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RESEARCH ARTICLE

B.Lakshmi et al, The *Experiment, January. 2013 Vol. .6(4), 365-371*

A NOVEL RP-HPLC METHOD FOR THE DETERMINATION OF DILTIAZEM IN PHARMACEUTICAL DRUG PRODUCTS

ABSTRACT

High resolution RP-HPLC method has been developed for the simultaneous determination of Diltiazem in pharmaceutical dosage forms. HPLC analysis was carried out by using a Chromosil C18 column (250 mm x 4.6 mm, 5µm) column. with the Isocratic mobile phase composed of Methanol:Acetonotrile (90:10v/v) and P_H is adjusted to 5.5 with 1% OPA. Mobile phase was run at 1ml/min with UV Detection wavelength at 245nm. The retention times of Diltiazem is 3.02 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness.The assay was linear over the concentration range of Diltiazem as 0.39ppm to 50ppm respectively. Limit of detection and limit of quantification were found to be 0.009ppm and 0.003ppm respectively and recovery of Diltiazem from tablet formulation was found to be 99.3%,99.6%, 101.33%. The proposed method was successfully applied for the quantitative determination of Diltiazem in tablet formulation.

KEY WORDS Diltiazem ,HPLC, Linearity, Validation.

INTRODUCTION

Diltiazem is in a group of drugs called calcium channel blockers belongs to a non-dihydropyridine (non-DHP class of compounds. Diltiazem is used to treat hypertension (high blood pressure), angina (chest pain), and certain heart rhythm disorders[1,2]. It works by relaxing the muscles of your heart and blood vessels. . By blocking the entry of calcium, Diltiazem decreases the force of contraction of the heart and its rate of contraction. It also relaxes the muscles surrounding the arteries, allowing the arteries to widen (dilate).

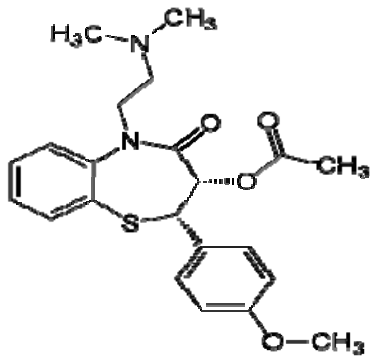

Fig 1: Diltiazem

IUPAC name : cis-(+)-[2-(2-dimethylaminoethyl)-5-(4-methoxyphenyl) -3-oxo-6-thia-2- azabicyclo[5.4.0]undeca-7,9, 11-trien-4-yl]ethanoate.

Methods for the simultaneous determination of Diltiazem in biological samples[3-11] and in pharmaceutical preparations[12,13] were also reported. Literature survey reveals the report of there was very few HPLC methods have been reported for simultaneous estimation of Diltiazem in pharmaceutical dosage form, which prompted to pursue the present work. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of Diltiazem.

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MATERIALS AND METHOD

Apparatus and Chromatographic conditions

Chromatographic separation was achieved on a A Series 200 HPLC system PEAK LC7000 equipped with isocratic HPLC with PEAK 7000 delivery system. PEAK LC7000 UV/Visible detector with soft ware, Rheodyne manual sample injector with switch (77251),Analytical column Chromosil 100-5 C18. 250×4.6mm, Electronic balance-DENVER (SI234) was used for separation. Injection volume is 20µL and UV absorbance measured at 245nm. Mobile phase consisting of Methanol : Acetonitrile (90:10 v/v) p_H adjusted to 5.5 with 1% Ortho Phosphoric acid. The mobile phase was filtered through a 0.45µ membrane filter and sonicated for 15min.

Reagents and Solutions

Pure (not less than 98.5%) standards of all active ingredients, HPLC grade acetonitrile and Methanol were used for this study. All other reagents used in this study were of AR grade. HPLC grade methanol, Acetonitrile was purchased from E. Merck (Mumbai, India).

Standard solution

Weighed accurately 50mg of pure standard and transferred into 100 ml of volumetric flask, dissolved the contents with 50ml of diluent, sonicated for 15min and diluted to 100ml volume with diluents. The above resulting solution diluted in to a suitable volumetric flask (50ppm for each active ingredient).

Sample solution

Market available dosage form were analyzed with a concentration of 50ppm for each ingredient.

RESULTS AND DISCUSSIONS

Method development

Method development trials were performed with different buffer salts, organic modifiers and columns. Finally the separation was achieved with Methanol: Acetonitrile (90:10 v/v) p_H adjusted to 5.5 with 1% Ortho Phosphoric acid. Standard solution represented in figure-2. The active ingredient were well separated and the peak shape, resolution (not less than 5.0) and tailing factor (not less than 1.5) were also within the limit.

System suitability

System suitability parameters were established by injecting the freshly prepared standard solution (each active 50ppm/five replicate injections) in to the chromatographic system. Calculated the percent relative standard deviation for peak area and retention time and results found to be satisfactory. System suitability results tabulated in table-1.

Method validation

Validated the finalized method as per ICH and FDA [14- 17] guidelines with parameters like specificity, precision, accuracy, linearity and range, ruggedness, robustness etc.

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Specificity

Different forced degradation studies were performed with acid, alkali, peroxide, UV and photo degradation conditions. The sample was passed the purity test. The purity angles for drug components in all stress conditions were found to be less than the threshold angle and no interference was observed with diluent and placebo.

Precision

Precision was evaluated by carrying out six different sample preparations for all individual and combination products. Percentage relative standard deviation (% RSD) was found (RSD-0.511) to be less than 1% for within a day and day to day variations, which proves that the developed method is precise. Results were tabulated in Table-2.

