



Spectrophotometric determination of Drugs and Pharmaceuticals based on Charge Transfer Complexation with *p*-CA

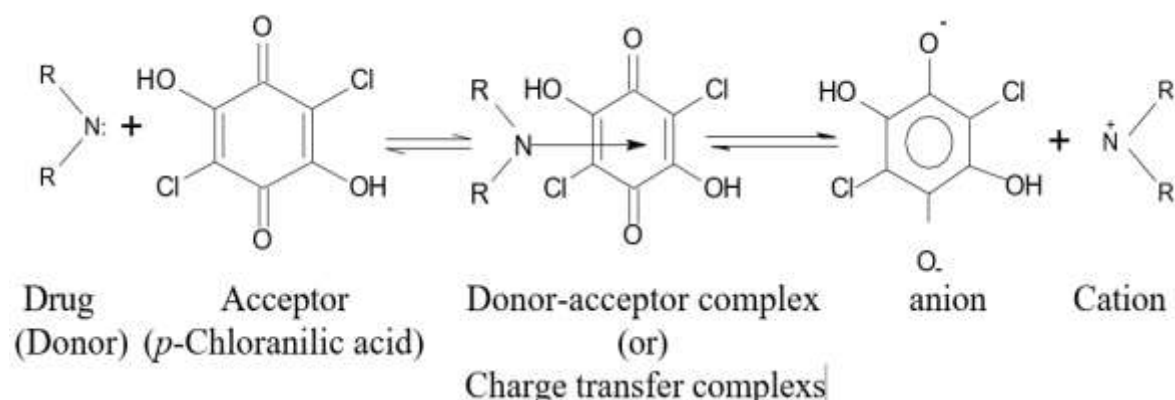
Dr. P.Adavi Raju *¹ Dr. R. Lavanya*²

1. Department of Chemistry, GDC- Malkajgiri, Medchal-500068, Telangana, India.

2. Department of Chemistry, Vivekananda GDC- (A) Vidhyanagar, Hyderabad-500044, Telangana, India.

ABSTRACT

Simple, sensitive, selective and Precise methods are developed for the UV-Visible Spectrophotometric methods have been developed for the estimation of five drugs VIZ., Aripiprazole (ARI), Astemizole (AST), Carvedilol(CRV), Esitalopram oxalate (ESC) and Ketoconazole (KET). The method involves the addition of *p*- chloranilic acid to the drugs possessing a lone pair of electrons results in the formation of a charge transfer complex of the $n-\pi$ type. The complex is formed by the lone pair of electrons donated by an n - donor to *p*- Chloranilic acid, a charge transfer reagent as an electron acceptor, through which a partial ionic bond (D^+A^-) is formed. *p*-CA solution in acetonitrile displays a maximum absorption peak at 450nm. The formation of colored solutions and the appearance of new peak are attributed to the formation of charge transfer ion pair complexes (D^+A^-).The broad peak with spike at 520nm is attributed to the radical anion of *p*-CA. In the present study Area Under Curve (AUC) has been considered for the purpose of quantification of drugs as AUC is more accurate and precise than selecting one wavelength because AUC combines optical densities at the all the wave length.This method has been applied for the estimation of drugs in their pure form as well as in tablet formulation. The results of analysis have been validated statistically for linearity, accuracy, precision, LOD and LOQ.



KEYWORDS: UV-Visible Spectrophotometer, Drugs, *p*- chloranilic acid, acetonitrile, Quantification, Validation.

