Development and Validation of a Stability Indicating Assay Method Using Hplc for Determinrtion of a Model Drug in Bulk and Pharmaceutical Dosage Form

Vijay Bhosale[1,*], AV. Chandewar[2], A. P Dewani[3], C K. Gadewar[4]

*[1]Pataldhamal Wadhwani college of pharmacy. Yavatmal Tal- Yavatmal, Dist. - yavatmal, Maharashtra, India- 445001...

***Corresponding Author:**
Email: Vijay28891@gmail.com

ABSTRACT

The aim of present work was to develop a RP-HPLC method for Determination of a model drug in bulk and pharmaceutical dosage form. Agilent Chromatographic system was optimized using a Hypersil BDS C18 column (250 x 4.60 x 5µm) with mobile phase comprising of 7.5 Phosphate buffer: Acetonitrile in the ratio of 300:700. The flow rate was adjusted to 2.0 ml/min with UV detection at 215 nm. DCL were eluted with retention times of 9.30± 0.3 min respectively. Beer's Lambert's Law was obeyed over the concentration ranges of 27.97- 51.94mcg/ml, for DCL. The high recovery and low coefficients of variation confirm the suitability of the method for quqntitative analysis of drug in a liquid dosage form. Statistical analysis proves that the method is sensitive and significant for the analysis of DCL in pure and in pharmaceutical dosage form without any interference from the excipients. The method was validated in accordance with ICH guidelines.

Keywords: *Dicyclomine Hydrochloride; Stability indicating study; HPLC.*

INTRODUCTION

Dicyclomine Hydrochloride (DCL) is 2-(diethylamino) ethyl bicyclohexyl-1-carboxylate hydrochloride [1]. It binds more firmly to M_1 and M_3 than to M_2 and M_4 receptors. It has one-eighth the neurotropic activity of atropine and approximately twice the musculotropic activity of papaverine. It is used for its spasmolytic effect on various smooth muscle spasms, particularly those associated with the gastrointestinal tract. It is also useful in dysmenorrheal, pylorospam and biliary dysfunction [2]. It is used to treat a certain type of intestinal problem called irritable bowel syndrome. It helps to reduce the symptoms of stomach and intestinal cramping. This medication works by slowing the natural movements of the gut and by relaxing the muscles in the stomach and intestines.

MATERIALS AND METHODS

Chemicals and reagents

Reference standard of DCL gifted by Alkem Research Centre Taloja (Mumbai), India and these were used as such without further purification. Agilent HPLC grade was obtained from a Milli-QRO water purification system. All the chemicals and reagents used were of analytical reagent grade.

Chromatography

Analysis was performed on HPLC system equipped with waters pump, UV-Visible detector, Hypersil BDS C18 column (250 x 4.60 x 5µm) was used for separation. Mobile Phase 7.5Phosphate Buffer: Acetonitrile (300:700) filtered through 0.45 µ filter paper. Then it was degassed by sonicator. The employed flow rate for analysis was 2.0 ml/min. After making suitable dilutions sample was scanned under UV range and maximum absorbance was found at λ_{max} 215 nm.

Standard solution Preparation

40 mg of Dicyclomine Hydrochloride was dissolve in 100 ml volumetric flask.add 40 ml diluent 1. (8.5 ml hydrochloric Acid in 1000 ml methanol) sonicate to dissolved, make up the volume with diluent 1. Further dialute 5 ml of this solution into 50 ml of volumetric flask with dialuent 2. (Methanol: Water; 80:20)

Preparation of calibration curve

Linearity of Dicyclomine Hydrochloride was performed using standard solution in range of 27.97 mcg/ml to 51.94 mcg/ml (i.e 70%-130% of test concentration)

System Suitability test

According to Indian Pharmacopoeia 2007 system suitability tests are an integral part of LC Method in the course of optimizing the conditions of the proposed Method. The system suitability test solution was injected and chromatographic parameters for DCL was evaluating for proving the system suitability.

Table 1 System Suitability Parameters

System Suitability Parameters	DCL*
Retention Time	9.30 + 0.3 min
Area Under Curve	35922
Theoretical Plates	5176
Tailing Factor	1.80

(* Results are average of 3 readings)

Validation

The method was validated according to ICH guidelines for linearity, selectivity, precision, accuracy, robustness. Selectivity was checked using drug sample and mixture of standards in order to optimize separation and detection. Linearity of method was performed by analyzing a standard solution of drugs by the method in the selected concentration range for both drugs. The accuracy of the proposed method was determined by a recovery study, carried out by adding standard in drugs. The samples were spiked with three different amounts of standard compounds. The samples were analyzed in triplicate by previously established optimal conditions. The obtained average contents of the target compounds were used to calculate the spike recoveries. Precision was determined by repeatability, inter day and intraday reproducibility experiments. A standard solution containing drugs were injected six times. The mean amount and standard deviation value of each constitute were calculated.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Optimum chromatographic conditions were obtained after running different mobile phase with reverse phase C18 column. Acetonitrile was preferred over potassium dihydrogen phosphate (7.5 Ph buffer Solution: Acetonitrile (300:700)) as a mobile phase. Many Trial of mobile phase was tried to achieve best separation of peaks. Selecting 215 nm as the detection wavelength resulted in an acceptable response. The temperature of column was maintained at 30 ^{0}C throughout analysis. Elution was carried out at a flow rate 2.0 ml/min with mobile phase

