IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

SPECTROPHOTOMETRIC DETERMINATION OF FEW COMMERCIAL DRUGS IN BULK DRUG AND TABLET DOSAGE FORM

Dr Rajitha Balusani*

1*Department of Chemistry, Government Degree College, Hayathanagar – 501 505, Rangareddy, Telangana.

ABSTRACT

Objective: Simple, sensitive, precise and accurate method for quantitative determination of drugs *viz.*, CetirizineDihydrochloride (CTZ), Imatinib Mesylate (ITM), Voriconazole (VCZ) have been developed.

Method: This method depends upon the oxidation of the drugs by a known excess N-Bromosuccinimide (NBS) in Hydrochloric acid medium and subsequent determination of unreacted NBS by using Rhodamine-B dye.

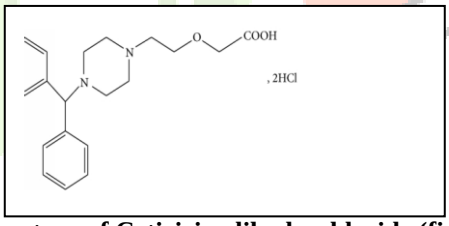
Results: The quantity of the drug present in commercial tablets with no interference of the excipients was assessed by applying this proposed method. This method has been validated in terms of LOD, LOQ, precision, accuracy, %RSD, robustness and ruggedness. Factors affecting the absorbance *viz.*, time of reaction and concentration of Hydrochloric acid are optimized. The presence of excipients has also been examined and found no effect. The calibration curves are found effective for the assessment of pure drug and pharmaceuticals. These curves also can be applied in bulk drug and pharmaceutical industries.

Key Words: Spectrophotometry, Drugs, N-Bromosuccinimide, Rhodamine-B Dye, Validation.

Email: rajitha.balusani@gmail.com, +91-91217-63791

INTRODUCTION

1. Cetirizinedihydrochloride (CTZ):

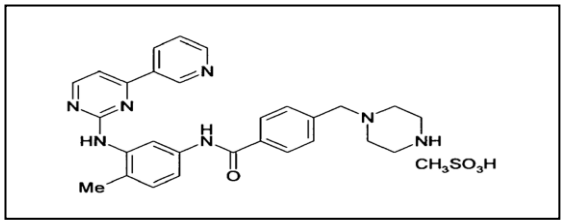


Structure of Cetirizinedihydrochloride (fig:1)

CTZ is a selective second generation anti histamine drug and chemically "(*RS*)-[2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy] acetic acid dihydrochloride." – IUPAC(fig.1), CTZ causes less side effects when compared it with other first generation antihistamines due to its less ability to pass through the blood-brain barrier and became a widely prescribed drug to treat allergic disorders like allergic rhinitis, chronic utricariaz etc.

Literature analysis confirmed that CTZ has been quantified by most of the available analytical methods in pharmaceutical preparations such as Spectrophotometry [1], HPLC [1], Spectrofluorimetry[2], Titrimetry[3], LC/MS[4] due to its significance and also acknowledged that no reports have been cited on this method of our interest for the quantification of CTZ.

2. Imatinib Mesylate:

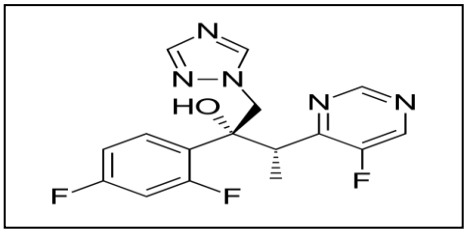


Structure of Imatinib Mesylate (fig:2)

Imatinib Mesylate (ITM) is the mesylate salt of imatinib. Chemically it is "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 developed cancer treatment drug under new class that selectively eliminates cancer cells instead of destroying all the rapidly dividing cells. ITM is used in the treatment of multiple cancers like most notably Philadelphia chromosome (Ph^+) chronic myelogenous leukemia (CML) and for other malignant pathologies such as lymphoblastic leukemia and gastrointestinal stromal tumors[5].

