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ORIGINAL ARTICLE

Method Development, Validation and Stability Study of Diltiazem by RP-HPLC Method

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ABSTRACT

Background: The main goal of this experiment was to create and test a reliable and fast HPLC method for measuring the amount of Diltiazem, a drug that lowers blood pressure, in large quantities and to conduct stability studies. **Methods:** For the method development we use column of octa decyl silane which was bonded to porous silica with particle size 5μ ODS C-18 (250×4.6mm, 5μm), methanol and water mixture v/v proportion (90:10) used as the mobile phase, a sample of 25 μL was inlet into the column and the outlet comes out at 1ml/min flowing capacity. The Peak of analyte was detected at a retention time of 2.037 min. **Results:** A perfect positive linear relationship between the concentration (10-70μg/mL) and correlation coefficient of 0.999 for Diltiazem was showed by the calibration curve of Diltiazem. This method had a LOD value of 1.276 and a LOQ value of 3.86 respectively. The absolute recovery was 96.52% for Diltiazem tablet. **Conclusion:** The assay was stability-indicating because the degradation product from stress studies does not affect the detection of diltiazem.

Keywords: Diltiazem; RP-HPLC; Methanol; Validation

INTRODUCTION

Method development measures the concentration of Active pharmaceutical ingredient (API) in a dosage form. During the process of development of drug, method development process can also be determining the multitude of constituent in formulation, Method validation is to verify the appropriateness of the analytical method used for a particular test, method validation is performed. [1,2] The outcome of method validation can help evaluate the accuracy, dependability and uniformity of the analytical data. Analytical Method Development aims to provide valuable data on the drug's strength, shelf life and impact. [3–5]

Diltiazem used as in the medications of different heart diseases like Hypertension and Angina by blocking the calcium channel. Diltiazem is chemically known as [(2S,3S)-5- [2-(dimethyl amino) ethyl]-2-(4- methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepin-3-yl] acetate and having a 414.5gm/mol of molecular weight. The physical properties of Diltiazem are white colour and crystalline powder in nature. The solubility of Diltiazem is possible in case of chloroform formic acid, water, and methanol but in case of ether it is insoluble in nature.

Based on pharmacokinetic activity Diltiazem taken by orally absorbed by git, having bioavailability 40-50%, diltiazem about 80% bound to plasma protein having half-life 3-4hrs. The liver metabolizes Diltiazem extensively in the first pass, and CYP3A4 mainly causes N-demethylation.

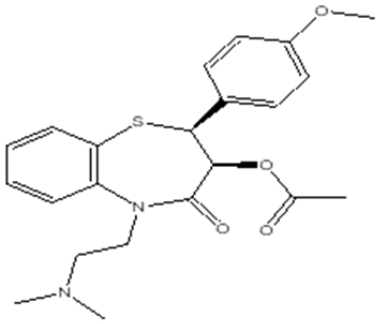

Fig. 1: Chemical structure of Diltiazem

KRUPANIDHI COLLEGE OF PHARMACY

During depolarization diltiazem inhibit the influx of calcium ion into the smooth muscle, decrease intra cellular calcium concentration and increase smooth muscle relaxation resulting vasodilation occur so, the myocardial ischemia occur.as a result both the Heart rate and blood pressure also reduced. [6–8]

Literature survey reveals few HPLC and UV method for the method development and validation. In the present investigated we try to develop a stability indicating assay method for Diltiazem that will analyse the drug with simple solvent system and in a cost-effective manner at reasonable pH, so the column will not be affected. [9–23].

MATERIAL METHOD AND EXPERIMENTAL WORK [24,25]

Drugs:

Pure Pharmaceutical sample of Diltiazem was obtained from sun Pharma Technologies Pvt Ltd, Diltiazem Tablet (DILZEM-30, Conventional release) containing Diltiazem Hydrochloride (30mg, Torrent Pharmaceuticals LTD) were procured from local market of Berhampur.

Chemicals:

Hydrogen peroxide, chloroform, NAOH, Methanol from Loba chemicals. Pvt. Ltd., India, Water from Royal college of pharmacy and health sciences laboratory, India

Instrument:

HPLC Shimadzu Japan Pump LC 20-AT, Detector SPD20A, syringe Hemilton Rheodyne- 25μl, column Shimadzu Japan (250×4.6mm, packed with5μ), UV-Vis Spectroscopy Shimadzu, Japan UV1700, Digital electronic balance Shimadzu Japan xp205, ph. meter Thermos fisher scientific USA Orion 5star, ultrasonic bath sonicator Pci Mumbai Rk102 CH Liter3,0, Hot air oven York scientific industry pvt. ltd., India, FTIR Perkin Elmer (spectrumV5.3.1) Different scanning calorimetry (DSC-60, Shimadzu, Japan)

