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RP-HPLC method development and validation of citalopram in pharmaceutical dosage form

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ABSTRACT

A simple, sensitive, precise and Reverse phase high performance liquid chromatographic method has been developed for the quantitative analysis of Citalopram drug present in drug substance. A suitable HPLC having a gradient system equipped with manual injector, UV detector is used for this work. The HPLC separation was achieved on HITACHI L2130 with D Elite 2000 software with Isocratic with UV-Visible detector (L2400).C_{18} Develosil ODS HG-5 RP 150 mm x 4.6 mm 5 µm particle size and column temperature 25ºc used as stationary phase. The mobile phase used in this analysis consists of a mixture of Phosphate Buffer 0.01M potassium dihydrogen phosphate (pH adjusted to 3.0 with ortho phosphoric acid) and acetonitrile in the ratio of 60:40. Stock sample is prepared by using acetonitrile. Working sample used is about 10 ppm. Flow rate maintained is about 1.0 ml/minute and wavelength is about 229nm. Sample colour is ambient. Injection volume injected about 20 µL with run time 10 minutes. The proposed method provided linear responses within the concentration range 10 ppm for Citalopram LOD and LOQ values for the active substance were 0.04 and 0.12 µg/mL respectively. Regression equations for the drug substance is about 0.994 in all cases. The precision of the method was demonstrated using intraday and inter day assay % RSD values which were in acceptance limit (≤ 2%) in all instances. The proposed method was found to be accurate, precise, reproducible and specific and it can also be used for routine quality control analysis.

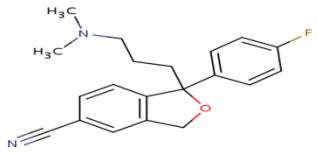
Keywords: HPLC, Method development, Validation, Reverse Phase, Citalopram.

INTRODUCTION

Today, the development of a method of analysis is usually based on prior art or existing literature, using the same or quite similar instrumentation. The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements of the method. It is necessary to consider the properties of the analyte(s) of interest that may be used to advantage and to establish optimal ranges of analyte parameter values [1-2]. Once the instrumentation has been assembled and analyte parameters have been considered, standards should be used for the

continued development, optimization, and preliminary evaluation of the method.

Citalopram hydrobromide belongs to a class of antidepressant agents known as selective serotonin-reuptake inhibitors (SSRIs). Citalopram and its N-demethylated metabolites exist as a racemic mixture but its effects are largely due to the S-enantiomer, S-citalopram and S-demthylcitalopram. Literature review reveals that few analytical methods have been evoked for the estimation of Citalopram by HPLC [3-8] method.



Citalpopram

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

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

This new method for the determination of Citalopram by RP-HPLC method was successfully developed and validated as per ICH guidelines [9-10], can be utilized for the validation of Citalopram in pharmaceutical dosage forms and it's stability indicative studies.

MATERIALS AND METHODS

Table 2.1: List of Instruments used

Sr. no.	Name of Instrument	Instrument Model	Name of manufacturer
1	UV-Visible spectrophotometer	UV 1800	Elico, corp. Japan.
2	HPLC	1575	Hitachi L2130
6	Ultra sonicator	--------	Wensar wuc-2L
7	Melting point apparatus	--------	

Table 2.2: chemicals / reagents used

		Specifications		
S.N.	**Name**	**Purity**	**Grade**	**Manufacturer/Supplier**
1.	Doubled distilled water	----	----	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	A.R.	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9	L.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9	L.R.	Sd fine-Chem ltd; Mumbai

METHOD DEVELOPMENT AND ITS VALIDATION FOR CITALOPRAM BY RP-HPLC

Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Citalopram, so that the same wave number can be utilized in HPLC UV detector for estimating the Citalopram. While scanning the Citalopram solution we observed the absorption maximz was 239 nm. The UV spectrum has been recorded on Elico, corp. make UV – VIS spectophotometer model UV-2450. The scanned UV spectrum is attached in

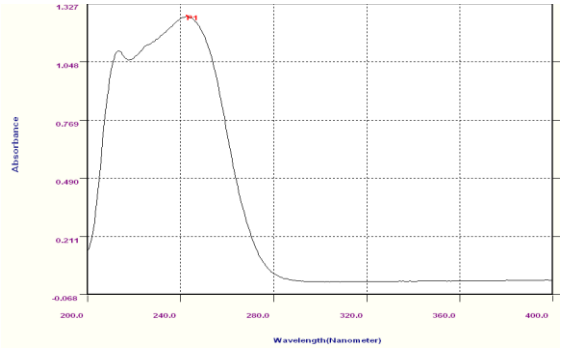


Figure 2.1.1: UV spectrum of Citalopram

Preparation of standard solution of Citalopram

25 mg of Citalopram was weighed accurately and transferred into 25 ml volumetric flask. About 10 ml of mobile phase was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 1000 µg/ml of Citalopram. From the stock solution again 0.1 ml was taken in a 10 ml volumetric flask & volume was make up to the mark by mobile phase. This solution contains 10 µg/ml of Citalopram which has been injected to HPLC.

Initialization of the instrument

The HPLC instrument was switched on. The column was washed with HPLC water for 45 minutes. The column was then saturated with mobile phase for 45 minute. The mobile phase was run to find the peaks. After 20 minutes the standard drug solution was injected in HPLC.

Different chromatographic conditions used and their Optimizations

The different HPLC chromatographic conditions were used to find out the optimum chromatographic condition for best elution of drugs.

Chromatographic conditions 1

Mobile phase	Water:ACN(80:20)
Wavelength	239nm
Flow rate	0.8 ml/ min.
Run time	10 min.
Column	Hiq Sil, C-18, V size(250mm*4.6mmØ)

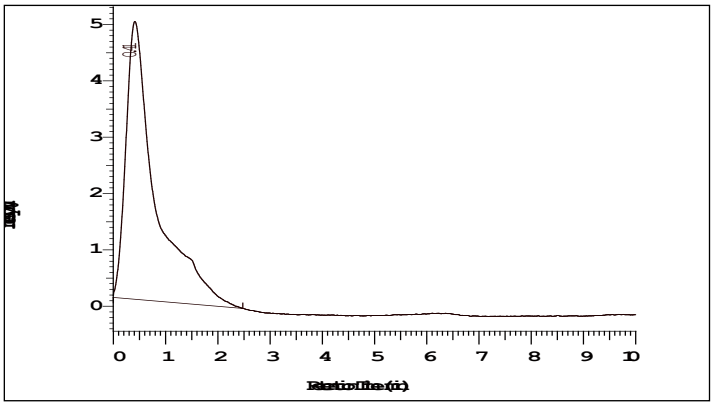


Figure 2.4.1: The chromatogram obtained after condition 1

Table 2.4.1: Results of condition-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	0.41	256412	36541	0.98

No peaks were found. Hence chromatogram was not acceptable.

