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Spectrophotometric determination of drugs by using Cerium (IV) and Rhodamine B couple as analytical reagent

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ABSTRACT: *Simple, sensitive, accurate and precise spectrophotometric methods for quantitative determination of five drugs viz., Moxifloxacin (MOX), Gemifloxacin(GEM), Sumatriptan succinate (SUM), Phenylephrine hydrochloride (PHE) and Duloxetine (DUL) were developed. The method for each drug depends upon oxidation of drugs by Ce (IV) (Excess) and estimating the amount of un reacted Ce (IV) by Rhodamine-B dye at 557 nm. The calibration curves obeyed Beer's law over the concentration range of 4-40μg ml^{-1} (MOX), 5-70 μg ml^{-1} (GEM), 8-100μg ml^{-1} (SUM), 10-100 μg ml^{-1} (PHE), & 10-70 μg ml^{-1} (DUL). The methods have been validated in terms of guidelines of ICH and has been applied to the analysis of pharmaceuticals.*

Keywords: *Cerium (IV), Rhodamine-B dye couple, drugs, Determination, UV-Vis Spectrohotometry.*

I. INTRODUCTION

Moxifloxacin (MOX) (Fig.1a) is chemically known as 1-cyclopropyl-7-[(1S, 6S)-2,8-diazabicyclo [4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-quinoline-3-carboxylic acid. It is an advanced-generation, 8-methoxyquinolone derivate of fluoroquinolone antibacterial agent that is synthetic. It was discovered in 1999. Moxifloxacin is a broad-spectrum antibiotic [1] that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerases, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell replication. Various methods cited in literature for its determinations involve, high performance liquid chromatography [2-4], liquid chromatography [5-7], voltammetry [8, 9], spectrophotometry [10, 11]. However, most of these methods involve time-consuming procedures, derivatization and/ or sophisticated instruments. Due to the fact that MOX is a compound of great pharmacological and analytical importance, in recent years, there has been an increased interest to develop accurate analytical methods which are valid for quantification of MOX in biological and pharmaceutical samples.

Gemifloxacin (GEM) (Fig.1b) is chemically known as 7-[(4Z)-3-(Aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo- 1, 8-naphthyridine-3-carboxylic acid. It is used to treat a variety of bacterial infections [12]. This medication belongs to a class of drugs called quinolone antibiotics. . Because of its physiological significance the drug has been quantitatively analyzed by different methods. A few analytical methods like HPLC [13-16], Spectrophotometry [17, 18], Spectrofluorimetry [19, 20] , LC-MS [21], and Chemiluminisence method [22] developed for the estimation of GEM are mention worthy.

Sumatriptan Succinate (SUM) (Fig.1c) is chemically known as 1-[3-(2-Dimethylaminoethyl)-1H-indol-5-yl]-N-methyl-methanesulfonamide It is a synthetic drug belonging to the triptan class, used for the treatment of migraine headaches [23]. Analytical methods available for the analysis of SUM include HPLC [24-28], Colorimetry [29], Spectrophotometry [30] and UPLC [31-33].

Phenylephrine Hydrochloride (PHE) (Fig.1d) chemically (R)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride is a direct sympathomimetic agent, a selective $\alpha_1$agonist, causing vasoconstriction. It is also a frequent constituent of orally administered nasal decongestant [34] preparations. Literature review reveals that a few methods have been published for analysis of PHE in the bulk form and in pharmaceutical preparations. Methods available include Spectrophotometry [35- 42], HPLC [43-45] and liquid chromatography [46].

Duloxetine (DUL) (Fig.1e) is chemically known as (+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine. The main uses of duloxetine are in major depressive disorder, general anxiety disorder, urinary incontinence, painful peripheral neuropathy, fibromyalgia, and chronic musculoskeletal pain associated with osteoarthritis and chronic lower back pain. [47]. several techniques have been reported in the literature for the

determination of DUL in pharmaceuticals and in biological samples include HPLC [48-50], UPLC [51] and Spectrophotometry [52, 53] in pharmaceuticals and in biological samples.