Chromatographic Condition:

Using a reverse phase octadecyl bonded silica gel C18 column, the solvent use for mobile phase was methanol with water (90:10) was equilibrated at a flowing capacity of 1ml/min. The eluent was detected at 236nm for Diltiazem. A 25μl sample was inserted with a fixed loop and the run time was 10min. This procedure was repeated daily for six days, and the outcomes were expressed as standard deviation and %RSD. [25]

Standard stock solution and mobile phase preparation method:

To prepare the mobile phase of total 300 ml, HPLC-grade methanol mixed with Mili-Q Water solution to a (90:10) v/v proportion respectively. Then the sonication of mobile phase was occurred for 10min. In a 10ml volumetric flask the powder drug was dissolve in the mobile phase and volume make up to the mark. [25]

From the prepared stock solution 1ml was taken and 10ml volume adjusted by adding mobile phase to get 100ppm concentration. The standard stock solution of 1000ppm of Diltiazem was prepared by dissolving 10mg of pure powder drug in mobile phase, in10mlvolumetric flask and the volume was made up to the mark. From the prepared stock solution 1ml taken and volume adjusted to 10 ml by adding mobile phase to get 100ppm concentration.

The 1000ppm conc. of standard solution was prepared by taking 1ml of solution from the mobile phase and dissolved with 10mg of pure powder drug of diltiazem. then the volume makes of up to the mark of 10ml volumetric flask. 1ml was taken from the above stock solution and volume makeup up to the mark of 10ml volumetric flask by adding the mobile phase then we got 100ppm concentration.

Calibration curve of Diltiazem:

The aliquots amount of stock solution of drug was taken in a 10ml volumetric flask and the volume was made up to the label by adding the mobile phase in a serial manner of 10-50μg/ml. Chromatograph was made under this above concentration by injecting the 25μl sample. Then the drug evaluation carried out and the area of peak was recorded at 236nm. The calibration curve was Plotted by taking the peak area on the x-axis and the concentration on the y-axis.

Assay Procedure:

Weighed accurately 6 tablets were used to calculate the average weight. After crushing, tablet powder equivalent to 180mg of diltiazem and 25mg of that was transfer to a 250ml conical flask. About 100ml methanol was added and sonicate for 30min, with continuous shaking (maintaining the temperature of sonication below 20^0C). The volume was made up to the mark with diluent and mixed. The solution was filtered through a 0.45μm PVDF filter. The filtrate was collected, with the top few millimetres discarded. Prepared 10ppm of that solution and again added in 100ml of diluents to obtain a typical chromatogram.

IR Analysis: [26]

The FTIR spectra of Diltiazem, carrier, and their binary solid dispersions (Spectrum BX) were captured by a Perkin Elmer FTIR spectrophotometer. Sample was mixed with spectroscopic grade potassium bromide and compressed

into discs using a hydraulic press before being scanned from 4000 to 500 cm^{-1}.Data analysis software from Perkin Elmer was employed. (spectrum V5.3.1).

Differential Scanning Colorimetry (DSC):[27]

The thermal behaviour of Diltiazem was examined using DSC. To obtain the spectra of DSC the instrument used was DSC-4000, PERKIN ELMER, all the samples weight 5mg and placed in an aluminium pan then to cover it place an aluminium lid, the scanning takes place at a temp rate of 40^0C/min from 30^0C to 300^0C.

VALIDATION OF RP-HPLC[28–30]

- **Accuracy:** The method's accuracy of the suggested approach was assessed for determining the standard drug by the percent recovery of the drug using the standard addition recovery method, The pre quantified sample solution were introduced that equivalent to 60%, 80%, 100% of the label claim. To estimate the quantity of drug the peak areas were then measured and fitted to the calibration curve's straight-line equation.[26]
- **Precision:** The Precision studies of the drugs were conducted by analysing the equivalent responses of single sample solution for six consecutive days, whereas inter-day studies were conducted six times on the same day for a single sample. and over six days period respectively. We determined the mean, standard deviation, and percent relative standard deviation (%RSD).[28]
- **Specificity :** Diltiazem was spiked with different concentration of excipient mix (magnesium stearate) and the sample was analysed for the % recovery.
- **Robustness**: By purposefully modifying analytical parameters such as the methanol content (90±2%) and flow rate (0.5±0.1 ml/min), the robustness approach is analyzed.[27]
- **Ruggedness:** The ruggedness is the result obtain variety of different condition and different method, which is denoted by %RSD. Here Different laboratory and different analysts is also included.[28]
- **Sensitivity:** The method's sensitivity was assessed in relation to LOD and LOQ, Detection Limit, and Quantification Limit. Plotting of calibration curves was done using drug concentrations within the predicted detection limit range (0.1–5μg/ml). The following formula was used to find the detection limit and quantification limit by substituting the standard deviation of the regression line's y-intercept. The quantification limit is 9.5 10 σ/s, and the detection limit is 2.8 σ/s. The standard deviation of the regression line's y-intercept is represented by σ, while the calibration curve's slope is denoted by s.

