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QUANTITATIVE ESTIMATION OF DRUGS AND PHARMACEUTICALS USING CHLORAMINE-T AND SAFRANIN-O DYE BY AN OXIDATIVE SPECTROPHOTOMETRIC METHOD

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Keywords:

Chloramine-T, Safranin-O, Cetirizine, Duloxetine, Esmolol, Gemifloxacin, Moxifloxacin, Hydrochloride

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ABSTRACT: Simple, sensitive and selective methods are developed for the spectrophotometric determination of drugs, *viz*., Cetirizine hydrochloride, Duloxetine hydrochloride, Esmolol hydrochloride, Gemifloxacin, Moxifloxacin and Phenylephrine Hydrochloride based on their reactivity towards Chloramine-T (ChL-T). The method is based on the oxidation of drugs by ChL-T (Excess amount) in presence of acidic medium and estimating the amount of un-reacted ChL-T by Safranin-O (SO) dye at λmax 524 nm. The amount of ChL-T reacted is equal to the drug concentration in this method. The concentration of ChL-T is 10 μgmL^{-1} and Safranin-O Dye is 10 μgmL^{-1}. The calibration curves obeyed Beer's law over the concentration range of 2.5-17.5 μgmL^{-1} (CET), 5-35 μgmL^{-1} (DUL), 12-84 μgmL^{-1} (ESM), 10-70 μgmL^{-1} (GEM) & 2.5-17.5 μgmL^{-1} (MOX). This method has been applied for the determination of drugs in their pure form as well as in tablet formulations. The method has been validated in terms of guidelines of International Conference on Harmonization.

INTRODUCTION: Cetirizine dihydrochloride chemically is [2-[4-[(4-chlorophenyl) phenyl methyl]-1 piperazinyl] ethoxy] acetic acid di HCl. Cetirizine di HCl is belonged to the group of the second-generation h1 antagonist, inhibits allergic reaction mediated by histamine. It used to the relief of itching of eyes, sneezing, itching of the nose or throat problems due to respiratory allergies. Some analytical techniques had been constructed for dugs quantification such as Reverse Phase-High Performance Chromatography (RP-HPLC) [1-3], HPLC [4-5], UV Spectrophotometry [6], Ion exchange resigns method [7], chemiluminescence [8] for Cetirizine di HCl.

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Duloxetine. HCl is chemically termed as N-methyl-3-(naphthalene-1-yloxy)-3-(thio phen-2-yl) propane -1 -amine HCl. DUL employed to major depressive disorders and effects on pain due to urinary infections. Nowadays, Duloxetine HCl is an alternative to therapeutic action drugs in the treatment of depression symptoms. Some of the analytical techniques have been constructed for quantification of several pharmaceutical samples such as UV spectrophotometric method [9-11], UV Spectrofluorometric method [12], RP-HPLC [13-14], High-Performance Thin Layer Chromatography (HPTLC) [15-16], Liquid chromatography-mass spectrometry [17], cyclic voltametry[18], photo fluorescent probe method [19] for Duloxetine HCl.

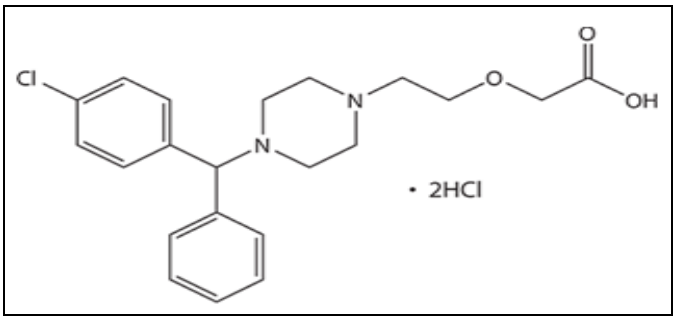
Esmolol HCl is chemically, methyl 3-{4-[2-hydroxy-3-(Iso propyl amino) propoxy] phenyl} propionate HCl. ESM is used in the rapid heart rate control in with atrial; it is essential to develop and validate different analytical methods for its quantification of drugs in pharmaceutical dosage

form. Some of the analytical techniques have been constructed for quantification of several drugs based on literature surveys such as on UV-Spectrophotometry [20-22], HPLC [23] and Capillary electrophoresis [24] for the determination of Esmolol HCl.

