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UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ESCITALOPRAM OXALATE AND CLONAZEPAM IN BULK AND IN TABLET DOSAGE FORM

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ABSTRACT

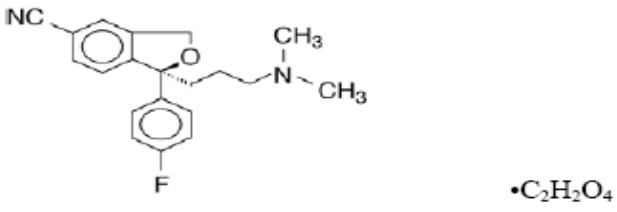
A simple, sensitive, accurate UV spectrophotometric method was developed for the simultaneous estimation of Escitalopram oxalate and Clonazepam in bulk and in pharmaceutical dosage form. In this communication simultaneous equation method was reported for the assay of both the drugs in the pharmaceutical dosage form. In simultaneous equation method the absorbance of the sample was measured at 246 nm and 230 nm respectively by using methanol which was further applied for determining the concentration of the both the drugs. The proposed method was validated statistically for specificity, linearity, accuracy and precision. The low values of standard deviation and percentage RSD indicated high precision of method. This method was successfully used for routine analysis of Escitalopram oxalate and Clonazepam in combination dosage form with good recoveries.

KEY WORDS: Escitalopram oxalate, Clonazepam, UV method, Validation.

INTRODUCTION

ESCITALOPRAM OXALATE (www.drugs.com, 2008, www. In-house pharmacy, Nagarjuna.A, *et al* 2004**):**

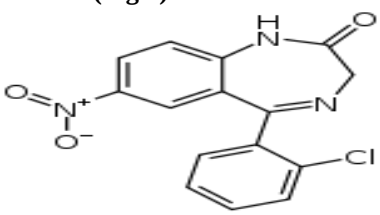
Chemical structure (Fig 1):



Molecular Formula is $C_{20}H_{21}FN_{20} \bullet C_2H_2O_4$, Molecular weight is 414.43, Chemical name (*S*)-1-[3- (dimethylamino) propyl]-1- (4fluorophenyl) 1,3dihydroiso benzofuran- 5-carbonitrile oxalate and Escitalopram oxalate is a fine, white to Slightly-yellow powder. Melting point 147–148°c. Escitalopram is a pure S-enantiomer of the racemic, bicyclic phthalates is citalopram derivative. Escitalopram is freely soluble in methanol and dimethylsulfoxide (DMSO), sparingly soluble in water and in ethanol, slightly soluble in ethyl acetate and insoluble in heptane. Bioavailability 80%, Protein binding was occur ~56%, Metabolism occur in Hepatic CYP3A4, Half-life 27–32 hours, Renal Excretion, used as a Anti-depressant, Adverse effects and dyskinesia, hypertonia, Mechanism of action was selective serotonin reuptake inhibition. Dosage 10 mg/day.

CLONAZEPAM (www.\MedlinePlus, 2007, www. Drug bank.com, www.medicine.com**):**

Chemical structure (Fig 2):



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Molecular Formula is $C_{15}H_{10}ClN_3O_3$, Molecular weight is 315.72, Chemical name 5-(o-chloro phenyl)- 7-nitro-1H-1,4- Benzodiazepin-2(3H)-one. It appears White, Light yellow crystal or crystalline Powder. No smell. Melting point 240°c (decomposition). Clonazepam is Slightly Soluble in Acetone, Chloroform, Acetic anhydride, hardly soluble in Methanol, Ethanol, Isopropanol, and Ether almost insoluble in Water. Drug Activity classification is Anticonvulsant. Bioavailability 90%, Protein binding 85%, Metabolism occur Hepatic CYP3A4. Renal excretion. Used as Anti convulsant, muscle relaxant, Anxiolytic. Adverse effects are drowsiness, cognitive impairment. Mechanism of action inhibition of synaptic transmission across the central nervous system.

