

## Spectrophotometric Determination of Drugs by Using Cerium (IV) and Indigocaramine Couple as Analytical Reagent

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**Abstract:** Simple, sensitive, accurate and precise spectrophotometric methods for quantitative determination of five drugs viz., Alfuzocin (ALF), Atrovastatin (ATV), Citalopram (CIT), Cetrizine (CET) and Duloxetine (DUL) were developed. The method for each drug depends upon oxidation of drugs by Ce (IV) (Excess) and estimating the amount of unreacted Ce (IV) by Rhodamine-B dye at 520 nm. The calibration curves obeyed Beer's law over the concentration range of 12-96  $\mu\text{g mL}^{-1}$  (ALF), 14-120  $\mu\text{g mL}^{-1}$  (ATV), 15-135  $\mu\text{g mL}^{-1}$  (CIT), 16-144  $\mu\text{g mL}^{-1}$  (CET), & 10-70  $\mu\text{g mL}^{-1}$  (DUL). The methods have been validated in terms of guidelines of ICH and have been applied to the analysis of pharmaceuticals.

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

### I. Introduction

**Alfuzocin** (ALF) (Fig.1a) is chemically known as N-[3-[(4-amino-6, 7-dimethoxy-quinazolin-2-yl)- methyl-amino]propyl] tetrahydrofuran- 2-carboxamide. It is a  $\alpha_1$  receptor antagonist used to treat benign prostatic hyperplasia (BPH). It works by relaxing the muscles in the prostate and bladder neck, making it easier to urinate.[1]. Analytical methods available for the determination of ALF include spectrophotometry [2-6], HPLC [7-8], LC [9], Conductometry [10], Colorimetry [11].

**Atrovastatin** (ATV) (Fig.1b) Atrovastatin (ATV) is (3R,5R)-7-[2-(4-fluorophenyl)-3- phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid [CAS no: 134523-03- 8] (Figure 1). It is a HMG-CoA (3-hydroxy-3-methyl- glutaryl-coenzymeA) reductase inhibitor [12]. This enzyme is involved in cholesterol biosynthesis by catalyzing the conversion reaction of HMG-CoA to mevalonate. The function of lowering the amount of cholesterol results in clearing the LDP (low-density lipoprotein) cholesterol in the blood by increased LDL receptors.

Various methods cited in literature for its determinations involve, spectrophotometry [13-18], high performance liquid chromatography [19-23], liquid chromatography [24], RP-LC [25] and spectrofluorimetry [26]. However, most of these methods involve time-consuming procedures, derivatization and/ or sophisticated instruments. Due to the fact that ATV 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 ATV in biological and pharmaceutical samples

**Citalopram** (CIT) (Fig.1c) Citalopram (CIT) (1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-,3-dihydro-5-isobenzofurancar-bonitrile, Figure 1) is a second generation antidepressant drug whose pharmacological activity is based on the selective serotonin reuptake inhibition. Its efficacy is comparable to tri cyclic antidepressants but it is better tolerated and is characterized by a lower risk of causing adverse effects [27]. Citalopram is effective and safe for the treatment of depressive symptoms even in children and adolescents.

Literature review reveals that a few methods have been published for analysis of CIT in the bulk form and in pharmaceutical preparations. Methods available include liquid chromatography [28-29], Spectrofluorimetry [30], Spectrophotometry [31- 33], Chemiluminiscence method [34], Ion selective electrode method [35] and Voltammetry [36].

**Cetizine** (CET) (Fig.1d) Cetirizine dihydrochloride (CET) is an orally active and selective H<sub>1</sub>-receptor antagonist. Its chemical name is ( $\pm$ )- [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid, dihydrochloride. It is a non-sedating type histamine H<sub>1</sub>-receptor antagonist used in symptomatic treatment of seasonal rhinitis, conjunctivitis, perennial allergic rhinitis, and pruritus and urticaria of allergic origin [38].

Various analytical techniques have been employed for the determination of CET in pharmaceutical preparations such as Capillary zone electrophoretic method [39], high performance liquid chromatography (HPLC) [40-43], liquid chromatography-mass spectrometry (LC/MS) [44,45], RP-HPLC [46,47], RP-LC [48] and UV spectrophotometry [49].

