



Research Article

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A Validated RP-HPLC Method for the Determination of Diltiazem in Raw Material and Pharmaceutical Dosage Form

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ABSTRACT

The objective of this work is to develop and validate a reverse phase high performance liquid chromatography (RP-HPLC) method for the quantitative analysis of Diltiazem in bulk and pharmaceutical dosage form. Chromatographic analyses were performed on RP C-18 column with a mobile phase consisting of 0.01M ammonium acetate in water, methanol and acetonitrile in the ratio 700:240:60 at a flow rate of 1 mL/min. The Diltiazem was detected and quantitated using a photodiode array detector at a wavelength of 295 nm with a retention time of 11.57 min. The detector response was linear in the concentration of 20-60 µg/ml, the respective linear regression equation being $Y=3000181x+356238.2$. The limit of detection and limit of quantification were 0.5 µg/ml and 0.15 µg/ml respectively. The assay of Diltiazem in bulk was found to be 99.85%. From the recovery studies it was found that about 101% on average of Diltiazem was recovered which indicates high accuracy of the method. The method was validated by determining its accuracy, precision and system suitability. The method fulfilled the requirements for reliability and feasibility for application to the quantitative analysis of Diltiazem in bulk and pharmaceutical dosage form.

Keywords: Diltiazem, calcium channel blockers, hypertension, method validation, ICH guidelines.

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INTRODUCTION

Calcium channel blockers (CCBs) are used as antihypertensive drugs, i.e., as medications to decrease blood pressure in patients with hypertension. CCBs are particularly effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients. Calcium channel blockers are also frequently used to alter heart rate, to prevent cerebral vasospasm and to reduce chest pain caused by

angina pectoris. Diltiazem is the widely used benzothiazepine class Ca^{2+} -blocking drugs (calcium channel blockers), used to treat cardiovascular diseases such as angina pectoris, hypertension, and cardiac arrhythmias. It lowers the blood pressure and has effect on cardiac conduction. [1-3] Diltiazem is almost completely absorbed by the gastrointestinal tract and undergoes extensive first-pass hepatic metabolism. [4] Diltiazem is chemically d-cis-3-acetyloxy-5-[2-(dimethyl

amino) ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)one) (Figure 1).

Literature survey reveals few spectrophotometric methods for estimation of Diltiazem in human plasma [5-6] and pharmaceutical dosage forms. [7-15] The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Diltiazem in bulk drug samples and in pharmaceutical dosage form.

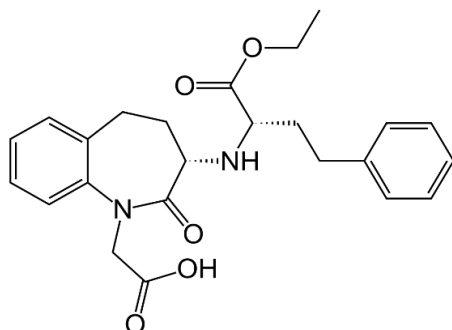


Fig. 1: Structure of Diltiazem

MATERIALS AND METHODS

Diltiazem was obtained as a gift sample from Hetero Drugs Ltd Hyderabad. Ammonium acetate, acetonitrile and methanol used were of HPLC grade (Qualigens). Commercially available Diltiazem tablets (Dilcontin® 60 mg) were procured from local market.

Instrument

Quantitative HPLC was performed on a Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with variable wave length PDA-Detector and powered with Empower-2 Software. The column used was Waters Spherisorb® RP C-18, 4.6 × 250 mm (250 × 4.6 mm i.d; particle size 5µm).

HPLC Conditions

The mobile phase comprises of 0.01M ammonium acetate in water, methanol and acetonitrile in the ratio 700:240:60. The contents were filtered through a 0.45µm membrane filter, pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min. The run time was set at 20.0 min and the column temperature was 45°C. Prior to the injection of the drug solution, the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The eluents were monitored at 295 nm.

Preparation of standard stock solution

A standard stock solution of the drug was prepared by dissolving 50 mg of Diltiazem in 100 ml volumetric flask containing 50 ml of water, sonicated for about 15 min and then made up to 100 ml with water to get approximately 500µg/ml.

Preparation of working standard solution:

5ml of the primary standard stock solution of 500µg/ml was taken in 50 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 50µg/ml.

Preparation of sample solution

Dilcontin® tablets equivalent to 50 mg of the active ingredient was mixed with 70 ml of mobile phase in 100 ml volumetric flask. The mixture was allowed to stand for 1 hour with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45µm membrane filter, followed by addition of mobile phase up 100 ml to obtain a stock solution of 500µg/ml. The resultant solution was further diluted by taking 5 ml of the stock solution with 50 ml of mobile phase to get the concentration of 50µg/ml.

