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OXIDATIVE SPECTROPHOTOMETRIC DETERMINATION OF DRUGS & PHARMACEUTICALS USING NBS AS OXIDANT & RHODAMINE-B DYE AS ANALYTICAL REAGENT

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ABSTRACT

Objective: Simple, sensitive, precise and accurate method for quantitative determination of drugs *viz.*, Alfuzosin (ALF), Atrovastatin Calcium (ATV) Ciprofloxacin (CIP) and Citalopram(CTP) 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**: This proposed method was applied for the determination of the quantity of the drug present in commercial tablets with no interference from the excipients. This method has been validated in terms of LOD, LOQ, precision accuracy, % RSD, robustness and ruggedness. Factors affecting the absorbance *viz.*, concentration of HCl and time of reaction are optimized. The effect of excipients has also been studied and found to have no effect. The calibration curves are found useful for determination of pure drug and can be applied to pharmaceuticals in bulk drug and pharmaceutical industries.

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

INTRODUCTION

Alfuzosin hydrochloride (ALF): Alfuzosin hydrochloride (ALF) is an alpha$_1$-adrenoreceptor blocker. It is used in the symptomatic treatment of urinary obstruction caused

by benign prostatic hyperplasia and has been tried in the treatment of hypertension[1] Alfuzosin Hydrochloride is chemically designated as N- {3- [(4-Amino-6,7-dimethoxyquinazolin -2-yl (methyl) amino] propyl} tetrahydro-2-furamide hydrochloride[2] It works by relaxing the muscles in the prostate and bladder neck, making it easier to urinate. It is used to treat the signs and symptoms of benign enlargement of the prostate, by increasing the flow in urine which is reduced by benign prostatic hypertrophy. Literature survey reveals that several methods reported like HPLC,[3,4] RP-HPLC,[5-7] LC-tandem mass spectroscopy,[8] Voltammetry,[9,10] Colorimetry,[11] Spectrophotometry,[12-14] of Alfuzosin in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage.

Atorvastatin Calcium (ATV)

Atorvastatin calcium (ATV) is a synthetic lipid-lowering agent. It is chemically known as [R-(R*, R*)]-2- (4-fluorophenyl)-β, δ-dihydroxy -5- (1-methylethyl)- 3-pheny l-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate (Fig.2) belongs to the group of statins.[1,2] All statins, including ATV reduce the production of cholesterol in the liver. ATV is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is a lipid regulating drug used to reduce LDL-cholesterol, apolipoprotein B and triglycerides and to increase HDL-cholesterol in the treatment of hyperlipidaemias. It is also used for prophylaxis of cardiovascular events in patients with multiple risk factors including diabetes mellitus. Several methods have been reported for quantitative determination of ATV like HPTLC,[15-17] RP-HPLC,[18-20] HPLC,[21,22] Spectrophotometric method,[23-26] Liquid chromatography-Electro spray ionization tandem mass spectrometry,[27] Capillary electrophoresis method,[28] and simultaneous equation method and absorbance ratio method,[29] in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage.

Ciprofloxacin Hydrochloride(CIP)

Ciprofloxacin (CIP) is a synthetic broad spectrum antimicrobial drug of a fluoroquinolone class. It is chemically known as 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid [fig3]. The bactericidal action of Ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV (both Type II topoisomerases) which are required for bacterial DNA replication, transcription, repair, and recombination.[1,2] CIP has been shown to be active against most isolates of the following bacteria both *in vitro* and in clinical infections. Assay of Ciprofloxacin in bulk drugs,

pharmaceuticals has previously been achieved by several analytical techniques such as HPLC,[30-35] RP- HPLC,[36-38] UPLC,[39] Spectrophotometry,[40-42] FTIR,[43] Solid-State FT-Raman Spectroscopy,[44] However, many of these techniques requires more analysis time. Literature survey revealed that there were no methods reported for the assay determination of Ciprofloxacin by this oxidative spectrophotometric determination technique which has more accuracy and requires less time in bulk drugs and pharmaceutical dosage forms.

