

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 α_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 H₁-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 H₁-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 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 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%).

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 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 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 H_2SO_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 (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 $8.0 \mu\text{g mL}^{-1}$ of the cited drugs, different volumes (0.5–2.5 mL) of 2.0 N H_2SO_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 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 $60 \pm 2^\circ\text{C}$ 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 H_2SO_4 with varying volumes of Ce (IV). The decrease in absorbance was found to be linear up to the 1 mL of $200 \mu\text{g mL}^{-1}$ 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 λ_{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 λ_{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

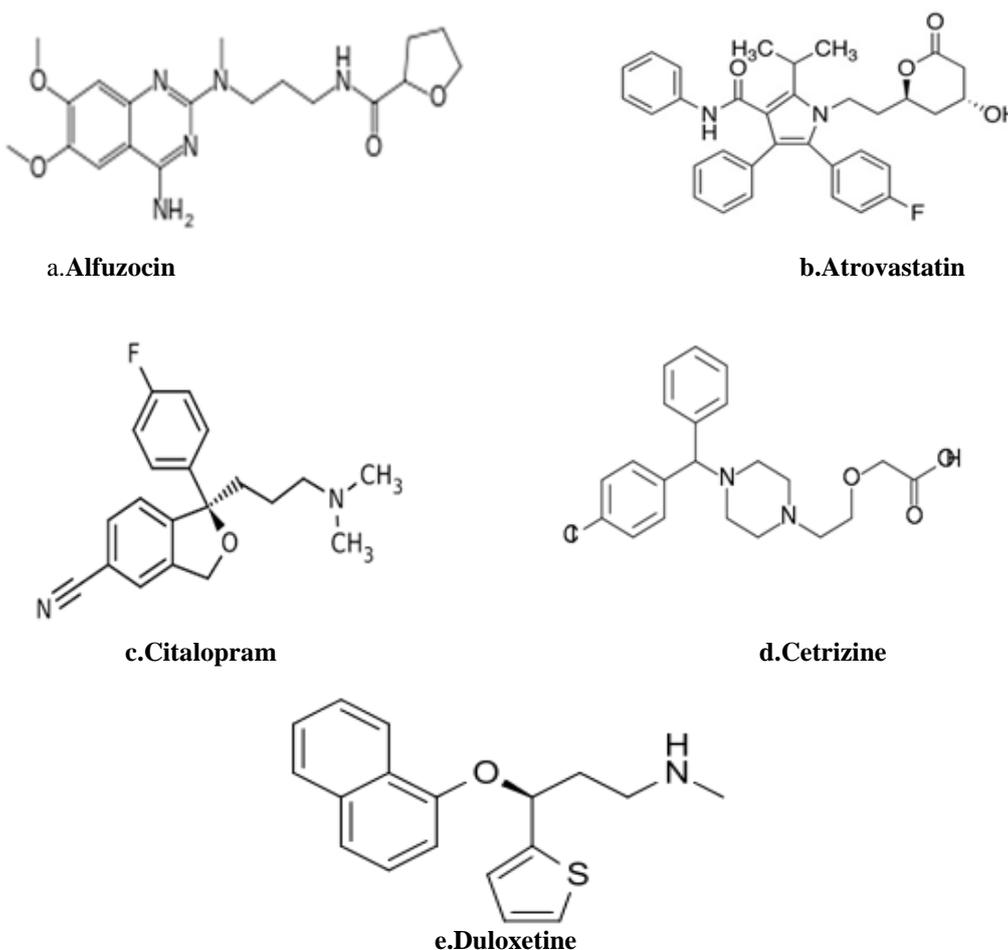


FIG.2 Calibration Curves

Fig. Calibration curve of Alfuzocin

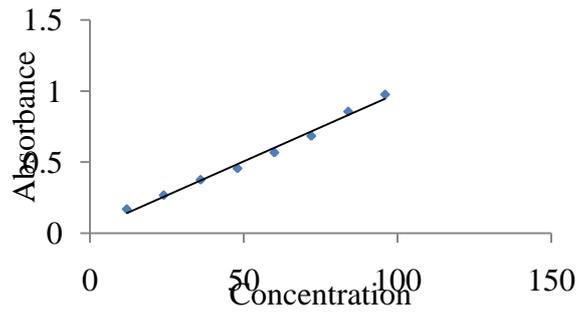


Fig. Calibration curve of Atrovastatin

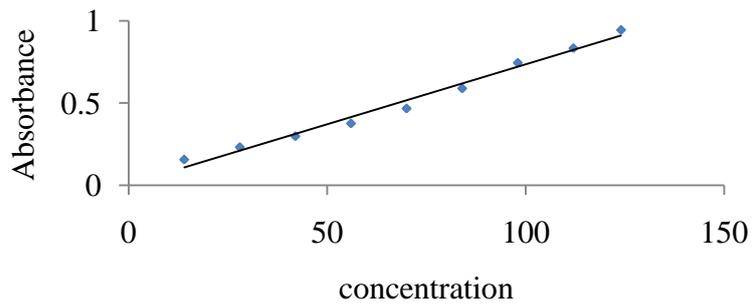


Fig. Calibration curve of cetirizine

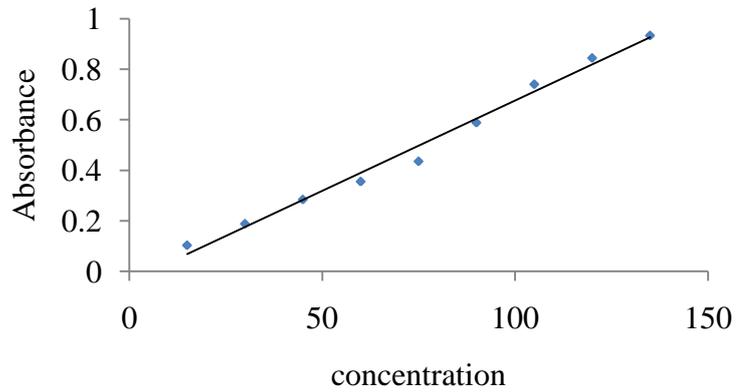


Fig. Calibration curve of citalopram

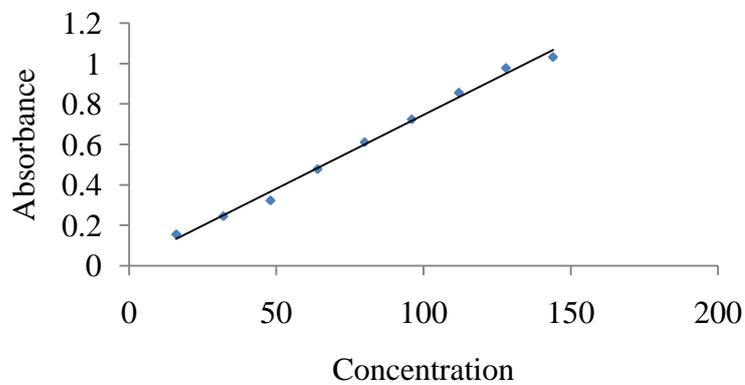


Fig. Calibration curve of duloxetine

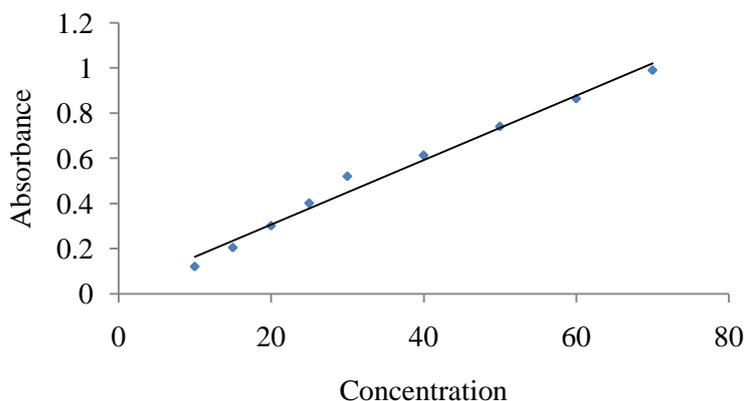


Table 1: Analytical Parameters For Determination Of Drugs By Oxidation With Cerium (Iv) And Indigo Caramine.