Literature studies disclosed that ITM has been estimated by few analytical methods in biological samples as well as pharmaceutical dosage forms like Spectrophotometry [6], HPLC [7], HPTLC [8], Spectro fluorimetry [9] and also confirms that so far no report has been published to quantify the ITM by using this method.

1. **Voriconazole:**



Structure of Voriconazole (fig:3)

VCZ is a second generation broad spectrum triazole antifungal drug used to treat serious fungal infections like invasive aspergillosis, esophageal candidiasis etc and also administered primarily to patients with progressive, possibly life-threatening infections. VCZ primary mode of action is by inhibition of the fungal cyochrome P $_{450}$-dependent 14 α- sterol demethylate, an essential enzyme in ergosterol biosynthesis. It is chemically known as "(αR,βS)-(2,4-difluorophenyl)-5-fluoro-β-methyl-α-(1H-1.2.4-triazol-1-yl-methyl)-4-pyrimideethanol" – IUPAC(fig. 3)[10].

An extensive literature review resulted that few methods like spectrophotometric [11], RP-UPLC [12], HPTLC [13] and colorimetry [14] were reported for the quantification of VCZ.

2. ABOUT THE METHOD

N-Bromo Succinimide has greater oxidation potential, versatility, strong oxidizing power, high stability in solution and perhaps it is a most important organic compound due to the presence of positive bromine group, so NBS has been used for the quantitative estimation of drugs and pharmaceuticals through oxidation of drugs by NBS. This oxidative spectrometric method is carried out by adding excess NBS to the drug solution and un reacted NBS is assessed by suitable dyes, which also should be oxidized by NBS viz,. Rhodamine –B, Indigo Caramine, Methyl Orange, Safranin –O, Malachite Green etc. To estimate unreacted NBS absorbance at 557nm Rhodamine-B dye is found suitable.

3. EXPERIMENTAL

3.1 Instrumentation:

The UV-VIS spectra of the study have been recorded on ELICO 210 double beam Spectrphotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-VIS single beam spectrophotometers using quartz cells of 10 mm path length.
Samples were weighed by using a Dhona 200 single pan electrical balance.

3.2 Materials and methods

Throughout the investigation distilled water was used and all reagents were used of analytical-reagent grade.

3.2.1 N-Bromosuccinimide:

0.01*M* N-Bromosuccinimide [1-Bromo-2,5-pyrrolidinedione], ($C_4H_4BrNO_2$, M.Wt. 177.98g mol^{-1}) solution was prepared by dissolving 1.8g of NBS (Himedia Laboratories Pvt.Ltd, Mumbai) in water with the aid of heat and diluted to one liter with water and standardized iodometrically. For all these tests 70 µg mL^{-1} NBS solution was used and it was prepared by diluting its stock solution with appropriate quantity of distilled water. The NBS stock solution was stored in an amber colored bottle and kept in a refrigerator when not in use.

3.2.2 Rhodamine-B

0.001*M* of Rhodamine-B dye [9-(2-Carboxyphenyl)-3,6-bis(diethylamino)xanthenium chloride was provided by S.D Fine Chem. Ltd, Mumbai] aqueous solution was prepared by dissolving an appropriate weight of 50 mg of Rhodamine-B dye in 100 ml of double distilled water. This solution was filtered by using glass wool. All the spectrophotometric tests were carried out by using 50 µg mL^{-1} of dye solution and it was prepared by further diluting the 0.001M dye solution.

3.2.3 Hydrochloric acid:

Prepared by diluting the concentrated acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) with water appropriately to get 1 *M* acid.

3.2.4 Preparation of drug solution:

To prepare 200 µg ml^{-1} drug solution, 20 mg of each drug accurately weighed and transferred into a 100ml standard flask and dissolved with suitable solvent up to the mark. The stock solutions of CTZ, ITB and VCZ were further diluted with the same solvent to obtain working concentrations.