Citalopram Hydrobromide(CTP)

Citalopram Hydrobromide(CTP) is a widely used antidepressant orally active drug comes under the class of selective serotonin reuptake inhibitors (SSRIs) having broad spectrum of therapeutic activity against central nervous system (CNS) abnormalities such as depressive disorders, obsessive-compulsive disorder and anxiety disorder, social phobia, and post traumatic stress.[45,46] It is chemically known as (±)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran -5-carbonitrile, hydrobromide.[1,2] ts of the body. Citalopram is metabolized in the liver mostly by CYP2C19, but also by CYP3A4 and CYP2D6. When compared its efficacy to other tricyclic antidepressants, it has lower risk of adverse effects and toxicity,[47] so it is widely prescribed as a antidepressant all over the world. Literature survey reveals that there are several methods have been reported for quantitative determination of Citalopram Hydrobromide like RP-HPLC method,[48-50] HPLC,[51,52] HPTLC,[53] Spectrophotometry,[54] Electrokinetic chromatographic method[55] in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage. Although much work has been published on the quantification of the above drugs, however many of these techniques requires more analysis time but the simple, sensitive oxidative spectrophotometric method using NBS as oxidizing reagent has not been reported yet. In the present communication we reported the oxidation of drugs using NBS as oxidant and Rhodamine-B dye as analytical reagent.

STRUCTURE OF DRUGS

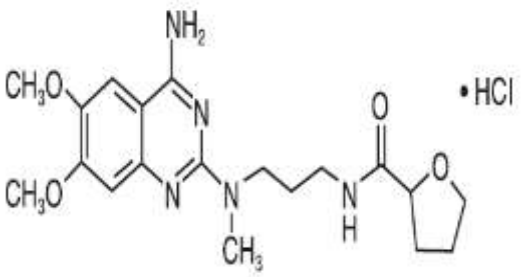

Fig 1. Alfuzosin

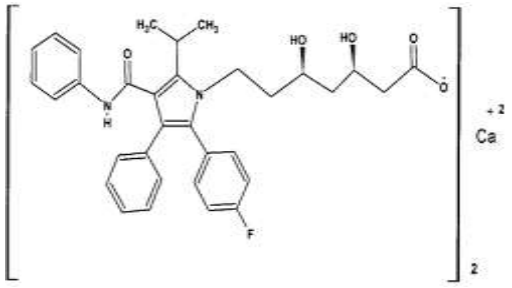

Fig 2. Atrovastatin Calcium

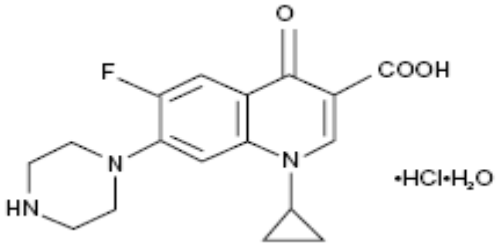

Fig 3. Ciprofloxacin

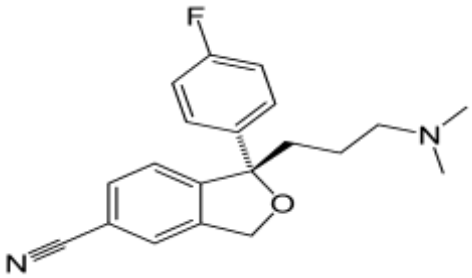

Fig 4. Citalopram

2. ABOUT THE METHOD

NBS has strong oxidizing power, versatility, high oxidation potential, high stability in solution and perhaps the most important positive bromine containing organic compound, so it has been used for quantitative determination of drugs based on the oxidation of drugs. This spectrophotometric method involved addition of excess NBS to the drugs and un reacted NBS is estimated by suitable dyes, which should be oxidized by NBS *viz.*, Methyl Orange, Orange G, Safranin-O, Indigo Caramine, Metanil Yellow and Malachite Green. Rhodamine-B dye is found suitable for estimation of unreacted NBS absorbance at 557 nm.

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. A Dhona 200 single pan electrical balance is used for weighing the samples.