INTRODUCTION

1. Aripiprazole: Aripiprazole (fig.1) is chemically 7-[4-[4-(2,3-dichloro) phenyl piperazine -1- yl] butoxy]-3,4-dihydro-1Hquinolin-2-one and the molecular formula is $C_{23}H_{27}Cl_2N_3O_2$ [488]. It is the sixth and most recent second-generation anti psychotic agent, used in the treatment of acute, manic and mixed episodes associated with bipolar disorder [489]. Aripiprazole appears to mediate its antipsychotic effect primarily by partial agonism at the D2 receptor that has been shown to modulate dopamine ergic activity in areas where activity may be high or low. It shows partial agonism at the 5-HT1A receptor and antagonist profile at the 5-HT2A receptor [490]. Only a few authors have described the validation methods for Aripiprazole among which UV and HPLC methods have been developed for drug in rat plasma, human plasma and pharmaceutical formulations. As these methods are expensive and not reproducible, we have made an attempt to develop a more precise, simple and economical spectrophotometric method with greater precision, accuracy and sensitivity for the analysis of Aripiprazole in bulk and dosage forms. It is important to emphasize that there are a large number of analytical procedures reported and their references cited almost all earlier methods of analytical of this drug such as UV Spectroscopy [293-303].

2. Astemizole: Astemizole, [Fig-2] chemically 1-[(4-fluorophenyl) methyl]-N-[1-[2-(4-Methoxyphenyl) ethyl]-4-piperidyl] benzoimidazol-2-amine [491] is a long-acting, selective histamine H1 receptor antagonist. It is a second generation anti histamine in that it does not readily cross the blood-brain barrier. Astemizole is used in the treatment of seasonal and perennial allergic rhinitis, allergic conjunctivitis, chronic urticarial and other chronic allergies [492]. Because of its physiological significance; it has been quantified by several methods which are enumerated in the recent reference such as UV Spectroscopy [304-312].

3. Carvedilol: Carvedilol (fig.3) is designated chemically as $(\pm)$ -1-(carbazol-4-yl oxy)-3-[[2-(o-methoxy phenoxy) ethyl] amino] -2-propanol [495]. It is a non-selective β adrenergic antagonist with no intrinsic sympatho mimetic activity and is widely used to treat essential hypertension and angina pectoris [496]. Carvedilol is also indicated for the treatment of mild to severe chronic heart failure, Left ventricular dysfunction following myocardial infarction in clinically stable patients. It also has multiple spectra of activities such as antioxidant property, inhibition of smooth muscle proliferation and calcium antagonistic blocking activity. UV- Spectrophotometry [321-329], HPLC [186-191], UPLC [276], GCMS [177], Fluorimetry [59], Fluorescence [56], NMR [93] appear in the literature for the determination of Carvedilol in bulk and pharmaceutical formulation.

4. Escitalopram oxalate: Escitalopram oxalate (fig.4) (S-(+)-1-[3-(dimethyl amino) propyl]-1-(p-fluorophenyl)-5-phthalan carbonitrile oxalate), the S-enantiomer of racemic citalopram, is a selective serotonin reuptake inhibitor and belongs to a group of medicines known as anti depressants [501]. These medicines help to normalize the levels of serotonin in the brain. Disturbances in the serotonin system of the brain are key factors in the development of depression and related disorders. Several analytical methods have been reported for the determination of escitalopram oxalate in pure form, in pharmaceuticals and in biological fluids. Most of the reported methods are chromatographic methods and no official methods have been reported for the determination of escitalopram oxalate. The reported methods include high performance thin layer chromatography (HPTLC), high-performance liquid chromatography with tandem mass spectroscopy (HPLC/MS/MS) [204-205], high performance liquid chromatography (LC/MS) [130-131]. The literature survey revealed that no spectrophotometric method has been reported for the determination of escitalopram. UV-Spectroscopy [344-348], FT-IR [82-83] and Calorimetry [297].

5. Ketoconazole: Ketoconazole (fig.5) is chemically 1-[4-(4-[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]ethan-1-one], an imidazole derivative, is a broad spectrum antifungal agent used in the treatment of superficial and systemic fungal mycoses, available as oral and topical preparations [502]. The main effect of imidazole is the inhibition of the sterol 14- α -demethylase, an enzymatic system dependent upon cytochrome P450, with a consequent inhibition of fungal development. UV-Spectroscopy [349-357], HPLC [206-207], LC/MS [132], UPLC [281] and Thermo gravimetry [54].

