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Simultaneous Estimation of Clonazepam and Escitalopram in Pure and Formulation by Using Reverse Phase High Performance Liquid Chromatography

M. Deepa [1], V. Sowmy [2]

[1]*Student, Surabhi Dayakar Rao College of Pharmacy, Gajwel, Siddipet, 502312, India*
[2]*Associate Professor, Surabhi Dayakar Rao College of Pharmacy, Gajwel, Siddipet, 502312, India*

A B S T R A C T

An accurate, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Escitalopram and Clonazepam in bulk and combined pharmaceutical tablet dosage forms. Escitalopram and Clonazepam were separated by using a Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size; Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of Methanol: 0.1% Orthophosphoric acid (64:36% v/v). The flow rate was set to 1ml/min with the responses measured at 224nm. The retention time of Escitalopram and Clonazepam was found to be 2.808min and 3.880min respectively with resolution of 5.68. Linearity was established for Escitalopram and Clonazepam in the range of 20-100µg/ml for Escitalopram and 60-140µg/ml for Clonazepam with correlation coefficient 0.999. The percentage recovery was found to be is 100.30% for Escitalopram and 100.21% for Clonazepam respectively. Validation parameters such as specificity, linearity, precision, accuracy and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification of bulk and active pharmaceutical ingredient present and in combined tablet dosage form.

Keywords: Escitalopram and Clonazepam, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

Fixed-dose combination (FDC) drugs containing two or more active pharmaceutical ingredients are growing rapidly since the last decade.FDC drugs have specific advantages which include improved medicaton compliance by reducing pill burden to the patients and providing the simpler overview on the pharmacokinetic profiles. Since FDC's are protected by patents, pharmaceutical companies may obtain exclusive rights to market a particular formulation, even though the individual active ingredients may be off-patent. Despite of many advantages, formulation scientists experience challenges such as compatibility issues, solubility and dissolution during the development of FDC drugs.

Escitalopram oxalte (ESO) is a novel selective serotonin reuptake inhibitor (SSRI) active pharmaceutical ingredient (API) approved by USFDA for handling the depressive disorders in adults and adolescents.ESO is marketed as tablets and as an oral solution. Through preventing neurotransmitter reuptake into presynaptic neurons, ESO increases levels of serotonin intrasynaptically. Compared to norepinephrine transporters (NET), escitalopram exhibits enhanced selectivity for serotonin transporters (SERT), and this nature shows the relatively mild side-effect profile.

The combination of escitalopram (EST) and clonazepam (CZP) is used for the treatment of anxiety disorder. EST is an antidepressant and CZP as an anticonvulsant, muscle relaxant, and anxiolytic agent. EST is a pure s-enantiomer of the racemic, bicyclic phthalates derivatives citalopram, belonging to class selective serotonin reuptake inhibitor have shown potent pharmacological effects [1,2]. Few pieces of literature are available for the simultaneous estimation of EST and CZP in dosages form based on spectrometric, colorimetric, and chromatographic analysis.

Review of literature for Escitalopram and Clonazepam gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other Escitalopram and Clonazepam. Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Escitalopram and Clonazepam and single method is available for such estimation by RP-HPLC.[3-6].

In view of the need for a suitable RP-HPLC method for routine analysis of Escitalopram and Clonazepam in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Escitalopram and Clonazepam and extend it for their determination in formulation.

MATERIALS AND METHODS

Materials

EST and CZP were procured from Sura labs, Telangana. HPLC grade methanol was procured from LICHROSOLV (MERCK). Acetonitrile for HPLC was purchased from Merck.

Instrumentation

Chromatographic conditions were developed for the analytical technique using Waters Alliance 2695 HPLC with PDA Detector 996 model. The column was Symmetry ODS C18 with dimension 4.6mm×150mm length and particle size packing 5µm.

