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Research Article

RP-HPLC METHOD FOR THE ESTIMATION OF ESCITALOPRAM IN BULK AND IN DOSAGE FORMS

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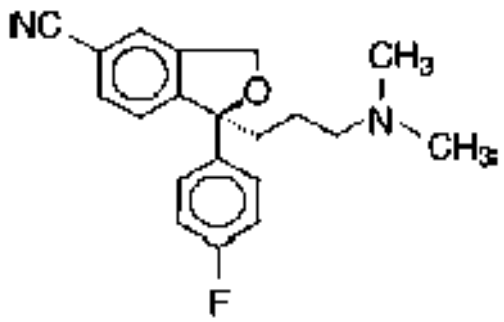
ABSTRACT:

The present study indicates a simple, accurate and precise RP-HPLC method for the estimation of Escitalopram in bulk and in pharmaceutical formulations. The mobile phase used was phosphate buffer with pH 7.0 and an organic mixture solvent (acetonitrile and methanol in the ratio of 1:1 v/v). Then the mobile phase was prepared by mixing buffer solution and mixture of organic solvents in the ratio of (55: 45 v/v) respectively. The specification of the chromatographic system 150 mm ×4.6 mm Xterra RP 18, 5 µm, flow rate 1.2ml/min, detection 238nm, injection volume 10µl and run time 10 min. Only very few HPLC procedures have been reported in the literature for the determination of Escitalopram in pharmaceutical formulations and biological fluids. There are no reports for the determination of Escitalopram by HPLC in pure form. Hence I have made an attempt to develop a HPLC method for the determination of Escitalopram in bulk and in pharmaceutical formulations.

Keywords: Escitalopram, RP- HPLC, Phosphate buffer, Acetonitrile and Methanol.

INTRODUCTION

Escitalopram is chemically[1] S (+)-1-[3-(dimethyl-amino) propyl]-1-(p-fluorophenyl)-5-phthalancarbonitrile



Escitalopram is a fine white to slightly yellow compound. Escitalopram soluble in methanol, dimethyl sulfoxide(DMSO), soluble in isotonic saline solution, sparingly soluble in water, ethanol, slightly soluble in ethyl acetate, and insoluble in heptane. It is having molecular formula is $C_{20}H_{21}FN_2O \cdot C_2H_2O_4$ and molecular weight is 414.40 g/mol[1]. The mechanism[1] of antidepressant action of escitalopram, the S-enantiomer of racemic citalopram, is presumed to be linked to potentiation of serotonergic activity in the central nervous system resulting from its inhibition of CNS neuronal reuptake of serotonin (5-HT). In vitro and in vivo studies in animals suggest that escitalopram is a highly selective serotonin reuptake inhibitor (SSRI) with minimal effects on norepinephrine and dopamine neuronal reuptake. Escitalopram is at least 100 fold more potent than the R-enantiomer with respect to inhibition of 5-HT reuptake and inhibition of 5-HT neuronal firing rate. Tolerance to a model of antidepressant effect in rats was not induced by long-term (up to 5 weeks) treatment with escitalopram. Escitalopram has no or very low affinity for serotonergic (5-HT_1-7) or other receptors including alpha- and beta-adrenergic, dopamine (D_1-5), histamine (H_1-3), muscarinic (M_1-5), and benzodiazepine receptors. Escitalopram also does not bind to or has low affinity for various ion channels including Na^+, K^+, Cl^- and Ca^{++} channels. Antagonism of muscarinic, histaminergic, and adrenergic receptors has been hypothesized to be associated with various anticholinergic, sedative, and cardiovascular side effects of other psychotropic drugs. Simultaneous estimation[2] of escitalopram and single estimation[3] of escitalopram has been done previously in UV and also in Colourometry[4]. Simultaneous determination of escitalopram oxalate and clonazepam in combined tablets by HPTLC[5] and RP-HPLC[6] has also been performed. Literature survey shows that no previous work has been performed using this mobile phase for the estimation of escitalopram.

MATERIALS AND METHODS

The instrument used for the study was HPLC (Agilent) having 1100 series HPLC pump, auto sampler equipped with a 20µL sample loop, dual absorbance detector, output signal was monitored and integrated using empower software. The reagents used in this work HPLC grade methanol (E. Merck India), HPLC grade Acetonitrile, Milli – Q water and Sodium dihydrogen phosphate anhydrous.

Method development

Selection of mobile phase: Various Mobile Phases were tried in different ratios for selection of Mobile Phase. The drug, Escitalopram was injected with different mobile phases at different ratios with different flow rates till a sharp peak, without any interference peaks containing spectrum was obtained. The different mobile phases were containing either one or the combinations of two or three of following solvents.

