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Method Development and Validation of Stability Indicating U-HPLC Method for Simultaneous Estimation for Escitalopram and Flupenthixol in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

This research work aimed to develop a simple, precise and rapid U-HPLC Method of analysis of Escitalopram and Flupenthixol in tablet dosage form. RP-HPLC method was developed for the estimation of Escitalopram and Flupenthixol in tablet dosage form. The proposed methods were applied for the determination of drug in tablet dosage form. A rapid and reliable RP-HPLC method was developed and validated estimation of Escitalopram and Flupenthixol in tablet dosage form. The U-HPLC method was performed C18-(100mm x 4.6 mm,) 2.5 µm particle size, and the sample was analysed using methanol 35 ml and 65 ml 0.1% Glacial acetic acid as a mobile phase at a flow rate of 0.7 ml/min and detection at 235 nm. By the retention time for Escitalopram and Flupenthixol was found 3.012 and 6.795 min. The method was applied to marketed tablet formulations. The tablet assay was performed was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with ICH guidelines. Validation related the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots by HPLC were linear over the 10-50 µg/ml for Escitalopram and Flupenthixol, and recoveries from tablet dosage form were between101.49 and 98.60% The method can be used for routine of the quality control in pharmaceutical The U-HPLC method was found to be simple, economical and rapid as compared to MS method was found to be more accurate, precise and robust. These methods can be used for routine analysis of Escitalopram and Flupenthixol in tablet dosage form.

DRUG PROFILE

Escitalopram

Structure:

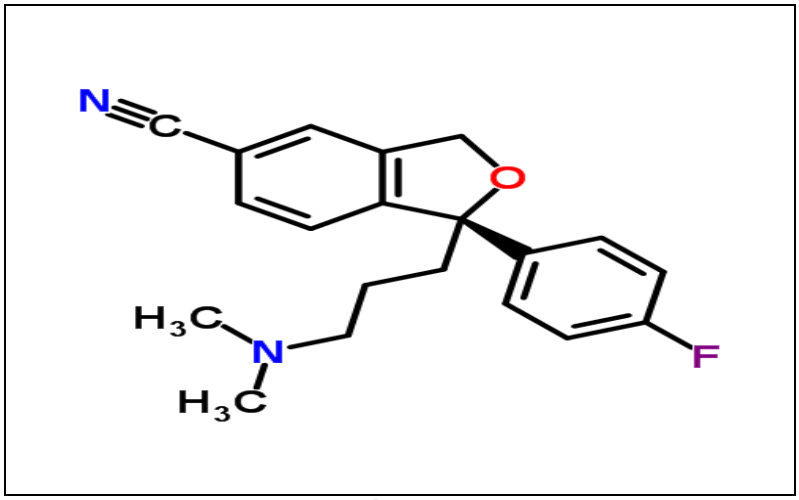

Fig. No. 1 Structure of Escitalopram

Iupac name :

(S)-1-[3-(Dimethylamino)propyl]-1-(4-flurophenyl)-1,3-dihydrobenzofuran-5-carbonitrile

Molecular Formula **:** $C_{20}H_{21}FN_2O$

Molecular Weight **:** 469.658 g/mol

Appearance **:** White to off-white powder.

Solubility **:** Freely soluble in Methanol and DMSO sparingly soluble In water and ethanol.

Category **:** Antidepressant.

Mechanism of action:

The 5-HT (5-hydroxytryptamine, serotonin) transporter (SERT) mediates the reuptake of 5-HT from the synaptic cleft into the neuron, and inhibition of this uptake is the target of selective serotonin reuptake inhibitors (SSRIs). Escitalopram (S-citalopram) is the most selective SSRI available, whereas the other enantiomer, R-citalopram, is less approximately 30–40 times more potent than the S-enantiomer.

Pharmacokinetics:

Citalopram and escitalopram are associated with dose-dependent QT interval prolongation and should not be used in those with congenital long QT syndrome or known pre-existing QT interval prolongation, or in combination with other medicines that prolong the QT interval. ECG measurements should be considered for patients with cardiac disease, and electrolyte disturbances should be corrected before starting treatment. In December 2011 the UK implemented new restrictions on the maximum daily dose. 10 mg for patients older than 65 years other doses remain unchanged.

Adverse drug reactions:

Escitalopram, like other SSRIs, has been shown to affect sexual functions causing side effects such as decreased libido, delayed ejaculation, genital anesthesia and anorgasmia. An analysis conducted by the FDA found a statistically insignificant 1.5 to 2.4-fold (depending on the statistical technique used) increase of suicidality among the adults treated with escitalopram for psychiatric indications. Similarly, the UK MHRA data indicate an 80% increase of suicide-related events, not reaching statistical significance, in the escitalopram vs. placebo patients. The authors of a related study note the general problem with statistical approaches: due to the rarity of suicidal events in clinical trials, it is hard to draw firm conclusions with a sample smaller than two million patients.

Drug–food interactions

Escitalopram, similarly to other SSRIs (with the exception of fluvoxamine), inhibits CYP2D6 and hence may increase plasma levels of a number of CYP2D6 substrates such as aripiprazole, risperidone, Tramadol, codeine, etc. Escitalopram can also prolong the QT interval and

hence it is not recommended in patients that are concurrently on other medications that can prolong the QT interval. Being a SSRI, escitalopram should not be given concurrently with MAOIs or other serotonergic medications

Escitalopram significantly alters the effectiveness of codeine as an analgesic. Much of the analgesic effect of codeine is attributable to its conversion (10%) to morphine. In most people, Escitalopram strongly inhibits this conversion, leading to reduced effectiveness of codeine; however, in a minority genetic group (known as CYP2D6 ultrarapid metabolizers), the opposite is the case, leading to increased morphine levels

Flupenthixol

Structure:

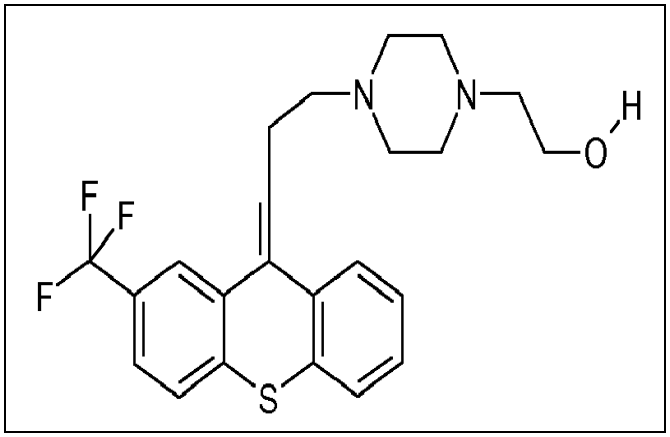

Fig.No. 2 Structure of Flupenthixol

Iupac name:

(EZ)-2-[4-[3-[2-(triflurомethyl)thioxanthine-9-ylidinw]propyl]piperazine-1-yl]ethanol

Molecular Formula **:** $C_{23}H_{25}F_3N_2OS$

Molecular weight **:** 434.5219g/mol

Appearance **:** White to off-white powder.

Solubility **:** Freely soluble in ethanol and methanol

Category : Antipsychotic

Mechanism of action:

Flupenthixol is a thioxanthene antipsychotic. The mechanism of action of Flupenthixol is not completely understood. Flupenthixol is a powerful antagonist of both D1 and D2 dopamine receptors, and an alpha-adrenergic receptor antagonist. Its antipsychotic activity is thought to be related to blocks postsynaptic dopamine receptors in the CNS

Pharmacokinetics:

Flupenthixol is an anxiolytic, antidepressive agent and a mood stabilizer. It inhibits the central monoamine receptors, particularly the dopamine D1 and D2 receptors. Therefore, it increases the amount of serotonin and noradrenaline that control mood and thinking, and improves mood.

Adverse drug reactions:

Unknown incidence adverse effects include

- Jaundice
- Abnormal liver function test results
- Tardive dyskinesia an often incurable movement disorder that usually results from years of continuous treatment with antipsychotic drugs, especially typical antipsychotics like flupenthixol. It presents with repetitive, involuntary, purposeless, and slow movements.
- Hypotension
- Confusional state
- Seizures
- Mania

Drug interactions:

It should be not used concomitantly with medications known to prolong the QTc interval (e.g. 5-HT3 antagonists, tricyclic antidepressants, citalopram, etc.) as this may lead to an increased risk of QTc interval prolongation Neither should it be given concurrently with lithium (medication) as it may increase the risk of lithium toxicity and neuroleptic malignant syndrome. It should not be given concurrently with other antipsychotics due to the potential for this to increase the risk of side effects, especially neurological side effects such as neuroleptic malignant syndrome. It should be avoided in patients on CNS depressants such as opioids, alcohol, and barbiturates.

AIM AND OBJECTIVES

The ever-growing number of drugs and their combinations in the market leads to the need for the development of analytical methods for their quality control. The methods must be such that it takes less time in their development as well as the best accurate and robust results should be obtained. The combination dosage form selected for the present study contains Escitalopram and Flupenthixol in solid oral dosage forms, recently this combination has been approved by USFDA (United States food drug administration).

The aim of work is to develop and validate a simple, precise, accurate and economical U-HPLC method as per ICH guidelines for the estimation of Escitalopram and Flupenthixol in bulk and pharmaceutical dosage forms.

Rationale

Literature survey revealed that, some analytical methods were reported for estimation of Escitalopram and Flupenthixol individually or in combination with other drugs by UV Spectroscopy, HPLC analytical methods.

No of stability indicating RP-HPLC method was reported for estimation of both these drugs. Now a day stability indicating method as important for regulatory & C-GMP point of view to assess the drug stability In the present study it was tried to developed stability indicating RP-HPLC method to determine possible degradation products of Escitalopram and Flupenthixol.

EXPERIMENTAL WORK

Chemicals used:

In method development and validation of preservatives following chemicals and reagents were used.

Table 1: List of chemicals

Ingredients	Grade	Suppliers
Escitalopram	API	R.S.I.T.C Jalgaon.
Flupenthixol	API	R.S.I.T.C Jalgaon.
Glacial Acetic Acid	HPLC	Merck Specialities Pvt. Ltd.
Ortho phosphoric acid(OPA)	HPLC	Avantor Performance material India Ltd. Thane, Maharashtra
MEOH	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai
Water	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager 'A' Worli, Mumbai

List of Marketed formulations

Table 2: List of brand names of combined formulations of Flupenthixol and Escitalopram

Sr. No	Brand name	Formulation	Available strength	company
1.	**Rexipra fx 10**	TABLET	Escitalopram 10 mg and 0.5 mg Flupenthixol	Intas Pharma

HPLC

Selection of Analytical Technique

HPLC was selected as analytical technique for estimation of Flupenthixol and Escitalopram.

Instruments

The analysis of the drug was carried out on Agilent (S.K.) Gradient System UV Detector. Equipped with Reverse Phase (Agilent) C18 column (4.6mm x 100mm; 2.5µm), a SP930Dpump, a 20µl injection loop and UV730D (DAD) Absorbance detector and running Chemstation software.

Table 3: List of Instruments

Sr. no.	Name of Instrument	Company Name
1	HPLC Instrument	Agilent 1100with auto sampler (Chemstation software)
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C18)	Agilent C18 (100mmX 4.6mm,5µm)
4	pH meter	VSI pH meter (VSI 1-B)
5	Balance	WENSAR™ High Resolution Balance.
6	Sonication	Ultrasonic electronic instrument

a) Chromatographic Conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table 4: Chromatographic conditions (HPLC) details used during method

Development

1.	HPLC	Agilent (S.K)Gradient System
2.	Software	Chemstation
3.	Column	(Agilent) C18 column (4.6mm x 100mm)
4.	Particle size packin	2.5 □m
5.	Stationary phase	C-18 (Agilent)
6.	Mobile Phase	MEOH: Water (0.1% with OPA)
7.	Detection Waveleng	235 nm
8.	Flow rate	0.7ml/min
9.	Temperature	25° C (Ambient)
10.	Sample size	20 □l
11.	pH	4.2
12.	Run Time	15 min
13.	Filter paper	0.45 □m

Study of Escitalopram and Flupenthixol on the chromatographic conditions used in method development of HPLC for the following mobile phase were tried

Method Development of HPLC

List of Trials

Table 5: Selection of Mobile Phase

Sr. No	Mobile Phase
1.	[35% MEOH +65% Water (pH 4.2 adjust with OPA) Flow 1 ml/min at 235 nm (column 100mm X 4.6, 2.5 µm)
2.	[25% MEOH +75% Water (pH 4.2 adjust with OPA) Flow 1 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)
3	[50% MEOH +50% Water (pH 4.2 adjust with OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)
4	[60% MEOH +40% Water (0.1% OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)
5	[65% MEOH +35% Water (0.1% OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)
6	[35% MEOH +65% Water (0.1 % Glacial Acetic Acid) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Analysis of standard drugs was done by following parameters

- Melting point
- Solubility
- UV spectra and λ_{max}

- HPLC chromatogram and retention time

Selection of wavelength by UV-Visible Spectrophotometry

Preparation of standard stock solution

Escitalopram standard stock solution (Stock I)

An accurately weighed quantity, 10 mg of Escitalopram (ESP) was dissolved in methanol in a 10 ml volumetric flask and volume made up to 10 ml to produce a solution of 1000 ug/ml.

Flupenthixol standard stock solution (Stock II)

An accurately weighed quantity, 0.5 mg of Flupenthixol (FLP) was dissolved in methanol in 10 ml volumetric flask and volume made up to 10 ml to produce a solution of 50 ug/ml.

Preparation of Stock Standard Combination Solution (Stock III) [FLP + EMPA

Accurately weight and transfer 10 mg Escitalopram and Flupenthixol 0.5mg working standard into 100 ml volumetric flask as about diluent methanol completely and make volume up to the mark with the same solvent to get 1000 & 50 µg/ml standard (stock solution) and 15 min sonicate to dissolve it and remove the unwanted gas, further an aliquots portion of Escitalopram and Flupenthixol stock solution in ratio of 1:20 were mixed in volumetric flask in 10 ml and volume was adjusted up to mark with mobile phase from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with MEOH :Water (0.1% Glacial Acetic Acid), prepared in (35 ml MEOH : 65ml Water (0.1% Glacial Acetic Acid) solvent.

HPLC used for chromatographic condition apply on the Preparation of standard solution

Preparation of Std. Escitalopram solution (Stock I)

An accurately weighed quantity, 10 mg of Escitalopram (ESP) was dissolved in methanol in a 10 ml volumetric flask and volume made up to 10 ml to produce a solution of 1000 ug/ml. From the freshly prepared standard stock solution (1000 ug/ml), 0.4ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 40 ug/ml.

Preparation of Std. Flupenthixol solution (Stock II)

An accurately weighed quantity, 0.5 mg of Flupenthixol (FLP) was dissolved in methanol in 10 ml volumetric flask and volume made up to 10 ml to produce a solution of 50 ug/ml from the freshly prepared standard stock solution (50ug/ml), 0.4 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 2 ug/ml.

Preparation of Std. Escitalopram and Flupenthixol solution (Stock III)

From the freshly prepared standard stock solution (1000 & 50ug/ml), 0.4 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 40& 2ug/ml.

Selection of mobile phase

Each mobile phase was vacuums degassed and filtered through 0.45µ membrane filter. The mobile phase was allowed to equilibrate until 0.1% Glacial Acetic Acid by baseline was obtained. The standard solution containing mixture of Escitalopram and Flupenthixol was run with different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing MEOH and Water (0.1% Glacial Acetic Acid) was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Escitalopram and Flupenthixol. Chromatograms of Escitalopram and Flupenthixol are shown in **(Table)** respectively.

Studies of Calibration plot.

Optimization of Chromatographic condition.

The following chromatographic conditions were established by trial and error and were kept constant throughout the analysis

- Column: C18 (100 mm× 4.6mm)
- Particle size packing: 2.5µm

- Detection wavelength: 235 n
- Flow rate: 0.7ml/min
- Temperature: Ambient
- Sample size: 20 µl
- Mobile phase: MEOH: Water (0.1% 0.1% Glacial Acetic Acid) (35:65)

Procedure for calibration curve of Escitalopram and Flupenthixol

The mobile phase was allowed to equilibrate with stationary phase until 0.1% Glacial Acetic Acid by baseline was obtained. From the freshly prepared standard stock solution, pipette out 10 mg Escitalopram and 0.5 mg Flupenthixol in 10 ml of volumetric flask and diluted with mobile phase. From it 0.4, 0.8, 1.2, 1.6 and 2 of solution were pipette out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 2,4,6, 8 and 10 µg/ml of Flupenthixol and 40, 80, 120, 160and 200 µg/ml of Escitalopram.

Sample was injected and peaks were recorded at 235 nm as the graph plotted as concentration of drug verses peak area is depicted in **(figure)** respectively.

Study of system suitability parameters:

The system suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from two replicate injections of standard solution.

Calibration Experiment

HPLC Method

a) Preparation of Calibration curve standard

The above standard stock solution (1: 20µg/ml) of Escitalopram and Flupenthixol was diluted with mobile phase to yield five calibration curve (cc) standards with concentrations of 2, 4, 6,8 and 10 µg/ml of Flupenthixol and 40, 80, 120, 160and

200 µg/ml of Escitalopram. (**Table) & (Table)** the calibration curve of Escitalopram and Flupenthixol is depicted in (**Figure and Figure).**

b) Selection of detection Wavelength.

Standard solutions were scanned in the range of 200-400nm, against 10 ml MEOH and volume make with water solvent system as reference Escitalopram **(Figure)** and Flupenthixol **(Figure)** were showed absorbance maxima (lambda max) at 237 nm and 233 nm respectively **(Figure**).

If Two Escitalopram and Flupenthixol sample Interact with this point is called Isosbestic point. Then detection of wavelength in Isosbestic point in 235 nm were selection wavelength is HPLC Method can be used.

c) Calibration standard drug and regression equation data.

From the standard stock solution of Escitalopram and Flupenthixol, different concentration were prepared respectively in the range of 40-200 µg/ml for Escitalopram (**Figure)** and 2-10 µg/ml for Flupenthixol and measured at 237 nm and 233 nm. The calibration curves were plotted **(Figure)** and Regression equation data presented in **(Table).**

d) Calibration runs and regression analysis.

These calibration standard solutions were analysed in three replicates using the under mentioned chromatographic conditions.

- Analytical column: AgilentC18 Column (100mm x 4.6mm, 2.5µm particle size).
- Injection volume: 20µl.
- Flow rate: 0.7ml/min.
- Mobile phase: MEOH: Water (0.1% Glacial Acetic acid) (35:65 % V/V).
- Detection: 235 nm.

Validation of method for analysis of Escitalopram and Flupenthixol

 T h e developed method was validated as per ICH guidelines.

Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range, The Result are shown in **Table.**

 Determination

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as functions of analyte concentration (Figure) Percentage curve fittings are calculated**.** The Result are shown in **(Table) (Figure).**

 Acceptance Criteria

The plot should be linear passing through the origin.

Correlation Coefficient should not be less than 0.999. The Result are shown in

 Preparation of standard stock solution for linearity

Average weight of tablet sample (equivalent to 10 mg of Escitalopram and 0.5 mg of Flupenthixol) were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling 0.1 ml of this solution diluted up to 10 ml volumetric flask with diluents was added to make up the volume.

 Preparation of linearity solution

A series of standard preparations of working standard of were prepared.

Table 6: Table of linearity for RP -HPLC Method

Concentration (µg/mL)	
Escitalopram	Flupenthixol
40	2
80	4
120	6
160	8
200	10

Accuracy (recovery)

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analysed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay, The RP-HPLC Result are shown in **Table.**

- **Acceptance Criteria:**

Mean recovery should be in the range of 98-102%.

The Relative Standard Deviation should not be more than 2.0%.

- **Preparation of standard stock solution**

10 mg of Escitalopram and 0.5 mg of Flupenthixol working standards were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume 0.4 ml of this solution diluted up to 10 ml with diluents.