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, viz., Indigo Carmine, Methyl Orange, Safranin-O and Xylene cyanol. We report Rhodamine-B dye is suitable for estimation of unreacted Ce (IV) absorbance at 557 nm.

Experimental

Apparatus: Spectral and absorbance measurements were made on a Elico 210 double beam spectrophotometer, Systronics 117 spectrophotometer 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.

II. MATERIALS AND METHODS

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

Cerium (Iv) Solution

Cerium (IV) sulphate ($CeSO_4.2H_2O$, 99.9 % pure) was prepared by dissolving 750 mg of chemical (Merck, Mumbai, India) in 2 N H_2SO_4 with the aid of heat and filtered using glass wool and diluted to 250 ml with the same acid and cerium is standardized by Ferrous Ammonium Sulphate uses 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%).

Rhodamine-B Dye

Aqueous solutions of 50µg ml^{-1} of Rhodamine-B dye was prepared by dissolving an appropriate weight of 0.050 grams in 100 ml bi distilled water.

Sulphuric Acid

Prepared by diluting the concentrated acid (Merck, Mumbai, India, and Sp. gr. 1.84, 98.0 %) with water appropriately to get 2 N acid.

Preparation Of Drug Solution

Standard drug solution (200 µg ml^{-1}) was prepared by dissolving 20 mg of drug with distilled water to the mark in 100 ml standard flask. The stock solution was diluted appropriately to get the working concentration.

Procedure

Aliquots containing 1.6 - 56.00 µg ml^{-1} of drug were transferred into a series of 10 ml standard flasks using a micro burette. To this, 1 ml of Ceric Ammonium Sulphate followed by 1 ml of 2N H_2SO_4 and contents were shaken well. After 30 minutes, 1 ml of 0.02% Rhodamine-B dye was added to the flask. 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.

Assay Of Drug Pure Sample

To the 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 4-40 µgml^{-1} of MOX, 5-70 µgml^{-1} of GEM, 8-100 µgml^{-1} of SUM, 10-100µgml^{-1} of PHE and 10-70 µgml^{-1}DUL have been taken. (Table 1) 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 Rhodamine-B dye.

Procedure For Analysis Of Pharmaceuticals

Moxifloxacin

For the analysis of pharmaceutical formulations three tablets (Crosmox – 400mg) were weighed and grounded. A quantity equivalent to 10mg of Moxifloxacin was transferred into a 100 mL calibrated flask and the volume was finally diluted to the mark with water, mixed well and filtered using a Whatmann No. 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion was diluted appropriately to get 3 µg mL^{-1} and the assay was completed according to the procedure described above.

Gemifloxacin

Five tablets (AdGem, 320 mg) were weighed and grounded. The powder equivalent to10mg gemifloxacin was stirred well with methanol, sonicated about 30 minutes. The solution was filtered through Whatmann filter paper in a 100ml volumetric standard flask and the residue was washed well with methanol for complete recovery of the drug and methanol was evaporated. The residue was dissolved in 100 mL of distilled water and it was further diluted to get required concentration for the analysis of the drug.

Sumatriptan

Ten tablets (Sumitrex, 25mg) were grounded and the powder equivalent to10 mg of sumatriptan succinate was weighed, dispersed in 25 mL of methanol, sonicated for 30 min and filtered through Whatmann filter paper No 42.The residue was washed thrice with methanol for complete recovery of drug and methanol was evaporated. The residue was dissolved in 100 mL of distilled water .It was used as stock sample solution and was further diluted with the same solvent to get working standard solution..

Phenylephrine Hydrochloride

Ten tablets (Dolgencorp, 10mg) were weighed powered and equivalent to about 10 mg of phenylephrine hydrochloride was transferred to 100 ml volumetric flask; 60.0 ml of distilled water was added and ultrasonicated for 20 min, then made up to the mark with distilled water. The resulting solution was mixed and filtered through Whatmann filter paper no. 42. From the filtrate solution was diluted appropriately with distilled water so as to obtain final concentration of drug and the resulting solution was used for the analysis.

