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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ESCITALOPRAM AND FLUPENTIXOL IN PURE AND MARKETED FORMULATION

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ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Escitalopram and Flupentixol, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA pH 4.2 (40:60) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 272 nm. The retention time of the Escitalopram and Flupentixol was 2.781, 4.048 ±0.02min respectively. The method produce linear responses in the concentration range of 7.5-37.5µg/ml of Escitalopram and 5-25µg/ml of Flupentixol . The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Key words: Escitalopram Oxalate, Flupenthixol Hydrochloride, Stability indicating RP-HPLC Method.

INTRODUCTION

Escitalopram oxalate is chemically name is (S) 1[3 (Dime thy lamino)propyl]1(4fluorophenyl) 1,3dihy droisobenzofuran5carbonitrile. It is an antidepressant, antiobsessivec ompulsive and antibulimic actions of Escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake serotonin at the serotonin reuptake pump of the neuronal membrane, enhancing the actions of serotonin on 5HT autoreceptors. SSRIs bind with significantly nor epinephrine receptors than tricyclic antidepressant drugs [1] fig.1.

Flupenthixol hydrochloride is chemically name is. 2-(4-{3-[2-(trifluoromethyl) 9,9-adihydro-4-aHthioxanthen-9-ylidene] propyl}piperazin-1-yl)ethan-1-ol..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 alphaadrenergic receptor antagonist. It's antipsychotic activity is thought to be related to blocks postsynaptic dopamine receptors in the CNS [2] fig 2.

MATERIALS AND METHODS

Chemical and reagents

Escitalopram oxalate & Flupenthixol hydrochloride were procured from Sura Pharma lab. Dilshuknagar, Hyderabad. Methanol, Acetonitrile (HPLC grade) were procured from Final chemicals Ltd. Hydrochloric Acid, Sodium Hydroxide, Potassium dihydrogen phosphate are AR grade. Marketed formulation was purchased from local market.

HPLC instrument and chromatographic condition

HPLC Model – WATERS 2695 equipped with PDA Detector was employed in this method. Empower-2 software was used for peak integration along with data processing. The column used for separation of analytes is Hypersil BDS Column C18 (250 mm × 4.6 mm, 5 µm). Mobile phase consist of buffer (pH 4.2) & Methanol in ratio of (40:60% v/v) at flow rate of 1.0 ml/min. Whatman filter (0.42 µ) is used for filtration activity. Sample was

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analyzed at 272 nm at injection volume of 20 µl [3].

Preparation of standard solution

Accurately weigh and transfer 10 mg of Flupentixol and Escitalopram working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15ml of Flupentixol and 0.225ml of Escitalopram from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer in proportion 40:60 v/v respectively [4].

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Symmetry C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow [5-9].

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used: Waters HPLC with auto sampler and PDA Detector 996 model.

Temperature : 40ºC
Column : Symmetry C18 (4.6×150mm, 5µ)
pH : 4.2
Mobile phase : Methanol:TEA buffer pH 4.2 (40:60v/v)
Flow rate : 1ml/min
Wavelength : 272nm
Injection volume : 10 µl
Run time : 6 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Triethylamine (TEA) buffer (pH-4.2)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Fliter and sonicate the solution by vaccum filtration and ultrasonication.

Preparation of mobile phase

Accurately measured 650 ml (65%) of Methanol and 350 ml of Phosphate buffer (35%) a were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration [10].

Diluent Preparation

The Mobile phase was used as the diluent.

Specificity Linearity and Range

Linearity was determined by plotting the standard curve in the concentration range of 10-30 µg/ml for Escitalopram oxalate and 0.5-1.5 µg/ml for Flupenthixol hydrochloride(fig.4-5) The linearity of the methods was evaluated by linear regression analysis, using least square method. Table 1-2.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD. The results are shown Table 3 & 4.

Precision

The precision (system, method) of the proposed method was evaluated by carrying out six independent assays of the sample. RSD (%) of six assay values obtained was calculated. The intermediate precision was carried out by analyzing the sample at different days and different analysts and the data is presented Table 3 & 4.

Accuracy

This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed for both Escitalopram oxalate and Flupenthixol hydrochloride at three different levels (80%, 100% and 120%), the mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery (%), standard deviation (SD) and relative standard deviation RSD (%) of the spiked drugs was calculated. Results are presented in Table 5-6.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Escitalopram: LOD=3.3 × 3762.7/10552 =1.1µg/ml
Flupentixol : LOD =3.3 × 4146/16592 =0.8µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
Escitalopram: LOQ= 10×3762.7/10552 = 3.5µg/ml
Flupentixol :LOQ =10×4146/16592 = 2.4µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Escitalopram and Flupentixol . The method is robust only in less flow condition and the method is

Asian J. Pharm. Res. Vol 8, Issue 1, 4-10, 2018.

robust even by change in the Mobile phase ±5%. The standard and samples of Escitalopram and Flupentixol were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) for Escitalopram oxalate and Flupenthixol hydrochloride were determined from standard deviation of the response and the slope.

