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STABILITY INDICATING CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ESCITALOPRAM OXALATE AND FLUPENTIXOL IN ITS PHARMACEUTICAL DOSAGE FORM BY HPLC

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ABSTRACT

Objective: An accurate, precise and simple stability indicating Chromatographic method for development and validation for the simultaneous estimation of Escitalopram Oxalate and Flupentixol in its pharmaceutical dosage form by HPLC and to perform stability study on developed method & validation method. **Experiment Work:** The best separation was achieved on a C18 Column (25 cm X 0.46 cm), C18 reversed phase column with Isocratic program, mobile phase A-Potassium Dihydrogen Phosphate Buffer, pH-5.5 &mobile phase B-Methanol (35: 65), Flow rate 1.0 ml/min., Injection volume 20 µl, UV detection was performed by using wavelength at 302 nm. **Result and Discussion:** The method was linear over the concentration range of 20-60 µg/ml (r^2-0.9995), with limits of detection and quantification of 1.326 µg/ml & 4.019 µg/ml for Escitalopram Oxalate (r^2-0.9995) and the method was linear over the concentration range of (1-3 µg/ml) (r^2-0.9995) limits of detection and quantification of 0.070 µg/ml & 0.211 µg/ml for Flupentixol (r^2-0.9995). Forced degradation study was carried out according to ICH guideline in all five conditions (Acid Degradation, Base Degradation, Oxidative Degradation, Thermal Degradation & Photo Degradation).

KEYWORDS: Escitalopram Oxalate, Flupentixol, RP-HPLC, Degradation Studies.

INTRODUCTION

Escitalopram Oxalate and Flupentixol

Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest. Also called major depressive disorder or clinical depression, it affects how you feel, think and behave and can lead to a variety of emotional and physical problems. You may have trouble doing normal day to day activities, and sometimes you may feel as if life isn't worth living. More than just a bout of the blues, depression isn't a weakness and you can't simply "snap out" of it. Depression may require long term treatment. But don't get discouraged. Most people with depression feel better with medication, psychological counseling or both.

MATERIALS AND METHODS

Standards and Reagents

❖ **Standard**

Standard	Source
Escitalopram Oxalate	Yash Pharmaceuticals
Flupentixol	Yash Pharmaceuticals

❖ **Sample**

Sample	Source
Detens	Archi care

❖ **REAGENTS USED IN EXPERIMENT**

Chemical/ Reagent	Grade	Manufacturer
Methanol	HPLC Grade	Merck specialties pvt, Ltd., Mumbai
Potassium Dihydrogen Phosphate	AR	Merck specialties pvt, Ltd., Mumbai
Water	HPLC Grade	Merck specialties pvt, Ltd., Mumbai
Acetonitrile	HPLC Grade	Merck specialties pvt, Ltd., Mumbai

Apparatus and Equipment used in experiment

Apparatus / Equipment

Components	Volume	Type
Volumetricflasks	10 ml, 25 ml, 50 ml,100 ml	Borosilicate glass type I
Pipettes	1 ml, 2 ml, 5 ml, 10 ml	Borosilicate glass type I
Measuring cylinder	100 ml	Borosilicate glass type I
Beaker	100 ml, 250 ml, 500 ml	Borosilicate glass type I
Whatmann Filter	-	Filter Paper No.42

Instrumentation

Instrumentation for HPLC

Component	Brand / Model / Software	Manufacturer/ Supplier
HPLC	Shimadzu LC-20 AT	Shimadzu
HPLC Column	C18 (25cm x 0.46 cm) Hypersil BDS	-
Detector	SPD-20A	-
Ultrasonic Water Bath	Fast Clean	Ultrasonic cleaner
pH meter	-	Electroquip's Digital pH meter
Analytical Balance	AUX-200	-

Instrumentation for UV spectrophotometer.

Component	Brand / Model / Software	Manufacturer/ Supplier
UV Visible spectrophotometer	Systronic 119	Systronic
Cuvette	Quartz cuvette	Shimadzu Corporation, Kyoto, Japan
Analytical Balance	AUX-200	-

Instrumentation for Melting Range

Component	Brand / Model / Software	Manufacturer/ Supplier
Melting pointApparatus	Thermocal	Analab

Preparation of standard solution of mixtures of Escitalopram Oxalate (40 ppm) and Flupentixol (2 ppm)

(A) Escitalopram Oxalate Standard stock solution: (400µg/mL)

A 40 mg of Escitalopram Oxalate was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase.

(B) Flupentixol Standard stock solution: (20µg/mL)

A 20 mg of Flupentixol was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with Methanol, Take 10ml from this solution and Transfer to 100ml volumetric flask and volume was made up with the Mobile phase

(C) Preparation of standard solution of binary mixtures of Escitalopram Oxalate (40µg/mL) and Flupentixol (2µg/mL)

Take 1 ml from the Escitalopram Oxalate stock solution and 1 ml from Flupentixol stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Table 1: RP-HPLC optimized chromatographic conditions.

