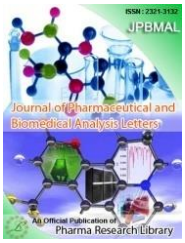

Journal of Pharmaceutical and Biomedical Analysis Letters
CODEN (USA): JPBAC9 | ISSN: 2347-4742
Home Page: https://pharmaresearchlibrary.org/journals/index.php/jpbmal
DOI: https://doi.org/10.30904/j.jpbmal.2025.4789
J. Pharm, Biomed. A. Lett., 2025, 13(1): 01-06

Development and Validation of a New RP-HPLC Method for the Estimation of Citalopram in Tablet Dosage Forms

K.S. Dhilip Kumar[1], Mahesh. M*[2]
[1]Department of Pharmaceutical Analysis, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu-515001, Andhra Pradesh, India.
[2]Assistant Professor, Department of Pharmaceutical Analysis, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu-515001, Andhra Pradesh, India

A b s t r a c t
A novel, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of citalopram in tablet dosage forms. The method utilized an Agilent Eclipse XDB C18 column (150 x 4.6 mm, 5 µm) with a mobile phase consisting of acetate buffer (pH 4.5) and acetonitrile in a 65:35 v/v ratio, pumped at a flow rate of 1.0 mL/min. Detection was carried out at 240 nm, and the retention time for citalopram was found to be 3.727 minutes. The method demonstrated excellent linearity in the concentration range of 25, 150 µg/mL (r^2 = 0.999). Validation studies confirmed the method's precision, accuracy, specificity, and robustness, with recovery values ranging from 98.65% to 101.72%. The limits of detection (LOD) and quantification (LOQ) were determined to be 1.125 µg/mL and 3.375 µg/mL, respectively. The method was successfully applied to quantify citalopram in commercial tablet formulations, with results showing 100.1% recovery of the labeled claim. Forced degradation studies under acidic, basic, oxidative, thermal, photolytic, and neutral conditions revealed that the method could effectively separate citalopram from its degradation products, indicating its stability-indicating capability. The proposed method offers advantages over existing methods, including shorter analysis time (8 minutes), higher sensitivity, and a wider linearity range. It is concluded that this RP-HPLC method is suitable for routine quality control analysis of citalopram in pharmaceutical formulations.

Keywords: Citalopram, RP-HPLC, Method Development, Method Validation, Tablet Dosage Form, Forced Degradation Studies.

A r t i c l e I n f o

Corresponding Author:
Mahesh. M
Assistant Professor, Department of Pharmaceutical Analysis
JNTUA-OTPRI, Ananthapuramu, A.P, India
Mail ID: meghavath9@gmail.com

Article History:
Received : 10 Mar 2025
Revised : 29 Mar 2025
Accepted : 18 April 2025
Published : 02 May 2025

Citation: *K.S. Dhilip Kumar, et al. (2025) Development and Validation of a New RP-HPLC Method for the Estimation of Citalopram in Tablet Dosage Forms. J. Pharm, Biomed. A. Lett., 13(1): 01-06.*

C o n t e n t s

1. Introduction

High-Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques in pharmaceutical, chemical, environmental, and biological research. It is a powerful tool for separating, identifying, and quantifying components in complex mixtures. Among the various HPLC modes, Reversed-Phase HPLC (RP-HPLC) is the most commonly employed due to its versatility, robustness, and wide applicability. This document provides a comprehensive introduction to HPLC principles, instrumentation, and method development, with

a special focus on RP-HPLC method development. The discussion spans 40 pages, covering:

Fundamentals of HPLC

RP-HPLC: Principles, Advantages Method Development Strategies Optimization and Validation**.** Applications in Pharmaceutical and Analytical Chemistry.