Quantification of drugs present in marketed formulation

The chromatograms showed complete separation of drug. The ingredient were also quantified with respect to the standard. The results obtained are showed in Table 2 and HPLC Chromatogram for DCL Fig. 1

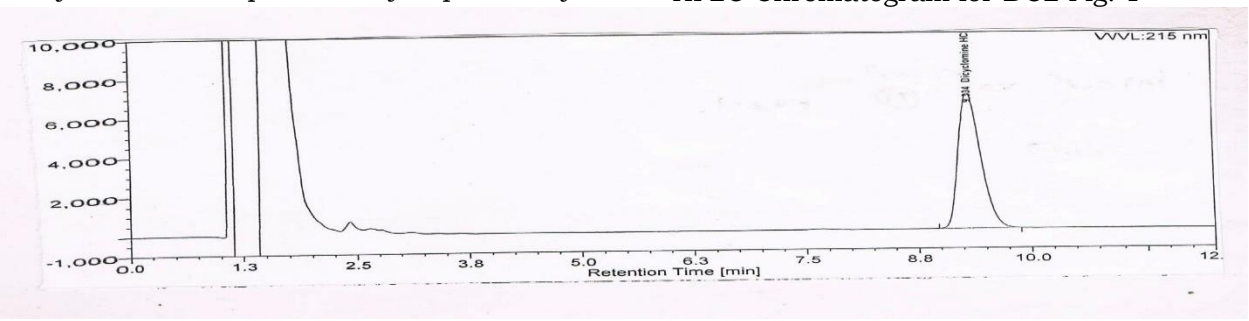

Fig 1: HPLC Chromatogram of standard

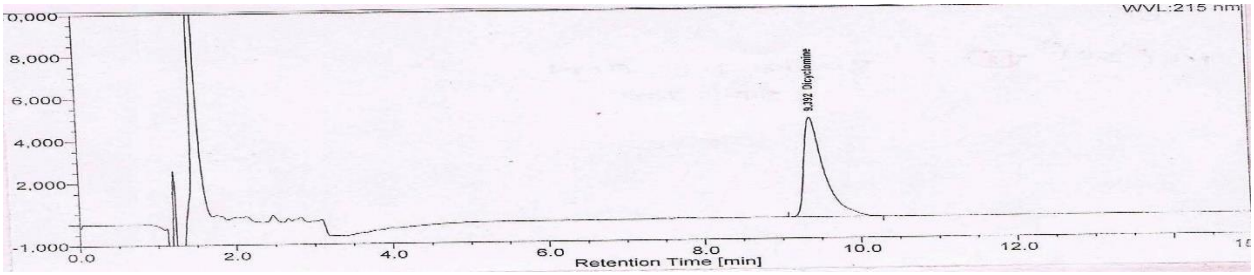

Fig 2: HPLC Chromatogram of test

Table 2: Analysis of DCL in Liquid Formulation

Std. Conc. mcg/ml	**DCL**
1	101.59
2	98.48
3	99.30
Mean	99.84
%found*	99.84
SD	1.467
%RSD	1.47

*Each reading is mean reading of three batch of formulation

Method validation for HPLC

The HPLC method was validated by defining the selectivity, linearity, accuracy, precision, robustness. For qualitative purposes, the method was evaluated by taking into account the precision in the retention time and selectivity of drugs eluted. A high repeatability in the retention time was obtained for standards and drugs even at high concentrations. For quantitative purpose, linearity, accuracy, precision, robustness were evaluated. Linear correlation was obtained between peak area and concentration of drug in their ranges. Values of the regression coefficients (r^2) for DCL higher than 0.99, thus conforming the linearity of the methods (Table 4). The high recovery values (99.50% - 98.13%) indicated a satisfactory accuracy.

Table 3: Recovery Studies of Formulation

Level of Recovery (%)	**70**	**100**	**130**
	DCL	**DCL**	**DCL**
Amount present (mg)	10	10	10
Amount of Std. added (mg)	7.12	10.24	13.75
Amount recovered (mg)	7.26	10.05	13.64
% Recovery	101.59	98.48	99.32

Relative standard deviation of all the parameters was less than 3.5% for the degree of repeatability of the developed method (Table 4). The low coefficient of variation values of intraday and inter day precision revealed that the method is precise (Table 4). Therefore this HPLC method can be regarded as selective, accurate and precise.

Table 4: Summary of Validation Parameters of Proposed method

PARAMFAERS	**DCL**
Linearity	
Range	27.97mcg/ml – 51.94mcg/ml
Slope	2118
Correlation Coefficient	0.99863
Equation of Line	y=211.8x – 110
Precision (%RSD)	
System	0.18
Method	0.47
Ruggedness (%RSD)	
Analyst 1 (n=3)	0.68
Analyst 2 (n=3)	0.58
Robustness (%RSD)	
Flow rate (-10%)	1.18
Flow rate (+10%)	0.75
Mobile phase ratio - 2 %	1.10
Mobile phase ratio + 2 %	0.99

DISCUSSION

This developed method is describes in detail the steps necessary to perform each parameter for validation. Interpretation of results of validation parameters study shows that results of method is directly proportional to the concentration of analyte within a given range shows linearity of method. Different environmental condition and minor change in chromatographic condition doesn't cause any significant change in results shows stability and reproducibility of developed method. There was no interference by excipients with analyte peak shows proposed method is specific for analyte. As well as recovery study shows developed method is highly accurate. Hence the proposed HPLC method has been evaluated and validated for the accuracy, precision, and linearity and found to be convenient, sensitive and specific for the quality control of DCL in Liquid dosage form.

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