Chromatographic conditions 2

Mobile phase	Water: Methanol (20:80)
Wavelength	239nm
Flow rate	0.8 ml/ min.
Run time	10 min.
Column	Hiq Sil, C-18, V size (250mm*4.6mm)

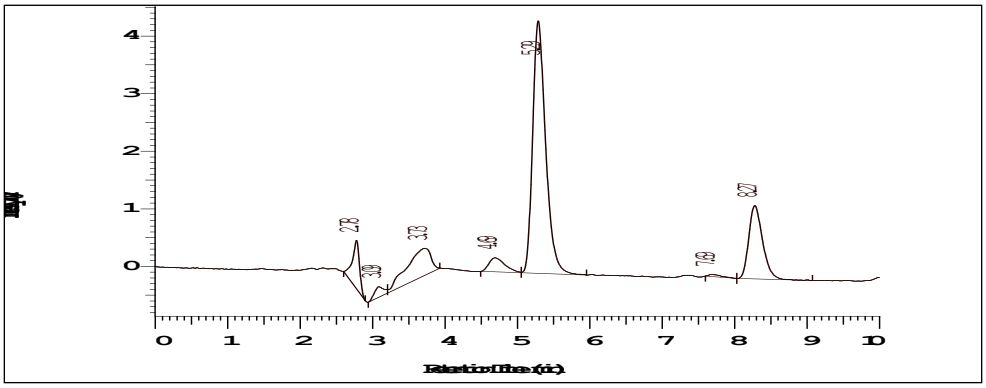


Figure 2.4.2: The chromatogram obtained after condition 2

Table 2.4.2: Results of condition-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	5.29	22134	254642	0.48

No peak was there and a negative peak were found. Hence chromatogram was not acceptable.

Chromatographic conditions 3

Mobile phase	Water: Methanol (40:60)
Wavelength	239 nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.

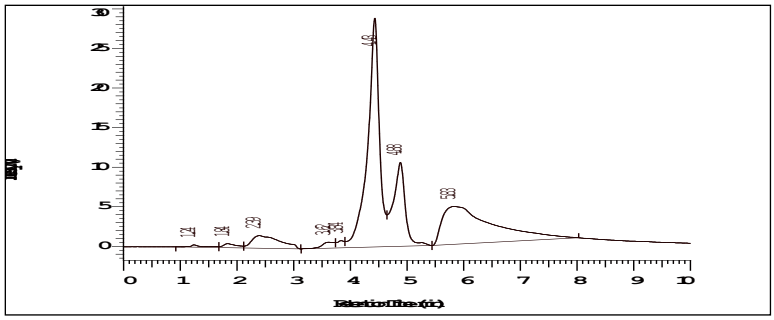


Figure 2.4.3: The chromatogram obtained after condition 3

Table 2.4.3: Results of condition-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	4.43	26542	38964	0.76

Peak was not good and also a third peak was found. Hence chromatogram was not acceptable.

Chromatographic conditions 4

Mobile phase	Water: Methanol (70:30)
Wavelength	239nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.

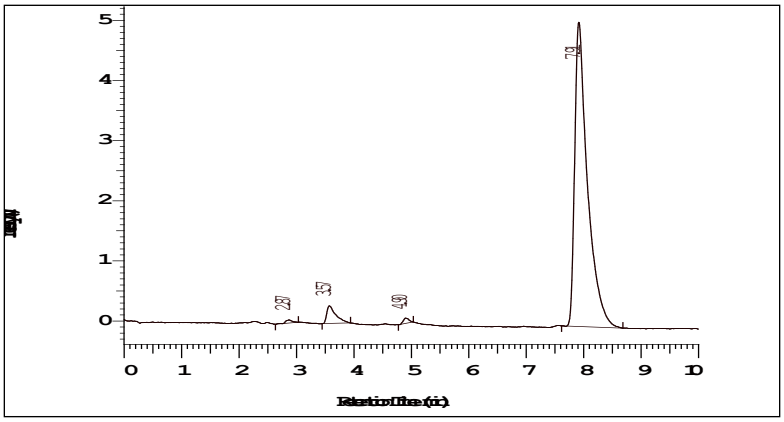


Figure 2.4.4: The chromatogram obtained after condition 4

Table 2.4.4: Results of condition-4

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	7.91	28464	456721	0.24

Peak was found but Rt was very late. Hence chromatogram was not acceptable.

Chromatographic conditions 5

Mobile phase	Potassium dihydrogen phosphate buffer:: Methanol (90:10)
Wavelength	239nm
Flow rate	0.8 ml/ min.
Run time	20 min.
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.

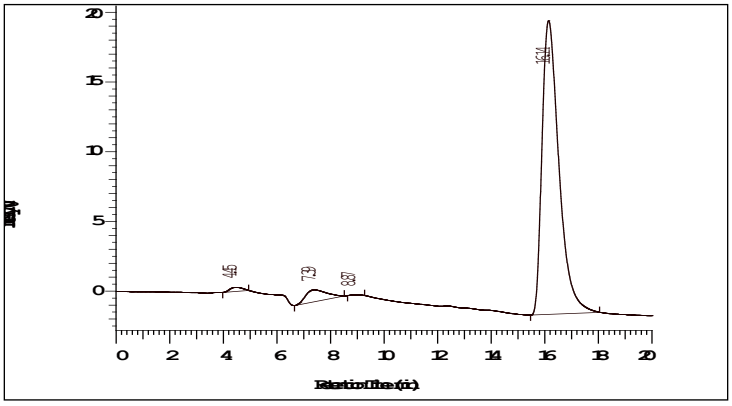


Figure 2.4.5: The chromatogram obtained after condition 5

Table 2.4.5: Results of condition-5

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	16.14	548764	645721	0.37

Peak was found but Rt was very late. Hence chromatogram was not acceptable.

