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Development and Validation of A Stability-Indicating RP-HPLC Method for Citalopram

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Abstract: A reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of citalopram in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved on an Agilent Eclipse XDB C18 column (150 × 4.6 mm, 5 µm) using acetate buffer (pH 4.5) and acetonitrile in a 65:35 (v/v) ratio as the mobile phase. The flow rate was 1.0 mL/min, column temperature was maintained at 30°C, injection volume was 10 µL and detection was performed at 240 nm. The retention time of citalopram was approximately 3.727 min and the total run time was 8 min. The method was evaluated for linearity, precision, accuracy, limit of detection, limit of quantitation, robustness, system suitability and applicability to tablet dosage forms. Linearity was demonstrated over 25–150 µg/mL with a reported regression equation of y = 15390x + 48483 and R^2 = 0.9991. Repeatability and intermediate precision produced %RSD values of 0.47% and 0.32%, respectively. Mean recoveries at 50, 100 and 150 µg/mL were approximately 99.60%, 101.40% and 99.57%, respectively. The reported LOD and LOQ were 1.125 and 3.375 µg/mL. Analysis of Celepra tablets gave 100.1% recovery of the labelled citalopram content. The method was also applied to stress-treated samples under acidic, alkaline, thermal, oxidative, photolytic and neutral conditions. The study data support the suitability of the proposed RP-HPLC procedure for quantitative analysis of citalopram in the studied pharmaceutical formulation.

Keywords: Citalopram; RP-HPLC; method development; validation; precision; accuracy; robustness; forced degradation; pharmaceutical formulation.

Introduction

High-performance liquid chromatography (HPLC) is a widely used liquid chromatographic technique for the separation and quantitative determination of components in complex mixtures. The source project describes HPLC as a method capable of providing reliable quantitative precision and accuracy for active pharmaceutical ingredients and related substances, with good reproducibility and broad applicability through appropriate selection of stationary phase and chromatographic conditions. Reversed-phase HPLC is particularly useful for pharmaceutical analysis because a polar mobile phase is passed through a relatively non-polar stationary phase such as C18 silica.

Development of an analytical method is undertaken when an adequate method is not available for a particular analyte and matrix, when existing methods have limitations in accuracy, precision, selectivity or sensitivity, or when newer instrumentation provides an opportunity to improve analytical performance.

Method development therefore involves deliberate selection and optimization of the separation mode, stationary phase, mobile phase, detector and operating conditions.

Citalopram hydrobromide is described in the source project as an orally active selective serotonin reuptake inhibitor (SSRI) used in the management of depression, obsessive-compulsive disorder and anxiety disorders. The project identifies its chemical name as (RS)-1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, molecular formula C20H21FN2O, molecular weight 324.39 g/mol and therapeutic category as antidepressant.

The present work was undertaken to develop a practical RP-HPLC method for estimation of citalopram in bulk drug and tablet dosage form and to demonstrate its analytical performance through validation studies. The project also investigated the ability of the chromatographic procedure to distinguish citalopram from products generated under forced-degradation conditions.

Analytical Background and Method Development Principles

Reversed-phase chromatography

In reversed-phase chromatography, the stationary phase is less polar than the mobile phase. Chemically bonded silica phases such as C18 are widely used. The source document notes that retention is governed by interactions among the solute, stationary phase and mobile phase, and that pH and organic-solvent composition can strongly influence retention and selectivity.

Components and operating variables of an HPLC system

The HPLC system described in the source comprises solvent reservoirs, a high-pressure pump, sample introduction system, analytical column, detector and data-recording/data-processing system. Important operating variables include mobile-phase composition, flow rate, injection volume, column temperature and detector wavelength. Degassing and membrane filtration of the mobile phase are used to reduce dissolved gases and particulate matter.

Method optimization

The source project identifies the principal method-development variables as separation mode, stationary phase, mobile phase and detector selection. For reversed-phase analysis, C18 was selected because of its retention characteristics. Mobile-phase optimization considered buffer type, buffer pH and organic-phase composition. The project states that experiments were performed with different buffer pH values and organic phases to obtain suitable separation and robustness.

Analytical validation

The source project follows the conventional analytical validation framework and discusses specificity, linearity and range, accuracy, precision, detection limit, quantitation limit, robustness and system suitability. Specificity is assessed by examining blanks, placebo and sample/standard responses. Linearity is assessed using multiple concentrations and regression analysis. Accuracy is expressed through recovery, while precision is expressed using standard deviation and relative standard deviation. Robustness is assessed by deliberate small changes in chromatographic conditions.

