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AN IMPROVED VALIDATED RP- HPLC METHOD FOR SEPARATION OF CITALOPRAM HBR IMPURITIES IN CITALOPRAM HBR TABLETS

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ABSTRACT
Objective: A New method was established for estimation of Citalopram by RP-HPLC method. **Methods**: Chromatogram was run through Hypersil BDS C18, 250 mm X 4.6 mm, 5 µm column. Mobile phase containing buffer and Methanol in the ratio of 60:40 was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 45^0C. Optimized wavelength for Citalopram was 239 nm. **Results**: Retention time of Citalopram was found to be 4.670 min. The % purity of Citalopram was found to be 100.4 %. The system suitability parameters for Citalopram such as theoretical plates and tailing factor were found to be 6.906.6, 1.67. The linearity study for Citalopram was found in concentration range of 20 µg-60 µg correlation coefficient (r2) was found to be 0.999 %, %RSD for repeatability was 0.86, % RSD for intermediate precision was 0.47. The precision study was precise, robust and repeatable. LOD value was 0.598, and LOQ value was 1.81. **Conclusion**: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Citalopram in pharmaceutical dosage form.

KEYWORDS: Citalopram, RP-HPLC, Method development, Validation.

INTRODUCTION

Citalopram belongs to a class of antidepressant agents known as selective serotonin-reuptake inhibitors (SSRIs) and is widely used to treat the symptoms of depression. Its chemical structure is unrelated to that of other SSRIs or of tricyclic, tetracyclic, or other prescribed antidepressants. Citalopram is also known as Celexa, and available in tablet and solution forms. This drug was initially approved by the FDA in 1998. The mechanism of action of citalopram results from its inhibition of CNS neuronal reuptake of serotonin (5-HT). The molecular target for citalopram is the serotonin transporter (solute carrier family 6 member 4, SLC6A4), inhibiting its serotonin reuptake in the synaptic cleft.[1-5] IUPAC name is 1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-3H-2-benzofuran-5-carbonitrile. Molecular formula $C_{20}H_{21}FN_2O$. Molecular Weight is 324.4. Citalopram HBr is sparingly soluble in water and soluble in ethanol. Celexa (citalopram hydrobromide) is available as tablets or as an oral solution.

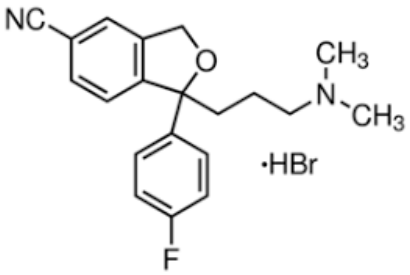

Figure 1: Structure of Citalopram HBr.

The literature survey revealed that There are very few methods reported in the literature for analysis of Citalopram HBr alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC.[6-9] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Citalopram HBr estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Citalopram HBr. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the estimation of Citalopram HBr in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH

guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS

Chemicals and Reagents: Citalopram HBr were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 239 nm with column Hypersil BDS C18, 250 mm X 4.6 mm, 5 µm, dimensions at 45^0C temperature. The optimized mobile phase consists of buffer and Methanol in the ratio of 60:40. Flow rate was maintained at 1 ml/min.

Preparation of Solutions

Preparation of buffer

0.7 g of Anhydrous Dibasic Sodium Phosphate was transferred into 500 ml volumetric flask and 250 ml of Water was added. Shaken the flask until the particles get dissolved and volume was made up to the mark with Water.

Preparation of standard stock solution

40 mg of standard Citalopram Hydrobromide was accurately weighed and transferred into a 100 ml clean dry volumetric flask, about 30 ml of diluent was added, sonicated for 5 minutes and diluted up to the mark with diluent (stock solution-I). From this stock solution-I (400 µg / ml), 5 ml of solution was transferred into a 50 ml volumetric flask and diluted with the diluent up to the mark and labeled as stock solution-II (40 µg / ml).

Preparation of sample stock solution

20 Tablets of Citalopram Hydrobromide were weighed and powdered in glass mortar. The powder equivalent to the amount of active ingredient present in 10 tablets was transferred into a 100 ml volumetric flask, 70 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for further dilution. 1 ml of upper clear solution was transferred to a 100 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter before injecting into HPLC system.

Preparation of Placebo

The amount of powdered inactive ingredient supposed to be present in 10 tablets was accurately weighed and transferred in to 100 ml volumetric flask, 70 ml of diluent was added and shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for dilution. 1 ml of upper clear solution was transferred to a 100 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter before injecting into HPLC system.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 30 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Hypersil BDS C18, 250 mm X 4.6 mm, 5 µm column, the mobile phase of composition buffer and Methanol in the ratio of 60:40 was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Citalopram in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method

Linearity: The linearity study was performed for the concentration of 20 ppm to 60ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3,4.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 75%, 100%,125%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Citalopram and calculate the individual recovery and mean recovery values. The results are shown in table 5.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day.

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 7.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1 ml/min. The results are shown in table 8,9.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 10.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve.