**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. [50]. several techniques have been reported in the literature for the determination of DUL in pharmaceuticals and in biological samples include HPLC [51-58], UPLC [59], Spectrofluorimetry[60], Vis-Spectrophotometry[61] and UV-Spectrophotometry [62] in pharmaceuticals and in biological samples.

#### **About The Method**

Cerium (IV) is a good oxidizing agent like  $\text{KMnO}_4$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$  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 unreacted cerium is estimated by suitable dyes, viz., Rhodamine-B, Methyl Orange, Safranin-O and Xylene cyanol. We report Indigo Carmine dye is suitable for estimation of unreacted Ce (IV) absorbance at 520 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 ( $\text{CeSO}_4 \cdot 2\text{H}_2\text{O}$ , 99.9 % pure) was prepared by dissolving 750 mg of chemical (Merck, Mumbai, India) in 2 N  $\text{H}_2\text{SO}_4$  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  $\text{H}_2\text{SO}_4$  to get working concentrations of  $4.0 \times 10^{-3}$  M (0.25%).

#### **Indigocaramine Dye**

A  $1000 \mu\text{g mL}^{-1}$  stock standard solution was first prepared by dissolving accurately weighed 112 mg of dye (Sigma-aldrich, 90% dye content) in water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 5.0-fold to get the working concentration of  $200 \mu\text{g mL}^{-1}$

#### **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 ( $200 \mu\text{g mL}^{-1}$ ) 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  $\mu\text{g mL}^{-1}$  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  $\text{H}_2\text{SO}_4$  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 520 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  $12-96 \mu\text{g mL}^{-1}$  (ALF),  $14-120 \mu\text{g mL}^{-1}$  (ATV),  $15-135 \mu\text{g mL}^{-1}$  (CIT),  $16-144 \mu\text{g mL}^{-1}$  (CET), &  $10-70 \mu\text{g mL}^{-1}$  (DUL) have been taken. (Table 1) To each of the solution 1 ml of  $250 \mu\text{g mL}^{-1}$  of cerium, 1 mL of 2 N of  $\text{H}_2\text{SO}_4$  were added and the unreacted cerium is analyzed as described above using Rhodamine-B dye.

## **Procedure for Analysis Of Pharmaceuticals**

### **Alfuzocin**

Ten tablets of Alfuzocin were weighed accurately and powdered. The powder equivalent to 50 mg was transferred into a 100 ml volumetric flask, containing a mixture of distilled water (10.0 ml) and HCl (2.0 ml). The flask was shaken for 5 minutes and the solution was filtered using Whatmann No.41 filter paper and further diluted with water to obtain working standard solution.

### **Atrovastatin**

Five tablets (X-Pil, 20 mg) were weighed and grounded. The powder equivalent to 10mg Atrovastatin was stirred well with methanol, sonicated about 30 minutes. The solution was filtered through Whatmann filter paper in a 100mL volumetric standard flask, 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.

### **Citalopram**

Ten tablets (Celexa, 20mg) were grounded and the powder equivalent to 10 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..

### **Cetirizine**

Ten tablets (Zyrtec, 10mg) were weighed powdered 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 a 100 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 ( $\text{H}_2\text{SO}_4$ ,  $\text{H}_3\text{PO}_4$  and  $\text{CH}_3\text{COOH}$ ) 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  $8.0 \mu\text{g mL}^{-1}$  of the cited drugs, different volumes (0.5–2.5 mL) of 2.0 N  $\text{H}_2\text{SO}_4$  and 1 mL of Ce(IV) ( $4.0 \times 10^{-3}\text{M}$ ) were added. After 5.0 min of heating time at  $60 \pm 2^\circ\text{C}$  in a water bath, the solution was cooled for about 3.0 min, 1.0 mL of Indigo caramine 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  $\text{H}_2\text{SO}_4$ . Above this volume, the absorbance decreased therefore, a volume of 1 mL of 2 N  $\text{H}_2\text{SO}_4$  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  $60 \pm 2^\circ\text{C}$  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  $200 \mu\text{g mL}^{-1}$  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  $200 \mu\text{g mL}^{-1}$  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 Indigo caramine dye, experiments were performed using 1 mL of 2 N  $\text{H}_2\text{SO}_4$  with varying volumes of Ce (IV). The decrease in absorbance was found to be linear up to the 1 mL of  $200 \mu\text{g mL}^{-1}$  Ce (IV) with optimum volume 1.0 ml of Indigo caramine 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(CAS) 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  $\lambda_{\text{max}}$  with increasing concentration of each drug. One ml of 2N acid was used in the reaction, as this concentration was found ideal.

