Determination of Citalopram by RP-HPLC & it's stability indicative studies

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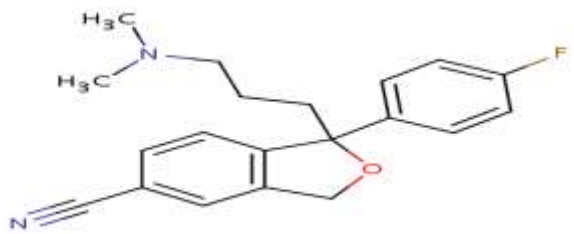
Abstract - In the present research, Determination of Citalopram by RP-HPLC & it's stability indicative studies was developed and developed method was validated as per ICH Q2R1 guidelines. The Chromatographic separation was carried out on a Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.with UV detection at 239 nm. The mobile phase contained ACN: phosphate buffer (pH 3.0) (20:80v/v). The mobile phase was run isocratically. The flow rate of the mobile phase was maintained at 1.0 ml/min. The linearity of the calibration curve was obtained in the concentration range of 5 to 20 µg/ml and coefficient of determination (R2) was found to be 0.9991. The % RSD value for intraday and interday precision was below 2 which indicated that the method was precise. Limit of detection and limit of quantification were 0.416 and 1.324µg/ml respectively. Forced degradation studies were performed under different conditions.

Index Terms - Citalopram, RP-HPLC, Method Development, Validation, Accuracy, Precision, Stability.

I. Introduction

The Citalopram denoted chemically as 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile. Citalopram hydrobromide belongs to a class of antidepressant agents known as selective serotonin-reuptake inhibitors (SSRIs).[1]

Fig.1 Structure of Citalopram



II.Literature survey

G.H. Ragab et al (2019)[25] A novel, fast and sensitive HPLC method has been developed for the simultaneous bioanalytical determination of Donepezil hydrochloride (DON) and Citalopram hydrobromide (CTP) in raw materials, spiked human plasma and tablets.Elution of both drugs was achieved with very good resolution using a RP-C18 chromatographic column, samples were analyzed using Hypersil Gold (100 mm × 4.6 mm), 5 µm particle size column and an isocratic binary mobile phase consists of phosphate buffer (0.05 M): acetonitrile (65:35). A Diode array detector at wavelength 232 nm was used. Chromatographic separation was within a short run time (less than 7 minutes) for both drugs.[2]

III.Aim, Objectives and Plan of Work

Aim

The existing physicochemical methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new methods for the assay & stability studies of Citalopram in pharmaceutical dosage forms adapting different available analytical techniques like UV spectrophotometry and HPLC.

Objectives

The objectives of the proposed method is to develop simple and accurate methods for the determination of Citalopram by RP-HPLC method in pharmaceutical dosage forms & it's stability indicative studies.

Plan of work

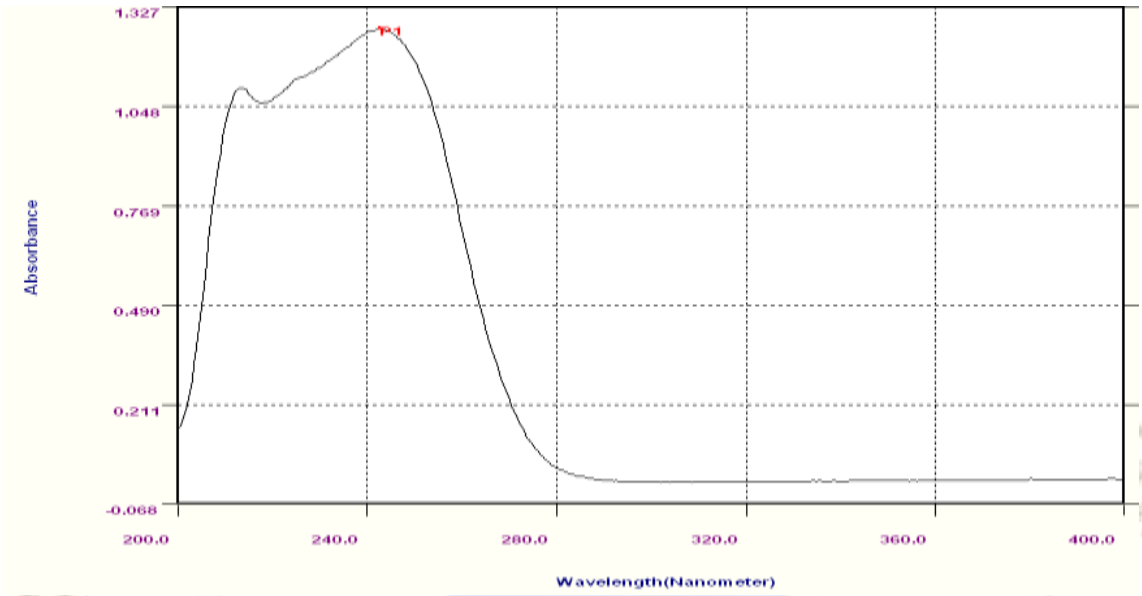
1. To undertake solubility study & analytical study of Citalopram & to develop initial UV & chromatographic conditions.
2. Optimisation of initial chromatographic conditions.
3. Analytical method validation of developed stability indicating method.
4. Quantitative determination of Citalopram in pharmaceutical dosage form using the method developed and validated.