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Buffer (pH 5.5): Methanol (35:65)
Column	C18 (25cm x 0.46 cm) Hypersil BDS
Flow rate	1ml/min
Runtime	9 min
Injection volume	20 µL
Detection wavelength	302 nm

Preparation of Calibration Graph

Linearity

The linearity for Flupentixol and Escitalopram Oxalate were assessed by analysis of combined standard solution in range of 1-6µg/ml and 20-60µg/ml respectively, 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Flupentixol (20µg/ml) and Escitalopram Oxalate (40µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 1,1.5,2,2.5 and 3µg/ml, and 20,30,40,50 and 60µg/ml for Flupentixol and Escitalopram Oxalate respectively. In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted. Correlation co- efficient for calibration curve Flupentixol and Escitalopram Oxalate was found to be 0.999 and 0.999 respectively. The regression line equation for Flupentixol and Escitalopram Oxalate are as following:

For Flupentixol y **= 551.41x - 12.387**and For Escitalopram Oxalate: **y = 39.499x - 14.706**

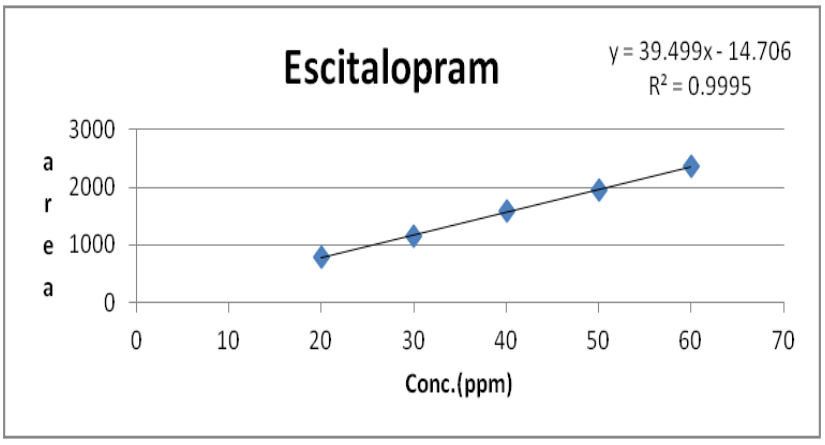

Figure1: Calibration Curve of Escitalopram Oxalate (20-60µg/ml).

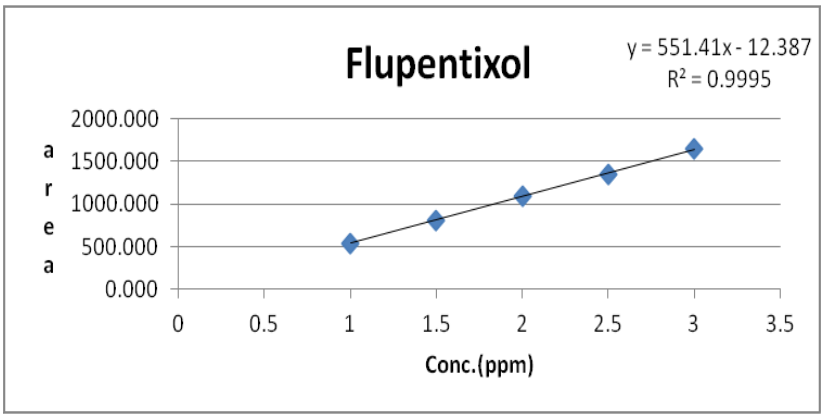

Figure 2: Calibration Curve of Flupentixol (1-3µg/ml).

Degradation Study of RP-HPLC Method

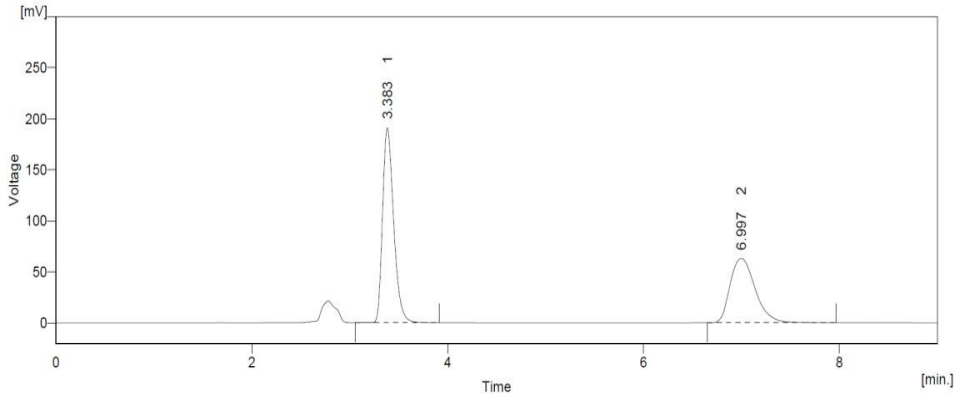

Figure 3: Escitalopram Oxalate and Flupentixol for sample.

1. Acid Degradation

Acid decomposition studies were performed by Transferring One ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N Hydrochloride solutions was added and mixed well and put for 5 hrsat Room Temperature. Then the volume was adjusted with diluent to get 2µg/ml for Flupentixol and 40 µg/ml for Escitalopram Oxalate.

