

A Validated RP-HPLC Method for the Estimation of Procyclidine Hydrochloride in Pharmaceutical Dosage Form

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Abstract – A simple, rapid and precise method was developed for the quantitative estimation of procyclidine hydrochloride in pharmaceutical dosage form. A chromatographic separation of procyclidine hydrochloride was achieved with Chiral-AGP, 100 x 4.0 mm, 5.0 μ m analytical column using aqueous solution of 10 mM sodium acetate pH 4.1 with acetic acid-acetonitrile (95:5 v/v). The instrumental settings include flow rate of 1.0 ml/min, column temperature at 30°C and detector wavelength of 210 nm using a photodiode array detector. Theoretical plates for procyclidine hydrochloride were 8232. Tailing factor for procyclidine hydrochloride was 1.33. The described method showed excellent linearity over a range of 2-200 μ g/ml for procyclidine hydrochloride. The correlation coefficient is 0.999. The relative standard deviation of peak area for six measurements is always less than 2%. Overall, the proposed method was found to be suitable and accurate for quantitative determination of procyclidine hydrochloride in pharmaceutical dosage form.

Keywords – Liquid chromatography, Method validation, Pharmaceutical preparation, Procyclidine hydrochloride.

1. Introduction

Procyclidine hydrochloride (Fig. 1) is a synthetic antispasmodic compound of relatively low toxicity. It has been shown to be useful for the symptomatic treatment of parkinsonism (paralysis agitans) and extrapyramidal dysfunction caused by tranquilizer therapy. Procyclidine hydrochloride was developed at The Wellcome Research Laboratories as the most promising of a series of antiparkinsonism compounds produced by chemical modification of antihistamines. Procyclidine hydrochloride is a white crystalline substance which is soluble in water and almost tasteless [1].

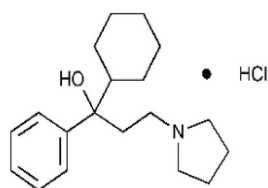


Figure 1. Chemical structure of Procyclidine hydrochloride

In the British Pharmacopoeia the indicated methods use spectrophotometric from the largest trough at about 257 nm to the largest peak at about 254 nm [2]. Moreover in the United States Pharmacopoeia it describes the method using spectrophotometric at 405 nm [3]. Literature survey reveals that there is no alternative method for the determination of Procyclidine Hydrochloride by any other method.

The HPLC method has been highly used for the quality control of drugs due to its sensitivity, reproducibility, and specificity. So The HPLC method can be suitable for the

investigation of the chemical stability than official methods. More over, this method has highly detection limit than official methods. The aim of this work was the development and validation of a new HPLC method. The present RP-HPLC method was validated following the ICH guidelines [4],[5].

2. Experimental

2.1. Chemicals and Reagents

Procyclidine hydrochloride reference standard was provided as a gift sample by GlaxoSmithKline Bangladesh Ltd., Chittagong, Bangladesh. HPLC grade acetonitrile was purchased from Rankem, India. Sodium acetate, acetic acid, chloroform and hydrochloric acid was purchased from Qualigens Fine chemicals, India; and Bromocresol purple and sodium hydroxide was purchased from Merck Ltd. India. High pure water was prepared by using Millipore Milli Q plus purification system. 0.45- μ m Pump nylon filter was obtained from Advanced Micro devices Pvt. Ltd. (Ambala Cantt, India). Commercial formulations Kemadrin tablet containing 5mg of procyclidine hydrochloride were purchased from the local market. Other chemicals used were of analytical or HPLC grade.

2.2. Method

2.2.1. Proposed Method

2.2.1.1. HPLC instrumentation and Chromatographic Conditions

The chromatographic system used was Shimadzu Prominence comprised of degasser, quaternary pump, auto injector, column compartment, photodiode array detector and the system was controlled through LC solution software. Chiral-AGP, 100 x 4.0 mm, 5 μ m (Regis Technologies, Inc.,

USA) column maintained at 30°C using column oven, eluted with mobile phase at the flow rate of 1.0 ml/min. The mobile phase consists of aqueous solution of 10 mM sodium acetate buffer pH 4.1 with acetic acid-acetonitrile (40:60 v/v). The mobile phase filtered through 0.45µm nylon filter and degassed in ultrasonic bath prior to use. Measurements were made with injection volume 10 µL and ultraviolet (UV) detection at 210 nm.

