Available online at www.icjpir.com ISSN: 2349-5448

INTERCONTINENTAL JOURNAL OF PHARMACEUTICAL INVESTIGATIONS AND RESEARCH

ICJPIR |Volume 4 | Issue 1 | Jan – Mar- 2017

Research Article

Method development and validation of escitalopram and estizolam in tablet dosage form by using new RP-HPLC method

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ABSTRACT

A simple and selective LC method is described for the determination of Escitalopram oxalate and Etizolam in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 30 volumes of ammonium acetate buffer, 40 volumes of acetonitrile and 30 volumes of Methanol with detection of 238 nm. Linearity was observed in the range 60-140 µg/ml for Escitalopram oxalate (r^2 =0.999) and 6-14 µg /ml for Etizolam (r^2 =0.996) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Escitalopram oxalate and Etizolam**,** Reverse phase HPLC.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books [1].

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification [2-4], purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis–Qualitative and Quantitative analysis [5-7]. Qualitative analysis is performed to establish composition of a

substance. It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half-life etc [8-10].

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the simultaneous estimation of Escitalopram oxalate and Etizolam bromide pharmaceutical dosage form.

Plan of work

- Solubility determination of Escitalopram oxalate and Etizolam bromide various solvents and buffers.
- Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

METHODOLOGY

Mobile Phase

A mixture of Ammonium acetate buffer (pH6): ACN: Methanol 30:40:30 were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately.

Preparation of standard stock solution of ESCITALOPRAM

10 mg of Escitalopram was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of ETIZOLAM

10 mg of ETIZOLAM was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS AND DISCUSSIONS

Solubility studies

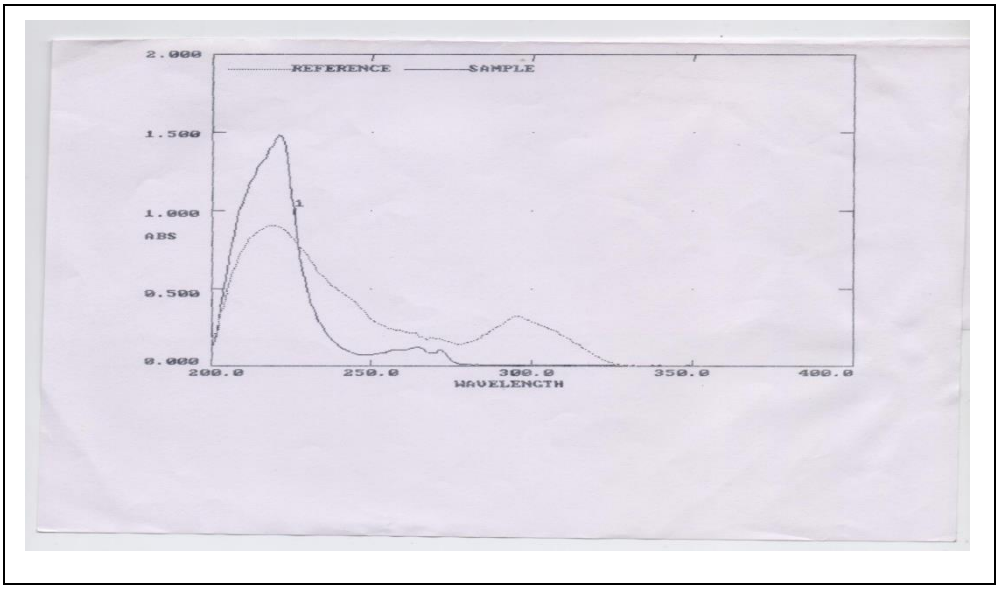
These studies are carried out at 25 ^{0}C

Escitalopram

Freely soluble in ethanol and methanol, and slightly soluble in acetone and isopropanol and very slightly soluble in water.

Etizolam

Freely soluble in methanol and water.



Results

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 8.1, and the isobestic point was found to be 227 nm for the combination.

Method development of escitalopram oxalate and etizolam bromide

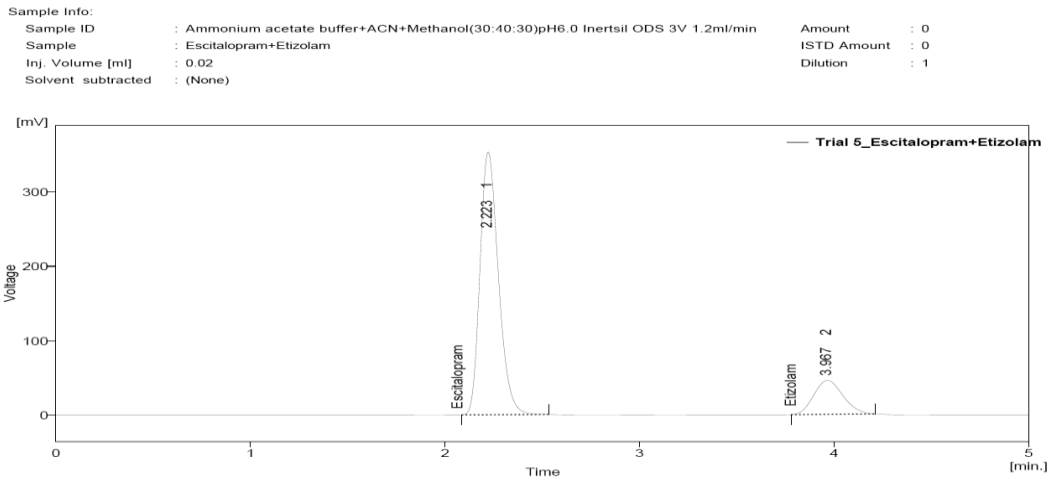
Trial- 1

Mobile phase : Ammonium acetate buffer+ ACN+ Methanol

pH : 6.0
Ratio : 30:40:30
Column : Inertsil ODS, (250×4.6× 5µ)
Wavelength : 227 nm
Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 10 mg of ESCITALOPRAM and ETIZOLAM in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of ESCITALOPRAM and ETIZOLAM is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.



Result Table (Uncal - Trial5_Escitalopram+Etizolam)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.223	2324.553	353.434	83.0	88.5	0.11
2	3.967	477.204	45.876	17.0	11.5	0.17
	Total	2801.758	399.310	100.0	100.0	

Column Performance Table (From 50% - Trial5_Escitalopram+Etizolam)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.223	0.107	1.417	0.00	2407	24069	-
2	3.967	0.167	1.244	0.00	3138	31381	7.506

Fig. 8.3.5: Chromatogram of ETIZOLAM and ESCITALOPRAM by using mobile phase

Observation

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both ETIZOLAM and ESCITALOPRAM.
- The efficiency was more than 2000 ETIZOLAM and ESCITALOPRAM.
- Resolution between two peaks >1.5.
- The details are given in the figure 8.3.8; hence this method was for optimized.

Table 8.3.8: Optimized chromatographic conditions

Mobile phase	Ammonium acetate buffer+ ACN+ Methanol (30:40:30)
Ph	6.0
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	227
Injection volume	20 µl
Run time	6 min
Retention time	About 2.323 min for ESCITALOPRAM and 3.967 min for ETIZOLAM.

Assay

Preparation of samples for Assay

Preparation of standard solution

Weigh accurately 10mg of ESCITALOPRAM and 10 mg of ETIZOLAM in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of ESCITALOPRAM and ETIZOLAM is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains ETIZOLAM-05 mg, ESCITALOPRAM -50 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of ETIZOLAM and ESCITALOPRAM (µg/ml) were prepared by dissolving weight equivalent to 10 mg of ETIZOLAM and ESCITALOPRAM and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of ETIZOLAM and ESCITALOPRAM was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Escitalopram oxalate and Etizolam present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