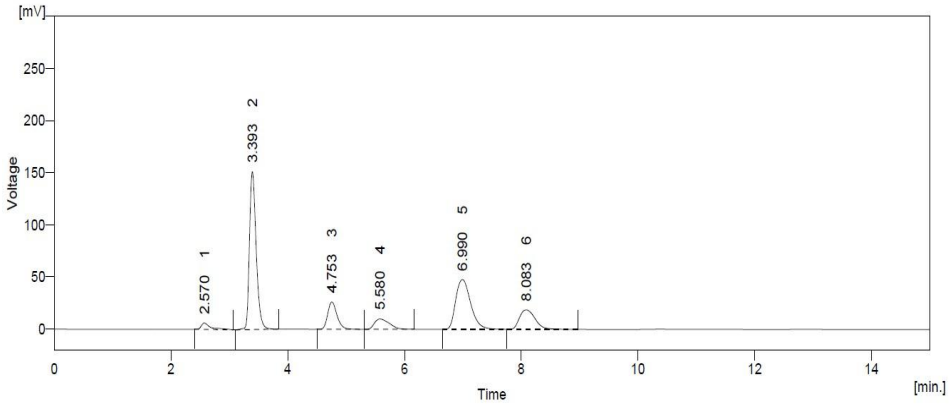

Figure 4: Flupentixol and Escitalopram Oxalate Acid Degradation Sample.

2. Base Degradation

Basic decomposition studies were performed by Transferring 1 ml of stocksolution in to 10 ml of volumetric flask. 2 ml of 0.1 N NaOH solutions was added and mixed well and put for 5 hrs at Room Temperature. Then the volume was adjusted with diluent to get 2 µg/ml for Flupentixol and 40 µg/ml for Escitalopram Oxalate.

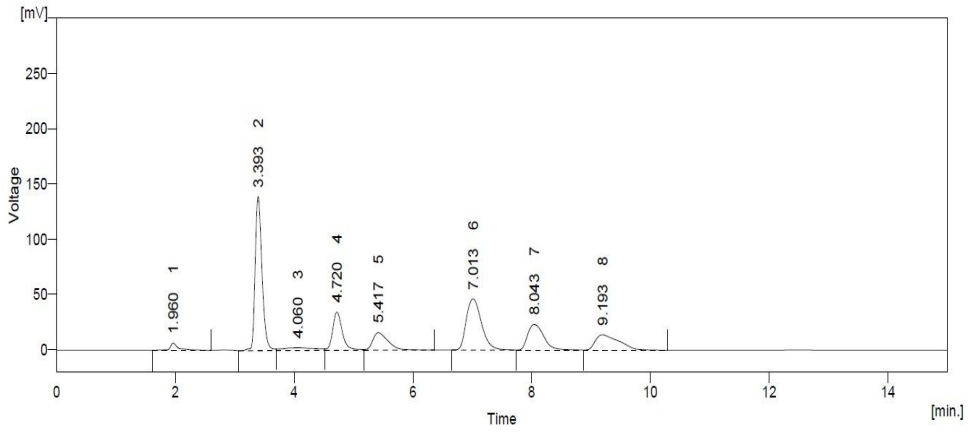

Figure 5: Flupentixol and Escitalopram Oxalate Base Degradation Sample.

3. Oxidative Degradation

Oxidative decomposition studies were performed by Transferring1 ml of stocksolution in to 10 ml of volumetric flask. 2 ml of 3% H_2O_2 solutions was added and mixed well and put for 5 hrs at Room Temperature. Then the volume was adjusted with diluent to get 2 µg/ml for Flupentixol and 40 µg/ml for Escitalopram Oxalate.

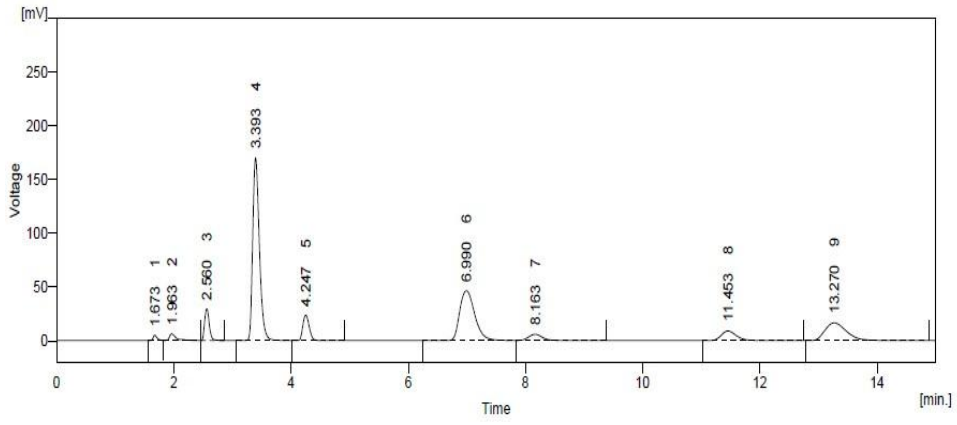

Figure 6: Flupentixol and Escitalopram Oxalate Oxidation Degradation sample.

4. Photo Degradation

Photo Degradation studies were performed by Transferring 1 ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was kept in UV Chamber for 24 hrs. Then the

volume was adjusted with diluent to get 2 µg/ml for Flupentixol and 40 µg/ml for Escitalopram Oxalate.