Most of the pharmaceutical companies are manufacturing combined drug formulations to meet the market demand and patient compatibility. It is a well-known fact that a combination of drugs has wider range to treat ailments as compared to the single drug component. Very few methods are available for estimation of combined drug formulations by simultaneous methods. Almost all pharmacopoeial methods available for the analysis of such formulations are applicable only after prior separation of drug components, hence making them tedious. This simultaneous estimation were time consuming and usage of solvent is minimized, analytical grade of solvents used for respective determinations and the solvent should be readily available and cheaper. The solvent should be completely extracting the active ingredient from formulation.

But there are few methods were reported for the estimation of Escitalopram oxalate and Clonazepam individually as well as in combination with some other drugs. But there are no methods were reported for the estimation of these drugs in combined dosage forms without prior separation. The non-availability of any UV-spectrophotometry method until now for simultaneous analysis of the combination made it a worth-while objective to pursue the present work.

Hence the present work, aim to develop a simple, precise and accurate method for the estimation of Escitalopram oxalate and Clonazepam in bulk and in combined pharmaceutical dosage form and to validate the developed method by UV spectrophotometry (Simultaneous Equation Method).

MATERIALS

Drug Samples (Raw material)

Escitalopram oxalate and Clonazepam were obtained as a sample from Siri Pharma Ltd., Hyderabad.

Formulation used

Snudep plus (Sun Pharmaceutical industries) containing Escitalopram oxalate 10mg and Clonazepam 0.5 mg was purchased from local pharmacy.

Chemicals and solvents used

Distilled water and Methanol (AR grade) were purchased from Qualigens India Pvt. Limited and Loba Chemie India Limited.

Instruments used

Different instruments used to carry out the present work,

a) Shimadzu AUX- 220 Digital balance.
b) Shimadzu- 1700 Double Beam- UV- Visible spectrophotometer.
c) Sonicator – Sonica ultrasonic cleaner – model 2200 MH.
d) Micropipette.
e) Shimadzu IR Affinity-1.
f) Buchi M-565.- Melting point

METHOD

In the present work an attempt was made to develop and validate simple, precise and accurate method for the estimation of Escitalopram oxalate and Clonazepam in pure form and in combined tablet dosage form by UV Spectrophotometry.

UV Spectrophotometry

Simultaneous Equation Method

SIMULTANEOUS EQUATION METHOD

A simple, accurate, rapid and precise simultaneous equation method was developed and validated.

Selection of solvent

The solubility of drugs was determined in a variety of solvents as per Indian pharmacopoeial standards. Solubility was carried out in polar to non polar solvents. The common solvent was found to be methanol for the analysis of Escitalopram oxalate and Clonazepam for proposed method.

Preparation of standard stock solution

50 mg of Escitalopram oxalate (A) and 2.5mg of Clonazepam (B) raw materials were weighed and transferred into 50 ml volumetric flasks separately and dissolved in methanol and made up to the volume with methanol. These solutions were observed to contain 1000 µg/ml (Solution-A) and 50 µg/ml (Solution-B).

Standard Preparation

Transferred 1 ml of solution A, 1 ml of solution B, into 10 ml volumetric flask and diluted with 10 ml of methanol, so that the concentration of Escitalopram oxalate and Clonazepam were 100 µg/ml and 5 µg/ml.

Selection of wavelengths for estimation and stability studies

The selection of wavelengths for the estimation Escitalopram oxalate and Clonazepam a suitable diluted stock solution contain 100 µg/ml and 5 µg/ml of the

solutions were scanned between 200 – 400 nm by using methanol as blank. From the overlain spectra, by the observation of spectral characteristics of Escitalopram oxalate and Clonazepam were selected for simultaneous estimation. The wavelengths selected were 246 nm and 230 nm. The two drugs were stable for 1hr at selected wavelengths.

Preparation of calibration graph

The aliquots of stock solution of Escitalopram oxalate and Clonazepam (0.2 – 1.2 ml of 1000 μg/ml and 0.2 – 1.2 ml of 50 μg/ml) were transferred into 10 ml volumetric flasks and made up to the volume with methanol. The absorbance of different concentration solutions were measured at 246 nm and 230 nm for Escitalopram oxalate and Clonazepam. The calibration curve was plotted at their corresponding wavelengths. So the Escitalopram oxalate and Clonazepam were linear with the concentration range of 20 – 120 μg/ml and 1 – 6 μg/ml at their selected wavelengths.

