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DEVELOPMENT AND VALIDATION OF ASSAY METHOD BY RP- HPLC FOR DETERMINATION AND QUANTITATION OF DILTIAZEM HYDROCHLORIDE ACTIVE PHARMACEUTICAL INGREDIENT

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ABSTRACT

A Revered Phase HPLC method was developed and validated for determination of Assay for Diltiazem HCl Active Pharmaceutical ingredient (API). The method was validated as per ICH Q2 (R1) and FDA guidelines and found to be simple, specific, linear, precise and robust which can be used for routine analysis purpose. The separation was achieved by using HPLC column Ascentis Express C18 column having dimension 7.5cm x 4.6mm and particle size 2.7µm. The mobile phase consists of mixture of 0.1% Triethylamine pH-3.0 (Previously adjusted with orthophosphoric acid) and Acetonitrile in the ratio of 65:35 v/v. The column temperature and mobile phase were constantly maintained at 50ºC and 1.0 ml/min respectively. The detection was performed at wavelength 236 nm using PDA detector. The retention time of Diltiazem peak was found to be about 1.5 minutes. The method was specific as no interference was observed at retention time of Diltiazem peak. The linear regression analysis data for the calibration curve shows a linear relationship over the concentration range of 50-150µg/mL for Diltiazem HCl and the correlation coefficient value obtained was 0.9999. The experimental data shows that the method was Specific, Linear, Precise and Robust for the Assay determination of Diltiazem Active Pharmaceutical ingredient.

***Keyword**: Diltiazem HCl, ICH guideline, Acetonitrile, RP-HPLC.*

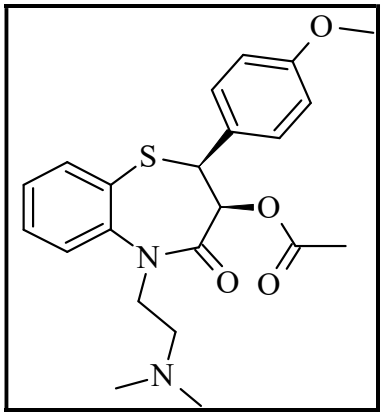
1. INTRODUCTION

Diltiazem (INN) is a nondihydropyridines (non-DHP) calcium channel blocker. Chemical name of Diltiazem Hydrochloride is cis-(+)-[2-(2-Dimethylaminoethyl)-5-(4-methoxyphenyl)-3-oxo-6-thia-2-azabicyclo [5.4.0] undeca-7, 9, 11-trien-4-yl] ethanoate hydrochloride salt. Molecular weight is 450.98 and molecular formula is $C_{22}H_{27}ClN_2O_4S$, for chemical structure, refer figure-1.

It is used in the treatment of hypertension [1], angina pectoris [2], and supraventricular tachyarrhythmia's [3]. It relaxes the smooth muscles in the walls of arteries, which opens (dilates) the arteries, allows blood to flow more easily, and lowers blood pressure. Additionally, it lowers blood pressure by acting on the heart itself to reduce the rate, strength, and conduction speed of each beat.

It is also used off-label as an effective preventive medication for migraine. It is a class-3 anti-anginal drug, and a class IV antiarrhythmic. It is a common adulterant of cocaine seized in the UK and has been found to reduce cocaine cravings in rats, indicating it may prolong the "high".

Diltiazem is metabolized by and acts as an inhibitor of the CYP3A4 enzyme which can cause it to interact with a variety of other medications.



. HCl

Figure-1: Chemical Structure of Diltiazem

The HPLC method for assay determination of Diltiazem HCl is official is USP [4,] BP [5,] also several method has been reported in literatures which includes a variety of analytical techniques such as Spectrophotometry [6–7], HPLC [8–11], HPTLC [12], Gas chromatography [13] , Capillary electrophoresis [14,15,18,19], Polarimetry [16] and Titrimetry [17]. Recently, HPLC–MS methods [20] have been reported to characterize the Diltiazem metabolites.