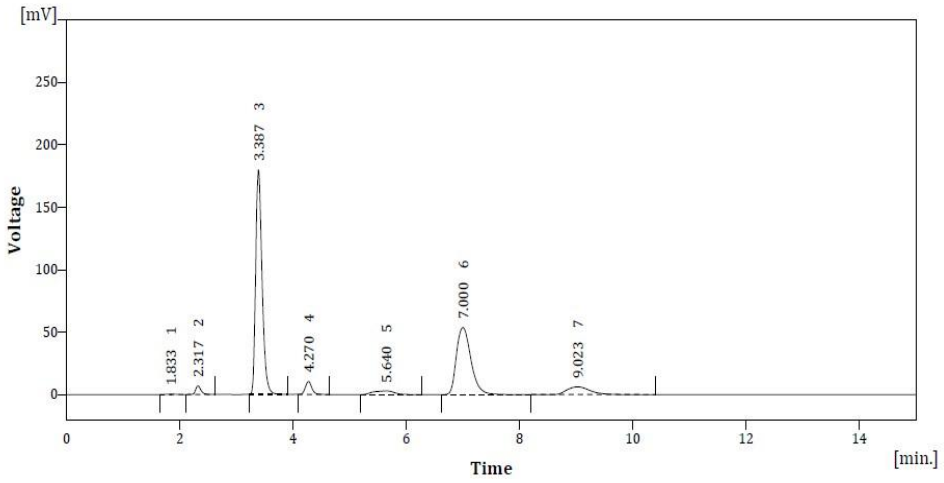

Figure 7: Escitalopram Oxalate and Flupentixol Photo Degradation Sample.

5. Thermal Degradation

Escitalopram Thermal Degradation: Taken 80mg of Escitalopram Oxalate in a Petri dish and Put it in the Oven at 70^0C for 3hrs, After Time Period the Escitalopram Oxalate was kept out and from this 40 mg of Escitalopram Oxalate was transferred in 100ml Volumetric Flask and Volume was Made up with Mobile phase, From this Solution Taken 1ml and Transferred to 10ml Volumetric flask to make Escitalopram Oxalate 40µg/ml.

Flupentixol Thermal Degradation: Taken 60mg of Flupentixol in a Petri dish and Put it in the Oven at 70^0C for 3hrs. After Time Period the Flupentixol was kept out and from this 20 mg of Flupentixol was transferred in 100ml Volumetric Flask and Volume was Made up with Mobile phase, From this Solution Taken 0.1ml and Transferred to 10ml Volumetric flask to make Flupentixol 2µg/ml.

Tablet Thermal Degradation: Tablet Powder taken in a Petri dish and put it in Oven at 70 °C for 4 hrs. After Time Period the Tablet Powder was equivalent to 2 mg Flupentixol and 14 mg Escitalopram Oxalate was transferred in 100ml Volumetric Flask and Volume was made up with Mobile phase. From this Solution Taken 1ml and Transferred to 10ml Volumetric flask to make Flupentixol 2µg/ml and Escitalopram Oxalate 40µg/ml.

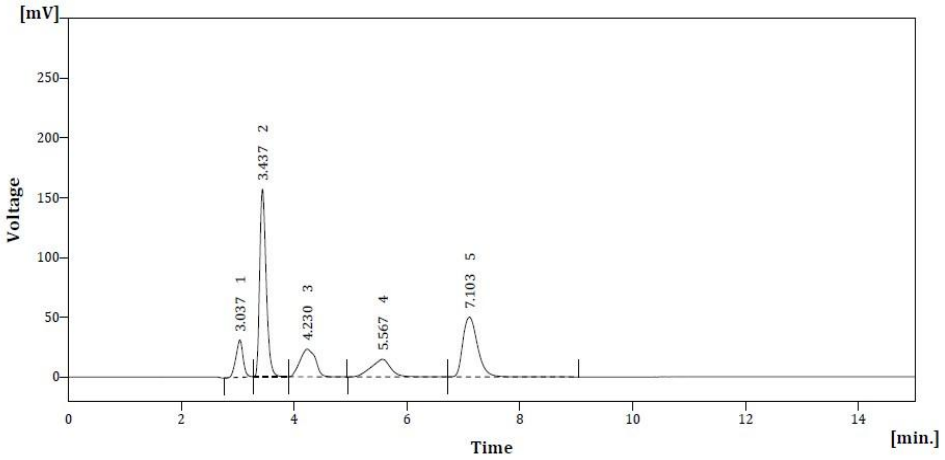

Figure 8: Escitalopram Oxalate and Flupentixol Thermal Degradation Sample.

Table 2: Calculation for Stability.

Drugs	Area
Escitalopram Oxalate	1610.381
Flupentixol	1121.245

Table 3: Flupentixol % Degradation.

Flupentixol				
Parameter	Standard		Sample	
	Area	%Degradation	Area	%Degradation
Acid	859.277	23.364	848.647	24.312
Base	813.754	27.424	823.520	26.553
Thermal	961.288	14.267	839.313	27.543s
Oxidation	811.379	27.636	826.722	26.267
Photo	944.533	15.761	950.486	12.52

Table 4: Escitalopram Oxalate % Degradation.

Parameter	Standard		Sample	
	Area	%Degradation	Area	%Degradation
Acid	1235.094	23.304	1203.210	25.284
Base	1137.596	29.359	1114.358	30.802
Thermal	1238.055	23.121	1166.847	25.145
Oxidation	1309.904	18.659	1341.292	16.710
Photo	1417.367	11.986	1408.773	15.23

Preparation of standard solution of mixtures of Escitalopram Oxalate (40 ppm) and Flupentixol (2 ppm)

(A) Escitalopram Oxalate Standard stock solution: (400µg/mL).

A 40 mg of Escitalopram Oxalate was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase.

(B) Flupentixol Standard stock solution: (20µg/mL).