Duloxetine

About ten to fifteen tablets (Ulozet, 40 mg) were powdered and equivalent to about 10 mg of Duloxetine hydrochloride had been taken in to a100 ml of volumetric flask and added about 30 ml of methanol, sonicated for 30 min and filtered through Whatmann filter paper No 42.The residue was washed thrice with methanol for complete recovery of drug and methanol was evaporated. The residue was dissolved in 100 mL of distilled water .It was used as stock sample solution .The aliquot portions of this stock solution were further diluted with distilled water to get the final concentration required for the determination of the drug.

Method Of Validation

The each method developed quantification of drugs has been validated in terms of precision, accuracy, limit of detection, limit of quantification, linearity, selectivity and ruggedness. Absorbance time curves were drawn, initial rate and fixed time methods were used to assess the recovery of the drug. To assess the precision each experiment was repeated at least 5 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. Further t-test and F-test values have also been calculated using a standard reference method. The closeness of t-test and F-test values is less than that they permissible range indicating high accuracy of the methods [Table 2].

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/S

Where s = 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/S.

Limits of linearity of calibration curves are mentioned in the [Fig. 2] 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.

Factors Effecting Absorbance

Effect Of Acid Concentration: To study the effect of acid concentration, 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. The reaction was performed in a series of

10 ml volumetric flask containing 8.0 µgml-1 0f the cited drugs, different volumes (0.5–2.5 ml) of 2.0 N H_2SO_4 and 1 ml of Ce(IV) (4.0x 10^{-3}M) were added. After 5.0 min of heating time at 60 ± 2°C in a water bath, the solution was cooled for about 3.0 min, 1.0 ml of Rhodamine-B dye were added, then complete to 10 ml total volume with water. It was found that the maximum absorbance was obtained at 1 ml 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.

Effect Of Heating Time

In order to obtain the highest and most stable absorbance, the effect of heating time on the oxidation reaction of drugs were catalyzed by heating in a water bath at 60 ± 2°C for the periods ranging for 2.5-20 min. the time required to complete the reaction and maximum absorbance was obtained after 5.0 min of heating. After oxidation process, the solution must be cooled at least for 3.0 min before addition of dye.

Effect Of Oxidant Concentration

When a study on the effect of Ce (IV) on color development was performed, it was observed that in both cases the absorbance increased with increase in the volume of Ce (IV). It reached maximum when 1 ml of 200 µg ml-1 Ce (IV) solution was added to a total volume of 10 ml for drugs solutions. The color intensity decreased above the upper limits. Therefore, 1 ml of 200 µg ml-1 Ce (IV) was used for all measurements.

Effect Of Dye Concentration

In order to ascertain the linear relationship between the volume of added Ce (IV) and the decrease in absorbance of Rhodamine-B dye, experiments were performed using 1 ml of 2 N H_2SO_4 with varying volumes of Ce (IV). The decrease in absorbance was found to be linear up to the 1 ml of 200 µg ml^{-1} Ce (IV) with optimum volume 1.0 ml of Rhodamine-B dye for fixed concentration drug solution. The color was found to be stable up to 24 hours.

Analysis Of Pharmaceuticals

To 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 2]. Further t-test and F-test values have also been calculated using a standard reference method. The closeness of t-test and F-test values is less than that they permissible range indicating excellent 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.

III. RESULTS AND DISCUSSION

The ability of cerium (IV) sulphate to oxidize drugs, and bleach the color of amaranth dye is the basis of the indirect spectrophotometric method developed 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 . 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, an concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective λmax with increasing concentration of each drug. One 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 + Rhodamine → oxidation product of rhodamine + unreacted rhodamine: (2)

Measured spectrophotometrically at λmax =557 nm

Scheme 1: Reaction Scheme of the indirect determination of drug by oxidation with Ce (IV) sulphate

Analytical Data

A linear correlation was found between absorbance at λmax and concentration ranges, and sensitivity parameters such as molar absorptivity, Sandal's sensitivity, detection limit and quantification limit are presented in Table 1. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and is also given in [Table 1].

