

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DILTIAZEM IN BULK AND TABLET DOSAGE FORMS

R. PRAVIN CUMAR^{*1}, DR. M. VASUDEVAN², DR. DEECA RAMAN³

¹Research Scholar, Department of Pharmaceutical Biotechnology, Dr. M.G.R Educational and Research Institute, Dr. M.G.R University, Maduravoyal, Chennai 600 09, (TN), India ²Dean, Department, ²President, Roxaane Research Ltd., Velachery, Chennai 600100, ³Dean, Department of Industrial Biotechnology, Dr. M.G.R Educational and Research Institute, Dr. M. G. R. University, Maduravoyal, Chennai 600 09, (TN), India. E-mail: cumar.pravin@gmail.com

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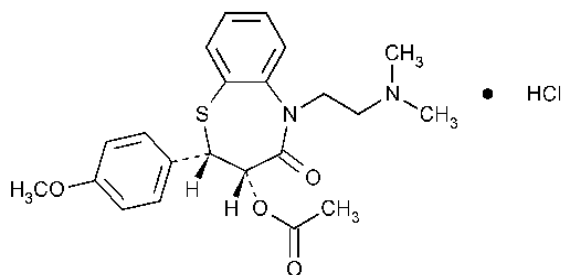
ABSTRACT

The present research work deals with the development of RP-HPLC method for the determination of Diltiazem in bulk and in formulation using UV detector. A mixture of 0.01% of Trifluoroacetic acid in water and Acetonitrile in the ratio of 40:60 was used as mobile phase. The flow rate was kept at 1.6 ml/min, the injection volume was 25 µl and the wavelength selected was 238 nm. The column used was Zorbax-SB Phenyl Column. The validation parameters used are System Suitability, Specificity, Precision, Accuracy, Linearity, Limit of detection (LOD), Limit of Quantification (LOQ), Solution Stability, Robustness and Ruggedness. All the validation parameters are carried as per ICH guidelines. The Retention time of Diltiazem was observed at 7.54 min. The proposed RP-HPLC method was found to be simple, sensitive and specific for the determination of Diltiazem in bulk and in tablet dosage forms.

Keywords: Diltiazem, ICH guideline, Acetonitrile, RP-HPLC

INTRODUCTION

Diltiazem is described chemically as 1,5-Benzothiazepin-4(5H)-one, 3-(acetyloxy)-5-[2(dimethylamino)ethyl]-2, 3-dihydro-2-(4-methoxyphenyl)-, mono hydrochloride. The empirical formula is C₂₂H₂₆N₂O₄S, and its molecular weight is 414.518. It is Soluble in water (50 mg/mL), Alcohol and formic acid.



Diltiazem is a non-dihydropyridine (non-DHP) member of the class of drugs known as calcium channel blockers, used in the treatment of hypertension, angina pectoris, and some types of arrhythmia. It is also an effective preventive medication for migraine. Diltiazem hydrochloride is a calcium channel blocking agent used to dilate coronary blood vessels. Diltiazem relaxes coronary vascular smooth muscles by inhibiting influx of calcium ions during depolarisation of the vascular smooth muscles and myocardium. It increases myocardial O₂ delivery in patients with vasospastic angina and inhibits cardiac conduction, particularly at the SA and AV nodes. Absorption: Rapidly and almost completely absorbed from the GI tract (oral).

Apparatus and chromatographic conditions

Working standards of Diltiazem was procured from Zydus- Cadila Ahmadabad, Distilled Water, Acetonitrile used where of HPLC grade. All the reagents used in the study are of AR grade. Chromatographic separation was performed on a Shimadzu LC-20 AT HPLC Detector UV visible (SPD 20A)

Chromatographic System and Conditions

Instrument: High Pressure Liquid Chromatography

Detector: UV-Visible detector

Column: 50 X 4.6mm; 1.8µm; Zorbax SB-Phenyl

Wavelength: 238 nm

Sample concentration: 1.0 mg/ml

Injection volume : 25 µL

Run time: 25 min

Flow rate: 1.6 ml/min

Column temperature: 30° C

Preparation of mobile phase

Acetonitrile and water were mixed in the ratio of 60:40 and 1 ml of Trifluoroacetic acid is added and is filtered through membrane filter, degassed in a sonicator for 15 minutes.

Preparation of standard solution

A stock solution of Diltiazem was prepared by weighing 5 mg of the reference standard transferred into 10 ml volumetric flask and diluted to volume using methanol

Preparation of sample solution

Weigh and powder 10 tablets, weight accurately a quantity of powder equivalent to 300 mg of Diltiazem and transferred it into a clean 1000 ml standard flask. Add few ml of methanol and dissolved, make up the volume with methanol. The solution is sonicated for 15 minutes and filtered through membrane filter, and marked as sample stock solution.