Acetonitrile (HPLC grade)

HPLC grade methanol (E. Merck India)

Milli – Q water

Preparation of mobile phase

Dissolve 3.8g of Sodium dihydrogen phosphate anhydrous in 1 L of water and pH adjusted with phosphoric acid and filtered through 0.45 µm pall pharma nylon 66 membrane filters. Mix acetonitrile and methanol in the ratio of 1:1 v/v. Then the mobile phase was prepared by mixing buffer solution and mixture of organic solvents in the ratio of (55: 45 v/v) respectively.

Standard stock solution

Stock solution of Escitalopram was prepared by dissolving 129mg in 100ml volumetric flask containing 75 ml of mobile phase, sonicate for about 10 min and then made up to mark with the mobile phase to get a concentration of 1mg/ml. This stock solution was further diluted to obtain a concentration.

Preparation of calibration curve

From the above stock solution (1000µg/ml) appropriate dilution were made to obtain a concentration of 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml and 300µg/ml. These different concentrations are injected into HPLC. A calibration curve was prepared taking concentrations in X-axis and Peak Area in Y-Axis (table no.1 & fig no.1).

Sample stock solution

Forty tablets weighed and powdered. A quantity of tablet powder equivalent to 100 mg was taken in a 100 ml volumetric flask and mobile phase was added up to the mark and filtered to get a concentration of 1mg/ml. The solution was sonicating for 10 min and filtered. This solution was further diluted to obtain a concentration.

PROCEDURE

Into a series of 10 ml volumetric flasks 0.5 – 3.0 ml of above stock standard solution (Escitalopram) was transferred. The total volume in each flask was made up to 10 ml with the mobile phase and filtered through 0.45 µ membrane filter. Initially the mobile phase was pumped for about 30 min to saturate the column thereby to get the baseline corrected. Then ten micro liters of Escitalopram or sample solutions were injected for six times. A quantitative determination of the active ingredient was made by comparison of the peak area from the sample injection to the corresponding peak area from a standard injection .The amount of Escitalopram present in a sample was calculated through the standard calibration curve (Fig). The retention time of Escitalopram was found to be 5.834.

Parameter fixation: In developing the method, a systematic study of the effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. In order to establish optimum conditions for good resolution, rapid, accurate, quantitative separation and estimation of Escitalopram, the author has performed control experiments varying one variable at a time and fixing all other variables such as mobile phase composition, flow rate, pH of the mobile phase etc., and the results are incorporated in table 2.

Method validation[7]:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity of Response:

To demonstrate the linearity of response, series of solutions ranging from 50 - 300 µg/ml were made and injected into the HPLC system following the described conditions. The graph (Fig.no.1) was constructed between concentration vs. peak area (table no.2) and it was found that correlation co-efficient and regression analysis were within the limits

Acceptance Criteria: The correlation co-efficient (R^2) should be not less than 0.98.

Accuracy

To establish the accuracy of the test method, sample solutions in triplicate by spiking the test solutions with Escitalopram at 80%, 100% and 120% of the specification were prepared and injected into the HPLC system as per the test procedure. The 'amount added', 'amount found' and average % recovery for Escitalopram at 80%, 100% and 120% spike levels were calculated and the results are summarized in the (table no.3).

Acceptance criteria: The mean recovery should be within 100±2%.

Precision: Analyzed a homogenous sample separately 3 times for 3 days using the same instrument employing the same analyst evaluated the same data for standard deviation relative standard deviation and coefficient of variance (table no.4, 5 & 6).

Acceptance criteria: %relative standard deviation (C.V.) of the determinations should not be more than 2.

Specificity: Amount of Escitalopram is spiked with 50%, 100%, and 150% of excipient mix and the sample is analyzed for Escitalopram recovery by H.P.L.C.

Acceptance criteria: % recovery of Escitalopram should be within 100±2%

Robustness

The robustness of an analytical procedure has been defined by the ICH as a "measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. The most important aspect of robustness is to develop methods that develop methods that allow for expected variations in method parameters. According to ICH guidelines, robustness should be considered early in the development stage of a method.

The typical variations studied under this parameter are Flow rate, Wavelength, Mobile phase composition, Temperature, pH of the mobile phase (table no.7).

Acceptance criteria

RSD for the peak areas of five replicate injections of the Standard is not more than 2.0%.