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.

IV. CONCLUSION

The present study described the successful development of new, simple, sensitive, selective, accurate and rapid spectrohotometric method for the accurate determination of drugs each one in its pharmaceutical forms 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.

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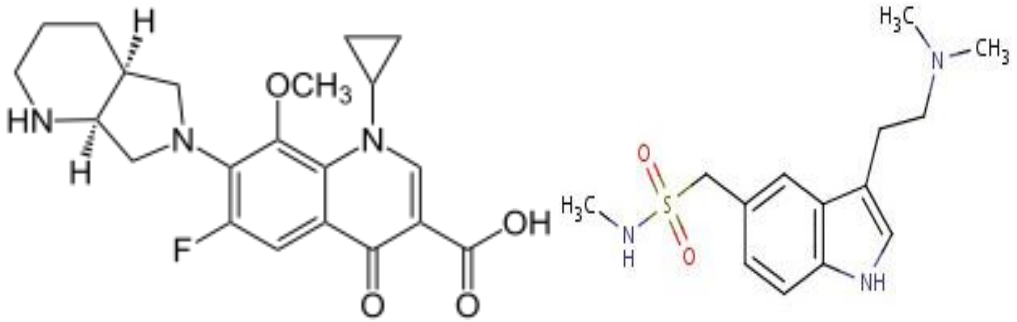
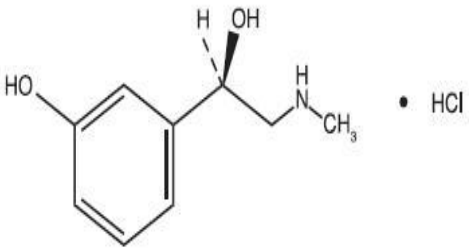
Fig.1 STRUCTURES OF DRUGS