Quantifications of formulation

Twenty tablets of formulation (Snudep plus containing 10 mg of Escitalopram oxalate and 0.5 mg of Clonazepam) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 20 mg of Escitalopram oxalate was weighed and transferred into 50 ml volumetric flask along with a minimum quantity of methanol to dissolve the substance and made up to the volume with the same. Then filter the solution by using membrane filter. Then take 0.25 ml of above solution and make up with 10 ml of methanol. The absorbance measurements were made the formulation at 246 nm and 230 nm. From the absorptivity values of Escitalopram oxalate and Clonazepam were used to determine the amount of Escitalopram oxalate and Clonazepam from tablet formulation by using simultaneous equation method.

Validation of developed method

Linearity

A calibration curve was plotted between concentration and absorbance. Escitalopram oxalate was linear with the concentration range of 20 – 120 μg/ml at 246 nm and 230 nm. Clonazepam showed the linearity in the range of 1 – 6 μg/ml at 230 nm and 246 nm.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. To the preanalyzed formulation, a known quantity of raw materials of Escitalopram oxalate and Clonazepam were added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and percentage RSD also calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

RESULTS AND DISCUSSION

Estimation of combined drug formulations have advantage that the method was less time consuming and usage of solvent is minimized. Simple, rapid, precise and accurate UV spectrophotometric method was developed and validated for the estimation of Escitalopram oxalate and Clonazepam in pure form and in combined tablet dosage form.

UV SPECTROPHOTOMETRIC METHOD

The drugs like Escitalopram oxalate and Clonazepam are identified by using FT-IR and Melting point. The results were shown in figure 3 and 4 and table 1.

The solubility of Escitalopram oxalate and Clonazepam was determined as per Indian Pharmacopoeia. Number of polar and non – polar solvents were tried to dissolve the drugs. From the solubility profile methanol was chosen as a common solvent for the estimation of Escitalopram oxalate and Clonazepam. The solubility data was shown in Table 2 and 3 for Escitalopram oxalate and Clonazepam respectively.

The sample solutions of 100 μg/ml of Escitalopram oxalate and 5 μg/ml Clonazepam in methanol prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank were shown in figures 5 and 6. The overlain spectrum of mixture of Escitalopram oxalate and Clonazepam was recorded as shown in Figure 7. From the spectrum, 246 and 230 nm was selected for the estimation of Escitalopram oxalate and Clonazepam for simultaneous estimation. To estimate the amount of Escitalopram oxalate and Clonazepam these wavelengths were used and the simultaneous equation method was applied to estimate titled drugs.

Different aliquots of Escitalopram oxalate and Clonazepam in methanol were prepared in the concentration

range of 20 – 120 µg/ml and 1 – 6 µg/ml. The absorbance of solutions were measured at 246 and 230 nm were shown in Table 4 - 5 for Escitalopram oxalate and Clonazepam. The calibration curve was plotted using concentration against absorbance. The calibration graph at 246 and 230 nm for each drug was shown in Figure 8 - 11 respectively. The preparation of calibration curve was repeated for six times for each drug at their selective wavelengths. The optical parameters like Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD and LOQ were calculated. The correlation coefficient for the two drugs was found to be 0.999. This indicates that the two drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The optical characteristics of two drugs at their selective wavelengths were shown in Table 6 - 7 for Escitalopram oxalate and Clonazepam. The tablet dosage form (Snudep plus containing 10 mg of Escitalopram oxalate and 0.5 mg of Clonazepam) was selected for the analysis. The concentration is 100 µg/ml of Escitalopram oxalate and it is also containing 5 µg/ml of Clonazepam in methanol. The absorbances of the solution were measured at their wavelengths. The percentage label claim present in tablet formulation was found to be 99.669 and 99.83 for Escitalopram oxalate and Clonazepam respectively. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 1.000 and 0.849 for Escitalopram oxalate and Clonazepam in respectively. The low % RSD values indicate that the method has good precision. The results of analysis are shown in Table 8.

