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Spectrophotometric determination of Drugs & Pharmaceuticals Using Cerium(IV) as oxidant and Amaranth dye as analytical reagent

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Abstract: *Simple, sensitive and accurate methods for determination of four drugs viz., Ciprofloxacin, Pantoprazole Sodium Sesquihydrate, Atrovastatin Calcium and Rosuvastine Calcium have been developed. The methods depend upon the oxidation of the drug by a known excess Ceric Ammonium Sulphate in sulphuric acid medium and subsequent determination of unreacted Ce (IV) using Amaranth dye. The methods have been validated in terms of LOD, LOQ, precision accuracy, %RSD, robustness and ruggedness. Factors affecting the absorbance viz., concentration of H_2SO_4 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.*
Key Words: *Spectrophotometry, Drugs, Ceric Ammonium Sulphate, Amaranth Dye, Validation*

I. Introduction

1.1. Ciprofloxacin:

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 [1,2] [fig 1]. 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 [3,4]. CIP has been shown to be active against most isolates of the following bacteria both in vitro and in clinical infections [5,6]. Assay of Ciprofloxacin in pharmaceuticals has previously been achieved by several analytical techniques such as HPLC [7], UV Spectrophotometry [8], RP- HPLC [9], Turbidimetry Method [10], UPLC [11], FTIR [12], Solid-State FT- Raman Spectroscopy [13]. 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 oxidative spectrophotometric determination technique with more accuracy and less time in bulk drugs and pharmaceutical dosage forms.

1.2 Pantoprazole Sodium Sesquihydrate

Pantoprazole Sodium Sesquihydrate (PNT) is the most frequently prescribed drug to treat and help heal duodenal and gastric ulcers of the drug class proton pump inhibitor. It is chemically known as sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl] sulfinyl]1H-benzimidazole sesquihydrate [1,2] [fig 2]. PNT is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by covalently binding to the (H^+,K^+)-ATPase enzyme system at the secretory surface of the gastric parietal cell [3,4]. This effect leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus [5,6]. The literature survey reveals that only few methods are available for the determination of PNT in dosage forms and include Voltammetry [14], A flow-injection biamperometric method [15], UV Spectrophotometry [16-19], RP-HPLC [20,21], HPLC [22] and Spectrofluorimetry [23].

1.3. Atorvastatin calcium

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-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate [fig 3] 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 in HPTLC [24-26], RP-HPLC [27-29], HPLC [30-31] Spectrophotometric method [32] and aqueous samples method [33] for bulk drug and Tablets.

1.4. Rosuvastatin calcium

Rosuvastatin calcium (ROSU) is a synthetic lipid lowering agent which is used in hypercholesterolemia and dyslipidemia [34]. It inhibits the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme that converts HMGCoA to mevalonate a precursor of cholesterol and thereby checks the synthesis of cholesterol [35]. It is chemically known as bis [(E)-7 [4-(4-fluorophenyl)-6 isopropyl- 2-[methyl (methyl-sulphonyl) amino] pyrimidin-5-yl] (3R, 5S) -3,5 dihydroxyhept-6-enoicacid] Calcium salt [1,2]. A survey of literature showed few LC–MS/MS with electrospray ionization method [36-38], UV spectrophotometric [39-42], few HPLC[43-46], RP- HPLC [47,48], HPTLC [49] and Voltammetry [50] methods are available for the estimation of Rosuvastatin in pharmaceutical preparation and in biological fluids.

Table 1: Comparison Of Various Existing Methods Used For Quantification Of Four Drugs

