RESEARCH PAPER

Validation And Stability Assessment Of Novel Rp-Hplc Method For Quantitative Determination Of Chlorpropamide In Api And Pharmaceutical Dosage Form

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Abstract

Objective: The present study aimed to develop and validate a simple, accurate, precise, and stability-indicating reverse phase high-performance liquid chromatographic (rp-hplc) method for quantitative estimation of chlorpropamide in api and pharmaceutical dosage form in accordance with international council for harmonisation (ich) guidelines.

Methods: Chromatographic separation was achieved using a inert sustain c18 column (4.6 × 250 mm, 5 µm) under isocratic conditions. The mobile phase consisted of methanol and water in the ratio of 80:20 v/v at a flow rate of 1.0 ml/min. Detection was carried out at 230 nm. Stress profile assessment were performed under various conditions to establish stability indicating capability and to study its degradation behaviour. The method was validated as per ich q2(r2) guidelines.

Results: The developed method exhibited excellent linearity over the concentration range of 5-15 µg/ml with a correlation coefficient (r^2) greater than 0.9957. The limit of detection (lod) and limit of quantification (loq) were found to be 0.33 µg/ml and 0.99 µg/ml respectively, indicating high sensitivity. Precision studies showed %rsd less than 2%. Degradation products were well resolved from the main drug peak with resolution greater than 2, confirming specificity and stability-indicating nature of the method.

Conclusion: The validated rp-hplc stability assessment method was sensitive, robust, and applicable for routine quality control analysis of chlorpropamide in api and for its pharmaceutical formulations.

Keywords: Chlorpropamide, Degradation Behaviour, Stability Assessment, Specificity.

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INTRODUCTION

Chlorpropamide (CPP), chemically 4-Chloro-N-[(propylamino) carbonyl] benzene sulfonamide (Figure 1), is the sulphonyl-urea derivatives, widely used as oral hypoglycemic drug in the treatment of diabetes.[1] CPP is found to stimulate pancreatic β-cell insulin production, which causes the reduction of glucose levels in blood. CPP belongs to BCS class 2 having high permeability low solubility.[2-5]

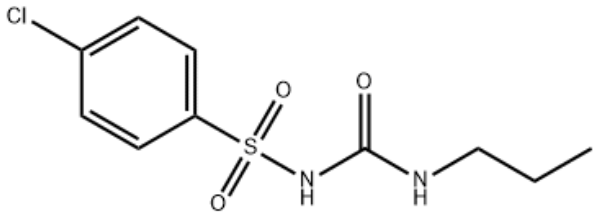

Fig.-1: Chemical Structure of Chlorpropamide

In recent years, the analysis of Chlorpropamide in pharmaceutical dosage forms has gained importance due to its therapeutic significance and regulatory requirements. Chlorpropamide is official in pharmacopoeia[6-8] and few approaches were documented, which includes bioanalysis[9-12] and simultaneous estimation.[13-16] To ensure the quality, efficacy, and stability of formulations, it is necessary to develop a reliable analytical method. Among various analytical techniques, Reverse Phase High-Performance Liquid Chromatography is the most preferred due to its high sensitivity and reproducibility. The current work sought to develop and validate a stability assessment RP-HPLC method for determining CPP in bulk and pharmaceutical dose form. The new method was validated using the ICH Q2 (R2) analytical method validation criteria.[17,18]

MATERIALS AND METHODS

Materials

Chlorpropamide (CPP) working standard was obtained as a gift sample from a reputed pharmaceutical manufacturer. The research study utilized Milli-Q water and HPLC-grade methanol sourced from Merck, India. AR grade reagents such as Orthophosphoric acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide were used for preparation of mobile phase and forced degradation studies.

Chromatographic analysis was achieved on Shimadzu LC-20AD UFLC system equipped with an Inertsustain C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) operating in isocratic mode. Detection was performed using an SPD detector, data acquisition and analysis were performed using LC solution workstation software for UFLC.

Methods

Selection of Mobile phase and diluent

Initially, a variety of mobile phase ratios were tested in order to estimate CPP in bulk and its pharmaceutical preparation. The mobile phase determined to be most appropriate for analysis of CPP was methanol and water in a ratio of 80:20 v/v, taking into account system appropriateness parameters such as RT, Tailing factor, number of theoretical plates, and HETP. After removing particulate matter using 0.45µm filter paper, the mobile phase was sonicated to degas it 1.0 ml/min was the flow rate used for analysis.

Preparation of Standard Solution

10 mg of CPP was precisely weighed, put into a 10 ml volumetric flask, dissolved in 5 ml of methanol, and sonicated for 10 minutes before the volume was adjusted to 10 ml using methanol. CPP concentration in methanol was 1000µg/ml (stock-A). Aliquots of the stock solution were further diluted with the mobile phase to obtain working standard solutions within the concentration range used for calibration studies.