Chromatographic conditions 6

Mobile phase	phosphate buffer(pH 3.0): Methanol (60:40)
Wavelength	239 nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.

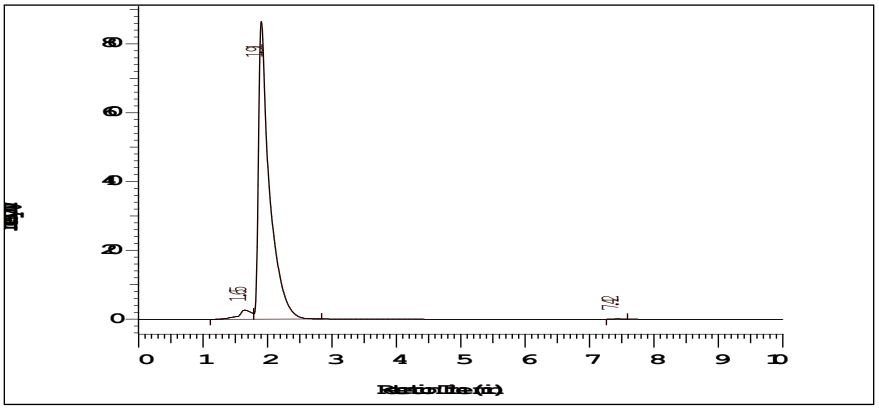


Fig 2.4.6: The chromatogram obtained after condition 6, Typical chromatogram of Citalopram.

Table 2.4.6: Results of condition-6

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	598746	652134	0.89

Here resolution was good, theoretical plate count and symmetry was appropriate. Also no unwanted little peaks were seen between two peaks. Hence it was acceptable.

FINAL RESULT & DISCUSSION

The selected and optimized mobile phase was Methanol: phosphate buffer (pH 3.0) (40:60v/v) and conditions optimized were: flow rate (1.0 ml/minute), wavelength (239 nm), Run time was 10 min. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs.

Preparation of mobile phase

Mobile phase was prepared by taking Methanol: phosphate buffer (pH 3.0) (40:60 v/v). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

Running the standard solution of Citalopram

1 ml of stock solution (100 ppm) was pipetted out into a 10 ml volumetric flask. The volume was made up to the mark with methanol. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in **fig 2.6.1.**

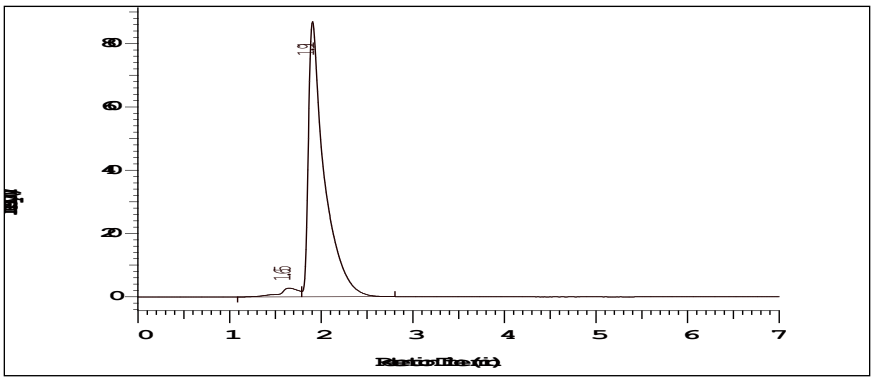


Figure 2.6.1: Chromatogram of Citalopram (Rt 1.91)

Table 2.6.1: Results of optimized condition of Citalopram

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	598746	652134	0.89

Result & Discussion

Retention time was found to be 1.91 min.

Result & Discussion

The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Citalopram for 07 min. The retention time were found to be 1.91 min.

FORCED DEGRADATION STUDIES

Following protocol was strictly adhered to for forced degradation of Citalopram Active Pharmaceutical Ingredient (API). The API (Citalopram) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body.

This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

ACID HYDROLYSIS

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that

0.1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions)

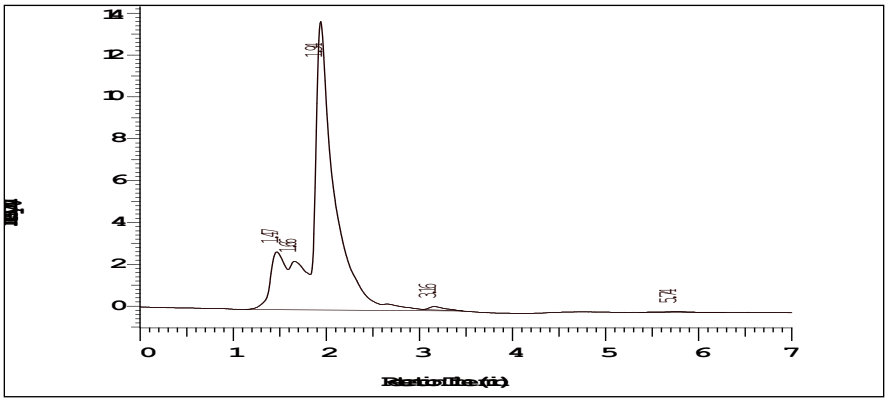


FIG 2.8.1: Chromatogram showing degradation for Citalopram in 0.1 N HCl

Table 2.8.1: Results of acid hydrolysis

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.94	36547	45321	0.46

BASIC HYDROLYSIS

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 0.1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of . NaOH (after all optimized conditions)