3.2 MATERIALS AND METHODS

All reagents used were of analytical-reagent grade and distilled water was used throughout the investigation.

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. The solution was kept in an amber colored bottle and was diluted with distilled water appropriately to get 70 µg mL^{-1} NBS for use in spectrophotometric method. The NBS solution was stored in a refrigerator when not in use.

3.2.2 Rhodamine-B dye

Aqueous solution of 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] was prepared by dissolving an appropriate weight of 50 mg in 100 ml by double distilled water and filtered using glass wool. Further the dye solution was diluted to 50 µg mL^{-1} for use in spectrophotometric method.

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

Standard drug solution (200 µg ml^{-1}) was prepared by dissolving accurately weighed 20 mg drug with suitable solvent to the mark in 100 ml standard flask. The stock solutions of ALF, ATV, CIP and CTP 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, 1 mL of Rhodamine-B (50 µg mL^{-1}) added to the contents. Then contents were shaken well and diluted with double distilled water up to the mark. The absorbance of each solution was measured at 557 nm against the corresponding reagent blank.

5. ASSAY OF PURE DRUG SAMPLE

To test the accuracy and precision of the methods developed, pure sample solutions containing drug in the Beer's Law limit were chosen. For this study 0.2-1.4 µg $mL^{-1}$of ALF, 0.4-2.8 µg mL^{-1} of ATV, 0.1-0.7 µg mL^{-1} of CIP and 0.6-4.2 of µg mL^{-1} of CTP has been taken. To each of the solution 1 mL of 70 µg mL^{-1} of NBS, 1 ml of 1 *M* of HCl were added and the un reacted NBS is analyzed as described above using Rhodamine-B dye. Calibration curves were constructed for all the drugs by plotting the absorbance versus the concentration of drugs. The absorbance data was collected for six replicate experiments and absorbance to concentration ratio called the relative response was determined. The relative responses

between 95% to 105% of average are only considered for construction of the Calibration curves.

5.1 Procedure For Assay Of Pure Drug

Sample solutions of each drug in the beer's law limits were chosen and recovery experiments were performed to check the accuracy and precision. The concentration chosen and % of recovery are tabulated in table2, for this purpose standard deviation method also adapted. Excellent recovery and %RSD being less than 2 speaks about the precision and accuracy of the drugs.