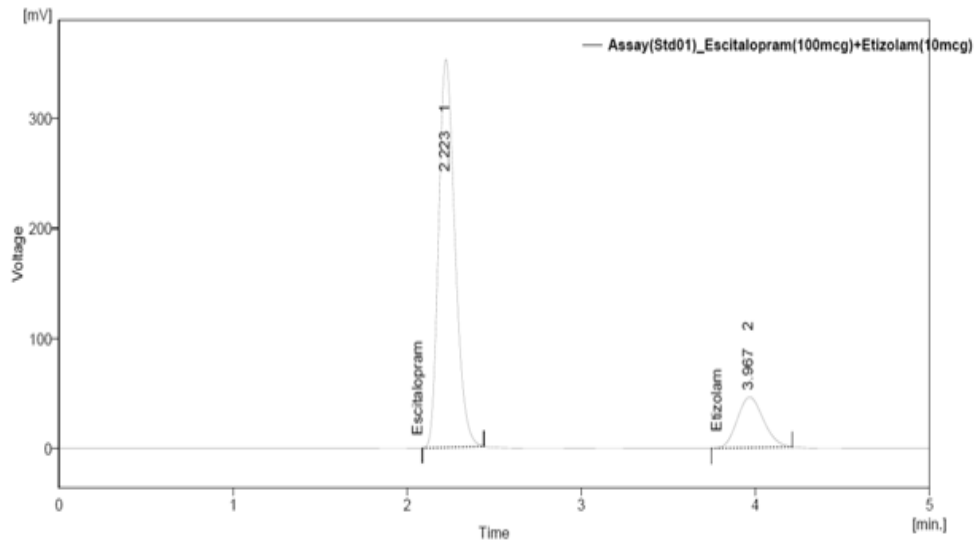


Fig: Chromatogram of Assay standard preparation-1

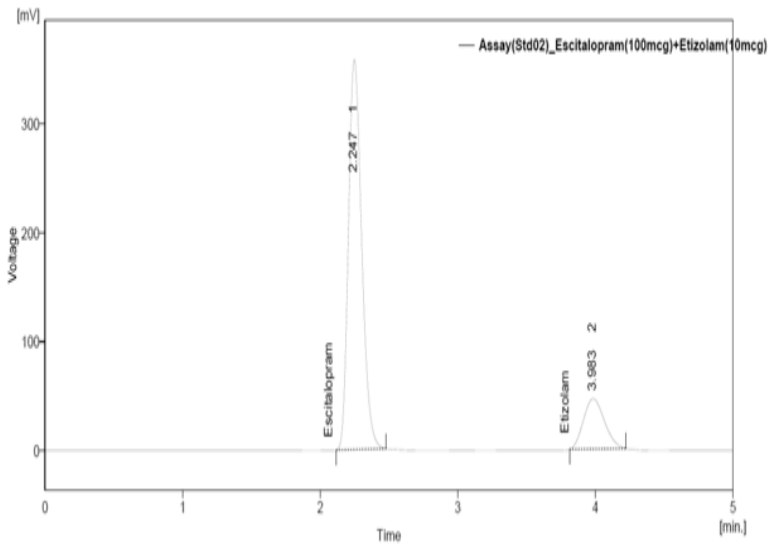


Fig: Chromatogram of Assay standard preparation-2

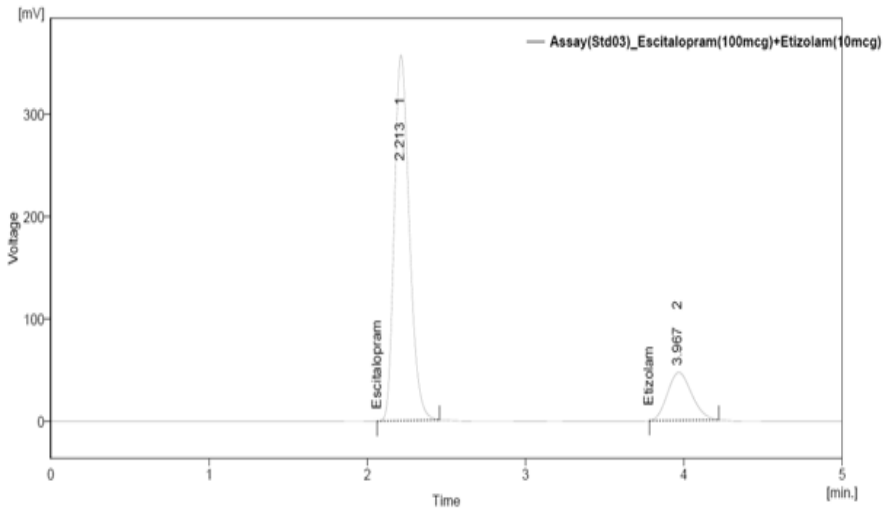


Fig: Chromatogram of Assay standard preparation-3

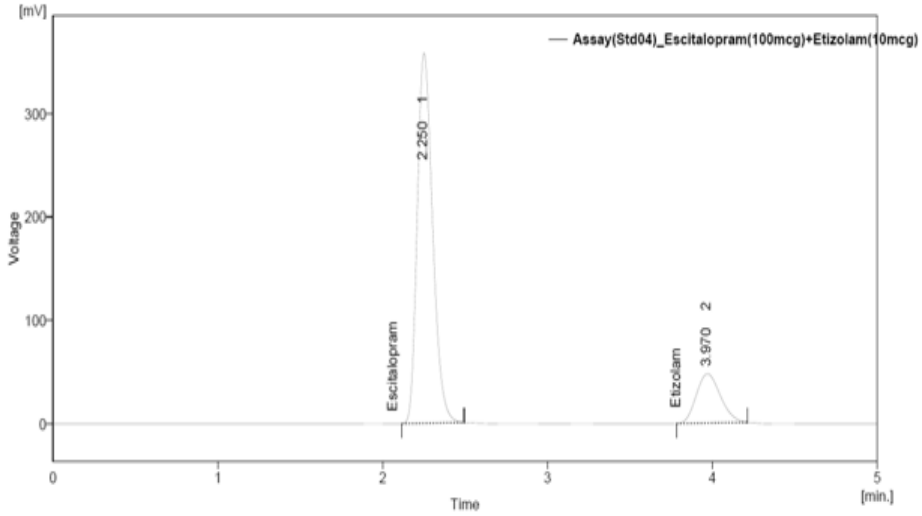


Fig: Chromatogram of Assay standard preparation-4

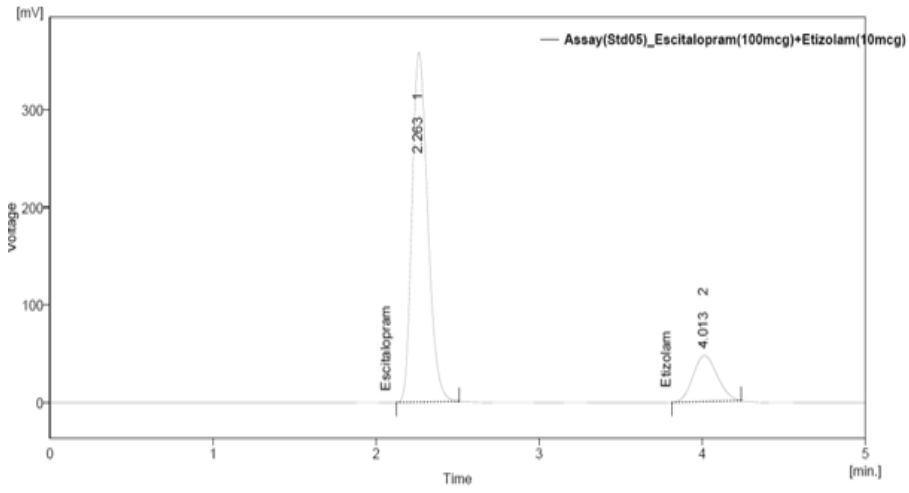


Fig: Chromatogram of Assay standard preparation-5

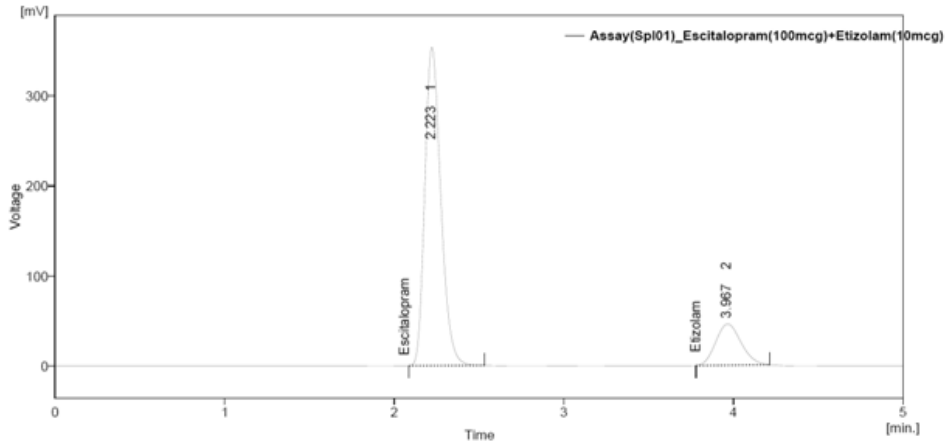


Fig: Chromatogram of Assay sample preparation-1

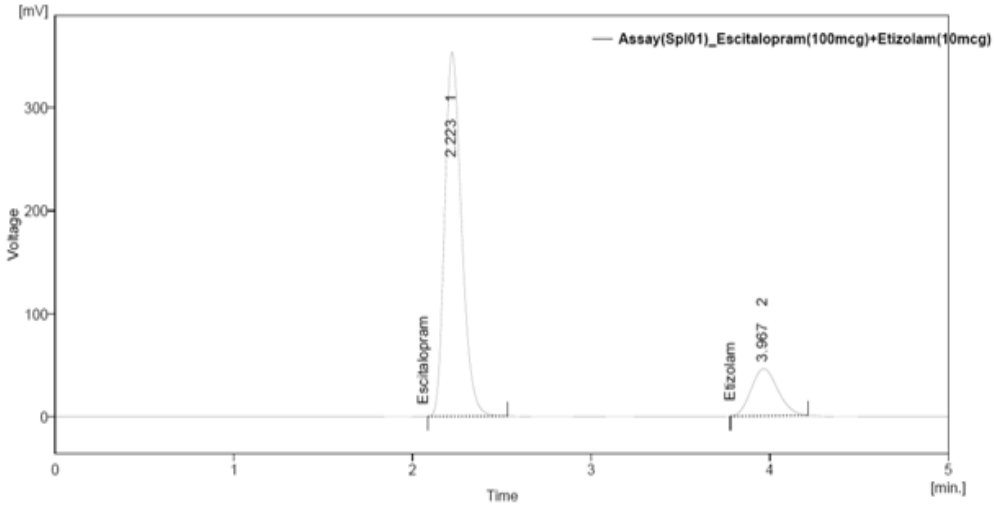


Fig: Chromatogram of Assay sample preparation-2

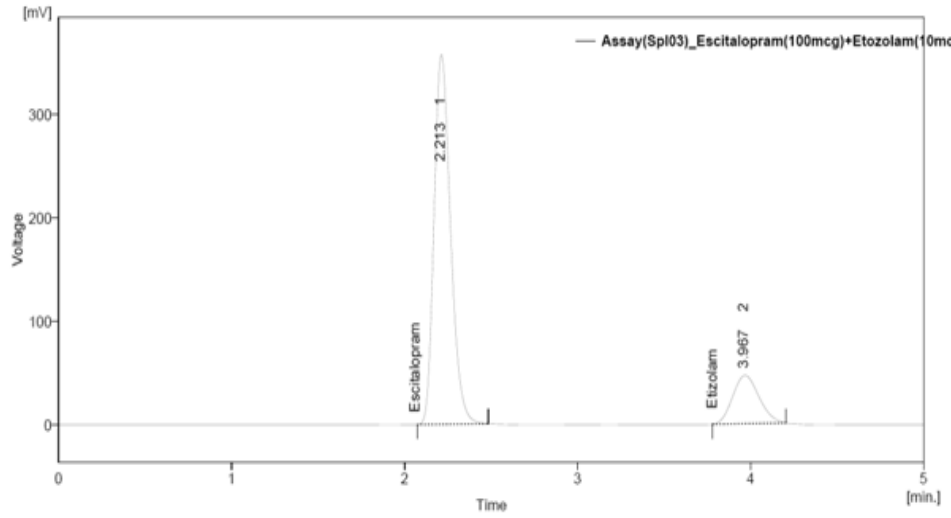


Fig: Chromatogram of Assay sample preparation-3

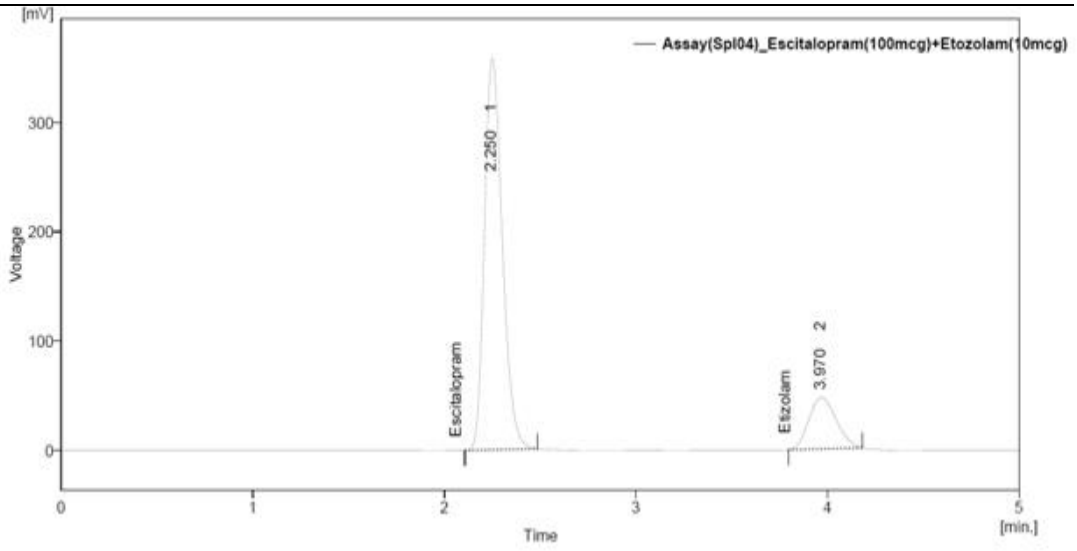


Fig: Chromatogram of Assay sample preparation-4