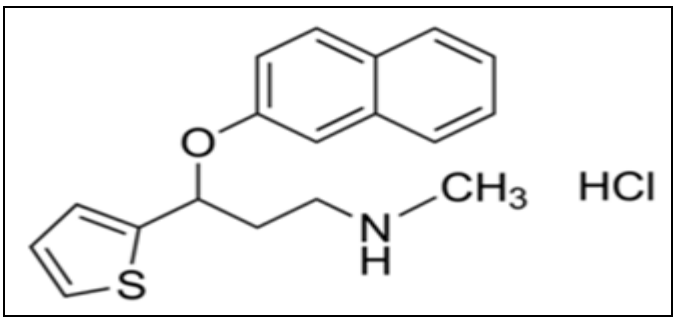
Gemifloxacin chemically is [{R,S}7-[{4Z}-3-{aminomethyl}- 4- {methoxyimino}- 1-pyrrolidinyl}- 1- cyclopropyl- 6- flouro- 1, 4-dihydro- 4- oxo- 1, 8-napthyridine-3-carboxylic acid. GEM is a new generation fluoroquinolone antibacterial drug. It is widely used for treatment of respiratory infections (bronchitis, pneumonia) and urinary infections. A few analytical techniques have been constructed for the quantification of Gemifloxacin in pure forms, such as, Spectro-photometric method [25-26], HPTLC [27], RP-HPLC [28-29], LC-MS [30], UV- Spectrofluorometric method [31] and Zone electrophoresis [32].

Moxifloxacin is chemically known as [1-Cyclopropyl-b-fluoro- 1, 4- dihydro-8-methoxy-7-[(4aS, 7aS)- octahydro- 6H- pyrrolo [3, 4–6] pyridine-6-yl]-4-oxo-3-quinoline carboxylic acid, is a fluoroquinolone broad-spectrum antibiotic agent and used in conjunctivitis. A few analytical techniques have been constructed for quantification of several drugs based on literature survey such as UV Spectrophotometry [33-36] RP-HPLC [37-40], HPLC [41-42], HPLC-MS [43-44], LC-MS [45], LC [46], chemiluminescence [47], Tanden mass spectrometry [48] methods for determination of Moxifloxacin have been reported.

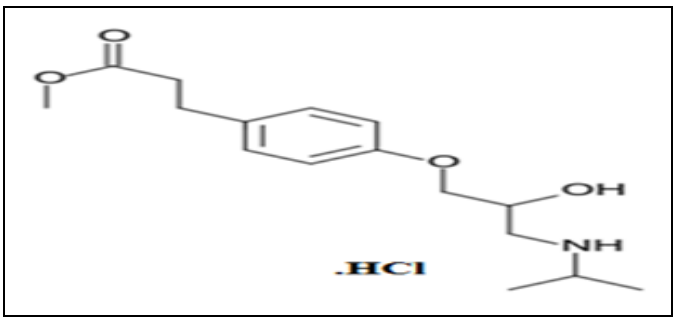
Through a survey of literature on the drugs mentioned above revealed that UV spectro-photometric quantification based on the use of Chloramine-T [49-53] as an oxidizing reagent and Safranin-O [54-56] dye as an analytical reagent had not been yet reported.