A RP-HPLC method using monolithic silica support for separation of Diltiazem and its impurities has been published [21]. Two validated stability indicating HPLC methods have also been reported for Diltiazem in bulk drug [22] and in tablets [23]. These stabilities indicating analytical methods are validated for assay of Diltiazem, and not for analysing the drug in the presence of its known impurities. Since there are no methods available with short run time for Assay determination, an attempt was made to develop a simple, rapid, RP-HPLC method for the estimation of Diltiazem HCl.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Working standard and Diltiazem HCl sample were received as gift from KP Labs Hyderabad, HPLC Grade water was obtained from Mill-Q Water system (Millipore), Acetonitrile HPLC grade from (Ranbaxy), Triethylamine (S.D fine), Ortho Phosphoric acid (S.D fine), Nylon membrane filters (0.45 µm)

2.2 Equipment's

Chromatographic separation was achieved by using HPLC System (Agilent 1100 series) having PDA detector. The software used was Chemstation Software® for capturing and processing chromatographic/Analytical data.

2.3 Selection of UV Wavelength

Structure determination of Active pharmaceutical ingredient confirmed the presence of multiple number of chromophores e.g. unsaturated double bonds as part of Benzene ring as well as presence of Carbonyl group. Based on this structural information we decided to select UV technique for detection purpose.

The standard solution of 10 ppm of Diltiazem Hydrochloride was scanned on UV spectrophotometer in the range of 200-400 nm using distilled water as blank. The λ max obtained of this solution was 236 nm, refer figure-2.

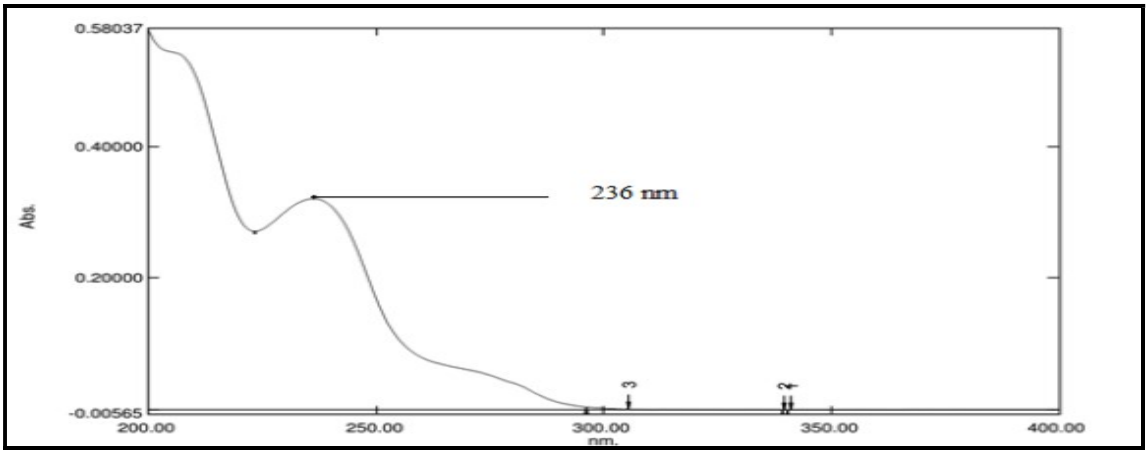


Figure-2: UV Absorption spectrum for Diltiazem HCl

2.4 HPLC instrumentation and analytical Conditions

The proposed method involves use of HPLC column Ascentis Express C18 having dimension 7.5cm x 4.6mm and particle size 2.7µ. The mobile phase consists mixture of 0.1%Triethylamine pH-3.0 (Previously adjusted with orthophosphoric acid) and Acetonitrile in the ratio of 65:35 v/v. The flow rate and column temperature were constantly maintained at 1.0 ml/min and 50ºC respectively throughout the experiment. The detection was performed at wavelength 236 nm using PDA detector. Injection volume was kept 5 µL.