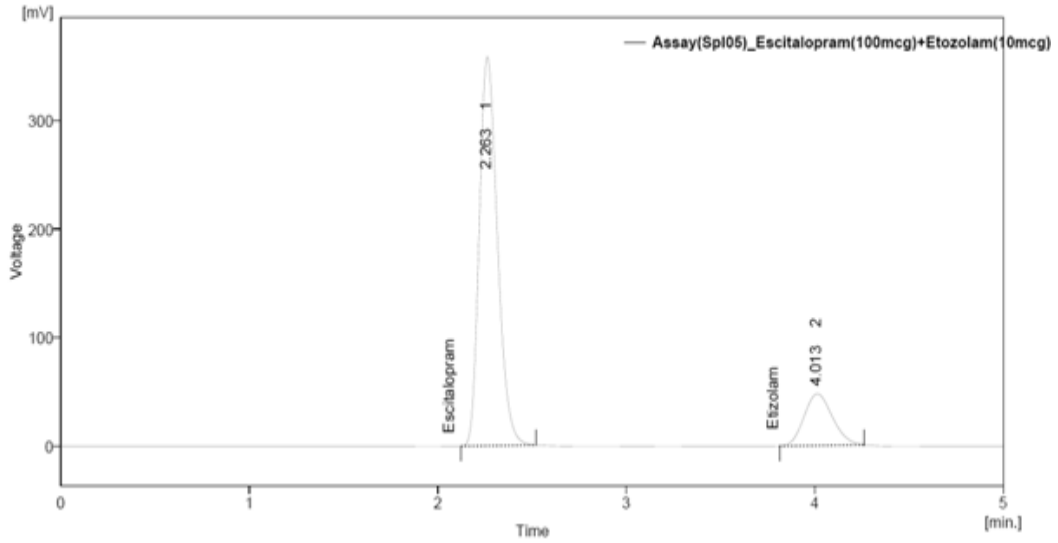


Fig: Chromatogram of Assay sample preparation-5

Table: Assay Results

ESCITALOPRAM			**ETIZOLAM**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	2306.966	2324.553	479.552	477.204
Injection-2	2393.229	2402.442	490.035	497.287
Injection-3	2368.996	2374.124	499.05	493.789
Injection-4	2324.494	2323.438	484.363	469.318
Injection-5	2398.839	2401.217	488.215	498.57
Average Area	2358.505	2365.155	488.243	487.2336
Standard deviatuion	39.24303		13.`17308	
%RSD	1.655898		1.551382	
Assay(%purity)	**100.08**		**98.50**	

Observation

The amount of ESCITALOPRAM and ETIZOLAM present in the taken dosage form was found to be 100.08 % and 98.50 % respectively.

VALIDATION

Specificity by Direct comparison method

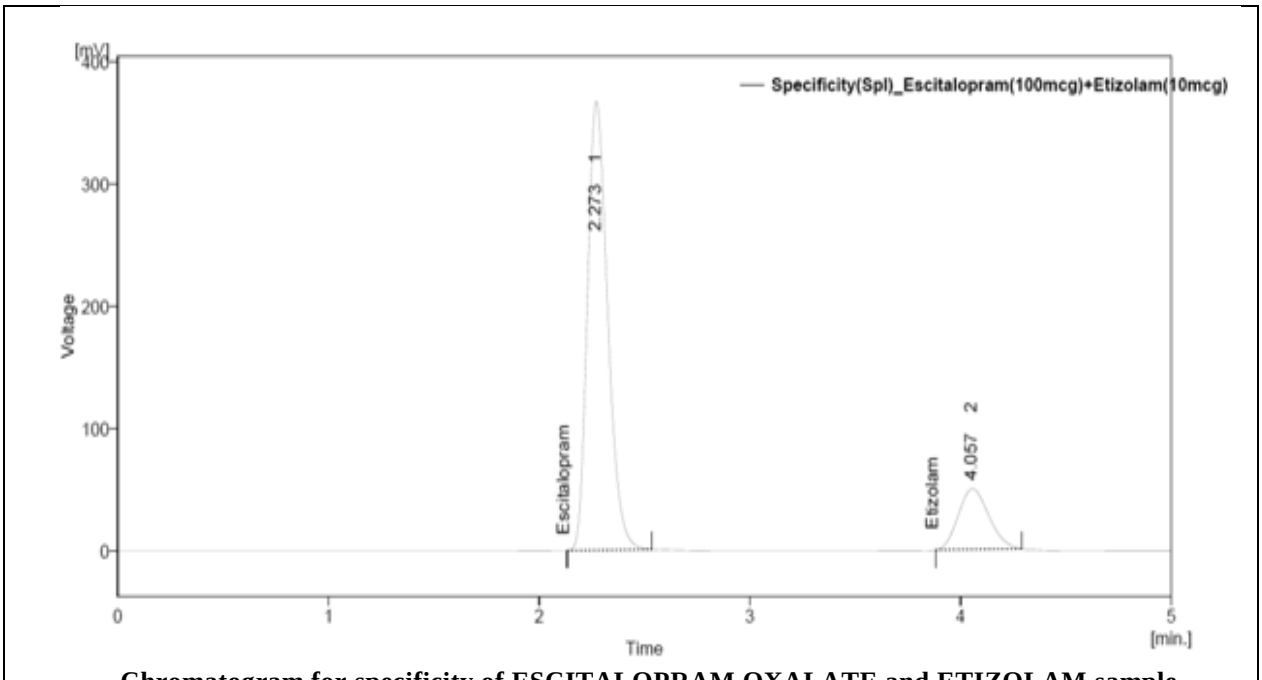
There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