Sample Solution Preparation

Twenty tablets containing the formulation was weighed and finely powdered. An amount equivalent to 10 mg of the drug was transferred into a volumetric flask containing methanol. The mixture was sonicated for 10 minutes to ensure complete extraction of the drug from the tablet matrix. The solution was filtered using a 0.45 µm membrane filter and diluted appropriately with the mobile phase.

RESULTS AND DISCUSSION

Chromatographic Conditions

Chromatographic separation was performed on a C18 reverse phase Inertsustain column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol and water in the ratio of 80:20 (v/v). The flow rate was maintained at 1.0 mL/min, and the injection volume was 20 µL. Detection was carried out using a SPD UV detector at an optimized wavelength of 230 nm. The column temperature was maintained at 30°C. Prior to analysis, both the mobile phase and sample solutions were filtered through 0.45 µm micropore membrane filters and degassed by sonication. Under these optimized conditions, CPP was eluted at a retention time of 2.685 ± 0.10 mins, producing a sharp and symmetrical peak. The system exhibited excellent baseline stability, reproducibility, and resolution, confirming the suitability of the method for routine analysis.

Method Validation

The suggested approach was validated using the ICH Q2 (R2) guidelines, which include system appropriateness was verified for analytical method validation.[17,18]

System Suitability Test

System suitability parameters including theoretical plates, tailing factor, resolution, and retention time were evaluated by injecting the standard solution (CPP) six times to ensure that the chromatographic system was performing properly. Retention time demonstrated excellent reproducibility and higher consistency with a very moderate standard deviation. Theoretical palates and asymmetry factor were within acceptable limits suggesting good column efficiency

and peak shape, the results were presented in table 1.

S. No	Concentration (µg/ml)	Peak Area
1	5	276270
2	7.5	405214
3	10	574821
4	12.5	675608
5	15	822663

Table 1: Optimized Chromatographic Condition

Parameters	Observation
Instrument used	Shimadzu LC-20AD HPLC
Mobile Phase	Methanol and Water (80:20)
Column	Intersustain C18 (250 mm x 4.6 mm; 5µm)
Detection Wavelength (nm)	230
Injection volume (µL)	20
Flow Rate (mL/min)	1.0
Runtime (min)	5
Temperature (°C)	Ambient
Mode of separation	Isocratic mode

Specificity

To determine the analyte presence of the components that would be predicted to be present, such as contaminants, degradation products, and matrix components, the specificity of the approach was tested. The drug was assessed in the presence of components expected to be present, and no interfering peaks were identified as depicted in table 2 and fig. 2.

Table-2: Results of System Suitability Studies

S.No.	Parameter	Observation
1	Retention time (min)	2.685 ± 0.10
2	Plate count	2525
3	Tailing factor	0.75
4	%RSD	0.72

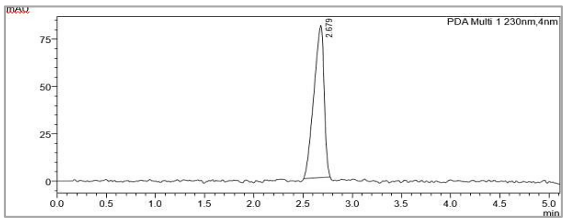

Fig. 2: Chromatogram of CPP

Linearity and Range

After passing system suitability parameter, linearity assessment was conducted by injecting 5 different concentrations of CPP working standard solution in UFLC 5-15 ug/ml respectively. The linearity of the method was assessed through regression analysis with a correlation coefficient (R^2) of 0.9957, indicating excellent linearity between peak area and drug concentration over the specified range. Calibration curve was plotted in figure 3 present's linearity profile and with its values in Table 3.

Table 3: Optimized Chromatographic Condition

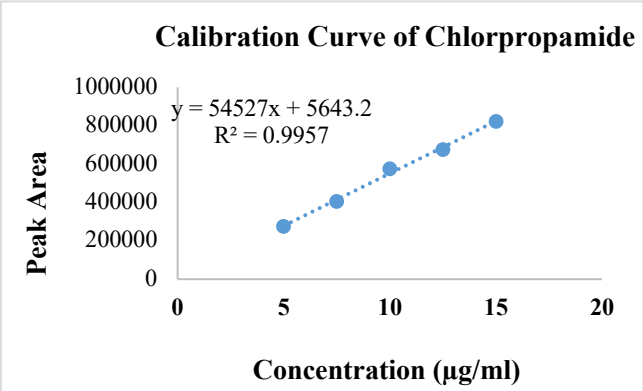

Fig. 2: Calibration Curve of CPP

Precision

Evaluations were conducted utilizing system precision, repeatability, and intermediate precision to meticulously assess the proposed HPLC method's precision in accordance with the criteria outlined in ICH Q2 (R2). The %RSD values were found to be below 2.0%, indicating good precision of the method.