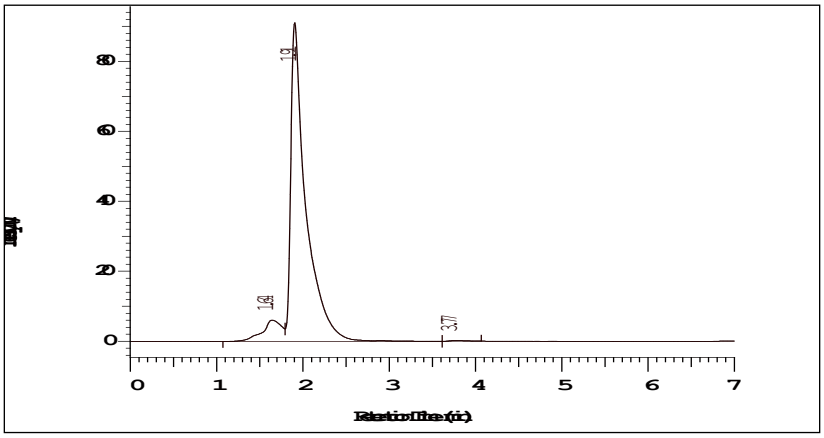


FIG 2.9.1: Chromatogram showing degradation related impurity in 0.1 N NaOH

Table 2.9.1: Results of basic hydrolysis

Sl. No	Rt	Theoretical Plates	sArea	Tailing factor
1	1.91	256421	39874	0.59

THERMAL DEGRADATION

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase. From this solution take 1 ml make up to the volume 10 ml & was maintained at 50 ^{0}C. for 24 hrs. then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

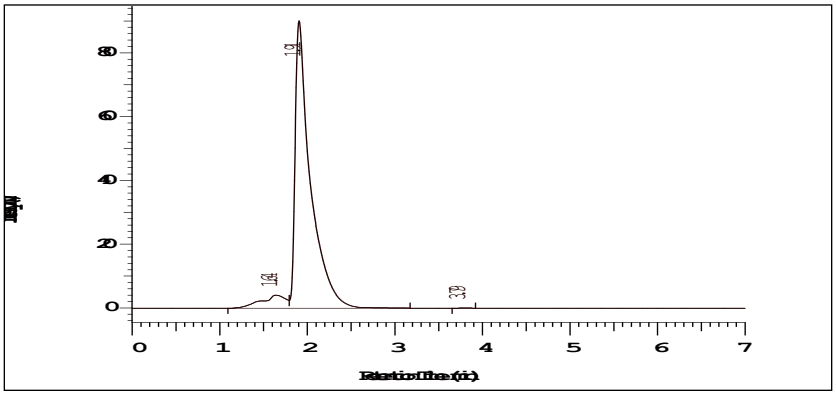


Fig 2.10.1: Chromatogram showing thermal degradation studies

Table 2.10.1: Results of thermal degradation

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	297847	352164	0.85

Photolytic Degradation

Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml. volumetric flask. First the UV exposed drug was dissolved in mobile phase & make up to the mark. than injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

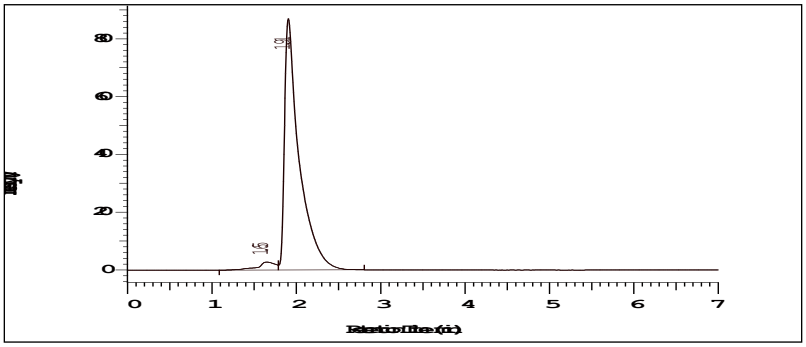


Fig 2.11.1: Chromatogram showing photolytic degradation.

Table 2.11.2 : Results of photolytic degradation

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	232415	54213	0.73

Oxidation with (3%) H_2O_2

Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H2O2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 ppm solution. The above sample was injected into the HPLC system.

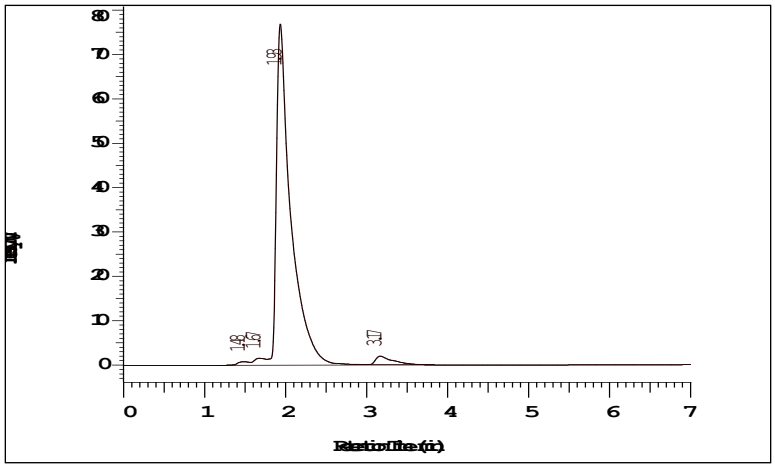


Fig. 2.12.1: Chromatogram showing oxidative degradation.

Table 2.12.1 : Results of oxidative degradation

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.93	31245	524311	0.65

Results of degradation studies

The results of the stress studies indicated the specificity of the method that has been developed. Citalopram was degraded only in 3% H_2O_2 & temperature stress conditions. The result of forced degradation studies are given in the following table.

Table 2.13.1: Results of force degradation studies of Citalopram API.

Stress condition	**Time**	**Assay of active substance**	**Assay of degraded products**	**Mass Balance (%)**
Acid Hydrolysis (0.1 M HCl)	24Hrs.	23.75	74.61	98.36
Basic Hydrolysis (0.I M NaOH)	24Hrs.	43.32	55.02	98.32
Thermal Degradation (50 ^{0}C)	24Hrs.	97.39	----------	97.39
UV (254nm)	24Hrs.	75.19	24.34	99.53
3 % Hydrogen peroxide	24Hrs.	78.75	20.28	99.03

METHOD VALIDATION

Linearity and Range

Linearity range was found to be 5-20 µg/ml for Citalopram. The correlation coefficient was found to be 0.999, the slope was found to be 47273 and intercept was found to be 11010 for Citalopram.