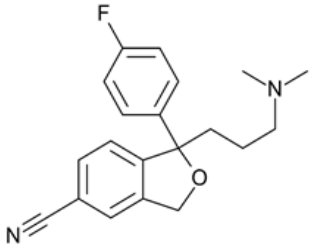

Fig.1

2. Methodology

Instrumentation

A Waters Alliance liquid chromatograph (Model 2695) equipped with an Eclipse employed for the study. Sample injections were done with an automatic injector. Empower2 software was used for data handling. Solubility of the substances was enhanced by sonication on an ultrasonicator (Ultrasonics 3.51). A Sartorius balance (Model CPA225D) was used for weighing different substances.

Drugs, chemicals and solvents

A pure sample of citalopram (purity-99.2%) obtained from Aurobindo Pharmaceuticals, Hyderabad, India was used as the reference standard. The commercial tablet formulation of citalopram "Celepra" (10 mg) manufactured by Micro Labs Ltd., Bangalore, India was used in this study. Ammonium acetate, glacial acetic acid, triethylamine, HPLC grade acetonitrile and HPLC grade methanol were purchased from Rankem Fine Chemicals Ltd., Mumbai. HPLC grade water was prepared by using Millipore Milli-Q system.

Preparation of the buffer solution (pH 4.5)

0.385 g of ammonium acetate and 0.5 mL of triethylamine were transferred into a 1000 mL beaker containing about 800 mL of water. The contents were mixed well and the volume was made up to 1000mL. The pH of the solution was then adjusted to 4.5 with glacial acetic acid. The solution was then filtered through a 0.45µ membrane filter.

Preparation of the mobile phase

A mixture of the above acetate buffer (pH 4.5) and acetonitrile in the ratio of 65:35 v/v was prepared by mixing 650 mL of buffer with 350 mL of acetonitrile in a one liter flask. It was degassed in an ultrasonic bath for 5 min and then filtered through a 0.45 µ membrane filter. This mixture was used as the mobile phase in the chromatography.

Stock and working standard solution of citalopram

About 50 mg of citalopram reference standard was accurately weighed and transferred into a 50 mL volumetric flask. To this, 25 mL of acetonitrile was added and sonicated for 5 min. The volume was made up with a further quantity of acetonitrile and mixed well. This solution was used as the stock solution. The working standard solution was prepared by transferring 1.0 mL of the stock solution into a 10 mL volumetric flask and diluting to volume with the diluent. This solution (100 µg/mL) was used as the working standard solution of citalopram.

Preparation of the diluent

A 50:50 v/v mixture of the acetate buffer (pH 4.5) and acetonitrile was used as the diluent for preparing drug solutions.

Optimization of Chromatographic Conditions and Method Development: After a number of trails a mixture of acetate buffer (pH 4.5) and acetonitrile in 65:35 v/v ratio was selected as the mobile phase for the separation of citalopram. The solvent was pumped at a flow rate of 1.0 mL/min. The injection volume was 10 µL and the column temperature was maintained at 30°C. The detector wavelength was set at 240 nm for monitoring the analytes. Prior to injection of the drug solution, the column was equilibrated for at least 20 min by pumping the mobile phase through it. Typical chromatogram of the working standard solution of citalopram is shown in Fig. 2.

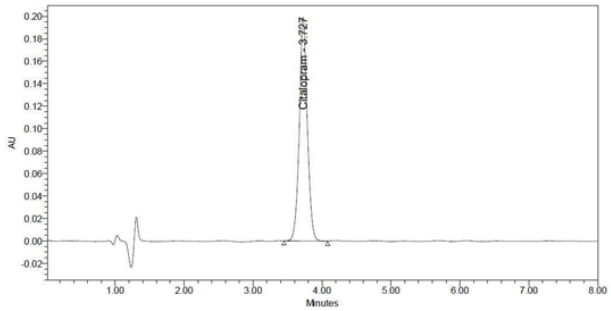

Fig. 2. A representative chromatogram of the working standard solution of citalopram.

3. Results and Discussion

Linearity

Solutions of citalopram at different concentration levels including the working standard concentration were prepared in the diluent. Each concentration was injected three times into the HPLC system (n=3). The response was read at 240 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas at the different concentration levels were calculated and the linearity plot of the mean peak areas over concentration was constructed. Linearity data for citalopram is presented in the Table 2. Linearity plot for citalopram is depicted in the Fig.3.

