International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 - 7587
Print ISSN 2249 - 7595

ANALYTICAL DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING HPLC METHOD FOR DETERMINATION OF DILTIAZEM HYDROCHLORIDE EXTENDED RELEASE CAPSULES FOR ITS RELATED SUBSTANCES

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ABSTRACT

A specific, precise, linear, accurate, rugged, robust, and stability indicating isocratic reversed phase high-pressure liquid chromatography method has been developed and validated as per ICH guidelines for estimation of related substances of Diltiazem Hydrochloride Extended Release Capsules 120,180,240,300,360mg USP. It was performed on a Waters µBondapak C18 (10µ particles size) (300 mm × 3.9 mm) column using mobile phase containing Acetate buffer Acetonitrile and Methanol in ratio 500:250:250 v/v/v (pH 6.3 adjusted with 0.1 N sodium hydroxide solution in buffer) at the flow rate 1.6 ml/min. Detection was performed at 240 nm and a sharp peak was obtained for Diltiazem hydrochloride at a retention time at about 20 min. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 0.5-18 µg/ml; the regression coefficient was 0.999. The detection (LOD) and quantification (LOQ) limits were 0.16 and 0.51µg/ml respectively and Desacetyl diltiazem hydrochloride at a retention time at about 14.3 min. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 0.4-5.3 µg/ml; the regression coefficient was 0.999. The detection (LOD) and quantification (LOQ) limits were 0.13 and 0.41µg/ml respectively. The HPLC method for the determination of related substances of Diltiazem Hydrochloride Extended Release Capsules 120,180,240,300,360 mg USP was validated. The stress sample solutions were assayed against the qualified reference standard of Diltiazem hydrochloride and the mass balance in each case was close to 99.9%, confirming its stability-indication capacity. The method was found to be specific, precise, linear, accurate, rugged, stability indicating and suitable for its intended use. The developed HPLC method to determine the related substances and assay determination of Diltiazem hydrochloride can be used to evaluate the quality of regular production samples. It can be also used to test the stability samples of Diltiazem hydrochloride.

Keywords: Diltiazem Hydrochloride, Desacetyl diltiazem hydrochloride, ICH, validation.

INTRODUCTION

Diltiazem is a calcium ion influx inhibitor (slow channel blocker or calcium antagonist). Chemically, diltiazem hydrochloride is 1, 5-Benzothiazepin-4(5H) one, 3-(acetyloxy)-5-[2-(dimethylamino) ethyl]-2, 3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride, (+)-cis-. Its molecular formula is C22H26N2O4S HCl and its molecular weight is 450.99. Diltiazem produces its antihypertensive effect primarily by relaxation of vascular smooth muscle with a resultant decrease in peripheral vascular resistance. The magnitude of blood pressure reduction is related to the degree of hypertension; thus hypertensive individuals experience an antihypertensive effect, whereas there is only a modest fall in blood pressure in normotensives. International Conference on Harmonization (ICH).[1-2] It is also recommended by ICH that all routine impurities at or above 0.1% level, should be identified through appropriate analytical methods no official method have been reported for related substance of

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this Extended release capsule in pharmacopoeia. In recent times, numerous analytical methods for the determination of Diltiazem in bulk drug as well as in formulations have been reported in HPLC–MS and CE methods have been reported to characterize the Diltiazem metabolites [3-4]An HPLC method for assay of Diltiazem Hydrochloride and its related substances in bulk drug and finished tablets is reported [5] . literature viz. spectrophotometry [6-7], gas chromatography [8], HPTLC [9],HPLC[10–12] ,Formulation evaluation[13–14] ,since all the methods were established only in tablet formulation A single HPLC method has been reported for determination of diltiazem hydrochloride and related substances in delay-onset sustained-release pellet capsules[15]. From preceding details of relevant literature it was apparent that a validated method is required to be developed which would be stability indicating.

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile	: HPLC grade
Methanol	: HPLC grade
d-10-camphorsulfonic acid	: LR grade
Sodium hydroxide	: GR grade
Sodium acetate trihydrate	: GR grade
Water	: HPLC grade

Chromatographic condition:

Column	: Waters µBondapak C18 (300 x 3.9 mm, 10 µm)
Column oven temperature	: Ambient
Flow rate	: 1.6 mL/min
Injection volume	: 10 µL
Wavelength	: 240 nm
Run time	: 50 minutes
Pump mode	: Isocratic

Preparation of buffer:

Weigh and dissolve 13.6 g of sodium acetate trihydrate and 1.16 g of d-10 camphorsulfonic acid in 1000 mL of water and adjust the pH to 6.20 ± 0.05 with 0.1N sodium hydroxide solution, filter through 0.45 µm membrane filter.