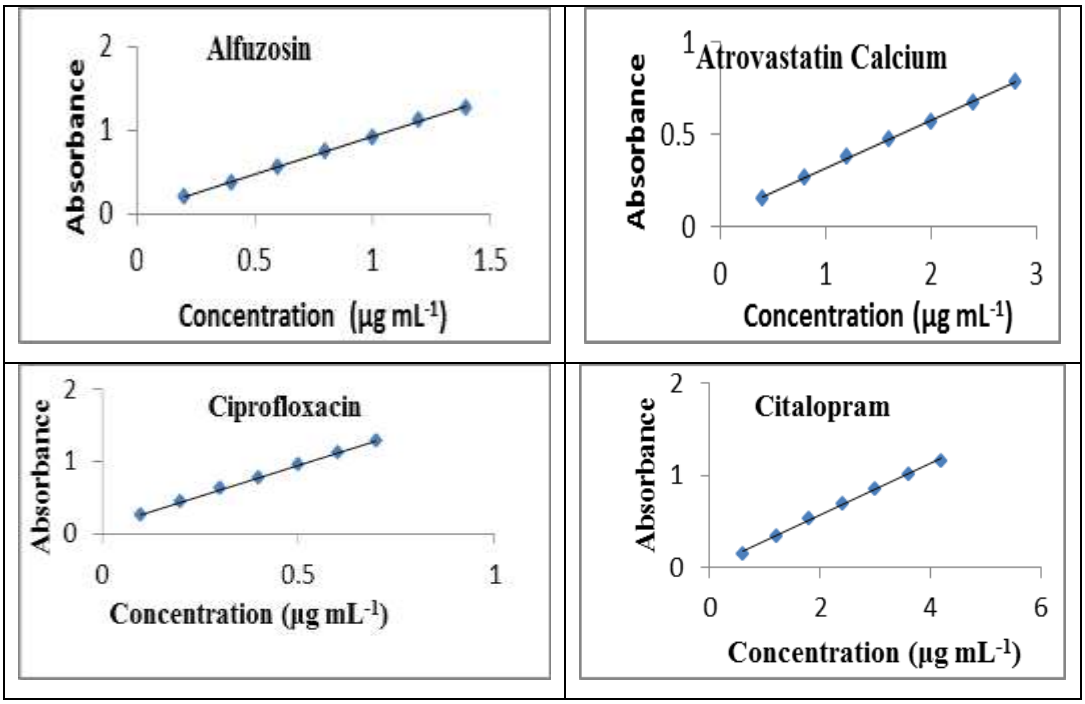

Fig.5 Calibration curves of drugs ALF, ATV, CIP and CTP.

6. PROCEDURE FOR ANALYSIS OF TABLETS

6.1 Alfuzosin

For the analysis of pharmaceutical formulations two tablets (Alfuzosin HCl –10 mg) were weighed accurately and grounded. A quantity equivalent to 10mg of Alfuzosin was weighed accurately, transferred into a 100 mL calibrated flask and the volume was finally diluted to the mark with double distilled water, mixed well and filtered using a Whatman No. 42 filter paper. It was used as stock sample solution and was further diluted with water to get working standard solution.

6.2 Atrovastatin Calcium

Two tablets (ATROVASTATIN CALCIUM-10mg) were crushed to a fine powder and the powder equivalent to 10 mg of Atrovastatin Calcium was weighed accurately, transferred into a 100 mL calibrated flask, dissolved in sufficient quantity of methanol, sonicated for 10 min and the volume was finally diluted to the mark with methanol. This solution was mixed well and filtered through Whatman filter paper No. 42. It was used as stock sample solution and was further diluted with the same solvent to get working standard solution**.**

6.3 Ciprofloxacin

One tablet (Cipro-500mg) were weighed accurately, crushed to a fine powder, the powder equivalent to 10mg of Ciprofloxacin was weighed accurately, transferred to 100 ml volumetric flask, dissolved in sufficient quantity of methanol, sonicated for 10 min and the volume was finally diluted to the mark with methanol. This solution was mixed well and filtered through Whatman filter paper No. 42. It was used as stock sample solution and was further diluted with the same solvent to get working standard solution.

6.4 Citalopram

For the analysis of pharmaceutical formulations two tablets (Celexa–20 mg) were weighed accurately and grounded. A quantity equivalent to 10mg of Citalopram HBr was weighed accurately, transferred into a 100 mL calibrated flask and the volume was finally diluted to the mark with double distilled water, mixed well and filtered using a Whatman No. 42 filter paper. It was used as stock sample solution and was further diluted with water to get working standard solution.

7. METHOD OF VALIDATION

The each method developed for quantification of drugs has been validated in terms of precision, accuracy, limit of detection, limit of quantification, linearity, selectivity 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].

As mentioned earlier limit of detection is the minimum limit that can be detected but not necessarily quantified is determined for each drug.

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 LOQ the minimum concentration of analyst using 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. To test the selectivity known excipients of each drug are added to the pure drug sample and recovery experiments were performed. 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 3 different instruments and 2 analysts, no significant changes were observed either by change of instrument or analyst hence the method may be taken 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 studies indicate that methods developed can be applied to pharmaceutical analysis without 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 measured spectrophotometrically at 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[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	ALF	ATV	CIP	CTP
λ_{max}, nm	557	557	557	557
Beer's law limits (µg mL^{-1})	0.2-1.4	0.4-2.8	0.1-0.7	0.6-4.2
Molar absorptivity	$4.2X10^5$	$4.6x10^5$	$9.3x10^5$	$1.0X10^5$
Sandell's sensitivity(µg cm^{-2})	0.00111	0.00384	0.00058	0.00359
Variance $(S_a)^2$	0.00767	0.0206	0.000339	0.00028
Limit of detection µg mL^{-1}	0.3807	1.8236	0.0357	0.0201
Limit of quantification µg mL^{-1}	1.1538	5.5262	0.1082	0.0609
Regression equation, Y**	Y=0.9005 x+0.0154	Y=0.2601x+0 554	Y=1.7x+0. 0999	Y=0.2785x+0 .0117
Intercept, (a)	0.0154	0.0554	0.0999	0.0117
Slope, (b)	0.9005	0.2601	1.700	0.2785
Correlation coefficient, (r)	0.9993	0.9992	0.9945	0.9987
Standard deviation of intercept (S_a)	0.0876	0.0110	0.0184	0.0016
Standard deviation of slope (S_b)	0.1772	0.0141	0.0264	0.0028