S.No.	Drug	Method	Quantification limit	%RSD
1	Ciprofloxacin	HPLC	4.0-24.0 (µg ml^{-1})	1.00
		HPLC	150-900 (µg ml^{-1})	0.19
		UPLC	6.33-50.69 (µg ml^{-1})	0.18
		Turbidimetry	14.0-56.0 (µg ml^{-1})	1.85
		RP-HPLC	3.0-18.0 (µg ml^{-1})	1.50
		UV Spectrophotometry	10.0-35.0(µg ml^{-1})	0.75
2	Pantoprazole Sodium Sesquihydrate	HPLC	1.0-10.0 (µg ml^{-1})	1.82
		RP-HPLC	15.0-75.0(µg ml^{-1})	0.73
		RP-HPLC	2.6-13.0 (µg ml^{-1})	0.
		UV Spectrophotometry	10.0-50.0(µg ml^{-1})	0.64
		UV Spectrophotometry	2.5-50.0 (µg ml^{-1})	1.85
		UV Spectrophotometry	2.0-20.0 (µg ml^{-1})	0.75
3	Atrovastatin Calcium	HPLC	4.0-24.0 (µg ml^{-1})	1.69
		HPTLC	200-1200(ng/spot)	0.38
		RP-HPLC	50.0-150.0(µg ml^{-1})	0.57
		RP-HPLC	8.13-23.77 (µg ml^{-1})	0.63
		UV Spectrophotometry	4.0-24.0 (µg ml^{-1})	0.39
4	Rosuvastatin Calcium	RP-HPLC	3.0-1602 (µg ml^{-1})	0.92
		RP-HPLC	1-160 (µg ml^{-1})	0.84
		HPLC	5-20 (µg ml^{-1})	0.79
		HPTLC	11-33.56(µg ml^{-1})	0.93
		UV Spectrophotometry	5-30 (µg ml^{-1})	0.56
		UV Spectrophotometry	5-35 (µg ml^{-1})	0.29

Although much work has been published on the quantification of the above drugs which is summarized in Table 1, but the simplest method using oxidative spectrophotometry has not been reported yet. In the present communication we report the oxidation of drug by Cerium(IV) - Amaranth dye couple to report the quantification of drug by Cerium(IV) as oxidant and Amaranth dye as analytical reagent.

II. About The Method

Cerium (IV) is a good oxidizing agent like $KMnO_4$, $K_2Cr_2O_7$ etc., it has been used for quantitative determination of drugs based on the oxidation of drugs. The spectrophotometric methods involved addition of excess Ce(IV) and un reacted Cerium is estimated by suitable dyes which should be oxidized by Cerium viz., Indigo Carmine, Methyl Orange, Safranin-O and Xylene cyanol. Amaranth dye is suitable for estimation of unreacted Ce (IV) absorbance at 523 nm. Cerium (IV) is a strong oxidizing agent due to its highest oxidation potential (Eo = 1.44 V).