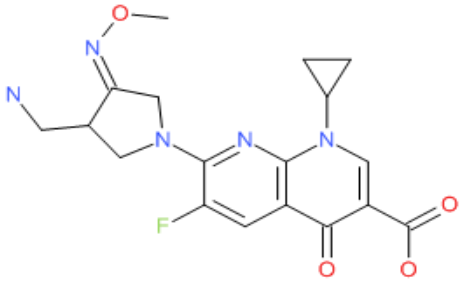


a. Moxifloxacin

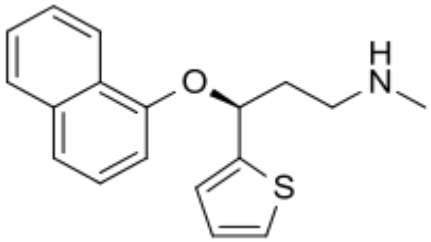
c. Sumatriptan



b. Gemifloxacin



d. Phenylephrine



e.Duloxetine

Fig.2 Calibration Curves

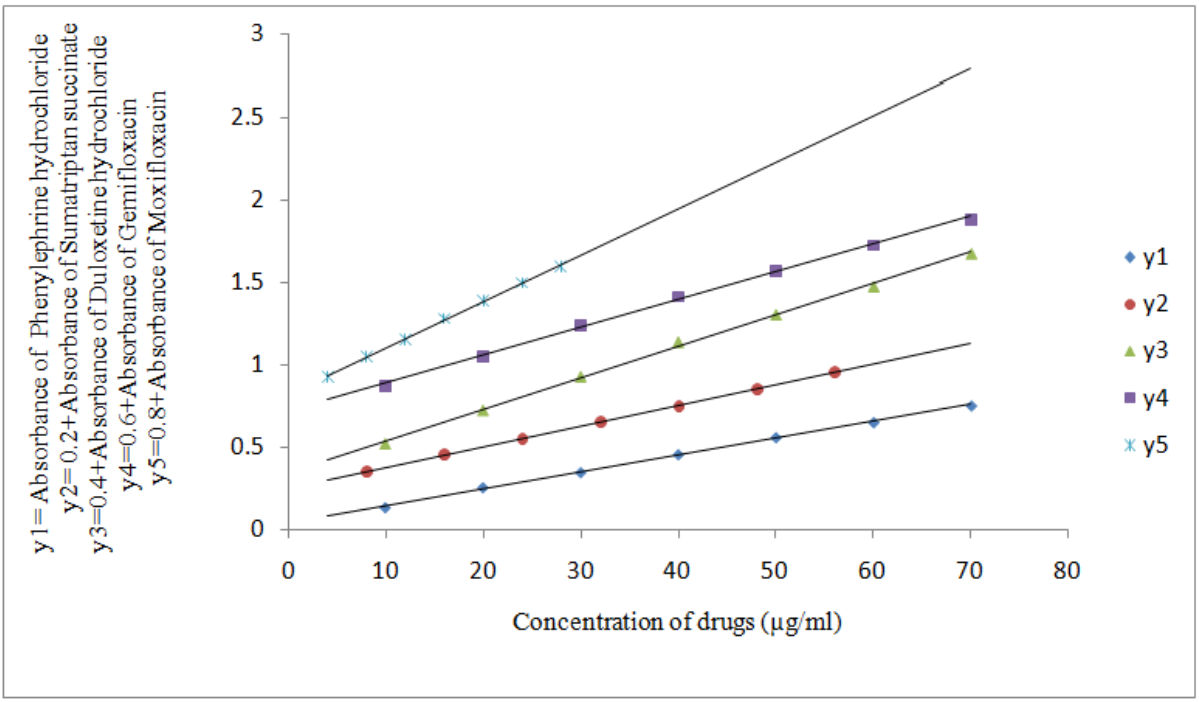


Table 1: Analytical Parameters For Determination Of Drugs By Oxidation With Cerium (Iv) And Rhodamine-B.

Parameter	MOX	GEM	SUM	PHE	DUL
λmax, nm	557	557	557	557	557
Beer's law limits µg mL^{-1}	4-40	5-70	8-100	10-100	10-70
Molar absorptivity, L mol^{-1} cm^{-1}	1.255×10^5	1.58×10^5	1.45×10^5	1.56×10^5	2.53×10^5
Sandell sensitivity* µg cm^{-2}	0029	0.0015	0.0023	0.0027	0.0024
Limit of detection µg mL^{-1}	0.0566	0.0847	0.5213	0.3691	0.2971
Limit of quantification µg mL^{-1}	0.0736	0.1856	1.1836	0.5074	0.5782
Regression equation, Y**					
Intercept, (a)	0.019	0.121	0.055	0.039	-0.052
Slope, (b)	0.028	0.016	0.012	0.010	0.019
Correlation coefficient, (r)	0.999	0.998	0.999	0.999	0.998
Standard deviation of intercept (Sa)	0.0042	0.0040	0.0271	0.012	0.0420
Standard deviation of slope (Sb)	0.0023	0.0037	0.0017	0.0015	0.0020

Table.2 Determination Of Accuracy And Precision Of The Methods On Pure Drug Samples.