Structure of Drugs:

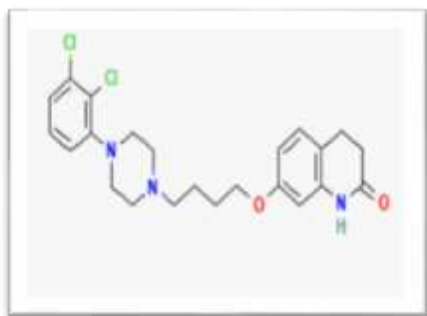


Figure.1 Aripiprazole

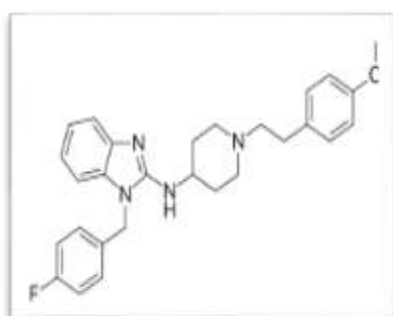


Figure.2 Astemizole

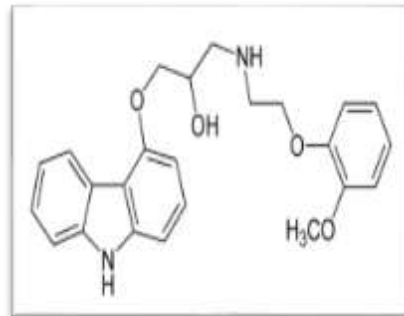


Figure.3 Carvedilol

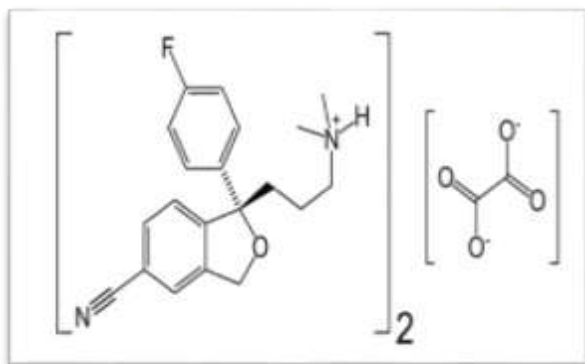


Figure.4. Escitalopram oxalate

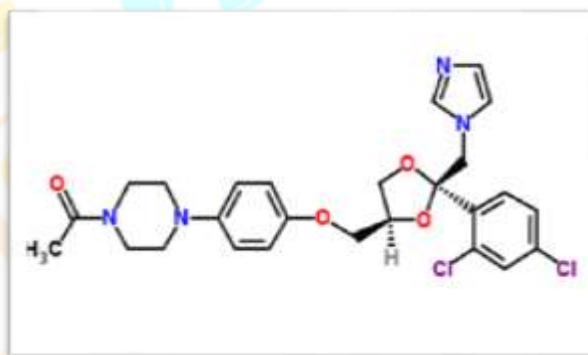


Figure.5. Ketoconazole

MATERIALS AND METHODS

Reagents and standards: The pharmaceutical grade drugs were supplied by Dr.Reddy's laboratory and Arabindo pharmaceutical, Hyderabad. *p*- Chloranilic acid, acetonitrile and Solvents purchased from S.D.fine chem. Pvt.Ltd, Mumbai, India. Whatman filter paper no.42 was used for filtration purpose. All the reagents used were of AR grade and triple distilled water was used throughout the investigation. Tablets were purchased from the Medplus and Apollo medical shops.

Instrumentation and Optical characteristics: All absorbance measurements were recorded on Shimadzu140 double beam spectrophotometer as well as on Elico 159 single beam and Elico SL-210 UV-Visible double spectrophotometers using matched pair of Quartz cells of 10mm path length. A high precision Analytical Dhona 200 balance was used for weighing the reagents.

Preparation of standard stock solution: *p*- chloranilic acid of ($9.569 \times 10^{-3}M$) stock solution was prepared by dissolving of sample in 100ml standard flask with in acetonitrile solvent. Standard stock solution of drugs was prepared by dissolving an accurately weighed 40mg drug to separate 100ml volumetric flasks. The stock solutions of ARI, AST, CRV, ESC and KET were further diluted with the same solvent to obtain working concentrations.