- **Application of proposed method for analysis of tablet formulation**

- **Accuracy**

The accuracy was determined by Escitalopram and Flupenthixol (equivalent to 10 mg of Escitalopram and 0.5 mg of Flupenthixol (80 %, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. This powder mixture containing 10 mg of Escitalopram and 0.5 mg of Flupenthixol were triturated and then subjected to chromatographic analysis using the described method. The resulting mixtures were analysed in triplicates over days. The % recovery of added drug was taken as a measure of accuracy**.**

The Result are shown in **Figure.**

Table 7: Table of Accuracy for HPLC Method

Sample	Amount added	
	ESP	FLP
Accuracy 80%	32	1.6
Accuracy 100%	40	2
Accuracy 120%	48	2.4

Repeatability

Precision of the system was determined with the sample of RP-HPLC for. Three replicates of sample solution containing 160 µg/ml of Escitalopram and 8 µg/ml Flupenthixol were injected and peak areas were measured and %RSD was calculated it was repeated for five times result are shown in **(Table) & (Figure)**

- **Application of proposed method for analysis.**

Weight of sample 160 µg/ml of Escitalopram and 8 µg/ml Flupenthixol were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling the above solution was filtered through 0.45µm membrane filter 1.6 ml

of this solution diluted up to 10 ml with diluents.

Precision

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test **(Figure).**

➢ **Result of Intraday and Inter day Precision studies on RP-HPLC and UV method for Escitalopram and Flupenthixol**

Intra-day Precision

Sample solutions containing 10 mg of Escitalopram and 0.5 mg of Flupenthixol three different concentration (80 µg/ml, 120 µg/ml, 160 µg/ml) Escitalopram and (4 µg/ml, 6µg/ml, 8 µg/ml) Flupenthixol. Escitalopram and Flupenthixol were analysed three times on the same day and % R.S.D was calculated. The Result is shown in **Table.**

Inter-day Precision

Sample solutions containing 10 mg of Escitalopram and 0.5 mg of Flupenthixol three different concentration (80 µg/ml, 120 µg/ml, 160 µg/ml) Escitalopram and 4 µg/ml, 6µg/ml, 8 µg/ml) Flupenthixol. Escitalopram and Flupenthixol were analyzed three times on the Next day and % R.S.D was calculated. The Result is shown in **Table.**

- **Acceptance criteria:**

The Relative Standard Deviation should not be more than 2% for test

Robustness

- **Preparation of Standard Stock Solution**

160 µg/ml of Escitalopram and 8 µg/ml Flupenthixol working standards were weighed and

transferred to 10 mL volumetric flask & diluents was added to make up the volume.1.6 ml of this solution diluted up to 10 ml with diluents. The mobile phase composition was changed in (±1 ml/ min^{-1}**)** proportion **(Figure)** of MEOH : Water (0.1 % Glacial acetic acid) in the mobile phase composition and the flow rate was (±1ml/ min^{-1}**)(Figure)** and the change in detection wavelength (±1 ml/ min^{-1}**)** and the effect of the results were examined **(Figure)** it was performed using 160 µg/ml and 8 µg/ml solution of Escitalopram and Flupenthixol in duplicate. The Result is shown in **Table.**

Detection Limit

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as,

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines. S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

The result is shown in **chapter 7**

Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as,

$$QL = \frac{10\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines. S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated

from the y-intercepts of regression line in calibration curve.

The result is shown in **chapter 7**

Analysis of Marketed Formulation

To determine the content of Escitalopram and Flupenthixol in marketed tablets (label claim 10mg of Escitalopram and 0.5 mg Flupenthixol), 20 tablets powder weighed in 0.38 gm sand average weight of powder was calculated in 0.38 Tablets were triturated and powder equivalent to weigh in 19 mg the drug was extracted from the tablet powder with 10 mL MEOH. To ensure complete extraction it was sonicated for 15 min.1.2 mL of supernatant was then diluted up to 10 mL with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted**. (Figure).**

Regression equation was generated using peak areas of standard solutions. Using the regression equation and peak area of the sample the amount of Escitalopram and Flupenthixol in the sample was calculated. The amount of Escitalopram and Flupenthixol Per tablet was obtained from the regression equation of the calibration curve as described in analysis of Tablet formulation are shown in **Table.**

Forced degradation studies:

Stress degradation of the method was performed to measure the analyst response in the presence of its potential impurities. Stress testing of the individual drug substance and the combination was performed to measure the resolution factors of the drug peak from its nearest resolving peak and from all other peaks. The drugs were subjected to acidic, alkaline, oxidizing, and photolytic conditions. For acidic degradation, the drugs were subjected to 0.1 N hydrochloric acid for 1 hr for the alkaline degradation the drugs treated with 1 N sodium hydroxide for 1hr . Oxidative studies were carried out using 3% hydrogen peroxide for 1 hr for heat.

Degradation behaviour

Forced degradation studies of both the drugs namely Escitalopram and Clonazepam were carried out individually and in under different stress conditions like acid hydrolysis, alkaline hydrolysis, hydrogen peroxide oxidation and photolysis.

1. Acid hydrolysis: The acid hydrolysis performed using 0.1N HCl for 1 hr for both Escitalopram and Clonazepam indicated degradation. The major degradation products for Escitalopram and Clonazepam were observed of 1 hr was16.57% and 10.25%respectivly degradation was observed. **(Fig no:).**

2. Alkaline hydrolysis: The alkaline hydrolysis condition was performed using 0.1N NaOH for 1 hr Escitalopram and Clonazepam. Degradation of Escitalopram and Clonazepam was found to Escitalopram and Clonazepam were observed 5.67% and 14.08% respectively **(Fig no :).**

3. Oxidation: In the oxidation condition with 3% H2O2 for 1 hr and 2 hr Escitalopram and Clonazepam show any oxidative stress degradation peak in the chromatogram. The major degradation products for Escitalopram and Clonazepam were observed 31.61% and 6.94% respectively (**Fig no :).**

4. Neutral: There was no major degradation observed for both Escitalopram and Clonazepam and hence they were not sensitive to light.**(Fig no :).**

RESULT AND DISCUSSION

Preliminary Studies on Escitalopram and Flupenthixol

Melting point

The procured reference standard of Escitalopram and Flupenthixol were found to melt in the range of 147-155^{o}C and >218^{0}C respectively.

Solubility

The drug was found to be Escitalopram soluble in water, soluble in methanol DMSO, ethanol sparingly soluble in alcohol very slightly soluble in acetone.

Insoluble in Heptane.

Flupenthixol Slightly soluble in water, freely soluble in chloroform, ether, methanol.

UV Spectroscopy

UV absorption of 20mcg solution of Escitalopram and Flupenthixol in MEOH was generated and absorbance was taken in the range of 200-400 nm λ max of Escitalopram and Flupenthixol in MEOH was found to be 237 nm and 233 nm respectively.

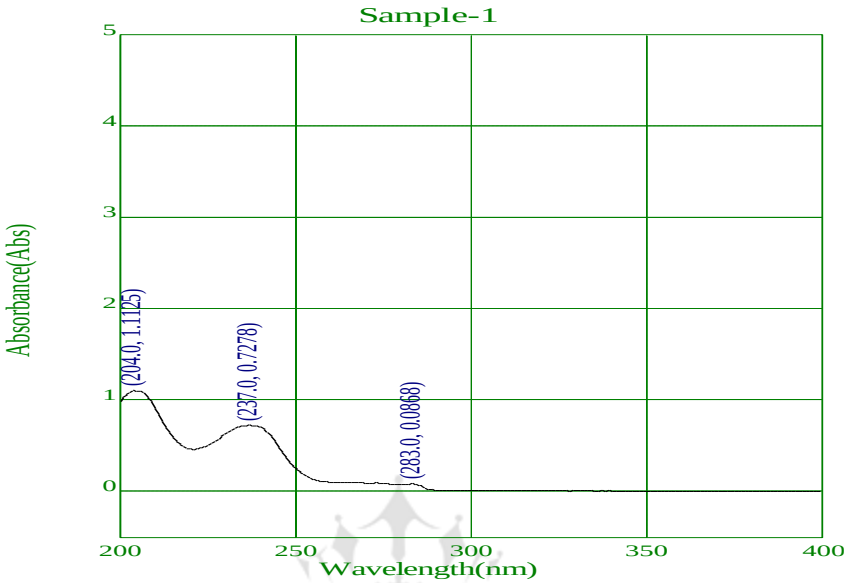

Figure 3: UV Spectrum of Escitalopra

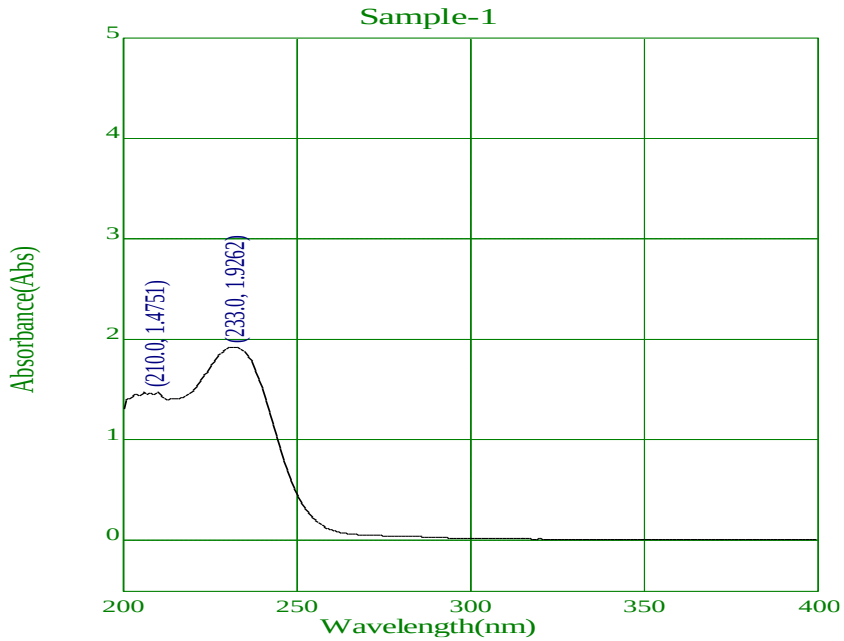

Figure 4: UV Spectrum of Flupenthixol

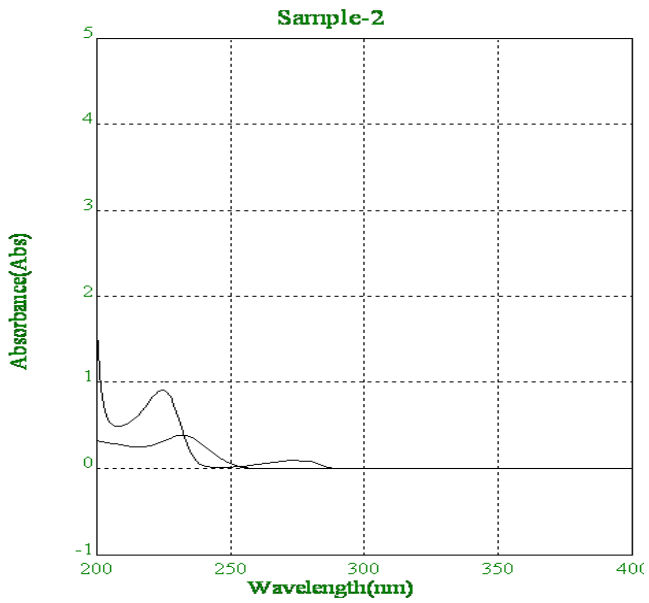

Figure 5: Iso-absorptive point of Escitalopram and Flupenthixol at 235nm

Studies on the chromatographic behaviour of Escitalopram and Flupenthixol

Table 8: Chromatographic behaviour of Escitalopram and Flupenthixol mobile phase of various compositions

Sr. No	Mobile Phase	Retention Time (min)		Remark
		ESP	FLP	
1.	[35% MEOH +65% Water (pH 4.2 adjust with OPA) 1 ml/min at 235 nm (column 100mm X 4.6, 2.5 µm)	2.120	4.633	No Sharpe Peak
2	[25% MEOH +75% Water (pH 4.2 adjust with OPA) 1 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm	2.135	7.634	No Sharpe Peak
3	[50% MEOH +50% Water (pH 4.2 adjust with OPA) 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µ	3.017	4.600	No Sharpe Peak
4	[60% MEOH +40% Water (0.1% OPA) Flow 0.7 ml/r absat 235 nm (column 100mm X 4.6, 2.5 µm)	3.002	4.113	No SharpePeak
5	[65% MEOH +35% Water (0.1% OPA) Flow 0.7 ml/r abs at 235 nm (column 100mm X 4.6, 2.5 µm)	2.990	3.964	No SharpePeak
6	[35% MEOH +65% Water (0.1% Glacial Acetic Acid 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)	3.043	6.480	Resolve Peak And Sharp

Conclusion, from the above, it has been observed that, using mobile phase of MEOH + Water (0.1% Glacial Acetic Acid) (35+65% v/v) 235 nm, 0.7 ml, gave adequate retention time at 3.043 min and 6.480 min. With good peak shape (Theoretical plates of 8527of Escitalopram& 10732 of Flupenthixol)

Chromatogram of Trial 1

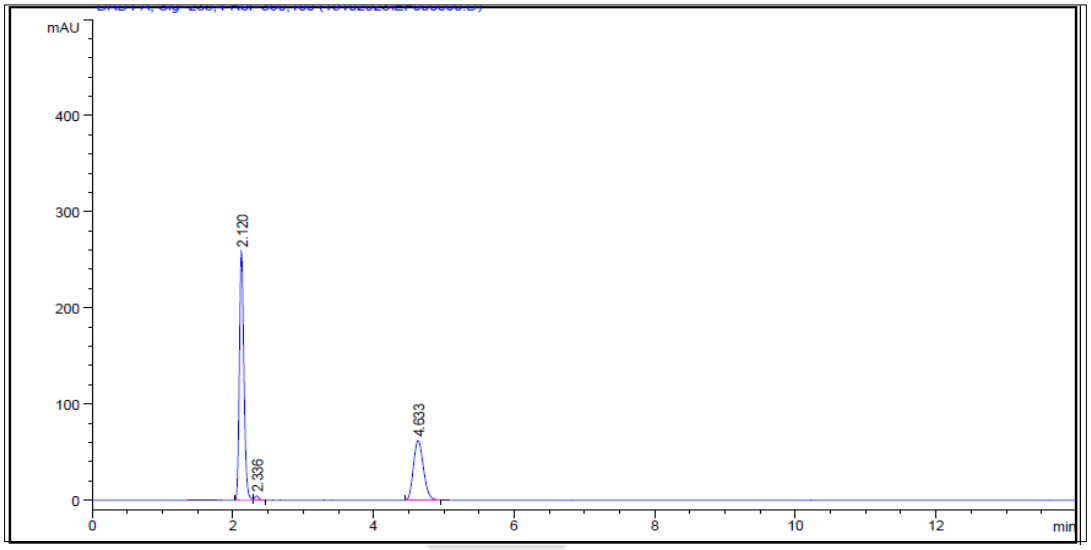

Figure 6: Representative Chromatogram of Escitalopram and Flupenthixol on 35% MEOH +65% Water (pH 3 adjust with OPA) Flow 1 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Table 9: Chromatogram result of Escitalopram and Flupenthixol on 35% MEOH

+65% Water (pH 3 adjust with OPA) Flow 1 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Drug	R.T	AREA	TH. PLATES	SYMM	Resolution
ESP	2.120	1159.6431	5479	0.78	--
	2.336	19.6085	7083	0.98	1.91
FLP	4.633	612.65076	5169	0.82	12.43

Conclusion for rejection of trial

Separations of peak are not clear, and retention time of both drug are very low resolve peak were not obtained. Unsatisfactory result.

Chromatogram of Trial 2:

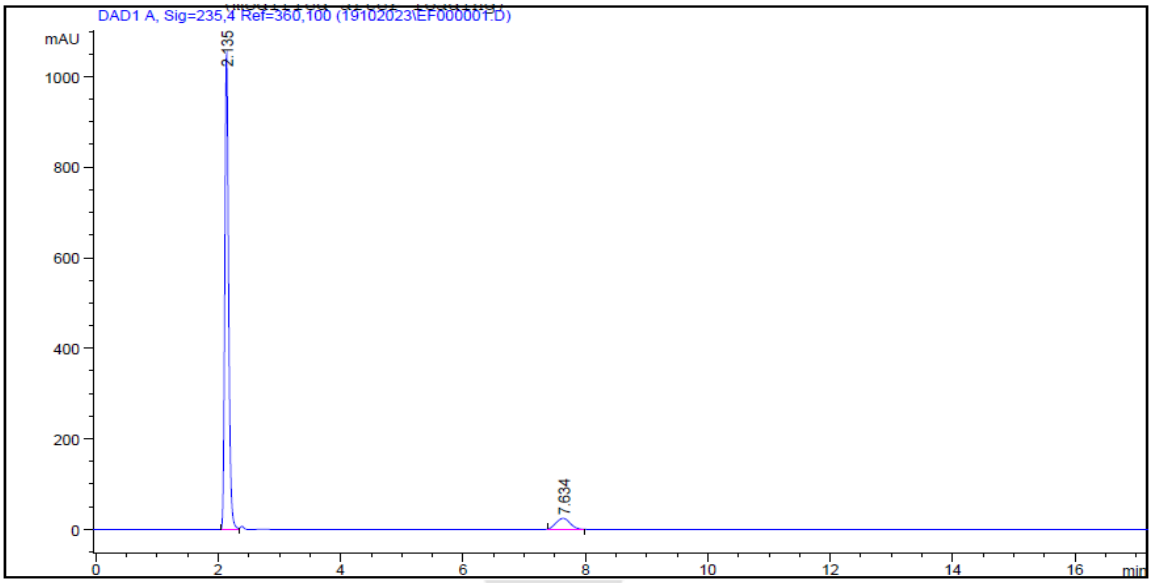

Figure 7: Representative Chromatogram of Escitalopram and Flupenthixol 25% MEOH +75% Water (pH 3.0 adjust with OPA) Flow 1 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Table 10: Chromatogram result of Escitalopram and Flupenthixol 25% MEOH +75% Water (pH 3.0 adjust with OPA) Flow 1 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Drug	R. T	AREA	TH. PLATES	SYMM	Resolution
ESP	2.135	4552.69531	6133	0.79	---
FLP	7.634	386.40689	5030	0.99	20.35

Chromatogram of Trial 3

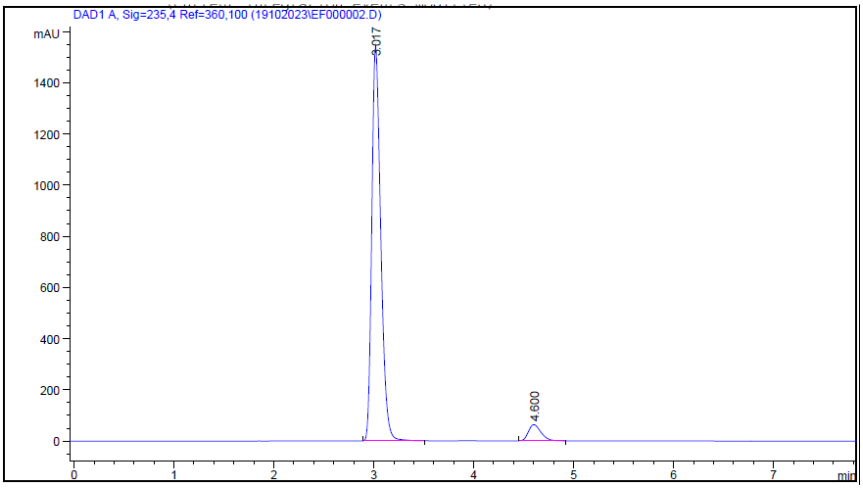

Figure 8: Representative Chromatogram of Escitalopram and Flupenthixol 50% MEOH +50% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Table 11: Chromatogram result of Escitalopram and Flupenthixol 50% MEOH +50% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Drug	R. T	AREA	TH. PLATES	SYMM	Resolution
ESP	3.017	9621.31934	5586	0.68	----
FLP	4.600	554.99152	6595	0.70	8.15

