electrophilic character of its bromine atom. These

features make it an effective reagent for the quantitative analysis of various pharmaceutical compounds through oxidation-based reactions. In the present spectrophotometric approach, an excess of NBS is first allowed to react with the drug, and the amount of unconsumed NBS is then measured indirectly. This is achieved by treating the reaction mixture with a dye that NBS readily oxidises. Several dyes, such as Rhodamine B, Indigo Carmine, Safranin O, Malachite Green, and Methylene Blue, can serve this purpose. Among them, Methylene Blue, which exhibits a strong absorbance at 665 nm, provides the best sensitivity and reliability for determining the remaining NBS.

3. EXPERIMENTAL

3.1 Instrumentation:

The UV–Visible spectra used in this work were obtained with an ELICO 210 double-beam spectrophotometer, a Thermo Nicolet 1000 instrument, and an ELICO 159 single-beam UV–Vis spectrophotometer, each operated with standard 10 mm quartz cuvettes. All sample mass measurements were carried out using a Dhona 200 single-pan electronic balance.

3.2 Materials and methods

Distilled water was used for all experimental procedures, and every reagent utilized in the study was of analytical reagent (AR) grade.

3.2.1 N-Bromosuccinimide:

A 0.01 M solution of N-Bromosuccinimide (NBS) was prepared by dissolving 1.8 g of NBS (Himedia Laboratories Pvt. Ltd., Mumbai) in distilled water with mild heating, followed by dilution to a final volume of one liter. The resulting solution was standardized using the iodometric method. For routine analysis, a working solution containing 70 μg mL^{-1} of NBS was prepared by suitable dilution of the stock solution with distilled water. To maintain its stability, the concentrated NBS solution was stored in an amber bottle and kept refrigerated when not in use.

3.2.2 Methylene blue:

A 0.002 M solution of Methylene Blue dye [9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthenium chloride], obtained from S.D. Fine Chem. Ltd., Mumbai, was prepared by dissolving 0.0639 g of the dye powder (BDH) in distilled water and making up the volume to 100 mL. The solution was passed through glass wool to eliminate any suspended particles. A working solution with a concentration of 1.8×10^{-4} mol L^{-1} was then obtained through suitable dilution of the stock solution using distilled water.

3.2.3 Hydrochloric acid:

A 2 M hydrochloric acid solution was prepared by suitably diluting concentrated HCl (S.D. Fine Chem., Mumbai, India; specific gravity 1.18) with distilled water.

3.2.4 Preparation of drug solution:

To obtain a 200 μg mL^{-1} stock drug solution, 20 mg of each drug was carefully weighed and transferred into a 100 mL volumetric flask. The contents were dissolved in an appropriate solvent and the volume was made up to the mark. These primary stock solutions of CTZ, ITM, and VCZ were subsequently diluted with the same solvent to prepare the required working concentrations.

4. PROCEDURE

Aliquots corresponding to 0.1–4.2 μg mL^{-1} of the drug were accurately measured using a microburette and transferred into a set of 10 mL volumetric flasks. Each flask received 1 mL of the 70 μg mL^{-1} NBS solution, followed by 1 mL of 2 M HCl, and the mixture was gently shaken. The reaction mixture was allowed to stand for 10 minutes with intermittent shaking to ensure complete oxidation. Subsequently, 1 mL of the 1.8×10^{-4} mol L^{-1} Methylene Blue solution was added, the contents were mixed thoroughly, and the flasks were finally made up to volume with double-distilled water.