RESULTS AND DISCUSSION

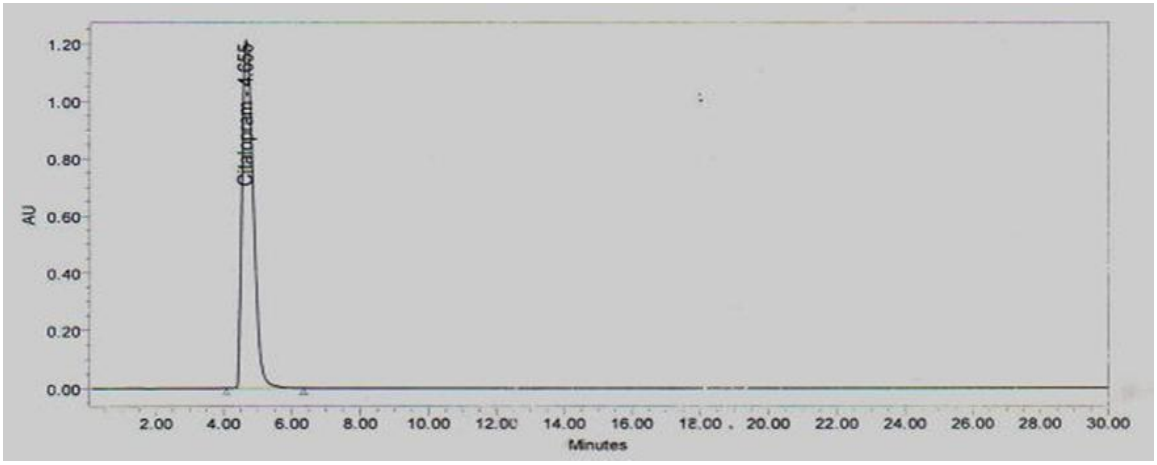

Figure 2: Standard chromatogram.

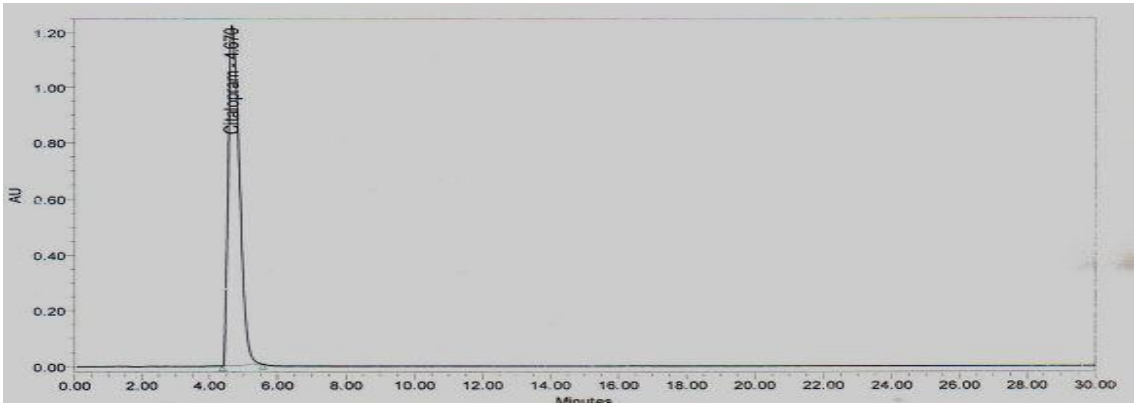

Figure 3: Sample chromatogram.

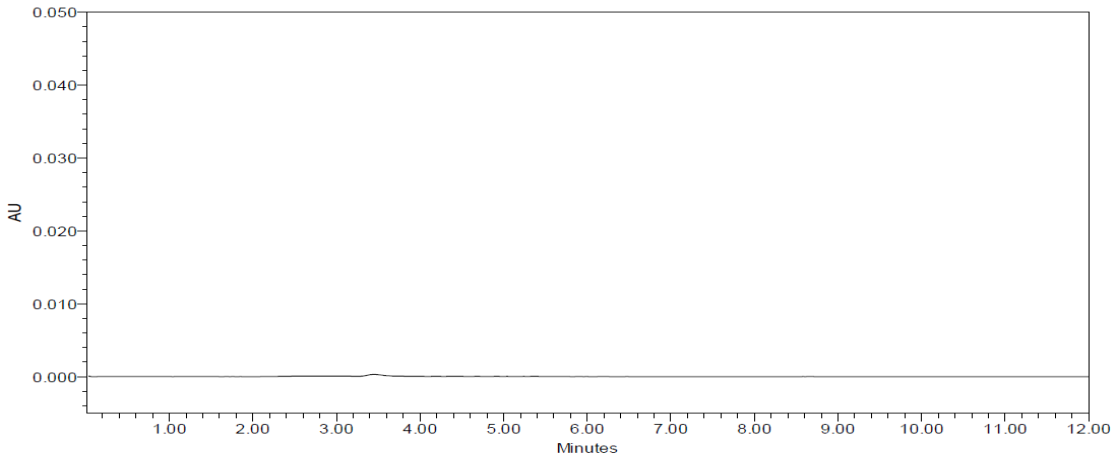

Figure 4: Blank chromatogram.