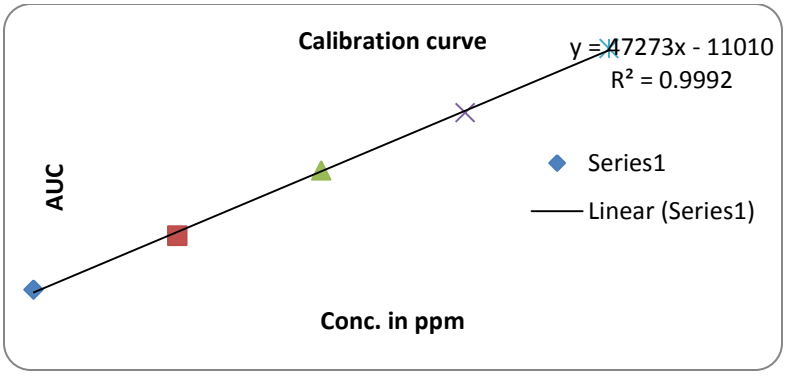


Fig 2.14.1: Standard curve for Citalopram

Table 2.14.1: Results of Linearity for Citalopram

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
5	210450
10	465728
15	690749
20	941680

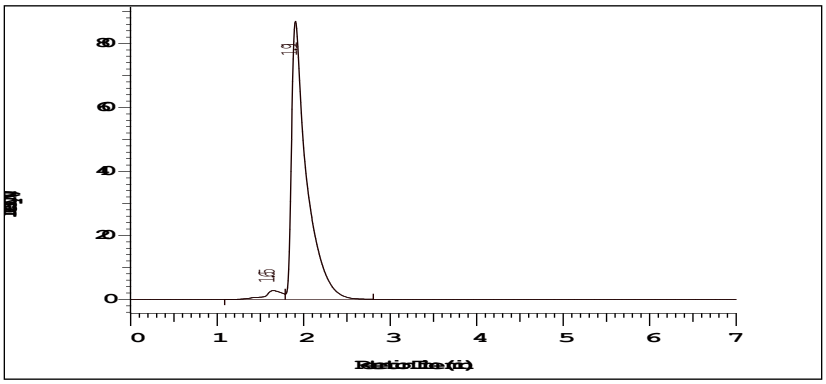


Fig 2.14.2: Chromatogram for 5 ppm

Table 2.14.2: Readings of Linearity-5 ppm

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	45243	210450	0.94

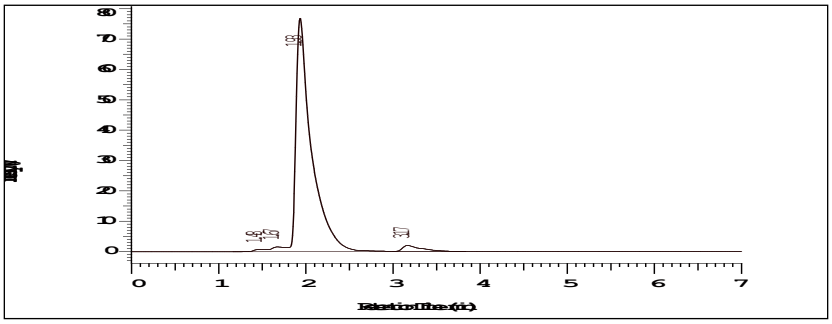


Fig. 2.14.3 : Chromatogram for 10 ppm

Table 2.14.3: Readings of Linearity-10 ppm

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.93	54213	465728	0.84

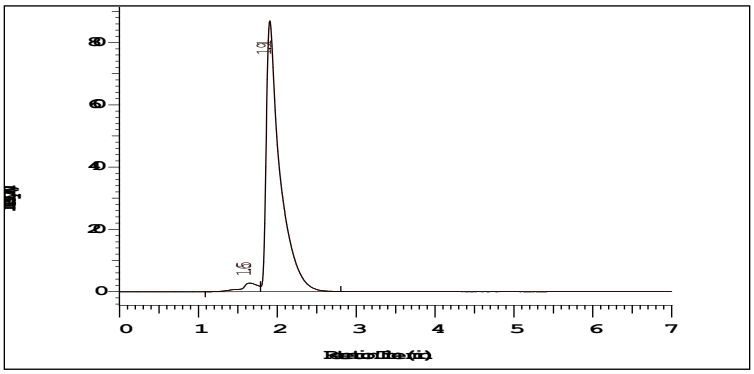


Fig 2.14.4: Chromatogram for 15 ppm

Table 2.14.4: Readings of Linearity-15 ppm

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	65421	690749	0.97

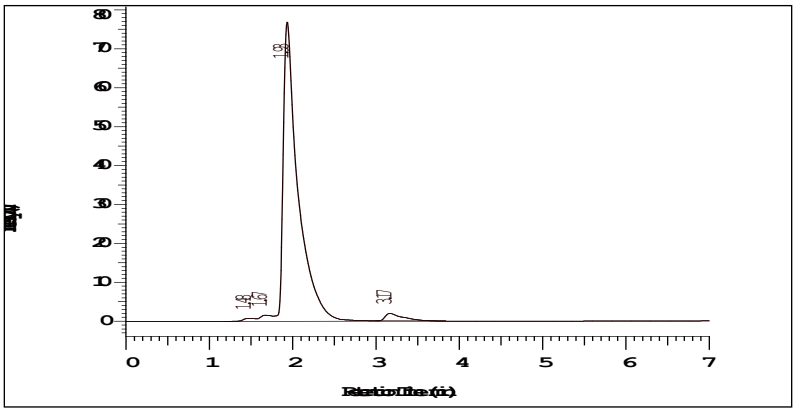


Fig. 2.14.5: Chromatogram for 20 ppm

Table 2.14.5: Readings of Linearity-20 ppm

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.93	54213	56213	0.82

Accuracy

Recovery study

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Citalopram were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-2.14.2.1.