2.2.1.2. Preparation of Standard Solution

The Standard stock solution (500µg/ml) was prepared by dissolving the drug in the diluents, and standard solution was prepared by diluting them to the desired concentration. Mobile phase was used as diluent.

2.2.1.3. Preparation of Test Solution

The 20 tablets were taken, and their average weight was calculated. The tablets were crushed to a fine powder; drug equivalent to 25 mg powder was transferred to a 50 ml volumetric flask. To this flask 25 ml of diluent was added and the solution was sonicated for 10 min. with intermittent shaking. The volume is makeup with diluent and centrifuged at 10,000 rpm for 10 min. The centrifuged solution was filtered through 0.45µ filter. From the filtered solution, 5 ml of solution was transferred into a 100 ml volumetric flask and diluted to requisite volume with diluent.

2.2.2. Official Method in United States Pharmacopoeia

The samples were prepared as official monographs in the United States Pharmacopoeia.

2.2.2.1. Preparation of bromocresol purple solution

250 mg of Bromocresol purple was dissolved in dilute glacial acetic acid (1 in 50) to make 1000 ml.

2.2.2.2. Preparation of Standard Solution

The standard solution was prepared by transferring 25 mg of USP Procyclidine Hydrochloride RS to a 100 ml volumetric flask; water was added up to volume, and mixed. 10.0 ml of this solution was transferred to a second 100 ml volumetric flask; was diluted with Bromocresol purple solution up to volume and mixed. The concentration of the standard solution was 25 µg per ml.

2.2.2.3. Preparation of Test Solution

20 tablets were weighed and finely powdered. An accurately weighed portion of the powder; equivalent to about 2.5 mg of Procyclidine hydrochloride, was transferred to a 100 ml volumetric flask, 10 ml of water was added, and mixed. The bromocresol purple solution was added up to the mark, mixed and allowed the undissolved particles to settle. The supernatant was used as directed in the procedure.

2.2.2.4. Procedure

5.0 ml each of the standard solution and test solution were transferred to individual 60 ml separators. 0.5 ml of water and 4.5 ml of bromocresol purple solution were transferred to a third separator to provide the blank. Each solution with 20 ml of chloroform was extracted and each extract was filtered, discarding the first 5 ml of filtrate. Concomitantly the absorbance of each subsequent filtrate was determined in a 1 cm cell at the wavelength of maximum absorbance at about

405 nm, with a suitable spectrophotometer, against the blank. The quantity was calculated, in mg, of C₁₉H₂₉NO.HCl in the portion of tablets taken by the formula 1:

$$0.1C(AU/AS) \quad (1)$$

in which C is the concentration, in µg per ml, of USP procyclidine hydrochloride RS in the standard solution, and AU and AS were the absorbances of the standard solution and test solution, respectively.

2.2.3. Official Method in British Pharmacopoeia

The samples were prepared as official monographs in the British Pharmacopoeia.

2.2.3.1. Preparation of Standard Solution

Standard solution was prepared by dissolving 25 mg of Procyclidine Hydrochloride in 0.1M hydrochloric acid and was diluted up to 100 ml with 0.1M hydrochloric acid.

2.2.3.2. Preparation of test solution

The 20 tablets were weighed and finely powdered. 80 ml of 0.1M hydrochloric acid was added to a quantity of the powdered tablets containing 25 mg of Procyclidine Hydrochloride, mixed with the aid of ultrasound for 15 minutes, cooled, diluted to 100 ml with 0.1M hydrochloric acid and filtered through a glass fibre paper having a maximum pore size of 0.7 µm (Whatman GF/F paper) discarding the first 10 ml of filtrate.

2.2.3.3. Procedure

The second-derivative ultraviolet absorption spectra of the standard solution and test solution were recorded in the range 220 to 280 nm. For each solution the amplitude from the largest trough at about 257 nm to the largest peak at about 254 nm was measured. The content of C₁₉H₂₉NO, HCl using the declared content of C₁₉H₂₉NO, HCl in procyclidine hydrochloride BPCRS was calculated.