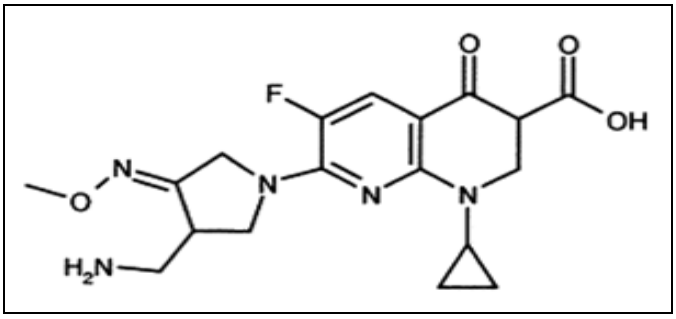
CETIRIZINE DIHYDROCHLORIDE



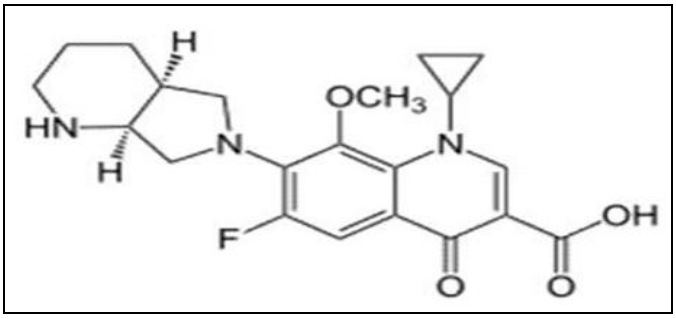
DULOXETINE HYDROCHLORIDE



ESMOLOL HCl



GEMIFLOXACIN



MOXIFLOXACIN

FIG. 1: STRUCTURE OF DRUGS

MATERIALS AND METHODS:

Instruments: All absorbance measurements were recorded on Shimadzu 140 double beam spectrophotometer as well as on Thermo Nicolet 100 & Elico double beam SL210 UV- Visible spectrophotometers using matched pair of Quartz cells of 10mm path length.

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

ChL-T solution (100 mg in 100 ml standard flask) was prepared by dissolving ChL-T (Himedia Laboratories Pvt. Ltd., Mumbai) in water with the aid of heat and standardized. The solution was diluted with distilled water appropriately to get 10 μgmL^{-1} of ChL-T for use in the spectrophotometric method.

A stock solution of Safranin-O (2.85×10^{-3} M) was prepared by dissolving the dye (s. d. Fine Chem. Ltd., Mumbai) in water and filtered using glass wool. The dye solution was diluted to 10 $\mu g\ mL^{-1}$.

Hydrochloric acid (1M): Concentrated hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 1M Hydrochloric Acid.

The pharmaceutical grade drugs were supplied by Dr. Reddy's pharmaceuticals and hetero drugs Pvt. Ltd., Hyderabad. A stock standard solution of drugs was prepared by dissolving accurately weighed 10 mg of pure drug in water and diluting to 100 mL in a calibrated flask with water. The solution was diluted stepwise to get working concentrations.

Assay Procedure: Aliquots containing 2.5-84 μgmL^{-1} of the drug were transferred into a series of 10 mL standard flasks using a micro burette. To this, 1 mL of ChL-T was followed by 1 mL of 1M HCl and contents were shaken well. After 30 min, 1 mL of Safranin-O dye added to the content. Then contents were shaken well and diluted up to the mark. The absorbance of each solution was measured at 524 nm against the corresponding reagent blank.