5. ASSAY OF PURE DRUG SAMPLE

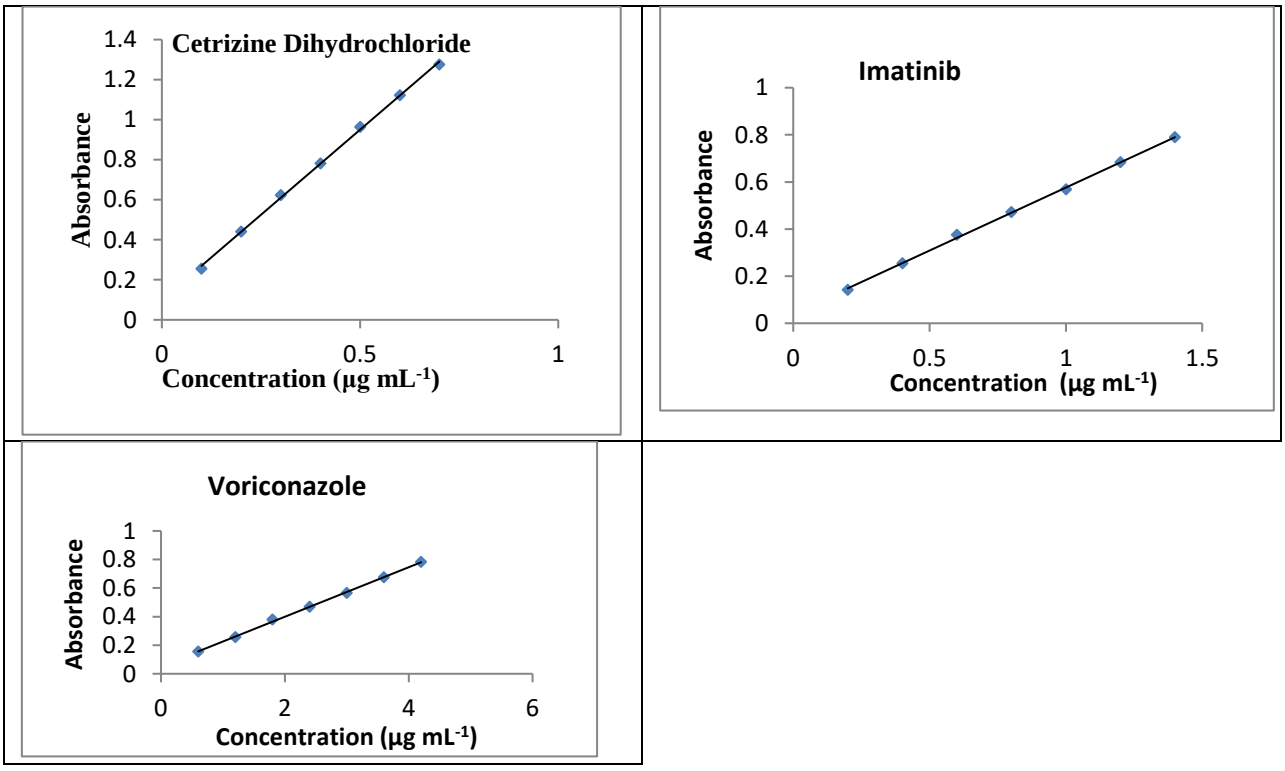
To assess the accuracy and precision of the developed spectrophotometric methods, pure drug solutions within their respective Beer's Law ranges were prepared. Concentration ranges of 2–14 µg mL^{-1} for CTZ, 0.2–1.4 µg mL^{-1} for ITM, and 0.8–4.87 µg mL^{-1} for VCZ were employed. Each solution was transferred into a 10 mL volumetric flask, followed by the addition of 1 mL of 70 µg mL^{-1} NBS and 1 mL of 1 M HCl. The unreacted NBS was subsequently quantified using Methylene Blue dye, as described in the developed procedure.

Calibration curves for each drug were plotted by correlating the drug concentration with the measured absorbance. Each measurement was performed in six replicates, and the relative response for each solution was calculated as the ratio of absorbance to concentration. Only relative response values within 95–105% of the mean were included in constructing the final calibration curves.

5.1 Procedure For Assay Of Pure Drug

The accuracy and precision of each drug solution were assessed by performing recovery experiments using concentrations within their respective Beer's Law ranges. The standard deviation approach was also employed to evaluate method reliability. The chosen concentrations and the resulting percent recovery values are summarized in Table 2. The low %RSD values (below 2%) in combination with the high recovery rates confirm that the developed method is both precise and accurate for the quantitative determination of these drugs.

Calibration curves of drugs (Fig. 4)



6. PROCEDURE FOR ANALYSIS OF TABLETS

6.1 Cetirizine Dihydrochloride:

Five tablets of Cetcip (10 mg each) were carefully powdered. A precisely weighed amount of 20 mg of CTZ was transferred into a volumetric flask, dissolved in double-distilled water, and allowed to stand for 10 minutes. The solution was thoroughly shaken and filtered to remove any insoluble residue. The residue was rinsed with the same solvent, and the combined filtrate was diluted to the calibration mark. Stepwise dilution of this stock solution was performed to prepare the required working standard solutions.