A 20mg of Flupentixol was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with Methanol, Take 10ml from this solution and Transfer to 100ml volumetric flask and volume was made up with the Mobile phase.

(C) Preparation of standard solution of binary mixtures of Escitalopram Oxalate (40µg/mL) and Flupentixol (2µg/mL).

Take 1 mL from the Escitalopram Oxalate stock solution and 1mL from Flupentixol stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Validation of RP-HPLC method: (1) Specificity

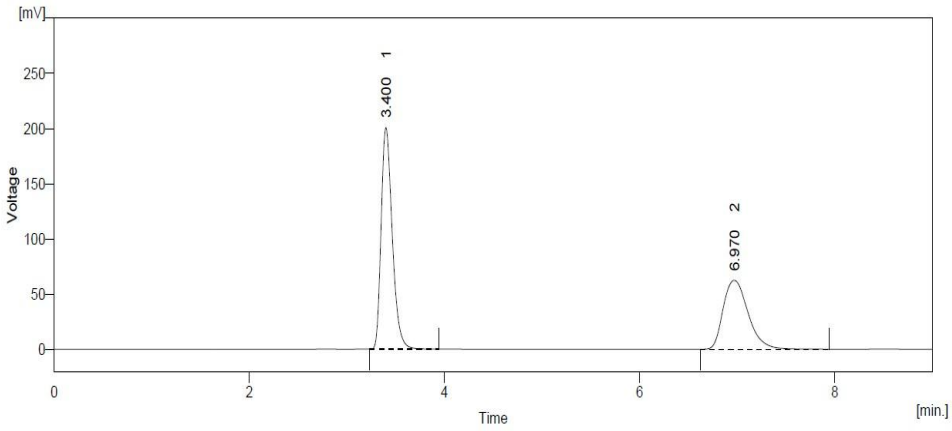

Figure 9: Chromatogram of Escitalopram Oxalate and Flupentixol Std.

(2) Linearity

The linearity for Flupentixol and Escitalopram Oxalate were assessed by analysis of combined standard solution in range of 1-6µg/ml and 20-60µg/ml respectively, 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Flupentixol (20µg/ml) and Escitalopram Oxalate (40µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 1,1.5,2,2.5 and 3µg/ml, and 20,30,40,50 and 60µg/ml for Flupentixol and Escitalopram Oxalate respectively.

In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Correlation co-efficient for calibration curve Flupentixol and Escitalopram Oxalate was found to be 0.999 and 0.999 respectively.

The regression line equation for Flupentixol and Escitalopram Oxalate are as following: For Flupentixol y = **551.41x - 12.387** and For Escitalopram Oxalate: **y = 39.499x - 14.706.**

Table5: Linearity data for Escitalopram Oxalate.

Sr.No	Concentration(µg/ml)	Area
1	20	782.724
2	30	1157.401
3	40	1578.097
4	50	1943.473
5	60	2364.659

Table 6: Linearity data for Flupentixol.

Sr.No	Concentration(µg/ml)	Area
1	1	544.787
2	1.5	804.528
3	2	1100.029
4	2.5	1354.552
5	3	1648.310

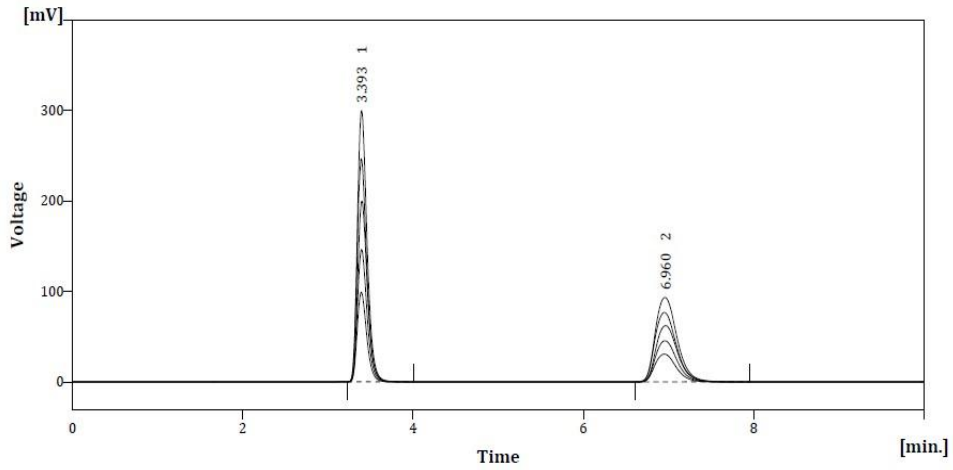

Figure 10: Overlay chromatogram of different concentrations of mixtures of Flupentixol and Escitalopram Oxalate.

Precision

1. Repeatability

The data for repeatability of peak area measurement for Flupentixol (2µg/ml) and Escitalopram Oxalate (40µg/ml) based on six measurements of samesolution of Flupentixol (2µg/ml) and Escitalopram Oxalate (40µg/ml). The % RSD for Flupentixol and Escitalopram Oxalate was found to be 1.014 and 0.855respectively.

Table 7: Repeatability data for Escitalopram Oxalate.

Escitalopram Oxalate				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	40	1571.550	1571.476±13.441	0.855
		1544.879		
		1578.109		
		1581.269		
		1578.105		
		1574.943		

Table 8: Repeatability data for Flupentixol.