LOD= σ/S X 3.3; LOQ= σ/S X 10

Robustness

The robustness of an analytical predure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage **Table**7-8.

Robustness of the method was investigated under a variety of conditions like change in flow rate by ± 0.2 ml/minute, temperature and change in mobile phase composition by ± 2%. The mixed standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % RSD of assay was calculated for each condition.

RESULTS AND DISCUSSION

Method development

Escitalopram oxalate and Flupenthixol hydrochloride were found to degrade significantly in acidic and alkaline conditions as well as in photolytic degradation and under oxidative condition. Resolution of drug and the degradation products formed under different stress studies were successfully achieved on a BDS hypersil C18 (250mm × 4.6 mm, 5µm (particle size) utilizing TEA Buffer (pH 4.2): Methanol (60:40 v/v) at Flow rate 1ml/min and at the detection wavelength of 256nm. The method was validated with respect to linearity, precision, accuracy, selectivity.

Method validation

RP-HPLC method was developed for simultaneous estimation of Escitalopram oxalate and Flupenthixol hydrochloride. In RP-HPLC method, good resolution and separation of two drugswas achieved. TEA Buffer (pH 4.2): Methanol (60:40 v/v) was used as mobile phase. Retention time of Escitalopram oxalate and Flupenthixol hydrochloride were found to be 3.423 min and 6.163 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 Flupenthixol hydrochloride Tablet dosage form.Table-10

FORCED DEGRADATION STUDIES

Acid degradation

Degradation was observed by the additon of 0.5N HCl.

Alkaline degradation

Degradation was observed by the additon of 0.5 N NaOH

Thermal degradation

Degradation was observed when the sample solution was kept under heat at 60-80^0 C for 3hours.

Peroxide degradation

Degradation was observed by the additon of 3% H_2O_2

Photolytic degradation

Degradation was observed by sunlight exposre.

Table 1. Linaerity data of Escitalopram

Concentration µg/ml	Average Peak Area
7.5	88464
15	166364
22.5	237423
30	319213
37.5	401317

Table 2. Linearity data of FLUPENTIXOL

Concentration µg/ml	Average Peak Area
5	80032
10	162782
15	241426
20	326009
25	417393

Asian J. Pharm. Res. Vol 8, Issue 1, 4-10, 2018.

Table 3. Results of repeatability for Escitalopram:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Escitalopram	2.766	2766870	294578	6684	1.3
2	Escitalopram	2.774	2771971	286541	6347	1.3
3	Escitalopram	2.770	2771958	302657	6674	1.3
4	Escitalopram	2.772	2780299	293412	6451	1.3
5	Escitalopram	2.771	2789695	283154	6678	1.3
Mean			2776159			
Std. Dev			8969.896			
% RSD			0.3			

Table 4. Results of method precession for Flupentixol

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Flupentixol	4.025	2534539	193240	5761	1.3	4.7
2	Flupentixol	4.040	2539247	201647	5489	1.3	4.6
3	Flupentixol	4.032	2544661	193472	5367	1.3	4.6
4	Flupentixol	4.041	2548839	196475	5845	1.3	4.6
5	Flupentixol	4.036	2558822	201394	5347	1.3	4.7
Mean			2545222				
Std. Dev			9329.852				
% RSD			0.3				

Table 5. The accuracy results for Escitalopram

%Concentration (at specification Level)	**Area**	**Amount Added (ppm)**	**Amount Found (ppm)**	**% Recovery**	**Mean Recovery**
50%	1382603	11.25	11.23	99. 8	99.3**%**
100%	2777270	22.5	22.1	98.2	
150%	41448756	33.75	33.73	99.9	

Table 6. The accuracy results for Flupentixol

%Concentration (at specification Level)	**Area**	**Amount Added (ppm)**	**Amount Found (ppm)**	**% Recovery**	**Mean Recovery**
50%	1306990	7.5	7.5	100	99.4**%**
100%	2510628	15	14.8	98.6	
150%	3777999	22.5	22.46	99.8	

Table 7. Results for Robustness data of Escitalopram

Parameter used for sample analysis	**Peak Area**	**Retention Time**	**Theoretical plates**	**Tailing factor**
Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
More Flow rate of 1.1 mL/min	2542012	2.516	6234	1.4
Less organic phase	2888515	3.326	6298	1.4
More organic phase	2541550	2.416	6287	1.2

Table 8. Robustness data of Flupentixol

Parameter used for sample analysis	**Peak Area**	**Retention Time**	**Theoretical plates**	**Tailing factor**
Actual Flow rate of 1.0 mL/min	2533532	4.048	5521	1.3
Less Flow rate of 0.9 mL/min	2750214	5.319	5643	1.6
More Flow rate of 1.1 mL/min	2254107	3.649	5782	1.5

Asian J. Pharm. Res. Vol 8, Issue 1, 4-10, 2018.