6.2 Imatinib Mesylate:

A single tablet of Imatib, containing 100 mg of Imatinib Mesylate, was finely powdered. An accurately weighed portion of 20 mg of ITM was transferred into a 100 mL volumetric flask and dissolved in double-distilled water. The solution was sonicated for several minutes to ensure complete dissolution, and then diluted to the mark with the same solvent to prepare the stock solution. Stepwise dilution of this stock solution was carried out to obtain the working standard solutions.

6.3 Voriconazole:

A single Voritek tablet, containing 200 mg of Voriconazole, was finely powdered. A precisely weighed portion of 20 mg of VCZ was transferred into a 100 mL volumetric flask and the volume was made up to the mark using double-distilled water. The solution was thoroughly stirred and filtered through filter paper to obtain the sample stock solution. This stock solution was then diluted stepwise with double-distilled water to prepare working solutions in the concentration range of 2–14 μg mL^{-1}, corresponding to the Beer's Law range.

7. METHOD OF VALIDATION

The developed quantification methods were validated for accuracy, precision, linearity, selectivity, limit of detection (LOD), limit of quantification (LOQ), and ruggedness. Calibration curves relating absorbance to concentration were constructed, and the fixed-time method was applied to evaluate drug recovery. Precision was assessed by performing each experiment at least six times, while accuracy was determined through percent recovery and %RSD calculations. High percent recovery values along with %RSD below 2% confirm the reliability and precision of the methods (Table 2).

For each drug, the limit of detection (LOD) was determined using the standard deviation of the y-intercepts of the regression lines obtained from replicate measurements.

The limit of detection (LOD) was calculated using the formula:

$$\mathrm{LOD} = \frac{3.3\, s_a}{S}$$

where s_a is the standard deviation of the intercept (n = 6) and S is the slope of the calibration curve.

The limit of quantification (LOQ), which represents the lowest concentration that can be reliably quantified, was determined using the formula:

$$\mathrm{LOQ} = \frac{10\, s_a}{S}$$

The linearity range for each calibration curve, corresponding to Beer's Law limits, is provided in Figure 4.

The selectivity of the developed methods was assessed by performing recovery experiments in which known quantities of excipients were added to the pure drug samples. Each drug was spiked with its corresponding excipients, and the recovery results confirmed that the method was free from interference.

Ruggedness, which measures the method's resilience to small, deliberate variations in experimental conditions, was evaluated by collecting absorbance data using two different analysts and three different instruments. No significant differences were observed due to changes in either the analyst or the instrument, demonstrating the robustness of the developed methods.

8. FACTORS AFFECTING ABSORBANCE

8.1 Effect of Acid Type: To evaluate the influence of different acids on the redox reaction, several acids—including HCl, H_2SO_4, H_3PO_4, and CH_3COOH—were tested to determine which provided the highest reaction yield. The results indicated that hydrochloric acid was the most effective oxidizing medium when using NBS.

Effect of Acid Concentration: The impact of HCl concentration on the reaction was studied by preparing a series of 10 mL volumetric flasks containing 0.6 μg mL^{-1} of each drug. Different volumes (0.5–2.5 mL) of HCl at concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 M were added along with 1 mL of 70 μg mL^{-1} NBS. After 10 minutes, 1 mL of 1.8×10^{-4} mol L^{-1} Methylene Blue dye was introduced, and the volume was adjusted to the mark with distilled water. Maximum absorbance was achieved using 1 mL of 2 M HCl, while higher volumes led to decreased absorbance. Consequently, 1 mL of 2 M HCl was used for all subsequent analyses.
8.3 Effect of Reaction Time: The influence of reaction time on drug oxidation was examined over a range of 2.5 to 20 minutes to determine the period required for maximum and stable absorbance. The reaction was found to reach completion at 10 minutes, which corresponded to the highest observed absorbance.
Effect of Sequence of Addition: The study revealed that the optimal order for adding reagents was drug → acid → NBS → dye. Deviations from this sequence led to lower absorbance values under identical experimental conditions.