Property Name of the Drug	ALF	ATV	CIT	CET	DUL
λ 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					

References

- [1]. Radha, G. V; Santosh Naidu, M. Design of transdermal films of alfuzocin HCl by using a natural polymer tamarind seed polysaccharide extract. *Scholars Research Library Der Pharmacia Lettre*, 5 (3), 2013, 457-464.
- [2]. Safwan Ashour, M.; Fawaz Chehna, and Roula Bayram. Spectrophotometric Determination of Alfuzosin HCl in Pharmaceutical Formulations with some Sulphonaphthalein Dyes. *Int J Biomed Sci.*, 2(3), 2006,273–278.
- [3]. D. Ravikumar , S.V.M. Vardhan, D. Ramachandran and C. Rambabu. Development of new spectrophotometric methods for the determination of Alfuzosin hydrochloride in bulk and pharmaceutical formulations, *Oriental Journal of Chemistry*, 24(2), 2008, 725-728.
- [4]. Kanchan Chauhan. First and Second Derivative Spectrophotometric Methods for Determination of Alfuzosin in Pharmaceutical Dosage. *American journal of pharmacy and health research*, 2(7), 2014, 291-98.
- [5]. Mona M. Abdel-Moety, Nagiba Y.M. Hassan, Samar G. Abdel-Hamid, Abdel-Aziz A. Abdel-Aleem. Spectrophotometric and Potentiometric Methods for the Determination of Alfuzosin Hydrochloride and Doxazosin Mesylate in Drug Substances and Drug products. *International Journal of ChemTech Research*, 6(5), 2014, 2875-2886.
- [6]. K. Damodar Reddy, B. Vijay Kumar, K. Sayanna & G.Venkateshwalu. Spectrophotometric determination of drugs based on oxidation by acidic KMnO₄. *World journal of pharmacy and pharmaceutical sciences*, 3(8),2014,812-824.
- [7]. Ashok K. Shakya , Tawfiq A. Arafat, Ahmad Abuawaad, Hamza Al-Hroub, Munther Melhim. Simple and Rapid HPLC Method for the Determination of Alfuzosin in Human Plasma. *Jordan Journal of Pharmaceutical Sciences*, 3(1), 2010.
- [8]. Salah Fayed A, Abdel-Aaty Shehata M, Yehia Hassan N, El-Weshahy S.A. Validated HPLC and HPTLC stability-indicating methods for determination of alfuzosin hydrochloride in bulk powder and pharmaceutical formulations. *J Sep Sci.*, 29(18),2006 ,2716-24.
- [9]. Noel Alex Gomes. Ashutosh Pudage , Santosh S. Joshi. Rapid and Sensitive LC–MS Method for the Simultaneous Estimation of Alfuzosin and Dutasteride in Human Plasma . *Chromatographia* , 69(1),2008,9-18.
- [10]. Safwan Ashour and Mouhammed Khateeb. Conductometric Titration Method for Determination of Alfuzosin Hydrochloride and Fexofenadine Hydrochloride Using Sodium Tetraphenylborate. *Canadian Chemical Transactions*, 1(4),2013, 292-304.
- [11]. B. Mohammed Ishaq, Dr. K. Vanitha Prakash, C. Hari Kumar, G. Usha Rani,P. Ramakrishna . Colorimetric Determination of Alfuzosin HCl in Pharmaceutical Formulations. *Journal of Pharmacy Research*, 4(1),2011,226-228.
- [12]. A.Rajasekaran, R.Sasikumar, J.Dharuman. Simultaneous RP-HPLC method for the stress degradation studies of atorvastatin calcium and ezetimibe in multicomponent dosage form. *Ars Pharm.* 52(3), 2011, 12-18.