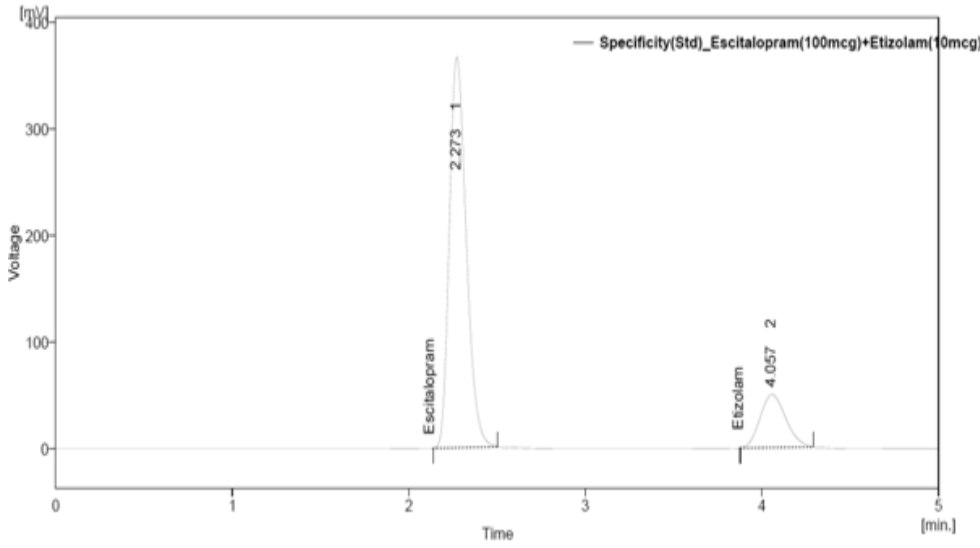
Weigh accurately 10mg of ESCITALOPRAM OXALATE and 10 mg of ETIZOLAM in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of ESCITALOPRAM OXALATE and ETIZOLAM is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains ETIZOLAM–0.5 mg, ESCITALOPRAM OXALATE -5 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of ETIZOLAM and ESCITALOPRAM OXALATE (µg/ml) were prepared by dissolving weight equivalent to 10 mg of ETIZOLAM and 20 mg of ESCITALOPRAM OXALATE and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of ETIZOLAM and ESCITALOPRAM OXALATE was made by adding 1 ml of stock solution to 10 ml of mobile phase.



Chromatogram for specificity of ESCITALOPRAM OXALATE and ETIZOLAM sample



Chromatogram for specificity of ESCITALOPRAM OXALATE and ETIZOLAM standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the ESCITALOPRAM OXALATE and ETIZOLAM peaks.

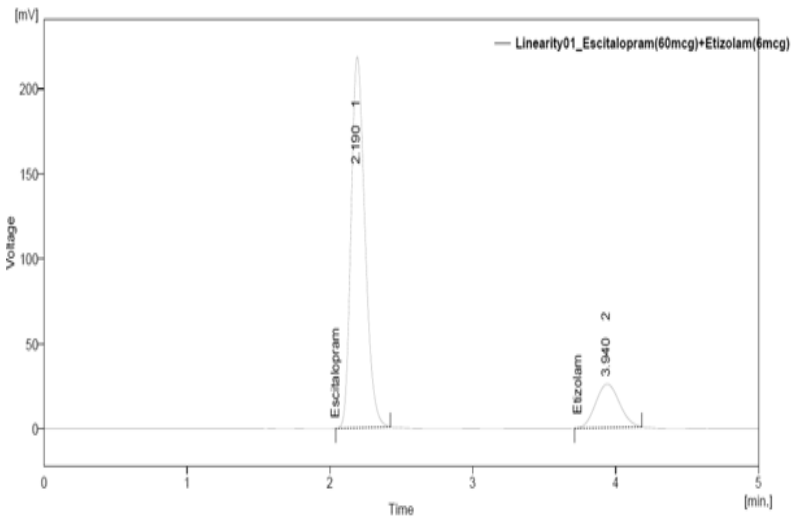
Linearity and range

Preparation of standard stock solution

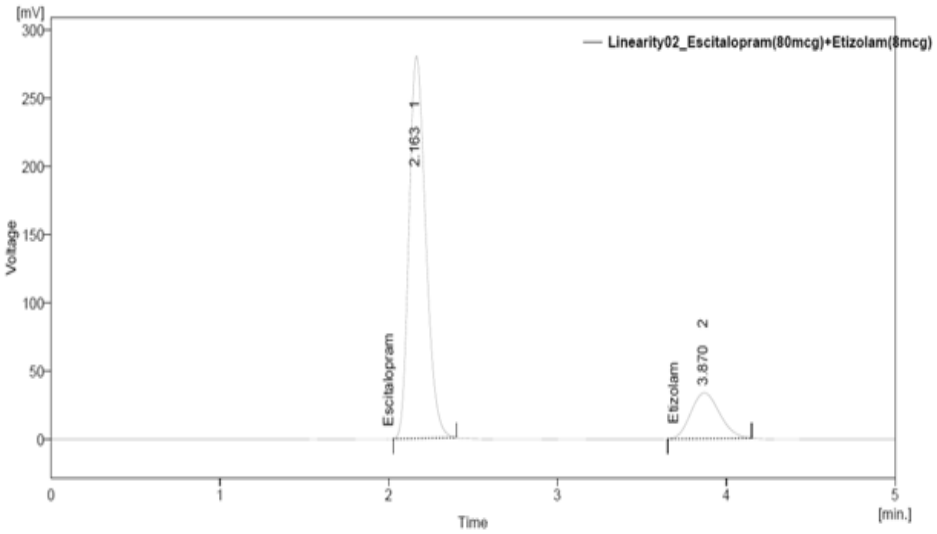
Standard stock solutions of ESCITALOPRAM OXALATE and ETIZOLAM (microgram/ml) were prepared by dissolving 10 mg of ESCITALOPRAM OXALATE and ETIZOLAM dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. Further dilutions were given in the table No 8.3.1.

Linearity Preparations

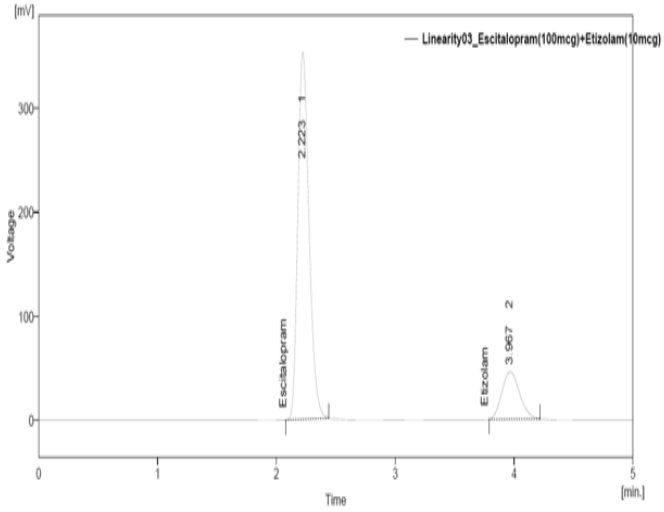
Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
			ESCITALOPRAM OXALATE	ETIZOLAM
Preparation 1	0.3	10	60	6
Preparation 2	0.4	10	80	8
Preparation 3	0.5	10	100	10
Preparation 4	0.6	10	120	12
Preparation 5	0.7	10	140	14



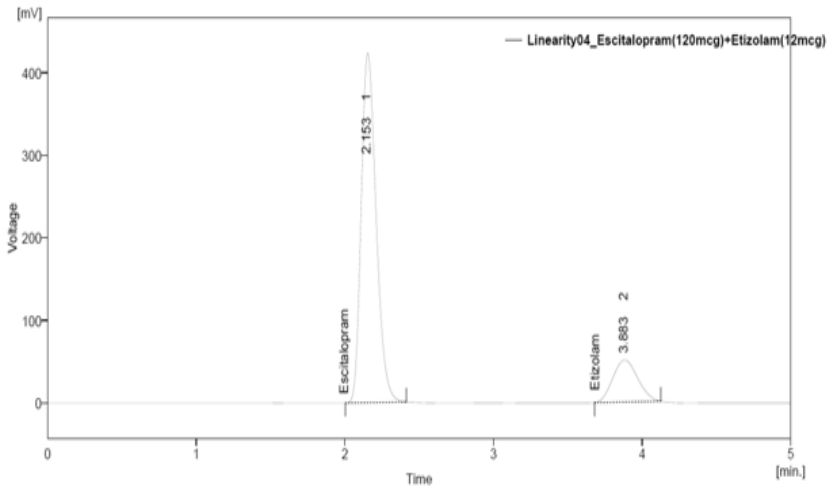
Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAMpreparation-1



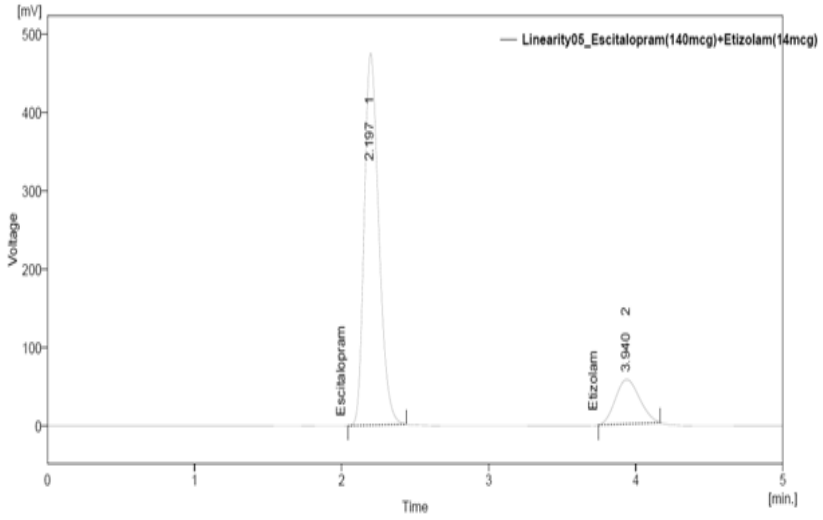
Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAMpreparation-2



Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAMpreparation-3



Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAM preparation-4



Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAM for preparation-5

Table: linearity of ESCITALOPRAM OXALATE

S.No.	Conc.(µg/ml)	Area
1	60	1479.248
2	80	1900.757
3	100	2407.394
4	120	2850.293
5	140	3271.353

Table linearity of ETIZOLAM

S.No.	Conc.(µg/ml)	Area
1	6	267.845
2	8	367.993
3	10	489.497
4	12	573.311
5	14	668.186

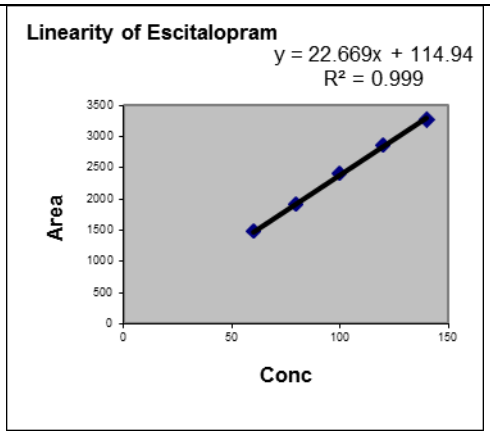


Fig. 9.3.9: Linearity graph of ESCITALOPRAM OXALATE

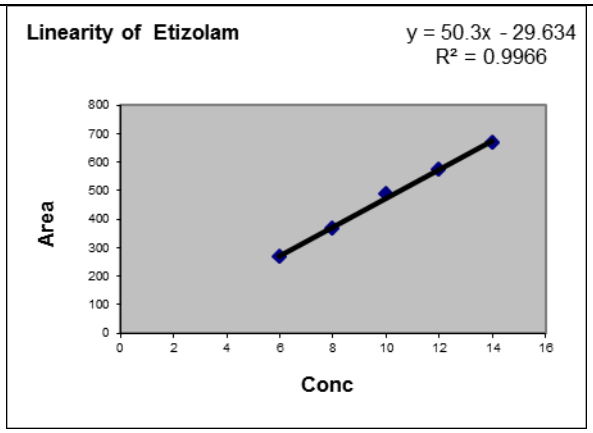


Fig. 9.3.9.1: Linearity graph of ETIZOLAM

Acceptance criteria

The relationship between the concentration of ESCITALOPRAM OXALATE and ETIZOLAM and area of ESCITALOPRAM OXALATE and ETIZOLAM should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of ESCITALOPRAM OXALATE and ETIZOLAM is 0.999 and 0.996. The relationship between the concentration of ESCITALOPRAM OXALATE and ETIZOLAM and area of ESCITALOPRAM OXALATE and ETIZOLAM is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 100%, 120%, 140%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 100%, 120%, 140%.

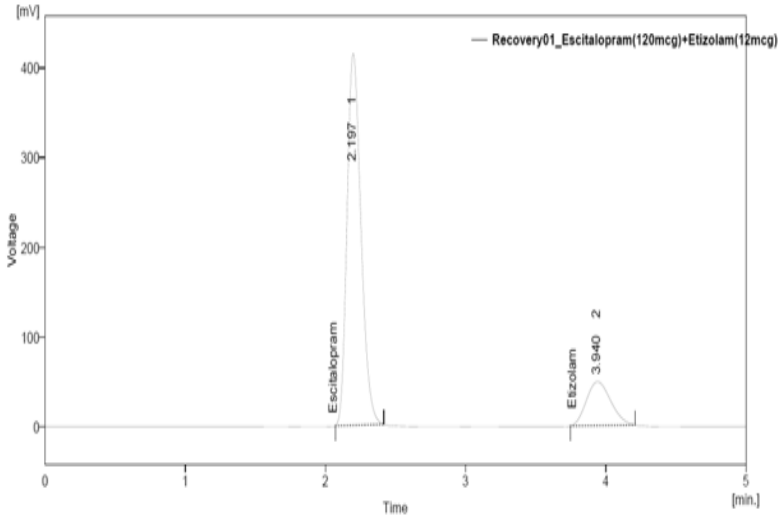


Fig: Chromatogram of 50% recovery (injection 1)

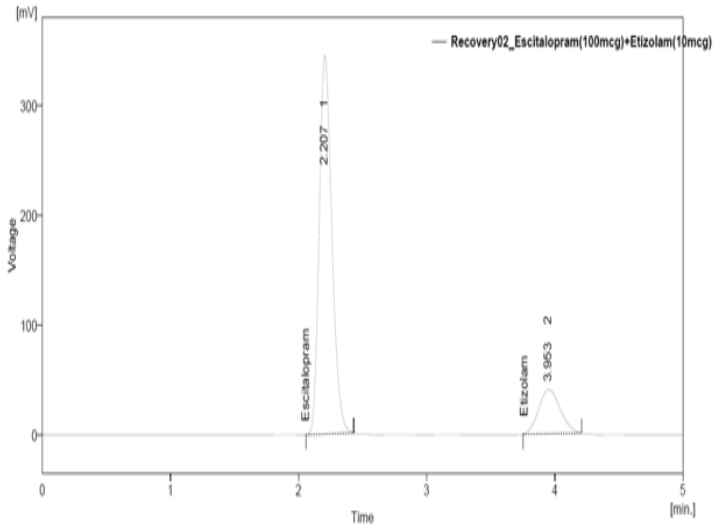


Fig: Chromatogram of 100% recovery (injection 2)

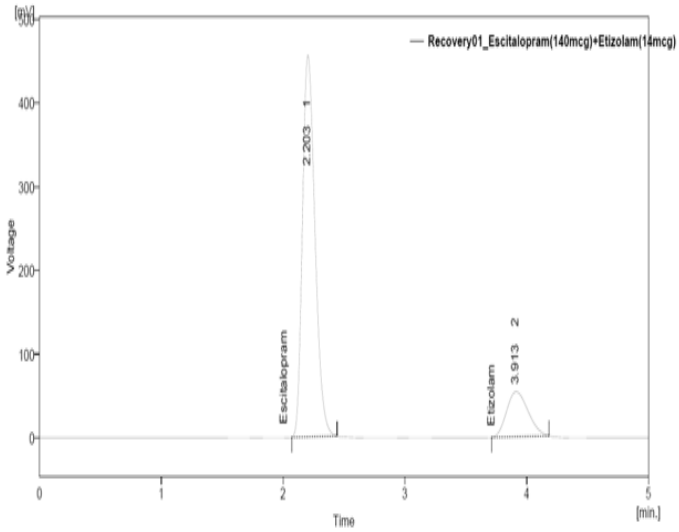


Fig: Chromatogram of 150% recovery (injection 3)

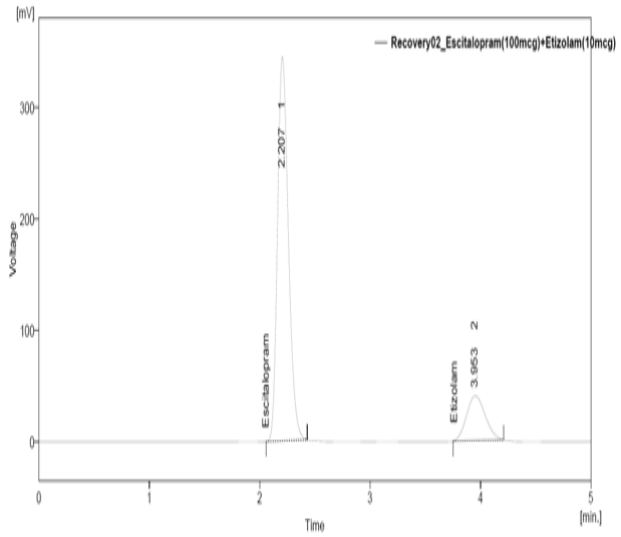


Fig: Chromatogram of 50% recovery (injection 1)

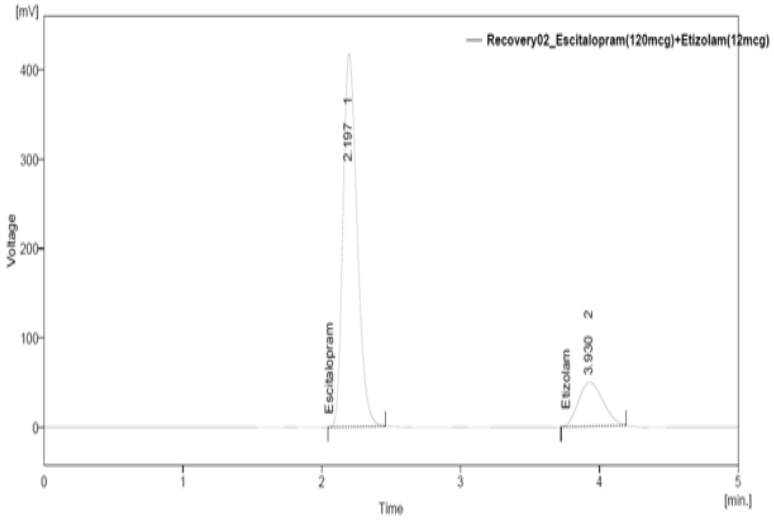


Fig: Chromatogram of 100% recovery (injection 2)