Limit of detection (LOD) and Limit of quantitation (LOD)

The LOD and LOQ for Escitalopram were predicted basing on the parameters of standard error of estimate (S_e) & Slope (S), calculated from linearity of the response data of Escitalopram. The predicted values in µg / ml can be obtained by using the formula (10 x S_e) / S for LOQ and (3.3 x S_e) / S for LOD. The values of LOD and LOQ were calculated.

Limit of detection (LOD)

The LOD of Escitalopram was found to be **1.856**µg/ml.

Limit of quantitation (LOQ)

The LOQ of Escitalopram was found to be **5.62**µg/ml.

RESULTS AND DISCUSSIONS

Preparation of calibration curve of Escitalopram

Conc. (µg/ml)	Average area
50	1141432
100	2262864
150	3404294
200	4505729
250	5706009
300	7980001

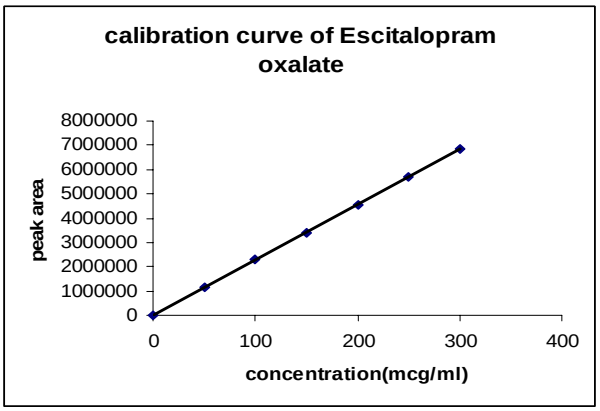


Fig. 1: Calibration curve Escitalopram oxalate

Slope: 29560, Intercept: 1345783.03, Correlation coefficient: 0.9999

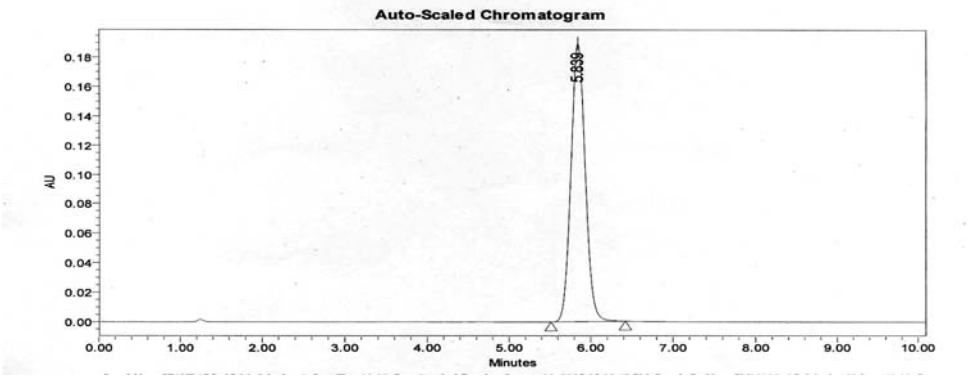


Fig. 2: Chromatogram of Standard Escitalopram

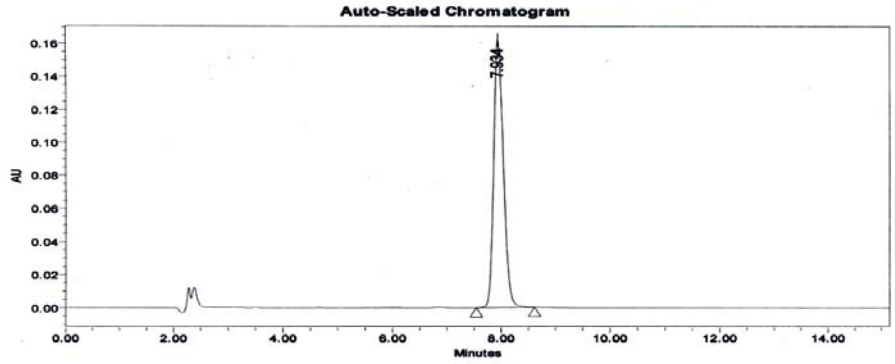


Fig. 3: Chromatogram of Sample Escitalopram

Table 2: Parameter fixation

Parameters	Optimum range	Conditions in procedure	Remarks
Mobile phase composition (% Of Acetonitrile)	35-65	50	Beyond the optimum range of % of Acetonitrile, the resolution factor and relative retention and asymmetry factor were decreased
PH of mobile phase	6.0 -7.5	7.0	Beyond the optimum range of pH of the mobile phase, better resolution was not found. When it is reduced or increased beyond optimum range asymmetry factor was increased.
Flow rate	0.8-1.2	1.2ml/min	At lower flow rates the asymmetry factor was increased and at higher flow rates the relative retentions was decreased