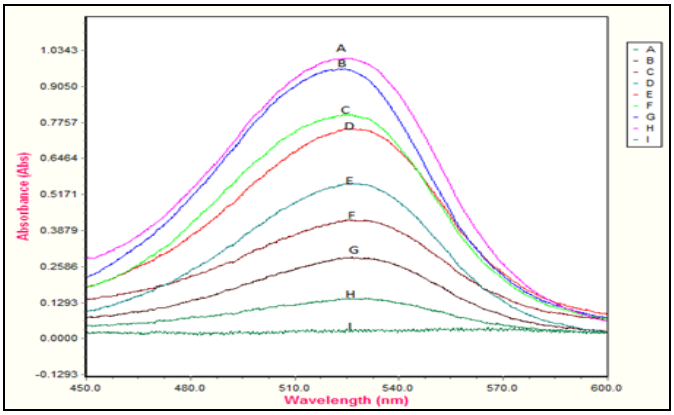
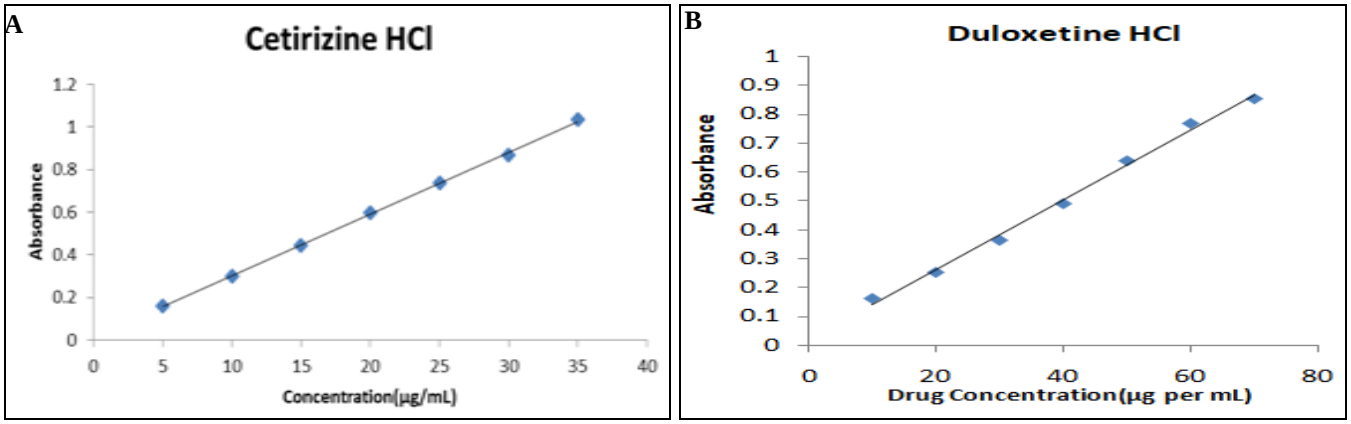


FIG. 2: OVERLAIN ABSORPTION SPECTRA OF A) SAFRANIN-O DYE (A). B) NEUTRALIZATION OF CHLORAMINE-T WITH SAFRANIN-O (I). C) GEMIFLOXACIN AND CHLORAMINE-T WITH SAFRANIN-O (B-H).

Calibration curves were constructed for all the drugs by plotting the absorbance versus the concentration of drugs. The absorbance data were collected for six replicate experiments, and absorbance to concentration ratio called the relative response was determined. The relative responses between 95-105% of average only being considered for construction of the Calibration curves **Fig. 3A-3E**.



