ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DILTIAZEM HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP- HPLC

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RESEARCH ARTICLE

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ABSTRACT

A simple, specific, precise and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the estimation of Diltiazem Hydrochloride in bulk and pharmaceutical dosage form. The chromatographic determination was performed on isocratic high performance liquid chromatography system of Agilent model no.1220. The separation was conducted by using column of Zorbax [C_8 (5µ, 4.6 mm×250)] with mobile phase consisting of buffer and Acetonitrile in the ratio of (60:40). The mobile phase was delivered at the flow rate of 1.0ml/min. The eluent was monitored at wavelength 240 nm and found a sharp and symmetrical peak with retention time of 4.66min. The method was validated for linearity, accuracy, precision, specificity, robustness. Recovery of Diltiazem Hydrochloride was found to be in the range of 98%-102%. The method was found to be linear over the concentration range 50-150 µg/ml with coefficient correlation r^2 = 0.995. After developing method, validation parameters were carried out successfully and obtained results were complied with USP monograph.

Keywords: Diltiazem Hydrochloride; Precision; Accuracy; Method Validation; Reverse phase chromatography.

INTRODUCTION

Diltiazem Hydrochloride is a calcium channel blocker, which has been used in the treatment of various cardiovascular disorders, particularly angina pectoris and systemic hypertension. Chemically it is 1,5-Benzothiazepin-4(5*H*)-one,3-(acetyloxy)-5-[2-(dimethylamino) ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride. (1)

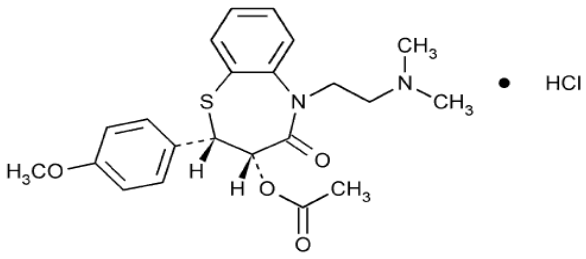

Figure 1 Structure of Diltiazem Hydrochloride

Literature survey revealed that methods have been developed and reported for estimation of Diltiazem HCL in bulk and in tablet dosage form and in combination also but no method was developed for estimation of Diltiazem HCL in capsule dosage form. The reported methods are HPLC and UV, (2-6) FT Raman spectroscopy, (7) Diltiazem HCl drug with combination of other therapeutic agents HPLC and UV (8-11) and Electrophoresis method. (12)

The present study was aimed to develop simple, precise, specific, accurate, linear, robust and less time consuming method.

MATERIALS AND METHODS

Diltiazem Hydrochloride (pure drug) was gifted by Wockhardt, Aurangabad. The formulation of Diltiazem Hydrochloride capsule USP containing 90 mg Diltiazem Hydrochloride was procured from local market.

Chemicals and Reagents:

Potassium monobasic phosphate(Analytical grade), Methanol (HPLC grade), Acetonitrile

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(HPLC grade), Triethylamine (Analytical grade), and Water (HPLC grade) were purchased from Research –Lab Fine Chem Industries, Mumbai.

Experimental Conditions:

Quantitative HPLC was performed on isocratic HPLC of Agilent model no. 1220 with Ezchrom elite software G 4286B-1220 infinity isocratic LC manual injector with variable wavelength detector. For method development several trials were carried out. After many trials, the chromatographic conditions were decided. The separation was conducted by using column of Zorbax [C_8 (5µ, 4.6 mm×250)] with mobile phase consisting of buffer and Acetonitrile in the ratio of (60:40). The mobile phase delivered at the flow rate of 1.0ml/min. The eluent was monitored at wavelength 240 nm and found a sharp and symmetrical peak with retention time of 4.66 min. The run time observed was 10 min.

Preparation of standard solution:

Transferred an accurately weighed quantity of about 60 mg of Diltiazem hydrochloride into a clean dry 50 ml volumetric flask & methanol was added to dissolve the content and make up the volume with methanol, 1ml of resulting solution was diluted to 50ml with methanol. (12 µg/ml)

Preparation of sample solution:

Accurately Weighed 5 Capsules and transferred the powder of capsule 213.28 mg. Grinded the contents thoroughly, and transferred an accurately weighed portion, equivalent to about 60 mg of Diltiazem hydrochloride to a 100 ml volumetric flask. Added methanol and make up the volume of volumetric flask, and shake by mechanical means for 30 minutes. Sonicated the resulting solution for 10 minutes to complete the extraction and then diluted 1.12 ml of resulting solution to 100 ml methanol (12µg/ml).

Diluent:

The methanol was used as diluent.

Method Validation

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, etc

System suitability test:

Standard solution of Diltiazem Hydrochloride injected five times into the HPLC system. The system suitability parameters were evaluated and found to be within the limits.