Further the precision of the method was confirmed by Intraday and Interday analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and interday analysis was found to be 0.06586 for Escitalopram oxalate and 0.204 for Clonazepam 5µg/ml. The results of analysis are shown in Table 9, 10. The results showed that the precision of the method was confirmed.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of Escitalopram oxalate, Clonazepam raw material solutions were added at different levels (80%, 100%, and 120%). The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.3 – 99.8% for Escitalopram oxalate, 99.2 - 100.1% for Clonazepam. The low % RSD value for two drugs indicates that this method is very accurate. The recovery data is shown in Table 11 - 12.

All the above parameters with the ease of operation ensure that the project method could be applied for the routine analysis of Escitalopram oxalate and Clonazepam in pure form and in tablet dosage forms.

Table 1. Identification of Drugs by Melting Point

S.No	Drugs	Melting point specification (ºc)	Observed specification (ºc)
1.	Escitalopram oxalate	147 – 148	147.2
2.	Clonazepam	240	239.8

Table 2. Solubility Profile of Escitalopram Oxalate

S.No.	Solvents	Extent of Solubility	Category
1	Distilled water	10 mg in 0.12 ml	Sparingly soluble
2	0.1M Hydrochloric acid	---	Insoluble
3	0.1M Sodium Hydroxide	---	Insoluble
4	Methanol	10 mg in 0.12 ml	Soluble
5	Ethanol	10 mg in 0.12 ml	Sparingly soluble
6	2 – Propanol	---	Insoluble
7	Chloroform	---	Insoluble
8	DMSO	10mg in 0.12ml	Soluble
9	Acetone	---	Insoluble
10	Ethyl acetate	---	Insoluble
11	Toluene	---	Insoluble
12	Butanol	---	Insoluble
13	Acetonitrile	10 mg in 0.12 ml	Soluble

Table 3. Solubility Profile of Clonazepam

S.No.	Solvents	Extent of Solubility	Category
1	Distilled water	---	Insoluble
2	0.1M Hydrochloric acid	---	Insoluble

3	0.1M Sodium Hydroxide	---	Insoluble
4	Methanol	10 mg in 0.09 ml	Freely soluble
5	Ethanol	10 mg in 0.09 ml	Freely soluble
6	2 – Propanol	10 mg in 0.09 ml	Hardly soluble
7	Chloroform	10 mg in 0.09 ml	Slightly soluble
8	DMF	10 mg in 0.09 ml	Soluble
9	Acetone	10 mg in 0.02 ml	Very soluble
10	Ethyl acetate	---	Insoluble
11	Toluene	---	Insoluble
12	Ether	10 mg in 0.02 ml	Soluble
13	Acetonitrile	10 mg in 0.02 ml	Soluble

Table 4. Linearity of Escitalopram Oxalate by Simultaneous Equation Method

Concentration (µg/ml)	Absorbance	
	230 nm	246 nm
20	0.030	0.041
40	0.066	0.079
60	0.108	0.126
80	0.151	0.159
100	0.190	0.201
120	0.218	0.235
Correlation coefficient(r)	0.9996	0.9998
Slope (m)	0.001	0.002
Intercept (b)	0.004	0.001

Table 5. Linearity of Clonazepam by Simultaneous Equation Method

Concentration (µg/ml)	Absorbance	
	230 nm	246 nm
1	0.032	0.025
2	0.061	0.049
3	0.095	0.078
4	0.129	0.107
5	0.169	0.143
6	0.199	0.173
Correlation coefficient(r)	0.9999	0.9995
Slope (m)	0.034	0.029
Intercept (b)	0.005	0.005

Table 6. Optical Characteristics of Esitalopram Oxalate by Simultaneous Equation Method at 230 nm and 246 nm

PARAMETERS	At 230 nm	At 246 nm
Beers law limit (µg/ml)	20– 120	20– 120
Molar absorptivity (L mol^{-1} $cm^{-1)}$	83.309	1406.989
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.0542	0.029411
Correlation coefficient (r)	0.9998	0.9999
Regression equation (y= mx+c)	y= 0.001x + 0.004	y= 0.034x + 0.005
Slope (m)	0.001	0.034
Intercept (c)	0.004	0.005
LOD (µg/ml)	0.23343	0.01372
LOQ (µg/ml)	0.7071	0.04159

Table 7. Optical Characteristics of Clonazepam by Simultaneous Equation Method at 230 nm and 246 nm