Chromatogram of Trial 4

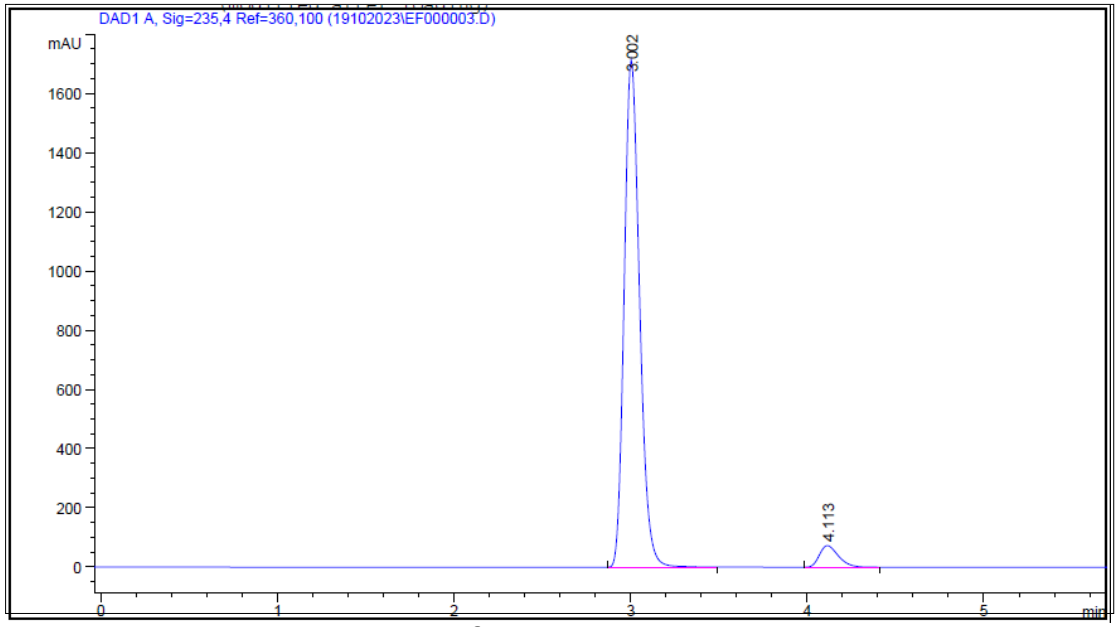

Figure 9: Representative Chromatogram of Escitalopram and Flupenthixol 60% MEOH +40% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Table 12: Chromatogram result of Escitalopram and Flupenthixol 50% MEOH +50% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Drug	R. T	AREA	TH. PLATES	SYMM	Resolution
ESP	3.002	10374.7	5944	0.78	----
FLP	4.113	546.26605	7298	0.67	6.37

Chromatogram of Trial 5

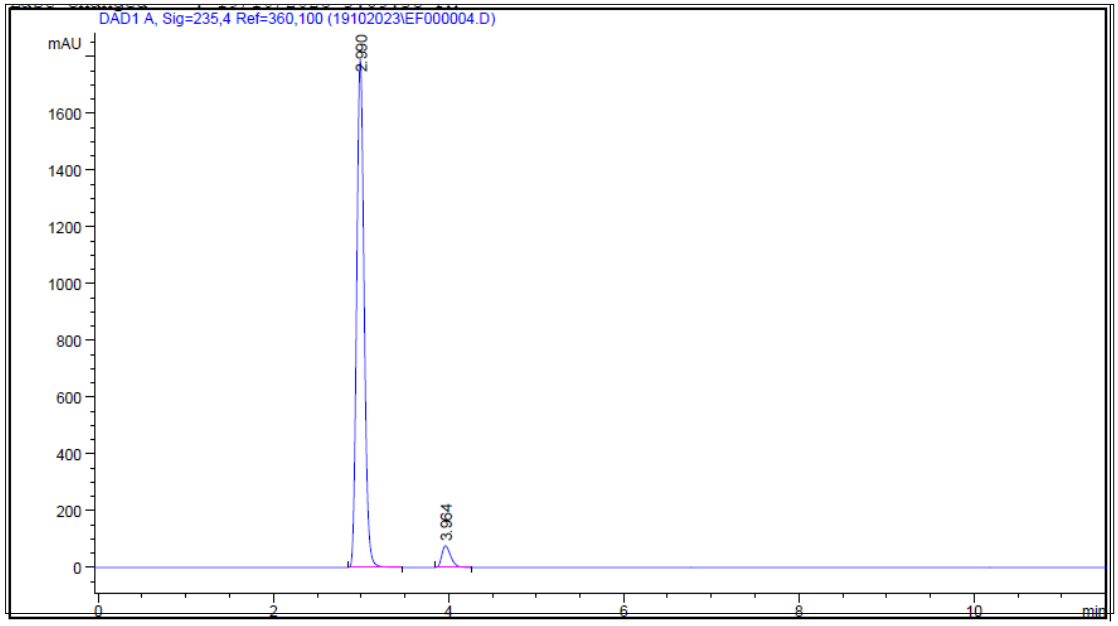

***Figure* 10: Representative Chromatogram of Escitalopram and Flupenthixol 65% MEOH +35% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)**

Table 13: Chromatogram result of Escitalopram and Flupenthixol 65% MEOH +35% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Drug	**R.T**	**AREA**	**TH. PLATES**	**SYMM**	**Resolution**
ESP	2.990	10586.8	6116	0.86	----
FLP	3.964	539.60840	7650	0.66	5.82

Chromatogram of Trial 6

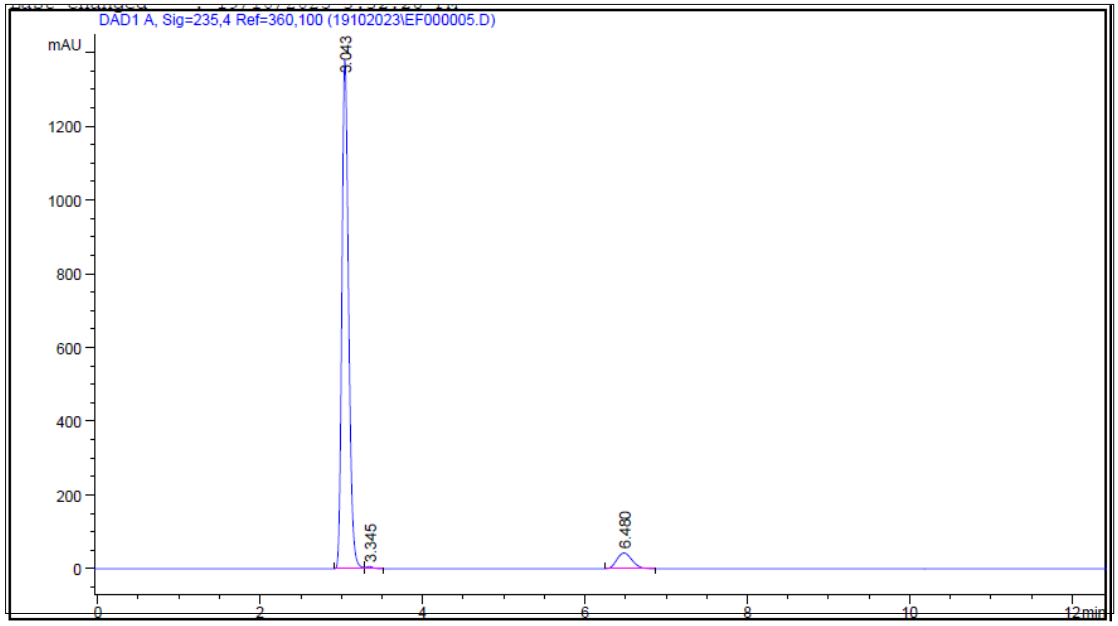

Figure 11: Representative Chromatogram of Escitalopram and Flupenthixol 35% MEOH +65% Water (pH 4.2 adjust with Glacial Acetic Acid) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Table 14: Chromatogram result of Escitalopram and Flupenthixol 35% MEOH +65% Water (pH 4.2 adjust with Glacial Acetic Acid) Flow 0.7 ml/min abs at 235 nm (column 100mm X 4.6, 2.5 µm)

Drug	R. T	AREA	TH. PLATES	SYMM	Resolution
ESP	3.043	7931.28125	6828	0.76	----
FLP	6.480	566.81885	5565	0.84	12.56

The Final Chromatographic Conditions Selected Were as Follow

DAD Detector Agilent (S.K) Gradient System

- Analytical column : (Agilent) C18 column (4.6mm x 100mm)
- Injection volume : 20µl
- Flow rate : 0.7 ml/min
- Mobile phase: MEOH +0.1 Glacial Acetic Acid (35+65% v/v)
- Detection : 235 nm
- Run Time : 15 min

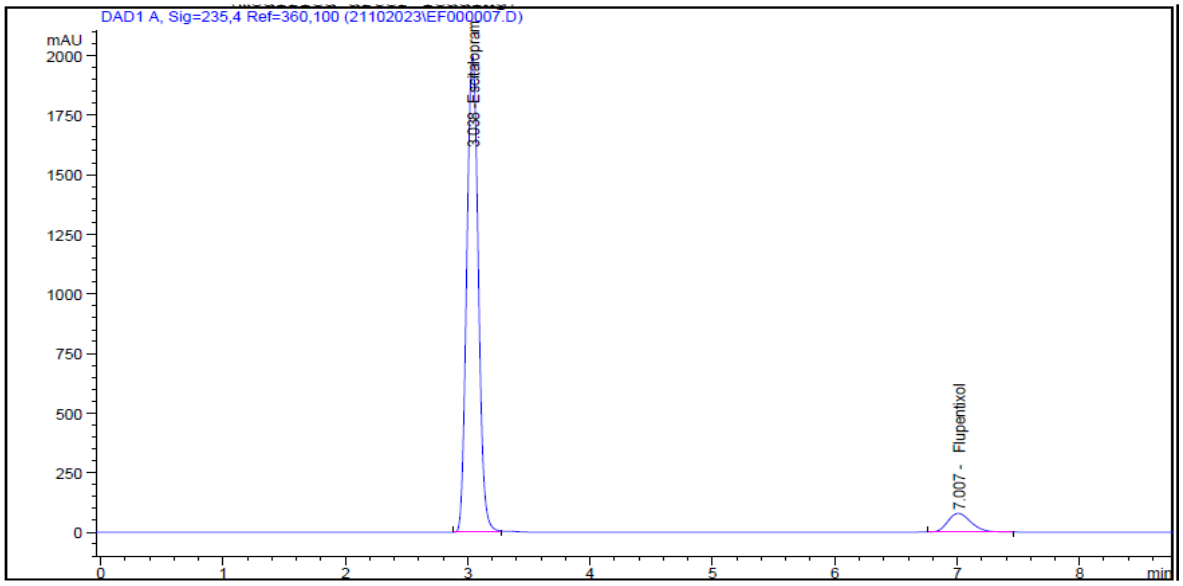

Figure 12: Chromatogram of standard Combination of Escitalopram and Flupenthixol

Table 15: Details of chromatogram of standard Combination containing

Drug	R. T	AREA	TH. PLATES	SYMM	Resolution
ESP	3.038	12164.1	5002	0.93	---
FLP	7.007	935.97363	6652	0.77	2.31

In the standard mixture of Escitalopram and Flupenthixol theoretical plates were found above 2000 i.e. for Escitalopram 5002 and Flupenthixol 6652 at minimum RT 3.038 and 7.007 respectively**.**

The Optimized chromatographic conditions selected were as follow

DAD Detector Agilent (S.K) Gradient System

- Analytical column: (Agilent) C18 column (4.6mm x 100mm)
- Injection volume: 20µl
- Flow rate: 0.7 ml/min
- Mobile phase: MEOH +0.1 Glacial Acetic Acid (35: 65 % v/v)
- Detection: 235 nm
- Run Time :15 min

After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity & accuracy. The optimized parameters for selected method are as below.

Calibration experiment

RP-HPLC Method

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range

40-200 µg/mL for Escitalopram and 2 – 10 µg/mL for Flupenthixol (**Table)** depict the calibration data of Escitalopram and Flupenthixol The respective linear equation for Escitalopram was y = 63.04 x + 2031 and Flupenthixol equation y = 114.7 x + 25.34 where x is the concentration and y are area of peak. The correlation coefficient was 0.999 and 0.999. The calibration curve of Escitalopram and Flupenthixol is depicted in (**Figure).**

Table 16: Linearity data for Escitalopram

	Conc µg/ml	Peak area(µV.sec)		Average peak (µV.sec)	S.D. of Peak	% RSD of Area
		1	2			
HPLC Meth	40	4450.369	4424.594	4437.48	18.23	0.41
	80	7188.318	7152.162	7170.24	25.57	0.36
	120	9690.231	9649.412	9669.82	28.86	0.30
	160	12164.1	12136.9	12150.50	19.23	0.16
	200	14499.4	14611.3	14555.35	79.13	0.54
	Equation		y =63.04 x +2031			
	R^2		0.999			

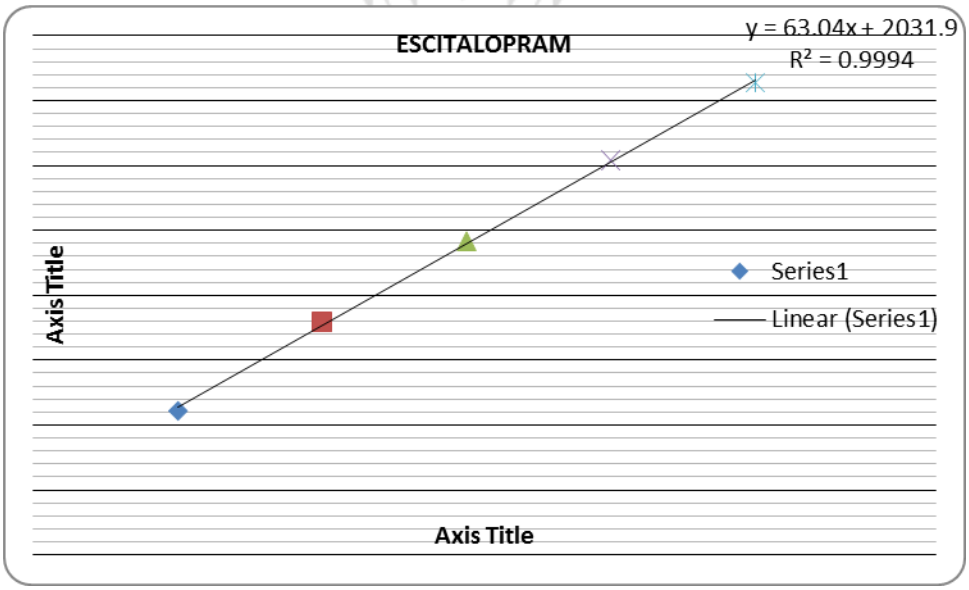

Figure 13: Calibration Curve of Escitalopram

The RP-HPLC Method for respective linear equation for Escitalopram was y = 63.04 x + 2031 where x is the concentration and y are area of peak. The correlation coefficient was 0.999.

The calibration curve of Escitalopram is depicted in **Figure.**

Table 17: Linearity Data for Flupenthixol

	Conc. µg/ml	Peak area (µV. sec)		Average peak (µV. sec)	S.D. of Peak	% RSD of Peak Area
		1	2			
HPLC Method	2	250.636	254.5312	252.58	2.75	1.09
	4	486.5102	498.6686	492.59	8.60	1.75
	6	710.11	706.5948	708.35	2.49	0.35
	8	935.9736	940.5142	938.24	3.21	0.34
	10	1177.852	1176.473	1177.16	0.98	0.08
	Equation		y = 114.7 X + 25.34			
	R^2		0.999			

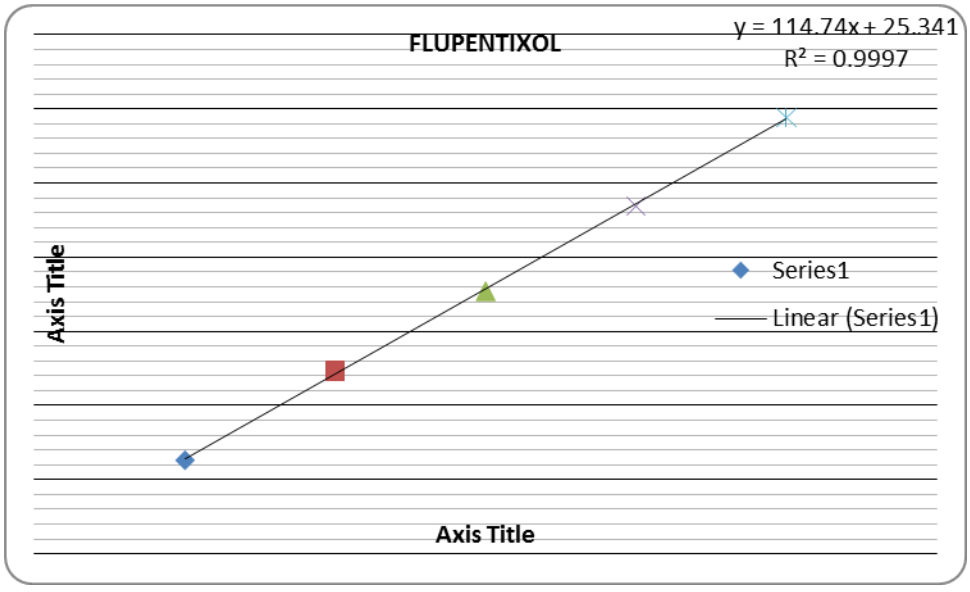

Figure 14: Calibration Curve of Flupenthixol

The RP-HPLC method for respective linear equation for Flupenthixol was y =114.7 X + 25.34 where x is the concentration and y are area of peak. The correlation coefficient was 0.999. The calibration curve of Flupenthixol is depicted in **Figure.**

Analytical of Method Validation:

1. Linearity

From Escitalopram standard stock solution, different working standard solution (40-200µg/ml) were prepared in mobile phase Likewise from Flupenthixol standard stock solution different working standard solution (2 to 10 µg/ml), were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. The areas for each concentration were recorded **(Table)**. The Calibration curves are shown in **(Figure).**

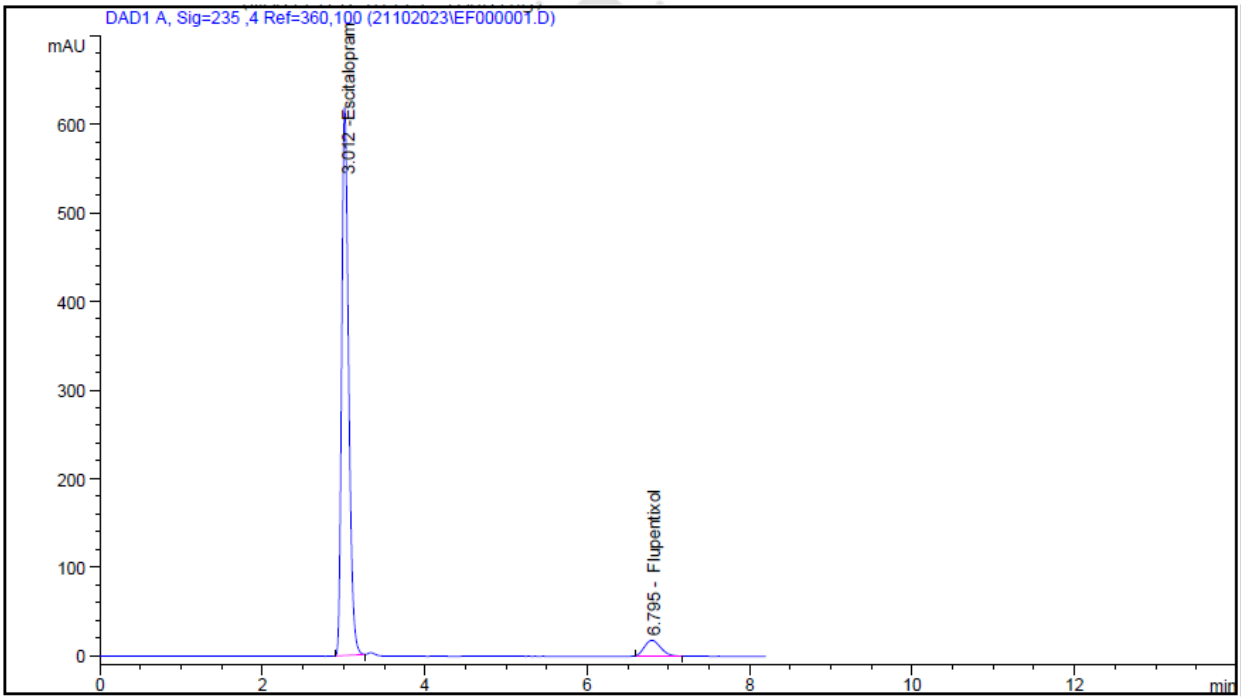

Figure 15: Chromatogram of Linearity LIN40+2 mcg microgram/ml-1

Table 18: Chromatogram of Linearity LIN 40+2 mcg microgram/ml-1

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.012	4450.36914	0.72	6205
FLP	6.795	250.63600	0.81	5393