3. Results and Discussion

3.1. Optimization of the chromatographic conditions

Procyclidine Hydrochloride is a chiral compound and basic in nature. α1-acid glycoprotein (AGP) is a very stable protein, which tolerates pure organic solvents, high temperatures and high and low pH. AGP is the chiral selector in the CHIRAL-AGP column. The selector has been immobilized on spherical 5µm particles. The column is used in the reversed-phase mode. The CHIRAL-AGP column can be used for the resolution of an extremely broad range of chiral compounds, such as amines (primary, secondary, tertiary and quaternary ammonium), acids, esters, sulphoxides, amides, alcohols etc. Cellobiohydrolase (CBH) is the chiral selector in the CHIRAL-CBH column. CBH is a very stable enzyme, which has been immobilized onto spherical 5µm silica particles. The column is used in the reversed-phase mode. The column is preferably used for the separation of enantiomers of basic drugs from many compound classes. To develop a stability-indicating method, it is necessary to separate the analyte peak from degradants peaks. To achieve this, different column like CHIRAL-AGP and CHIRAL-CBH were tried.

The enantioselectivity and the retention were regulated by the pH of the mobile phase, the buffer concentration and the nature and the concentration of the organic modifier. The most important tool in method development is the pH. The reason is that by changing the pH the net charge of the chiral selector as well as the charge of the solute can be changed, which affects the way the analyte interacts with the chiral selector. AGP has a low isoelectric point of 2.7. This means that using the column at pH 2.7 gives a net charge of zero of the chiral stationary phase. By increasing the pH from 2.7 up to 7 means that the degree of net negative charge of the chiral selector increases. This gives the prerequisites for ionic binding of positively charged solutes, resulting in a high affinity and high retention of the solute. Reducing the pH towards the iso- electric point reduces the negative charge of the stationary phase, resulting in lower retention of the solute. A change of the net charge of the chiral selector strongly affects the interaction between the solute and the chiral stationary phase. It has been demonstrated that ionic binding of amines to the AGP column is a very important type of interaction for retention of this category of compounds. The solutes are also retained by hydrophobic interaction and hydrogen bonding. When chromatography hydrophobic amines a pH of 4-5 is preferred compared to a pH of 7. The explanation to this finding is that chromatography of the amine at a pH of 7, where the protein has a strong degree of net negative charge and the analyte is positively charged, gives a strong ionic binding of the analyte. However, reducing the pH to the range 4-5 reduces the degree of net negative charge of the protein (the analyte is still fully ionized) which gives a reduction of the ionic bonding of the analyte and the retention is strongly reduced.

2-propanol, acetonitrile, methanol, ethanol and 1-propanol were used as organic modifiers. The retention and the enantioselectivity for procyclidine hydrochloride were reduced with higher modifier concentration. By changing from one organic modifier to another with different hydrogen bonding properties, i.e. from acetonitrile (hydrogen accepting properties) to 2-propanol (hydrogen accepting and donating properties), it was possible to strongly affect the enantioselectivity for procyclidine hydrochloride. There was no chiral selectivity by using 1-propanol while acetonitrile a complete base-line resolution was found.

Our objective of chromatographic method development was to achieve peak tailing factor <2, retention time in between 3 to 12 min. The chromatographic separation of procyclidine hydrochloride from its degradants was achieved using CHIRAL-AGP (100 x 4.0 mm, 5 μ m.) column. In all aforementioned trials, CHIRAL-AGP column shows better performance as compared to CHIRAL-CBH column. It was determined that aqueous solution of 10 mM sodium acetate buffer pH 4.1 with acetic acid and acetonitrile in the ratio of 95:5 (v/v), the flow rate of mobile phase at 1.0 ml/min and column temperature at 30°C is the optimal condition. It was observed that pH 4.1 of buffer solution helps to reduce the tailing of analyte peak and to increase the efficiency in terms of theoretical plates. The analyte peak shape with less tailing resolved from degradants and the chromatographic analysis time was found to be less than 15 min. The typical retention time of procyclidine peak is about 9.9 min which is shown in figure 2 and figure 5. Method development results are shown in Table 1.