Table .1The range of concentration of drugs used for Charge Transfer Complexation with *p*-CA

Drugs	Working Concentration	Range
Aripiprazole	60 $\mu\text{g mL}^{-1}$	60-540 $\mu\text{g mL}^{-1}$
Astemizole	50 $\mu\text{g mL}^{-1}$	50-450 $\mu\text{g mL}^{-1}$
Carvedilol	40 $\mu\text{g mL}^{-1}$	40-360 $\mu\text{g mL}^{-1}$
Escitalopram oxalate	40 $\mu\text{g mL}^{-1}$	40-360 $\mu\text{g mL}^{-1}$
Ketoconazole	60 $\mu\text{g mL}^{-1}$	60-540 $\mu\text{g mL}^{-1}$

Assay procedure: Into a series of 10mL volumetric flasks, different volume of standard solution of drug were transferred. To each flask, 1mL of ($9.569 \times 10^{-3}M$) *p*-CA solution in acetonitrile was added and remaining volume was made up to the mark by solvent. When the pale yellow colored solution of *p*-CA mixed with drugs purple colors were observed. The absorbance of the solution was measured after 2 or 3 min of mixing against blank at 520nm. The spectra of each sample at 2 or 3 different concentrations have been recorded on scan mode and for the remaining optical density were noted on fixed wavelength mode at 520 nm.

RESULTS AND DISCUSSION

Effect of concentration of reagent: When various concentrations of 2% of *p*-Chloranilic acid (0.2,0.4,0.6,0.8,1.0,1.2,1.4,1.6,1.8,2.0,2.2,2.4mL) was added to fixed concentration of various drugs viz., 60 $\mu\text{g mL}^{-1}$ of ARI, 50 $\mu\text{g mL}^{-1}$ of AST, 40 $\mu\text{g mL}^{-1}$ of CRV, 40 $\mu\text{g mL}^{-1}$ of ESC, 60 $\mu\text{g mL}^{-1}$ of KET. A plot of volume of reagent and the absorbance showed that 1.8mL of reagent solution is enough ($9.569 \times 10^{-3}M$) to develop the purple color to its maximum intensity after that a plateau was observed. Therefore an excess of reagent i.e. 1mL of reagent in a total volume of 10 mL was used throughout the work.

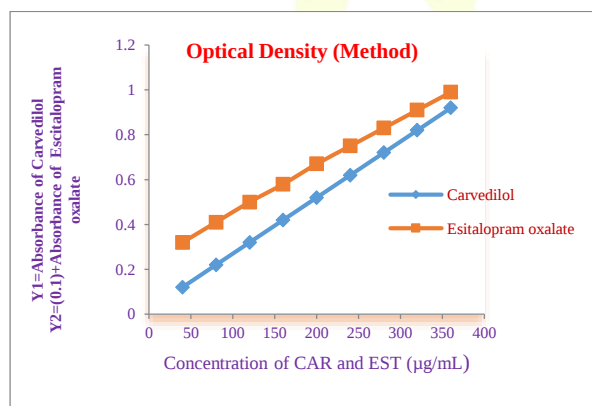


Fig 6. Calibration curves of CAR and EST (OD)

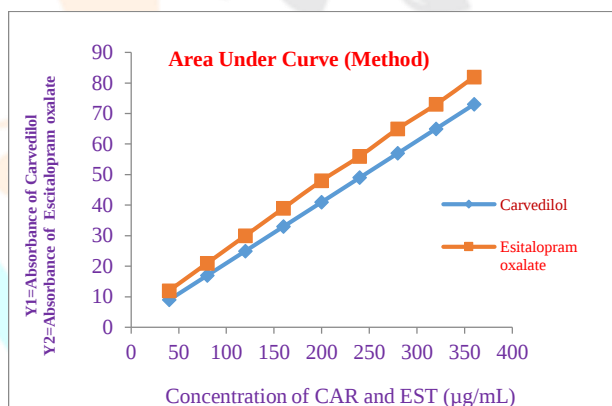


Fig 7. Calibration curves of CAR and EST (AUC)