$\text{D} + \text{Ce (IV)excess} \rightarrow \text{D oxidation product} + \text{Ce (III)} + \text{Ce (IV)unreacted} : (1)$

$\text{Ce (IV) unreacted} + \text{Indigocaramine} \rightarrow \text{oxidation product of rhodamine} + \text{unreacted Indigocaramine} : (2)$

Measured spectrophotometrically at  $\lambda_{\text{max}} = 520 \text{ nm}$

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  $\lambda_{\text{max}}$  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 spectrophotometric 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

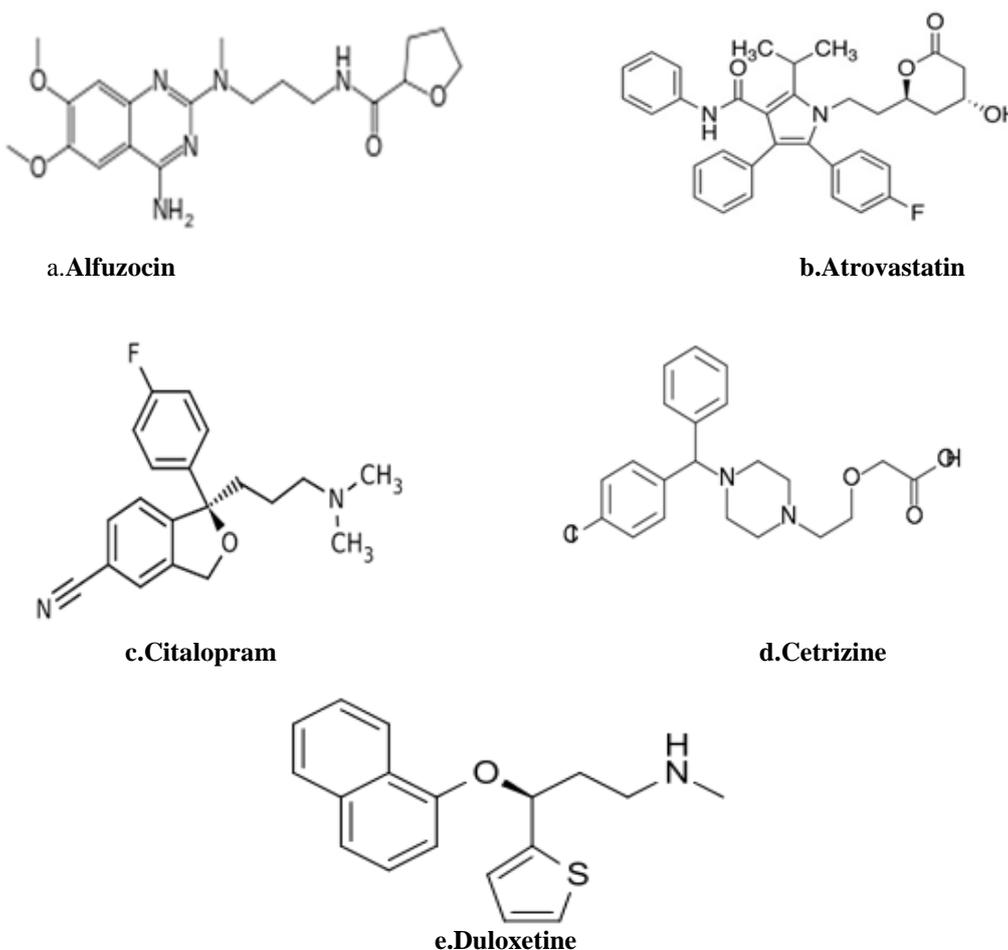
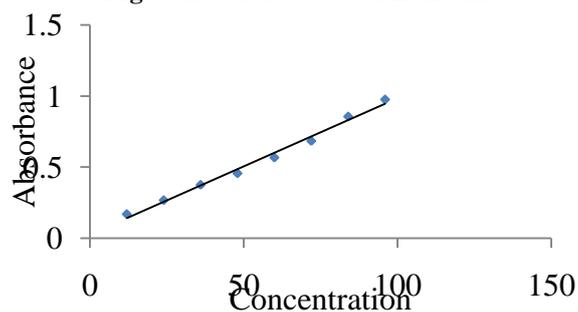
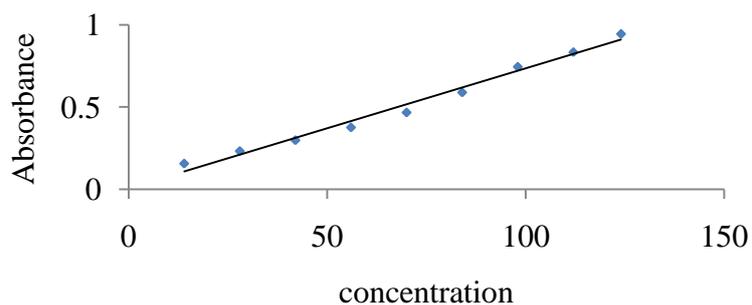