2.5 Preparation of solutions

Standard and sample solution were prepared in mobile phase and injected on the HPLC system.

2.6 Standard solution

Weighed accurately about 20 mg of Diltiazem HCl standard into a 20 ml volumetric flask, added about 10 mL of mobile phase and sonicated to dissolve. Allowed the solution to attain the room temperature and finally diluted up to mark with mobile phase (1000 ppm). Further 1 ml of above solution was diluted to 10 ml with mobile phase (100 ppm).

2.7 Sample solution

Weighed accurately about 20 mg of Diltiazem HCl sample into a 20 ml volumetric flask, added about 10 mL of mobile phase and sonicated to dissolve, Allowed the solution to attain the room temperature and finally diluted up to mark with mobile phase (1000 ppm). Further 1 ml of above solution was diluted to 10 ml with mobile phase (100 ppm).

2.8 Calculations

The percentage of Diltiazem HCl in the portion of sample taken is calculated by using below formula,

$$\% \text{ Assay} = \frac{\text{Sample Area} \times \text{Standard dilution factor} \times \text{Purity of standard} \times 100}{\text{Average standard Area} \times \text{Sample dilution factor} \times 100}$$

3. RESULTS AND DISCUSSION

3.1 Method development

The initial development was started with simple mobile phase comprising of water and Acetonitrile in the ratio of 50:50 v/v and HPLC column selected was Zorbax SB C8, 15cm x 4.6mm, 5 µm. The flow rate was 1 ml/ min and column temperature was set at 25°C. 10 µL of 1000 ppm Diltiazem HCl standard was prepared in Acetonitrile and injected on the chromatographic system. The peak eluted at about 4 minutes and had tailing. So, Various trails were taken to improve the peak shape of Diltiazem first by changing the column to Inertsil C18 ,25cm x 4.6mm, 5µ and second by modifying the diluent to Acetonitrile: Water 50:50 v/. But still tailing problem persists.

Further mobile phase was modified to 0.1%TEA whose pH was adjusted to 3.0 with Ortho Phosphoric acid to improve the peak shape. A broad peak shape was observed with high retention time (About 6 minutes). To further optimise the peak broadening, a mixture of Acetonitrile and buffer (0.1% TEA pH 3.0) was employed in the mobile phase, comparatively a sharp peak was observed at about 4 minutes. Since attempt was taken for assay method development method for shorter run time the conventional column was replaced with new generation column having fused core technique. So Ascentis Express C18 column with dimension of 7.5cm x4.6 mm, 2.7 µ was used. The peak shape obtained after the usage of new column shows considerable improvement in the peak shape and in retention time.

Literature survey revealed that the method available in the official Compendia (USP) using HPLC for analysing Diltiazem hydrochloride. The present method was compared with the reported method in literature is summarized in table-1.

3.2 Method validation [24]

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, Validation of Analytical Procedures: Q2(R1)[7], for the parameters like system suitability, Specificity, linearity and range, precision (repeatability), Intermediate precision (Ruggedness) and robustness.

3.3 System suitability

The system suitability test performed according to USP39. The standard solution was injected six times and results were recorded to find the adequate peak shape, percentage relative standard deviation for area and retention time, peak asymmetry and theoretical plates. The results obtained were compiled in Table-2.

Table-1: Comparison of the performance characteristics of the present method with the published methods

Sr. No	Method	Reagents/ Column used	Detection wavelength	Remark	Reference
1	HPLC	d-10- camphorsulphonic acid, Sodium acetate, Sodium hydroxide, Acetonitrile, Methanol/ 30 cm x 3.9 mm, L1 packing	240 nm	Tedious mobile phase preparation and High analysis time and solvent consumption	USP-39
2	HPLC	Triethylamine, Acetonitrile, orthophosphoric acid/ Ascentis Express C18 having dimension 7.5cm x 4.6mm and particle size 2.7µ	236 nm	Simple mobile phase preparation, cost effective and time saving method.	Present work

Table 2: System suitability results

Parameter	Observed value	Acceptance Limit
% RSD for Area for replicate injection of standard solution	0.1	**NMT 2.0%**
Theoretical plates	2605	**NLT 1500**
Tailing factor	1.4	**Should be Between 0.8 to 2.0**

Results: **T**he percentage Relative standard deviation (% RSD) for Area, tailing factor and Theoretical plates for analyte peaks were within the acceptance limit which shows that the method has good system suitability.