9. ANALYSIS OF PHARMACEUTICALS

To evaluate the **applicability** of the developed methods, solutions of pharmaceutical tablets containing the drug within the **Beer's Law range** were selected. To assess **precision**, each tablet analysis was repeated **at least six times**, while **accuracy** was determined in terms of **percent recovery** and **%RSD**. Excellent percent recovery values, with **%RSD less than 2**, demonstrate the suitability of the methods for **pharmaceutical analysis** (Table 3). These results confirm that the developed methods can be confidently applied to the analysis of pharmaceutical formulations.

10. RESULTS AND DISCUSSION

The proposed indirect spectrophotometric approach exploits the oxidative properties of N-Bromosuccinimide (NBS) to both react with the target drugs and decolorize Methylene Blue dye. In this method, a known excess of NBS is added to the drug solution in an acidic medium, and the remaining unreacted NBS is quantified by its reaction with Methylene Blue, with absorbance measured at 665 nm (Scheme 1). The observed absorbance shows a direct linear relationship with drug concentration. As the drug quantity increases relative to a fixed amount of NBS, more NBS is consumed, reducing its residual concentration. Upon addition of a constant amount of Methylene Blue, the unreacted dye increases proportionally, producing a linear rise in absorbance at 665 nm with increasing drug levels. The reaction was carried out using 1 mL of 2 M hydrochloric acid, which provided an optimal acidic environment for the process.

Reaction Scheme:

1. **Oxidation of drug:**

Drug + Excess NBS → Oxidized drug product + Unreacted NBS

2. **Reaction with dye:**

Unreacted NBS + Fixed amount of Methylene Blue
→ Oxidized Methylene Blue + Unreacted Methylene Blue

The amount of Methylene Blue remaining unreacted was determined spectrophotometrically by measuring the absorbance at its characteristic wavelength of 665 nm.
Scheme 1: Proposed indirect method for drug estimation through NBS-mediated oxidation.

11. ANALYTICAL DATA

A direct linear relationship was established between the absorbance at λ_max and the studied concentration ranges. Key sensitivity parameters, such as Sandell's sensitivity, the limit of detection (LOD), and the limit of quantification (LOQ), were determined following ICH guidelines [15] and are summarized in Table 1, reflecting the high sensitivity of the proposed methods. Furthermore, regression analysis of the Beer's Law data was carried out using the least squares method to determine the slope (b), intercept (a), and correlation coefficient (r), which are also included in Table 1.

Table 1: Analytical and Regression parameters of Spectrophotometric Method

Name of drug Property	**CTZ**	**ITM**	**VCZ**
λ_{max}, nm	665	665	665
Beer's law limits (μg mL^{-1})	2-14	0.2-1.4	0.8-5.6
Molar absorptivity	$3.6031X10^4$	$3.5036X10^5$	$4.5857X10^4$
Sandell's sensitivity(μg cm^{-2})	0.019241	0.001863	0.00649
Variance $(S_a)^2$	0.000023	0.002253	0.000127
Limit of detection μg mL^{-1}	0.1313038	0.293988	0.234746.
Limit of quantification μg mL^{-1}	0.948621	0.890833	0.71148
Regression equation, Y**	Y=0.053x+0.057	Y=0.524x+0.042	Y=0.157x + 0.013
Intercept, (a)	0.057	0.042	0.013
Slope, (b)	0.053	0.534	0.157
Correlation coefficient, (r)	0.996394	0.0985	0.996484
Standard deviation of intercept (S_a)	0.01125	0.047561	0.01134
Standard deviation of slope (S_b)	0.014154	0.066199	0.014154

*The limit of determination is defined as the concentration of the drug in μg/mL that produces an absorbance of 0.001 in a cuvette with a 1 cm² cross-sectional area and a 1 cm path length. The calibration relationship is expressed as Y** = a + bX, where Y represents the measured absorbance and X denotes the drug concentration in μg/mL.*

12. ACCURACY AND PRECISION

The developed methods were assessed for accuracy and precision by testing pure drug solutions at six distinct concentration levels within the established working range. Accuracy was expressed as relative error (%), while precision was represented by the percentage relative standard deviation (%RSD), as summarized in Table 2. The findings confirm that the methods exhibit excellent accuracy and precision.