Flupentixol				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	2	1095.647	1094.282±11.094	1.014
		1097.830		
		1072.097		
		1102.243		
		1100.031		
		1097.846		

2. Intraday precision

Standard solution containing (20,40,60 µg/ml) of EscitalopramOxalateand (1,2,3 µg/ml) of Flupentixolwere analyzed three times on the same day and % R.S.D was calculated.

Table 9: Intraday precision data for estimation of Escitalopram Oxalates.

	Escitalopram Oxalate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	20	776.163± 6.707	0.864
2	40	1565.797± 9.036	0.577
3	60	2346.963± 16.705	0.712

Table 10: Intraday precision data for estimation of Flupentixol.

	Flupentixol		
SR. NO.	**Conc.(µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1	540.466± 3.543	0.655
2	2	1089.221± 8.454	0.776
3	3	1632.374± 15.290	0.937

3. Interday precision

Standard solution containing (20,40,60 µg/ml) of Escitalopram Oxalate and (1,2,3 µg/ml) of Flupentixol were analyzed three times on the different day and % R.S.D was calculated

Table 11: Interday precision data for estimation of Escitalopram Oxalate.

	Escitalopram Oxalate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	20	773.234± 5.163	0.668
2	40	1561.685± 7.314	0.468
3	60	2340.688± 10.701	0.457

Table 12: Interday precision data for estimation of Flupentixol.

	Flupentixol		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1	538.161± 3.876	0.720
2	2	1084.214± 11.298	1.042
3	3	1632.222± 13.574	0.832

Accuracy

For Escitalopram Oxalate

20 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 302 nm. The amount of Flupentixol was calculated at each level and % recoveries were computed.

For Flupentixol

1 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 302 nm. The amount of Flupentixol was calculated at each level and % recoveries were computed.

Table 13: Recovery data for Escitalopram Oxalate.

SR. NO.	Conc. Level (%)	Sample amount(µg/ml)	Amount Added (µg/ml)	Amountrecovered (µg/ml)	% Recovery	% Mean Recovery± S.D
1	80 %	20	16	15.838	98.989	99.890 ± 0.780
2		20	16	16.057	100.356	
3		20	16	16.052	100.324	
4	100 %	20	20	19.839	99.197	99.468 ± 0.304
5		20	20	19.959	99.797	
6		20	20	19.882	99.409	
7	120 %	20	24	23.951	99.795	99.429 ± 0.409
8		20	24	23.757	98.989	
9		20	24	23.881	99.504	

Table 14: Recovery data for Flupentixol.

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1	0.8	0.791	98.908	100.046 ± 1.060
2		1	0.8	0.808	101.004	
3		1	0.8	0.802	100.228	
4	100 %	1	1	0.991	99.087	99.719 ± 0.650
5		1	1	1.004	100.385	
6		1	1	0.997	99.685	
7	120 %	1	1.2	1.202	100.207	99.722 ± 0.492
8		1	1.2	1.191	99.224	
9		1	1.2	1.197	99.737	

LOD and LOQ

Limit of Detection

Table 15: Limit of Detection data for Escitalopram Oxalate and Flupentixol.

Escitalopram Oxalate	Flupentixol
LOD = 3.3 x (SD / Slope) = 3.3 x (15.874/39.499) = 1.326µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (11.625/551.41) = 0.070µg/ml

Table 16: Limit of Quantitation data for Escitalopram Oxalate and Flupentixol.

Escitalopram Oxalate	Flupentixol
LOQ = 10 x (SD / Slope) = 10 x (15.874/39.499) = 4.019µg/ml	LOQ = 10 x (SD / Slope) = 10 x (11.625/551.41) = 0.211µg/ml

Robustness

Table 17: Robustness data for Escitalopram Oxalate.

SRNO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobilephase (-2)	Area at Mobilephase (+2)
1	1617.449	1521.123	1591.681	1493.440	1606.246	1519.728
2	1644.730	1541.811	1620.678	1510.276	1615.806	1538.721
3	1651.033	1551.267	1630.222	1516.632	1625.285	1549.716
% R.S.D	1.090	1.002	1.243	0.795	0.589	0.988

Table 18: Robustness data for Flupentixol.

SRNO.	Area at Flowrate (- 0.2ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobilephase (-2)	Area at Mobilephase (+2)
1	1136.351	1068.168	1123.090	1048.304	1124.216	1066.027
2	1121.026	1062.832	1106.071	1036.880	1095.558	1050.014
3	1133.255	1081.320	1136.351	1057.186	1132.939	1080.236
% R.S.D	0.717	0.889	1.353	0.972	1.750	1.419

Analysis of marketed formulation by developed method

Table 19: Analysis on marketed formulation.

Tablet (Detens)	**Drugs**	
Label claim	**Escitalopram Oxalate (10mg)**	**Flupentixol (0.5mg)**
Assay (% of label claim*) Mean ± S. D.	94.562±0.785	101.02±0.941

The assay results were comparable to labelled value of each drug in combined dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

RESULT AND DISCUSSION

Development of RP-HPLC Method

1. Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of Escitalopram Oxalate (40 µg/ml) and Flupentixol (2 µg/ml) were prepared in Methanol. These drug solutions were than scanned in UV region of 200-400 nm and overlay spectrums were recorded.

Escitalopram Oxalate solution: 40mg- →100ml with methanol. Further 1ml to a 10ml and make up with methanol (40 µg/ml in methanol).