Parameters	At 230 nm	At 246 nm
Beers law limit (µg/ml)	1– 6	1– 6
Molar absorptivity (L mol^{-1} $cm^{-1)}$	520.938	440.3
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.0303	0.0344
Correlation coefficient (r)	0.9998	0.9996
Regression equation (y=mx+c)	y= 0.033x + 0.002	y= 0.029x + 0.005
Slope (m)	0.033	0.029
Intercept (c)	0.002	0.005
LOD (µg/ml)	0.03535	0.04022
LOQ (µg/ml)	0.1039	0.1218

Table 8. Analysis of Tablet Formulation (Snudep Plus) by Simultaneous Equation Method

Sample	Label claim	Amount taken	Amount found	% Assay Avg± S.D	%R.S.D
Snudep Plus	Escitalopram oxalate- 10 mg	20mg	10.068 mg 9.927 mg 9.926 mg 10.062 mg 10.066 mg 10.068 mg	99.669±0.0997	1.000
	Clonazepam - 0.5mg	20mg	0.502 mg 0.496 mg 0.499 mg 0.499 mg 0.501 mg 0.498 mg	99.83±0.8482	0.849

(n=6) * Mean of six observations

Table 9. Precision Studies for Escitalopram Oxalate by Simultaneous Equation Method

Standard concentration of Escitalopram oxalate	Absorbance	
	Intra day (n=3)	Inter day (n=3)
100µg/ml	0.213	0.215
100µg/ml	0.212	0.214
100µg/ml	0.213	0.215
Mean	**0.2146**	**0.2148**
Standard deviation	**0.00014142**	
% R.S.D	**0.06586**	

Table 10. Precision Studies for Clonazepam by Simultaneous Equation Method

Standard concentration of clonazepam	Absorbance	
	Intra day (n=3)	Inter day (n=3)
5µg/ml	0.169	0.174
5µg/ml	0.171	0.173
5µg/ml	0.172	0.173
Mean	**0.1725**	**0.173**
Standard deviation	**0.0003535**	
% R.S.D	**0.204**	

Acceptance criteria: %RSD of 6 replicate preparations of assay should not be more than 2%.

Table 11. Accuracy Data for Escitalopram Oxalate by Simultaneous Equation Method

S.No	Spiked level	Amount of drug (µg/ml)	Amount added (µg/ml)	Amount recovered	%Recovery Avg ± S.D	%R.S.D
1	80%	80	10	89.67 89.71 89.72	99.6 ± 0.0264	0.0265
2	100%	100	10	109.79 109.60 109.82	99.75 ± 0.107	0.108
3	120%	120	10	129.72 128.81 128.89	99.32 ± 0.398	0.400

(n=3)

Table 12. Accuracy Data for Clonazepam by Simultaneous Equation Method

S.No	Spiked level	Amount of drug (µg/ml)	Amount added (µg/ml)	Amount recovered	%Recovery Avg ± S.D	%R.S.D
1	80%	4	0.5	4.53 4.45 4.42	99.23 ± 1.228	1.237
2	100%	5	0.5	5.44 5.48 5.45	99.20 ± 0.378	0.381
3	120%	6	0.5	6.52 6.51 6.49	100.08 ± 0.230	0.230

(n=3) Acceptance criteria: The mean % recovery of the each spiked level should not be less than 98.0 % and not more than 102.0 %.