Table 2.14.2.1: Data of recovery studies

Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis
	Pure drug	Formulation	Pure drug	
S_1 : 80 %	8	10	101.61	Mean= 101.13%
S_2 : 80 %	8	10	100.17	S.D. = 0.831384
S_3 : 80 %	8	10	101.61	% R.S.D.= 0.822095
S_4 : 100 %	10	10	99.133	Mean= 99.14633%
S_5 : 100 %	10	10	99.133	S.D. = 0.02309
S_6 : 100 %	10	10	99.173	% R.S.D.= 0.023293
S_7 : 120 %	12	10	99.327	Mean= 99.35367%
S_8 : 120 %	12	10	99.357	S.D. = 0.25166
S_9 : 120 %	12	10	99.377	% R.S.D. = 0.02533

RESULT & DISCUSSION

The mean recoveries were found to be 101.13, 99.14633, and 99.35367% for Citalopram. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

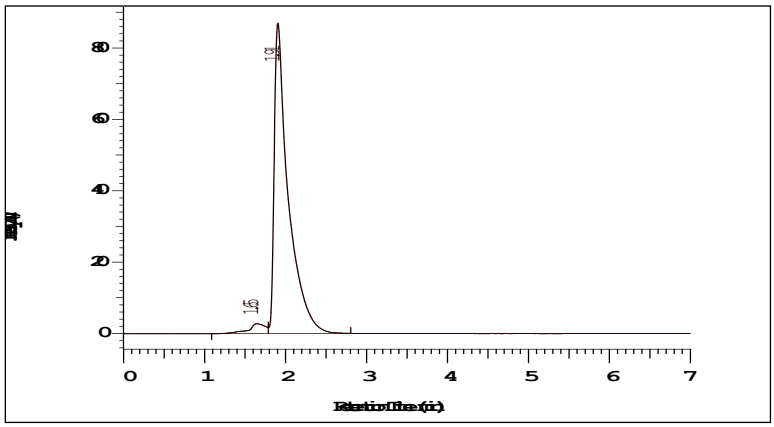


Fig. 2.15.1: Chromatogram for accuracy-1 replicate-1

Table 2.15.1: Accuracy Readings of accuracy-1 replicate-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	35687	36542	0.79

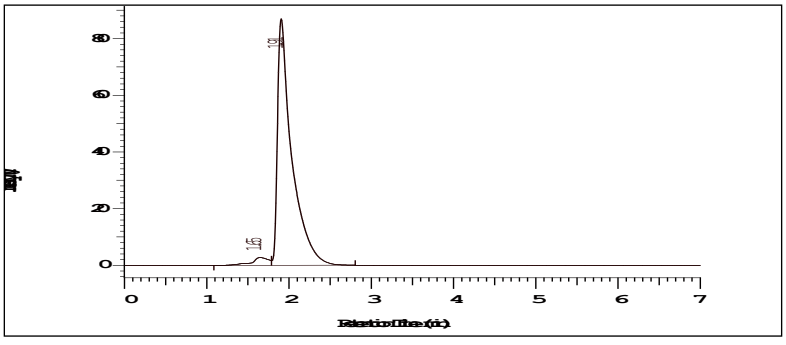


Fig. 2.15.2: Chromatogram for accuracy-1 replicate-2

Table 2.15.2: Accuracy Readings of accuracy-1 replicate-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	39756	56487	0.59

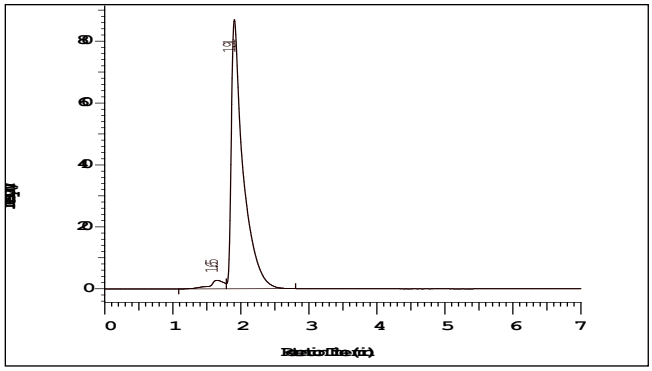


Fig 2.15.3: Chromatogram for accuracy-1 replicate-3

Table 2.15.3: Accuracy Readings of accuracy-1 replicate-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	46786	65784	0.58

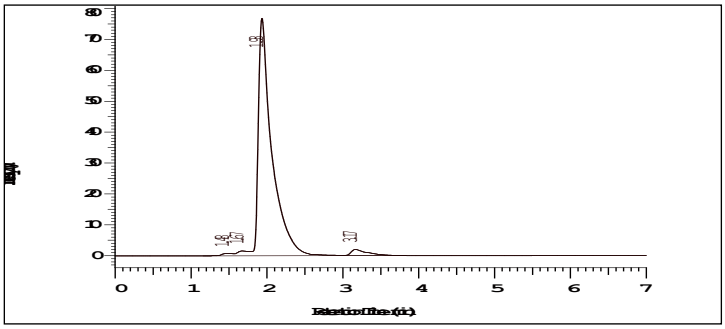


Fig 2.15.4: Chromatogram for accuracy-2 replicate-1

Table 2.15.4: Accuracy Readings of accuracy-2 replicate-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.93	54231	45425	0.65

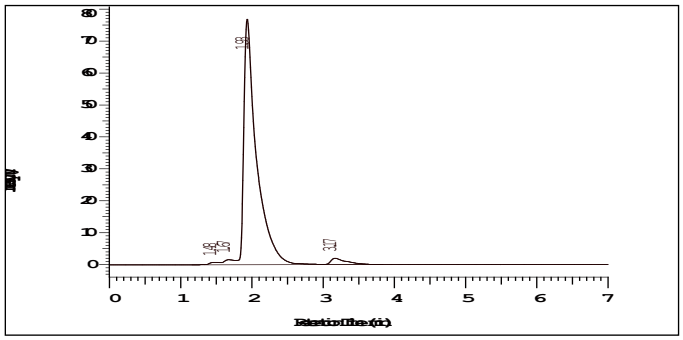


Fig 2.15.5: Chromatogram for accuracy-2 replicate-2

Table 2.15.5: Accuracy Readings of accuracy-2 replicate-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.93	54213	45312	0.54