FIG.2 Calibration Curves

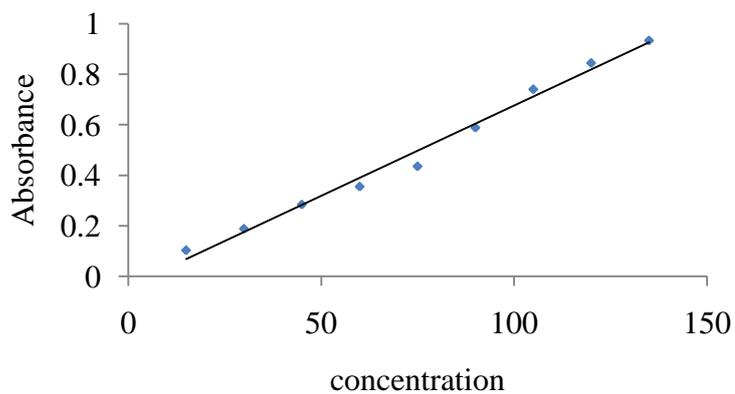
**Fig.** Calibration curve of Alfuzocin



**Fig.** Calibration curve of Atrovastatin



**Fig.** Calibration curve of cetirizine



**Fig.** Calibration curve of citalopram

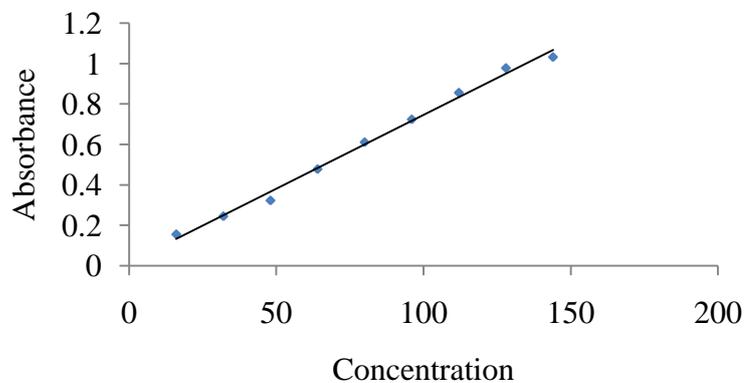


Fig. Calibration curve of duloxetine

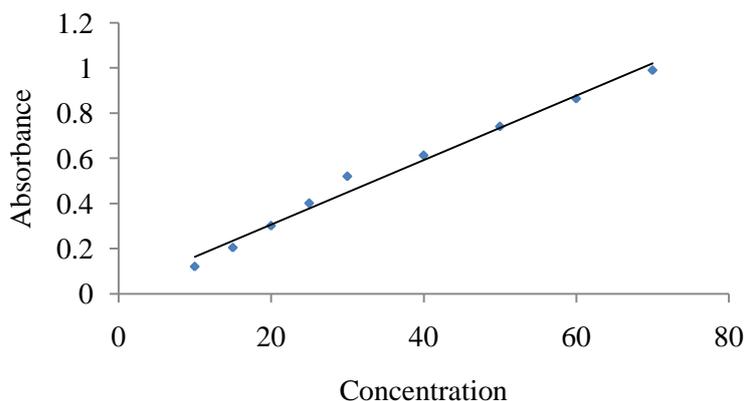


Table 1: Analytical Parameters For Determination Of Drugs By Oxidation With Cerium (Iv) And Indigo Caramine.

Property Name of the Drug	ALF	ATV	CIT	CET	DUL
$\lambda$ max (nm)	520	520	520	520	520
Beer's law limits( $\mu\text{g mL}^{-1}$ )	12-96	14-124	15-135	16-144	10-70
Sandell Sensitivity( $\mu\text{g cm}^{-2}$ )	0.111	0.142	0.142	0.142	0.0714
Slope (a)	0.009	0.007	0.007	0.007	0.014
Intercept (b)	0.027	0.07	-0.038	0.015	0.045
Correlation coefficient (r)	0.990	0.984	0.988	0.993	0.986
Standard deviation of intercept ( $S_b$ )	0.0075	0.0073	0.0079	0.0085	0.0099
Limit of detection ( $\mu\text{g mL}^{-1}$ )	2.768	3.474	3.741	4.01	2.30
Limit of quantification ( $\mu\text{g mL}^{-1}$ )	8.388	10.53	11.33	12.14	6.97
Regression equation $Y=b+ax$	0.027 +0.009X	0.07 +0.007X	-0.038+ 0.007X	0.015+ 0.007X	0.045+ 0.014X

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

| Name of the Drug |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Alf              | Alf              | Alf              | Alf              | Alf              | Alf              |
| Atr              | Atr              | Atr              | Atr              | Atr              | Atr              |