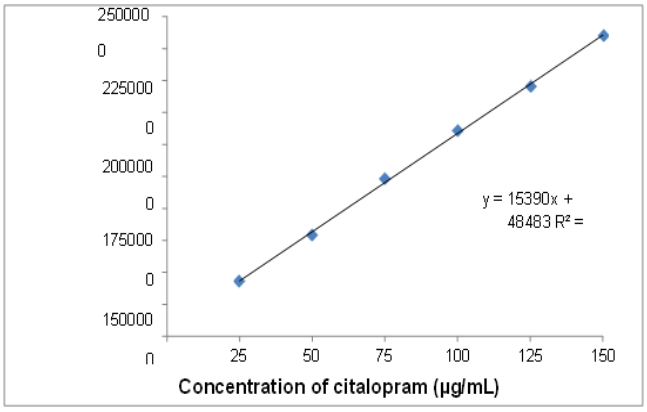

Fig.3. Linearity plot for citalopram

Precision

Repeatability and intermediate precision were assessed by analyzing standard solutions of citalopram on the same day (n=6) and on two consecutive days, respectively. The results of repeatability and intermediate precision studies are depicted in the tables 3 and 4.

Accuracy

The accuracy of the method was determined by analyzing in triplicate a known concentration of the drug corresponding to 50, 100 and 150 % levels of citalopram (50, 100, 150 µg/mL). The percent recovery was calculated. The results are incorporated in the table 5.

Limit of detection (LOD) and Limit of quantitation (LOQ): LOD and LOQ were calculated by using residual standard deviation of the response and the slope of the regression line. The LOD and LOQ of citalopram was found to be 1.125 and 3.375 µg/mL respectively.

Robustness study

The robustness of the method was determined as per ICH guidelines under three conditions i.e. flow rate, temperature and mobile phase composition. The results obtained by deliberate variation in method conditions are summarized showed in table.6.

Specificity of the proposed method

The specificity of the method was evaluated with regard to interference due to presence of excipients in tablet formulation. The HPLC chromatograms recorded for the drug matrix did not show any interfering peak within retention time ranges. Fig.4 show the representative chromatograms obtained from the analysis of citalopram from working standard solution and the formulation sample solution. The figures show that the selected drug was clearly separated.

System suitability

For finding out system suitability, six replicates of the working standard sample were injected and the parameters like peak retention time, tailing factor, number of theoretical plates (N), HETP of the peak were generated. These results are shown in Table 7.

Estimation of the drug from tablet dosage forms

Fifteen tablets of "Celepra" were crushed into a fine powder. A quantity of tablet powder equivalent to 100 mg of citalopram was accurately weighed and transferred into a 100 mL volumetric flask. 70 mL of the diluent was added to it and sonicated for 25 min. Then, the volume was made up with the diluent and the contents filtered through a 0.45µ nylon filter. From the filtered solution, 1.0 mL was transferred into a 10 mL volumetric flask and diluted to mark with the diluent and mixed well to get a final concentration of 100µg/mL. This solution was chromatographed six times. From the chromatograms obtained, the average drug content in the formulation was calculated. A typical chromatogram obtained from the analysis of Celepra tablet is shown in the fig.4.

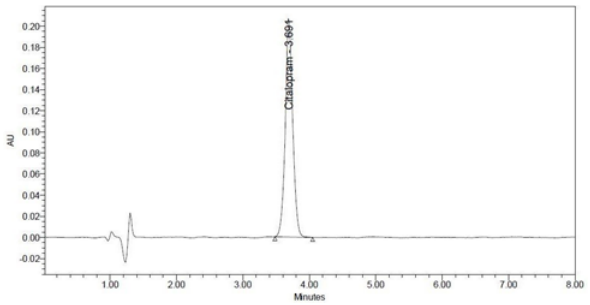

Fig.4. Representative Chromatogram obtained from analysis of citalopram from formulation sample solution

Method suitability

The commercial tablet formulation, "Celepra" was analyzed by the proposed method and the results are shown in Table 4.9. The values were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of the drug in pharmaceutical dosage forms.