Less organic phase	2754017	5.318	5309	1.4
More organic phase	2215870	3.233	5580	1.51

Table 9. Results of degradation studies

S.No	Type of degradation	Weight of sample (µg/ml)	Area of sample		Assay content (% w/w)	
			Escitalopram	Flupentixol	Escitalopram	Flupentixol
1	Acid (0.5N HCl)	15µg/ml of Flupentixol and 22.5µg/ml of Escitalopram	197361	207465	97.7%	98.9%
2	Base (0.5N NaOH)	15µg/ml of Flupentixol and 22.5µg/ml of Escitalopram	198745	208741	91.6%	92.9%
3	Peroxide (3%H_20_2)	15µg/ml of Flupentixol and 22.5µg/ml of Escitalopram	199632	217452	96.3%	99%
4	Thermal (at 60^0 c)	15µg/ml of Flupentixol and 22.5µg/ml of Escitalopram	198664	217465	91.3%	99.3%
5	Photolytic(sunlight)	15µg/ml of Flupentixol and 22.5µg/ml of Escitalopram	197835	228461	99.5%	95.7%

Fig 1. Chemical structure of Escitalopram

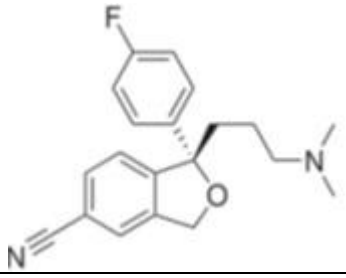


Fig 2. Chemical structure of Flupenthixol

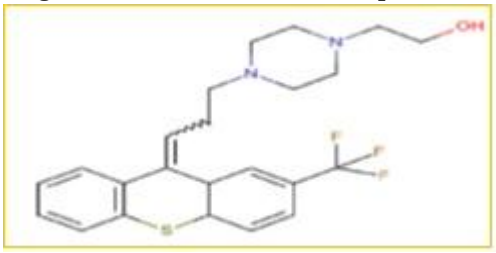


Fig 3.Typical chromatogram of mixed standard solution

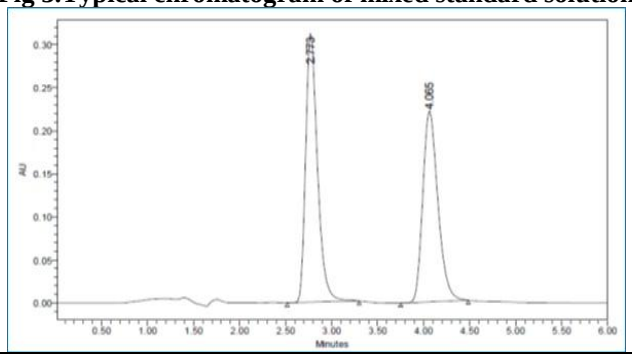


Fig 4. Calibration graph for Escitalopram

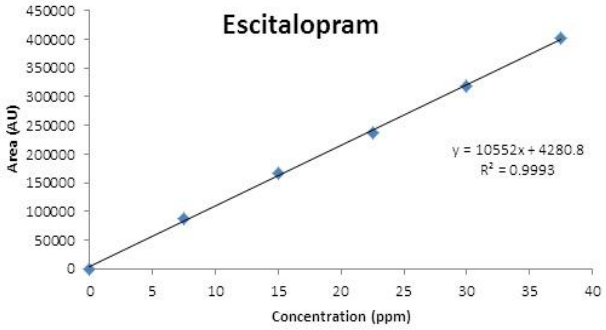


Fig 5. Calibration graph for Flupentixol

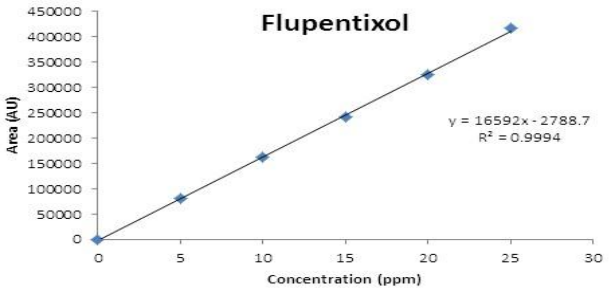
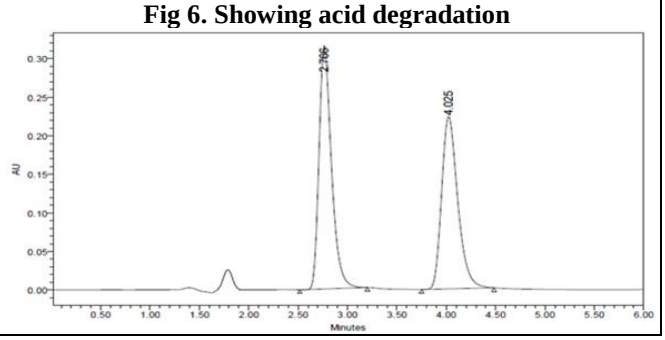


Fig 6. Showing acid degradation