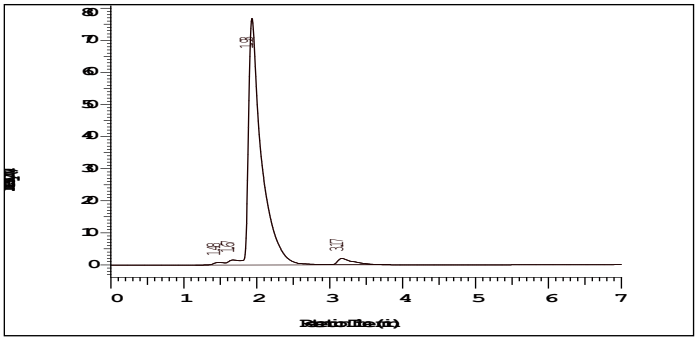


Fig 2.15.6: Chromatogram for accuracy-2 replicate-3

Table 2.15.6: Accuracy Readings of accuracy-2 replicate-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.93	54213	65875	0.96

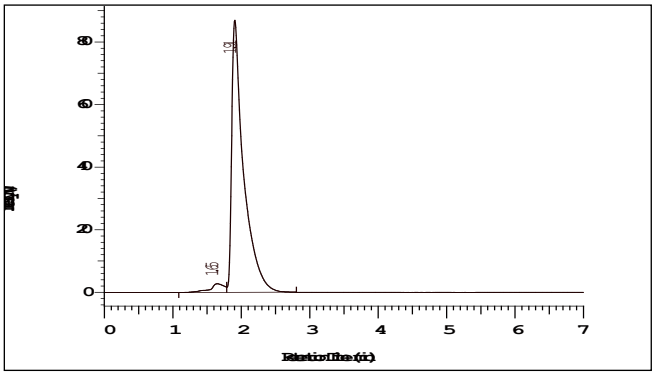


Fig 2.15.7: Chromatogram for accuracy-3 replicate-1

Table 2.15.7: Accuracy Readings of accuracy-3 replicate-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	67544	56421	0.48

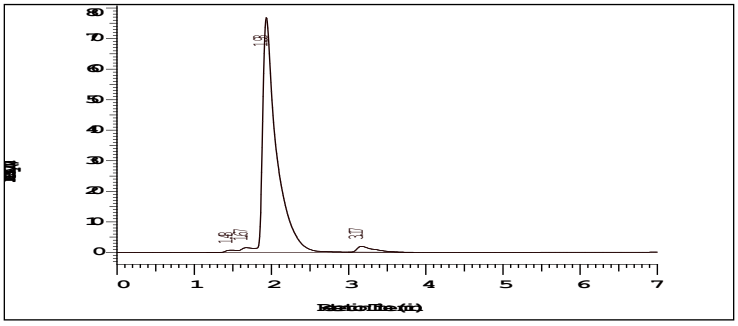


Fig 2.15.8: Chromatogram for accuracy-3 replicate-2

Table 2.15.8: Accuracy Readings of accuracy-3 replicate-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.93	56874	65421	0.89

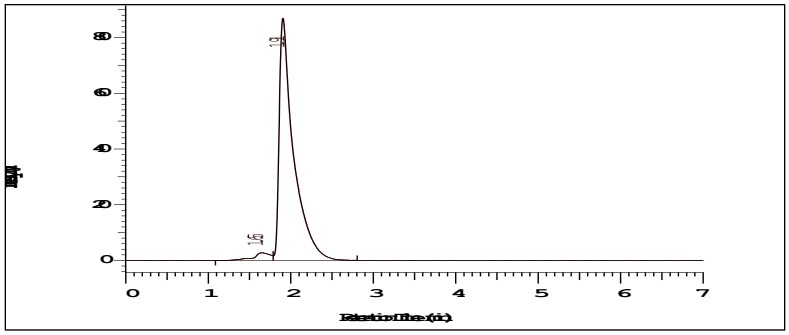


Fig 2.15.9: Chromatogram for accuracy-3 replicate-3

Table 2.15.9: Accuracy Readings of accuracy-3 replicate-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	49875	56874	0.79

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Citalopram. The percent relative standard deviations were calculated for Citalopram are presented in the table-2.15.1.

Table 2.15.1: Data showing repeatability analysis

HPLC Injection Replicates of Citalopram	Retention Time	Area
Replicate – 1	1.91	465728
Replicate – 2	1.91	465429
Replicate – 3	1.91	458963
Replicate – 4	1.91	469853
Replicate – 5	1.91	469852
Average	1.91	465965
Standard Deviation	0.008944	4460.851
% RSD	0.467308	0.957336

The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml of Citalopram showed a RSD of 0.957336%. It was concluded that the analytical technique showed good repeatability.