3.4 Specificity

Specificity was performed to detect the presence of interference peak at the retention time of the analyte peak. The specificity of the method was checked by comparison of chromatograms obtained from test sample solution and the blank solution. The representative chromatogram shown in figure-3

Results: No interference at retention time of analyte peak due to blank was observed.

3.5 Precision (Repeatability) and Ruggedness (Intermediate Precision)

Method precision was evaluated by carrying out six different test sample solution preparation. Intermediate precision was performed on different day in the same laboratory by carrying out six different test sample solution preparation. The assay of these samples was determined. Precision and intermediate precision of the method was evaluated by calculating the %RSD. The values were given in Table 3.

Results: The percentage Relative standard deviation (%RSD) obtained was found to be less than 2 .0 % for day-1 and day-2.

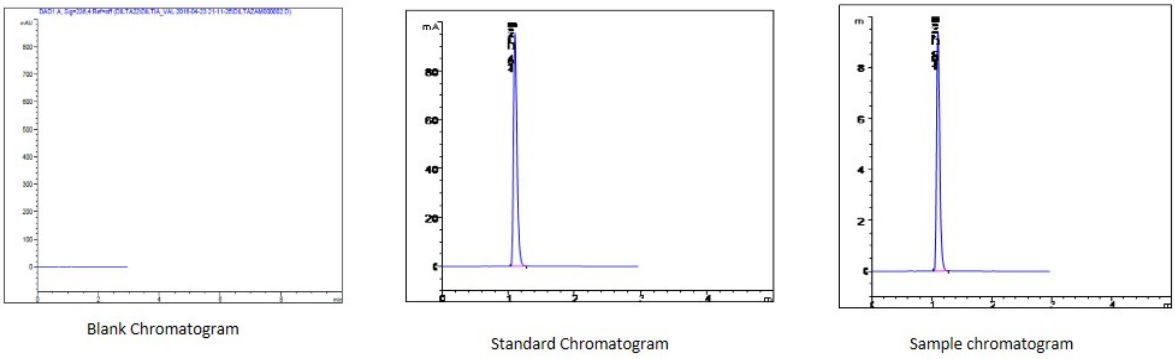


Figure-3: A typical chromatogram of Blank, Standard and Sample solution for Assay determination.

Table 3: Precision and Intermediate Precision results

Parameter	Precision (Day -1)	Intermediate Precision (Day-2)	Average for Precision (Day-1 and Day-2)	% RSD for Precision (Day-1 and Day-2)	Acceptance Criteria
Average % Assay	99.73	99.86	99.8	1.43	%RSD should not be more than 2.0% for day-1 and day-2.
% RSD	1.39	1.59			

3.6 Linearity and Range

The linearity of detector response was determined by preparing a series of solution of the working standards over the range of 50 to 150 % of specification level targeted concentration. These solutions were injected onto the chromatographic system and response area were recorded. Calibration curve was constructed by plotting area against concentration and regression equation was computed. The linearity plots with values were shown in Figure 4.