Linearity

The linearity of method was evaluated by analyzing different concentrations (0.39ppm to 50ppm for each ingredient) of the standard solution. Calibration graph was plotted against peak area and concentration of solution. The Intercept value (0.998) found to be within the limit 0.999. The linearity linearity results tabulated in table-3 and linearity plots were represented in graph-1.

Accuracy

Accuracy of the method was carried out with a known quantity of the pure drug was added to the placebo sample at the levels of 50%, 100% and 150% of the test concentration. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The mean recoveries were (101.33, 99.3, 99.68**)** in range of 98.0-102.0 % which shows that there is no interference from excipients. Table-4 represents the recovery results.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different different analysts using different columns of similar types. The percent RSD of six different preparations assay values with two different analysts and columns were 1.6- 0.9, 1.8- 1.3 respectively.

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as flow rate and column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The robustness limit for mobile phase variation, flow rate variation and temperature variation were well within the limit, which shows that the method is having good repeatability under given set of conditions and results were within the limit. Robustness results were tabulated in table-5.

CONCLUSION

The complete study results reveals that the developed and validated method has applicable for the determination of Diltiazem in pharmaceutical drug products. The developed method has potential application for all ingredients and applicable for routine quality control analysis.

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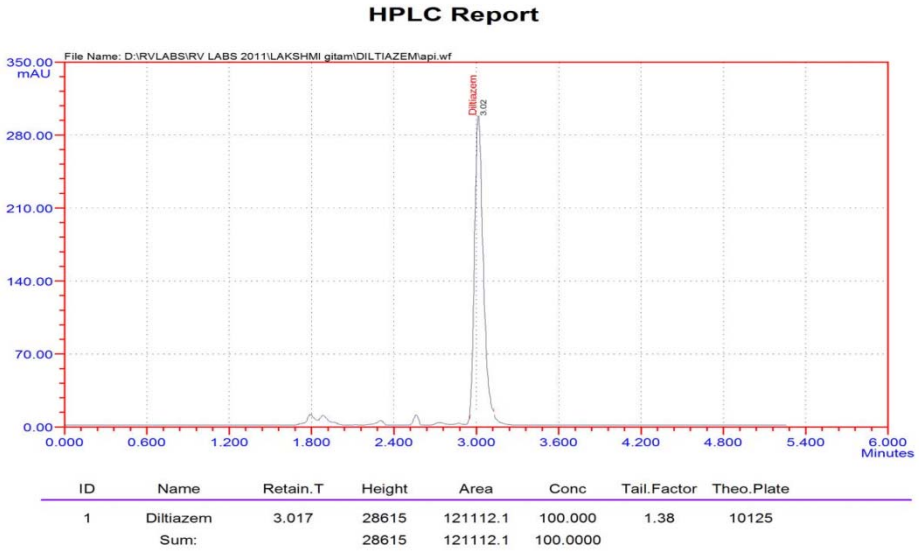

Fig 2 : Chromatogram of Diltiazem API

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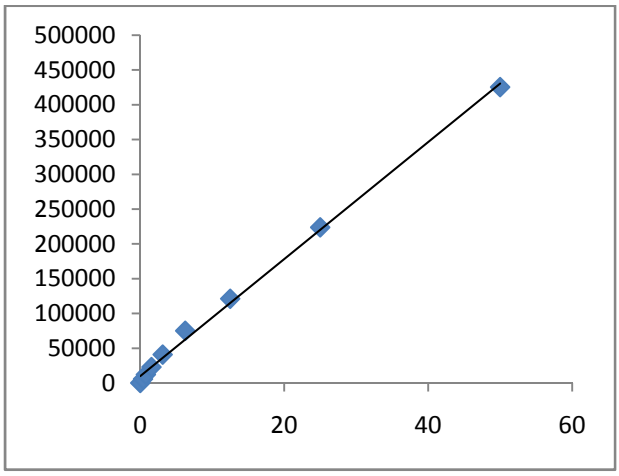
Parameter	**Diltiazem**
Retention time	3.02 min
Tailing factor	1.32
Theoretical plates	10124

Table 1: Statistical analysis of parameters required for system suitability testing of the HPLC method

TEST.1	PRECISSION 1			
	CONC 5 ppm			
INTRA	INJECTION	AREA		R.S.D = 0.511
	1	58695		
	2	58391		
	3	58564		
	4	58449		
	5	58147		
	6	58073		
	7	57851		

Table 2: Statistical analysis of parameters of Precision.



Graph 1: Linearity.

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TEST-2	LINEARITY			
	S.NO	CONC ppm	AREA	INTERCEPT = 0.9989 SLOPE = 8353.40 C.C = 11852.49
	1	0.39	5644	
	2	0.78	12062	
	3	1.56	22901	
	4	3.12	40852	
	5	6.25	75034	
	6	12.5	121112	
	7	25	223757	
	8	50	425457	

Table 3: Statistical analysis of parameters of Linearity.

Amount taken ppm	**Amount added ppm**	**Amount found ppm**	**% Recovery ± S.D(n=3)**
10	**5**	**15.20**	**101.33**
10	**10**	**19.86**	**99.3**
10	**15**	**24.92**	**99.68**

Table 4: Data of recovery study for Diltiazem by HPLC method

Parameter	**System suitability**	
	Tailing factor	**Percent (%) RSD**
Standers solution	1.0-1.4	1.2-1.0
Flow Rate		
+0.1mL per min	1.2-1.0	1.3-1.2
-0.1mL per min	0.9-1.5	0.9-1.2
pH		
5.7	1.1-1.0	1.0-0.9
5.3	1.2-1.1	1.1-0.7

Table-5: Robustness Results.

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