Forced degradation

Forced degradation is used to deliberately stress a drug substance or formulation using conditions such as acid, base, heat, oxidizing agents, light and neutral hydrolysis. The purpose described in the project is to evaluate the stability of citalopram and the ability of the chromatographic method to separate the principal analyte response from degradation-related responses.

Materials and Methods

Instrumentation

A Waters Alliance liquid chromatograph (Model 2695) equipped with an Agilent Eclipse XDB C18 column (150 × 4.6 mm; 5 µm) and diode-array detector (Model 2996) was used. Samples were introduced through an automatic injector and data were processed using Empower2 software. An ultrasonicator was used to enhance solubility and a Sartorius CPA225D balance was used for weighing.

Drugs, chemicals and solvents

Citalopram reference standard of reported purity 99.2% was obtained from Aurobindo Pharmaceuticals, Hyderabad, India. Celepra tablets containing 10 mg citalopram and manufactured by Micro Labs Ltd.,

Bangalore, India were used as the commercial formulation. Ammonium acetate, glacial acetic acid, triethylamine, HPLC-grade acetonitrile and HPLC-grade methanol were obtained from Rankem Fine Chemicals Ltd., Mumbai. HPLC-grade water was prepared using a Millipore Milli-Q system.

Preparation of acetate buffer (pH 4.5)

Approximately 0.385 g ammonium acetate and 0.5 mL triethylamine were transferred into a 1000 mL beaker containing approximately 800 mL water. The contents were mixed and the volume was made up to 1000 mL. The pH was adjusted to 4.5 using glacial acetic acid. The resulting solution was filtered through a 0.45 µm membrane filter.

Preparation of mobile phase

Acetate buffer at pH 4.5 and acetonitrile were mixed in a 65:35 (v/v) ratio. For one litre, 650 mL buffer was mixed with 350 mL acetonitrile. The mixture was sonicated for 5 min and filtered through a 0.45 µm membrane filter before use.

Standard and working solution

Approximately 50 mg citalopram reference standard was accurately weighed into a 50 mL volumetric flask. About 25 mL acetonitrile was added and the solution was sonicated for 5 min. The volume was made up with acetonitrile and mixed. A working standard was prepared by transferring 1.0 mL of stock solution into a 10 mL volumetric flask and making up to volume with diluent to obtain 100 µg/mL citalopram.

Diluent

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

Chromatographic optimization

After trials with chromatographic conditions, acetate buffer (pH 4.5) and acetonitrile in a 65:35 (v/v) ratio were selected. The flow rate was 1.0 mL/min, injection volume 10 µL, column temperature 30°C and detection wavelength 240 nm. Before sample injection, the column was equilibrated for at least 20 min with the mobile phase.

Parameter	Optimized condition
Stationary phase	Agilent Eclipse XDB C18 (150 × 4.6 mm, 5 µm)
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
Reported retention time	3.727 min

Method Validation

Linearity

Citalopram solutions at 25, 50, 75, 100, 125 and 150 µg/mL were prepared in diluent. Each concentration was injected three times. Mean peak areas were calculated and plotted against concentration.

Conc. (µg/mL)	Peak areas	Mean area	SD	%RSD
25	431987; 431258; 430721; 431065	431258	654.62	0.15
50	793675; 791900; 792480; 789546	791900	2124.66	0.27
75	1237854; 1233089; 1232449	1233089	4478.96	0.36
100	1604278; 1609081; 1609057	1609081	4814.54	0.30
125	1959731; 1955447; 1954555	1955447	3915.40	0.20
150	2350967; 2350330; 2348610	2350330	1506.52	0.06

The reported regression equation was $y = 15390x + 48483$ with $R^2 = 0.9991$, indicating a strong linear response across 25–150 µg/mL.

Precision

Repeatability was assessed using six injections of the standard solution under the same analytical conditions. The six peak areas were 1617890, 1621056, 1612908, 1603209, 1603742 and 1617460. The reported average was 1612711, standard deviation 7612.93 and %RSD 0.47%.

Parameter	Result
Repeatability, n	6 injections
Average peak area	1612711
SD	7612.93
%RSD	0.47%
Intermediate precision: Day 1 average	1609002
Intermediate precision: Day 2 average	1610545
Overall average	1609774
SD	5108.73
%RSD	0.32%

Accuracy

Accuracy was evaluated by analyzing citalopram at 50, 100 and 150 µg/mL in triplicate and calculating recovery.