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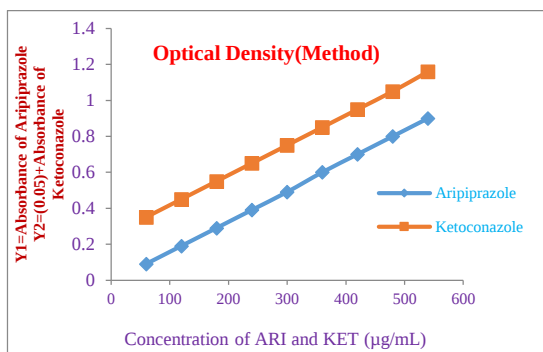


Fig 8. Calibration curves of ARI and KET (OD)

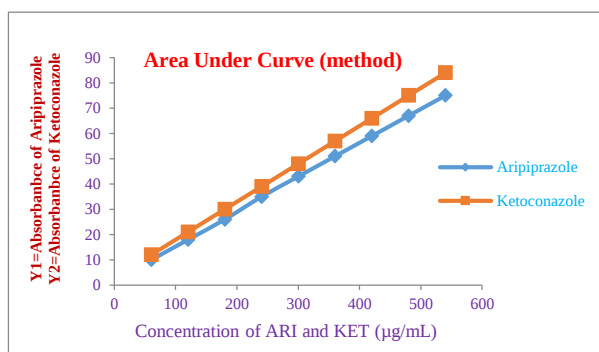


Fig 9. Calibration curves of ARI and KET (AUC)

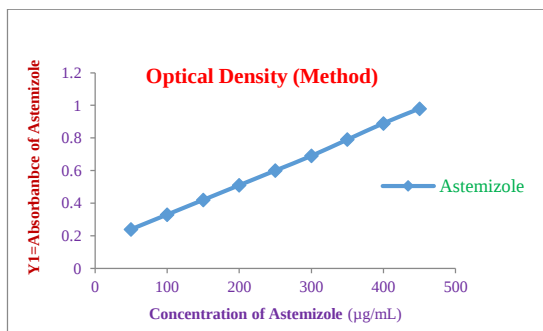


Fig 10. Calibration curve of AST (OD)

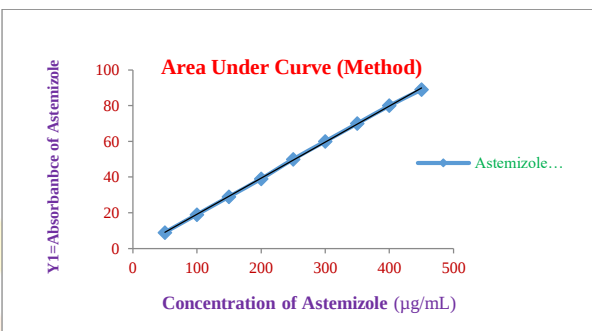


Fig 11. Calibration curve of AST (AUC)

Effect of concentration of Drug: Different volumes of drug of random concentration were added to fixed volumes of acceptor solutions developed coloration. Absorbance of solutions was measured at 520nm. Beer's law was obeyed up to certain extent of concentration above which linearity was not observed. This concentration was taken as optimum concentration and stock was prepared. The stock was further diluted to get a minimum of 8 to 10 points in the range of Beer's law plot. Similarly when the concentration is below certain limit points scattered. This was taken roughly a measure of limit of detection which is further checked by following the procedure for the determination of LOD and LOQ.

Effect of time: The interaction of *p*-CA with drugs resulted in the formation of colored product which stabilized within 2 mints of mixing. The developed color remained stable at room temperature for about an hour. After two hours many solutions turned purple. After a day all solutions turned black hence the measurements were made immediately after mixing the solutions.

Effect of organic solvent: Various solvents such as carbon tetrachloride, chloroform, 1, 2 di chloro ethane, methanol and acetonitrile have been tried to select suitable solvent for the analysis of the drug. Acetonitrile is found to be the suitable solvent as it produces maximum optical density with a fixed concentration of drug while other solvents mentioned above are found to be unsuitable as they produced lower absorbance due to incomplete dissociation of complex. Hence acetonitrile is used throughout the work.