Preparation of mobile phase:

Accurately measured 640ml of Acetonitrile (64%) of and 360ml of HPLC Water (36%) were mixed and degassed in a digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

System Suitability

Accurately weigh and transfer 10 mg of Escitalopram and Clonazepam working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette out 0.6ml of Escitalopram and 1ml of Clonazepam from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was determined.

Linearity

Accurately weigh and transfer 10 mg of Escitalopram and Clonazepam working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Solutions were prepared containing 20ppm, 40ppm, 60ppm, 80ppm, 100ppm, concentrations of Escitalopram and 60ppm, 80ppm, 100ppm, 120ppm, 140ppm, concentrations of Clonazepam. Inject each level into the chromatographic system and measure the peak area.

Precision

Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of An analytical method is usually expressed as the standard deviation correlative standard deviation (coefficient of variation) of seriesof measurements

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150 % of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For 50%, 150% concentration five sets and for 100% three sets were prepared and injected.

Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), variation of mobile phase i.e. Methanol: 0.1% Orthophosphoric acid (64:36% v/v) was taken in the ratio and 69:31, 59:41 instead of 64:36 remaining conditions are same

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae LOD=3.3*σ/slope, LOQ=10*σ/slope (Where σ=the standard deviation of the response and S= Slope of calibration curve).

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of Escitalopram and clonazepam. The mobile phase containing Methanol: 0.1% Orthophosphoric acid (64:36% v/v) was found ideal to resolve the peak of Escitalopram and clonazepam. Retention time of Escitalopram and clonazepam were 2.808 and 3.880 min respectively. System suitability parameters were evaluated and results shown in (Table-2), which were within acceptance criteria. Result of assay is shown in Table3. Results of intraday and interday precision were shown in the (Table-4&5). LOD and LOQ values were placed in Table-6. The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and mobile phase. The result obtained implies method is robust for routine qualitative analysis (Table-7 &8).

Table 1 - Observations of sample Chromatogram.

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Escitalopram	2.808	65258	4326		1.08	5685.4
2	Clonazepam	3.880	8659854	659823	5.68	1.42	6895.7

Table 2-: Results of system suitability parameters for Escitalopram and Clonazepam

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Escitalopram	2.816	65358	4536		1.08	5689.6
2	Clonazepam	3.893	8658746	658985	5.69	1.42	6892.4

Table 3-: Results of Assay

S.No.	Name of Compound	% Purity
1	Escitalopram	99.68%
2	Clonazepam	99.46%

Table 4 -: Results of Intermediate precision for Escitalopram

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Escitalopram	2.808	66895	4468	5784.2	1.09
2	Escitalopram	2.808	66986	4523	5835.1	1.09
3	Escitalopram	2.808	66258	4475	5864.4	1.10
4	Escitalopram	2.808	66457	4514	5864.6	1.09
5	Escitalopram	2.808	66539	4489	5784.9	1.10
6	Escitalopram	2.808	66298	4565	5748.5	1.10
Mean			66572.17			
Std. Dev			304.536			
% RSD			0.457452			

Table 4a-: Results of Intermediate precision for Clonazepam

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Clonazepam	3.882	8758568	669583	6982.4	1.43	
2	Clonazepam	3.882	8756982	665984	6935.3	1.44	5.69
3	Clonazepam	3.882	8746925	665345	6984.7	1.44	
4	Clonazepam	3.882	8723654	665325	6952.8	1.43	5.70
5	Clonazepam	3.882	8754982	669852	6898.9	1.44	
6	Clonazepam	3.882	8754698	665874	6976.5	1.43	5.69
Mean			8749302				
Std. Dev			13188.56				
% RSD			0.150738				

Table 5-: Results of method precision for Escitalopram

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Escitalopram	2.808	65898	4365	5682.2	1.08
2	Escitalopram	2.808	65487	4375	5628.6	1.09
3	Escitalopram	2.808	65324	4395	5649.7	1.08
4	Escitalopram	2.808	65982	4328	5638.4	1.09
5	Escitalopram	2.808	65248	4371	5698.3	1.08
6	Escitalopram	2.808	65734	4391	5682.7	1.09
Mean			65612.17			
Std. Dev			304.8425			
% RSD			0.464613			