FORCED DEGRADATION STUDIES[31–33]

Through forced degradation studies was occurs using UV, alkaline, acid, thermal, oxidative, and photolytic degradations on the sample, the specificity of the procedure may be shown. After the sample was subjected to these circumstances, the primary peak's peak purity was analysed, revealing whether or not the product peak was changing.

Degradation in Natural Condition

A minimal volume of methanol was added to a 10ml volumetric flask containing the precisely weighted 10mg equivalent drug. After that, add liquid to make up the volume and maintain the temperature at 700^0C. The solutions were made at various intervals of time, and the HPLC equipment was filled with 10 ppm of the sample solutions.

Acidic degradation

The accurately weighed 10mg equivalent drug was ingested in a 10ml volumetric flask and dissolved in minimum volume of methanol. Then the volume was made up to the mark by using 1N HCL and kept at 700^0C. The solutions were prepared at different time interval and 10ppm of the sample solutions were inserted into the HPLC apparatus.

Basic degradation

In a 10ml volumetric flask, the precisely weighted 10mg equivalent drug was taken in and dissolved in the smallest amount of methanol. The volume was then increased to the required level using 1N NaOH and maintained at 700^0C.A 10ppm sample solution was added to the HPLC equipment after the solution was made at various interval.

Oxidative Degradation

In a 10 ml volumetric flask, the precisely weighted 10mg equivalent drug was taken in and dissolved in the smallest amount of methanol. After that make volume up to the mark by using 6%w/v H2O2 and kept at 700^0C. The solutions were prepared at different time interval and 10ppm of the sample's solution were loaded into the HPLC Apparatus.

Thermal Degradation

In three different, clean petridishes,100mg 100mg of pure drugs were taken. The drugs were then dried at 700^0C, and sampling was done every 10days, 20days, 30days. Drug solutions were produced, and the HPLC apparatus was loaded with 10ppm of the sample solutions.

RESULTS & DISCUSSIONS

Optimization:

Solubility: Solubility of Diltiazem was performed using various solvents and the results are shown below.

Table 1: Solubility of Diltiazem

Solvents	Drug Solubility
Methanol	Soluble
Dichloromethane	Soluble
Acetonitrile	Sparingly soluble
Acetone	Sparingly soluble
Ethanol	Soluble
Distilled water	Slightly soluble

Method:

Determination of working wavelength:

UV-VIS spectrophotometer used to scan the drug solution of diltiazem within the 200-400 nm wavelength region against methanol- water (90:10) as blank. The maximum wavelength of absorption (λ_{max}) of Diltiazem was found to be 236nm in methanol-water (90:10). The resulting overlay spectra were shown in Figure 2.

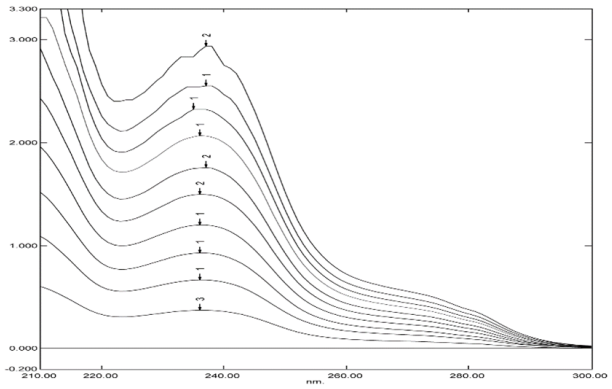

Fig. 2: Overlay UV-Vis Spectra of Diltiazem

Preparation of calibration curve:

Primary stock solution of 1000μg/ml was prepared for the preparation of calibration curve, from which a secondary stock solution of 100μg/ml was prepared. Separate concentrate was then prepared in a serial dilution manner from 5-50μg/ml and measured the absorbance at 236 nm. The concentration and corresponding absorbance were given in Table 2.

In a linearity range of 5-50μg/ml the calibration curve was plotted with regression coefficient 0.999.