Structure activity relationship: From the slopes of Calibration curve and from stability constants and it is clear that the donor ability of the drug is in the order: CRV>AST>ARI>ESC>KET. From the structures of the drugs it is clear that **CRV** has two secondary amines, first is most probable donarsite, and second is involved in resonance with neiburing benzene ring. **AST** has four nitrogen atoms 1 is atertiary, 2 is secondary amine but involved in resonance with neiburing group 3&4 are sp² nitrogen's. Hence 1 is more donarsite. **ARI** has three nitrogen's 1 is a tertiary nitrogen most probable donarsite, 2&3 are involved in resonance with neiburing group. **ESC** has two nitrogen's 1 is tertiary, 2 is sp nitrogen. **KET** has four nitrogen's 1 is resonance with benzene, 2 is adjacent to the Carbonyl group, 3 is resonance with ring and 4 is sp² nitrogen. 1 is most probable donarsite.

Validation of the proposed methods: The AUC (Area Under Curve) was calculated using Excel programme. This is taken between 400 to 700 nm as this area is minimally interfered by *p*-CA absorption. After recording optical densities and calculating AUC for four replicates a commonly used analytical parameter called "relative response" (O.D / Conc. ($\mu\text{g mL}^{-1}$) and AUC / Conc. ($\mu\text{g mL}^{-1}$) have been calculated. The points between 95% and 105% of average relative response only are considered for construction of calibration. The calibration graphs are shown in (figures 6). The different concentrations of donors have been mentioned (Table.1). Calibration curves were linear for all the drugs whose limits are mentioned. The methods developed have been validated in terms of guidelines of international conference of harmonization (ICH) [486,487] viz., selectivity, precision, accuracy, linearity, LOD, LOQ and robustness. The methods are selective and can differentiate the analyte from the excipients. The precision is tested by repeating each experiment at least 6 times while the accuracy has been tested by taking known weight of sample and performing recovery experiments.(Table 4).Limits of calibration, LOD, LOQ, have been determined as mentioned earlier. The robustness of the methods is examined by performing the experiments on three different spectrophotometers with excellent absorbance values. The methods developed have also been applied for the analysis of pharmaceuticals. The recovery experiments performed show high accuracy and precision and the results are compared to the available validated reported methods on each drug. The values % RSD and t-and F tests are in the permissible range of experimental errors (Table 5) and show that the methods can be used in both pharmaceutical and drug industries.

Table 2. Analytical parameters for the determination of drugs by Charge Transfer Complexation with *p*-CA (AUC Method)

Parameter	ARI	AST	CAR	ESC	KET
$\lambda_{\text{max}}(\text{nm})$	520	520	520	520	520
Beer's Law Limits ($\mu\text{g mL}^{-1}$)	60-540	50-450	40-360	40-360	60-540
Molar absorptivity ($\text{L mol}^{-1}\text{cm}^{-1}$)	0.1092×10^7	0.4640×10^7	0.1824×10^7	0.3391×10^7	0.1508×10^7
Sandall sensitivity ($\mu\text{g cm}^{-2}$)	0.0068	0.0047	0.5	0.0045	0.0062
LOD ($\mu\text{g mL}^{-1}$)	0.3286	0.2830	0.0988	0.5449	0.1065
LOQ ($\mu\text{g mL}^{-1}$)	0.9959	0.8576	0.2996	1.6513	0.3227
Intercept, (A)	0.294	1.653	0.805	1.889	+0.009
Slope, (B)	0.147	0.209	0.203	0.222	0.159
Correlation Coefficient, (R)	0.998	0.994	0.998	0.997	0.998

StandardDeviation of Intercept (Sa)	0.0146	0.0179	0.0060	0.0366	0.0051
StandardDeviation of Slope (Sb)	0.0045	0.0101	0.0058	0.003	0.0087
Regression equation,(y) Y=bx+a	0.147x +0.294	0.209 +1.653	0.203x +0.805	0.222x +1.889	0.159x +0.009