Preparation of mobile phase:

Mix buffer, acetonitrile and methanol in the ratio of 50:25:25 % v/v/v and degas.

Diluent: Methanol

Preparation of standard solution

Weigh accurately about 24.0 mg Diltiazem hydrochloride reference/working standard and 24.0 mg of Desacetyl hydrochloride reference/working standard and transfer into a 100 mL volumetric flask. Add about 60 mL of methanol, sonicate to dissolve the contents and make the volume up to the mark with methanol and mix. Transfer 5 mL of this solution into a 100 mL volumetric flask and make the volume up to the mark with methanol

Preparation of placebo solution

Weigh accurately and transfer placebo equivalent to 120 mg of Diltiazem hydrochloride (Subtract the 120 mg from the obtained equivalent weight) into a 100 mL volumetric flask, add about 60 mL of methanol and sonicate for 10 minutes. Cool the solution to room temperature and make the volume up to the mark with methanol. Mix and filter the solution through 0.45µm nylon filter and discard first 5 mL of the filtrate.

Preparation of sample solution

Weigh not less than 20 capsules and empty the contents. Weigh the empty capsules and calculate the average fill weight. Crush the pellets in to fine powder. Weigh accurately the sample equivalent to about 120 mg of Diltiazem hydrochloride and transfer into a 100 mL volumetric flask. Add about 60 mL of methanol and sonicate for 10 minutes. Cool the solution to room temperature and make the volume up to the mark with methanol. Mix and filter the solution through 0.45µm nylon filter and discard first 5 mL of the filtrate.

RRT and RRF of Impurity

S.No	Name of the impurity	RRT	RRF
1.	Desacetyl diltiazem	0.68	1.14

Calculations:

Calculation for known impurity:

% known impurity =

$$\frac{AT_1}{AS} \times \frac{STDw}{100} \times \frac{5}{100} \times \frac{100}{Samw} \times \frac{Avg.\ Fill\ wt}{LC} \times \frac{1}{RRF} \times P$$

Where,

AT_1	:	Area of the known impurity peak in the sample chromatogram.
AS	:	Average area of Diltiazem hydrochloride peak in standard chromatogram.
STDw	:	Weight of Diltiazem hydrochloride reference/working standard taken in mg.
Samw	:	Weight of sample taken in mg.
RRF	:	Relative response factor
Avg. Fill wt	:	Average fill weight of the capsules in mg
LC	:	Label Claim in mg.
P	:	Percentage potency of Diltiazem hydrochloride reference/working standard (as is basis) (99.2).

Calculation for unknown impurity:

$$\% \text{ Unknown impurity} = \frac{AT_2}{AS} \times \frac{STDw}{100} \times \frac{5}{100} \times \frac{100}{Samw} \times \frac{\text{Avg. Fill wt}}{LC} \times P$$

Where,

AT_2	:	Area of the unknown impurity peak in the sample chromatogram.
AS	:	Average area of Diltiazem hydrochloride peak in standard chromatogram.
STDw	:	Weight of Diltiazem hydrochloride reference/working standard taken in mg
Samw	:	Weight of sample taken in mg.
Avg. Fill wt	:	Average fill weight of the capsules in mg
LC	:	Label Claim in mg.
P	:	Percentage potency of Diltiazem hydrochloride reference/working standard (as is basis) (99.2).

Total impurities:

% Total impurities = % of known impurities + % of unknown impurities

Chemical Name of impurities: Desacetyl Diltiazem Hydrochloride – purity 99.8

RESULTS AND DISCUSSIONS

Precision

System Precision

Six replicate injections of standard solution were injected. The mean and percentage relative standard deviation (% RSD) for peak areas of Diltiazem hydrochloride were calculated. The results are tabulated in Table - 1.

Acceptance criteria

Percentage relative standard deviation (% RSD) is not more than 10.0 for peak areas of standard solution.

Method Precision

Prepared the blank, standard solution and six samples of 360 mg Capsules spiked with known impurities at specification level and analyzed as per testing procedure. The percentage of impurities and percentage relative standard deviation (% RSD) for percentage of impurities were calculated and the results are tabulated in Table - 2.

Acceptance Criteria

Percentage relative standard deviation (% RSD) for percentage of known and total impurities is not more than 10.0.