Table 3: Column Performance

S. No.	PARAMETERS	RESULTS
1	Retention time in minutes (t)	5.837
2	Column Length in cm. (L)	25
3	Theoretical plates (n)	5380
4	Theoretical plates per meter (N)	21250
5	Ht equivalent to Theoretical. Plates (HETP) (mm)	0.057
6	Tailing Factor	1.1
7	Peak asymmetry (T)	1.3

Table 4: Linearity of Response

Concentration (µg/ml)	Average area	Statistical Analysis	
50	1141432	Slope	29560
100	2262864	Intercept	1345783.03
150	3404294	Correlation coefficient	0.9999
200	4505729		
250	5706009		
300	7980001		

Table 5: Accuracy of Escitalopram

Concentration	Amount added (mg)	Amount found(mg)	% Recovery	Mean recovery	Mean added	Mean found
50%Sample 1	0.101	0.099	98.09	99.01	0.100	0.099
50%Sample 2	0.099	0.101	102			
50%Sample 3	0.102	0.098	96.07			
75 % Sample1	0.151	0.149	98.67	98.9	0.151	0.149
75%Sample 2	0.152	0.1479	97.39			
75%Sample 3	0.151	0.152	100.6			
100%Sample1	0.201	0.199	99	98.72	0.200	0.199
100%Sample2	0.199	0.192	99.04			
100%Sample3	0.202	0.202	99			

Table 6: Precision Results Showing Repeatability for Escitalopram

Observation / Results			
Concentration (µg/ml)	**Injection**	**Area**	**Statistical Analysis**
150	1	3404197	Mean = 3437128
	2	3403191	SD = 50954.93
	3	3404291	%RSD = 1.48%
	4	3405280	
	5	3503215	
	6	3502594	

Table 7: Intra-assay precision

OBSERVATION / RESULTS					
Concentration (µg/ml)	**Injection**	**Area 1**	**Area 2**	**Area 3**	**Mean %RSD**
	1	3404791	3504720	3501092	
	2	3404552	3408392	3404289	
150	3	3404336	3503948	3405195	
	4	3404170	3404197	3394793	
	5	3404226	3404098	3398698	
	6	3401253	3501872	3404182	
Statistical Analysis	**SD**	1311.03	53680.45	40885.94	
	% RSD	**0.0385%**	**1.5539%**	**1.1961%**	**0.929%**

Table 8: Inter-assay precision

Concentrations (µg/ml)	%RSD	Average %RSD		
	Day 1	**Day2**	**Day3**	
150	1.162%	1.167%	0.078%	0.802%

Table 9: Robustness of Escitalopram

pH – 10.5			**pH – 10**		
Conc. (µg/ml)	Area	Statistical Analysis	Conc. (µg/ml)	Area	Statistical Analysis
	3404296	Mean= 3420078		3404092	Mean= 3404151
	3404191	SD = 39705.22		3404091	SD = 59.47352
150	3403991	%RSD = 1.160%	150	3404191	%RSD = 0.0017%
	3404280			3404110	
	3501115			3404225	
	3402594			3404194	
Flow Rate – 0.5ml/min			**Flow Rate – 0.7ml/min**		
Conc. (µg/ml)	Area.	Statistical Analysis	Conc. (µg/ml)	Area	Statistical Analysis
	3404197	Mean= 3437128		3403997	Mean= 3403855
	3403191	SD = 50954.93		3404191	SD = 630.5397
150	3404291		150	3404191	%RSD = 0.0185%
	3405280	%RSD = 1.482%		3404240	
	3503215			3403915	
	3502594			3402594	

Table 10: Results showing all the validation parameters of Escitalopram

Validation Parameters		**Results**
Linearity	Correlation Coefficient	0.9999
	Slope	29560
	Intercept	1345783.03
	Regression	Y= 1345783.03X +29560
Accuracy	Spiked Concentration	%Recovery
	50 %	98.72
	75%	98.9
	100%	99.01
Precision	%RSD	0.253
LOD	Concentration(µg/ ml)	1.856
LOQ	Concentration(µg/ ml)	5.62

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Escitalopram in pure form. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement within the limit. Hence, this method can be easily and conveniently adopted for routine analysis of Escitalopram in pure form and can also be used for dissolution or similar studies.

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