Table.3Analytical parameters for the determination of drugs by Charge Transfer Complexation with *p*-CA (OD Method)

Parameters	ARI	AST	CAR	ESC	KET
$\lambda_{\text{max}}(\text{nm})$	520	520	520	520	520
Beer's Law Limits ($\mu\text{g mL}^{-1}$)	60-540	50-450	40-360	40-360	60-540
Molar absorptivity ($\text{Lmol}^{-1}\text{cm}^{-1}$)	0.1092×10^7	0.4640×10^7	0.1824×10^7	0.3391×10^7	0.1508×10^7
Sandalsensitivity ($\mu\text{g cm}^{-2}$)	1	0.5	1	0.5	0.5
LOD ($\mu\text{g mL}^{-1}$)	28.0661	8.4671	11.5892	11.5892	12.4936
LOQ ($\mu\text{g mL}^{-1}$)	25.6580	85.0490	35.1188	35.1188	37.8593
Intercept,(A)	-0.025	+0.096	+0.016	+0.131	+0.03
Slope,(B)	0.001	0.002	0.002	0.002	0.001
Correlation Coefficient,(R)	0.992	0.989	0.995	0.987	0.994
StandardDeviationof Intercept (Sa)	0.0085	0.0051	0.0035	0.0070	0.0075
StandardDeviationof Slope (Sb)	0.0015	0.0020	0.0025	0.0015	0.0015
Regression equation,(y) Y=bx+a	0.001x -0.025	0.002x +0.096	0.002x +0.016	0.002x +0.131	0.001x +0.03

Procedure for analysis of Pharmaceuticals:

Five tablets of (Arena-30mg) were weighed and ground in to fine powder. Weight equivalent to 50mg of Aripiprazole was transferred in 100mL volumetric flask and 50mL of Ethanol was added and shaken well for 5mints. The content was filtered using whatman No. 42 filter paper in the beaker. The residue is washed with 20 mL of Ethanol. Ethanol was evaporated by heating on water bath. To that content Acetonitrile was added and serial dilutions are done accordingly.

Four tablets of (Stemiz 40mg) were weighed and ground in to fine powder. Weight equivalent to 40mg of Astemizole was transferred in 100mL volumetric flask and 50mL of Chloroform was added and shaken well for 5 mints. The content was filtered using whatman No. 42 filter paper in the beaker. The residue is washed with 20 mL of Chloroform. Chloroform was evaporated by heating on water bath. To that content Acetonitrile was added and the solution is diluted for the analysis.

Three tablets of (Coreg 100mg) were weighed and ground in to fine powder. Weight equivalent to 50mg of Carvedilol was transferred in 100mL volumetric flask and 100 mL of Methanol was added and shaken well for 10 mints. The content was filtered using whatman No.42 filter paper in the beaker. The residue is washed with 20mL of Methanol. Methanol was evaporated by heating on water bath. To that content Acetonitrile was added and serial dilutions are done accordingly.

Three tablets of (Escitalentplus-10mg) accurately weighed and powdered well. Powdered tablets equivalent to 40 mg of escitalopram oxalate was transferred into a 100 mL volumetric flask, 25 mL of 0.2N HCl was added and sonicated for a few min. The extraction was repeated three times and all extracts were mixed and made up to 100 mL with the same solvent. The solution was filtered using a whatman No. 42 filter paper. The resultant of the solution was further diluted with Acetonitrile to get a required concentration for the analysis of the drug.

Three tablets of (Ketoazole-200mg) were weighed and ground in to fine powder with the help of a mortar and pestle. Weight equivalent to 50mg of Ketoconazole was transferred in 100mLvolumetric flask and 100 mL of Methanol was added and shaken well for 5 mints. The content was filtered using whatman No. 42 filter paper in the beaker. The residue is washed with 30 mL of Methanol. Methanol was evaporated by heating on water bath. To that content Acetonitrile was added and serial dilutions are done accordingly.

