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Development and Validation of the Method Estimation for Escitolapram and Etizolam in Formulated dosage form using RP-HPLC

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Abstract - A refined and selective RP-HPLC method has been established and validated for the analysis of escitalopram and etizolam active pharmaceutical ingredients (APIs). The peak purity results derived from the analysis of samples using the described method suggest that the lack of co-eluting peaks alongside the primary peaks of escitalopram and etizolam confirms the specificity of the developed method for their estimation. The calibration curve exhibited a linear relationship across a concentration range of 25-150 µg/ml for escitalopram and 2.5-15 µg/ml for Etizolam, with correlation coefficients of 0.998 for both substances. The method's accuracy was validated at levels corresponding to 80%, 100%, and 120% of the specified limit.The minimum detection limits identified were 0.1 µg/ml for escitalopram and 0.15 µg/ml for Etizolam. The minimum quantification limits established were 0.3 µg/ml for escitalopram and 0.45 µg/ml for Etizolam.

***Key Words*:** Escitolapram,Etizolam,RP-HPLC

1.INTRODUCTION

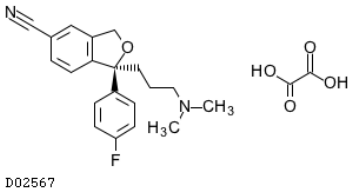
Escitalopram, the S-enantiomer of citalopram, is classified as a selective serotonin reuptake inhibitor (SSRI), a category of antidepressant medications. Although there are notable structural variations among the compounds within this class, they exhibit comparable pharmacological effects. Similar to other antidepressants, it may take several weeks of treatment before a therapeutic effect is observed. SSRIs are highly effective in inhibiting the reuptake of serotonin in neurons, while having minimal to no impact on the reuptake of norepinephrine or dopamine. Additionally, they do not block α- or β-adrenergic receptors, dopamine D2 receptors, or histamine H1 receptors. In the initial phase of treatment, SSRIs inhibit serotonin reuptake, thereby enhancing serotonin activity at somatodendritic 5-HT1A and terminal autoreceptors.2.

Etizolam is primarily utilized for the management of insomnia and anxiety disorders, including panic disorder and generalized anxiety disorder. This medication is effective in providing immediate relief from panic attacks and anxiety symptoms. Etizolam functions by activating the same benzodiazepine receptors as other conventional medications in this category, such as diazepam. Consequently, it binds to GABAa receptors, enhancing GABA transmission and resulting in sedation of the central nervous system. Additionally, etizolam exhibits potent sedative properties, which can impair judgment and reduce inhibitions.

DRUG PROFILE

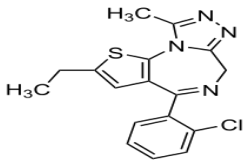
Escitalopram:

Chemical formula :$C_{20}H_{21}FN_2O$



IUPAC :(1S)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile
Class: Benzofurans

Etizolam



Chemical formula : $C_{17}H_{15}ClN_4S$.

IUPAC:7-(2-Chlorophenyl)-4-ethyl-13-methyl-3-thia 1,8,11,12tetraazatricyclo[8.3.0.02,6]trideca-2(6),4,7,10,12-pentaene

Materials and Method: The Hitachi LaChrome 1575 instrument is equipped with a Develosil ODS HG-5 RP C18 column, measuring 5 micrometers in particle size and 15 cm x 4.6 mm in dimensions. This setup utilizes an HPLC auto sampler and a UV detector, operated through Elite software. Additionally, a UV double beam spectrophotometer, model UV 1800, is sourced from Elico India. The reagents, including methanol, dipotassium hydrogen phosphate, acetonitrile, orthophosphoric acid, and potassium dihydrogen orthophosphate, were procured from Sd Fine-Chem Ltd. and Loba Chem, both located in Mumbai.

Method Development

Sélection wavelength: The maximum wavelength (λmax) of the two components, Escitalopram and Etizolam, was determined to be 239 nm and 249 nm, respectively, when analyzed in the mobile phase as the solvent system. Additionally, the isobestic point for these drugs was identified at 249 nm..

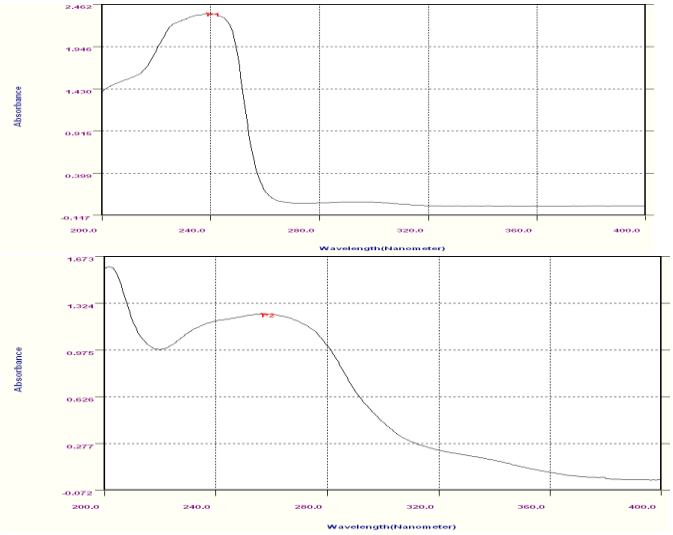


Fig 02: UV-Spectrum for Etizolam

Optimazation chromatographic conditions:

Mobile phase : Potassium dihydrogen phosphate buffer+Dipotassium hydrogrn phosphate (0.02 M, pH 3.0): acetonitrile (20:80)

Wavelength : 249 nm

Flow rate : 1.0 ml/ min.