Discussion

This study aimed to develop a precise, accurate, and sensitive HPLC method for analyzing citalopram in bulk and pharmaceutical dosage forms. The optimal mobile phase was acetonitrile and acetate buffer (pH 4.5) in a 35:65 v/v ratio, using an Agilent Eclipse XDB C18 column, which provided sharp, well-resolved peaks with minimal tailing. The retention time was consistently 3.727 minutes across replicates, with reproducible peak areas and a strong linearity (r = 0.999), described by the regression equation

$$Y = 15390X + 48483$$

The method showed excellent precision, accuracy, and recovery, with no interference from excipients. Tablets contained an average of 100.1% of the labeled citalopram content, and system suitability parameters were within acceptable limits. Robustness was confirmed through consistent performance under varied conditions, while low LOD and LOQ values indicated high sensitivity. Compared to existing methods, the proposed technique offers advantages such as shorter retention time, higher quantitation limit (150µg/mL), and reduced run time. Overall, this HPLC method is reliable and efficient for routine analysis of citalopram in dosage forms.

Table 1: Optimized chromatographic conditions of the proposed method

Stationary phase	Agilent Eclipse XDB C18 (150 x 4.6 mm, 5µm
Mobile phase	acetate buffer (pH 4.5) : acetonitrile - 65:35 v/v
Flow rate	1.0 mL/min
Column temperature	30°C
Injection volume	10 µL
Detection wavelength	240 nm
Run time	8 min

Table 2: Linearity data for citalopram

Conc. of citalopram (µg/mL)	Peak area	Mean peak area	SD	%RSD
25	431987	431258	654.62	0.15
	430721			
	431065			
50	793675	791900	2124.66	0.27
	792480			
	789546			
75	1237854	1233089	4478.96	0.36
	1232449			
	1228965			
100	1604278	1609081	4814.54	0.30
	1609057			
	1613907			
125	1959731	1955447	3915.40	0.20
	1954555			
	1952054			
150	2350967	2351414	1506.52	0.06
	2351414			

Table 3: Repeatability data

S. No.	Peak areas of citalopram
1	1617890
2	1621056
3	1612908
4	1603209
5	1603742
6	1617460
Average	1612711
SD	7612.93
%RSD	0.47

Table 4: Intermediate precision data

Day	Average peak areas of citalopram (n=6)
1	1609002
2	1610545
Overall average	1609774
SD	5108.73
%RSD	0.32

Table 5. Recovery data of citalopram

Concentration (µg/mL)	Peak area	Recovery	Mean recovery	SD of recoveries	% recovery
50	810256	49.50	49.60	0.34	99.00
	817609	49.98			99.95
	807628	49.33			98.65
100	1604278	101.09	101.40	0.31	101.09
	1609057	101.40			101.40
	1613907	101.72			101.72
150	2350967	149.61	149.57	0.10	99.74
	2351414	149.64			99.76
	2348610	149.46			99.64

Table 6. Robustness data

Chromatographic condition	Value	Retention time (min)	Tailing factor	Number of theoretical plates
Flow rate (mL/min)	0.9	4.703	1.11	4934
	1.0 (O)	3.727	1.07	4385
	1.1	2.979	1.04	3842
Temperature (°C)	23	3.433	1.03	2852
	30 (O)	3.727	1.07	4385
	35	3.361	1.08	4285
Mobile phase composition (acetate buffer : acetonitrile (% v/v))	66:34	3.143	1.03	4242
	65:35 (O)	3.727	1.07	4385
	64:36	3.972	1.11	4304

Table 7. System suitability parameters of the proposed method

S. No.	Parameter	Result
1	Retention time (min)	3.727
2	Tailing factor	1.07
3	Number of theoretical plates	4385
4	HETP	0.0177

Table 8. Recovery of the drugs from the tablet dosage form "Celepra"

Drug	Labeled amount (mg)	Amount recovered (mg) (n=6)	% Recovery
Citalopram	10	10.01	100.1

4. Conclusion

The proposed RP-HPLC method is precise, accurate, sensitive, and robust for the quantitative analysis of citalopram in pharmaceutical formulations. Its advantage include a short analysis time (8 minutes), wide linear range, and minimal interference from excipients or degradation products. The method complies with ICH validation guidelines and offers a reliable alternative for routine quality control and stability testing of citalopram in bulk and dosage forms.