Structure of Drugs

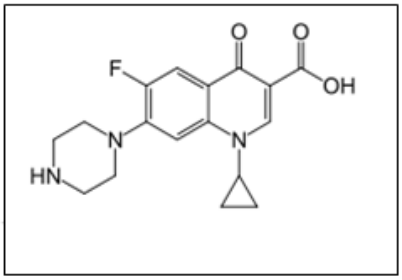

Fig 1 Ciprofloxacin

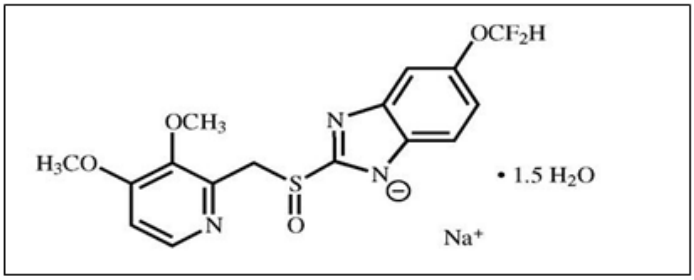

Fig. 2 Pantoprazole Sodium Sesquihydrate

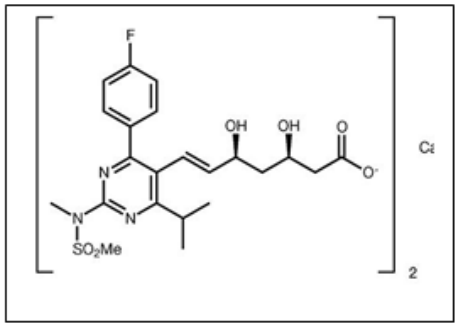

Fig.3 Atrovastatin Calcium

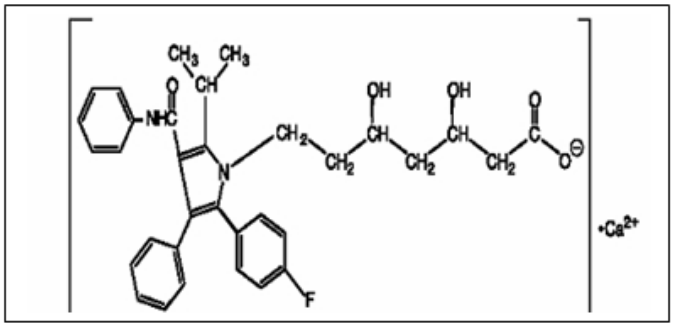

Fig.4 Rosuvastatin Calcium

III. 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. Cerium (IV) solution:

Prepared by dissolving 750 mg of Cerium (IV) sulphate ($CeSO_4.2H_2O$, 99.9 % pure) (Merck, Mumbai, India) in 2 N $H_2SO_4$with the aid of heat, filtered using glass wool, and diluted to 250 ml with the same acid and standardized. Cerium is standardized by ferrous ammonium sulphate and ferroin indicator. The solution was then diluted appropriately with 2 N H_2SO_4 to get working concentrations of 4.0×10^{-3} M (0.25%).

3.2.2. Amaranth dye:

Aqueous solution of 0.8×10^{-3} M of Amaranth dye was prepared by dissolving an appropriate weight of 0.0483 grams in 100 ml by distilled water.

3.2.3. Sulphuric acid:

Prepared by diluting the concentrated acid (Merck, Mumbai, India, Sp. gr. 1.84, 98.0 %) with water appropriately to get 2 N 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 CIP, PNT, ATV and ROSU were further diluted with the same solvent to obtain working concentrations.

IV. Procedure

Aliquots containing 1.2 - 8.4 µg ml^{-1} (CIP), 1.6 -11.2 µg ml^{-1} (PNT), 1.8-12.6 µg ml^{-1} (ATV), 5.1-10.5 µg mL^{-1} (ROSU) of drugs was transferred into a series of 10 ml standard flasks using a micro burette. To this, 1 mL of CAS was added followed by 1 mL of 2N H_2SO_4 and contents were shaken well. After 15 minutes, 1 mL of 0.02% of amaranth added to the contents. Then contents were shaken well and diluted up to the mark. The absorbance of each solution was measured at 523 nm against the corresponding reagent blank.

V. 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 1.2-8.4 µg mL^{-1} of CIP, 1.6-11.2 µg mL^{-1} of PNT, 3.0 -21.0 µg mL^{-1} of ATV, 1.5 -10.5 µg mL^{-1} of ROSU.

To each of the solution, 1 mL of 250 µg mL^{-1} of Cerium, 1 ml of 2 N of H_2SO_4 were added and the un reacted Cerium is analyzed as described above using Amaranth 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.

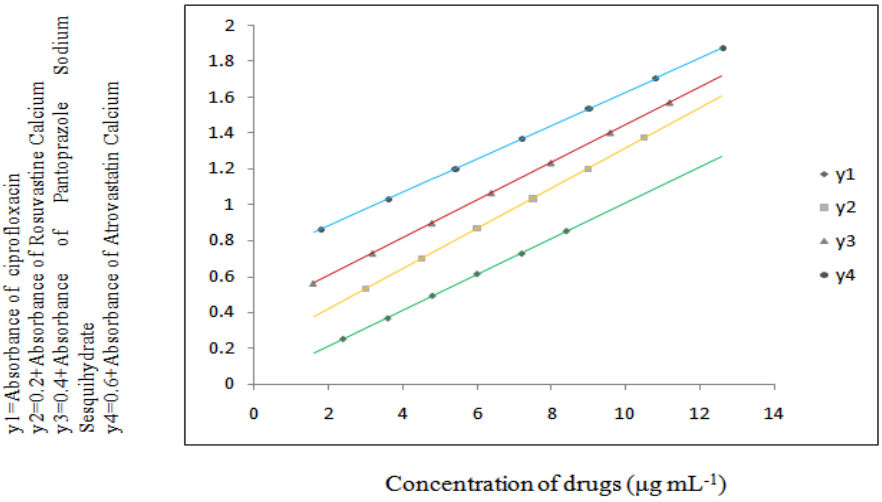

Fig.5 Calibration curves of CIP, ROSU, PNT and ATV.

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 table3.For this purpose standard deviation method also adapted. Excellent recovery and %RSD being less than 2 speaks about the precision and accuracy of the method.