Fig: 3. Identification of Escitalopram Oxalate by IR

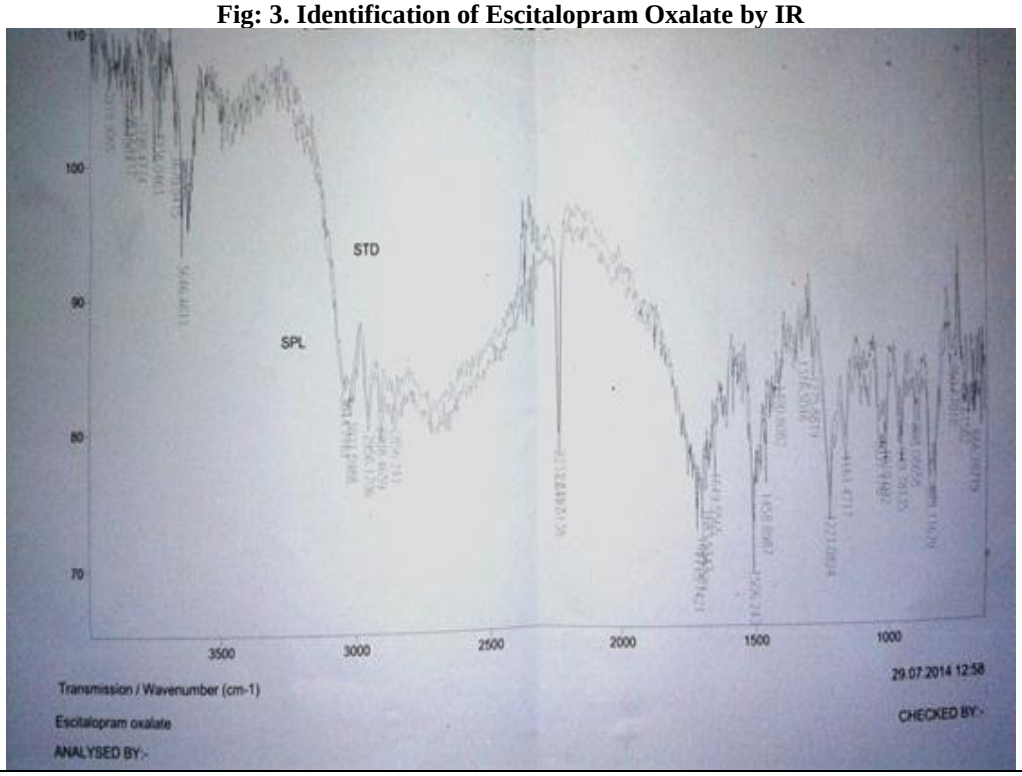


Fig: 4. Identification of Clonazepam by IR

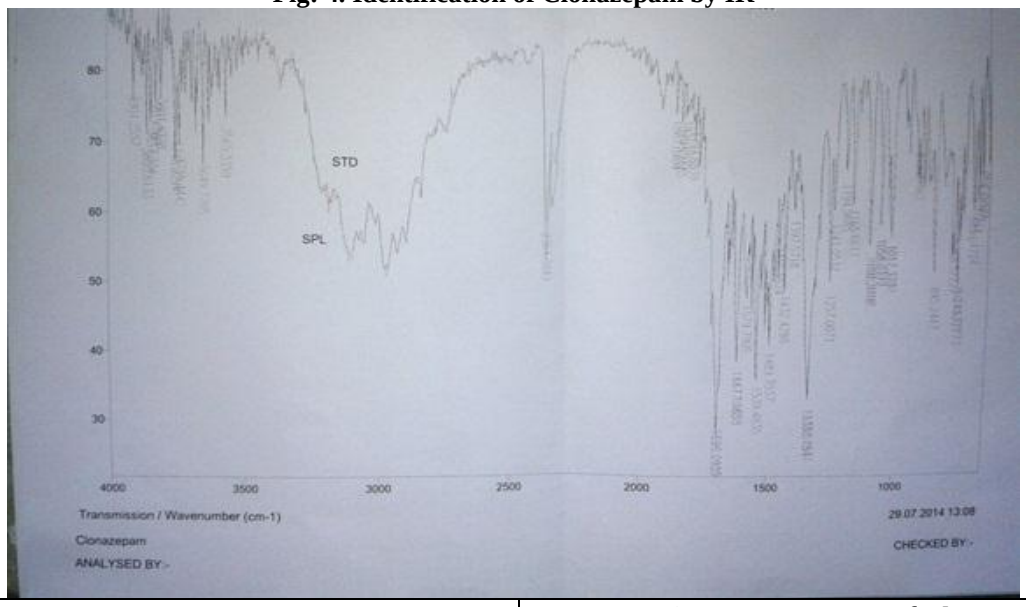


Fig: 5. UV spectrum of Escitalopram Oxalate

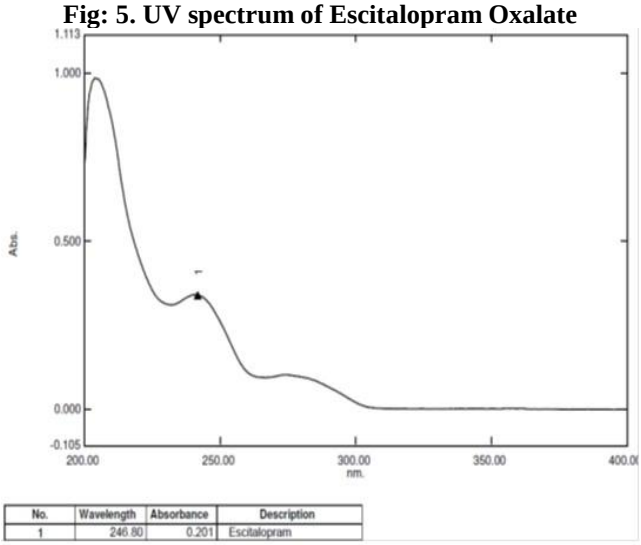