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 established by analyzing the pure drug solution at 6 different levels (with 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 evaluate the robustness of the methods, volume of Hydrochloric acid was slightly altered. The reaction time (after adding NBS, time varied was 10 ±2min) and the time after addition of dye is slightly changed. To check the ruggedness, analysis was performed by three different analysts and on three 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
ALF	0.2 0.4 0.6	0.20 0.40 0.61	0.00 0.00 1.66	100.0 100.0 101.6	0.956	100.55±0.9622
ATV	0.4 0.8 1.2	0.40 0.81 1.19	0.00 1.25 0.83	100.0 101.2 99.16	1.020	100.12±1.022
CIP	0.1 0.2 0.3	0.10 0.20 0.31	0.00 0.00 3.33	100.0 100.0 96.66	1.946	98.88±1.924
CTP	0.6 1.2 1.8	0.60 1.21 1.79	0.00 0.83 0.55	100.0 100.83 99.44	0.696	100.09±0.697

14. APPLICATION TO FORMULATIONS

The proposed methods were applied to the determination 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[14,20,42&50] 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 analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method at the 95% confidence level with respect to accuracy and precision.

Recovery experiment was performed via standard addition technique to ascertain the accuracy and validity of the proposed 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 was found by the proposed 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
Alfuzosin (ALF)	0.50	0.2	0.70	0.00	100.0	0.9231	100.56 ±0.739	99.68 ±0.9202
	0.50	0.4	0.89	1.11	98.88			
	0.50	0.6	1.11	0.90	100.9			
	0.20	0.0	0.20	0.00	100.0			
	0.40	0.0	0.40	0.00	100.0			
	0.60	0.0	0.59	1.66	98.33			
Atrovastatin Calcium (ATV)	0.50	0.4	0.90	0.00	100.0	0.4971	100.03 ±0.409	99.42 ±0.4942
	0.50	0.8	1.29	0.76	99.23			
	0.50	1.2	1.69	0.58	99.41			
	0.40	0.0	0.40	0.00	100.0			
	0.80	0.0	0.79	1.25	98.75			
	1.20	0.0	1.19	0.83	99.16			
Cipro-500 (CIP)	0.50	0.1	0.50	0.00	100.0	1.3317	102.5± 1.76	98.99 ±1.3183
	0.50	0.2	0.69	1.42	98.57			
	0.50	0.3	0.79	1.25	98.75			
	0.1	0.0	0.10	0.00	100.0			
	0.2	0.0	0.20	0.00	100.0			
	0.3	0.0	0.29	3.33	96.6			
Celexa-20 (CTP)	0.50	0.6	1.10	0.00	100.0	0.5494	99.37 ± 0.95	99.92 ±0.5490
	0.50	1.2	1.69	0.58	99.41			
	0.50	1.8	2.31	0.43	100.4			
	0.6	0.0	0.60	0.00	99.16			
	1.2	0.0	1.19	0.83	99.44			
	1.8	0.0	1.79	0.55	100.0			

Table 4: F-test and t-test values.

	Alfuzosin (ALF)	Atrovastatin Calcium(ATV)	Ciprofloxacin (CIP)	Citalopram (CTP)
F-test*	0.8467 (4.2839)	0.2442 (4.0990)	3.0976 (4.3874)	0.9025 (4.7571)
t-test**	0.3472 (2.447)	0.3128 (2.262)	0.4632 (2.571)	0.6767 (3.182)

*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.

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