- [13]. R Savithri, N Sai Sree Bindu, P Shiva Bhargavi, D H H Theja and P Ramalingam. Dual Wavelength UV - Spectrophotometric Method for Simultaneous Estimation of Atorvastatin and Ezetimibe in bulk and their combined Tablet Dosage Form. *Der Pharmacia Sinica*, 2(5),2011, 251-258
- [14]. Balaha R. G., Patel Vandana. B.and Mayank Bapna. Simultaneous Spectrophotometric Determination of Atorvastatin Calcium and Ezetimibe in Tablet Dosage Form. *Int.J. PharmTech Res.* 1(2),2009,233-236.
- [15]. S. Dey, S. Sarkar, J. Malakar, A. Ghosh, A. Gangopadhyay, B. Mazumder. Spectrophotometric method for simultaneous determination of atenolol and atorvastatin in tablet dosage forms. *Int JPharm Biomed Res.* 3(1), 2012, 40-43.
- [16]. R Savithri, N Sai Sree Bindu, P Shiva Bhargavi, D H HTheja and P Ramalingam.Dual Wavelength UV - Spectrophotometric Method for Simultaneous Estimation of Atorvastatin and Ezetimibe in bulk and their combined Tablet Dosage Form. *Der Pharmacia Sinica*, 2 (5),2011, 251-258.
- [17]. P. Nagaraju, Pasumarthy, NV Gopal, VDN Srinivas, and SVN Padma. Spectrophotometric Methods for the Determination of Atorvastatin Calcium in Pure and It's Pharmaceutical Dosage Forms. *Asian J. Research Chem*, 1(2),2008,64-66.
- [18]. George M.Mekhaail, Amany O.Kamel, Gehanne A.S.Awad, Nahed D.Mortada. Anticancer effect of atorvastatin nanostructured polymeric micelles based on stearyl-grafted chitosan. *InternationalJournal of Biological Macromolecules.* 51,2012,351-363.
- [19]. A.Zarghi, A.Shafaati , S.M.Foroutan, A.Khoddam. A simple and rapid HPLC method for the determination of atorvastatin in human plasma with UV detection and its application to pharmacokinetic studies. *Arzneimittelforschung.* 55(8),2005,451-4.
- [20]. Zahid Zaheer, M.N.Farooqui, A.A.Mangle, A.G.Nikalje. Stability-indicating high performance liquid chromatographic determination of atorvastatin calcium in pharmaceutical dosage form, *African Journal of Pharmacy and pharmacology* 2(10),2008,204-210.
- [21]. A.Patel , C.Macwana , V.Parmar , S.Patel. Simultaneous determination of atorvastatin calcium, ezetimibe, and fenofibrate in a tablet formulation by HPLC. *J AOAC Int.* 95(2), 2012,419-23.
- [22]. Sandeep S. Sonawane, Atul A.Shirkhedkar, Ravindra A. Fursule, Sanjay J. Surana. Application of UV-Spectrophotometry and RP-HPLC for Simultaneous Determination of Atorvastatin Calcium and Ezetimibe in Pharmaceutical Dosage Form. *Eurasian J. Anal. Chem.*1(1),2006,31-41.
- [23]. Zahid Zaheer, M.N.Farooqui, A.A.Mangle, A.G.Nikalje. Stability-indicating high performance liquid chromatographic determination of atorvastatin calcium in pharmaceutical dosage form, *African Journal of Pharmacy and pharmacology.*2(10), 2008,204-210.
- [24]. Srinivasa Rao Polaganian, Nageswara Rao Pilli, Ramakrishna Gajula, Venkateswarlu Gandu. Simultaneous determination of atorvastatin, metformin and glimepiride in human plasma by LC-MS/MS and its application to a human pharmacokinetic study. *Journal of Pharmaceutical Analysis.* 3(1), 2013,9-19.
- [25]. G.Bharat Chaudhari, M. Natvarlal Patel, B.Paresh Shah, J.Laxman Patel, P.Vipul Patel. Stability-Indicating Reversed-Phase Liquid Chromatographic Method for Simultaneous Determination of Atorvastatin and Ezetimibe from Their Combination Drug Products, *Journal of AOAC International*, 90(6), 2007, 1539-1546.
- [26]. Mohie M.K. Sharaf El-Dina,Fathy M.M. Salamab,Mohamed W.I Nassarb,Khalid A.M. Attiab,Mohamed M.Y. Kaddaha. Validated spectrofluorimetric method for the determination of atorvastatin in pharmaceutical preparations. *Journal of Pharmaceutical Analysis.*2(3),2012,200-205.