4. PROCEDURE

Aliquots containing 0.1-4.2 µg ml^{-1} of drug were transferred into a series of 10 ml standard flasks using a micro burette. To this, 1 mL of NBS solution (70 µg mL^{-1}) was added followed by 1 mL of 1*M* HCl and contents were mixed and the flasks were set aside for 10 min under occasional shaking. After 10 minutes, each flask was added with 1 mL of Rhodamine-B (50 µg mL^{-1}). All the contents in each flask were shaken thoroughly and diluted upto the mark with double distilled water. Each solution absorbance was measured against the corresponding reagent blank at 557.

5. ASSAY OF PURE DRUG SAMPLE

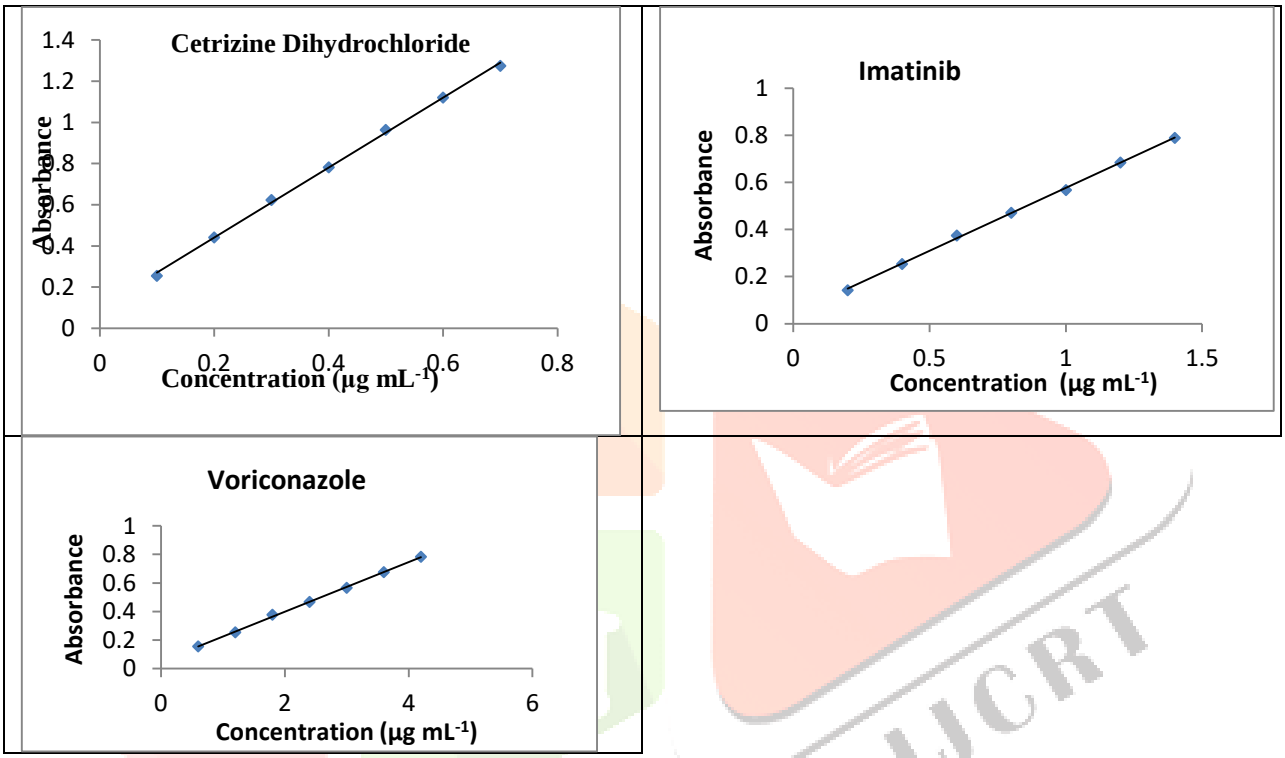
To assess the accuracy and precision of the methods developed, pure sample solutions containing drug in the Beer's Law limit were chosen. For this study 2-14 µg $mL^{-1}$of CTZ, 0.2-1.4 µg mL^{-1} of ITM and 0.8-4.87 of µg mL^{-1} of VCZ has been taken. Each drug solution was taken in 10 ml standard flasks and 1 mL of 70 µg mL^{-1} of NBS and 1 ml of 1 *M* of HCl were added to it. The un reacted NBS is analyzed by using Rhodamine-B dye as described in this developed method.