Table 2: Calibration for the Diltiazem UV-VIS Spectrophotometric method

Drug Concentrations (μg/ml)	Absorbance (at 236 nm)
5	0.371
10	0.665
15	0.927
20	1.200
25	1.498
30	1.756
35	2.069
40	2.323
45	2.552
50	2.937

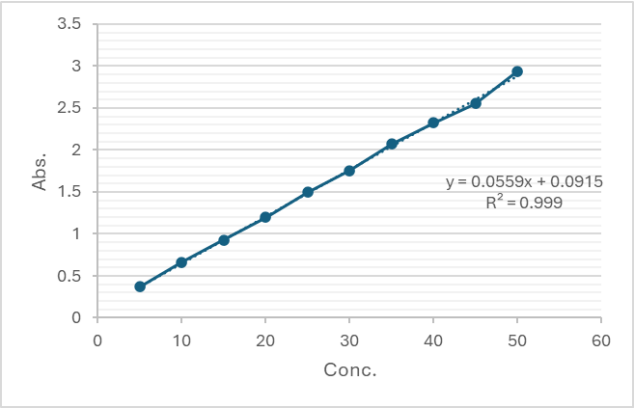

Fig. 3: Calibration **Curve of Diltiazem**

Table 3: Optical Characteristics of Diltiazem

Limit of Beer's Law	5-30μg/ml
Maximum absorption (λ_{max})	236nm
Regression Formula (Y)	Y=0.0559x +0.0915
Incline (a)	0.0559
Intercept (b)	0.0915
Correlation value (r^2)	0.999

Table 4: Results of UV-VIS Precision data for Diltiazem

Sl. No	Concentration	Abs.	Calc. Amt.	Statistical Analysis
1	25	1.498	25.161	
2	25	1.500	25.197	Mean = 25.128
3	25	1.495	25.107	SD = 0.083
4	25	1.502	25.232	%RSD = 0.332
5	25	1.490	25.017	
6	25	1.492	25.053	

Table 5: Results of UV-VIS Intraday precision data for Diltiazem

Sl. No	Concentration	Abs. 1	Abs. 2	Abs. 3	Statistical Analysis
1	25	0.148	0.149	0.146	
2	25	0.147	0.141	0.152	Mean = 24.973
3	25	0.149	0.142	0.147	SD = 0.001
4	25	0.143	0.146	0.149	%RSD = 0.153
5	25	0.142	0.148	0.141	
6	25	0.141	0.145	0.151	
Mean		0.145	0.145	0.148	
Cal. Amt. (μg/ml)		24.957	24.960	24.004	

Table 6: Results of UV-VIS Interday precision data for Diltiazem

	Mean Absorbance			Statistical Analysis
	Day 1	Day 2	Day 3	
25	0.152	0.142	0.141	Mean = 24.957
25	0.142	0.148	0.142	SD = 0.109
25	0.141	0.145	0.148	%RSD= 0.099
Mean	0.149	0.146	0.145	
Calc. Amt. (μg/ml)	25.082	24.903	24.886	

Table 7: Results on UV-VIS Ruggedness data of the Diltiazem by different Analyst

Conc. (μg/ml)	Analyst-1		Statistical Analysis	Conc. (μg/ml)	Analyst-2		Statistical Analysis
	Abs.	Calc. Amt.			Abs.	Calc. Amt.	
25	1.496	25.125	Mean= 25.119	25	1.495	25.125	Mean= 25.119
25	1.493	25.071	SD = 0.053	25	1.493	25.071	SD = 0.053
25	1.495	25.107	%RSD =0.214	25	1.496	25.125	%RSD =0.214
25	1.5	25.196		25	1.5	25.196	
25	1.498	25.161		25	1.498	25.161	
25	1.492	25.053		25	1.492	25.053	

Table 8: Results on UV-VIS Ruggedness data of the Diltiazem by different Instruments

UV-1700				UV-1800			
Conc. (μg / ml)	Abs.	Calc. Amt.	Statistical Analysis	Conc. (μg / ml)	Abs.	Calc. Amt.	Statistical Analysis
25	1.496	25.125	Mean= 25.119	25	1.496	25.125	Mean= 25.119
25	1.493	25.071	SD = 0.053	25	1.493	25.071	SD = 0.053
25	1.492	25.053	%RSD =0.214	25	1.495	25.107	%RSD =0.214
25	1.5	25.196		25	1.5	25.196	
25	1.498	25.161		25	1.498	25.161	
25	1.495	25.107		25	1.492	25.053	

Table 9: Results on UV-VIS Robustness data of Diltiazem by different solvent composition

(45:55)				(35:65)			
Conc. (μg/ml)	Abs.	Calc. Amt.	Statistical Analysis	Conc. (μg/ml)	Abs.	Calc. Amt.	Statistical Analysis
25	1.495	25.125	Mean= 25.128	25	1.496	25.125	Mean= 25.119
25	1.493	25.071	SD = 0.056	25	1.492	25.054	SD = 0.054
25	1.498	25.161	%RSD =0.222	25	1.495	25.107	%RSD =0.214
25	1.5	25.196		25	1.5	25.197	
25	1.498	25.161		25	1.498	25.161	
25	1.492	25.053		25	1.493	25.0715	