Nominal conc. (µg/mL)	Individual recovery (%)	Mean recovery (%)	SD	Reported % recovery
50	99.00; 99.95; 98.65	99.60	0.34	99.00–99.95 (individual values)
100	101.09; 101.40; 101.72	101.40	0.31	101.09–101.72 (individual values)
150	99.74; 99.76; 99.64	99.57	0.10	99.64–99.76 (individual values)

The source data demonstrate recoveries close to the nominal values across the tested concentration range.

Limit of detection and quantitation

LOD and LOQ were calculated using the residual standard deviation of the response and the slope of the regression line. The reported LOD and LOQ were 1.125 µg/mL and 3.375 µg/mL, respectively.

Robustness

Robustness was evaluated by deliberate changes in flow rate, column temperature and mobile-phase composition. The observed retention time, tailing factor and theoretical plate count are presented below.

Condition	Value	Rt (min)	Tailing	Theoretical plates
Flow rate	0.9 mL/min	4.703	1.11	4934
Flow rate	1.0 mL/min (O)	3.727	1.07	4385
Flow rate	1.1 mL/min	2.979	1.04	3842
Temperature	23°C	3.433	1.03	2852
Temperature	30°C (O)	3.727	1.07	4385
Temperature	35°C	3.361	1.08	4285
Mobile phase	66:34	3.143	1.03	4242
Mobile phase	65:35 (O)	3.727	1.07	4385
Mobile phase	64:36	3.972	1.11	4304

Specificity and system suitability

System suitability was evaluated using six replicate injections of the working standard. The reported retention time was 3.727 min, tailing factor 1.07, theoretical plates 4385 and HETP 0.0177.

System-suitability parameter	Result
Retention time	3.727 min
Tailing factor	1.07
Theoretical plates	4385
HETP	0.0177

For formulation analysis, the source describes the absence of additional interfering peaks as evidence that common tablet excipients did not interfere with citalopram determination under the selected chromatographic conditions.

Assay of Citalopram in Tablet Dosage Form

Fifteen Celepra tablets were crushed to a fine powder. Tablet powder equivalent to 100 mg citalopram was accurately weighed into a 100 mL volumetric flask. Seventy millilitres of diluent were added and the mixture was sonicated for 25 min. The volume was made up with diluent and the solution was filtered through a 0.45 µm nylon filter. One millilitre of filtrate was transferred into a 10 mL volumetric flask and diluted to volume to obtain a final concentration of 100 µg/mL. The resulting solution was chromatographed six times.

Formulation	Label claim (mg)	Amount recovered (mg), n=6	% Recovery
Celepra	10	10.01	100.1

Forced Degradation Studies

The proposed method was applied to samples subjected to acidic, alkaline, thermal, oxidative, photolytic and neutral stress conditions. The source project provides chromatograms for the standard and each stress condition and summarizes the chromatographic parameters and percentage degradation.

Stress condition	Retention time (min)	Area	USP plates	USP tailing	Purity angle	Purity threshold
Acidic	3.743	1424209	3611	1.5	0.122	0.316
Basic	3.791	1429351	4015	1.3	0.544	0.877
Thermal	3.617	1457202	3973	1.5	0.291	0.641
Oxidative	4.181	1447207	5416	1.4	0.614	6.519
Photolytic	3.504	1535186	3849	1.5	0.091	0.310
Neutral	3.491	1551002	3657	1.5	0.088	0.305
Standard drug	3.696	1567081	4839	1.46	—	—

Stress study	Reported degradation (%)
Acidic	8.9
Basic	8.6
Thermal	6.8
Oxidative	7.5
Photolytic	1.8
Neutral	0.8

Stress conditions

For acid stress, 1.0 mL standard solution was treated with 1.0 mL of 2 N hydrochloric acid and refluxed for 30 min at 60°C. For alkaline stress, 1.0 mL standard solution was treated with 1.0 mL of 2 N sodium hydroxide and refluxed for 30 min at 60°C. Oxidative stress used 1.0 mL of 20% hydrogen peroxide with 1.0 mL standard solution for 30 min at 60°C. Thermal degradation involved placing standard drug solution in an oven at 105°C for 6 h. Photolytic stress involved exposing a 100 µg/mL solution to UV light in a photostability chamber for 7 days or 200 Wh/m². Neutral degradation was studied by refluxing the drug in water for 6 h at 60°C. Stress-treated solutions were diluted to approximately 100 µg/mL and chromatographed according to the project procedure.