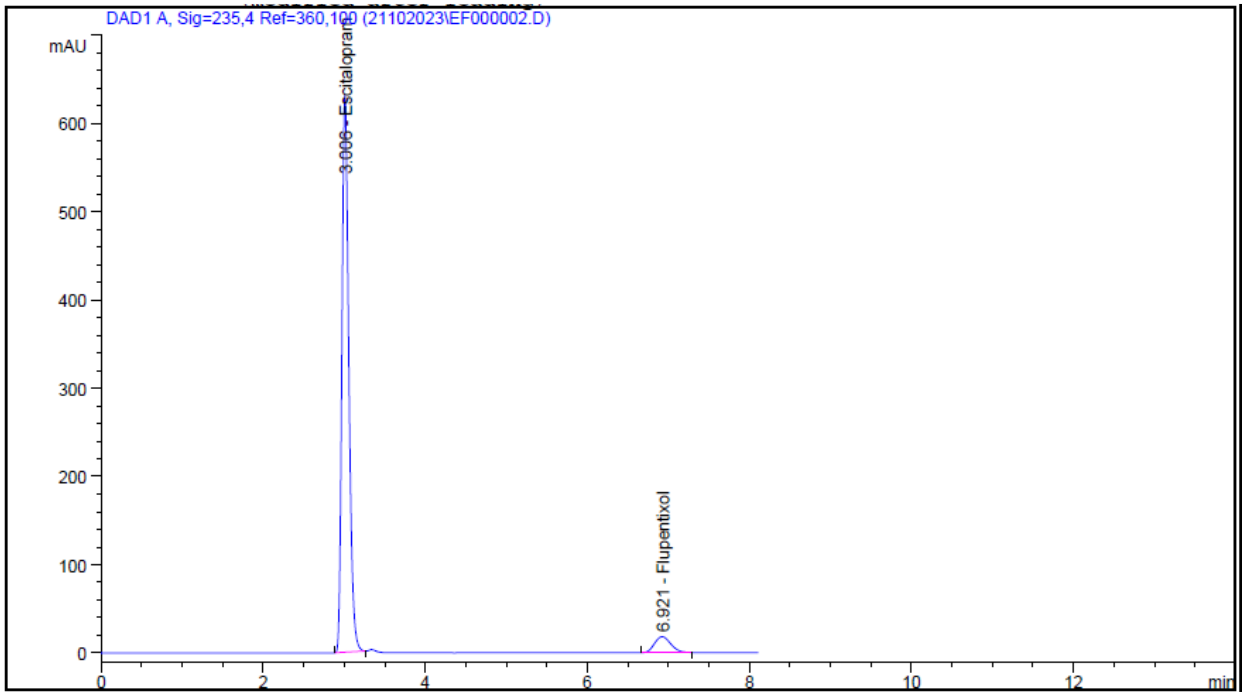

Figure 16: Chromatogram of Linearity LIN 40+2 mcg microgram/ml-2

Table 19: Chromatogram of Linearity LIN 40+2 mcg microgram/ml-2

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.006	4424.59448	0.73	6338
FLP	6.921	254.53125	0.83	5595

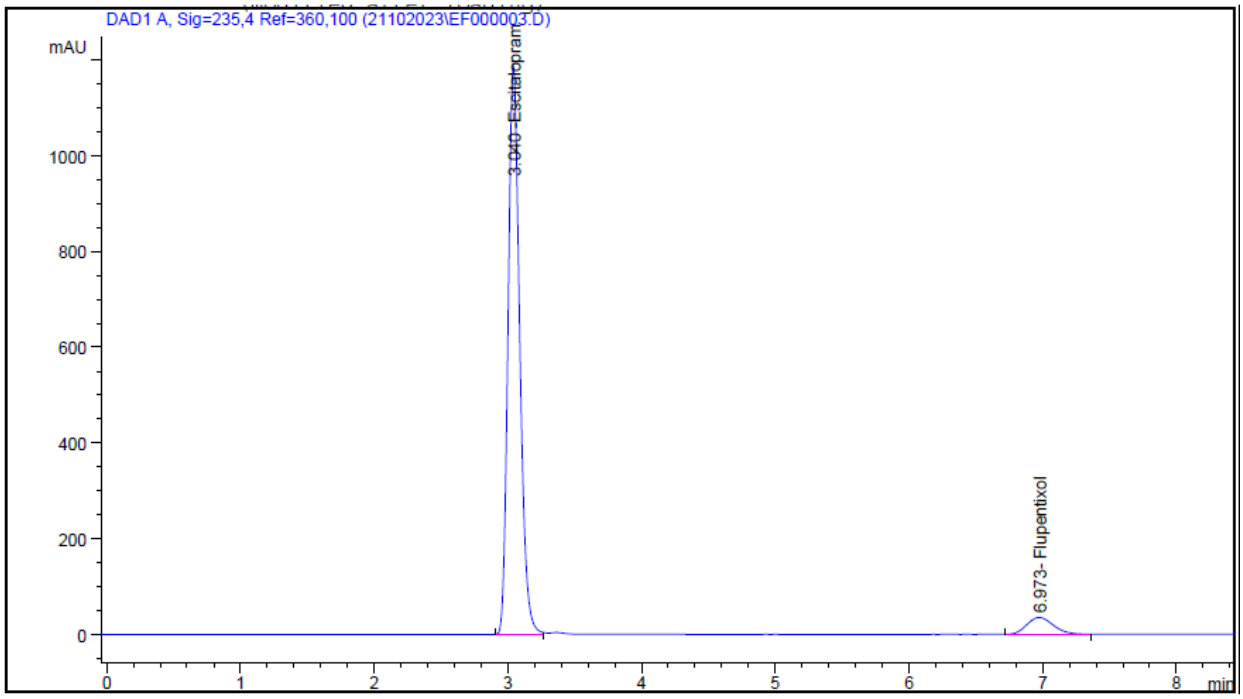

Figure 17: Chromatogram of Linearity LIN 80+4 mcg microgram/ml-1

Table 20: Chromatogram of Linearity LIN 80+4 mcg microgram/ml-1

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.040	7188.3183	0.77	6168
FLP	6.973	486.51025	0.83	5798

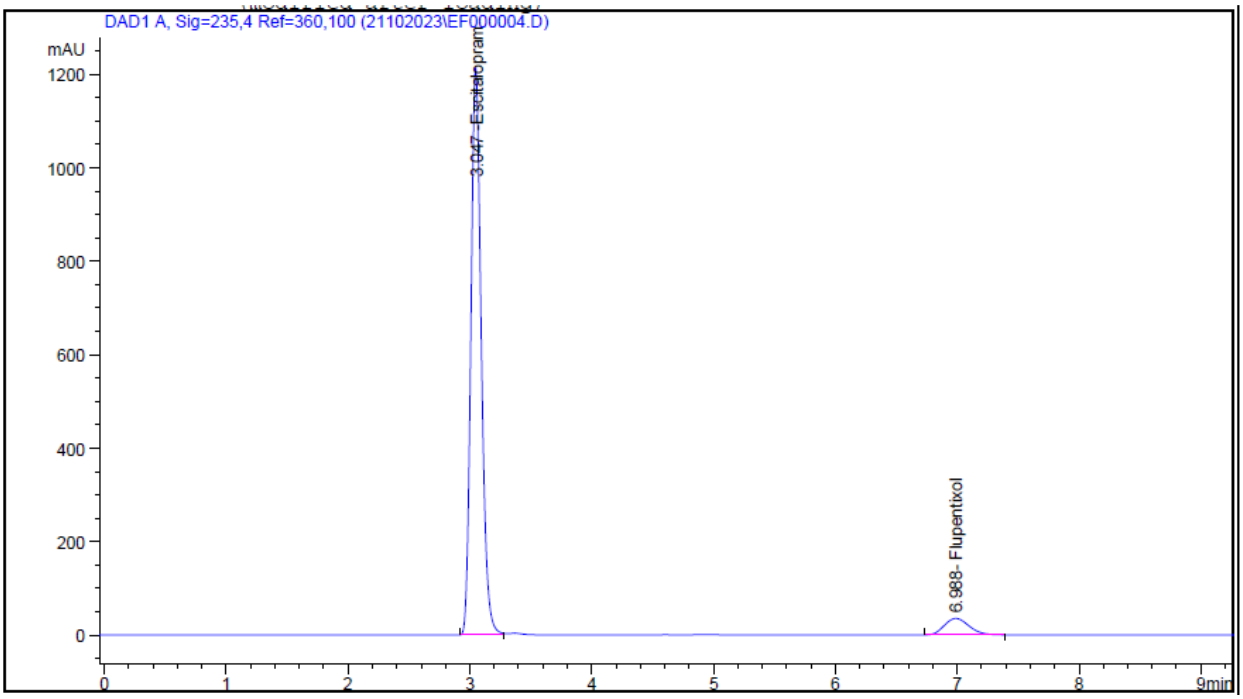

Figure 18: Chromatogram of Linearity LIN 80+4 mcg microgram/ml-2

Table 21: Chromatogram of linearity LIN 80+4 mcg microgram/ml-2

Drug name	R.T	AREA	SYMM	TH. PLATES
ESP	3.047	7152.16162	0.79	6509
FLP	6.988	498.66861	0.83	5589

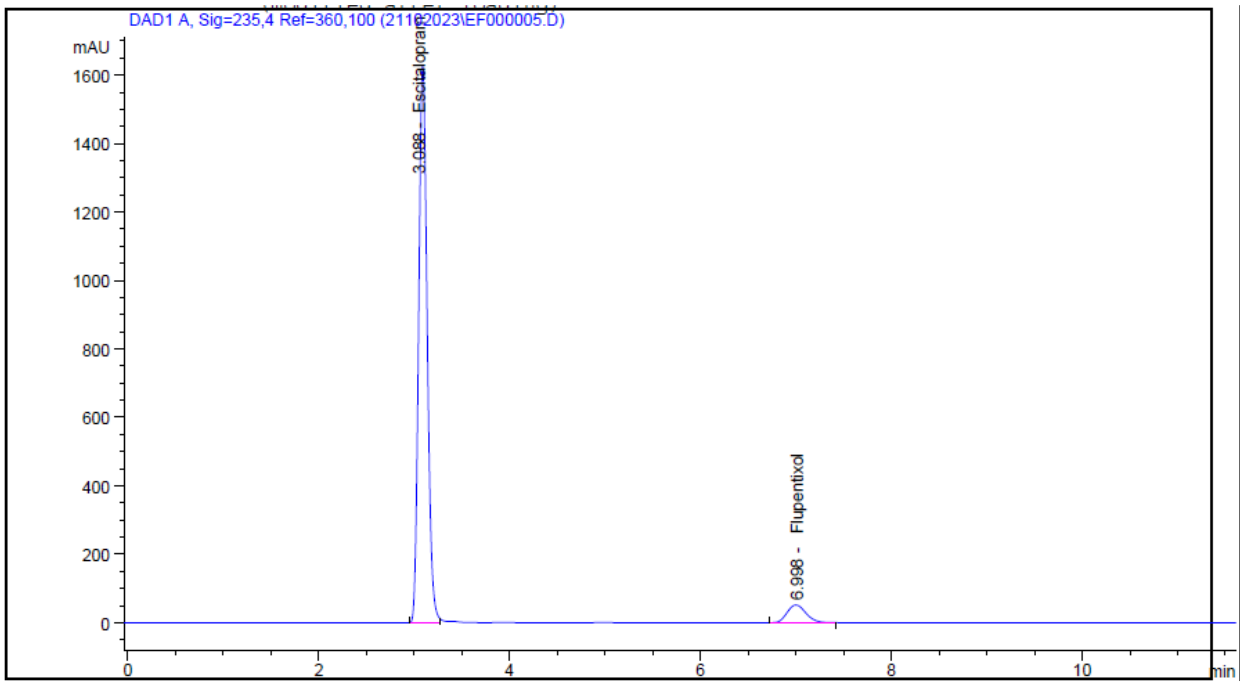

Figure 19: Chromatogram of Linearity LIN 120+6 mcg microgram/ml-1

Table 22: Chromatogram of Linearity LIN 120+6 mcg microgram/ml-1

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.088	9690.016	0.83	5922
FLP	6.998	710.11993	0.83	6088

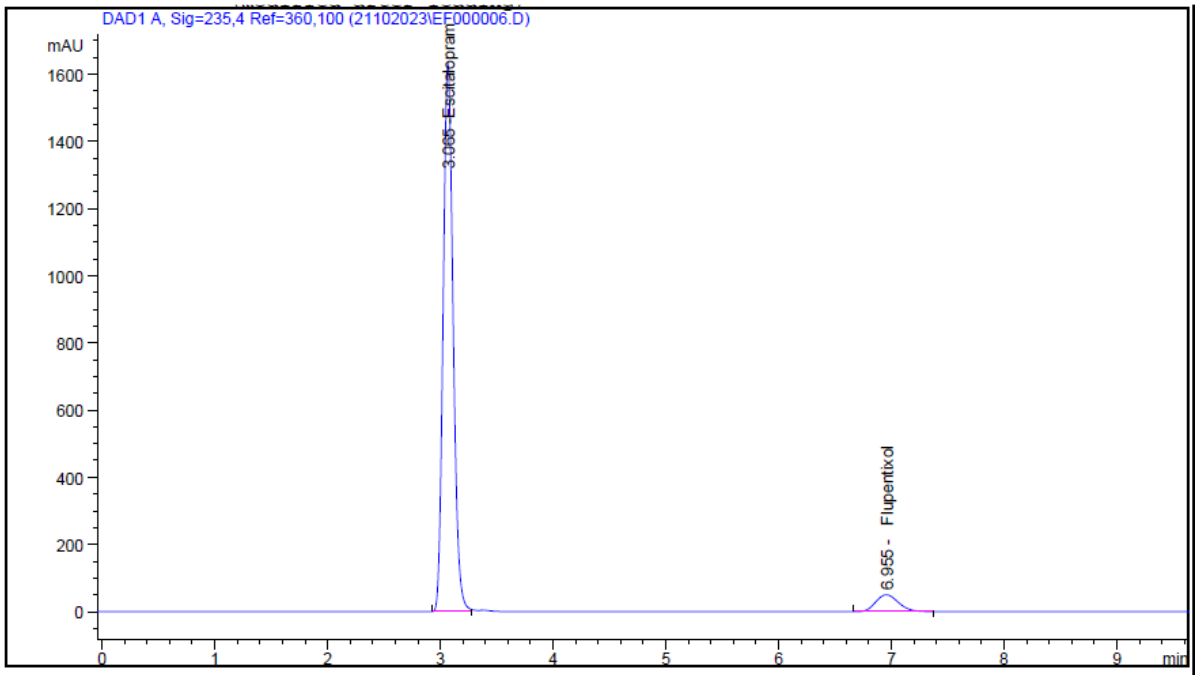

Figure 20: Chromatogram of Linearity LIN 120+6 mcg microgram/ml-2

Table 23: Chromatogram of Linearity LIN 120+6 mcg microgram/ml-2

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.066	9649.494	0.83	5836
FLP	6.955	706.59485	0.84	5650

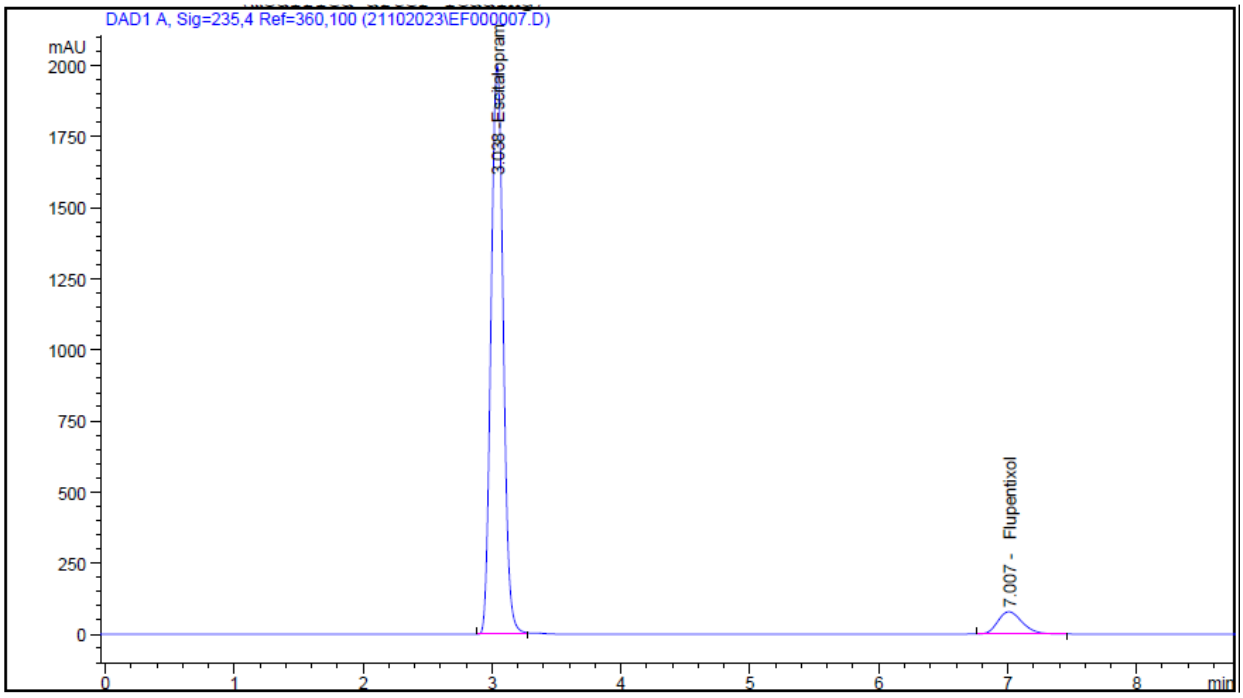

Figure 21: Chromatogram of Linearity LIN 160+8 mcg microgram/ml-1

Table 24: Chromatogram of Linearity LIN 160+8 mcg microgram/ml /ml-1

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.038	12164.1	0.93	5002
FLP	7.007	935.97363	0.77	6652

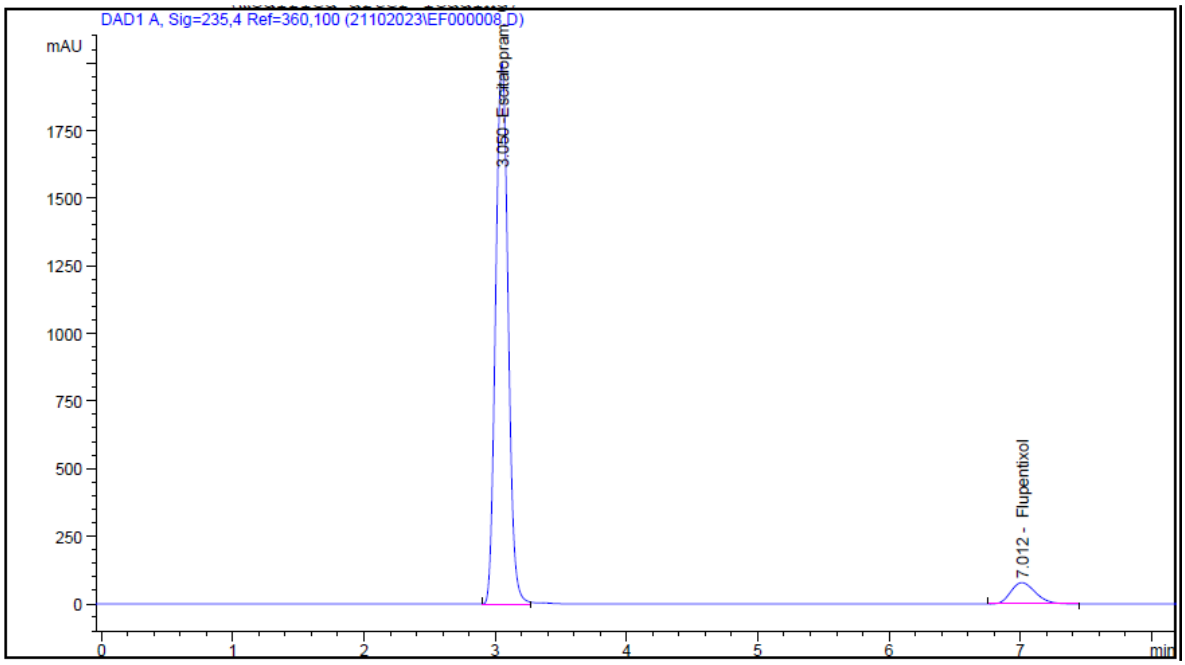

Figure 22: Chromatogram of Linearity LIN 160+8 mcg microgram/ml-2

Table 25: Chromatogram of Linearity LIN 160+8 mcg microgram/ml-2

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.050	12136.9	0.93	4888
FLP	7.012	940.51428	0.77	6660