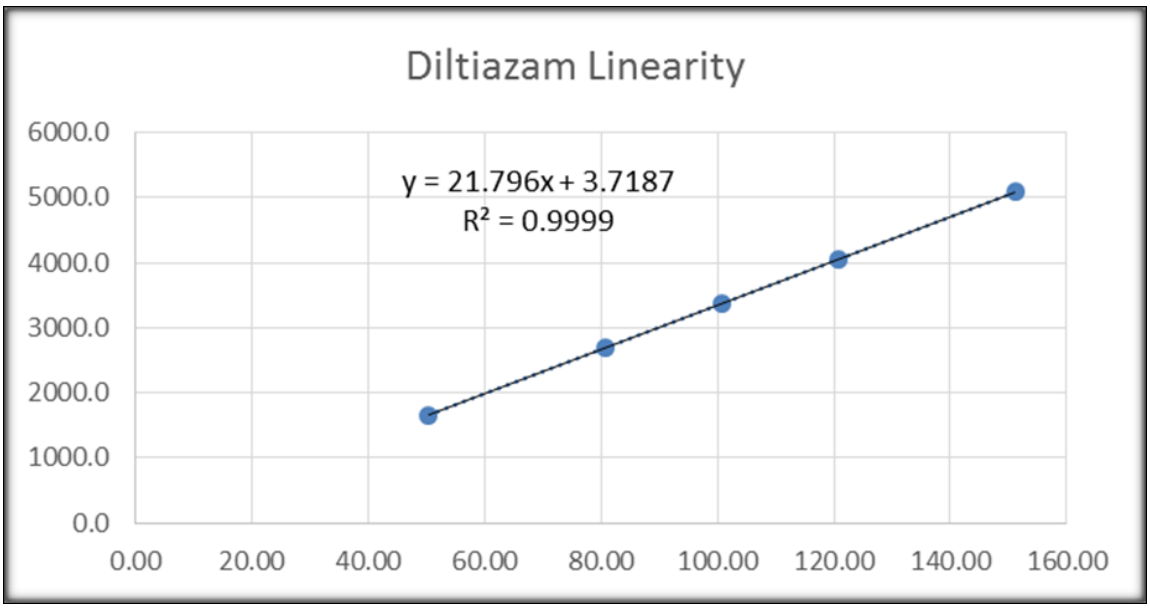


Figure-4: Calibration curve for Diltiazem HCl showing the Linearity

Table 4: Linearity study results

Parameter	Observed value	Acceptance Limit
Correlation coefficient	0.9999	0.999
% y-Intercept	0.11	Should be between ± 2 %.

Results: The results obtained for correlation coefficient and % y-intercept were within acceptance limit.

3.7 Robustness: Effect of variation in Flow rate, Column temperature and pH of Mobile Phase Buffer

To study robustness of the test method, small, deliberate changes were made to the chromatographic condition. A study was performed by changing the Flow rate, column temperature and pH of Mobile Phase Buffer. Standard solution prepared as per the test method and was injected into the HPLC system at 45°C and 55°C temperature. The pH of mobile phase buffer was altered to 2.5 and 3.5. The system suitability parameters were evaluated on the modified conditions.

Table 5: Results of Robustness study

Sr. No	Parameter		Theoretical plates (Limit NLT 1500)	Tailing Factor (NMT 2.0)
1	**Normal condition**		2605	1.4
2	**Flow rate**	**0.9 ml/min**	2235	1.3
		1.1 ml/min	1787	1.2
3	**Column temperature**	**45°C**	2142	1.3
		55°C	2082	1.2
4	**Mobile phase Buffer pH variation**	**2.5**	2111	1.4
		3.5	1922	1.4

Results: The results obtained from the above deliberate changes, refer table-5, it was concluded that there was no impact on the system suitability test parameter which demonstrates robustness of the method.

4. CONCLUSION

The HPLC method was developed for Assay determination of Diltiazem is simple, specific, linear, sensitive, precise, efficient and robust. The method developed has short run time, thus ensuring maximum usage of the HPLC system. Also, shorter run time requires less consumption of solvents there by reducing cost per analysis and also generating lesser solvent waste. Further the proposed method is on HPLC with shorter run time obtained generally with advance and costly techniques. The shorter run time will help in faster the analysis of multiple batches in short duration thus increasing the out-put of batch analysis.

The method was validated as per ICH Q2 (R1) guidelines, showing satisfactory data for all the method validation parameters tested. Hence, the proposed method can be employed for assessing the Assay determination of Diltiazem for Routine and R& D analysis.

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