Run time : 10 min.

Column : Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm.

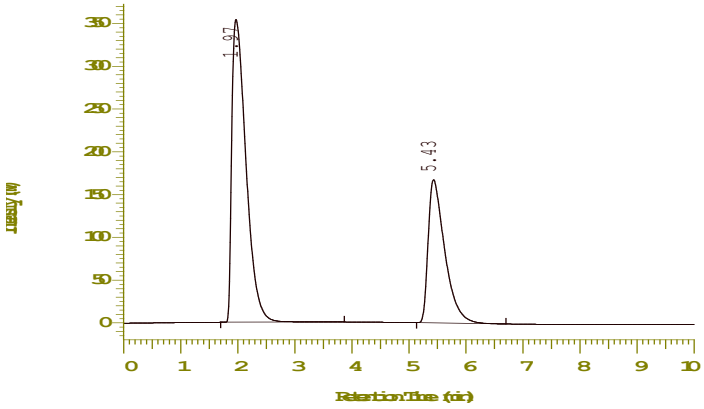


Fig 03: Optimization Chromatogram

Method Validation

Linearity :

The linearity range for Escitalopram was determined to be 0-45 µg/ml, while for Etizolam, it was established as 0-75 µg/ml. The correlation coefficients for both substances were calculated to be 0.998. The slopes were measured at 210870 for Escitalopram and 64545 for Etizolam, with intercepts recorded at 22039 and 2189, respectively.

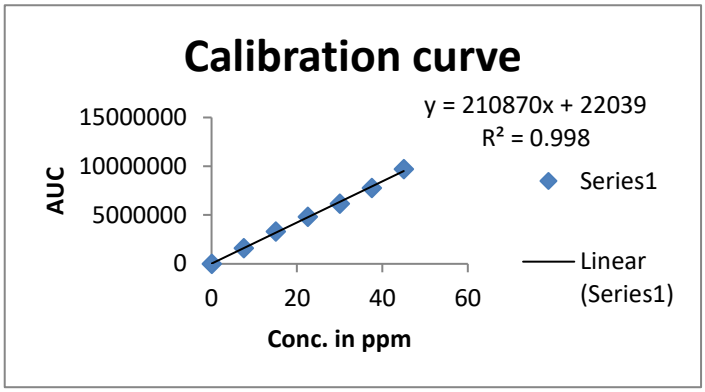


Fig.04 Standard curve for Escitalopram

CONC	AUC
25	1599571
50	3307873
75	4831264
100	6164471
125	7765523
150	9698441

Table:01 Linearity data of Escitalopram

Etizolam:

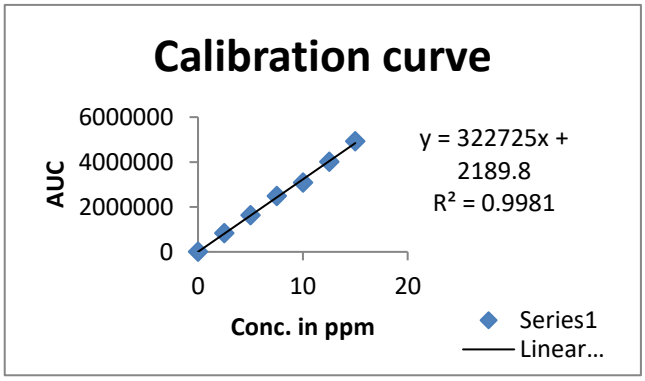


Fig.05 Standard curve for Etizolam

CONC	AUC
2.5	830382
5	1623373
7.5	2489997
10	3081595
12.5	4005827
15	4927209

Table.02 Linearity data of Etizolam

Accuracy :

To assess the accuracy of the proposed method for Escitalopram, recovery studies were conducted by incorporating varying quantities (80%, 100%, and 120%) of pure Escitalopram. Each amount was injected three times, and the percentage recovery values were subsequently calculated.