For all the drugs calibration curves were constructed by plotting the concentration versus the absorbance of drugs. For each solution absorbance data was collected with six replicate experiments and relative responses of each solution were calculated from the absorbance to concentration ratio. Calibration curves were constructed by considering the relative response values which are only in between 95% to 105% of average.

5.1 Procedure For Assay Of Pure Drug

Accuracy and precision of the sample solutions of each drug was checked through performing recovery experiments by choosing them in beer's law limit. Also standard deviation method adapted for this purpose, concentration chosen and % of recovery of each is tabulated in table2. Calculations of %RSD values (less than 2) and excellent recovery values disclose the Precision and accuracy of these drugs by developed method.

Calibration curves of drugs



6. PROCEDURE FOR ANALYSIS OF TABLETS

6.1 CetirizineDihydrochloride:

Five tablets of Okacet-10 mg were grounded finely. A small quantity of the powder amounting to 20 mg of CTZ weighed carefully and transferred into a volumetric flask, dissolved in double distilled water, kept aside for10 minutes, shaken well and filtered the residue. Same solvent was used to wash the residue and to adjust the volume up to the mark and again added the same solvent to this stock solution step wise to give a working standard solution.

6.2 Imatinib Mesylate:

One tablet of Imatib which contains 100 mg of Imatinib Mesylate was grounded finely. A small quantity of the powder amounting 20 mg of ITM was taken, transferred into a 100 ml volumetric flask and dissolved in double distilled water. This solution was sonicated for few minutes to ensure complete dissolution and then filled the flask up to the mark with the same solvent and further used it as stock solution. Same solvent was added to this stock solution step wise to give a working standard solution.

6.3 Voriconazole:

One tablet of Voritek containing 200 mg of VCZ was grounded finely. A small quantity of the powder amounting 20 mg of Voriconazole was taken into a 100mL calibrated flask and the volume finally diluted by adding double distilled water up to the mark. This solution was stirred thoroughly, filtered by using a filter paper and used as sample stock solution. It was further diluted with double distilled water stepwise to obtain desired working concentrations 2 µg/mL-14 µg/mL in the range of Beers law limit.

7. METHOD OF VALIDATION

Each developed quantification method has been verified in terms of accuracy, precision, limit of quantification, limit of detection, selectivity, linearity and ruggedness. Absorbance-Concentration curves were drawn, fixed time method was used to assess the recovery of the drug. To assess the precision each experiment was repeated at least 6 times, accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery, RSD being less than 2 for each drug demonstrates accuracy and precision of the methods[Table2].

For each drug, limit of detection (LOD) is determined from the standard deviation of y-intercepts of regression lines of replicate determinations.

LOD = 3.3 s_a / S

Where s_a = standard deviation of intercept (n=6)

S = slope of linearity plot

Limit of Quantification (LOQ) is the minimum concentration used by an analyst to construct the calibration curve is also determined.

LOQ = 10s_a/S.

Limits of linearity of calibration curves [Fig5] are mentioned in the under the title Beer's law limit.

Selectivity was tested through performing recovery experiments by adding known excipients of each drug to its pure sample.

To test the selectivity of these developed methods, each pure drug sample was added with its known excipients and also performed recovery experiments. Ruggedness is resistance of method for a small change in variables like instrument, analyst or both to test the ruggedness of the method absorbance data was collected using 2 analysts and 3different instruments, no considerable changes were observed either by change of analyst or instrument hence the developed may be considered as robust.