Table 10: LOD & LOQ Data of UV Method of Diltiazem

Conc. (μg/ml)	Abs.	
0.5	0.03	
1	0.058	
1.5	0.089	Mean=2.361
2	0.115	Sd=1.369
2.5	0.148	Rsd=0.58
3	0.179	LOD=1.2767
3.5	0.21	LOQ=3.86
4	0.245	

Limit of Detection (LOD) = 3.3× (SD/S) = 3.3× 1.369/0.58= 1.276
Limit of Quantification (LOQ) =10(SD/S) = 10× 1.369/0.58 = 3.86

Validation:

RP-HPLC METHOD DEVELOPMENT FOR DILTIAZEM

Optimization:

As per solubility parameter of diltiazem, the different mobile phase system individually and in combination ratios with different rate of flow and pH were tried to get a symmetric and sharp peak. Finally, Methanol:Water in the ratio (90:10) was found to be ideal mobile phase system for the drug.

Table 11: Optimized Chromatographic Conditions of Diltiazem

Parameters	Conditions
Stationary Phase (Column)	Phenomenex ODS C-18 (250 x 4.6 mm, packed with 5 micron)
Mobile phase	HPLC Grade Meth:H_2O (90:10),
Flow rate (ml/min)	1 ml/min
Run time (minutes)	10min
Column temperature (^{0}C)	Ambient
Volume of injection loop (μl)	25μl
Detection Wavelength (nm)	236nm
Drug R_t (min)	2.037min

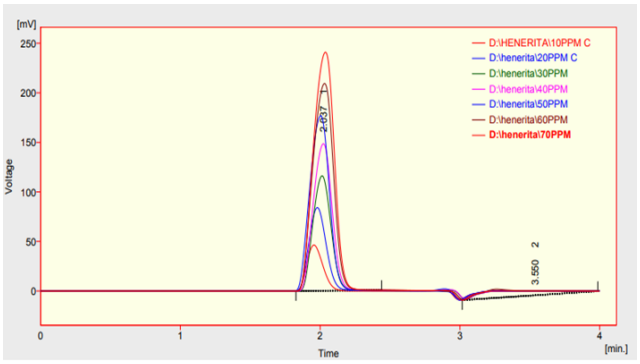

Fig. 4: HPLC Overlay Chromatogram of Diltiazem

Method:

Table 12: Calibration Table of Diltiazem for RP-HPLC Method

Drug Concentrations (PPM)	Peak area
10	377.061
20	723.101
30	1048.041
40	1394.259
50	1772.105
60	2146.728
70	2502.214

A calibration curve was plot by using the concentration on X-axis and peak area on Y-axis.

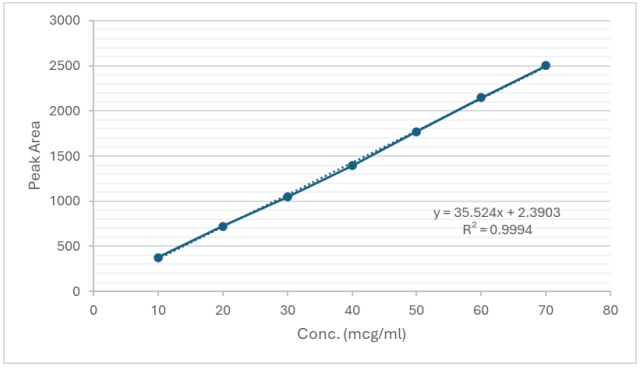

Fig. 5: HPLC Overlay Chromatogram of Diltiazem

From the calibration curve it was found that it shows linearity in the range of 10-60μg/ml with regression coefficient 0.999.

Validation:

Calculation for Percentage of Purity of Diltiazem:

For the determination of % of purity, we can calculate by formula: y = mx + c

Table 13: Results on RP-HPLC Precision Data for Diltiazem

Sl. No.	Concentration (μg/ml)	Area	Calc. Amt.	Statistical Parameter
1	30	1048.041	29.435	Mean= 29.431 SD= 0.0316 %RSD= 0.107
2	30	1047.029	29.406	
3	30	1049.091	29.464	
4	30	1048.041	29.435	
5	30	1049.029	29.462	
6	30	1046.221	29.383	

Table 14: Results on RP-HPLC Intraday Precision Data of Diltiazem

Sl. No.	Concentration (μg/ml)	Area	Calc. Amt.	Statistical Parameter
1	30	1048.041	29.435	Mean= 29.421 SD= 0.028 %RSD= 0.0959
2	30	1047.029	29.406	
3	30	1046.221	29.384	
4	30	1048.041	29.435	
5	30	1049.029	29.463	
6	30	1046.993	29.405	