Acknowledgement

I am very thankful to Director, JNTUA-OTPRI, Ananthapuramu for providing the laboratory facilities, chemicals to carryout entire research work.

Conflict of Interest

We affirm that there are no conflicts of interest.

5. References

[1] Baumann P. Pharmacology and pharmacokinetics of citalopram and other SSRIs. International Clinical Psychopharmacology, 1996, 11, 5-11.

[1] Pato M. T. Beyond depression: citalopram for obsessive-compulsive disorder. A International Clinical Psychopharmacology, 1999, 14, 19-26.

[2] Bolton JM, Sareen J and Reiss JP. Genital anaesthesia persisting six years after sertraline discontinuation. J. Sex Marital Ther., 2006, 32, 327-30.

[3] Csoka AB, Csoka A, Bahrick A and Mehtonen OP. Persistent sexual dysfunction after discontinuation of selective serotonin reuptake inhibitors. J. Sex Med., 2008, 5, 227? 33.

[4] Ishak WW, Christensen S, Sayer G, Ha K, Li N, Miller J, Nguyen JM and Cohen RM. Sexual satisfaction and quality of life in major depressive disorder before and after treatment with citalopram in the STAR D study. J. Clin. Psychiatry, 2013, 74, 256- 61.

[5] Rampello L, Alvano A, Chiechio S, Malaguarnera M, Raffaele R, Vecchio I and Nicoletti F. Evaluation of the prophylactic efficacy of amitriptyline and citalopram, alone or in combination, in patients with comorbidity of depression, migraine, and tension-type headache. Neuropsychobiology, 2004, 50, 322?28.

[6] Chakole RD, Charde MS, Bhavsar N and Marathe RP. Simultaneous estimation of escitalopram and clonazepam by RP-HPLC in pharmaceutical formulation. Int. J. Phytopharmacy, 2012, 2, 25-29.

[7] Dilip Kumar V, Mahesh NM, Gurupadayya BM and Ravi V. Simultaneous estimation of citalopram hydrobromide and dothiepin hydrochloride in human plasma by HPLCmethod. J. Pharmacy Res., 2011, 4, 50-52.

[8] Menegola J, Steppe M and Schapoval EE. Development and validation of column high-performance liquid chromatographic and ultraviolet spectrophotometric methods for citalopram in tablets. J. AOAC Int., 2008; 91, 52-58.

[9] Gandhi SV, Dhavale ND, Jadhav VY and Sabnis SS. Spectrophotometric and reversed-phase high-performance liquid chromatographic methods for simultaneous determination of escitalopram oxalate and clonazepam in combined tablet dosage form. J. AOAC Int., 2008, 91, 33-38.

[10] Rao RN, Raju AN and Narsimha R. Isolation and characterization of degradation products of citalopram and process-related impurities using RP- HPLC. J. Sep. Sci., 2008, 31, 1729-38.

[11] Tadi S, Nikoli K and Agbaba D. Development and optimization of HPLC analysis of citalopram and its four non chiral impurities using experimental design methodology. J. AOAC Int., 2012, 95, 733-43.

[12] Greiner C, Hiemke C, Bader W and Haen E. Determination of citalopram and escitalopram together with their active main metabolites desmethyl (es-) citalopram in human serum by column-switching high performance liquid chromatography (HPLC) and spectrophotometric detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 2007, 848, 391-94.

[13] ICH Harmonized Tripartite Guideline; Validation of analytical procedures: Text and Methodology Q2(R1), European Union, Japan and USA (2005).