Tablets	Drug in tablet µg mL^{-1}	Drug added µg mL^{-1}	Total found µg mL^{-1}	er (%)	Recovery (%)	RSD (%)	Reference method Mean ± SD	Propose method ± SD	t-test	F-test
Cambidol (TDH)	1.5 1.5 1.5 1.0 2.0 3.0	1.0 2.0 3.0 0.0 0.0 0.0	2.49 3.51 4.52 0.98 2.02 3.03	1.40 0.28 0.44 1.00 1.00 1.00	99.66 100.28 100.44 98.00 101.00 101.00	1.516	101.70 ±1.70	100.06 ±1.516	0.145 (2.571)	1.26 (4.95)
Dobusol (DOB)	1.0 1.0 1.0 2.0 3.0 4.0	0.5 1.0 1.5 0.0 0.0 0.0	1.50 2.04 2.50 1.99 3.02 4.01	0.00 2.00 0.00 0.50 0.66 0.25	100.00 102.00 100.00 99.55 100.66 100.25	0.881	101.00 ± 1.0	100.41 ±0.885	0.23 (2.571)	1.29 (4.95)
Trivedon (TRMZ)	1.0 1.0 1.0 2.0 4.0 6.0	1.0 2.0 3.0 0.0 0.0 0.0	2.02 3.02 4.02 2.01 4.01 6.02	1.00 0.66 0.50 0.50 0.25 0.33	101.00 100.66 100.50 100.50 100.25 100.33	0.265	99.80 ±1.26	100.54 ±0.267	1.754 (2.571)	0.042 (4.95)
Terazen (TRZ)	1.0 1.0 1.0 1.0 3.0 5.0	1.5 3.0 4.5 0.0 0.0 0.0	2.48 4.02 5.51 1.02 3.05 4.99	1.00 0.50 0.02 2.00 1.66 0.20	99.20 100.50 100.18 102.00 101.66 99.80	1.022	99.90 ±0.470	100.55 ±1.028	1.145 (2.477)	0.212 (4.28)
Miniblok (ESM)	1.0 1.0 1.0 3.0 4.0 5.0	0.5 1.0 1.5 0.0 0.0 0.0	1.51 1.99 2.49 3.02 4.04 5.02	0.66 0.50 0.40 0.66 1.00 0.40	100.66 99.50 99.60 100.66 101.00 100.40	0.641	100.45 ±0.573	100.30 ±0.643	0.142 (3.182)	1.045 (4.75)

Table 3 Results Of Assay Of Tablets By The Proposed Methods And Statastical Evaluation And Recovery Experiments By Standara Addition Method.

Tablets	Drug in tablet µg mL^{-1}	Drug added µg mL^{-1}	Total found µg mL^{-1}	er (%)	Recovery (%)	RSD (%)	Reference method Mean ± SD	Propose method ± SD	t-test	F-test
Cambidol (TDH)	1.5 1.5 1.5 1.0 2.0 3.0	1.0 2.0 3.0 0.0 0.0 0.0	2.49 3.51 4.52 0.98 2.02 3.03	1.40 0.28 0.44 1.00 1.00 1.00	99.66 100.28 100.44 98.00 101.00 101.00	1.516	101.70 ±1.70	100.06 ±1.516	0.145 (2.571)	1.26 (4.95)
Dobusol (DOB)	1.0 1.0 1.0 2.0 3.0 4.0	0.5 1.0 1.5 0.0 0.0 0.0	1.50 2.04 2.50 1.99 3.02 4.01	0.00 2.00 0.00 0.50 0.66 0.25	100.00 102.00 100.00 99.55 100.66 100.25	0.881	101.00 ± 1.0	100.41 ±0.885	0.23 (2.571)	1.29 (4.95)
Trivedon (TRMZ)	1.0 1.0 1.0 2.0 4.0 6.0	1.0 2.0 3.0 0.0 0.0 0.0	2.02 3.02 4.02 2.01 4.01 6.02	1.00 0.66 0.50 0.50 0.25 0.33	101.00 100.66 100.50 100.50 100.25 100.33	0.265	99.80 ±1.26	100.54 ±0.267	1.754 (2.571)	0.042 (4.95)
Terazen (TRZ)	1.0 1.0 1.0 1.0 3.0 5.0	1.5 3.0 4.5 0.0 0.0 0.0	2.48 4.02 5.51 1.02 3.05 4.99	1.00 0.50 0.02 2.00 1.66 0.20	99.20 100.50 100.18 102.00 101.66 99.80	1.022	99.90 ±0.470	100.55 ±1.028	1.145 (2.477)	0.212 (4.28)
Miniblok (ESM)	1.0 1.0 1.0 3.0 4.0 5.0	0.5 1.0 1.5 0.0 0.0 0.0	1.51 1.99 2.49 3.02 4.04 5.02	0.66 0.50 0.40 0.66 1.00 0.40	100.66 99.50 99.60 100.66 101.00 100.40	0.641	100.45 ±0.573	100.30 ±0.643	0.142 (3.182)	1.045 (4.75)

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