Table 15: Results on RP-HPLC Interday Precision Data of Diltiazem

Sl. No	Concentration (μg/ml)	Day 1	Day 2	Day 3	Statistical Analysis
1	30	1048.041	1049.029	1048.041	Mean= 29.427 SD= 0.006 %RSD= 0.019
2	30	1047.029	1047.029	1047.029	
3	30	1049.091	1046.221	1046.221	
4	30	1048.041	1048.041	1048.041	
5	30	1049.029	1049.029	1049.029	
6	30	1046.221	1048.041	1046.993	
	Mean Peak Area	1047.909	1047.898	1047.559	
	Calc. Amt. (μg/ml)	29.431	29.431	29.426	

we got y value which is the area for 20ppm of drug sample from the Table 18, and we have to calculate x 9 conc. of drug)

X = y - c/m

By put the values we get, X = y - 2.3901/35.313

= 854.582-2.3901/35.313

= 852.191/35.313

=24.132

For 25ppm concentration of drug is and calculate for 100ppm

= 24.132/25×100

= 96.52%

So, we concluded % purity of Diltiazem (marketed drug) is 96.52%.

Table 16: Results on RP-HPLC Ruggedness Data of by Different Analysts

Analyst-1				Analyst-2			
Conc. (mcg/ ml)	**Peak Area**	**Measured quantity**	**Statistical Parameter**	**Conc. (mcg/ ml)**	**Peak Area**	**Measured quantity**	**Statistical Parameter**
30	1046.221	29.384	Mean = 29.418 SD = 0.0318 %RSD = 0.108	30	1048.041	29.435	Mean =29.421 SD =0.028 %RSD = 0.095
30	1047.029	29.406		30	1047.029	29.406	
30	1046.221	29.384		30	1046.221	29.383	
30	1048.041	29.435		30	1048.041	29.435	
30	1049.029	29.462		30	1049.029	29.463	
30	1048.041	29.435		30	1046.993	29.405	

Table 17: Results on RP-HPLC Robustness Data of the Diltiazem at different pH

pH-5.3				pH-5.7			
Conc. (μg/ml)	**Peak Area**	**Measured quantity**	**Statistical Parameter**	**Conc. (μg/m l)**	**Peak Area**	**Calc. Amt.**	**Statistical Parameter**
30	1046.221	29.383	Mean = 29.412 SD = 0.0309 %RSD = 0.105	30	1048.041	29.435	Mean = 29.426 SD = 0.027 %RSD = 0.093
30	1047.029	29.406		30	1047.029	29.406	
30	1046.221	29.383		30	1046.221	29.384	
30	1048.041	29.435		30	1048.041	29.435	
30	1049.029	29.462		30	1049.029	29.463	
30	1046.993	29.405		30	1048.041	29.435	

Table 18: Results on RP-HPLC Robustness Data of Diltiazem at different Flow Rate

Flow Rate 0.9ml/min				Flow Rate 1.1ml/min			
Conc. (μg/ml)	**Peak Area**	**Measured quantity**	**Statistical Parameter**	**Conc. (μg/m l)**	**Peak Area**	**Calc. Amt.**	**Statistical Parameter**
30	1048.041	29.435	Mean = 29.426 SD = 0.0274 %RSD = 0.0932	30	1048.041	29.435	Mean = 29.421 SD = 0.028 %RSD = 0.096
30	1047.029	29.406		30	1047.029	29.406	
30	1046.221	29.384		30	1046.221	29.384	
30	1048.041	29.435		30	1048.041	29.435	
30	1049.029	29.463		30	1049.029	29.463	
30	1048.041	29.435		30	1046.993	29.405	

Table 19: Results on RP-HPLC % of Purity Data of Diltiazem

Sl. No.	Retention time [min]	Area [mV. s]	Height [mv]	Area [%]	Height [%]	W05 [min]
1	2.867	13.560	0.897	1.6	1.4	0.27
2	2.907	16.321	1.682	1.3	1.1	0.14
3	3.977	10.763	0.727	0.8	0.5	0.21
4	3.617	1169.502	139.546	91.9	92.5	0.12
5	3.377	54.951	6.432	0.4	0.4	0.15
6	3.127	8.067	1.034	0.6	0.7	0.12
7	4.533	854.582	64.337	98.4	98.6	0.19
	Total	2127.746	214.655	195	195.2	12

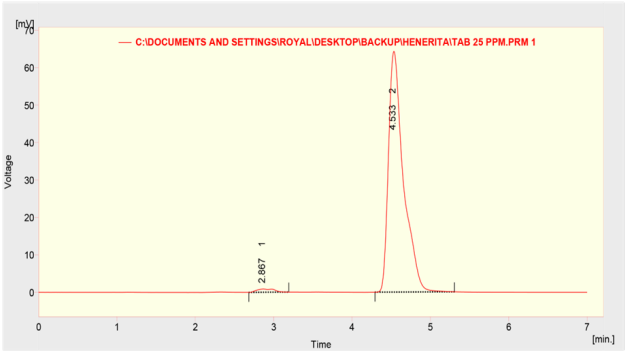

Fig. 6: HPLC % purity Assay of Chromatogram of Diltiazem

FORCED DEGRADATION STUDIES: [3–5]

Degradation in Neutral Condition:

According to the protocol the sample were withdrawn. 50μg/ml solution were prepared and loaded for analysis after drawn from the Sample. The result it indicates that, there was no change in peak area, peak height, and retention time of drug peak in the above condition. It indicates that no degradation takes place in this condition.