IV.Experimental Work

Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a Urea and diluted with Acetonitrile for UV analysis for the final concentration 10µg/ml. While scanning the Citalopram solution we observed the absorption maxima was 239 nm. The scanned UV spectrum is attached in Figure 2.

Figure 2: UV spectrum of Citalopram



Method Development of Citalopram

Preparation of mobile phase

Mobile phase was prepared by taking ACN: Phosphate buffer (pH 3.0) (20:80 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 3.

Figure 3: Chromatogram of Citalopram (Rt 1.91)

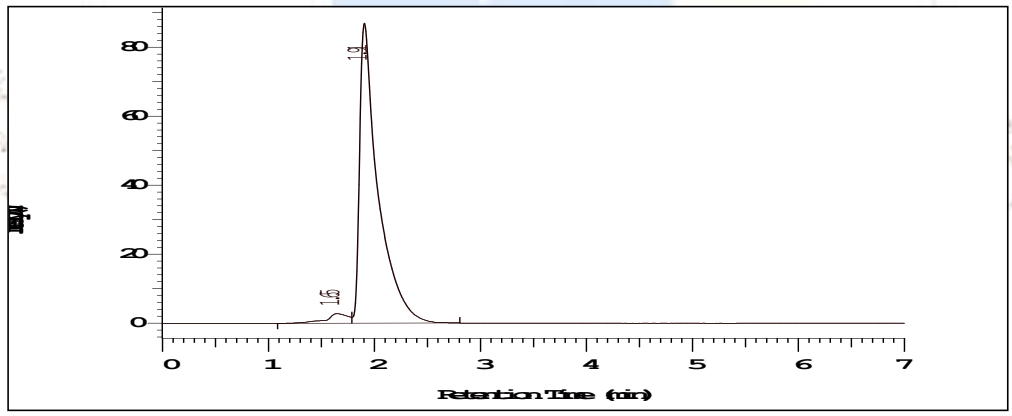


Table-1: Results of optimized condition of Citalopram

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	57874	654279	0.73

FORCED DEGRADATION STUDIES:

The API (Citalopram) was subjected to stress Trial 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 a 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.

The results of the stress studies indicated the specificity of the method that has been developed. The result of forced degradation studies are given in the following table.

Table – 2 : Results of force degradation studies of Citalopram API.

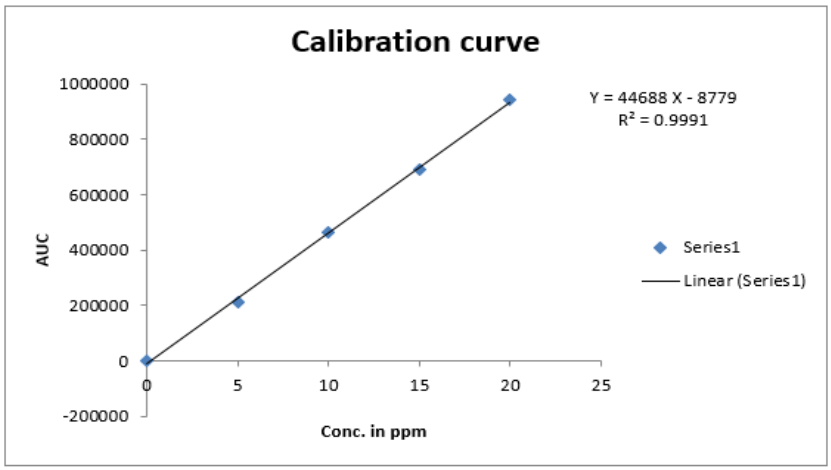
Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	28.12	78.2	98.56
Basic Hydrolysis (0.I M NaOH)	24Hrs.	45.36	55.02	98.49
Thermal Degradation (50 ^{0}C)	24Hrs.	98.39	----------	98.31
UV (254nm)	24Hrs.	81.26	29.64	98.53
3 % Hydrogen peroxide	24Hrs.	79.15	25.42	98.03