VI. Procedure For Analysis Of Tablets

6.1 Ciprofloxacin

For the analysis of pharmaceutical formulations one tablets (CIPRO – 250mg) were weighed accurately and grounded. A quantity equivalent to 10mg of Ciprofloxacin 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 Pantoprazole Sodium Sesquihydrate

Two tablets (Pantoprazole, 40mg) were weighed accurately and crushed into a fine powder. A quantity equivalent to10 mg of Pantoprazole Sodium Sesquihydrate 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.

6.3 Atrovastatin Calcium

Three tablets (Atrovastatin calcium 10mg) were weighed accurately and crushed to a fine powder and the powder equivalent to 10mg of Atrovastatin calcium was weighed accurately and 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 Rosuvastatin Calcium

Two tablets (Rosuvas 20mg) were crushed to a fine powder and the powder equivalent to 10mg of Rosuvastatin calcium was weighed accurately and 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.

VII. 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 and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates accuracy and precision of the methods. [Table 3].

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 are mentioned in the [Fig. 5] 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 and analyst or both to test the Ruggedness of the method absorbance data was collected using 3 different instrument and 2 analysts no significant changes were observed either by change of instrument or analyst hence the method may be taken as robust.

VIII. Factors Effecting Absorbance And Selection Of Acid

8. 1. Selection of acid:

To study the effect of acid, different types of acids were examined (H_2SO_4, $H_3PO_4$and CH_3COOH) to achieve maximum yield of redox reaction. The results indicated that the Sulphuric acid was the preferable acid with Ce (IV) as oxidant.

8.2 Selection of volume of acid and concentration:

To study the effect of acid concentration, different concentrations of H_2SO_4 were examined. The reaction was performed in a series of 10 ml volumetric flask containing 12.0 µg mL^{-1} of the cited drugs, different volumes (0.5–2.5 mL) of 0.5 N , 1.0 N , 1.5 N ,2.0 N, 2.5 N H_2SO_4 and 1 ml of Ce(IV) ($4.0x\ 10^{-3}$M) were added. After 15 min of time, 1.0 ml of amaranth dye and water added upto the mark. It was found that the maximum absorbance was obtained with 1mL of 2 N H_2SO_4. Above this volume the absorbance decreased therefore a volume of 1 mL of 2 N H_2SO_4 was used for all measurements.

8.3. Effect of time:

In order to obtain the highest and most stable absorbance, the effect of time on the oxidation reaction of drugs were catalyzed by the time periods ranging for 2.5-20 min. the time required to complete the reaction and maximum absorbance was obtained after 15 min.

IX. 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 and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates applicability of the methods for pharmaceutical analysis [Table 4]. The excellent recovery studies indicate that methods developed can be applied to pharmaceutical analysis without hesitation.

X. Results And Discussion

The ability of Cerium (IV) sulphate to oxidize drugs and bleach the color of amaranth dye is the basis to develop indirect spectrophotometric method here. In this method the drugs were reacted with a measured excess of Cerium(IV) sulphate in acidic medium and the unreacted oxidant was determined by reacting with Amaranth followed by absorbance measurement at 523 nm (scheme1). The absorbance increased linearly with increasing concentration of drug, when increasing amounts of each drug were added to a fixed amount of 0.25% of CAS, consumed the latter and there occurred a concomitant fall in its concentration. When fixed amount of the dye was added to decreasing amount of oxidant, a concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective λ_{max} with increasing concentration of each drug. 1 ml of 2N acid was used in the reaction as this concentration was found ideal.
D + Ce (IV) excess → D oxidation product + Ce (III) + Ce (IV)unreacted : (1)
Ce (IV) unreacted + amaranth → oxidation product of amaranth + unreacted amaranth: (2)
Measured spectrophotometrically at λ_{max} =523 nm
Scheme 1: Tentative reaction scheme of the indirect determination of drug by oxidation with Ce (IV) sulphate.

XI. 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[51] and presented in Table 2 which reveals very high sensitivity of this method. Regression analysis of Beer's law data by using the method of least squares was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and also given in Table 2.

XII. 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 3 and reveal the high accuracy and precision of the method.

XIII. Robustness And Ruggedness

To evaluate the robustness of the methods, volume of Sulphuric acid was slightly altered. The reaction time (after adding CAS, 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.