Table 1. Results of method development

Column	Retention time (min)	Theoretical Plates	USP tailing
CHIRAL-CBH; 10cm x 4.0 mm d.,5 μ	9.7	3523	1.6
CHIRAL-AGP; 10cm x 4.0 mm d.,5 μ	9.9	8232	1.3

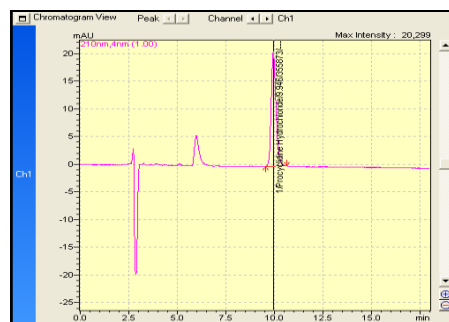


Figure 2. Chromatogram of Procyclidine in Standard Solution

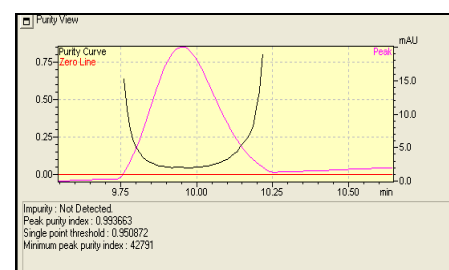


Figure 3. Purity Curve of Procyclidine in Standard Solution

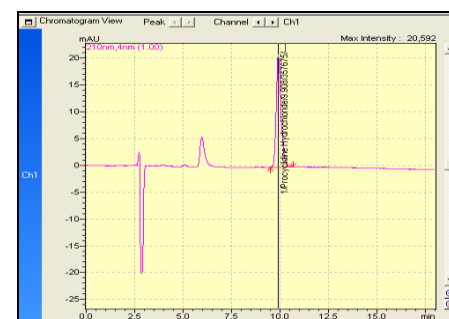


Figure 4. Chromatogram of Procyclidine in Test Solution

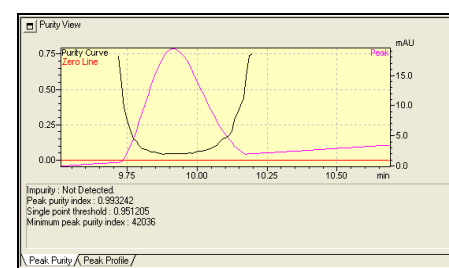


Figure 5. Purity Curve of Procyclidine in Test Solution

3.2. Method validation

3.2.1. Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It was found that the proposed method was specific because there is no interference of diluent and excipients ensuring that the peak response is due to only a single

component. Photodiode array detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of the analyte peak. The peak purity values that are more than 999 for drug product show that the peaks of analyte were pure which is shown in figure 3. Formulation excipients and degradants were also not interfering with the analyte peak which is shown in figure 5.

3.2.2. Calibration and linearity

Linearity experiment was tested from range of 8 to 800% of the targeted level assay concentration 25 µg/ml. The linearity solutions were injected in triplicate. The calibration graph was obtained by plotting peak area against the concentration of the drug. The equation of the calibration curve $y = 9.327x + 0.7701$. The calibration graph was found to be linear in the aforementioned concentrations with correlation coefficient 0.999.

3.2.3. Precision (repeatability)

The precision of the proposed method was evaluated by carrying out six independent (25 µg/ml) assays of test sample. RSD (%) of six assay values was calculated. Intermediate precision was carried out by analyzing the samples by different analyst on another instrument. Table 2 indicates the results of the precision study, which shows the method is reliable (RSD % < 2).

Table 2. Precision data

Analyst	Assay(%) (n=6)	%RSD
Analyst-1	99.23	0.59
Analyst-2	99.63	0.86

3.2.4. Accuracy (recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiment was performed by adding known amounts of drug in the placebo. The recovery was performed at three levels: 80%, 100% and 120% of the label claim of the tablet (5 mg of procyclidine hydrochloride). The recovery samples were prepared as per the procedure mentioned in preparation of sample. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated. The recovery value for procyclidine hydrochloride ranged from 99.20 to 100.66%. The average recovery of three levels (nine determinations) was 99.87%. Results are shown in Table 3.