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 47542 and intercept was found to be 11245 for Citalopram. The Standard curve obtained is shown in fig 4.

Figure 4: Standard curve for Citalopram



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- 3.

Table 3: Data of recovery studies

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	102.41	Mean= 101.64%
S_2 : 80 %	8	10	101.27	S.D. = 0.667008
S_3 : 80 %	8	10	101.24	% R.S.D.= 0.656
S_4 : 100 %	10	10	99.56	Mean= 99.58%
S_5 : 100 %	10	10	99.32	S.D. = 0.275741
S_6 : 100 %	10	10	99.87	% R.S.D.= 0.277
S_7 : 120 %	12	10	99.84	Mean= 99.65%
S_8 : 120 %	12	10	99.31	S.D. = 0.297714
S_9 : 120 %	12	10	99.81	% R.S.D. = 0.299

Precision

Intraday and interday precision studies were conducted by taking final concentration of 10µg/ml of standard and injected into HPLC. It shows 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.

Table 4: Data for Citalopram analysis

Conc.of Citalopram (API) (µg/ml)	Observed Conc. of Citalopram (µg/ml) by the proposed method					
	Intra-Day		Inter-Day for Two days			
			Day-1		Day-2	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.003	1.210	10.012	0.891	10.014	0.931

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LOD & LOQ: The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ= 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.416 & 01.324 µg/ml 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. The data are shown in Table 5.

Table 5: 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. The data are shown in Table 6 ,7 and the chromatogram obtained is shown in fig 5.

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 6 : Assay Data for estimation Citalopram in Celexa-10mg tablet

Brand name of Tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	% RSD
Celexa-10mg Tablet {Cyril Pharmaceuticals}	10	9.82 (±0.498)	99.82 (±0.343)

Fig 5: Chromatogram for assay sample

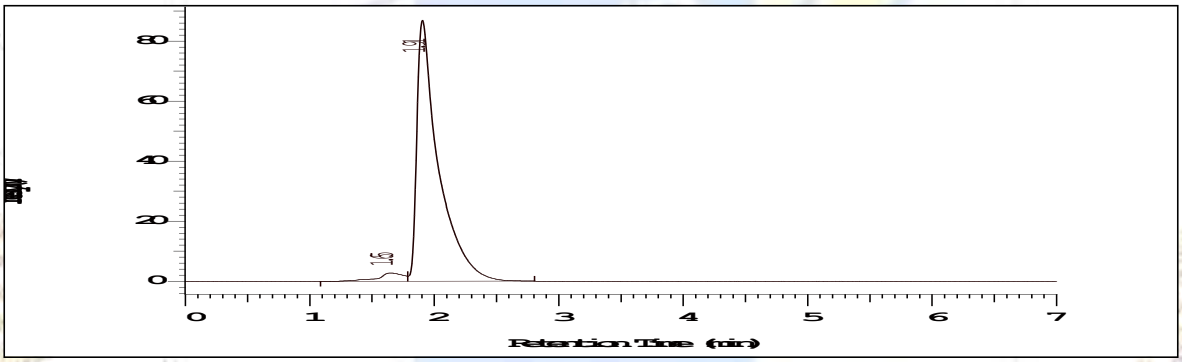

Table 7 : Assay chromatogram results of sample

Sl. No	Rt	Theoretical Plates	Area	Tailing factor
1	1.91	55673	664563	0.62

The amount of drug Celexa-10mg Tablet was found to be 9.82 (±0.343) mg/tab for Citalopram & % assay was 99.82.

V. Results & discussion:

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.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). 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 20µ 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.

VI. Conclusions:

A sensitive & selective stability indicating RP-HPLC method has been developed & validated for the analysis of Citalopram API. Further the 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.

VII.References

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