After 10 days, the result it indicates that, there was no change in peak area, peak height, and retention time of drug peak in the above condition. It indicates that no degradation takes place in this condition.

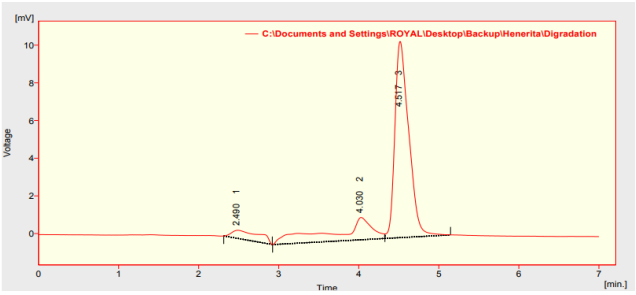

Fig. 7: Forced Degradation Studies in Neutral Condition of Diltiazem

Acidic Degradation:

According to the protocol the samples were withdrawn. 50μg/ml of prepared solution were subjected for analysis after drawn from the sample. The result it indicates that, there was no change in peak area, peak height, and retention time of drug peak in the above condition. It indicates that no degradation takes place in this condition.

After 10 days, the result it indicates that, there was no change in peak area, peak height, and retention time of drug peak in the above condition. It indicates that no degradation takes place in this condition.

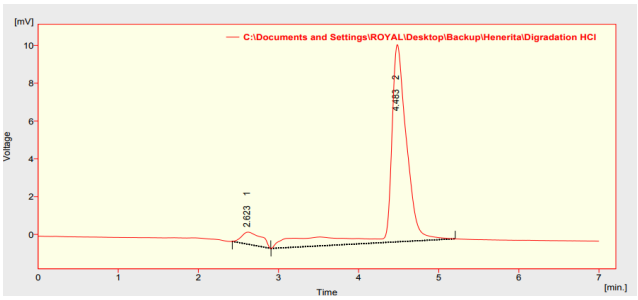

Fig. 8: Forced Degradation Studies in Acidic Condition of Diltiazem

Basic Degradation:

According to the protocol the samples were withdrawn. 50μg/ml of prepared solution were subjected for analysis after drawn from the sample. The result it indicates that, there was no change in peak area, peak height, and retention time of drug peak in the above condition. It indicates that no degradation takes place in this condition.

After 10 days, the result it indicates that, there was change in peak area, peak height, and retention time of drug peak in the above condition. It indicates that no degradation takes place in this condition.

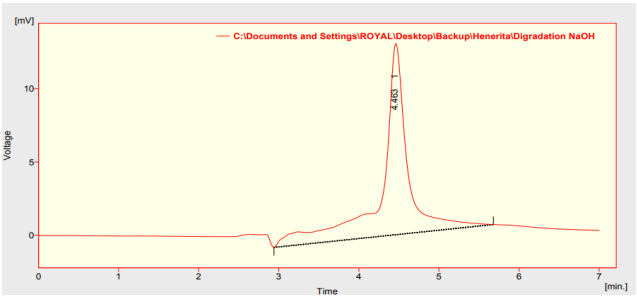

Fig. 9: Forced Degradation Studies in Basic Condition of Diltiazem

Oxidative Degradation:

According to the mentioned protocol in experimental part, samples were withdrawn. From 50μg/ml of prepared solution were subjected for analysis after drawn from the sample. There are no degradation takes place after 7 days that indicated by the representative chromatogram. The extra peak is for H_2O_2.

Thermal Degradation:

According to the protocol the Samples were withdrawn according to the protocol. 50μg/ml solutions were prepared and loaded for analysis after drawn from the sample. The result it indicates that, there was no change in peak area, peak height, and retention time of drug peak in the above

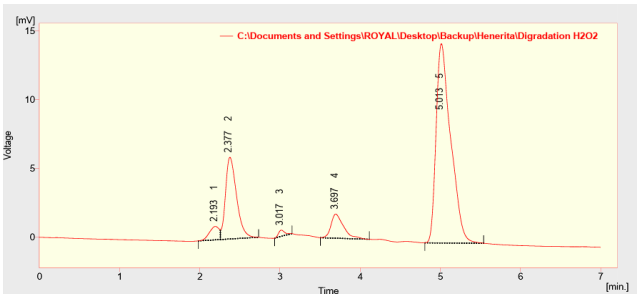

Fig. 10: Forced Degradation Studies in Oxidative Condition of Diltiazem

condition. It indicates that no degradation takes place in this condition.