Method validation

Once the method development was over the method was validated as per ICH guidelines (ICH Guidelines; 2006). The parameters like Linearity, precision, accuracy, specificity, ruggedness, robustness, stability, repeatability etc. are performed. For all parameters % relative SDV were calculated

Linearity

Linearity was demonstrated by analyzing six different concentrations of the standard solutions of Diltiazem and was found to be linear in the range of 50-150 µg/ml. Coefficient of correlation was 0.9987. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area against concentrations of Diltiazem ad regression equation was computed. The results were tabulated in table 1. The slope, intercept and correlation coefficient were found to be 2.11, 1335 and 0.998 respectively. This shows an exceptional correlation subsists between concentration of the drug and the peak area within the

concentration range as mentioned above regression graph was mentioned at Fig: 2

Precision

Precision of the method was determined by carrying out three independent samples prepared at three different concentrations as described in sample preparation above. The percentage RSD was found to be less than 1% for interday and intraday, which demonstrates that the method developed is precise. The results are tabulated in table 2

Repeatability

Ten injections of standard solution of Diltiazem was injected repeatedly and the corresponding peak areas are recorded. The % RSD was calculated and found to be within limits i.e. less than 1%. The results are tabulated in table 3.

Specificity

The percentage of the organic solvent, the pH of the buffer solution flow rate etc. where changed and there were no additional peaks found. The RT of the peak was changing but no additional peaks found.

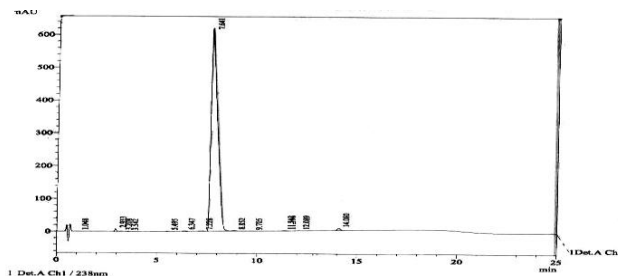


Fig 1 Chromatogram showing the peak of Diltiazem

System suitability

A system suitability test was performed to ensure the validity of the method developed. Six injections, 25 ul of the standard solution containing 75 ug/ml was injected and the data are collected. The system suitability was evaluated by the parameters like asymmetry, number of theoretical plates, RT and area.

Limit of Detection and Limit of Quantification

The LOD and LOQ is determined by injecting progressively low concentrations of the standard. LOD is the minimum concentration that gives a peak (signal to noise ratio of 3) of the analyte and LOQ is the minimum measurable concentration (signal to noise ratio of 10). The LOD was 15 µg/ml and LOQ was 25 µg/ml for Diltiazem.

Assay

The analysis of the tablet form of Diltiazem containing 10mg was carried out by the proposed HPLC method against the reference standard.

RESULT AND DISCUSSION

The proposed RP HPLC method was found suitable for the estimation of Diltiazem in solid dosage form. A chromatographic peak of the proposed drug is shown in fig. 1 where it shows a well separated symmetrical peak at 7.51 min. and this proves that the method to be a rapid and the best chromatographic conditions were adequately selected.

Diltiazem show linearity in the range of 50 to 150µg/ml, and the coefficient was found to be 0.998 which shows an excellent linearity of the method as shown in fig 2. Recovery studies were done at the levels of 50 and 100%. The stability in refrigerator and room temperature was found to be 10 hrs and 4 hours respectively. The low recovery and low % RSD shows the reproducibility and accuracy of the method. The intraday precision and interday precision was found to be within limits for conc. 75, 115 and 150 µg/ml.

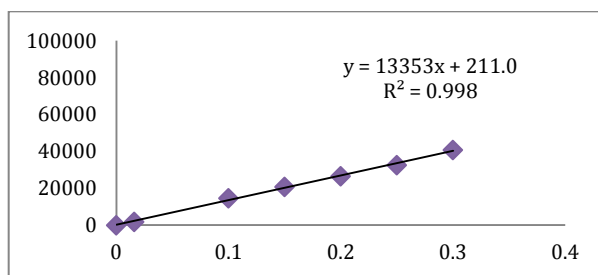


Fig 2: showing the linearity of Diltiazem.

Table 3: Inter day precision

Conc. (ng/ml)	Area	%RSD
75	11497	0.2464
	11488	
	11541	
115	15031	0.2932
	15041	
	15112	
150	21025	0.3031
	20898	
	20958	

Table 3: Intraday precision.

Conc. (ng/ml)	Day	Area	%RSD
75	1	10967	0.4490
		10983	
115	2	10891	0.3123
		15202	
		12171	
150	3	15109	0.3932
		21925	
		22001	
		21829	

Table 4: Repeatability.

Sample	Concentration	Peak area	% RSD
1	115	159880	
2	115	158589	
3	115	157981	
4	115	159749	
5	115	158941	
6	115	159561	0.609
7	115	159757	
8	115	159086	
9	115	159987	
10	115	158123	
11	115	159990	
12	115	159880	

Table 6: Assay.

Amount of drug		Label claim
Labeled	Estimated	
30 mg	29.58 mg	98.6%

CONCLUSION

A suitable and rapid RP-HPLC method was developed and the method was validated. The developed method was also used for the estimation of the drug in tablet dosage form. The validation data shows that the method is accurate, robust and linear. Low interday and intraday percentage RSD shows excellent recovery. This shows that this method is simple, accurate and rapid and can be used for the estimation of Diltiazem in bulk as well as in solid dosage forms.

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