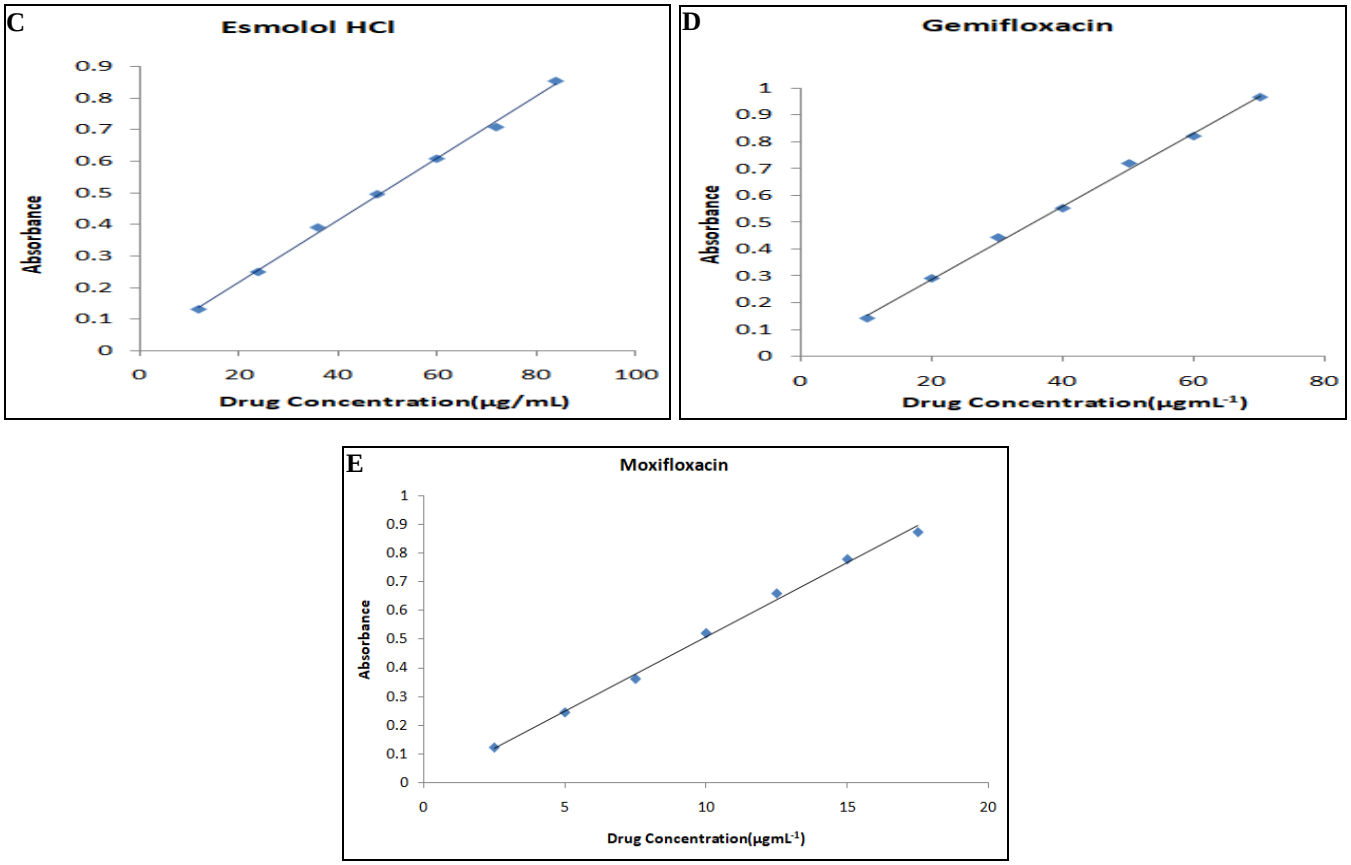


FIG. 3: CALIBRATION CURVES OF DRUGS

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 was chosen and recovery are tabulated in **Table 2**. For this purpose, the standard deviation method is also adapted. Excellent recovery and % RSD being less than 2 speaks about the precision and accuracy of the method **Table 2**.

TABLE 1: DRUGS CONCENTRATION RANGES USED FOR OXIDATION WITH ChL–T AND SAFRANIN O DYE

Name of the Drugs	Concentration (working solution) (µg/mL)	Range of working concentration (µg/mL)
Cetirizine Di HCl	2.5	2.5-17.5
Duloxetine HCl	5	5-35
Esmolol HCl	12	12-84
Gemifloxacin	10	10-70
Moxifloxacin	2.5	2.5-17.5

TABLE 2: DETERMINATION OF ACCURACY AND PRECISION OF THE METHODS ON PURE DRUG SAMPLES

Drug sample	Drug took (µg/mL)	Drug found (µg/mL)	Percentage of error	Percentage of drug recovery	Regression SD of drug	Mean ± SD of the proposed method
	4	3.98	0.5	99.5		
CET	8	7.96	0.5	99.5	0.301	99.75 ± 0.30
	12	12.01	0.08	100.08		
	6	5.96	0.66	99.33		
DUL	12	11.9	0.41	99.58	0.307	99.69 ± 0.306
	18	17.97	0.16	99.83		
	15	14.96	0.27	99.77		
ESM	30	29.98	0.07	99.93	0.133	99.91 ± 0.132
	45	45.02	0.04	100.04		
	12	11.9	0.17	99.83		
GEM	24	24.01	0.04	100.04	0.09	99.93 ± 0.090
	36	35.96	0.11	99.89		
	3	2.98	0.67	99.33		
MOX	6	6.01	0.17	100.07	0.343	99.78 ± 0.342
	9	8.98	0.23	99.77		

Procedure for Tablets:

Cetirizine Hydrochloride: Three tablets of GLOCET each containing 10 mg were collected and crushed into powder. 15 mg equivalent of Cetirizine Hydrochloride was weighed from tablet powder and transferred into 150 mL volumetric standard flask, completely dissolved in bidistilled water by sonication technique for 30 min and filtered with Eisco qualitative filter paper. After that, the solution converted to working concentration on dilution with bidistilled water for oxidative indirect spectrophotometric determination of Cetirizine Hydrochloride drug with Chloramine-T and Safranin-O dye couple.