XIV. Application To Formulations

The proposed method was applied to the determination of drugs in tablets. The results in Table 4 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 [52-55] 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 summarized in Table 5 and 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 4) was within the permissible limits showing the absence interference by the inactive ingredients in the assay.

XV. Conclusion

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

Acknowledgement

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Table 2: Analytical And Regression Parameters Of Spectrophotometric Method

Name of drug Property	CIP	PNT	ATV	ROSU
λ_{max}, nm	523	523	523	523
Beer's law limits (μg mL^{-1})	1.2-8.4	1.6-11.2	1.8-12.6	1.5-10.5
Molar absorptivity	$5.11x10^4$	$4.41x10^4$	$7.27x10^4$	$1.29X10^4$
Sandell's sensitivity (μg cm^{-2})	0.0380	0.3226	0.017637	0.101523
Variance $(S_a)^2$	0.0320	0.0021	0.0009	0.0010
Limit of detection μg mL^{-1}	1.8063	1.5100	1.8256	1.1681
Limit of quantification μg mL^{-1}	5.4737	4.5758	5.5321	3.8125
Regression equation, Y**				
Intercept, (a)	0.0028	0.0031	0.0216	0.0939
Slope, (b)	0.0268	0.1006	0.0567	0.0104
Correlation coefficient, (r)	0.9997	0.9998	0.9946	0.9906
Standard deviation of intercept (S_a)	0.0143	0.0460	0.0313	0.0320
Standard deviation of slope (S_b)	0.0012	0.0102	0.0074	0.0013

*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.

Table 3: 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
CIP	1.2 2.0 3.0	1.18 2.0 3.02	1.66 0.00 0.66	98.33 100.00 100.66	1.200	99.66 ±1.201
PNT	2.0 4.0 6.0	2.01 3.9 6.02	0.50 2.50 2.00	100.50 97.50 100.33	1.382	99.44 ±1.375
ATV	3.0 6.0 9.0	2.95 6.01 9.02	1.66 0.16 0.22	98.33 100.16 100.22	1.074	99.57 ±1.074
ROSU	1.5 3.0 6.0	1.51 2.98 5.92	0.66 0.66 1.33	100.66 99.33 98.66	1.018	99.55 ±1.018

Table 4 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 ± **SD**
CIPRO (CIP)	0.50 0.50 0.50 1.2 2.4 3.0	1.0 2.0 3.0 0.0 0.0 0.0	1.49 2.49 3.5 1.21 2.41 3.0	0.66 0.40 0.00 0.83 0.41 0.00	99.33 99.60 100.00 100.83 100.41 100.00	0.5444	100.15 ±0.18	100.01 ±0.5405
PANTO PRAZOLE (PNT)	0.50 0.50 0.50 2.0 4.0 6.0	0.3 0.6 0.9 0.0 0.0 0.0	0.80 1.11 1.41 2.01 3.99 6.03	0.00 0.90 0.71 0.50 0.25 0.50	100.00 100.90 100.71 100.50 99.75 100.50	0.4333	98.16 ±1.15	100.39 ±0.4350
ATRO VASTATIN CALCIUM (ATV)	0.50 0.50 0.50 3.0 6.0 9.0	1.0 2.0 3.0 0.0 0.0 0.0	1.50 2.49 3.52 2.98 6.01 9.02	0.000 0.40 0.57 0.66 0.16 0.22	100.0 99.60 100.5 98.34 100.16 100.22	0.4450	100.03 ±0.409.	99.98 ±0.4455
ROSUVAS (ROSU)	0.50 0.50 0.50 2.0 3.0 4.5	1.0 2.0 3.0 0.0 0.0 0.0	1.504 2.49 3.52 1.985 2.98 4.45	0.27 0.40 0.57 0.75 0.66 1.11	100.27 99.60 100.57 99.25 99.33 98.88	0.7817	100 ±1.070	99.92 ±0.7810

Table 5: F-Test And t-Test Values

	CIPRO (CIP)	PANTOPRAZOLE (PNT)	ATROVASTATIN CALCIUM (ATV)	ROSUVAS(ROSU)
F-test*	1.478 (3.182)	1.314 (2.571)	0.158 (2.262)	0.534 (2.447)
t-test**	0.29 (4.75)	0.18 (4.38)	0.19 (4.09)	0.61 (4.28)

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

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