There is no degradation after 10 days at 70^0C. The resulted Peak also reveals that the drug can be analysed in presence of their degradants.

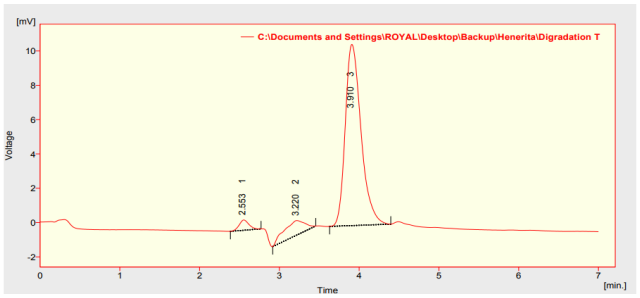

Fig. 11: Forced Degradation Studies in Thermal Condition of Diltiazem

Differential scanning colorimetry (DSC):

Differential Scanning Calorimetry (DSC) By analysing the thermal behaviour of the preparation, differential scanning calorimetry (DSC) was utilized to assess the melting point, crystallinity, breakdown, and drug-excipient interaction. By maintaining a heating rate of 1^0C/min, the DSC verified the presence of the medication Diltiazem showed a pronounced endothermic peak with an onset temperature of 215.75^0C and a peak temperature of 225.26^0C, which is the same as its melting point.

Fourier-transform infrared spectroscopy (FTIR):

Fourier-transform infrared spectroscopy (FTIR) The FTIR spectra of Diltiazem and their solid dispersions were captured. using FTIR spectrophotometer. Samples were mixed with spectroscopic grade potassium bromide and compressed into discs using a hydraulic press before being scanned from 4000 to 500 cm^{-1}. Data analysis software from Perkin Elmer was employed.

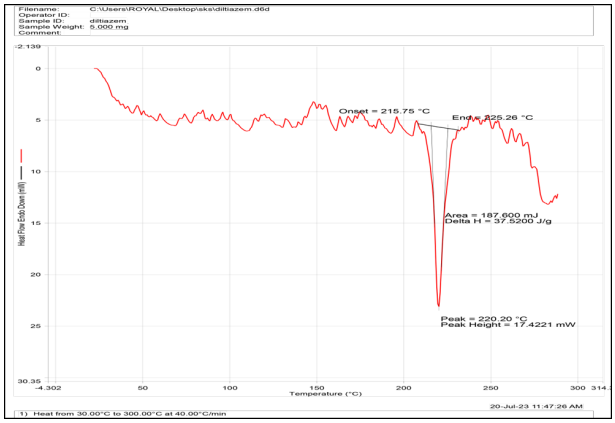

Fig. 12: DSC - Diltiazem

Fig. 13: IR of Diltiazem

CONCLUSION

The objective of ICH guidelines is to harmonize internationally the scientific necessities to make sure that harmless, successful, with good class medicines are generated at a low cost. The ICH guideline which deals with "steadiness checking of latest Drug and Products" plays a pivotal role in establishing the stability of bulk drugs as well as the pharmaceutical formulations. Here the aim is to use emergent RP-HPLC method for Diltiazem and to carry out stability studies at different stress conditions.

From the results of method development it is found that the developed methods are simple, reliable, sensitive, economic and accurate. The Developed UV-Vis Spectrophotometric method can be used for routinely, rapid, and precise analysis of Diltiazem. The optical characteristics of the proposed UV-Vis spectrophotometric method showed that the drug follows linearity in the range of 5-50 μg /ml. The result of validation shows that the method is good. The development of RP-HPLC method was found to be suitable for the analysis of Diltiazem, in their pure drug. The method was found to be fast, simple, reliable, sensitive, economical, accurate and precise. In RP-HPLC method the

drug follows linearity within the range of 10-70μg/ml. The method successfully validated in the optimized conditions. The validation parameters were within the limit.

In the present investigation the Diltiazem was subjected for its stability studies under different conditions as per the ICH guidelines. From the degradation study of Diltiazem, it was found that there was no degradation taking place by using different conditions like in neutral conditions 2 weeks, Acidic conditions (1N) at 2 weeks and Basic conditions (1N) at 2 weeks. The thermal study indicates there was no degradation taking place by placing the Diltiazem in thermal conditions (10 days). From the stability studies of Diltiazem, it concluded that by using the above degradation conditions, there was no degradation taking place by using the above all conditions.

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