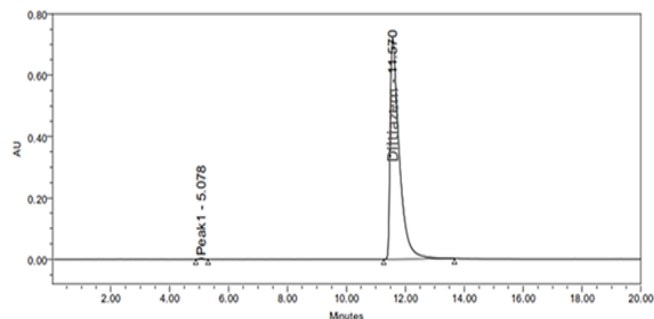


Fig. 2: Typical System suitability Chromatogram of Diltiazem standard solution.

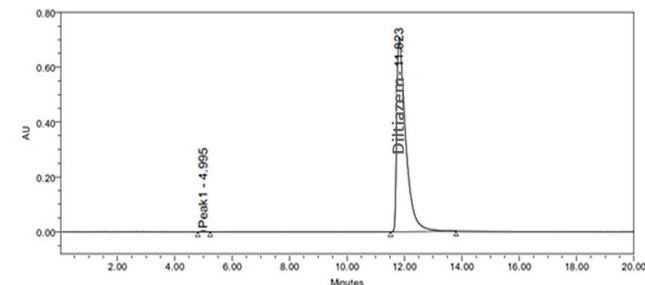


Fig. 3: Typical Chromatogram of Diltiazem working sample

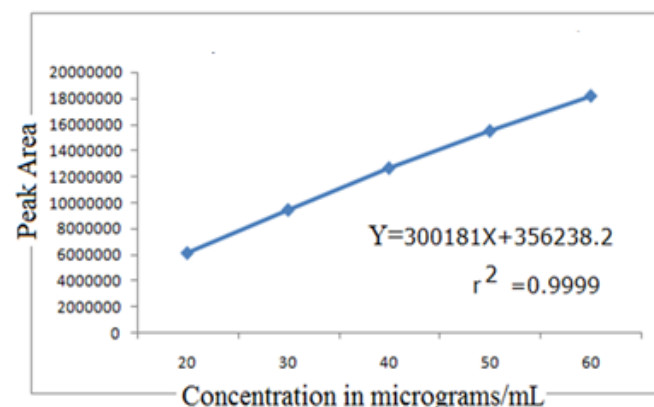


Fig. 4: Standard Calibration Curve of Diltiazem

RESULTS AND DISCUSSION

Analytical method validation

The HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines (2005). [16] The following characteristics were considered for validation: specificity, linearity, range, accuracy, precision, LOD, LOQ and robustness.

The specificity was evaluated by comparing the representative chromatograms of samples containing possible interfering substances and samples containing

Diltiazem. Linearity was determined from plot peak area vs. concentration for the five concentrations (20-60 µg/ml). The regression equation and regression coefficient were calculated using least square methodology. The accuracy was tested by calculating the percent recovery of the mean concentration of Diltiazem at three different concentration levels (40, 50 and 60 µg/ml), and the relative standard deviation (RSD) was determined.

The precision of the method was ascertained separately from the peak area obtained by actual determination of 6 replicas of a fixed amount of drug and formulation. The results were reported as %RSD. The LOD and LOQ were determined from the specific calibration curve obtained using six standard solutions that were the closest to the LOQ.

Robustness was evaluated by deliberately varying the flow rate and using similar columns.

System suitability

The system suitability tests were carried out on freshly prepared stock solution of Diltiazem standard and sample. The system was suitable for use, the tailing factors for Diltiazem were 1.85 and USP theoretical plates were found to be significantly high around 7245 (Figure 2, 3).

Precision

The precision of the method was ascertained separately from the peak area obtained by actual determination of 6 replicas of a fixed amount of drug and formulation. The HPLC systems was set up at described Chromatographic conditions, mentioned as above and follow the system to equilibrate, injected 50 µg/ml of Diltiazem standard 6 times and recorded the response (peak area). The proposed method was extended to the pharmaceutical dosage forms by injecting the 50 µg/ml of Diltiazem sample with the formulated sample from Dilcontin containing Diltiazem of same concentration 6 times and recorded the response (peak area). The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated and presented in Table 2.

Linearity

Aliquots of standard Diltiazem stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase to obtain the final concentrations of Diltiazem in the range of 20-60 µg/ml. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded (Table 3, 4). Evaluation was performed with PDA detector at 295 nm and a calibration graph was obtained by plotting peak area versus concentration of Diltiazem. The linearity chromatograms presented in Figure 4.

Assay and recovery studies

Recovery studies were conducted by analyzing pharmaceutical formulation in the first instance for the active ingredient in the working standard of concentrations 80% (40 µg/ml), 100% (50 µg/ml) and 120% (60 µg/ml) by the proposed method. Each

concentration was injected 3 times and the peak area recorded. Known amounts of pure drug 80% (40 µg/ml), 100% (50 µg/ml) and 120% (60 µg/ml) was then added to each 3 previously analyzed formulation and the total amount of the drug was once again determined by the proposed method (each concentration was again injected 3 times) after keeping the active ingredient concentration within the linearity limits. The Recovery data is given in Table 5.