Flupentixol solution: 20mg- →100ml with methanol. Further 1ml to a 10ml and make up with methanol. (2 µg/ml in methanol).

All solutions were scanned between 200 - 400 nm.

Wavelength was selected from the overlay spectra of above solutions.

2. Selection of Mobile Phase

Trail contains various mobile phase which are considered of Acetonitrile, Water and Methanol in different proportions and different volumes at different flow rate were tried. On the basis of various trails the mixture of Buffer (pH 5.5): Methanol (35:65), at 1.0mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry.

Mobile Phase was selected based on the review of literature. Various mobile phases were tried. Trial contains various mobile phases which consisted of Methanol, Water, in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. On the basis of various trials the mixture of Buffer (pH 5.5): Methanol (35:65).

3. Optimization of flow rate

1ml/min flow rate, proved to be better than the other in terms of resolution, peak shape and shorter retention time.

CONCLUSION

- Escitalopram oxalate is an antidepressant of the selective serotonin reuptake inhibitor class. It has been use in depression disorder.
- Flupentixol is an antipsychotic neuroleptic drug .it has been investigated for use in Schizophrenic disease.
- RP-HPLC method was developed for simultaneous estimation Escitalopram oxalate and flupentixol. In RP-HPLC method, good resolution and separation of two drug was achieved potassium Dihydrogen phosphate: methanol (35:65) was used as mobile phase.
- Retention time of Escitalopram oxalate and flupentixol were found to be 3.860 and 6.990 min respectively with a flow rate of 1ml/min. the proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Escitalopram oxalate and flupentixol in tablets.
- Forced degradation study of Escitalopram oxalate and Flupentixol was performed by RP-HPLC method which includes Acid, Base, oxidative, photo and thermal degradation.
- Results of degradation were found within limit.

REFERENCES

1. Introduction to Depression", Sept-2016, http://www.mayoclinic.org/diseasesconditions/depression/basics/causes/con20032977
2. FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration," Rockville, US Department of Health and Human Services, 2000.
3. Dong WM. Modern HPLC for Practicing Scientists; A Wiley- Inter science publication, USA, 2006; 1-9.
4. Kazakevich Y and LoBrutto R. HPLC for pharmaceutical Scientists; A John Wiley and sons, 2007; 1-6.
5. Snyder LR, Kirkland JJ and Glajch LJ. Introduction to Modern Liquid Chromatography; 2nd Edn; A Wiley- Inter science publication, NY, USA, 1997; 5-42.
6. Snyder LR., Kirkland JJ and Glajch LJ. Practical HPLC Method Development; 2nd Edn; A Wiley- Inter science publication, NY, USA, 1997; 3-35.

7. Bajaj S, Singla D, Sakhuja N, "Stability Testing of Pharmaceutical Products", *J. app. Pharm. Sci.*, 2012; 2(3): 129-138.
8. ICH, Validation of Analytical Procedures; Methodology, Q2(R1), International Conference on Harmonization, IFPMA, Geneva, 1996.
9. "Drug profile for Escitalopram", Sept.-2016, www.drugbank.ca/drugs/DB01175.
10. Drug profile for Flupentixol", Sept.-2016, www.drugbank.ca/drugs/DB00875.
11. Indian Pharmacopoeia Food and drug administration, Ghaziabad, 2010; II: 1293-1294.
12. United state pharmacopoeia, Pending Monograph L: Escitalopram.
13. Samanta T, Dey S, Samal HB, Kumar DB, Mohanty DL, "RP-HPLC Method For The Estimation Of Escitalopram In Bulk And In Dosage Forms" *Int. j. of chem. Res.*, 2011; 2(2): 11-15.
14. Lasan VM, Patel SA, "Analytical Method Development and Method Validation for Escitalopram Oxalate in Pharmaceutical Dosage Forms by HPLC Method" *Int. j. pharm. res. sch.*, 2015; 4(1): 19-25.
15. Syama SB, Suneetha A, "Development And Validation Of Liquid Chromatographic Method For Estimation Of Escitalopram Oxalate In Tablet Dosage Forms" *Int. j. pharm. and bio. sci.*, 2011; 2(1): 140-146.
16. Ramathilagam N, Padmaja N, Amarnath HS, "Development and Validation of HPLC Method for the estimation of Escitalopram Oxalate in Tablets, "*Int. j. of pharm. and anal. Res.*, 2013; 2(1): 65-69.
17. Bhimananduni CN, Garikapati DR, Pasupuleti U, "Development and validation of an RP-HPLC method for the simultaneous determination of Escitalopram Oxalate andClonazepam in bulk and its pharmaceutical formulations" *Int. current pharm. J.*, 2012; 1(8): 193-198.
18. Chakole RD, Charde MS, Bhavsar N, Marathe RP, "Simultaneous Estimation Of Escitalopram And Clonazepam ByRP-HPLC In Pharmaceutical Formulation" *Int. J. phyto.*, 2012; 2(1): 25-29.
19. Dighe VV, Pawaskar P, Adhyapak S, Shambhu N, "Development of normal phase chiral liquid chromatographic method for estimation of escitalopram oxalate and determination of R-citalopramenantiomer from escitalopram oxalate in bulk drug and tablet" *j. of chem.. and pharm. Res.*, 2012; 4(11): 4804-4809.
20. Shalini S, Ajitha A, Rao VU, "Analytical Method Development And Validation For Simultaneous Estimation Of Escitalopram Oxalate And Etizolam In A Combined Dosage Form By RP-HPLC" *Int. j. inn. pharm. sci. and Res.*, 2014; 2(9): 2169-2178.