Precision:

The Precision of the method was demonstrated by system precision, method precision and intermediate precision. In system precision, the standard solution injected five times as per procedure. In method precision six replicate injections of the standard solution and sample solution prepared as per the proposed method and chromatograms were recorded. In Intermediate precision was performed on different day by using different column of same dimensions.

Accuracy:

Accuracy is calculated for the percentage recovery by the assay of the known amount of analyte in the sample or as the difference between the mean and the accepted true value together with confidence intervals. For assay method, spiked samples are prepared in triplicate at three intervals over a range of 50-150 % of the target concentration**.**

Linearity:

It is the ability of an assay to obtain test results, which are directly proportional to the concentration of an analyte in the sample. For the establishment of linearity, a minimum of 5 concentrations required. For assay of a drug substance or a finished product 50-150 % of the concentration should be taken.

Specificity:

Specificity study was carried out by injecting blank, standard and sample solution of

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Diltiazem Hydrochloride. It showed no interference of standard and sample in the blank preparation.

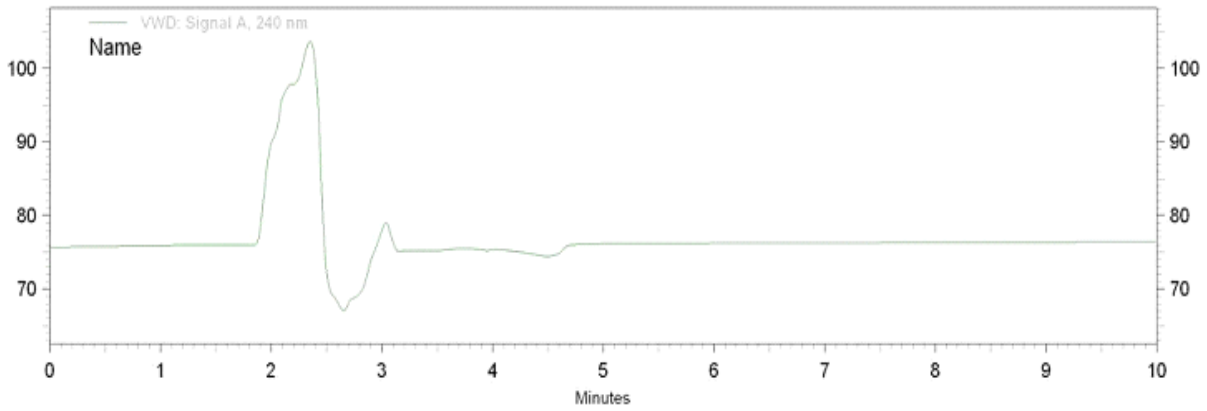

Figure 2: Chromatogram for diluent

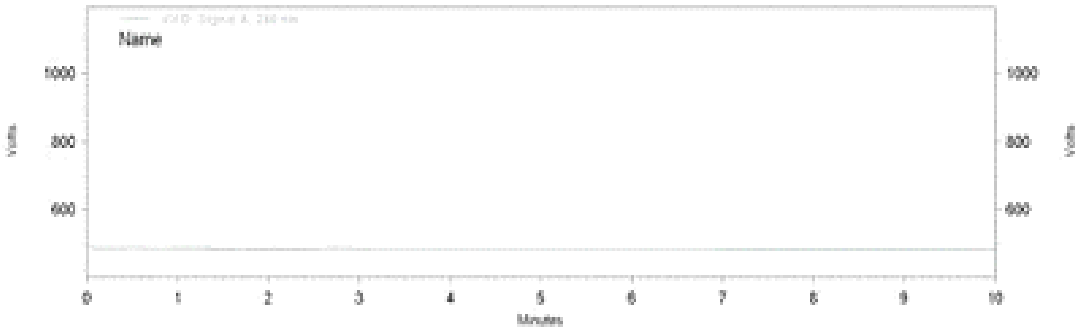