Table 5a-: Results of method precision for Clonazepam

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Clonazepam	3.880	8659824	658784	6859.4	1.42	5.68
2	Clonazepam	3.880	8658547	657489	6824.6	1.43	5.69
3	Clonazepam	3.880	8659824	652368	6829.3	1.42	5.68
4	Clonazepam	3.880	8659875	658745	6892.7	1.43	5.69
5	Clonazepam	3.880	8658745	658213	6875.2	1.42	5.68
6	Clonazepam	3.880	8659862	652354	6859.8	1.42	5.69
Mean			8659446				
Std. Dev			623.2924				
% RSD			0.007198				

Table 6: LOD and LOQ

S.No.	Name of Compound	LOD (µg/ml)	LOQ (µg/ml)
1	Escitalopram	0.97	2.91
2	Clonazepam	2.06	6.18

Table 7-: Robustness – variation in flow-System suitability results for Escitalopram

S.No	Flow Rate (ml/min)	System Suitability Results USP Plate Count	USP Tailing
1	0.9	5784.6	1.06
2	1.0	5685.4	1.08
3	1.1	5869.5	1.09

System suitability results for Clonazepam

S.No	Flow Rate (ml/min)	System Suitability Results USP Plate Count	USP Tailing
1	0.9	6698.3	1.46
2	1.0	6895.7	1.42
3	1.1	6983.6	1.49

Table 8-: Robustness – variation in mobile phase-System suitability results for Escitalopram

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results USP Plate Count	USP Tailing
1	10% less	5895.3	1.12
2	*Actual	5685.4	1.08
3	10% more	5964.2	1.16

Robustness – variation in mobile phase-System suitability results for Clonazepam

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results USP Plate Count	USP Tailing
1	10% less	6785.2	1.46
2	*Actual	6895.7	1.42
3	10% more	6982.4	1.49

Table 9: Summary of validation data for Escitalopram

S.NO.	PARAMETER	RESULT	ACCEPTENCE CRITERIA
	System suitability		
	Theoretical plates	5685.4	Not less than 2500
1	Tailing factor	1.08	Not more than 2
	Retention time	2.808	
	%RSD	0.4	
	Specificity		
	a) Blank interference		
2	b) Placebo interference	Specific	Specific
	Method precision (%RSD)	0.464	Not more than 2.0%
3			
	Linearity parameter	20-100 µg/ml	
4	Slope	1163	≤1
	Correlation coefficient(r^2)	0.999	
	Accuracy		
5	Mean % recovery	100.30	97 - 103%
	Robustness	All the system suitability	
	a) Flow rate variation	parameters are within the	
6	b) Temperature variation	limits.	

Table 10: Summary of validation data for Clonazepam

S.NO.	PARAMETER	RESULT	ACCEPTENCE CRITERIA
	System suitability		
	Theoretical plates	6895.7	Not less than 2500
1	Tailing factor	1.42	Not more than 2
	Retention time	3.880	
	%RSD	0.3	
	Specificity		
	c) Blank interference		
2	d) Placebo interference	Specific	Specific
	Method precision		
3	(%RSD)	0.007	Not more than 2.0%
	Linearity parameter	60-140 µg/ml	
4	Slope	84268	≤1
	Correlation coefficient(r^2)	1	
	Accuracy		
5	Mean % recovery	100.21	97 - 103%
	Robustness	All the system suitability	
	c) Flow rate variation	parameters are within the	
6	d) Temperature variation	limits.	

CONCLUSION

The proposed RP-HPLC method was used for the simultaneous estimation of Escitalopram and clonazepam was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, in vitro dissolution study of combinational dosage formulations containing Escitalopram and clonazepam.

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