Table 3. Results of Accuracy experiment using proposed method

Level (%)	Amount of drug spiked (mg)	Found (mg)	Recovery% (n=3)
80	4.02	4.01	99.75
100	5.01	4.97	99.20
120	6.03	6.07	100.66

3.2.5. Robustness

The robustness is the ability of method to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were purposely altered and assay, peak tailing, theoretical plates and peak area %RSD were evaluated. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate

it was changed to 0.1 units from 1.0 to 0.9 ml/min and 1.1 ml/min. The effect of column temperature was studied at 28°C and 32°C instead of 30°C, while other mobile phase components were kept constant. The effect of mobile phase composition was studied in aqueous solution of 10 mM sodium acetate pH 4.1 with acetic acid: acetonitrile (95.5:4.5 v/v) and (94.5:5.5 v/v). At all conditions sample was assayed in triplicate. The effect of detection wavelength was studied at 208 nm and 212 nm. Assay % at all deliberate conditions within 98.81 to 99.88 %. Results are shown in table 4.

Table 4. Results of robustness study

Sr. No.	Parameter	Variation	Assay % (n=3)
1	Flow rate (± 10% of the set flow)	a) At 0.9 ml/min b) At 1.1 ml/min	99.56 99.18
3	Mobile phase composition (± 2°C of set temperature)	a) At 28°C b) At 32°C	98.81 99.23
3	Mobile phase composition (± 10% of organic modifier)	a) At 4.5 ml b) At 5.5 ml	98.97 99.32
4	Wavelength (± 2 nm)	a) At 208 nm b) At 212 nm	99.88 99.75

3.2.6. Determination of limit of quantification and limit of detection (LOQ & LOD)

The detection and quantification limits were evaluated from calibration curves plotted in concentration range of detection level 2 µg/ml to 200 µg/ml. The formula used were $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$ (where σ = standard deviation of response and S = slope of calibration curve). LOD and LOQ for this method were found to be 0.6 and 2.0 µg/ml, respectively. These values indicated the method was very sensitive to quantify the drug. The standard drug solutions for each value of LOD and LOQ concentration were injected 6 times. % RSD values for the area of replicate injections were calculated.

3.2.7. Solution stability

The stability of standard solution was tested at the intervals of 12 and 24 h. The stability of solutions was determined by comparing results of area% and peak purity of procyclidine hydrochloride. The area% values were found to be within 0.5 % after 24 h. The results indicate that the solution was stable for 24 h at ambient temperature as there was no formation of any unknown peak and solution remains stable. The RSD of peak area% and peak purity were 0.32%, 99.983, respectively.

3.3. Comparison between proposed HPLC and official methods

The proposed analytical method was compared with official methods using statistical analysis. ANOVA was applied and revealed no significant difference between the experimental values obtained in the sample analysis by proposed HPLC and official methods. The calculated F-value ($F_{calc} = 0.12$) was found to be less than the tabled F-value ($F_{tab} = 6.36$) at 1 % significance level. Results are shown in table 5.

Table 5 : Comparison between proposed HPLC and official methods

Method followed As per	Observations						F _{calc}
	1	2	3	4	5	6	
BP	100.2	100.3	99.6	99.8	100.1	99.5	0.12
USP	100.1	99.6	100.4	99.7	99.8	99.9	
Proposed HPLC	99.9	100.3	100.2	100.1	99.7	99.8	

3.4. Abbreviations and Acronyms

nm	Nanometer
HPLC	High Performance of Liquid Chromatography
μ	Micron
mg	Milligram
AGP	α-acid glycoprotein
mm	Millimeter
°C	Degree Celsius
ml	Milliliter
min	Minute
mM	Millimolar
v/v	Volume/volume
UV	Ultraviolet
USP	United States Pharmacopoeia
RS	Reference Standard
M	Molarity unit
GF/F	Glass micro fiber filter
CBH	Cellobiohydrolase
RSD	Relative standard deviation
LOD	Limit of detection
LOQ	Limit of quantification
h	Hour
AVONA	Analysis of variance
F value	A ratio of two mean squares
F _{calc}	Obtained F-ratio
F _{tab}	Critical value of F for the significance level
P value	Cumulative probability

4. Conclusion

The proposed HPLC method was found to be reliable, simple, fast, precise, accurate and sensitive. Results of HPLC method showed no significant difference from those obtained with the official methods ($P > 0.01$). The purpose of the new HPLC method is not to replace the available methods for the analysis of procyclidine hydrochloride in Pharmaceutical Dosage Form, but to serve as an alternative method to be used for routine analysis. More over, this method can be used in dissolution studies.

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