Table 1: Performance & Detection Characteristics of HPLC method

Parameter	Results of the proposed HPLC method	
	Diltiazem Standard solution	Diltiazem sample solution
Retention time (min)	11.570	11.823
Theoretical plates (n)	7245	7430
Plates per meter (N)	28980	29720
HETP	3.45065×10^{-5}	3.36473×10^{-5}
Peak asymmetry (T)	1.85	1.92
Linearity range (µg/mL)	20-60	
Limit of Detection (µg/mL)	0.05	
Limit of Quantification (µg/mL)	0.15	

Table 2: Precision of Standard drug with statistics

Injection No.	Name of the drug & conc. (50 µg/ml)	Retention time in min.	Peak Area
1	Diltiazem sample-1	11.597	15589654
2	Diltiazem sample -2	11.605	15153698
3	Diltiazem sample -3	11.616	15874596
4	Diltiazem sample -4	11.622	15658794
5	Diltiazem sample -5	11.630	15658974
6	Diltiazem sample -6	11.637	15356894
Mean		11.618	15548768.33
% RSD.		0.015	254944.14
Std. Deviation		0.13	1.63

Table 3: Linearity data of Diltiazem

S. No	Concentration(µg/ml)	Area
1	20	6125691
2	30	9965893
3	40	13125612
4	50	16003642
5	60	18904934

Table 4: Optical & Regression Characteristics of HPLC method

Parameter	Results of HPLC Method
Detection wavelength (nm)	295
Linearity range (µg/ml)	20-60
Regression Equation (y=mx + c)	Y=3000181x+356238.2
Slope (m)	3000181
Intercept (c)	356238.2
Correlation coefficient	0.9999
Relative Standard deviation*	0.1
% error in bulk samples	1.63

Robustness

A method is robust if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at two different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the mobile phase flow rate was varied by $\pm 10\%$ and different column had no significant effect on

the retention time and chromatographic response of the method, indicating that the method was robust. When the chromatographic conditions were deliberately altered, system suitability results remained within acceptance limits and selectivity for individual substance was not affected. The results of the study prove the robust nature of the method (Table 6 and Table 7).

Limit of detection [LOD] and Limit of quantification [LOQ]

The detection limit of the method was investigated by injecting standard solutions of Diltiazem into the HPLC column. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. A signal-to-noise ratio (S/N) of 3 is generally accepted for estimating LOD and signal-to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise.

The limit of detection (LOD) and limit of quantification (LOQ) for Diltiazem were found to be 0.05 µg/ml and 0.15 µg/ml respectively.

The author has developed a sensitive, accurate and precise RP-HPLC for the estimation of Diltiazem in bulk drug and in pharmaceutical dosage form. From the typical chromatogram of Diltiazem it was found that the retention time was 11.570 min. The mobile phase consisting of 0.01M ammonium acetate in water, methanol and acetonitrile in the ratio 700:240:60 at a flow rate of 1.0 ml/min and eluents were monitored at 295 nm. The method was found to be most suitable to obtain a peak well defined and free from tailing. In the present RP- HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship ($r^2=0.999$) was observed between the concentration range of 20-60 µg/ml. The assay of Diltiazem in bulk was found to be 99.85%. From the recovery studies it was found that about 101% on average of Diltiazem was recovered which indicates high accuracy of the method.

Table 5: Recovery Peak areas of Diltiazem by Accuracy studies

S. No	Recovery at 80% dilution level		Recovery at 100% dilution level		Recovery at 120% dilution level	
	Standard	Spiked	Standard	Spiked	Standard	Spiked
1	12105542	13995406	15166296	16982253	18393278	19937765
2	12062082	13993842	15149180	16975559	18204944	20143657
3	12063724	13994795	15192077	16994139	18223729	20046329
Avg	12077116.0	13994681.0	15169184.3	16983983.7	18273983.7	20042583.7
SD	24631.3	788.2	21593.9	9410.1	103738.0	102997.1
%RSD	0.2	0.0	0.1	0.1	0.6	0.5
% Recovery	104.5		98.08		99.41	

Table 6: Robustness study of Diltiazem Standard solution at 100 % level (50 µg/ml)

Parameter	Peak areas of Diltiazem in Flow increase study	Peak areas of Diltiazem in Flow decrease study	Peak areas of Diltiazem in Variable column Study
Injection-1	14069361	17028319	15322433
Injection-2	13979535	17091603	15357329
Injection-3	13951021	17129794	15365196
Mean	13999972.3	17083238.7	15348319.3
% RSD	61760.4	51252.0	22760.7
Std. Dev	0.4	0.3	0.1

Table 7: Robustness study of Diltiazem sample solution at 100 % level (50 µg/ml)

Parameter	Peak areas of Diltiazem in Flow increase study	Peak areas of Diltiazem in Flow decrease study	Peak areas of Diltiazem in Variable column Study
Injection-1	13970242	17016395	15274637
Injection-2	14009808	17138352	15282147
Injection-3	13985154	17099070	15320559
Mean	13988401.3	17084605.7	15292447.7
% RSD	19981.9	62251.8	24633.0
Std. Dev	0.1	0.4	0.2

The validation results demonstrate that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, can be easily used for the routine quality control of bulk and pharmaceutical dosage form of Diltiazem within a short analysis time. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations.

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