Asian J. Pharm. Res. Vol 8, Issue 1, 4-10, 2018.

Fig 7. Showing Alkaline degradation

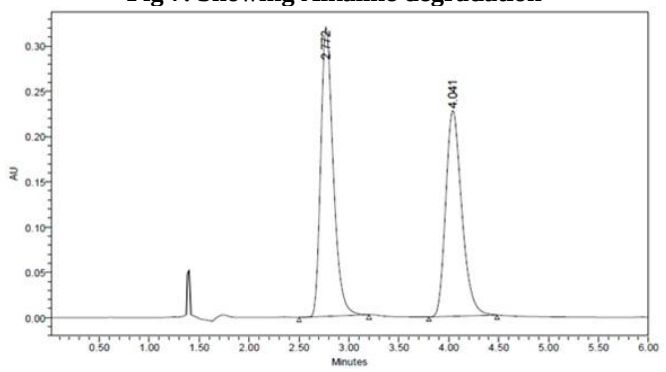


Fig 8. Showing Thermal degradation

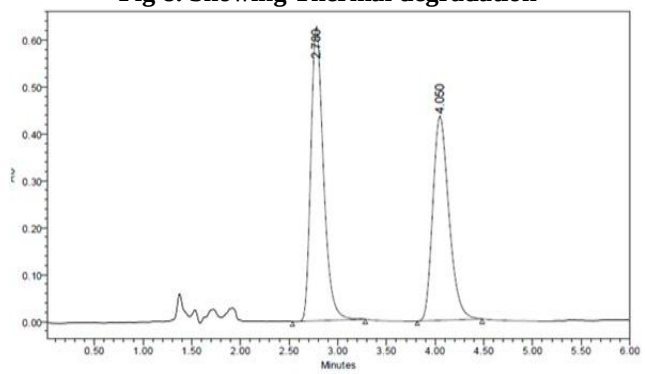


Fig 9. Showing peroxide degradation

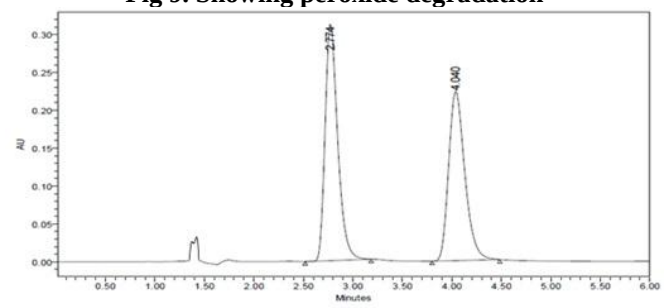
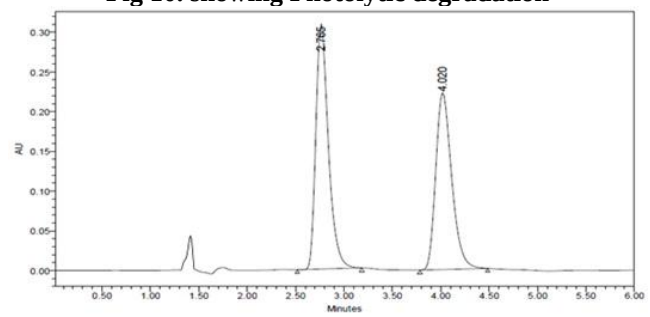


Fig 10. showing Photolytic degradation



SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 272 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry C_{18} because it was giving good peak.40ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: TEA pH 4.2 (40:60) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 6min because analyze gave peak around 2.781, 4.048 ±0.02min respectively and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range 7.5-37.5mg/ml of Escitalopram and 5-25 mg/ml of Flupentixol of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Escitalopram and Flupentixol in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Escitalopram and Flupentixol was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA pH 4.2 (40:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Escitalopram and Flupentixol in bulk drug and in Pharmaceutical dosage forms.

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