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 20-60µg/ml, the respective linear regression equation being Y=3000181x+356238.2. The limit of detection and limit of quantification were 0.5µg/ml and 0.15µg/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 Ca2+-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-benzoazepin-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 C18, 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.

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 (20-60μg/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 60μg/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

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 50μg/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 50µg/ml, 100% (50µg/ml) and 120% (60µg/ml) of Diltiazem standard 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 20-60μg/ml. Each of these drug solutions (20µL) 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% (40μg/ml), 100% (50μg/ml) and 120% (60μg/ml) by the proposed method. Each concentration was injected 3 times and the peak area recorded. Known amounts of pure drug 80% (40μg/ml), 100% (50μg/ml) and 120% (60μg/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.
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.

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 | Standard | Spiked |
| 1     | 12105542 | 13995406 | 15166296 | 16982253 | 18393278 | 19937765 |
| 2     | 12062882 | 13993842 | 15149180 | 16975559 | 18204944 | 20143657 |
| 3     | 12063724 | 13994795 | 15192377 | 16991349 | 18257292 | 20046329 |
| Avg   | 12077116.0 | 13994681.0 | 15169184.3 | 16983983.7 | 18273983.7 | 20042983.7 |
| SD    | 24631.3 | 788.2 | 21559.3 | 9410.1 | 103738.0 | 21909.7 |
| %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 (50µg/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 | 13997935 | 17091603 | 15357329 |
| Injection-3 | 13951021 | 17129794 | 15365196 |
| Mean       | 13990972.3 | 17083238.7 | 15340831.3 |
| % 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 (50µg/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 | 13988154 | 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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