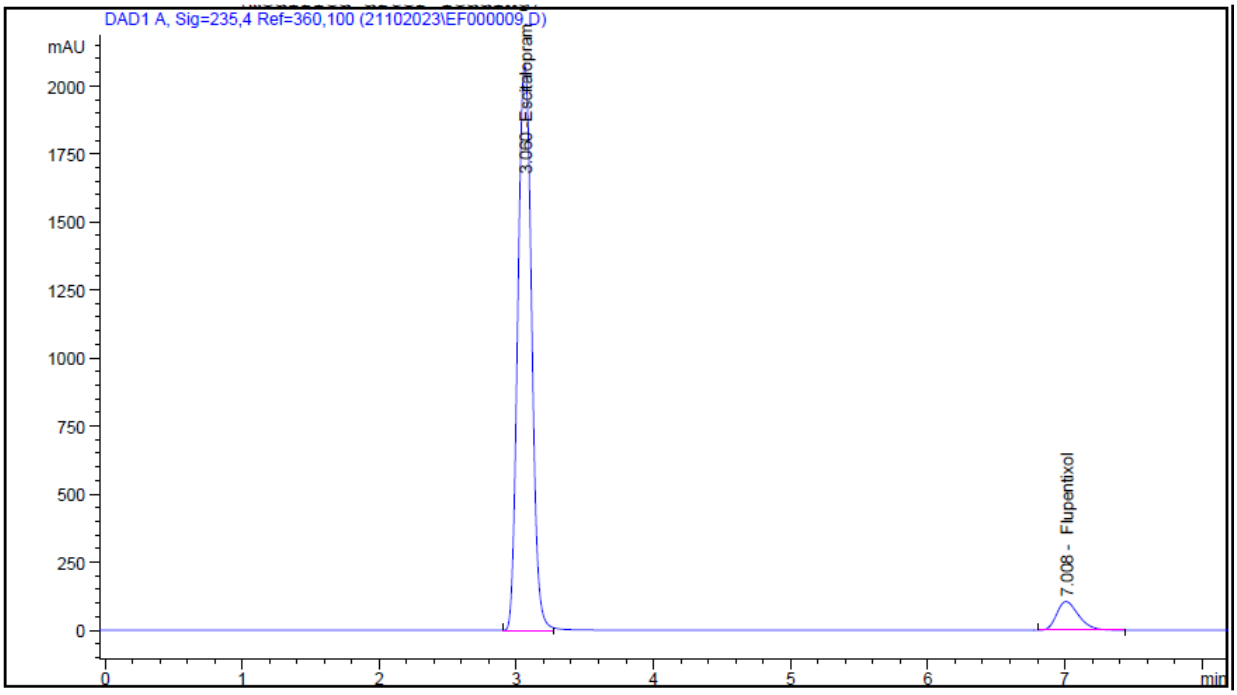

Figure 23: Chromatogram of Linearity LIN 200+10 mcg microgram/ml-1

Table 26: Chromatogram of Linearity LIN 200+10 mcg microgram/ml-1

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.060	14499.4	0.93	4340
FLP	7.008	1177.8518	0.69	9601

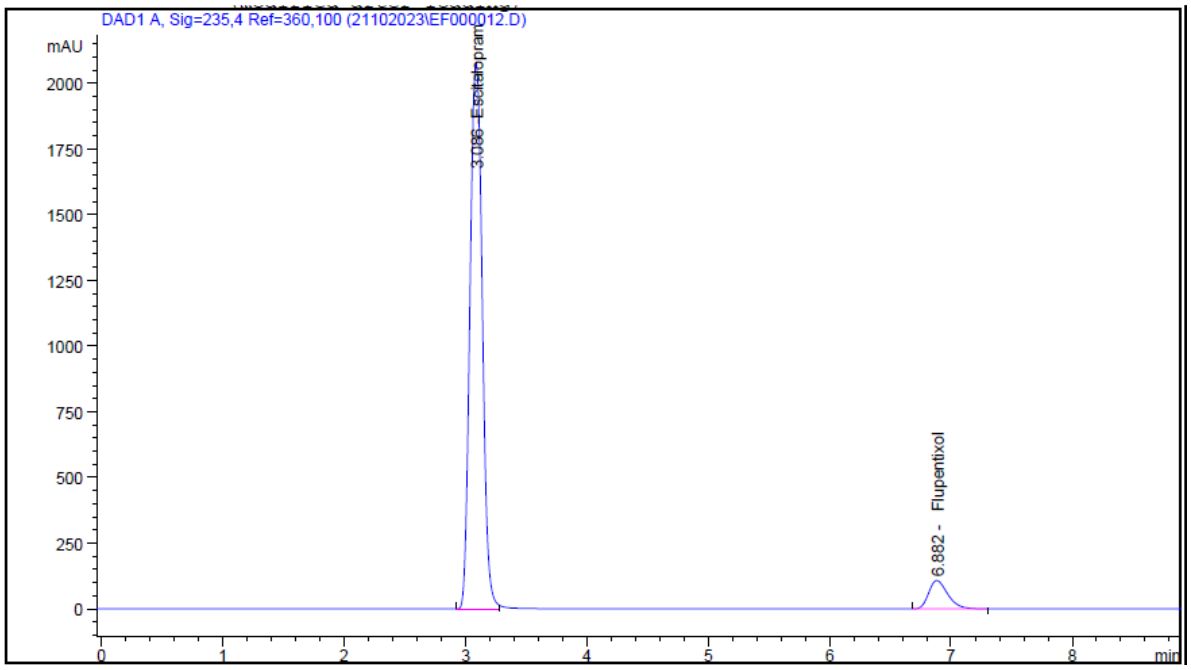

Figure 24: Chromatogram of Linearity LIN 200 + 10 mcg microgram/ml-2

Table 27: Chromatogram of Linearity LIN 200+10 mcg microgram/ml-2

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.086	14611.3	0.93	4413
FLP	6.881	1176.47253	0.69	9836

Table 28: Linearity of Escitalopram

Concentration ug/ml	Area Escitalopram
Method	**HPLC**
40	4437.48
80	7170.24
120	9669.82
160	12150.50
200	14555.35

Table 29: Regression Equation Data for Escitalopram

Regression Equation Data Y= mx + c	**For HPLC**
Slope(m)	63.04
Intercept(c)	2031
Correlation Coefficient	0.999

Table 30: Linearity of Flupenthixol

Concentration	**Area Flupenthixol**
Method	**HPLC**
2	252.58
4	492.59
6	708.35
8	938.24
10	1177.16

Table 31: Regression Equation Data for Flupenthixol

Regression Equation D	**For HPLC**
Slope(m)	114.7
Intercept(c)	25.34
Correlation Coefficien	0.999

Linearity of Escitalopram and Flupenthixol was observed in both methods the range of 40-200 ug/ml and 2-10 ug/ml. Detection wavelength used was 235 nm. **(Table).**

The plot should be linear passing through the origin; Correlation Coefficient should not be less than 1 that concluded. **(Table).**

2. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed **(Table).** Statistical validation of recovery studies shown in **(Table).**

Accuracy 80%

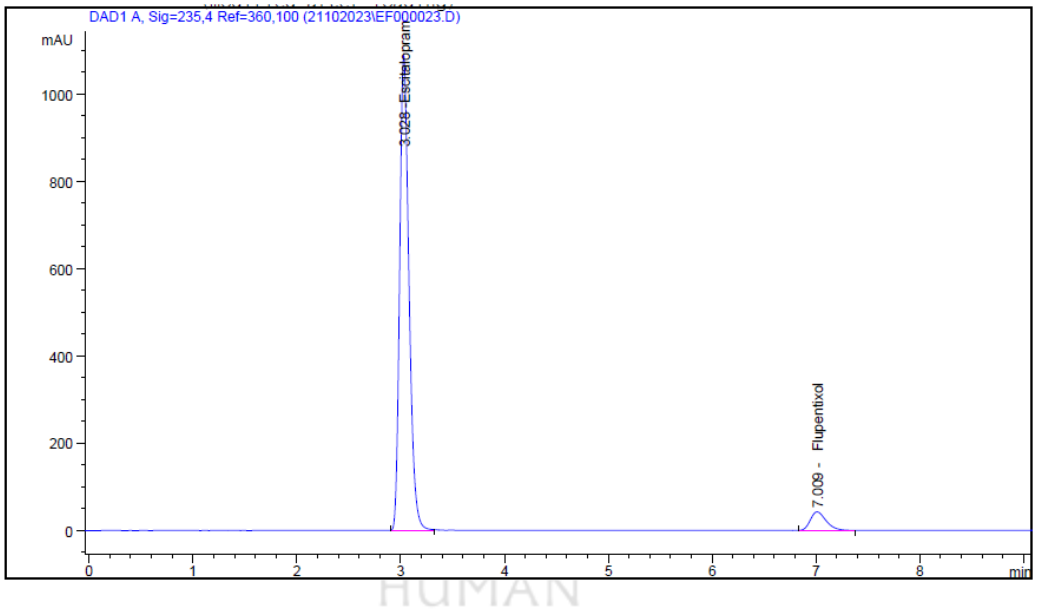

Figure 25: Chromatogram of Accuracy 80%-1

Table 32: Chromatogram of Accuracy 80% -1

Drug name	R.T	AREA	SYMM	TH. PLATES
ESP	3.028	6579.8212	0.72	5832
FLP	7.009	440.35367	0.70	10631

Accuracy 100%

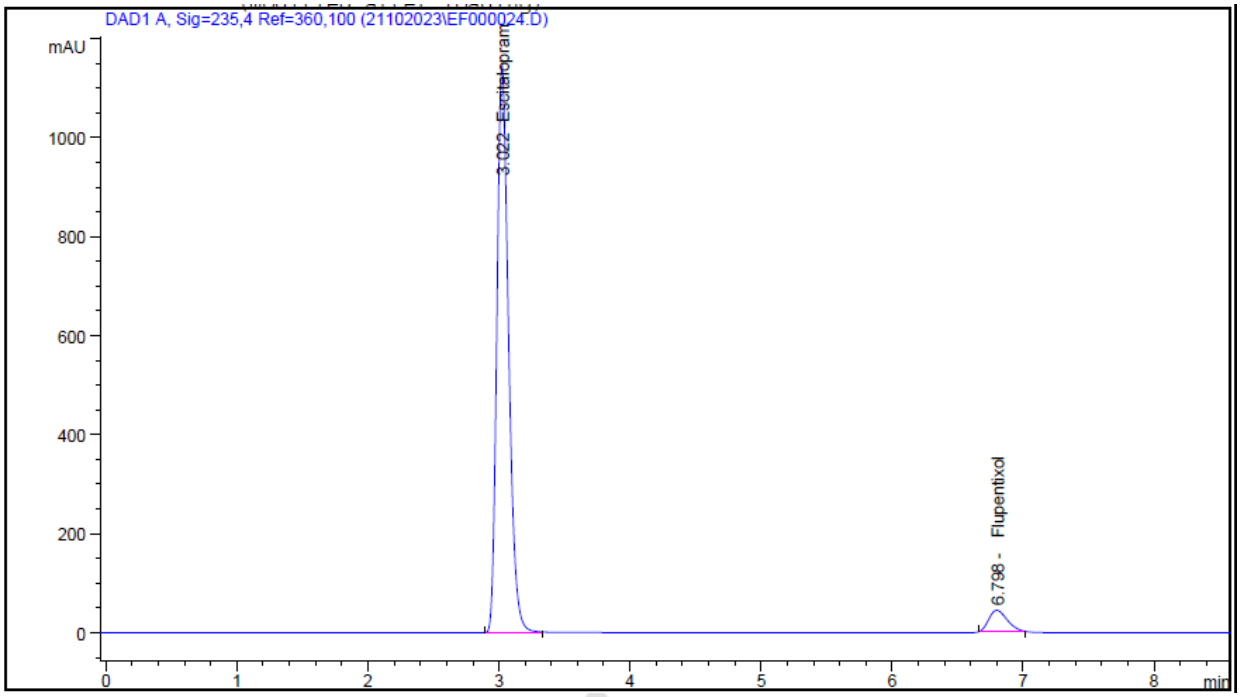

Figure 26: Chromatogram of Accuracy 100%

Table 33: Chromatogram of Accuracy 100%

Drug name		R.T		AREA		SYMM		TH. PLATES	
ESP	NAP	3.022	2.69	7125.96739	3802.83	0.72	0.77	5948	6641
FLP	PAN	6.798	5.04	488.8996	352.64	0.71	0.67	10888	9036

Accuracy 120%

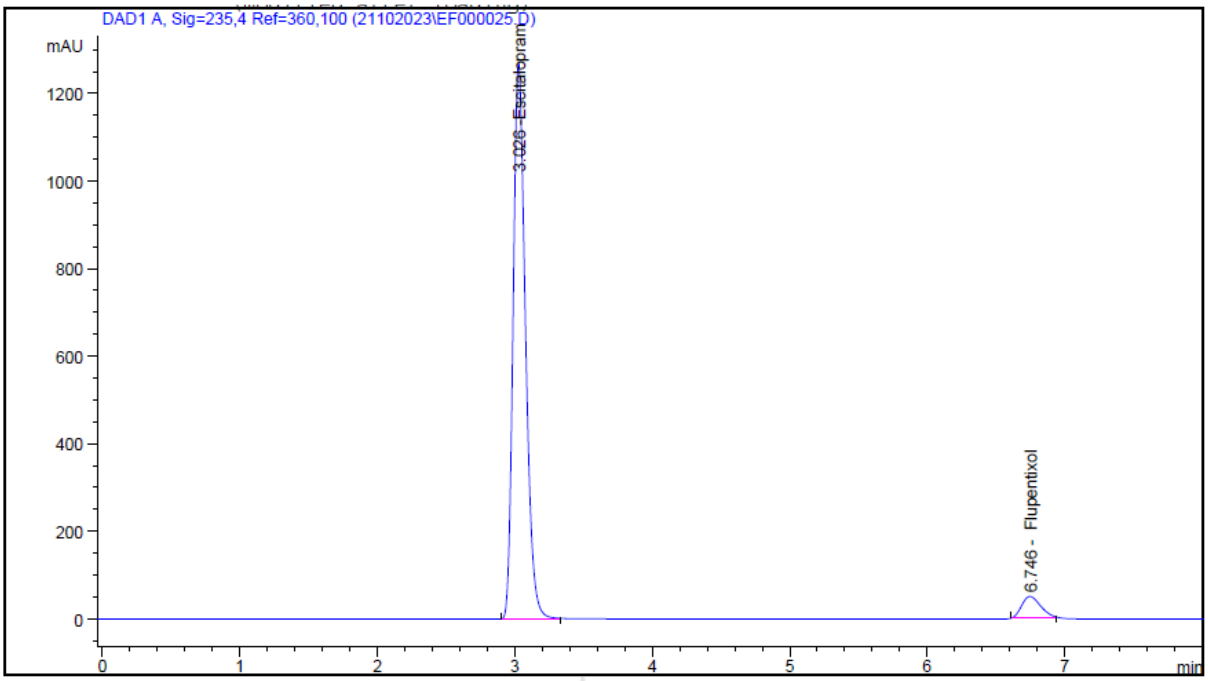

Figure 27: Chromatogram of Accuracy 120%-1

Table 34: Chromatogram of Accuracy 120%-1

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.026	7523.3774	0.72	5824
FLP	6.746	530.5412	0.71	10961

Table 35: Result of Recovery data for Escitalopram and Flupenthixol

METH OD	Drug	Level (%)	Amt. taken (ug/ ml	Amt. Adde d (ug/ml	AREA Mean* ± S.D.	Amt.recovere Mean* ± S.D	%Recovery Mean * ± S.D.
RP-HPLC Method	ESP	80%	40	32	72.08±0.109	32.08±0.109	100.25±0.34
		100%	40	40	80.78±0.064	40.78±0.06	101.94±0.16
		120%	40	48	87.20±0.111	47.20±0.111	98.34±0.23
		80%	2	1.6	3.60±0.026	1.60±0.026	100.00±1.62
		100%	2	2	4.03±0.010	2.03±0.010	101.71±0.51
		120%	2	2.4	4.40±0.006	2.40±0.006	101.71±0.51

*Mean of each 3 reading for RP-HPLC method

Table 36: Statistical Validation of Recovery Studies Escitalopram and Flupenthixol

METHOD	Level of Recovery (%)	Drug	% RSD	Standard Deviation*	Mean Recovery
Rp-HPLC Me	80%	ESP	0.34	0.109	100.25
		FLP	1.62	0.026	100.00
	100%	ESP	0.16	0.064	101.94
		FLP	0.51	0.010	101.71
	120%	ESP	0.23	0.111	98.34
		FLP	0.25	0.006	100.01

*Denotes average of three determinations for RP-HPLC

Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-101% **(Table).**

3. System suitability parameters (Repeatability):

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Escitalopram and Flupenthixol system suitability parameters were studied. The result shown in below **(Table).**

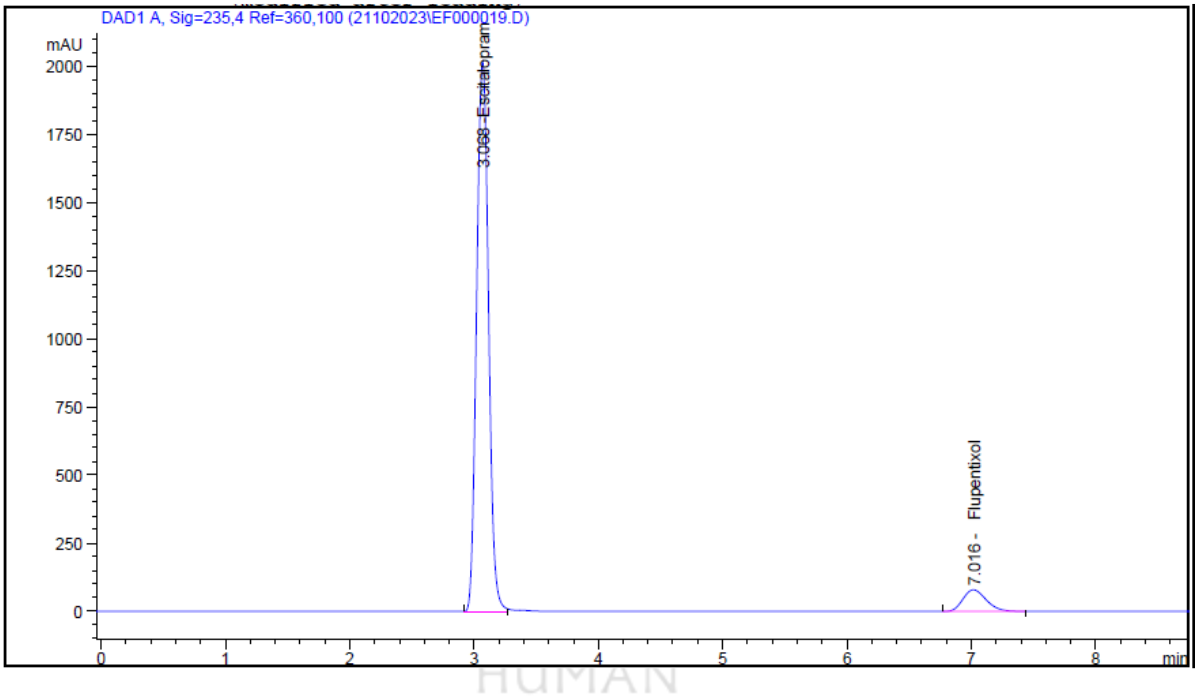

Figure 28: Chromatogram of System suitability (160+8)-01

Table 37: Chromatogram of System suitability (160+8)-01

Drug name	R.T	AREA	SYMM	TH. PLATES
ESP	3.06	12412.5	0.94	4948
FLP	7.01	935.7775	0.77	6818