13. ROBUSTNESS AND RUGGEDNESS

The robustness of the developed methods was examined by making slight variations in the volume of hydrochloric acid, the reaction time after adding NBS (10 ± 2 min), and the time following the addition of the

dye. Ruggedness was evaluated by conducting the analyses with three different analysts and by performing measurements on three separate spectrophotometers using the same analyst.

Table 2 Determination of accuracy and precision of the methods on pure drug Samples.

Drug	Taken (µg/ml)	Found (µg/ml)	error (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD
CTZ	2 4 6	1.92 3.96 5.92	3.5 1.0 1.33	96.5 99.0 98.67	1.38	98.06 ± 1.36
ITM	0.2 0.4 0.6	1.99 3.97 5.98	0.5 0.75 1.67	99.5 99.25 98.33	1.29	99.03 ± 0.62
VCZ	0.8 1.6 2.4	0.8 1.57 2.38	0.0 1.88 2.92	100 98.12 97.08	1.50	98.4 ± 1.48

14. APPLICATION TO FORMULATIONS

The proposed methods were utilized to analyze the drug content in various tablet formulations. The results, shown in Table 3, indicate that these methods provide accurate drug estimation and that common excipients present in the tablets do not interfere with the measurements. The outcomes were compared with previously reported validated methods [16–19] and were found to be consistent with both the labeled claims and literature data. Statistical analysis using Student's t-test for accuracy and F-test for precision showed no significant differences between the proposed and literature methods at the 95% confidence level, confirming the reliability and validity of the developed methods.

The accuracy and validity of the developed methods were further confirmed through recovery studies using the standard addition approach. Known amounts of pure drug, corresponding to 50%, 100%, and 150% of the drug content in the tablet powder, were added to a fixed quantity of the tablet sample. The total drug content was then determined using the proposed methods. Each measurement was performed in six replicates, and the percent recovery values of the added drug (Table 3) fell within acceptable limits, demonstrating that the excipients present in the tablets did not interfere with the assay.

Table 3 Results of assay of tablets by the proposed methods and statistical evaluation and recovery experiments by standard addition method.

Tablets	Drug in tablet µg mL^{-1}	Drug added µg mL^{-1}	Total found µg mL^{-1}	Error (%)	Recovery (%)	RSD (%)	Reference method Mean± SD	Proposed method Mean± SD
Cetcip (CTZ)	0.50 0.50 0.50 2.0 4.0 6.0	2.0 4.0 6.0 0.0 0.0 0.0	2.48 4.48 6.47 2.00 3.99 5.96	0.80 0.22 0.46 0.00 0.25 0.97	99.20 99.78 99.54 100.0 99.75 99.03	0.37	99.93 ±0.657	99.55 ± 0.37

Imatib (ITM)	0.50 0.50 0.50 0.2 0.4 0.6	0.2 0.4 0.6 0.0 0.0 0.0	0.69 0.89 1.08 0.20 0.40 0.59	1.43 1.10 1.91 0.00 0.00 1.57	98.57 98.90 98.09 100.0 100.0 98.43	0.826	98.70 ±3.80	99.00 ± 0.82
Voritek (VCZ)	0.50 0.50 0.50 0.8 1.6 2.4	0.8 1.6 2.4 0.0 0.0 0.0	1.30 2.08 2.89 0.80 1.59 2.38	0.00 0.94 0.35 0.00 0.63 0.36	100.0 99.06 99.65 100.0 99.37 99.64	0.3694	99.72 ±1.254	99.62 ±0.3670

Table 4: F-test and t-test values

	Cetcip (CTZ)	Imatib (ITM)	Voritek (VCZ)
F-test*	1.679349 (2.447)	1.725436 (2.571)	1.662853 (2.447)
t-test**	0.09054 (4.2839)	0.04563 (4.3874)	0.08575 (4.2839)

*t- test and **F-test values from literature.

15. CONCLUSION

In this study, a simple, rapid, sensitive, selective, accurate, and cost-effective spectrophotometric method was successfully developed for the determination of the selected drugs in pharmaceutical formulations using N-Bromosuccinimide (NBS) as the oxidizing agent. The method demonstrated high specificity, showing no interference from common excipients or additives, and proved suitable for analyzing both pure drug substances and their dosage forms. Overall, this approach provides a reliable and practical alternative to existing analytical techniques for these drugs.

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