| Cit              | Cit              | Cit              | Cit              | Cit              | Cit              |
| Cet              | Cet              | Cet              | Cet              | Cet              | Cet              |
| Dul              | Dul              | Dul              | Dul              | Dul              | Dul              |
| Name of the Drug |
Alf	Alf	Alf	Alf	Alf	Alf
Atr	Atr	Atr	Atr	Atr	Atr
Cit	Cit	Cit	Cit	Cit	Cit
Cet	Cet	Cet	Cet	Cet	Cet

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

Name of the Drug	Amount Taken ( $\mu\text{g ml}^{-1}$ )	Amount Found ( $\mu\text{g ml}^{-1}$ )	% Recovery	RSD %	Proposed method Mean $\pm$ SD	Ref method Mean $\pm$ SD	t-test (*)	F-test (**)
Alf	15	15.09	100.617	0.255	100.425 $\pm$ 0.255	[193] 99.98 $\pm$ 0.152	0.7254 (2.45)	2.855 (4.28)
	30	30.2	100.673					
	45	44.69	100.231					
	60	60.11	100.176					
Atr	20	19.9	99.52	0.604	100.08 $\pm$ 0.605	[164] 101.5 $\pm$ 0.41	0.604 (2.57)	2.175 (4.95)
	40	40.23	100.57					
	60	59.76	99.60					
	80	80.51	100.63					
Cit	25	25.18	100.73	0.509	100.329 $\pm$ 0.511	[136] 98.73 $\pm$ 0.37	0.516 (0.745)	1.912 (0.711)
	50	50.16	100.32					
	75	75.62	100.82					
	100	99.7	99.70					
Cet	30	29.47	99.11	0.658	99.932 $\pm$ 0.657	[214]		
	60	60.28	100.46					
	90	89.72	99.69					
	120	120.6	100.47					
Dul	15	14.88	99.22	0.7024	99.93 $\pm$ 0.702			
	30	30.19	100.64					
	50	49.73	99.46					
	65	65.27	100.42					

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