21. Kakde RB, Satone DD, Gadapayle KK, Kakde MD, "Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Escitalopram Oxalate and Clonazepam" *J. chrom. Sci.,* 2012; 2(1): 6.
22. Mondal P, Santosh B, Satla SR, Raparla R, " A new validated simultaneous RP- HPLC method for estimation of escitalopram oxalate and etizolam in bulk and table dosage form" *Der Pharma chemical.,* 2013; 5(3): 26-32.
23. Nikam R, Garge V, Vilasrao K, "Analytical Method Development And Validation For Simultaneous Estimation of Escitalopram Oxalate And Fenofibrate Using RP- HPLC Method" *Ind. ame. j. pharm. Res.,* 2015; 5(7): 2497-2502.
24. Kalimullah T, Ahmed M, Sharma HK, Rajput P, "Reverse Phase Liquid Chromatographic Method for the Quantification of Di-*P*-Toluoyl-*D*-Tartaric Acid in Escitalopram Oxalate Drug Substance" *Eur. j. anal. Chem..,* 2011; 6(3): 197-205.
25. SakhreliyaBD,TrivediPD,ModiDK,"DevelopmentandValidationof Spectrophotometric Methods for Simultaneous Estimation of Escitalopram oxalate and Etizolam in their Combined Tablet Dosage Form" *J. of bio. sci. and pharm. res.,* 2012; 2(5): 195-200.
26. British Pharmacopoeia, Official Monograph of Flupentixol, 2009; 1-5.
27. Sheikh IA, Charde MS, Kasture AV, "Estimation of Flupenthixol HCl in single dosage form by RPHPLC method" *Int. J. Pharm. Sci.,* 2009; 1(2): 11-19.
28. Chhalotiya UK, Bhatt KK, Shah DA, Chauhan GR, Baldania SL, "Development of LC Method for the Simultaneous Determination of Antidepressant Drug Combination Melitracen Hydrochloride and Flupentixol Di hydrochloride in their Combined Dosage Form" *Hindawi,* 2011.
29. Nagar A, Chugh NN, "Simultaneous estimation method development as analytical method for flupentixol dihydrochloride and melitracen hydrochloride from their combine pharmaceutical dosageforms by RP-HPLC" *The pharm, inn.,* 2015; 4(1): 81-86.
30. Sharma MC, "Densitometric Method for the Quantification of Melitracen hydrochloride and Flupentixol dihydrochloride in Pharmaceutical Dosage Form" *J. World app. Sci.,* 2014; 31(2): 165-170.
31. Hussain MS, Aqeel M, Hussain SA, "Validated Spectrophotometric Method for Simultaneous Estimation of Flupenthixol and Melitracen in Combined Pharmaceutical dosage form" *Int. J, pharmtech. Res.,* 2013; 5(4): 1493-1497.
32. Acharjya SK, Panda P, Mallick P, Sarvani Y, Annapurna M, "Spectrophotometric methods for simultaneous estimation of Flupentixol Dihydrochloride and Melitracen

Hydrochloride incombined tablet dosage form" *J. Chem. And pharm. Res.*, 2010; 2(3): 158-171.

33. PatelK,MohanS,"Development&ValidationofSimultaneousEquation Spectrophotometric Method for Estimation of Flupentixol & Melitracen in Combined Dosage Form" *Ame. J. Pharm. Tech. Res.*, 2013; 3(3): 684-693.
34. Yunus M, Siddiqui SS, Bagga P, Ali A, Singh K, "A Simple Uv Spectrophotometric Method For The Determination Of Flupenthixol Dihydrochloride In Bulk And Pharmaceutical Formulations" *Int. J. Pharm. Sci. And res.*, 2011; 2(8): 2152-2155.
35. Patel SC, Maheshwari DG, "Development and Validation of UV Spectrometric and HPLC Method for Estimation of Escitalopram Oxalate and Flupentixol Dihydrochloride in Combined Dosage Form" *Asi. J. Pharm. Tech. And inn.*, 2016; 4(17): 59-70.
36. Singh P, Patel D, Meshram D, Desai S, "First Order Derivative Spectrophotometric Method For Simultaneous Estimation Of Escitalopram Oxalate And Flupentixol Dihydrochloride In Pharmaceutical Dosage Form" *Ind. Ame. J. Pharm. Tech. Res.*, 2016; 6(2): 4544-4552.
37. Khan MU, Sundaran MS, Kaushik V, "An improved process for the preparation of Escitalopram" European Patents, EP20060765643, 2008.
38. Dancer R, Peterson H, Neilsen O, Rock M, "Crystalline base of escitalopram and orodispersible tablets comprising escitalopram base" , US Patents, US, 2006; 11/425: 522.
39. Olsen FE, "Flupentixol compositions", European Patents, EP20100159137, 2012.
40. Liang G, Bo W, Min Z, Li Fan, Haoxi H, Fu L, "A pharmaceutical grade hydrochloric acid droperidol preparation of thioxanthone" Chinese Patents, CN 201510786453, 2016.
41. Yuhuna W, Wang Y, Gold X, "Method for preparing flupentixol and melitracen capsule" Chinese Patents, CN 200710045583, 2008.