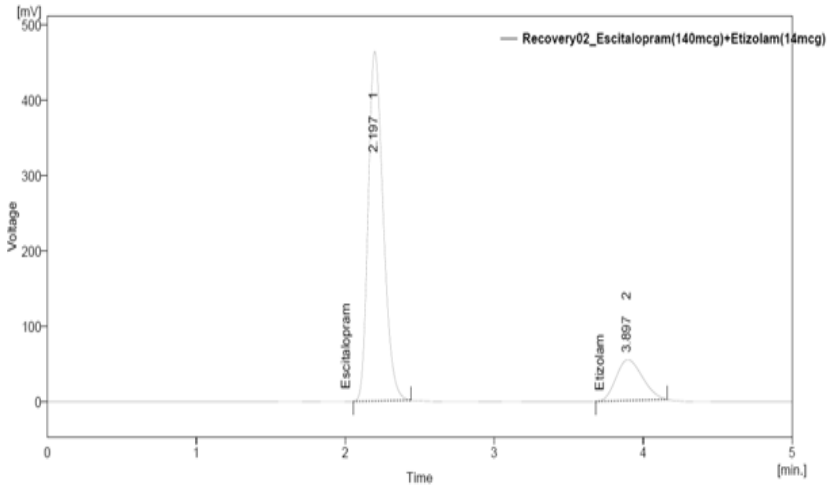


Fig: Chromatogram of 150% recovery (injection 3)

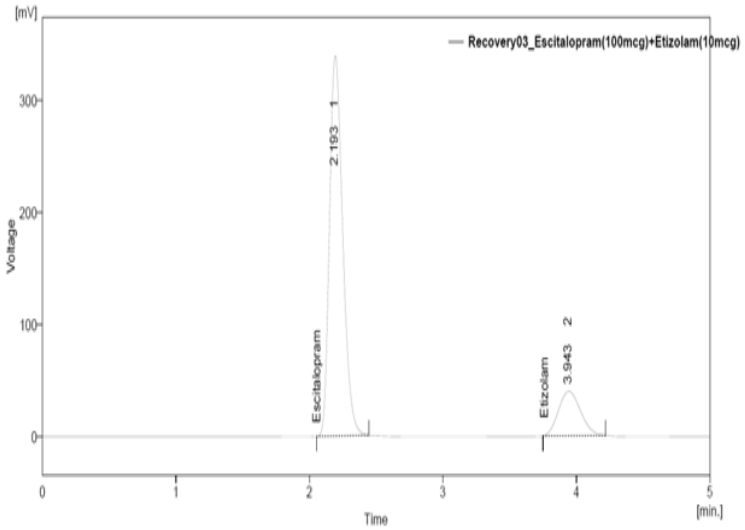


Fig: Chromatogram of 50% recovery (injection 1)

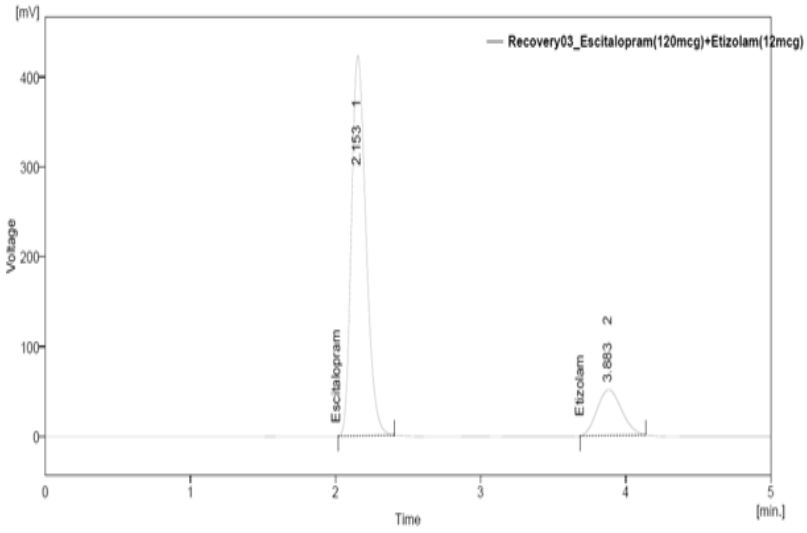


Fig: Chromatogram of 100% recovery (injection 2)

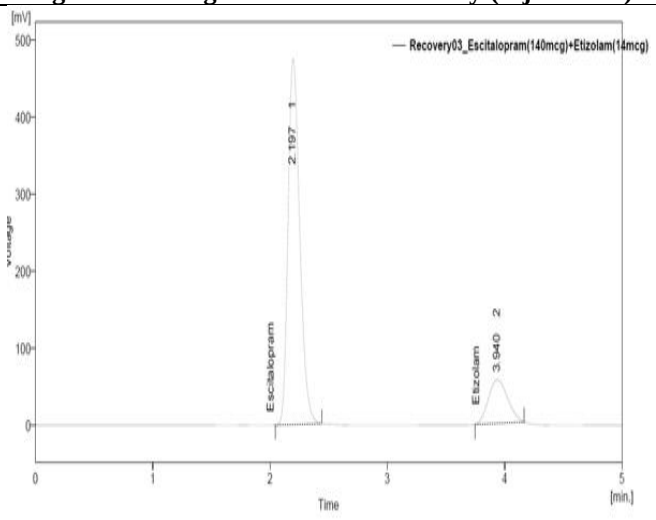


Fig: Chromatogram of 150% recovery (injection 3)

Acceptance criteria

The % recovery of ESCITALOPRAM OXALATE and ETIZOLAM should lie between 98 % and 102%.

Recovery results for ESCITALOPRAM OXALATE

Recovery level	Accuracy ESCITALOPRAM OXALATE					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recoverd	%Recovery	
100	**100**	**2323.152**	**2335.832**	98.31	98.31	99.19
	100	**2357.226**				
	100	**2327.117**				
120	**120**	**2861.207**	**2922.183**	121.38	101.15	
	120	**2960.687**				
	120	**2944.655**				
140	**140**	**3262.124**	**3262.966**	137.37	98.12	
	140	**3255.422**				
	140	**3271.353**				

Table 9.4.9.2: Recovery results for ETIZOLAM

Recovery level	Accuracy ETIZOLAM					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
100	10	**437.297**	**456.928**	9.93	99.33	99.89
	10	**472.154**				
	10	**461.334**				
120	12	**591.067**	**598.477**	12.23	101.89	
	12	**606.831**				
	12	**597.534**				
140	14	**660.340**	**658.550**	13.78	98.46	
	14	**667.125**				
	14	**648.186**				

Observation

The percentage mean recovery of ESCITALOPRAM OXALATE and ETIZOLAM is 99.19 % and 99.89 % respectively. .

Precision

Method precision

Prepared sample preparations of ETIZOLAM and ESCITALOPRAM OXALATE as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of ETIZOLAM and ESCITALOPRAM OXALATE should be not more than 2.0%.