Duloxetine Hydrochloride: Two tablets of DUMOOD each containing 20 mg were collected and crushed into powder. 20 mg equivalent of Duloxetine Hydrochloride was weighed from tablet powder and transferred into 200 mL volumetric standard flask, completely dissolved in bidistilled water by sonication technique for 35 min and filtered with Eisco qualitative filter paper. After that, the Solution converted to working concentration on dilution with bidistilled water for oxidative indirect spectrophotometric determination of Duloxetine Hydrochloride drug with Chloramine-T and Safranin-O dye couple.

Esmolol Hydrochloride: One tablet of NEOTACH - 100 mg were collected and crushed into powder. 20 mg equivalent of Esmolol Hydrochloride was weighed from tablet powder and transferred into 200 mL volumetric standard flask, completely dissolved in bidistilled water by sonication technique for 30 min and filtered with Eisco qualitative filter paper. After that, the Solution converted to working concentration on dilution with bidistilled water for oxidative indirect spectrophotometric determination of Esmolol Hydrochloride drug with Chloramine-T and Safranin-O dye couple.

Gemifloxacin: One tablet of GEMITAB each containing 320 mg were collected and crushed into powder. 20 mg equivalent of Gemifloxacin was weighed from tablet powder and transferred into 200 mL volumetric standard flask, completely dissolved in bidistilled water by sonication technique for 30 min and filtered with Eisco qualitative filter paper. After that, the Solution converted to working concentration on dilution with bidistilled water for oxidative indirect spectrophotometric determination of Gemifloxacin drug with Chloramine-T and Safranin-O dye couple.

Moxifloxacin: One tablet of TALEMOX -400 mg were collected and crushed into powder. 20 mg equivalent of Moxifloxacin was weighed from tablet powder and transferred into 200 mL volumetric standard flask, completely dissolved in bidistilled water by sonication technique for 30 min and filtered with Eisco qualitative filter paper. After that, the Solution converted to working concentration on dilution with bidistilled water for oxidative indirect spectrophotometric determination of Moxifloxacin drug with Chloramine-T and Safranin-O dye couple.

RESULTS AND DISCUSSION: Each method developed for quantification of drugs has been validated regarding precision, accuracy, Limit of detection, Limit of quantification, Linearity, Selectivity, and Ruggedness. The Beer's law limits, Slope, Intercept, Correlation coefficient, Sandell's sensitivity and Regression equations for each drug are tabulated in **Table 3**. To assess the precision, each experiment was repeated at least 6 times, and accuracy is estimated regarding percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates accuracy and precision of the methods.

Factors Affecting Absorbance:

Effect of Acid Concentration: To study the effect of acid concentration, 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 ChL-T as the oxidant. The reactions were performed in a series of 10 mL volumetric flasks containing 8.0 μgmL^{-1} of the cited drugs, different volumes (0.5–2.5 mL) of 1M HCl and 1 mL of ChL-T. 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 mL of Safranin-O 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 1M HCl. Above this volume, the absorbance decreased. Therefore, a volume of 1 mL of 1M HCl was used for all measurements.

TABLE 3: ANALYTICAL AND REGRESSION PARAMETERS DERIVATION FOR DRUGS: ChL-T WITH SAFRANIN-O DYE- OXIDATIVE SPECTROPHOTOMETRIC METHOD