Fig: 6. UV spectrum of Clonazepam

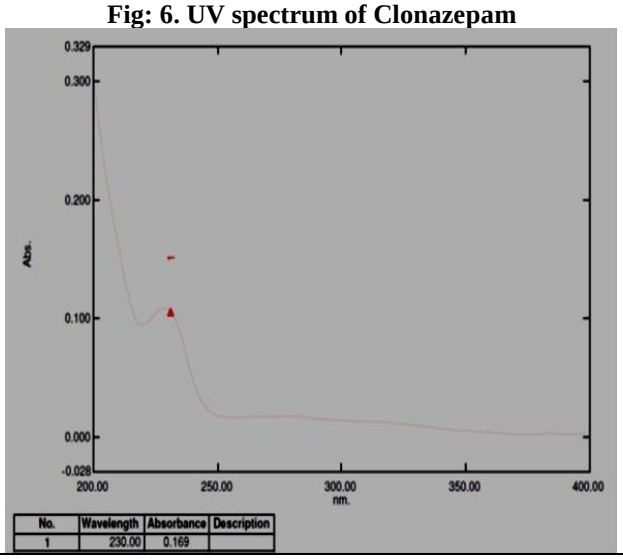


Fig: 7. Overlain UV spectrum of Escitalopram Oxalate and Clonazepam

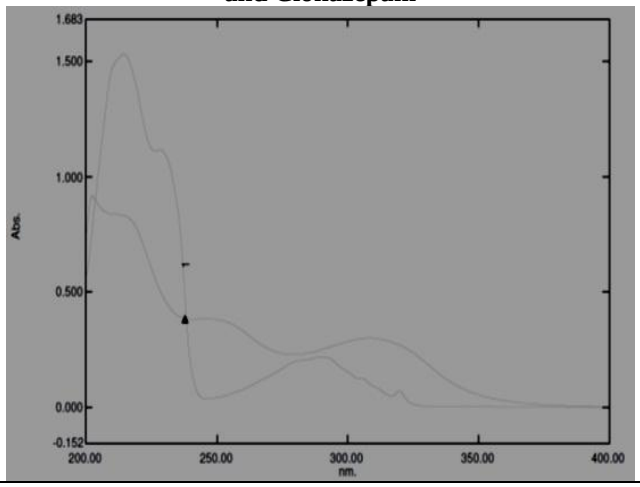
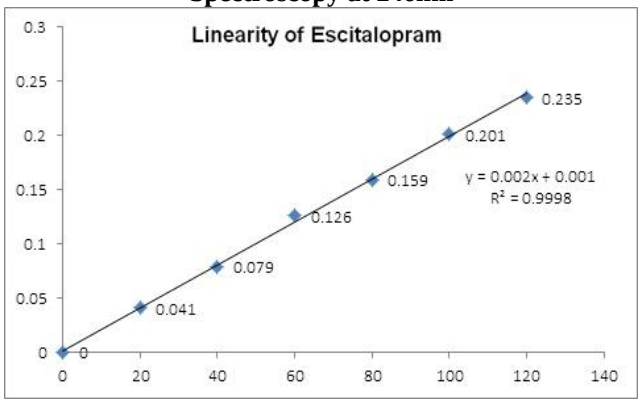
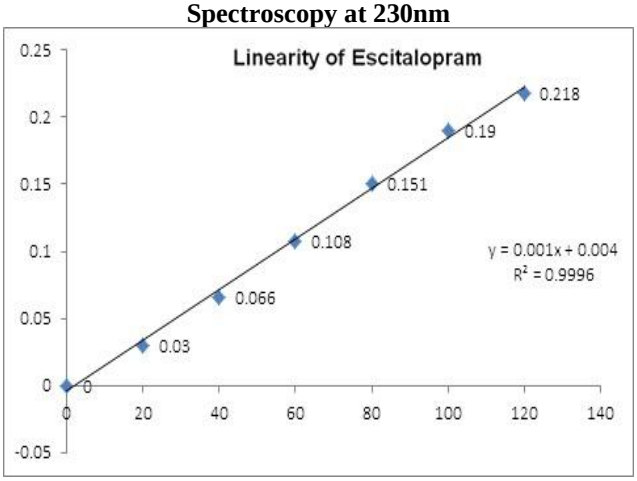