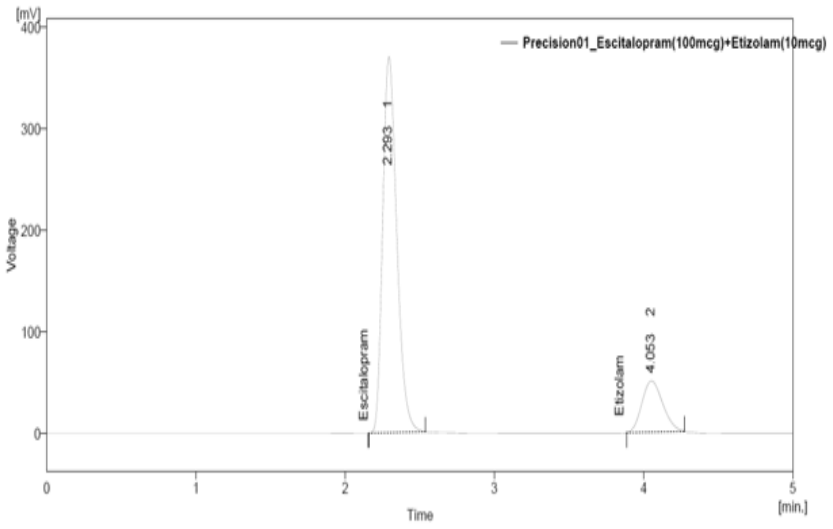


Fig: Chromatogram of precision injection 1

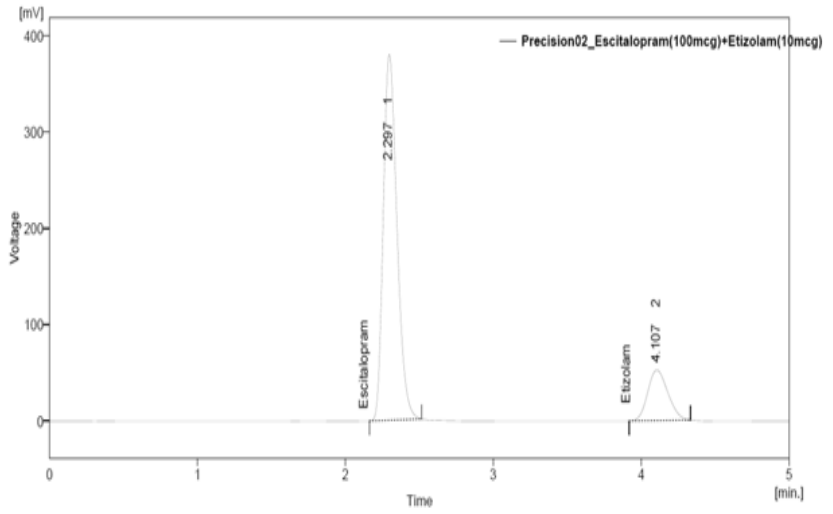


Fig: Chromatogram of precision injection 2

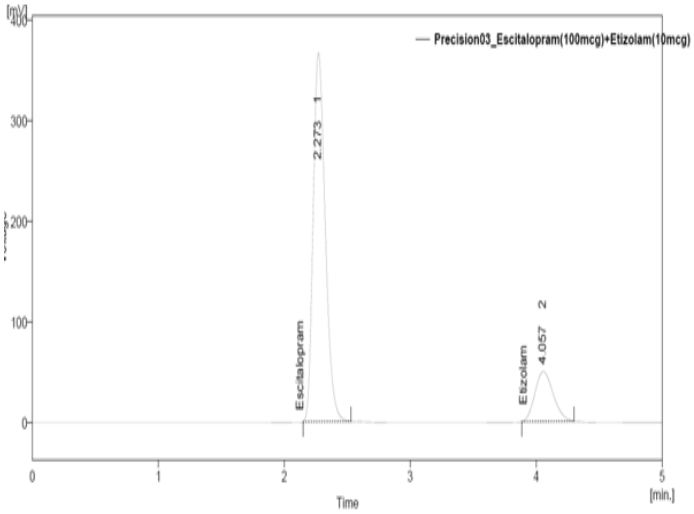


Fig: Chromatogram of precision injection 3

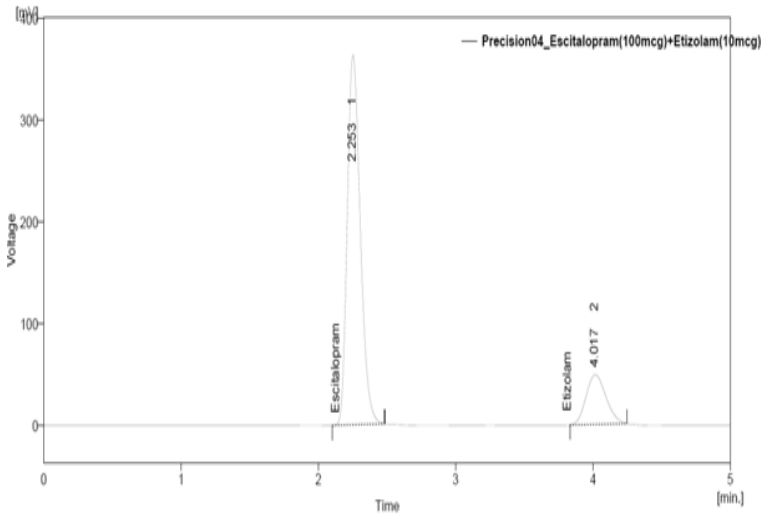


Fig: Chromatogram of precision injection 4

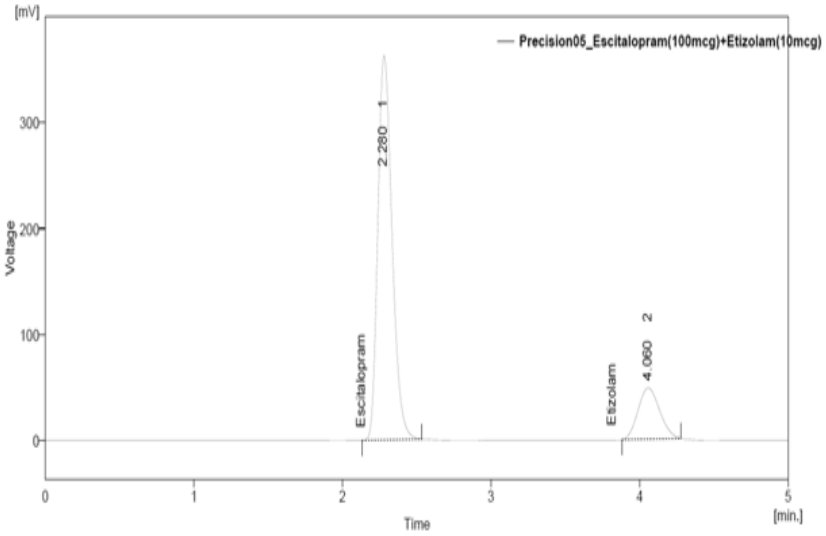


Fig: Chromatogram of precision injection 5

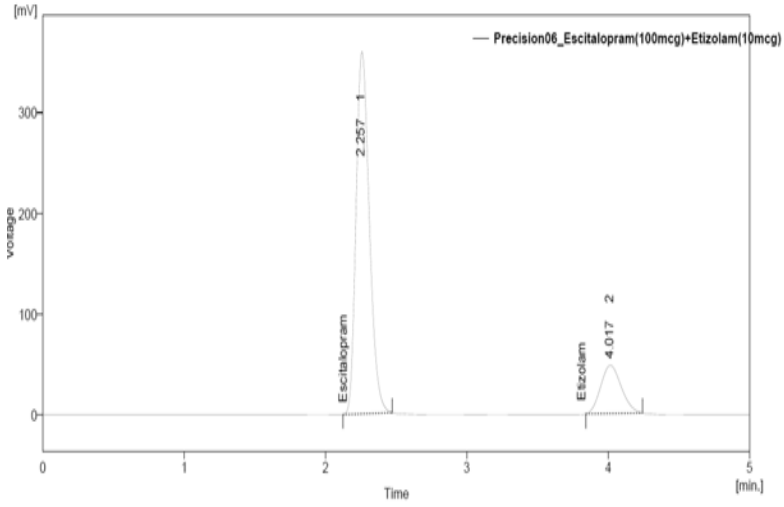


Fig: Chromatogram of precision injection 6

Results for Method precision of ESCITALOPRAM OXALATE and ETIZOLAM

ESCITALOPRAM OXALATE			ETIZOLAM		
S. No.	Rt	Area	S. No.	Rt	Area
1	2.293	2423.957	1	4.053	490.117
2	2.297	2438.926	2	4.107	495.49
3	2.273	2440.250	3	4.057	503.271
4	2.253	2396.625	4	4.017	492.889
5	2.28	2411.747	5	4.060	487.714
6	2.257	2353.154	6	4.017	483.477
avg	2.2755	2410.777	avg	4.052	492.160
stdev	0.0181	32.730	stdev	0.033	6.847
%RSD	**0.80**	**1.36**	**%RSD**	**0.82**	**1.39**

Observation

Test results for ETIZOLAM and ESCITALOPRAM OXALATE are showing that the %RSD of Assay results are within limits.

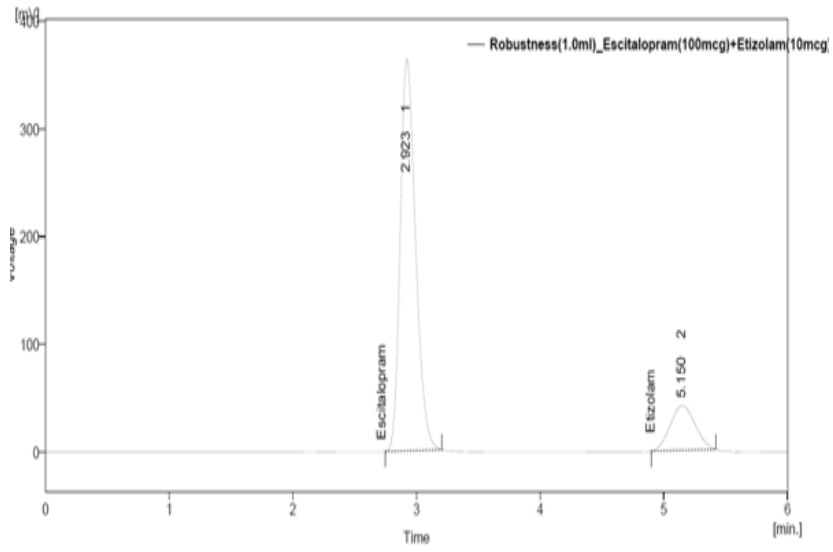
Robustness

Chromatographic conditions variation

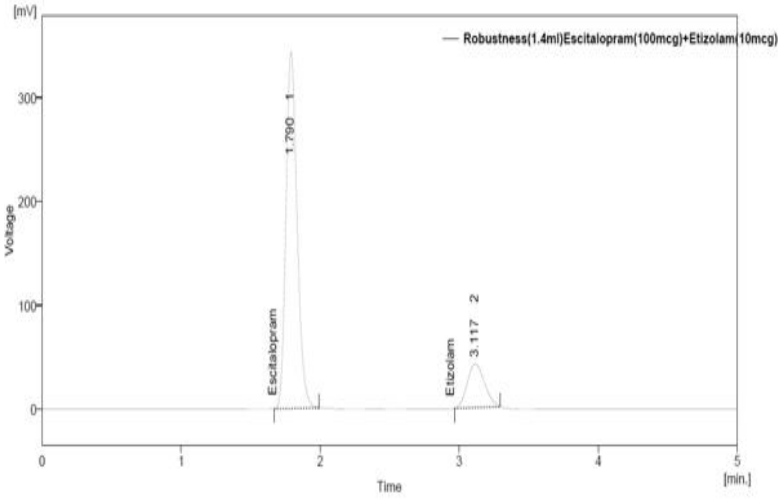
To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