Accuracy:

The correctness of the procedure was determined using the usual addition method in triplicate levels at three different concentrations. The Known amounts of the reference standard solution were added to the working solution. For every accuracy level and mean %, three shots were administered. The mean % recovery was found within the acceptance limits. The results obtained are reported in Table 4.

Table 4: Recovery Studies

S.No	Spike level (%)	Peak Area	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	% Mean Recovery
1	50	859249	5	5.11	102.25	100.72
2		848599		4.92	98.34	

3		857381		5.08	101.57	
4	100	1131480	10	10.11	101.05	100.24
5		1120969		9.91	99.12	
6		1128733		10.05	100.54	
7	150	1417134	15	15.34	102.29	101.48
8		1405296		15.13	100.84	
9		1409198		15.20	101.32	

Sensitivity

The sensitivity of the proposed method for CPP was determined using the standard deviation of the response and the slope obtained from the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.33 µg/mL and 0.99 µg/mL, respectively. The method effectively detects and quantifies the drug within the tested range, indicate the high sensitivity of the developed analytical method.

Robustness

The robustness of the developed RP-HPLC method was evaluated by introducing deliberate, minor variations in analytical parameters to assess the method's reliability under slightly altered conditions. The changes included variations in flow rate (±0.2 mL/min), column temperature (±5°C) and wavelength (±2 nm) relative to the optimized chromatographic. After each modification, parameters such as retention time (RT), tailing factor (TF) and theoretical plates (N) were studied. The results are summarized in Table 5, showing that all variations produced %RSD values below 2%, with negligible shifts in RT and consistent system suitability metrics. This confirms that the method is robust and unaffected by small operational variations and were remain within acceptable limits (%RSD < 2%), confirming the reproducibility of the proposed method.

Table 5: Results showing Robustness study

S. No	Robustness Parameter	Altered Condition	Rt (min)	Peak Area	Theoretical Plates	% Assay
1	Flow rate	0.8	3.2	698281	3416	102.2
2	(± 0.2 ml/min)	1.0*	2.6	574821	2268	99.63
3		1.2	2.1	482213	2696	88.19
4	Column Temperature (± 5ºC)	20	2.6	627453	3018	97.07
5		25*	2.6	574821	2268	99.63
6		30	2.5	594393	3214	92.90
7	Wavelength (± 2 nm)	228	2.5	624140	2949	85.88
8		230*	2.6	574821	2268	99.63
9		232	2.6	617602	2945	102.10

Stability Assessment Profile (SIAM)

The stability studies of CPP, under various stress conditions was performed to assess its degradation behaviour. The forced degradation study was conducted for predetermined time intervals under different stress environments to simulate potential degradation pathways. Acidic and basic hydrolysis were performed by exposing the drug to 0.1 N HCl and 0.1 N NaOH, respectively, at 60°C for 3 h. Oxidative degradation was carried out using 3.0% hydrogen peroxide at room temperature for 3 h. For photolytic degradation, the samples were exposed to ultraviolet (UV) and visible light for 24 h, as recommended by ICH. The drug exhibited controlled degradation under these stress conditions, confirming the stability-indicating nature of the developed RP-UFLC method. The results were presented in table 6.

Table 6: Results of Stress degradation profile

S.No	Stress Conditions	% Purity	% Degradation
1	0.1N HCl	92.22	7.77
2	0.1N NaOH	92.61	7.38
3	Oxidative	89.95	10.05
4	Thermal	89.57	10.42
5	Photolytic	91.50	9.50

CONCLUSION

A streamlined, precise, and robust reversed-phase HPLC (RP-HPLC) method was developed and comprehensively validated per ICH Q2(R2) for

quantification of Chlorpropamide encompassing the stability assessment. The system accuracy produced %RSD values of <2.0, indicating remarkable consistency. The method shown exceptional accuracy, achieving mean recovery rates of 101.81%. The assay demonstrated superior linearity (r^2 > 0.999) and sensitivity. Stability assessment was conducted on the drug & its drug product, and samples remained within permissible limits, demonstrating the method' efficacy confirming no co-elution, establishin stability-indicating attributes. The proposed method i efficient and suitable for routine quality contro assessments, as evidenced by the reduced retentio time and robust validation metrics.

Conflict of Interest

The authors declare that there is no conflict of interes regarding the research work presented in this report.

Consent for Publications

All authors have read and approved the final version o this manuscript for publication.

Availability of Data and Material

All data generated or analyzed during this study ar included in the manuscript and are available within th document.

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