8. FACTORS EFFECTING ABSORBANCE

8.1 Effect of acid: To study the effect of acid, different types of acids were examined (HCl, H_2SO_4, H_3PO_4 and CH_3COOH) to achieve maximum yield of redox reaction. The results indicated that the Hydrochloric acid was the preferable acid with NBS as oxidant.

8.2 Effect of acid concentration: To study the effect of acid concentration different concentrations of HCl were examined. The reaction was performed in a series of 10 ml volumetric flask containing 0.6 µg mL^{-1} of the cited drugs, different volumes (0.5–2.5 mL) of 0.5 *M* , 1.0 *M* , 1.5 *M* ,2.0 *M*, *2.5 M* HCl and 1 ml of NBS (70 µg mL^{-1}) were added. After 10 min of time, 1ml of Rhodamine-B (50 µg mL^{-1}) dye and water added upto the mark. It was found that the maximum absorbance was obtained with 1mL of 1*M* HCl. Above this volume, the absorbance decreased therefore, a volume of 1 mL of 1 *M* HCl was used for all measurements.

8.3 Effect of time: In order to obtain the highest and most stable absorbance with the effect of time on the oxidation reaction of drugs were catalyzed by the time periods ranging for 2.5-20 minutes. The time required to complete the reaction and maximum absorbance was obtained after 10 min.

8.4 Effect of sequence of addition: Drug-acid-NBS-dye is optimum sequence of addition and other sequences gave lower absorbance values under same experimental conditions.

9. ANALYSIS OF PHARMACEUTICALS

To the test the applicability of the method developed solution of pharmaceutical tablets solutions containing drug in the Beer's Law limit were chosen. To assess the precision each tablet analysis was repeated at least 6 times, accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery, RSD being less than 2 for each drug demonstrates applicability of the methods for pharmaceutical analysis [Table 3]. The excellent recovery observations reveals that these developed methods can be implemented to pharmaceutical analysis without any hesitation

10. RESULTS AND DISCUSSION

The ability of NBS to oxidize drugs and bleach the color of Rhodamine-B dye is the basis of the indirect spectrophotometric method developed here. This method makes use

of bleaching action of NBS on the dye and discoloration being caused by the oxidative destruction of the dyes. In this method the above drugs were reacted with a measured excess of NBS in acidic medium and the unreacted NBS was determined by reacting with Rhodamine-B followed by absorbance measurement at 557 nm (scheme1).

The absorbance increased linearly with increasing concentration of drug, when increasing amounts of each drug were added to a fixed amount of NBS, consumes the latter proportionally and there occurs a concomitant fall in NBS concentration. When fixed amount of the dye was added to decreasing amount of NBS, a proportional increase in the concentration of dye resulted. This was observed as a linear increase in absorbance at the respective λ_{max} (557) with increasing concentration of each drug. One ml of 1*M* Hydrochloric acid was used in the reaction, as this acid was found suitable medium for this method and concentration was found ideal.

Drug + known excess of NBS → oxidation product of Drug + unreacted NBS

Unreacted NBS+fixed amount of Rhodamine-B→oxidation product of Rhodamine-B+unreacted Rhodamine-B

(Unreacted Rhodamine-B was estimated spectrophotometrically at it's λ_{max} =557 nm)

Scheme1: Tentative reaction Scheme for the indirect determination of drug by oxidation with NBS.

11. ANALYTICAL DATA

A linear correlation was found between absorbance at λ_{max} and concentration ranges and sensitivity parameters such as Sandal's sensitivity, detection limit and quantification limit calculated according to ICH guidelines[15 35] are also presented in table 1 which reveal the very high sensitivity of the methods. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) is also given in table 1.