Table 4.Determination of accuracy and precision of the method on pure Drug sample

Drug	Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Er(%)	Recovery (%)	RSD (%)	Proposed method mean $\pm$ SD
ARI	2.5	2.49	0.40	99.60	0.365	99.97
	3.0	3.01	0.33	100.33		± 0.364
	3.5	3.5	0.00	100.00		
AST	1.0	1.0	0.00	100.00	0.705	99.97
	3.0	3.02	0.66	100.66		± 0.704
	4.0	3.97	0.75	99.25		
CAR	2.0	1.98	1.0	99.00	0.665	99.66
	4.0	4.01	0.33	100.33		± 0.662
	6.0	5.99	0.33	99.66		
ESC	3.0	3.01	0.33	100.33	0.500	99.75
	3.5	3.48	0.57	99.43		± 0.499
	4.0	3.98	0.50	99.50		
KET	3.5	3.46	1.14	98.86	0.970	99.88
	4.0	4.0	0.00	100.0		± 0.969
	5.0	5.04	0.79	100.79		

Table 5. Results of assay of tablets by the proposed method and statistical evaluation and recovery experiment by standard addition method.

Tablets	Drug in tablet ($\mu\text{g mL}^{-1}$)	Drug added ($\mu\text{g mL}^{-1}$)	Total found ($\mu\text{g mL}^{-1}$)	Error (%)	Recovery (%)	RSD (%)	Referencemeth od mean $\pm$ SD	Proposed method mean $\pm$ SD	t-test	F-test
Arena (ARI)	0.50	0.3	0.79	1.25	98.75	0.638	99.346 ± 0.874	99.57 ± 0.638	0.725	0.763
	0.50	0.5	1.0	0	100					
	0.50	0.6	0.09	0.91	99.09					
	2.0	0.0	2.01	0.5	100.5					
	3.0	0.0	2.98	0.66	99.33					
Stemiz (AST)	4.0	0.0	3.99	0.25	99.75	0.722	100.86 ± 0.630	100.22 ± 0.722	1.630	0.397
	0.50	0.3	0.81	1.25	101.25					
	0.50	0.5	1.01	1	101					
	0.50	0.7	1.20	0	100					
	2.0	0.0	1.99	0.5	99.5					

	6.0	0.0	5.99	0.17	99.83					
Corez (CAR)	0.50	0.1	0.59	1.66	98.33					
	0.50	0.3	0.81	1.25	101.25					
	0.50	0.5	1.01	1	101	1.057	99.8	100.05		
	3.0	0.0	2.99	0.33	99.66		±0.896	±1.057	0.438	0.802
	3.5	0.0	3.51	0.28	100.28					
	4.0	0.0	3.99	0.25	99.75					
Escitalant plus (ESC)	0.50	0.5	0.99	1	99					
	0.50	1.0	1.49	0.66	99.33					
	0.50	1.5	2.01	0.5	100.5	0.510	100.31	99.63		
	3.0	0.0	2.99	0.33	99.66		±0.820	±0.508	1.719	0.672
	4.0	0.0	3.98	0.50	99.5					
	5.0	0.0	4.99	0.20	99.8					
Ketozone (KET)	0.50	2.0	2.49	0.40	98.6					
	0.50	4.0	4.51	0.22	100.22					
	0.50	6.0	6.48	0.31	99.69	0.263	99.86	99.71		
	3.5	0.0	3.49	0.29	99.71		±0.783	±0.262	0.433	0.613
	4.0	0.0	3.98	0.50	99.5					
	4.5	0.0	4.48	0.44	99.56					

APPLICATION

These methods are economical compared to other sophisticated analytical instruments, hence can be used for routine analysis of commercially available formulation.

CONCLUSIONS

The obtained results from the methods for the determination of above mentioned drugs indicate that methods are simple, accurate and precise. We observed the quantification of drugs as AUC is more accurate and precise than selecting one wavelength because AUC combines optical densities (OD) at the all the wave length. The methods are economical compared to other sophisticated analytical instruments, hence can be used for routine analysis of commercially available formulations. The method is suitable for the determination of these drugs in tablet formation without interference from commonly used recipients. The solvent used for the method are inexpensive and simple to prepare, and could be used in a quality control laboratory for routine drug analysis.

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