Recovery studies for Escitalopram

Conc	Rt	peak area	Conc. Found	% Recovery
80	1.99	4831264	22.8	101.333333
80	1.99	4887585	23.07	102.533333
80	1.99	4859800	22.94	101.955556
			Avg.	101.940741
			SD	0.60013716
			%RSD	0.58871179
100%				
Conc	Rt	peak area	Conc. Found	% Recovery
100	1.97	6191960	29.25	97.5
100	1.96	6164471	29.12	97.0666667
100	1.96	6143413	29.02	96.7333333
			Avg.	97.1
			SD	0.38441875
			%RSD	0.39589985
120%				
Conc	Rt	peak area	Conc. Found	% Recovery
120	1.97	7765523	36.72	97.92
120	1.97	7788156	36.82	98.1866667
120	1.97	7768426	36.73	97.9466667
			Avg.	98.0177778
			SD	0.14686855

Table 03: Data of Accuracy studies

Recovery studies for Etizolam

80%				
Conc	rt	peak area	Conc. Found	% Recovery
8	5.39	2489997	38.54	102.773333
8	5.39	2524308	39.07	104.186667
8	5.39	2509012	38.83	103.546667
			Avg.	103.502222
			SD	0.70771411
			%RSD	0.68376707
100%				
Conc	rt	peak area	Conc. Found	% Recovery
10	5.42	3294919	51.01	102.02
10	5.43	3281595	50.8	101.6
10	5.43	3242166	50.19	100.38
			Avg.	101.333333
			SD	0.85189984
			%RSD	0.84069063
120%				
Conc	rt	peak area	Conc. Found	% Recovery
12	5.37	4005827	62.02	99.232
12	5.37	4109382	63.63	101.808
12	5.35	4072536	63.06	100.896
			Avg.	100.645333
			SD	1.30616589
			%RSD	1.29779081

Table04: Date of Accuracy

Precision

Repeatability of Escitolapram

Repliacate	Rt	Area
Replicate – 1	1.97	6130775
Replicate – 2	1.97	6022268
Replicate – 3	1.97	6164471
Replicate – 4	1.96	6043413
Replicate – 5	1.96	6191960
Avg	1.966	6110577
SD	0.005477	74574.12
%RSD	0.278597	1.22041

Table 05 Data showing repeatability analysis for Escitalopram

Repeatability of Etizolam

Repliacate	Rt	Area
Replicate – 1	5.43	3389698
Replicate – 2	5.43	3481595
Replicate – 3	5.42	3394919
Replicate – 4	5.43	3442166
Replicate – 5	5.43	3342926
Avg	5.428	3410261
SD	0.004472	53147.64
%RSD	0.08239	1.558463

Table 06 Data showing repeatability analysis for Etizolam

Results: The repeatability study performed on the solution with concentrations of approximately 30 g/ml for Escitalopram and 50 g/ml for Etizolam (n = 5) demonstrated a relative standard deviation (RSD) of 1.5584% for Escitalopram and 1.22041% for Etizolam. It was determined that the analytical method exhibited satisfactory repeatability.

Intermediate précision

Conc	Observed Conc. Of Escitalopram (µg/ml) by the proposed method			
	Intra Day		**Inter day**	
	Mean	**%RSD**	**Mean**	**%RSD**
80	79.95	0.5	80.01	0.24
100	100.98	0.55	100.051	0.41
120	119.84	0.18	119.95	0.19

Table 07 Data for Escitalopram analysis

Conc	Observed Conc. Of Etizolam (µg/ml) by the proposed method			
	Intra Day		Inter day	
	Mean	%RSD	Mean	%RSD
8	10.01	0.86	10.03	0.87
10	20.02	0.30	20.03	0.32
12	29.97	0.13	29.95	0.11

Table 08 Data for Etizolam analysis

Results: Intraday and interday analyses indicate that the average relative standard deviation (RSD) percentage remained within the acceptable limit of 2%. Consequently, it can be concluded that there was no significant difference observed in the assay results, whether tested on the same day or across different days.

Limit of detection and limit of quantification

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Results: The LOD was found to be 0.1 µg/ml and 0.3 µg/ml and LOQ was found to be 0.15 µg/ml and 0.45 µg/ml for Escitalopram & Etizolam respectively.

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	3.15
2	Asymmetry	T ≤ 2	Escitalopram =0.14 Etizolam =0.19
3	Theoretical plate	N > 2000	Escitalopram =3971 Etizolam= 4861

Table:09Data of System Suitability Parameter

Assay Of Escitalopram & Etizolam In Dosage Form:

Brand name of tablets	Labeled amount of Drug (mg) Escitalopram & Etizolam	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Etilaam DS (Intas Pharmaceuticals Ltd.)	(50+0.5)	50.04 (±0.13) 0.49 (±0.09)	100.08 (±0.39) 98.00 (±0.42)

Table 10: Data of Assay tablets

The assay of Etilaam DS tablets containing Escitalopram was found to be 100.08% Etizolam was found to be 98.00 %.

CONCLUSIONS

In order to perform analysis escitalopram and etizolam API, a sensitive and selective RP-HPLC method has been validated. Based on the peak purity results from the sample analysis using stated method , it can be concluded that the absence of coeluting peak along with the main peak of Escitolapram and Etizolam indicates that the developed method is specific for the estimation of Escitolapram and Etizolam .Furthermore the suggested RP-HPLC method has excellent sensitivity, precision and reproducibility.

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