Table 1: Analytical and Regression parameters of Spectrophotometric Method

Name of drug Property	CTZ	ITM	VCZ
λ_{max}, nm	557	557	557
Beer's law limits (µg mL^{-1})	2-14	0.2-1.4	0.8-5.6
Molar absorptivity	$3.6021X10^4$	$3.5046X10^5$	$4.5847X10^4$
Sandell's sensitivity(µg cm^{-2})	0.019231	0.001873	0.006329
Variance $(S_a)^2$	0.000024	0.002263	0.000126
Limit of detection µg mL^{-1}	0.1313048	0.293978	0.234756.
Limit of quantification µg mL^{-1}	0.948631	0.890843	0.71138
Regression equation, Y**	Y=0.052x+0.056	Y=0.534x+0.041	Y=0.158x + 0.012
Intercept, (a)	0.056	0.041	0.012
Slope, (b)	0.052	0.534	0.158
Correlation coefficient, (r)	0.996494	0.0995	0.996494
Standard deviation of intercept (S_a)	0.01124	0.047571	0.01124
Standard deviation of slope (S_b)	0.014154	0.066199	0.014154

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 cm2 and path length of 1 cm. Y = a+bX, where Y is the absorbance and X concentration of drugs in µg per mL.*

12. ACCURACY AND PRECISION

The accuracy and precision of the methods were evaluated by analyzing the pure drug solution at 6 different levels by choosing them in working limits. The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in Table 2 and reveal the high accuracy and precision of the methods.

13. ROBUSTNESS AND RUGGEDNESS

To test the robustness of these developed methods, volume of Hydrochloric acid was slightly changed. The reaction time (after adding NBS, time varied was 10 ±2min) and the time after addition of dye is slightly changed. Ruggedness of these developed methods were evaluated by performing same analysis with 3 different analysts and also performing analysis on 3 different spectrophotometers by 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	1.97 3.99 5.98	1.5 0.25 0.33	98.5 99.75 99.67	0.705	99.307 ±0.6997
ITM	0.2 0.4 0.6	0.2 3.99 5.99	0.00 2.5 1.67	100.00 97.5 98.33	1.29	98.61 ±1.27
VCZ	0.8 1.6 2.4	0.78 1.6 2.39	2.5 0.00 0.42	97.5 100.0 99.58	1.35	99.028 ±1.34

14. APPLICATION TO FORMULATIONS

These developed methods were applied for the estimation of drugs in tablets.

The results in Table3 showed that the methods are successful for the determination of drugs and that the excipients in the dosage forms do not interfere. The results are compared to the available validated reported [16, 17, 18 &19] methods on each drug and the results agree well with the claim and also are in agreement with the results obtained by the literature method.

Statistical evaluation results were used to test the accuracy by Student's t-test and precision by F-test which revealed no significant change was observed between the literature method and proposed method at the 95 % confidence level with reference to accuracy and precision.

Recovery experiments were carried out via standard addition technique to calculate the accuracy and validity of these developed methods. To a fixed and known amount/concentration of drug in tablet powder, pure drug was added at three levels (50, 100 and 150% of the level present in the tablet) and the total amount of drug was found by these developed methods. Each experiment was repeated six times and the percent recovery of pure drugs added (Table 3) was within the permissible limits showing the absence interference by the inactive ingredients in 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
Okacet (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	2.49 4.48 6.49 2.00 3.98 5.99	0.40 0.44 0.15 0.00 0.50 0.17	99.60 99.56 99.85 100.0 99.50 99.83	0.1984	99.93 ±0.657	99.72 ±0.1978
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 1.11 1.81 0.00 0.00 1.67	98.57 98.89 98.19 100.0 100.0 98.33	0.8208	98.70 ±3.80	98.99 ±0.8126
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 0.95 0.34 0.00 0.62 0.35	100.0 99.05 99.66 100.0 99.38 99.65	0.3684	99.72 ±1.254	99.62 ±0.3670

Table 4: F-test and t-test values

	Okacet (CTZ)	Imatib (ITM)	Voritek (VCZ)
F-test*	1.639349 (2.447)	1.725336 (2.571)	1.662863 (2.447)
t-test**	0.09064 (4.2839)	0.04573 (4.3874)	0.08565 (4.2839)

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

15. CONCLUSION

The present study described the successful development of new, simple, sensitive, selective, accurate cost-effective and rapid spectrophotometric method for the accurate determination of the above drugs in its pharmaceutical form by using NBS as the oxidizing reagent. There is no interference from additives and excipients. The method thus can be used in the quantitative determination of these drugs in pure and pharmaceutical formulations. So, it is the good alternative to the reported methods for the quantitative determination of these drugs.