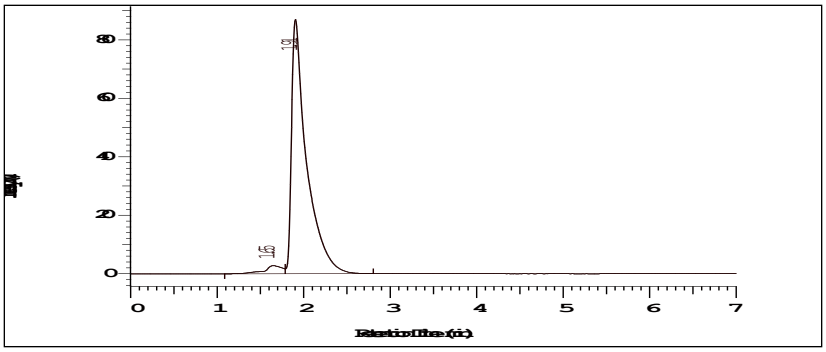


Fig 2.15.: Chromatogram for repeatability-1

Table 2.15.2: Results for Repeatability-1

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	32456	465728	0.46

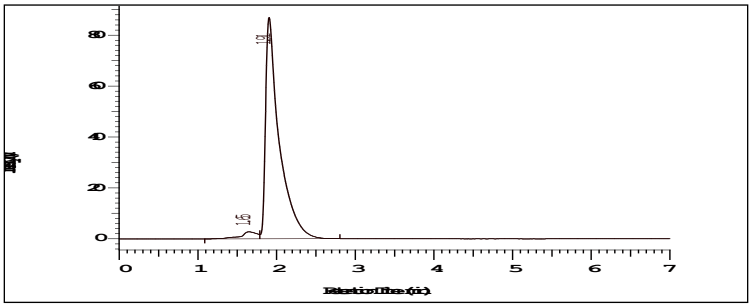


Fig 2.15.2: Chromatogram for repeatability-2

Table 2.15.3: Results for Repeatability-2

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	29245	465429	0.64

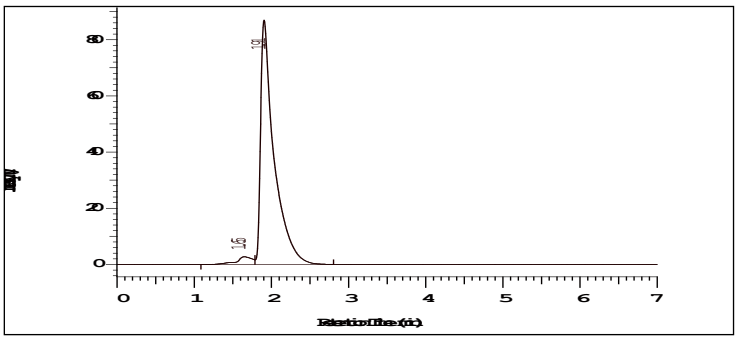


Fig 2.15.3: Chromatogram for repeatability-3

Table 2.15.4: Results for Repeatability-3

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	35644	458963	0.76

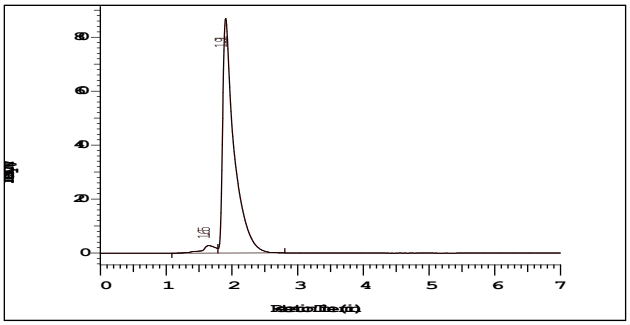


Fig 2.15.4: Chromatogram for repeatability-4

Table 2.15.5: Results for Repeatability-4

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	38764	469853	0.82

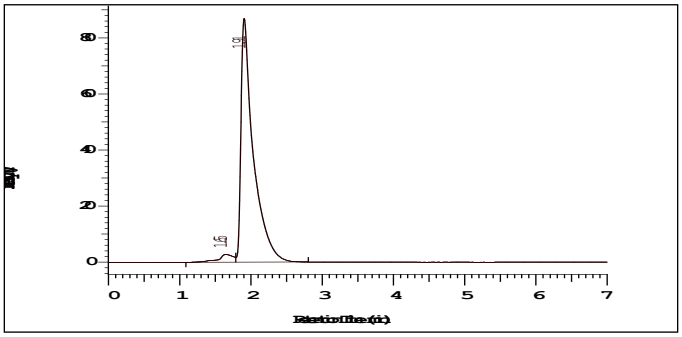


Fig 2.15.6: Chromatogram for repeatability-5

Table 2.15.7: Results for Repeatability-5

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	46121	469852	0.82

Intermediate precision

For intra-day studies the drug having concentration value 80%, 100 % & 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days. Data were subjected to statistical treatment for the calculation of SD and RSD. The data are shown in **Table 2.15.2.1.**

Table 2.15.2.1: Data for Citalopram analysis

Conc. Of Citalopram (API) (µg/ml)	Observed Conc. Of Citalopram (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
08	08.005	1.05	08.006	0.24
10	10.003	0.55	31.084	0.41
12	11.84	0.18	11.95	0.18

Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

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

The LOD was found to be 0.452 µg/ml and LOQ was found to be 1.356 µg/ml for Citalopram which represents that sensitivity of the method is high.

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. The data are shown in Table 2.15.2.2.

Table 2.15.2.2: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.15
2	Asymmetry	T ≤ 2	Citalopram=0.12
3	Theoretical plate	N > 2000	Citalopram=3246

Estimation of Citalopram in Tablet Dosage Form

Label claim: Each tablet contains: 10 mg

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded.

ASSAY

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Table 2.15.3.1: Recovery Data for estimation Citalopram in C-Talo tablet

Brand name of Tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	% RSD
C- talo {Alkem Laboratories Ltd (Pentacare)}	10	9.82 (±0.498)	99.82 (±0.343)

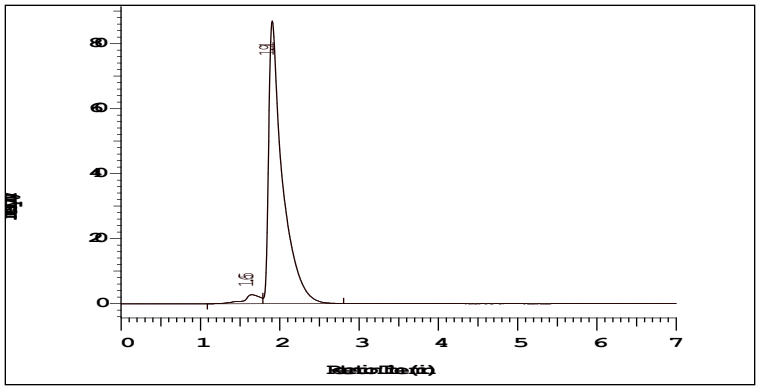


Fig 2.15.3.1: Chromatogram for assay sample

Table 2.15.3.2: Assay chromatogram results of sample

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	65421	58422	0.68

The amount of drugs in C-Talo tablet was found to be 9.82 (±0.343) mg/tab for Citalopram & % assay was 99.82.

SUMMARY & CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Citalopram, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx 4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be highly soluble in acetonitrile & dichloromethane and partially soluble in methanol. Drug was insoluble in water. Using these solvents with appropriate composition newer methods can be developed and validated. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Citalopram it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 l were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Citalopram in different formulations. A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Citalopram API.

Further he proposed RP-HPLC method has excellent sensitivity,precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Citalopram in different formulations.

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