- [27]. L.Kristoffersen, A.Bugge, E.Lundanes, L.Sjrdal. Simultaneous determination of citalopram, fluoxetine, paroxetine and their metabolites in plasma and whole blood by high-performance liquid chromatography with ultraviolet and fluorescence detection. *J Chromatogr B Biomed Sci Appl.* 734(2),1999,229-46.
- [28]. XQ Cao, XY Chen, YF Zhang, DF Zhong. Determination of citalopram in human plasma with LC-MS/MS method and its bioequivalent evaluation. *Yao Xue Xue Bao.* 42(4), 2007,450-4.
- [29]. Tao Jianga, Zhengxing Ronga, Liang Penga, Bing Chen, Yifan Xiea, Congying Chena, Jing Sun, Yiping Xu, Yang Lua, Hongzhuan Chena. Simultaneous determination of citalopram and its metabolite in human plasma by LC-MS/MS applied to pharmacokinetic study. *Journal of Chromatography B.* 878(5-6), 2010, 615-619.
- [30]. SG Vasantharaju, S Lakshmana Prabu, A Jacob. Spectrofluorimetric method for determination of citalopram in bulk and pharmaceutical dosage forms. *Indian Journal of pharmaceutical sciences.* 70(5), 2008 , 647-648.
- [31]. Badiadka Narayana and Kunnummel Veena . Spectrophotometric Determination of Citalopram Hydrobromide in Pharmaceuticals. *J. Mex. Chem. Soc.* 54(2), 2010, 98-102.
- [32]. Raza, TM.Ansari. Spectrophotometric determination of citalopram hydrobromide in tablet dosage form using chloranil. *Pak J Pharm Sci.* 27(2), 2014 , 255-60.
- [33]. Asad Raza and Tariq Mahmood Ansari . Spectrophotometric determination of citalopram hydrobromide in tablet dosage form using chloranil. *Pak. J. Pharm. Sci.,* 27(2),2014,255-260.
- [34]. M.N.Khan, MR.Jan, J.Shah, SH.Lee. A validated silver-nanoparticle-enhanced chemiluminescence method for the determination of citalopram in pharmaceutical preparations and human plasma. *Luminescence.* 29(3), 2014 , 266-74.
- [35]. Tamer Awad Ali, Gehad G. Mohamed, A. M. Al-Sabagh, M. A. Migahed. A New Screen-printed Ion Selective Electrode for Determination of Citalopram Hydrobromide in Pharmaceutical Formulation. *Chin J Anal Chem,* 42(4), 2014, 565-572.
- [36]. Henri P. A. Nouwsa, Cristina Delerue-Matosa and Aquiles A. Barros. Electrochemical Determination of Citalopram by Adsorptive Stripping Voltammetry-Determination in Pharmaceutical Products. *Analytical Letters.* 39(9), 2006, 1907-1915.
- [37]. SEVGI TATAR ULU. Extractive Spectrophotometric Determination of CetirizineDihydrochloride in Pure and Pharmaceutical Preparations. *Journal of Food and Drug Analysis,*18(6), 2010, 440-446.
- [38]. F. Sattary Javid, A. Shafaat, A. Zarghi. Determination of cetirizine and its impurities in bulk and tablet formulation using a validated capillary zone electrophoretic method. *Journal of Analytical Chemistry.* 69(5),2014, 442-447.
- [39]. A.M.Y. Jaber, H.A. A Sherifeb, M.M. A Omarib, A.A. Badwanb. Determination of cetirizine dihydrochloride, related impurities and preservatives in oral solution and tablet dosage forms using HPLC. *Journal of Pharmaceutical and Biomedical Analysis .* 36(2), 2004,341-350.
- [40]. C.K.Kim, K.J. Yeon, E.Ban, M.J.Hyun, J.K.Kim, M.K.Kim, S.E.Jin and J.S.Park. Narrow-bore high performance liquid chromatographic method for the determination of cetirizine in human plasma using column switching. *J. Pharm. Biomed. Anal.*37, 2005, 603-609.
- [41]. Effat Souri, Ali Hatami, Nazanin Shabani Ravari, Farhad Alvandifar, and Maliheh Barazandeh Tehrani. Validating a Stability Indicating HPLC Method for Kinetic Study of Cetirizine Degradation in Acidic and Oxidative Conditions. *Iran J Pharm Res.* 12(2), 2013,287-294.
- [42]. S.N.Makhija and P.R.Vavia. Stability indicating HPTLC method for the simultaneous determination of pseudoephedrine and cetirizine in pharmaceutical formulations. *J. Pharm. Biomed. Anal.* 25,2001,663-667.