The reported degradation values were 8.9% under acidic conditions, 8.6% under basic conditions, 6.8% under thermal conditions, 7.5% under oxidative conditions, 1.8% under photolytic conditions and 0.8% under neutral conditions.

Results and Discussion

The developed RP-HPLC method uses a conventional C18 reversed-phase stationary phase and an acetate-buffer/acetonitrile mobile phase. The selected conditions produced a citalopram retention time of 3.727 min and an 8 min run time. The method therefore provides a relatively short chromatographic cycle for the studied application.

The linearity experiment demonstrated a strong relationship between concentration and chromatographic response over 25–150 µg/mL, with a reported R^2 of 0.9991. The regression equation, $y = 15390x + 48483$, provides the calibration relationship used in the study. The replicate response variability at the tested concentrations was reported as low.

Precision results were expressed through repeatability and intermediate precision. The repeatability %RSD of 0.47% and intermediate-precision %RSD of 0.32% indicate low variability in the reported experimental measurements. Accuracy testing gave recovery values around the nominal concentrations at 50, 100 and 150 µg/mL.

The reported LOD and LOQ of 1.125 and 3.375 µg/mL characterize the sensitivity of the method under the calculation procedure used in the project. Robustness testing showed changes in retention time with deliberate variations in flow rate, temperature and mobile-phase composition, while the reported tailing factors and plate counts remained within the range observed during the study.

Application to Celepra tablets resulted in a reported recovery of 100.1% of the labelled citalopram content. The source project interprets the absence of additional chromatographic peaks in the relevant elution zone as evidence of limited interference from common tablet excipients.

The forced-degradation study further demonstrates application of the method to stressed citalopram solutions. Acidic and basic conditions produced the highest reported degradation percentages among the tested conditions, while photolytic and neutral conditions produced lower reported degradation. The chromatographic parameters recorded for the stressed samples provide supporting evidence that the principal citalopram peak could still be monitored after stress treatment. The project does not provide structural identification of individual degradation products; therefore, the present study should be described as an assay/stability-indicating chromatographic investigation rather than a structural characterization study of degradants.

Comparison with Reported Methods

Method	Column / conditions	Mobile phase	Flow	Rt	Linearity	Run time
Proposed	Eclipse XDB C18, 150×4.6 mm, 5 µm	Acetate buffer pH 4.5:ACN 65:35	1.0 mL/min	3.727 min	25–150 µg/mL	8 min
Chakole et al.	C18, 250×4.6 mm, 5 µm	Methanol:phosphate buffer pH 4.0 90:10	1.0 mL/min	3.22 min	2.5–80 µg/mL	10 min
Dilip Kumar et al.	Phenomenex C18, 250×4.6 mm, 5 µm	ACN:water 35:65	1.0 mL/min	—	50–250 µg/mL	—
Menegola et al.	ACE C18	Triethylamine solution/acetonitrile 55:45	1.0 mL/min	—	10–70 µg/mL	—
Gandhi et al.	HiQ SiL C18	ACN:tetrabutyl ammonium hydrogen sulfate 55:45	—	—	10–60 µg/mL	—

The comparison is reproduced from the source project's comparison table. It indicates that the proposed method combines a C18 column, a 25–150 µg/mL working range, a 3.727 min reported retention time and an 8 min total run time. The source project presents these characteristics as useful for routine pharmaceutical analysis.

Conclusion

A new RP-HPLC method was developed for the estimation of citalopram in bulk drug and tablet dosage form using an Agilent Eclipse XDB C18 column with acetate buffer (pH 4.5) and acetonitrile (65:35, v/v). The method was operated at 1.0 mL/min with detection at 240 nm and produced a reported citalopram retention time of 3.727 min. Validation studies covered linearity, precision, accuracy, LOD, LOQ, robustness and system suitability. The method showed a reported R^2 of 0.9991, repeatability %RSD of 0.47%, intermediate precision %RSD of 0.32%, LOD of 1.125 µg/mL and LOQ of 3.375 µg/mL. The assay of Celepra tablets gave 100.1% recovery of the labelled amount. Stress studies under acidic, basic, thermal, oxidative, photolytic and neutral conditions further demonstrated the method's applicability to monitoring stressed citalopram samples. On the basis of the supplied project data, the method is suitable for the studied quantitative pharmaceutical application.

Limitations and Further Work

The supplied project does not include independent laboratory reproducibility across multiple institutions, structural identification of individual degradation products, or a full impurity-characterization workflow. Future studies may therefore focus on identification and characterization of degradation products using complementary chromatographic and spectrometric techniques and on inter-laboratory transfer of the method.

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