Analytical Parameters	CET	DUL	ESM	GEM	MOX
SO dye λ_{max} (nm)	524	524	524	524	524
Beer's law Concentrations range (µg mL^{-1})	2.5-17.5	5-35	12-84	10-70	2.5-17.5
Solution Molar absorptivity, ($Lmole^{-1}cm^{-1}$)	2×10^{4}	9.7×10^{3}	3.7×10^{3}	5.5×10^{3}	2.1×10^{4}
SS (µg /cm^{2})	2×10^{-2}	3.6×10^{-2}	1.12×10^{-1}	7.7×10^{-2}	2×10^{-1}
LOD (µg per mL)	0.816	0.785	1.34	1.61	0.276
Limit of quantification (µg mL^{-1})	2.47	2.378	4.05	4.89	0.837
Intercept of curve(b)	8×10^{-3}	1.3×10^{-2}	2×10^{-2}	2×10^{-2}	8×10^{-3}
Slope of curve(a)	5.1×10^{-2}	2.8×10^{-2}	9×10^{-3}	1.3×10^{-2}	5.1×10^{-2}
Correlation coefficient, (R^2)	9.91×10^{-1}	9.99×10^{-1}	0.997	0.995	0.996
Regression equation of curve	Y= 0.051X-8×10^{-3}	Y= 0.028X+ 1.3 $\times10^{-3}$	Y= 0.009X+ 2×10^{-2}	Y= 0.013X-1.7×10^{-2}	Y= 0.051X-8×10^{-3}
SD (intercepts) (σ)	1.26×10^{-2}	6×10^{-3}	3.6×10^{-3}	9.97×10^{-1}	4.2×10^{-3}

Effect of Heating Time: 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 5-10 min the time required to complete the reaction and maximum absorbance was obtained after 5.0 min of heating.

After the oxidation process, the solution must be cooled at least for 3.0 min before addition of Safranin-O dye.

Effect of Oxidant Concentration: When a study on the effect of ChL-T on color development was performed, it was observed that in both cases the absorbance increased with increase in the volume of ChL-T. It reached maximum when 1 mL of 10 µg mL^{-1} ChL-T 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 10 µg mL^{-1} ChL-T was used for all measurements.

Effect of Dye Concentration: To ascertain the linear relationship between the volume of added ChL-T and the decrease in absorbance of Safranin-O dye, experiments were performed using 1 mL of 1M HCl with varying volumes of ChL-T.

The decrease in absorbance was found to be linear up to the 1 mL of ChL-T with optimum volume 1.0 mL of Safranin-O dye for fixed concentration drug solution. The color was found to be stable up to 24 h.

Application to Formulations: The proposed methods were 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 recipients in the dosage forms do not interfere.

TABLE 4: RESULTS OF ASSAY OF TABLETS BY THE PROPOSED METHODS AND STATISTICAL EVALUATION AND RECOVERY EXPERIMENTS BY STANDARD ADDITION METHOD

Name of the Tablet sample	Drug taken in tablet (µg/mL)	Drug found in tablet (µg/mL)	% Error	% Recovery	Regression SD of drug	Mean± SD (Reference method)	Mean ± SD (Proposed method)	t-test	F-test
CET	6	5.98	0.33	99.67		99.612 ±	99.715	0.588	0.498
(GLOCET-	10	10.02	0.2	100.2	0.317	0.637	± 0.441		
10mg)	16	15.92	0.5	99.5					
DUL	12	12.01	0.08	100.08		100.8 ±	99.880	1.71	0.0759
(DUMOOD	15	14.96	0.27	99.73	0.219	0.249	± 0.154		
-20mg)	19	18.94	0.31	99.69					
ESM	14	13.98	0.14	99.86		99.23 ±	99.96	1.79	0.059
(NEOTACH	20	20.01	0.05	100.05	0.082	0.015	± 0.082		
-100mg)	32	32	0	100					
GEM	13	12.98	0.15	99.85		99.47 ±	99.926	1.97	0.032
(Gemi tab-	16	15.98	0.13	99.87	0.092	0.147	± 0.092		
320mg)	18	18.01	0.05	100.05					
MOX	4	4.01	0.25	100.25		99.45 ±	99.85	1.67	0.082
(TALEMO	8	7.98	0.25	99.75	0.266	0.274	± 0.266		
X -400 mg)	12	11.96	0.33	99.67					

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 concerning accuracy and precision **Table 4**.

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, the 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 was within the permissible limits showing the absence of interference by the inactive ingredients in the assay.

CONCLUSION: This is a simple, rapid, and cost-effective methods for the determination of drugs have been developed and validated. The proposed method is more sensitive, and the methods depend on the use of simple and cost-effective chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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CONFLICT OF INTEREST: Nil

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