- [43]. S.Rudaz, S. Souverain, C.Schelling, M.Deleers, A.Klomp, A. Norris, T. L.Vu, B.Ariano and J. L. Veuthey. Development and validation of a heart-cutting liquid chromatography-mass spectrometry method for the determination of process-related substances in cetirizine tablets. *Anal. Chim. Acta* 492(1-2),2003, 271-282.
- [44]. Q. Song, H.Junga, Y. Tang,A.C. Li, T. Addison, M.McCort-Tipton,B.Beato, and W.Naidong. Automated 96-well solid phase extraction and hydrophilic interaction liquid chromatography–tandem mass spectrometric method for the analysis of cetirizine (ZYRTEC®) in human plasma-with emphasis on method ruggedness. *J. Chromatogr. B* 814, 2005, 105-114.
- [45]. T.Pradeep Kumar ,M. Akiful Haque , K.Prashanth Kumar , G.Nivedita , V.Prakash Diwan. Simultaneous Determination of Cetirizine Hydrochloride and Ambroxol Hydrochloride in Combined Dosage form by Using RPHPLC Method. *Am. J. PharmTech Res.* 2(6). 2012.
- [46]. Paloma Flórez Borges, Pilar Pérez Lozano, Encarna García Montoya, Montserrat Miñarro, Josep R Ticó, Enric Jo and Josep M Suñe Negre. Determination of stress-induced degradation products of cetirizine dihydrochloride by a stability-indicating RP-HPLC method. *DARU Journal of Pharmaceutical Sciences* 22,2014, 82 -5
- [47]. M.F Zaater, Y.R.Tahboub, and N.M.Najib. RP-LC method for the determination of cetirizine in serum. *J. Pharm. Biomed. Anal.* 2000, 22, 739-744.
- [48]. F. M.E Waliily, M. A.Korany, A. E Gindy and M. F. Bedair. Spectrophotometric and high performance liquid chromatographic determination of cetirizine dihydrochloride in pharmaceutical tablets. *J. Pharm. Biomed. Anal.*17, 1998, 435-442.
- [49]. S.Pankaj, T.T.Mariappan, U.C.Banerjee. High performance liquid chromatographic method for the simultaneous estimation of the key intermediates of duloxetine. *Talanta*, 67, 2005, 975-978
- [50]. S.Lakshmana Prabu, S.Shanawaz, A.Karthik, C.Dinesh Kumar, S.G. Vasatharaju. High performance liquid chromatography method for the quantification of duloxetine in rat plasma. *ARS Pharm.*, 49(4), 2008, 283-292.
- [51]. S.Lakshmana Prabu, M.Srinivasan, S.Thiyagarajan, Queeni Marina. Determination of duloxetine hydrochloride in pharmaceutical formulation by HPLC with UV detection. *International Journal of ChemTech Research*, 2(3), 2010, 1441-1444.
- [52]. Sheikh Shahnawaz, Abdul Wadood Siddiqui, Mir Tariq Masroor, and Vandana Arora. Stability-indicating HPTLC method for determination of duloxetine hydrochloride in bulk drug and tablet formulation. *Chromatography Research International*, 2011 (2011), Article ID 404189, 5 pages.
- [53]. Sejal K Patel, NJ Patel, KM Patel, PU Patel, BH Patel. Estimation of duloxetine hydrochloride in pharmaceutical formulations by RP-HPLC method. *Indian J Pharm Sci.* , 70(6), 2008, 825-827.
- [54]. P.B.Battu. Validation and Stability indicating Reverse Phase-High Performance Liquid Chromatography for the Determination of Duloxetine in Tablets. *Int. J. ChemTech Research.*, 1(3), 2009, 602-605.
- [55]. V.R.Sinha, R.Anamika,Kumria, J.R.Bhinge. Stress Degradation Studies on Duloxetine Hydrochloride and Development of an RP-HPLC Method for its Determination in Capsule Formulation. *J Chromatogr. Sci.*, 47(7), 2009, 589-593.
- [56]. P.B.Battu. Validation and Stability indicating Reverse Phase-High Performance Liquid Chromatography for the Determination of Duloxetine in Tablets. *Int. J. ChemTech Research.*, 1(3), 2009, 602-605.
- [57]. V.R.Sinha, R.Anamika,Kumria, J.R.Bhinge. Stress Degradation Studies on Duloxetine Hydrochloride and Development of an RP-HPLC Method for its Determination in Capsule Formulation. *J Chromatogr. Sci.*, 47(7), 2009, 589-593.
- [58]. Navneet Kumar, D. Sangeetha and P. Balakrishna. Development and validation of a UPLC method for the determination of duloxetine hydrochloride residues on pharmaceutical manufacturing equipment surfaces. *Pharm Methods*, 2(3),2011,161–166.
- [59]. SL Prabhu, S Shahnawaz, C Dinesh Kumar, A Shirwaikar. Spectrofluorimetric method for determination of duloxetine hydrochloride in bulk and pharmaceutical dosage forms. *Indian Journal of Pharmaceutical Sciences.* 70(4),2008, 502-503.
- [60]. T. Srinivasa Reddy, U. Viplava Prasad,M. L. N. Acharyulu, B. V. Srinivas, C. S. P. Sastry. Visible spectrophotometric methods for the determination of duloxetine hydrochloride in bulk and dosage forms. *Der Pharma Chemica*, 4(6), 2012, 2427-2433.
- [61]. K Susmitha,G Venkateswarlu.Extractive spectrophotometric methods for determination of duloxetine hydrochloride in pharmaceutical formulations using acidic triphenyl methane dyes. *International Journal of ChemTech Research.* 3(3), 2011, 1246-1254.