Figure 3: Chromatogram for blank

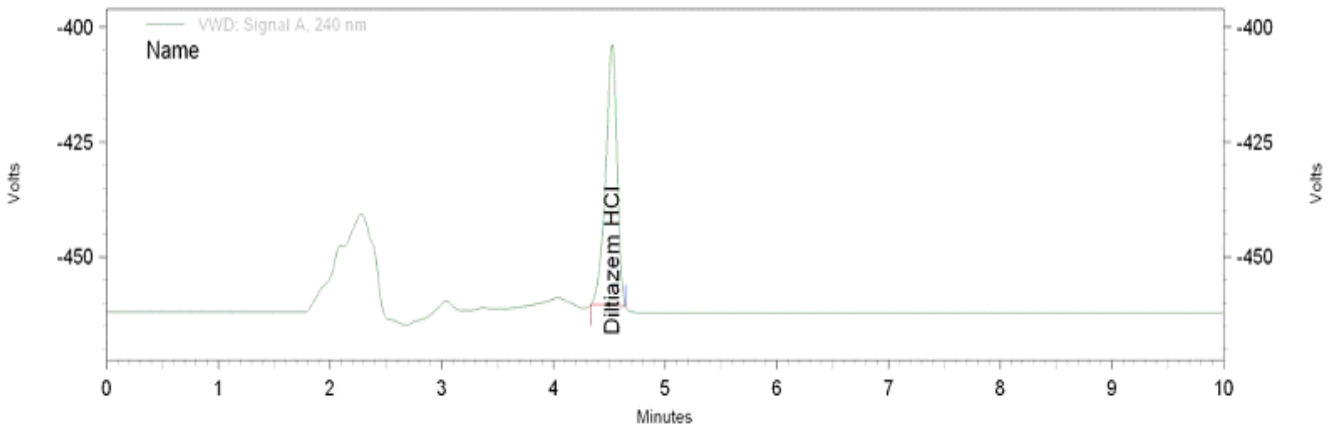

Figure 4: Specificity Standard chromatogram of Diltiazem Hydrochloride

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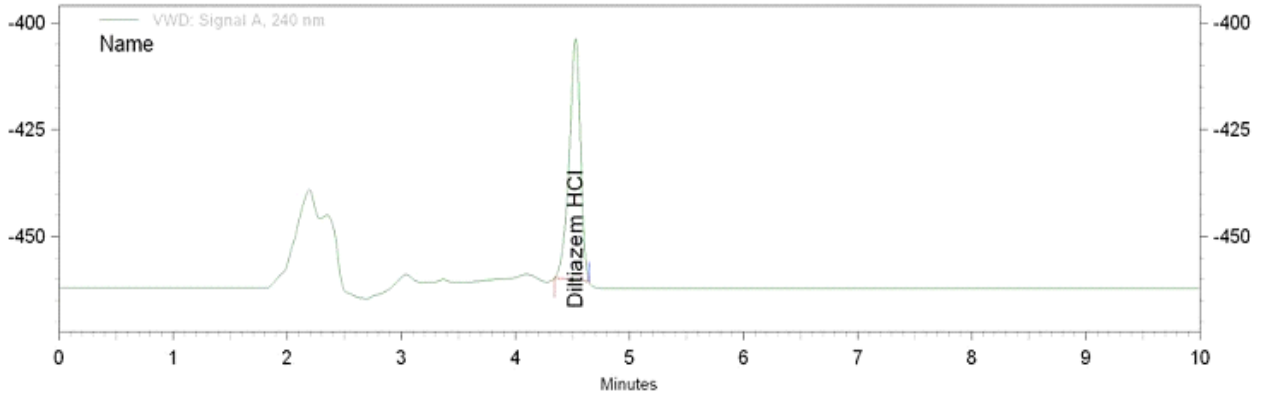

Figure 5: Specificity Sample chromatogram of Diltiazem Hydrochloride

Robustness:

The robustness of the methods was determined by performing the assay of the triplicate by deliberately alternating parameters and that the results are not influenced by different changes in the above parameters.

Change in wavelength: ± 2nm

Change in flow rate: ± 0.2ml/min.

Change in pH: ± 0.2

Stability of analytical solution:

Evaluate the stability in analytical solution by injecting the standard preparation and sample preparation of Diltiazem Hydrochloride at regular interval. The stability of solution is carried out after 0, 3, 6, 12, 24, hrs.

RESULTS AND DISCUSSION

For the development of accurate and precise method for the quantitative estimation of Diltiazem Hydrochloride in bulk and pharmaceutical dosage form several trials were carried out. After many trials, the chromatographic conditions were finalized. The separation was conducted by using column of Zorbax [C_8 (5µ, 4.6mm×250)] with mobile phase consisting of buffer and Acetonitrile in the ratio of (60:40). The mobile phase was delivered at the flow rate of 1.0ml/min. The eluent was monitored at wavelength 240 nm and found a sharp and symmetrical peak with retention time of 4.66min. The run time was 10 min.