Table 1: System suitability parameters.

Injection	t_R	Peak Area	USP Plate count	USP Tailing
1	4.665	27554861	6859	1.64
2	4.654	27631819	6865	1.65
3	4.643	27617916	6911	1.69
4	4.647	27622416	6932	1.69
5	4.655	27644753	6890	1.65
6	4.660	27723581	6983	1.69
Mean	4.654	27632558	6906.6	1.67
SD	0.008	54384.6	46.418	0.02
% RSD	0.2	0.2	0.672	1.43

Table 2: Assay results for Citalopram.

	Label Claim (mg)	% Assay
Citalopram	40	100.4

Table 3: Linearity results of Citalopram.

InjectionNo	Solution 1	Solution 5
1	13816279	41428137
2	13826255	41384875
3	13719289	41483134
4	13718879	41478191
5	13918279	41366139
Avg. Area	13799796	41428095
SD	83724.14	53022.2
% RSD	0.6	0.12

Table 4: Linearity Range and Average area values.

Solution No.	Conc. (µg / ml)	Avg Area
1	20	13799796
2	30	20532237
3	40	27994172
4	50	34890435

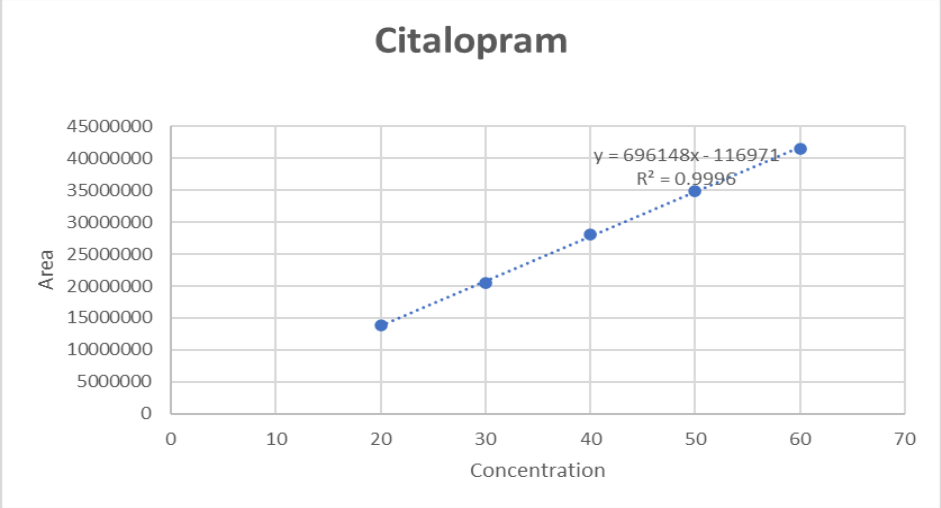

Figure 5: Linearity graph for Citalopram.

Table 5: Showing accuracy results for Citalopram.

SampleNo.	SpikeLevel	Amount (µg / ml)added	Amount (µg / ml)found	% Recovery	Mean % Recovery
1	50 %	20	19.84	99.20	99.22
	50 %	20	19.85	99.28	
	50 %	20	19.84	99.20	
2	75 %	30	29.93	99.78	99.94
	75 %	30	30.01	100.05	
	75 %	30	30.00	100.00	
3	100 %	40	39.92	99.80	99.83
	100 %	40	39.92	99.80	
	100 %	40	39.96	99.90	
4	125 %	50	50.08	100.16	100.11
	125 %	50	49.61	99.92	
	125 %	50	50.12	100.21	
5	150 %	60	59.83	99.73	99.62
	150 %	60	59.87	99.69	
	150 %	60	59.67	99.46	

Table 6: Precision results for Citalopram.

Injection No	Peak Area	% Recovery
1	27818714	99.87
2	28074488	101.06
3	27424386	98.78
4	27622416	98.91
5	27644753	99.01
6	27703581	99.23
Mean	27718056	99.47
SD	218159.9	0.865648
% RSD	0.78	0.86

Table 7: Ruggedness results of Citalopram.

InjectionNo	Peak Area	% Recovery
1	27723581	99.56
2	27644753	99.17
3	27622416	99.13
4	27424386	98.91
5	28074488	100.17
6	27818714	99.79
Avg	27718056	99.455
SD	218159.9	0.472938
% RSD	0.78	0.47

Robustness results

Table 8: Flow variation results for Citalopram.

Sl. No	Change in flowrate	t_R	Asymmetry
01	0.9 ml / min	4.650	1.675
02	1.1 ml / min	4.654	1.680

Table 9: System suitability results for Citalopram.

Sl. no	Composition of mobile phase (Methanol: Buffer)	t_R	Asymmetry
01	68: 32	4.6365	1.675
02	72: 28	4.6665	1.705

Table 10: LOD, LOQ of Citalopram.

Drug	LOD	LOQ
Citalopram	0.598	1.81

CONCLUSION

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of citalopram in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Citalopram in pure and its pharmaceutical dosage forms.

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