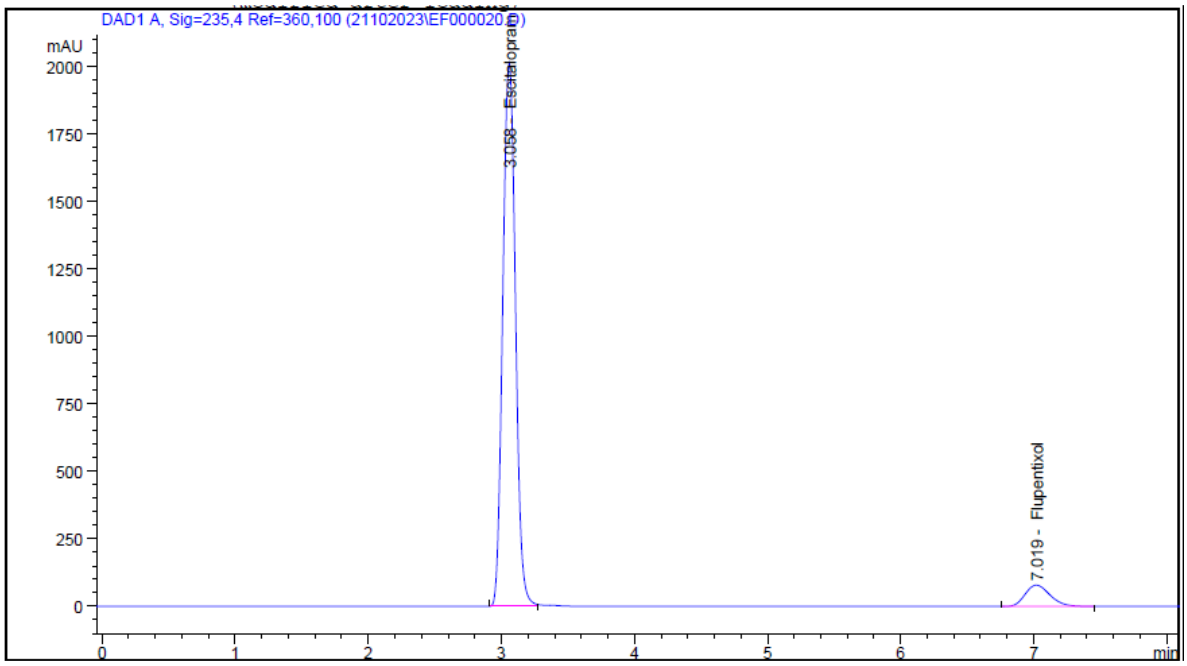

Figure 29: Chromatogram of System suitability (160+8)-02

Table 38: Chromatogram of System suitability (160+8)-02

Drug name	R.T	AREA	SYMM	TH. PLATES
ESP	3.05	12338.0	0.94	4915
FLP	7.01	931.237	0.77	6823

Table No.39: Repeatability studies on RP-HPLC for Escitalopram and Flupenthixol

METHOD	Concentration of Escitalopram Flupenthixol (mg/ml	Peak area	Amount (mg)	% Amount found
HPLC ESP	160	12164	160.74	100.46
METHOD	160	12166		
		Mean	12164.00	
		SD	0.001	
		%RSD	0.001	
HPLC FLP	8	935.7775	7.92	98.97
	8	931.2379		
METHOD		**Mean**	933.51	
		SD	3.21	
		%RSD	0.34	

Repeatability studies on RP-HPLC for Escitalopram and Flupenthixol was found to be The %RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded .**(Table).**

4. **Precision**

The method was established by analyzing various replicates standards of Escitalopram and Flupenthixol. All the solution was analyzed thrice to record any intra-day & inter- day variation in the result that concluded. The result obtained for intraday is shown in **(Table)** respectively.

Chromatogram of Precision

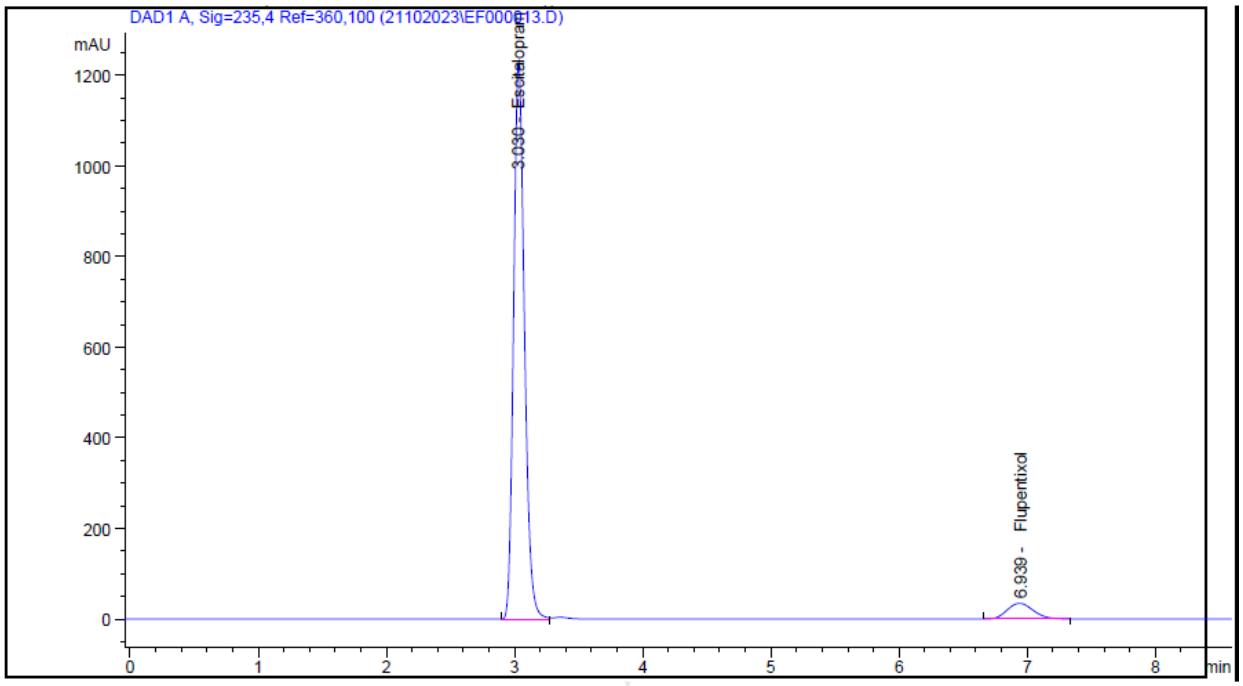

Figure 30: Chromatogram of Intraday Precision (80+4)-1

Table 40: Chromatogram of Precision (80+4)-1

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.030	7130.3237	0.79	6439
FLP	6.939	479.4401	0.84	5402

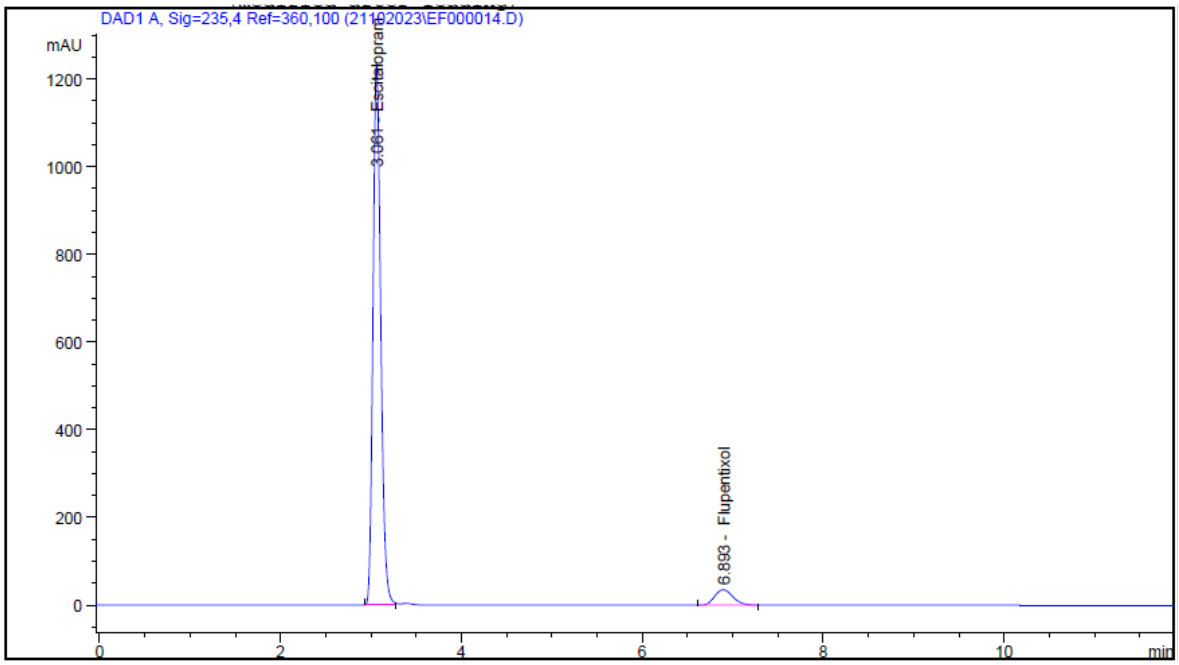

Figure 31: Chromatogram of Interlay Precision (80+4)-2

Table 41: Chromatogram of Precision (80+4)-2

Drug name	R.T	AREA	SYMM	TH. PLATES
ESP	3.061	7161.94580	0.79	6409
FLP	6.893	471.80814	0.83	5550

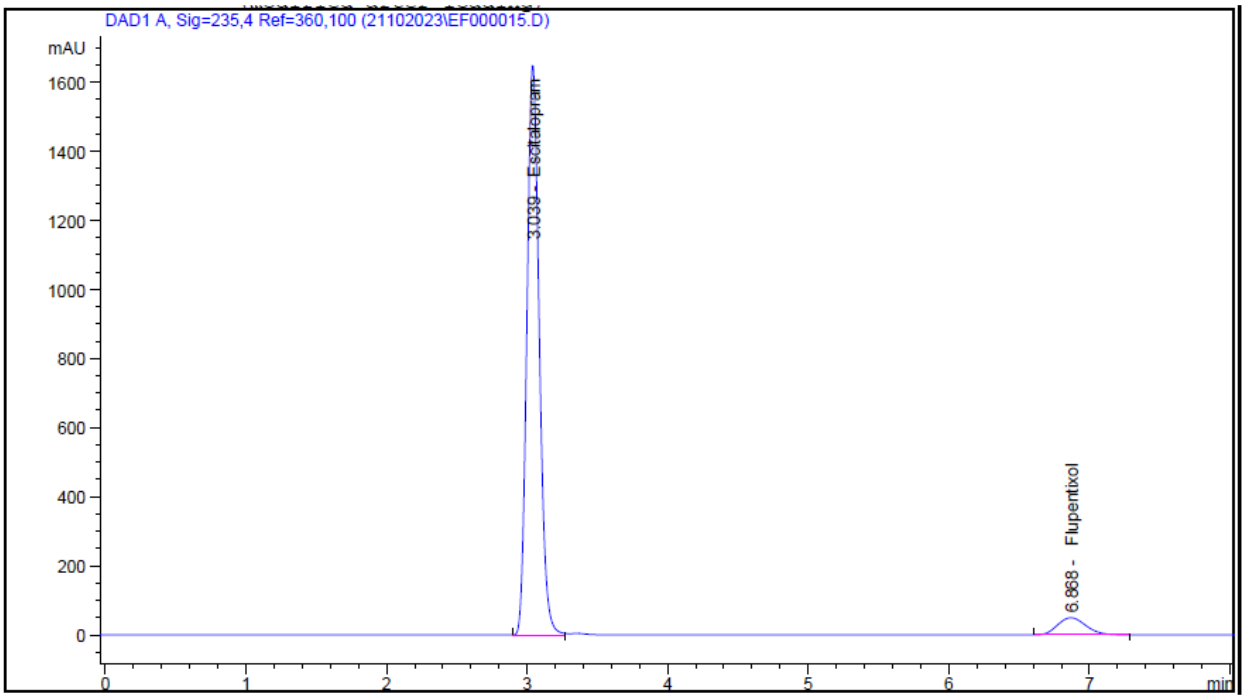

Figure 32: Chromatogram of Intraday Precision (120+6)-1

Table 42: Chromatogram of Precision (120+6)-1

Drug name	**R. T**	**AREA**	**SYMM**	**TH. PLATES**
ESP	3.039	9642.15	0.84	5875
FLP	6.868	701.5968	0.84	5509

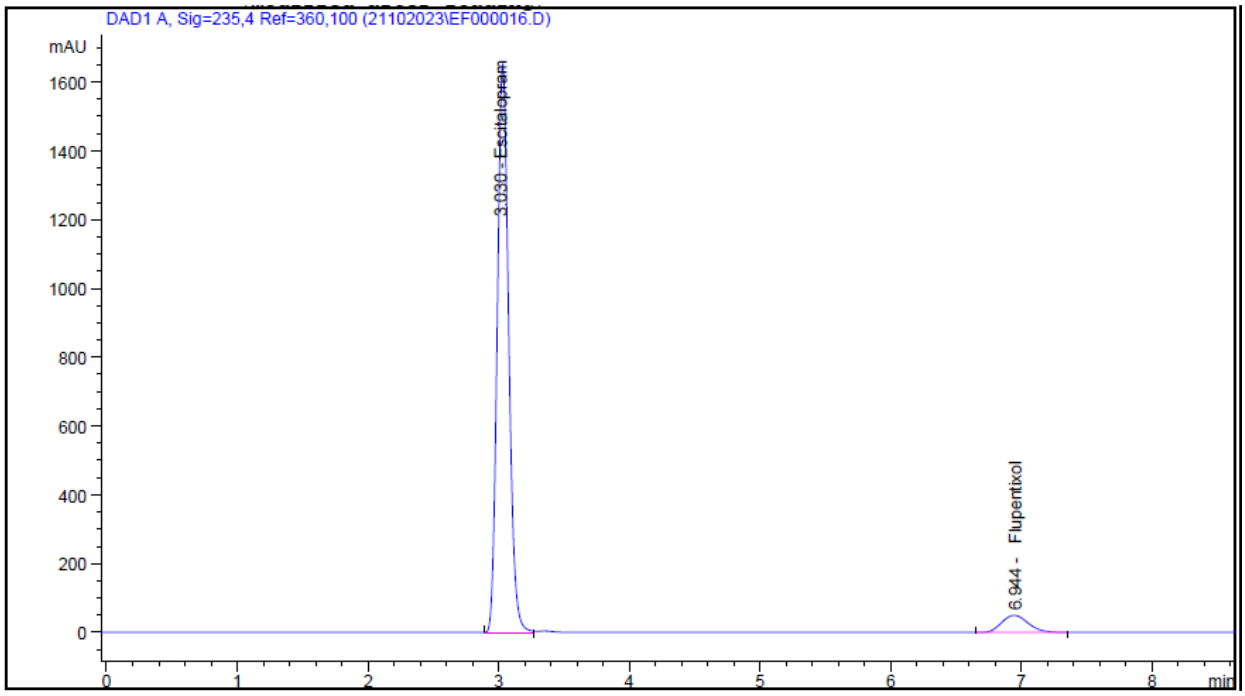

Figure 33: Chromatogram of Interday Precision (120+6)-2

Table 43: Chromatogram of Precision (120+6)-2

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.030	9997.6923	0.85	5982
FLP	6.944	704.6033	0.84	5519

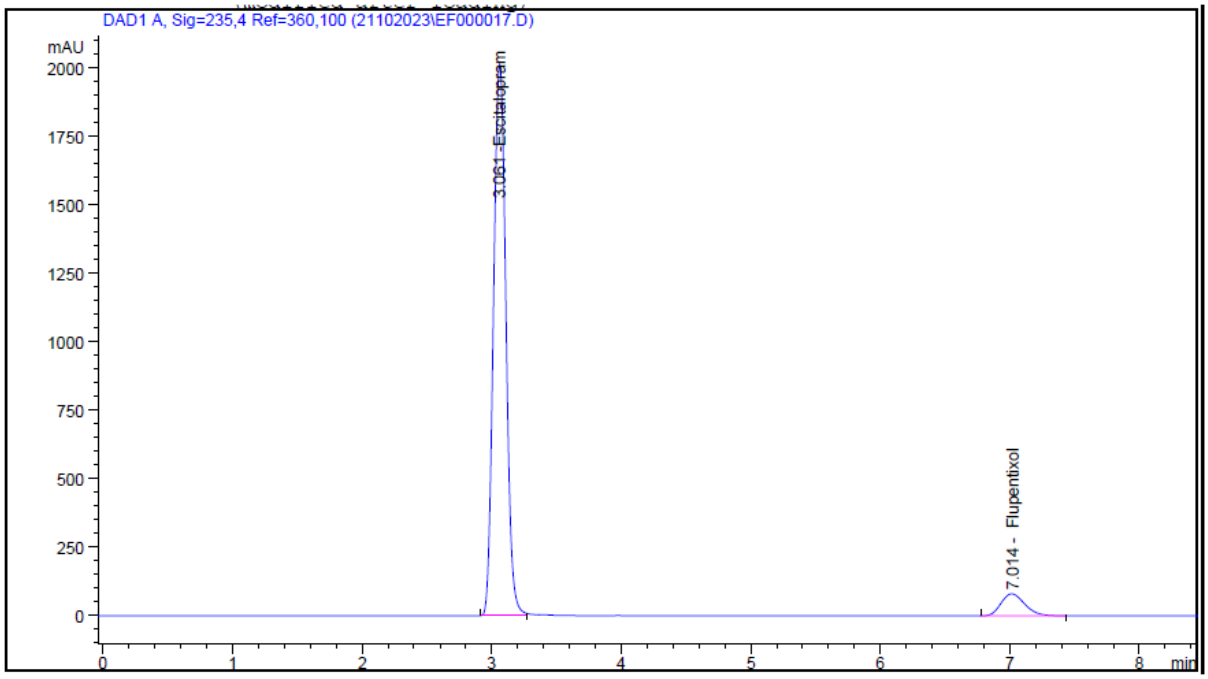

Figure 34: Chromatogram of Intraday Precision (160+8)-1

Table 44: Chromatogram of Precision (160+8)-1

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.061	12375.6	0.94	4925
FLP	7.014	927.07568	0.77	7126

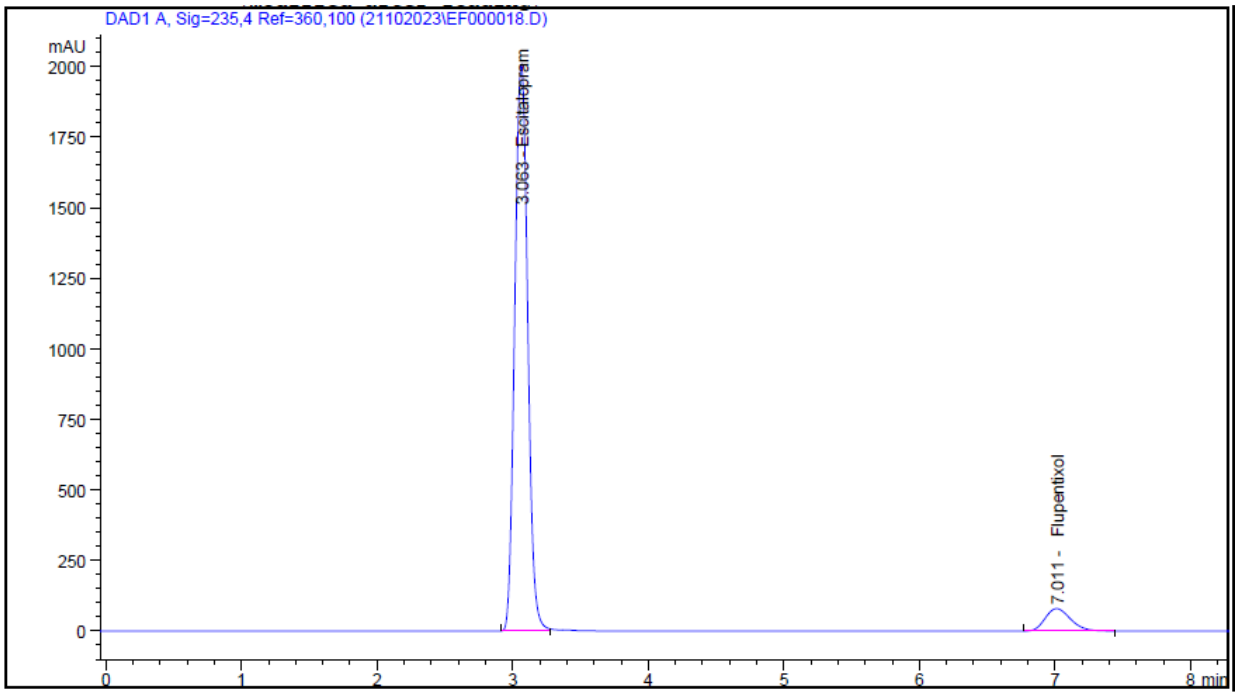

Figure 35: Chromatogram of Interday Precision (160+8)-2

Table 45: Chromatogram of Precision (160+8)-2

Drug name	R.T	AREA	SYMM	TH. PLATES
ESP	3.063	12329.1	0.94	4931
FLP	7.011	935.72363	0.77	6807

Table 46: Result of Intraday and Inter day Precision studies on RP-HPLC method for Escitalopram and Flupenthixol

METHOD	Drug	Conc. (µg/ml)	Intra day Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt
Rp - HPLC METHOD	ESP	80	7146.13±22.36	101.43	7131.42±1.27	101.13
		120	9670.10±39.03	100.98	9643.24±1.80	100.63
		160	12352±32.88	102.33	12353.28±35.78	102.34
	FLP	4	475.62±5.40	98.14	478.07±0.24	98.68
		6	703.10±2.13	98.48	701.01±0.63	98.18
		8	931.40±6.12	98.74	957.63±2.14	101.60

*Mean of each 3 reading for RP-HPLC method

Intraday and Inter day Precision studies on RP-HPLC method for Escitalopram and FLP which shows the high precision % amount in between 98% to 102% indicates to analytical method that concluded.

5. Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in (±1 ml/min^{-1}) proportion and the flow rate was varied by (±1ml/min^{-1}), and wavelength change (±1 ml/min^{-1}) of optimized chromatographic condition. The results of robustness studies are shown in **(Table)**.Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

1) **Flow Rate Change 0.6 ml & 0.8 ml**

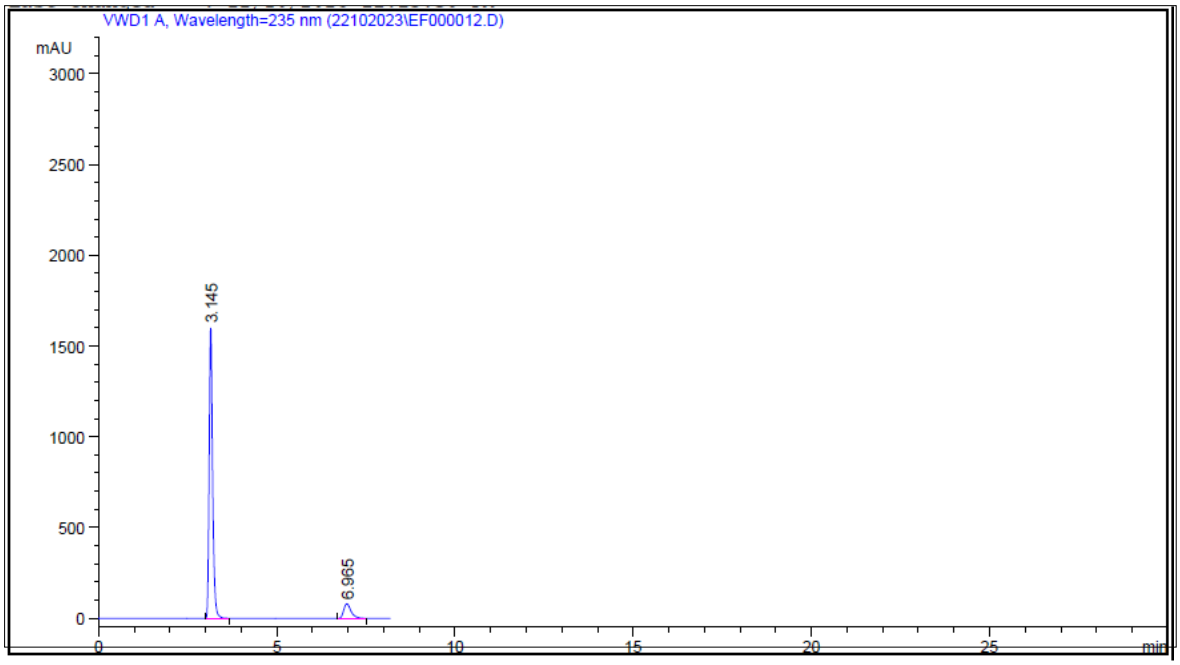

Figure 36: Chromatogram of flow change 0.6 ml (160+8 mcg/ml)

Table 47: Chromatogram of flow change 0.6 ml (160+8mcg/ml)

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.145	10778.4	0.76	5816
FLP	6.965	1163.0017	0.69	5893

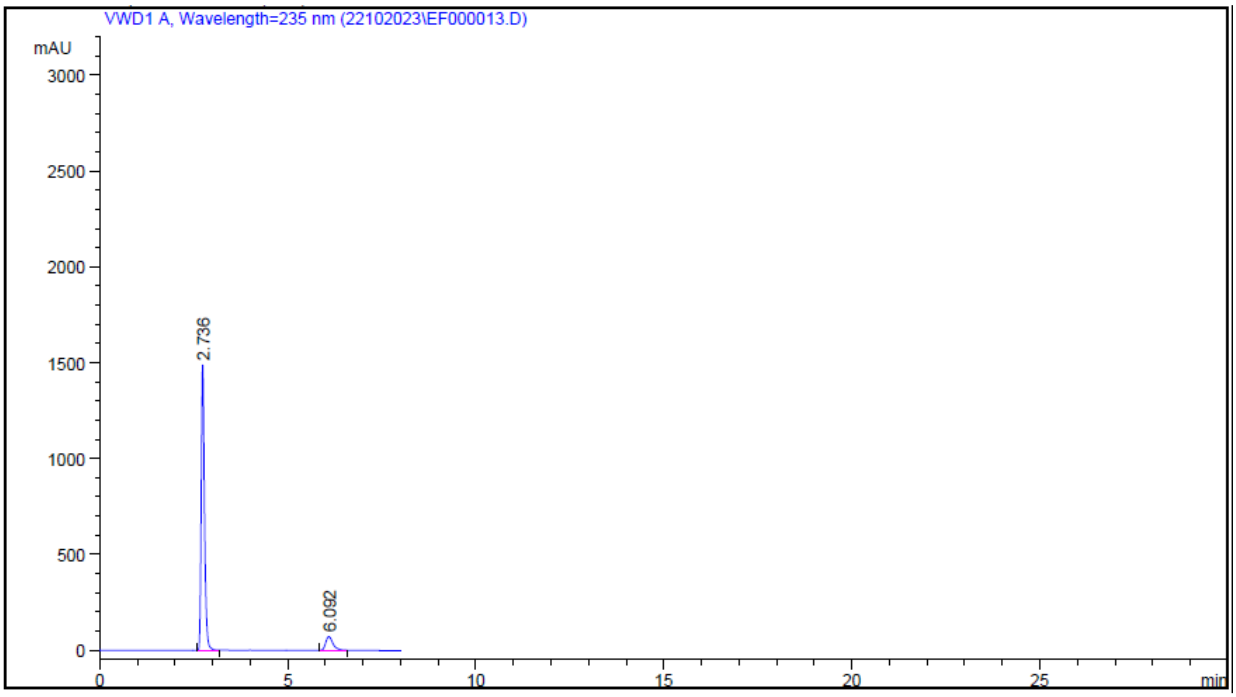

Figure 37: Chromatogram of flow change 0.8ml (160+8 mcg/ml)

Table 48: Chromatogram of flow change 0.8 ml (160+8 mcg/ml)

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	2.27	6393.18	0.77	5664
FLP	4.14	579.02	0.64	8346

2) **Robust wavelength change (234) (160+8mcg/ml)**

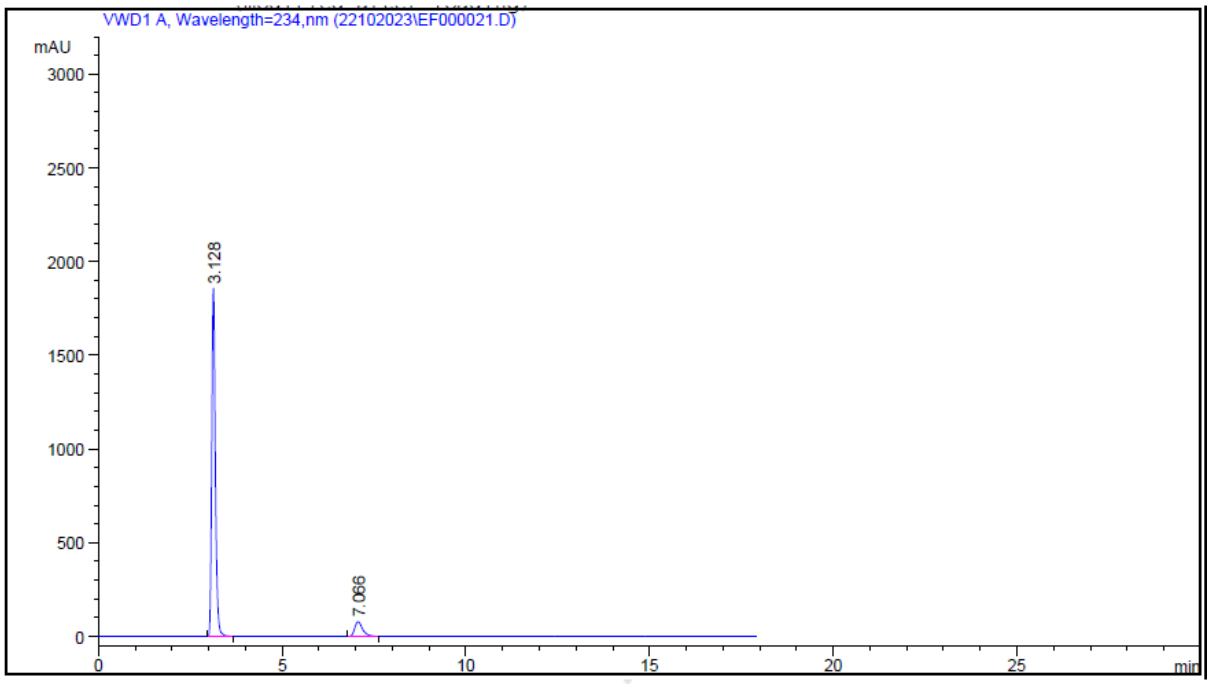

Figure 38: Chromatogram of wavelength change (234) (160+8 mcg/ml)

Table 49: Chromatogram of wavelength change (234) (160+8mcg/ml

Drug name	R.T	AREA	SYMM	TH.PLATES
ESP	3.12	12613.5	0.80	5567
FLP	7.06	1178.4637	0.68	5799

Robust wavelength change (236nm) (160+8 mcg/ml)

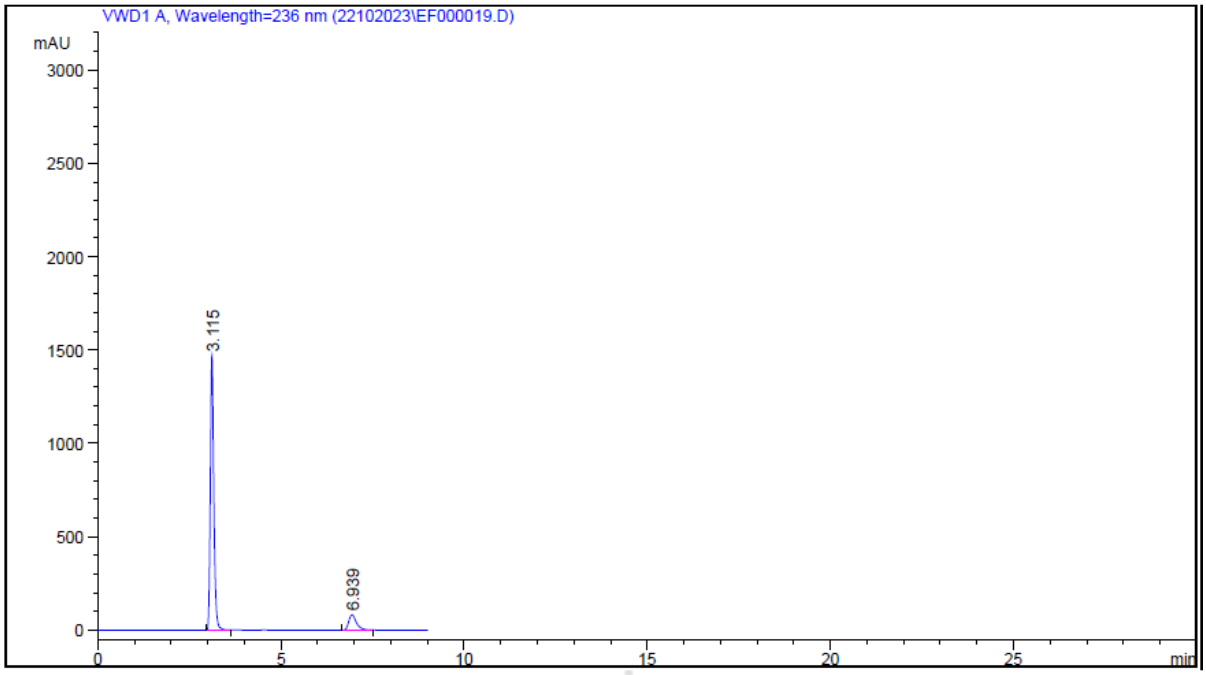

Figure 39: Chromatogram of wavelength change (236nm) (160+8 mcg/ml)

Table 50: Chromatogram of wavelength change (236nm) (40+2mcg/ml)

Drug name	R.T	AREA	SYMM	TH.PLATES
ESP	3.115	9650.0752	0.80	6104
FLP	6.939	1199.4163	0.68	5718

3) Chromatogram of mobile phase change (34 MEOH + 66 Buffer 0.1% WATER) (160+8mcg/ml)

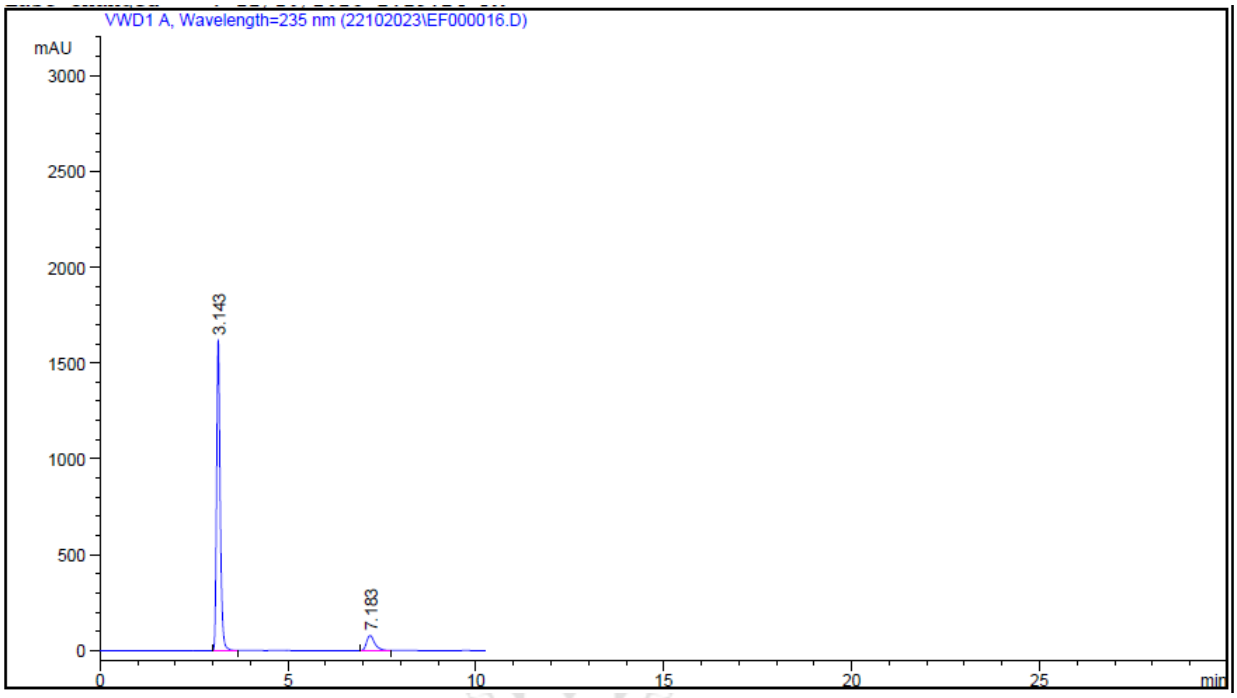

Figure 40: Chromatogram of mobile phase change (34 MEOH + 66 Buffer 0.1% WATER) (160+8mcg/ml)

Table 51: Chromatogram of mobile phase change (34 MEOH + 66 Buffer 0.1% WATER) (160+8 mcg/ml)

Drug name	R.T	AREA	SYMM	TH. PLATES
ESP	3.143	10818.8	0.78	5808
FLP	7.183	1178.92834	0.68	5862

Chromatogram of mobile phase change (36 MEOH + 64 OPA 0.1% Buffer) (160+8mcg/ml)

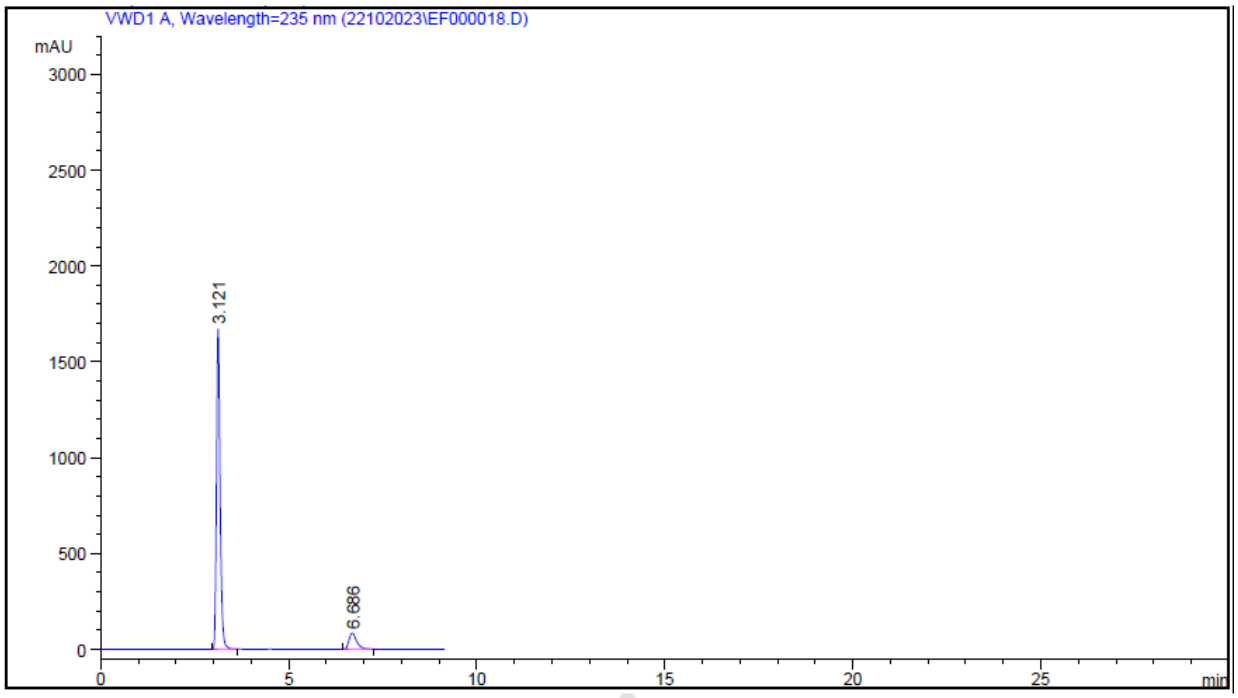

Figure 41: Chromatogram of mobile phase change (36 MEOH + 64 Buffer 0.1% WATER) (160+8 mcg/ml)

Table 52: Chromatogram of mobile phase change (36 MEOH + 64 OPA 0.1% WATER) (160+8 mcg/ml)

Drug name	R. T	AREA	SYMM	TH. PLATES
ESP	3.121	11177.2	0.78	5726
FLP	6.686	1193.9401	0.67	5821

Table 53: Result of Robustness Study of Escitalopram

Parameters	Conc. (µg/r	Amount of Detected (me an ±SD)	%RSD	Amount of detected (mean±SD)	%RSD
		For Escitalopram		For Flupenthixol	
Chromatogram of flow change 0.6 ml	160+8	10781±4.9	0.05	1168.52±8.0	0.68
Chromatogram of flow change 0.8 ml	160+8	8976.±21.4	0.24	955.46±5.98	0.63
Chromatogram of comp change wavelength change nm	160+8	12585.8±39.	0.31	1184.8±1.41	0.12
Chromatogram of comp change wavelength change nm	160+8	9709.42±83.	0.86	1208.27±12.5	1.04
Chromatogram of mobile phase chang 34+66 ml	160+8	10871±74.6	0.69	1178.56±0.5	0.04
Chromatogram of mobile phase change 36+64 ml	160+8	11036.1±19	1.81	1185.3±12.2	1.03

Robustness Study of Escitalopram

The changes were doing flow rate (±1 ml/ min^{-1}), PH of mobile phase composition (±1 ml/ min^{-1}), and Wavelength (±1 ml/ min^{-1}). %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded. **(Table).**

Robustness Study of Flupenthixol

The changes were doing flow rate (±1 ml/ min^{-1}), PH of mobile phase composition (±1 ml/ min^{-1}), and Wavelength (±1 ml/ min^{-1}). %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded. **(Table).**

6. Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as.

$$LOD = 3.3 (SD)/S$$

Where,

SD = Standard deviation of Y intercept

S = Slope

Limit of detection = 0.5178 (ug/mL)

Limit of Quantitation = 1.56 (ug/mL)

The LOD and LOQ of Flupenthixol was found to be 0.5178 (ug/mL) and 1.56 (ug/mL), analytical method that concluded.

7. Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the

S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

$$LOQ = 10 (SD)/ S \text{ Where,}$$

SD = Standard deviation Y intercept

S = Slope

Limit of detection = 3.3X34.20/63.04=4.47 (ug/mL)

Limit of Quantitation = 1 0 X34.20/ 63.04=13.55 (µg/mL)

The LOD and LOQ of Escitalopram was found to be 4.47 (ug/mL) and 13.55 (ug/mL), analytical method that concluded.

Analysis of tablet formulation

Procedure

Weigh 20 Escitalopram and Flupenthixol combination tablets and calculated the average weight, accurately weigh, and transfer the sample equivalent to 10 mg Escitalopram and 0.5 mg Flupenthixol into 10 ml volumetric flask. Add about 10 ml MEOH of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.1ml of the above stock solution into a

10 ml volumetric flask and dilute up to the mark with diluents. (120+6 µg/ml) The simple chromatogram of test Escitalopram and Flupenthixol Shown in (**Figure).**

The amounts of Escitalopram and Flupenthixol per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Label claim for %RSD Calculated, Result was shown in (**Table)**

Brand Name: Rexipra fx 5 (Intas)

Total weight of 20 tab wt . . . . =0.38 Gms -

Avg. Weight = 0.19 Gms. /Tab

Eq. wt for 10 mg= 10X19/ 10 =19 mg

Take 1.2 mg sin 10 ml water sonicate 10 min

I.e.120 µg/ml Escitalopram and 6 µl/ml Flupenthixol ----- STOCK -I

Take 19 mgs in 10 ml MEOH = 50 µg/ml FLP and1000 µg/ml ESP

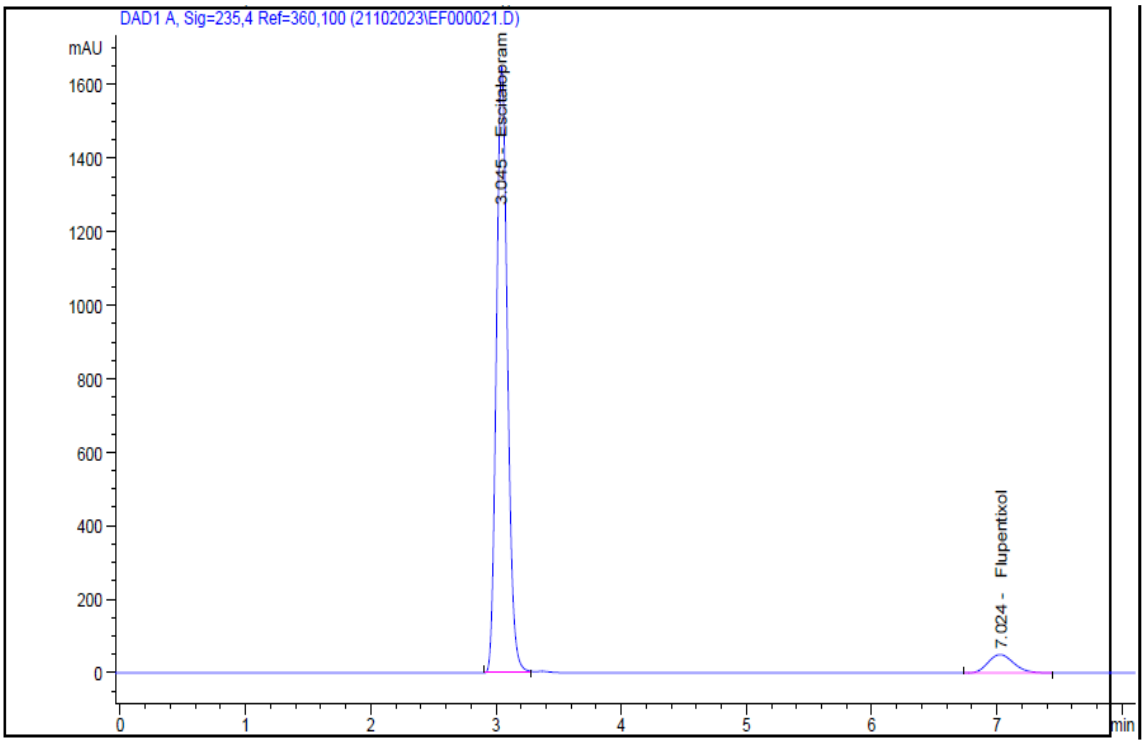

Figure 42: Chromatogram for Marketed Formulation (120+6)

Table 54: Analysis of marketed formulation

	Drug	Amt. Found	%Label Claim	SD	%RSD
Rp-HPLC Metho	ESP	121.84	101.54	0.087	0.073
	FLP	5.94	99.13	0.751	0.762
	ESP	121.72	101.44	0.073	0.072
	FLP	5.88	98.07	0.045	0.762

Analysis of marketed formulation were also % Label Claim was found to be 98-102% Satisfactory are concluded. **(Table).**

Stability study of Escitalopram and Flupenthixol

Degradation study at 0.1N HCL heating after 1 hour

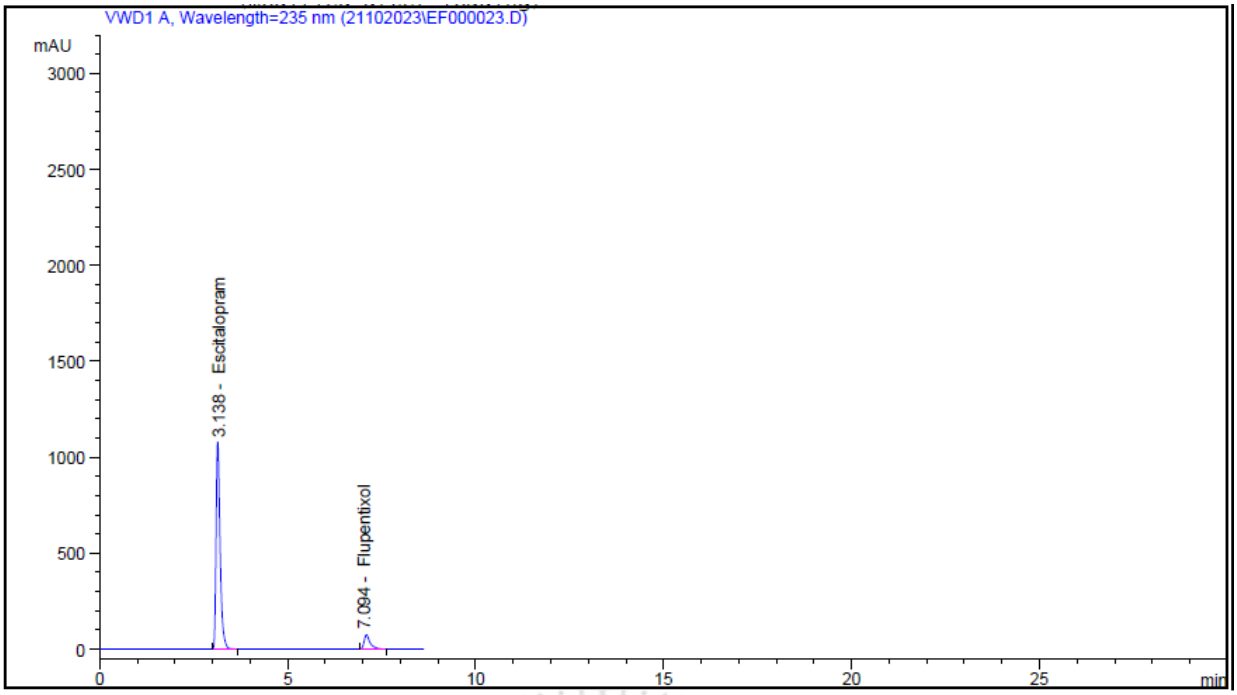

Figure 43: Chromatogram of at 0.1N HCL heating after 1 hour.

Table 55: Result of Degradation Study of Escitalopram and Flupenthixol at 0.1N HCL heating after 1 hour

Ret. Time [min]	Area [mAU*a]	Area % [mAU*a]	Name
3.138	8067.36426	90.6131	ESP
7.094	635.72357	9.3869	FLP

Degradation study at 0.1N NaOH heating after 1 hours

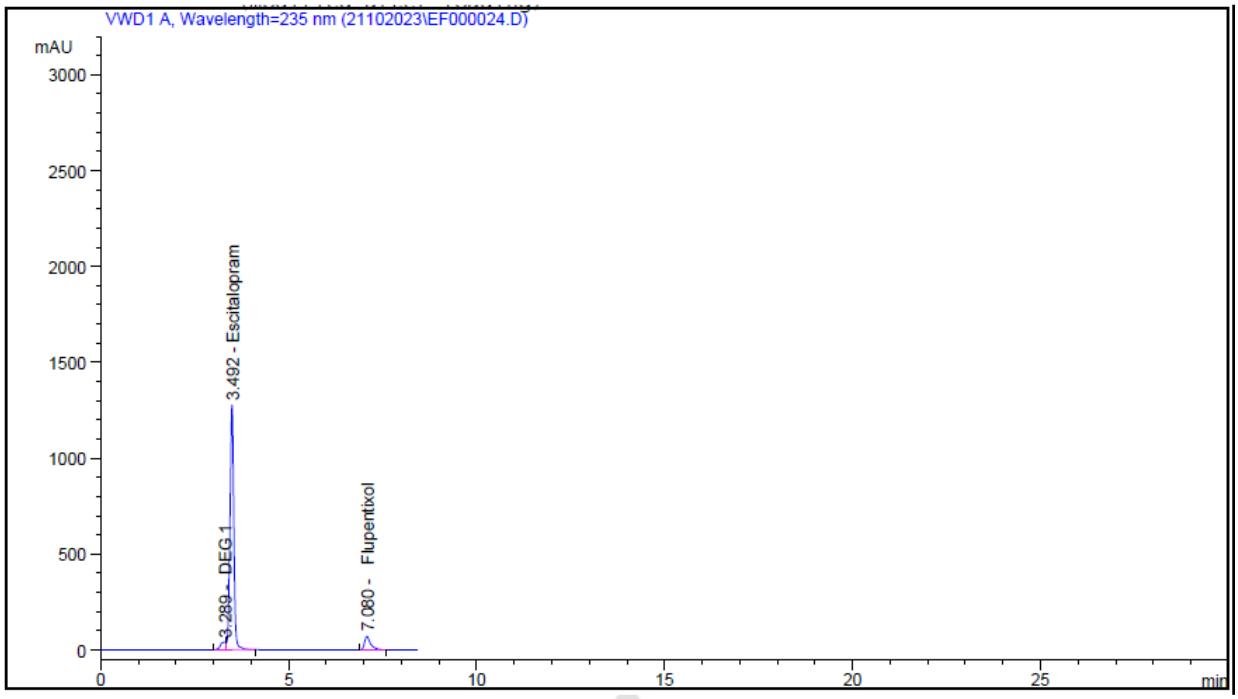

Figure 44: Chromatogram of comp change at 0.1N NAOH heating after 1 hour.

Table 56: Result of Degradation Study of Escitalopram and Flupenthixol at 0.1N NaOH heating after 1 hour

Ret. Time[min]	Area[mAU*a]	Area % [mAU*a]
3.289	408.75266	3.9537
3.492	9121.16504	88.2250
7.080	608.60669	7.8213

Degradation study at 3% H2O2 heating after 1 hour.

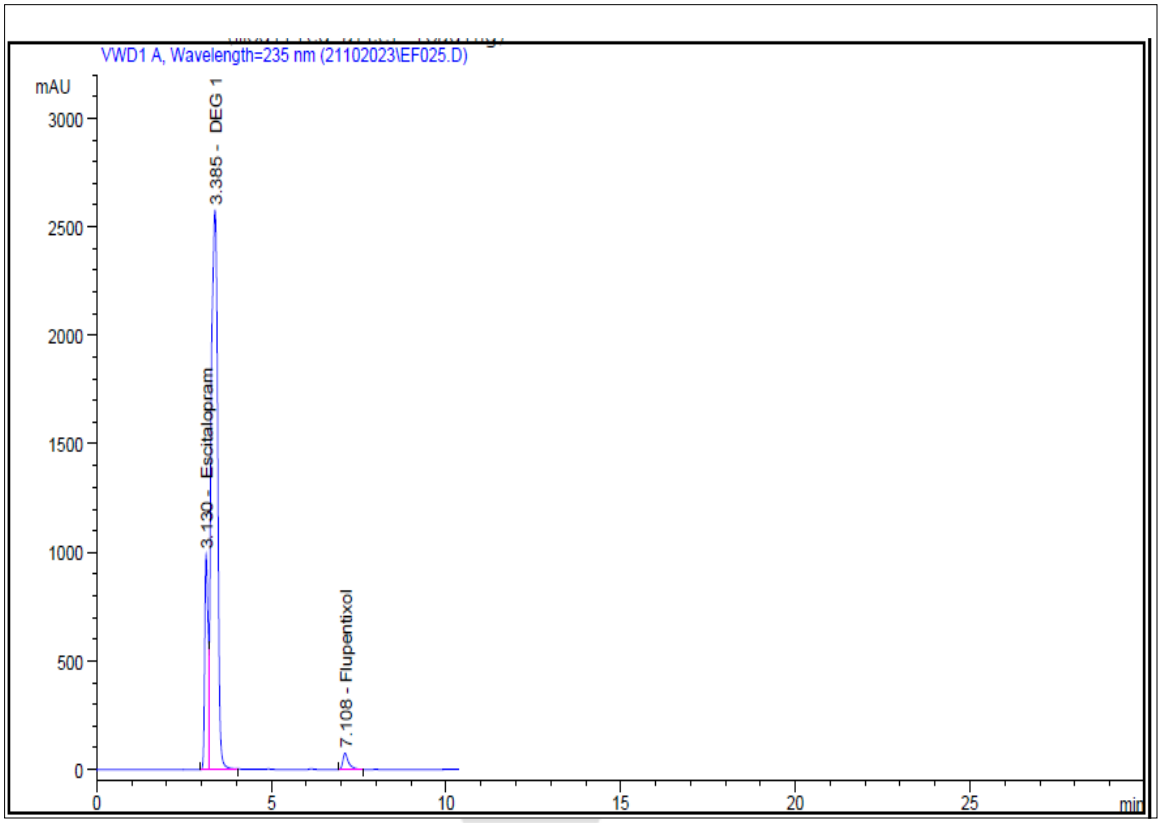

Figure 45: Chromatogram of comp. change at 3% H2O2 heating after 1 hour

Table 57: Result of Degradation Study of Escitalopram and Flupenthixol at 3% H2O2heating after 1 hour

Ret. Time[min]	Area[mAU*a]	Area %[mAU*a]
3.130	6613.0078	16.4224
3.385	32795.9	81.4438
7.108	659.21576	2.1337

Degradation Study at Neutral heating after 1 hour.

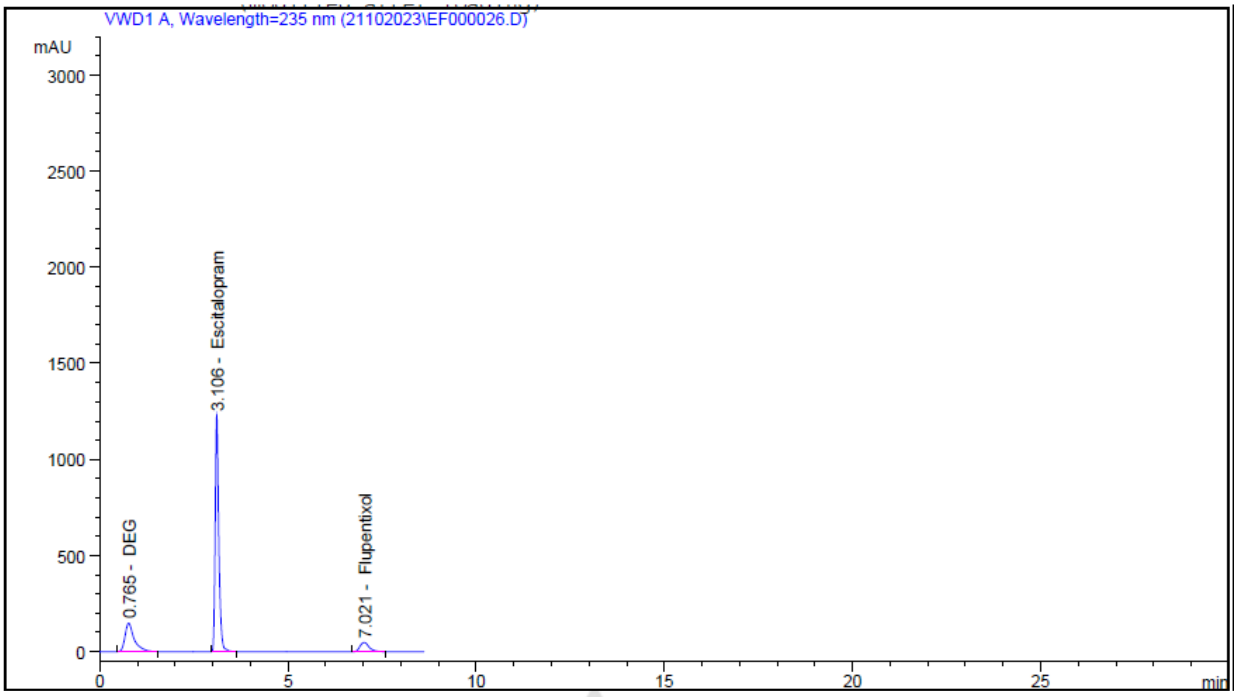

Figure 46: Chromatogram of comp change at Neutral heating after 1 hour

Table 58: Result of Degradation Study of Escitalopram and Flupenthixol at Neutral heating after 1 hour

Ret. Time[min]	Area [mAU*a]	Area % [mAU*a]	Name
0.765	2524.06250	22.0225	
3.106	9621.8168	70.8631	ESP
7.021	705.3999	7.1144	FLP

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