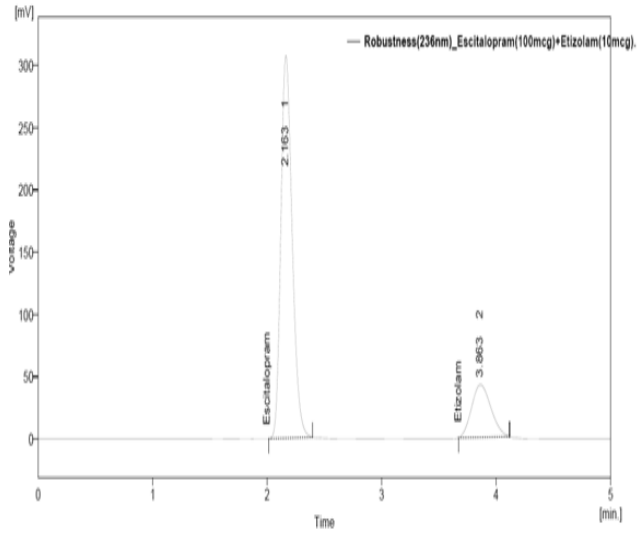
The system suitability should pass as per the test method at variable conditions.



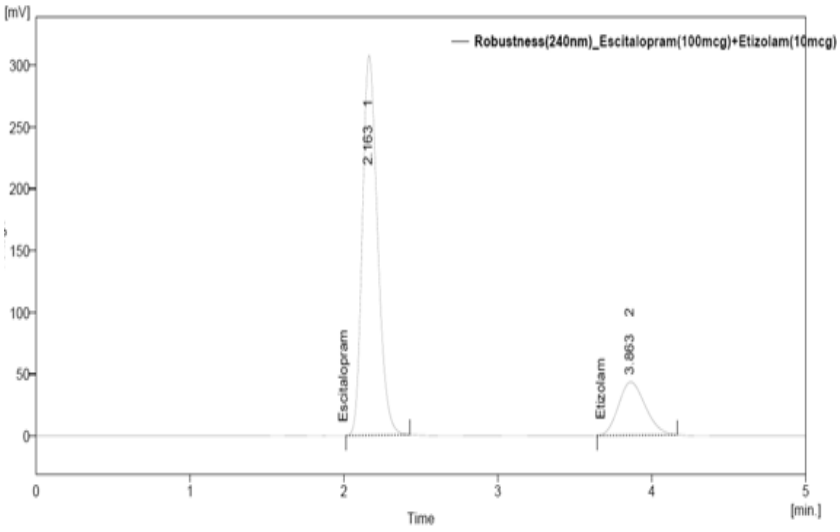
Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAM Robustness (1.0 ml/min)



Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAM Robustness (1.4 ml/min)



Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAM for Robustness (236nm)



Chromatogram of ESCITALOPRAM OXALATE and ETIZOLAM for Robustness (240nm)

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

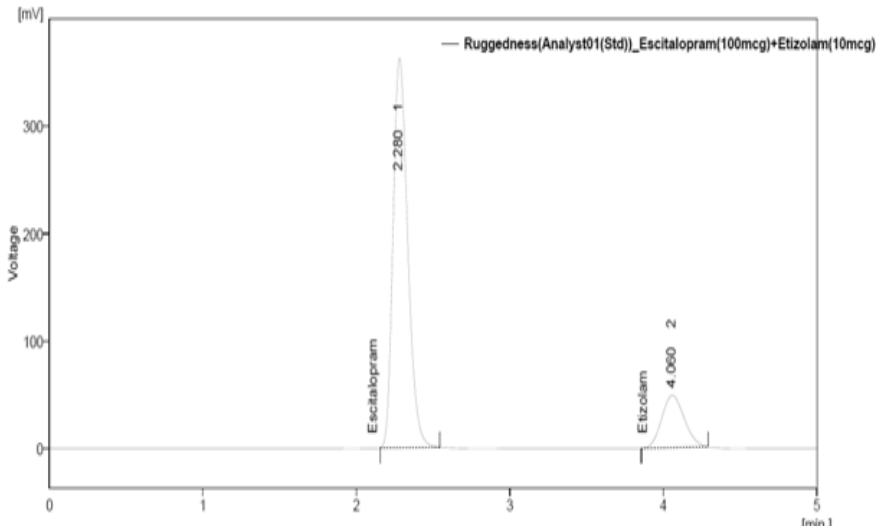


Fig: Chromatogram of Analyst 01 standard preparation

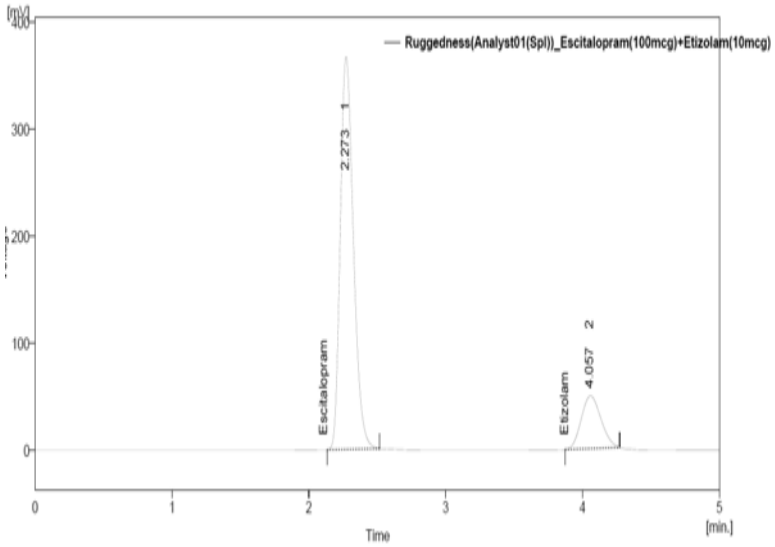


Fig: Chromatogram of Analyst 01 sample preparation

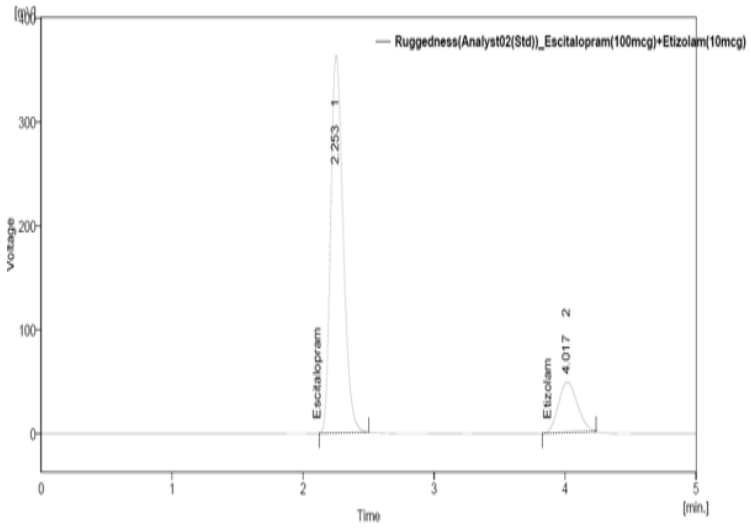


Fig: Chromatogram of Analyst 02 standard preparation

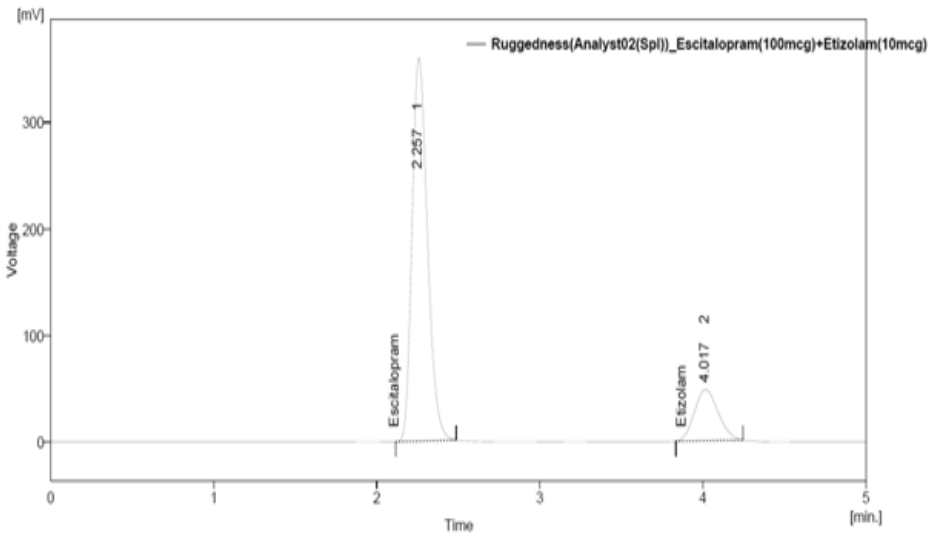


Fig: Chromatogram of Analyst 02 sample preparation

Results for Ruggedness

ESCITALOPRAM OXALATE	%Assay	ETIZOLAM	%Assay
Analyst 01	101.45	**Analyst 01**	99.00
Anaylst 02	98.34	**Anaylst 02**	100.02

Observation

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

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