Fig: 8. Calibration Curve of Escitalopram Oxalate by UV Spectroscopy at 246nm



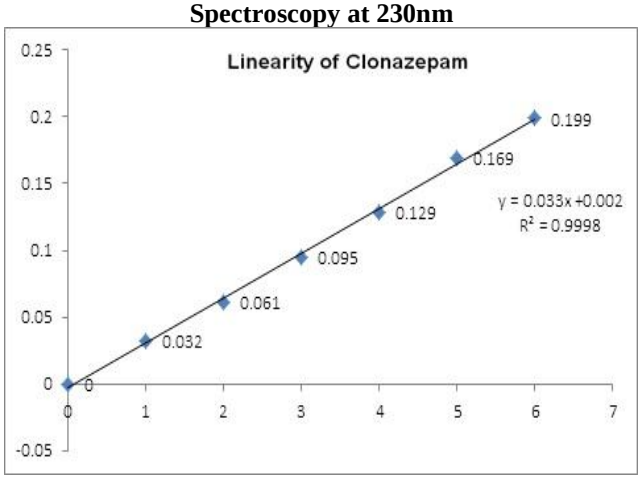
Relation Between Concentration (µg/ml) And Absorbance

Fig: 9. Calibration Curve of Escitalopram Oxalate by UV Spectroscopy at 230nm



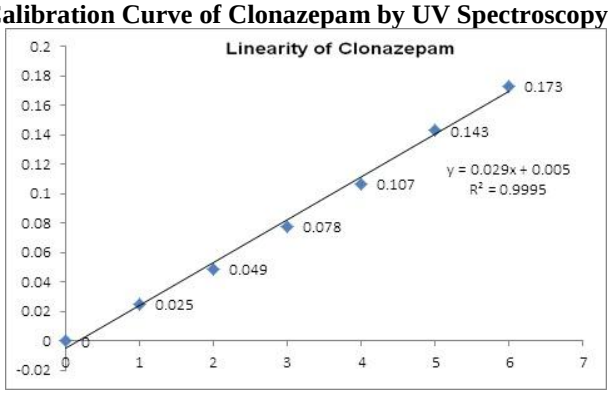
Relation Between Concentration (µg/ml) And Absorbance

Fig: 10. Calibration Curve of Clonazepam by UV Spectroscopy at 230nm



Relation Between Concentration (µg/ml) And Absorbance

Fig: 11. Calibration Curve of Clonazepam by UV Spectroscopy at 246nm



Relation Between Concentration (µg/ml) And Absorbance

CONCLUSION

Simple, rapid and accurate UV spectrophotometric (Simultaneous Equation method) method was developed for the determination of Escitalopram oxalate and Clonazepam in bulk and in tablet formulation by using UV spectrophotometer with a simple solvent (Methanol). The method showed excellent sensitivity, reproducibility, accuracy and repeatability, which is evidenced by low percentage relative standard deviation. The results obtained in recovery studies were indicating that there is no interference from the excipients used in the formulation. UV spectrophotometric method (Simultaneous Equation method) was found to be economic when compared to RP-HPLC. Hence it is suggested that the proposed UV spectrophotometric method can be effectively applied for the routine analysis of Escitalopram oxalate and Clonazepam in bulk and in tablet formulation and the obtained results will be presented elsewhere.

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