ACKNOWLEDGEMENT

The authors are thankful to Prof. M. Sayaji Rao (retd), Department of Chemistry, University College of Science, Osmania University for helpful discussions.

References

1. Fatma Ahmed Aly, Nahed EL-Enany, Heba Elmansi and Amany Nabil., Chemistry Central Journal; (2017), 11, 99.
2. F. Ibrahim, M. K. Sharaf El- Din, M. Eid and M. E. K. Wahba*.,International Journal of Pharmaceutical Sciences and Research; (2011), 21, 2056-2072.
3. Rizk, N.M., Abbas, S.S., El-Sayed, F.A., Abo-Bakr, A., International Journal of Electrochemical Science; (2009), 4, 396-406.
4. Divyesh Patel, Samir Patel, Yogendra Parmar, Kamlesh Chauhan, Pooja Sannigrahi, A. S. Rawat, Anand Vardhan., International Journal of Pharma Research & Review; (2013), 2(9), 10-15.
5. Yan Ma, Jeff Hirst, J., Margaret von Mehren, Scott Weir, J., Andrew Godwin, K., Ziyan Pessetto, Y., Molecular Cancer Therapeutics; **(2014)**, 13, 2276.
6. M. Sasikala, B. Vijay Kumar and G. Venkateshwarlu., Journal of Chemistry and Chemical Sciences; (2015), 5(8), 463-475.
7. P. Ravisankar, A. Niharika, K. Anusha Rani, S.M. Neeha and G. Pavan., Der Pharmacia Letters; (2015), 7(7), 102-112.
8. Mahesh S. Wajurkar, Manjusha N. Dole., Journal of Advanced Scientific Research; (2015), 6(3), 51-54.
9. Dalia W. Zidan, Wafaa S. Hassan, Manal S. Elmasry, Abdalla A. Shalaby., Luminescence; (2017), 33(1).
10. Sweetman, S.C., Martindale: The Complete Drug Reference, 35 Edition Pharmaceutical Press:London; **(2009)**, 550-551.
11. Mohamed A. Zayed, MAnal A. El-shal and Marwa A. Addallah., Egypt J.Chem; (2017), 60(6), 1177-1188.
12. Pavani P, Palanichamy, S. Ramasubramaniyan. P, Srinag. T, LakshmiM Solairaj. P., World Journal of Pharmacy and Pharmaceutical Sciences; (2013), 2(6), 5491-5497.
13. Manali W. Jain, Atul A. Shirkhedkar, Sanjay J. Suran., Arabian Journal of Chemistry; (2017), 10(1), S355-360.
14. Raval Kashyap and Kelvin Makavana., International Journal of Research in Pharmacy and Chemistry; (2013), 3(3), 712-722.
15. Luciana Pereira Lourenço[1], Fernando Armani Aguiar[1], Anderson Rodrigo Moraes de Oliveira[2] and Cristiane Masetto de Gaitani[1] .; Journal of Analytical Methods in Chemistry (2015), 294270 /10.1155.
16. Safwan Ashour*, Haitham Aboudan.; International Journal of Pharmacy and Chemistry (2018), 4(1): 8-15.
17. Felix, F.S., Angnes, L., Journal of pharmaceutical sciences; **(2010)**, 99(12), 4784-4804.
18. Moussa, B.A., El-Zaher, A.A., Mahrouse, M.A., Ahmed, M.S., Analytical Chemistry Insights; **(2013)**, 8, 107–115.
19. Sibel, A., Ozkan, Cemal Akay, Semsettin Cevheroglu, Zuhre Senturk., Journal of Liquid Chromatography & Related Technologies; **(2001)**, 24(7), 983–991.