Table 1: Result of Assay

Sample	Amount taken	Area	Assay
Standard	60 mg	836126	98.44%
Sample	213mg	864991	

Table 2: Results of System suitability Test

Sr. No	Sample	Area	R.T.	Theoretical plates	Tailing factor
1.	Blank	-	-	-	-
2.	Std. 1	857171	4.643	12643	1.08
3.	Std. 2	874529	4.653	15479	0.73
4.	Std. 3	860542	4.650	12840	1.08
5.	Std. 4	884768	4.653	8631	0.82
6	Std. 5	897971	4.660	8701	0.84
Average		**874996.2**	**4.651**	**11658.8**	**0.91**
%RSD		**1.93**			

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The blank, standard solution and sample solution are injected. There should be no interferences of standard and sample in blank preparation

Table 3: Results of System Precision

Sr. No	Sample	Area	R.T	Theoretical plates	Tailing factor
1.	Std. 1	934671	4.627	7740	0.80
2.	Std. 2	950554	4.627	8328	0.86
3.	Std. 3	934881	4.630	8432	0.82
4.	Std. 4	956217	4.627	7653	0.81
5.	Std. 5	908143	4.627	7823	0.82
6	Std. 6	932430	4.630	7574	0.82
Average		936149	4.628	7925	0.82
% RSD		1.79			

The precision was performed to check for the consistent results and which are in the limits. The method and intermediate precisions are showing the results within the limits.

Table 4: Results of Method Precision and Intermediate Precision

Sr. No	Standard (% assay)	Sample (% assay)
1	99.23	101.69
2	99.65	98.66
3	98.56	99.72
4	98.89	99.05
5	99.12	98.78
6	99.63	100.66
Average	**99.18**	**99.76**
%RSD	**0.42**	**1.26**

Table 5: Results of specificity

Sr. No	Sample	Area	R.T	Theoretical plates	Tailing factor
1.	Blank				
2.	Std. 1	897971	4.660	8701	0.84
3.	Std. 2	900018	4.673	9011	0.87
4.	Std. 3	890884	4.680	8074	0.83
5.	Std. 4	896700	4.687	8005	0.82
6	Std. 5	894887	4.627	8421	0.82
Average		**896092**	**4.665**	**8442**	**0.836**
%RSD		**0.38**			

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Table 6: Results of Linearity

Concentration (µg/ml)	Peak Area
6	3119245
9	5438299
12	6925899
15	8684575
18	9735335

The proposed method was found linear and the range is 6µg/ml to 18µg/ml and correlation coefficient is 0.998.

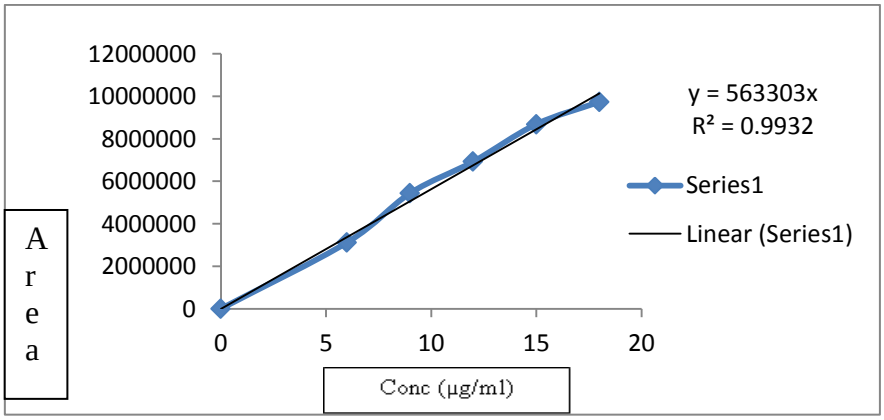

Figure 6: Graph of linearity

The accuracy of the method was determined by the recovery studies, carried out at different levels 50%, 100% and 150%.

Table 7: Results of Accuracy

Spike Level in %	Area	Amt. Added	Amt. Found	%recovery	Mean	SD	%RSD
50%	4142188	0.006	0.0060	100.66	98.89	1.55	1.57
	3938906	0.006	0.00585	97.70			
	4048460	0.006	0.00597	98.33			
100%	5110526	0.0012	0.00122	101.66	101.38	0.47	0.472
	5285208	0.0012	0.00121	100.83s			
	5314775	0.0012	0.00122	101.66			
150%	8651678	0.0018	0.00177	98.33	98.51	0.32	0.32
	8722465	0.0018	0.00178	98.89			
	8519110	0.0018	0.00177	98.33			

Results of stability solution

Evaluate the stability in analytical solution by injecting the standard preparation and sample preparation at regular interval.

Table 8: Results of stability solution

Stability in hours	% Assay
0	98.50
3	99.90

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6	99.93
12	101.25
24	101.75
Average	**100.26**
%RSD	**1.27**

CONCLUSION

The developed method was found to be simple, accurate, precise, specific and robust and this method can be applied for routine quantitative analysis of Diltiazem Hydrochloride in bulk and pharmaceutical formulations like capsule.

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CONFLICT OF INTEREST

Author declares that there are no conflict of interest.

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