Intermediate Precision (Ruggedness)

Ruggedness of the method was verified by analyzing the six samples of 100 mg Capsules spiked with known impurities at specification level of same batch which was used for method precision. The study was performed by different analyst using different instrument and different column on different day.

The percentage of impurities was determined. Calculated percentage relative standard deviation (% RSD) for percentage of impurities in six samples and also calculated overall percentage relative standard deviation (% RSD) for ruggedness results and method precision results. The results are tabulated in Table-3.

Acceptance criteria

Percentage relative standard deviation (% RSD) for percentage of known and total impurities is not more than 10.0

Overall percentage relative standard deviation (% RSD) is not more than 10.0 for method precision and ruggedness results.

Specificity

Blank, placebo, standard sample solution of 360 mg capsules (unspiked) and sample solution of 360 mg capsules spiked with known impurity at specification level were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of known impurity and main peak. Peak purity data reveals that known impurity and Diltiazem hydrochloride peaks were homogeneous and there were no co-eluting peaks at the retention time of known impurity and Diltiazem hydrochloride peaks.

The peak purity data of known impurity and Diltiazem hydrochloride peaks in spiked sample are summarized in Table-4a. The retention time of Diltiazem hydrochloride and relative retention time of known impurity from spiked sample are compiled in Table-4b.

Refer figure 3 to 6 for the chromatograms of blank, placebo, standard, individual impurity at specification level, sample (unspiked) and impurity spiked sample.

Acceptance criteria

No peak elutes at the retention time of known impurities and main peak in the blank and placebo.

Peak purity for main peak and known impurities passes in spiked sample.

[Waters Empower software: Purity angle should be less than purity threshold].

Forced Degradation

Forced degradation study was carried out by treating the sample under the following conditions.

a) Degradation by hydrochloric acid (Acid stressed sample)

Sample of 360 mg Capsules was treated with 5 mL of 0.2N Hydrochloric acid and kept for 24 hours. Treated sample solution was analyzed as per the testing procedure.

b) Degradation by sodium hydroxide (Alkali stressed sample)

Sample of 360 mg Capsules was treated with 5 mL of 0.1 N Sodium hydroxide and kept 1hours. Treated sample solution was analyzed as per the testing procedure.

c) Degradation by hydrogen peroxide (Peroxide stressed sample)

Sample of 360 mg capsules was treated with 5 mL solution of 30% Hydrogen peroxide solution and kept on bench top for 3 hours. Treated sample solution was analyzed as per the testing procedure.

d) Degradation by thermal (Heat stressed sample)

Sample of 360 mg Capsules was kept in oven at 105°C for 24 hours. Treated sample was analyzed as per the testing procedure.

e) Degradation by photo light [Controlled condition (wrapped in aluminum foil)]

Sample of 360 mg Capsules was exposed to light of 1.2 million lux hours in protected condition. Treated sample was analyzed as per the testing procedure.

f) Degradation by photo light [Uncontrolled condition]

Sample of 360 mg Capsules was exposed to light of 1.2 million lux hours. Treated sample was analyzed as per the testing procedure. The results of forced degradation studies are summarized in Table - 5. Refer figure 9 to 14 for the chromatograms of stressed samples.

Acceptance criteria

Peak purity for main peak passes. [Waters Empower software: Purity angle should be less than purity threshold]Degradation is not more than 30 % in each condition.

Limit of Detection and Limit of Quantitation (LOD and LOQ)

The limit of detection and (LOD) and limit of quantitation (LOQ) is determined by signal to noise ratio method by using the formula.

Signal to noise ratio (S/N) = 2H/h

H - Height of the analyte peak

h - Height of the noise.

LOD and LOQ value was verified by giving six replicate injections of solution containing known impurities and Diltiazem hydrochloride at this level. The percentage relative standard deviation (% RSD) calculated for the peak areas and tabulated in Table-6a, 6b.LOQ and LOD values are summarized in Table 6c.

Acceptance criteria

Signal-to-noise ratio 10:1 at the level of LOQ and 2 or 3:1 at the level of LOD. Percentage relative standard deviation (% RSD) for peak areas at LOQ level is not more than 10.

Linearity

The linearity of known impurity and Diltiazem Hydrochloride were performed in the range of LOQ to 150 % of specification limit. A graph was plotted with concentration (in µg/mL) on x-axis and peak areas on y-axis. Slope, y-intercept, correlation coefficient (r-value) and residual sum of squares (RSS) were determined. The results are tabulated in Table - 7a 7b graphically represented in figure 1 and 2.The relative response factor value is tabulated in Table – 7c

Acceptance criteria

The correlation coefficient (r) is not less than 0.97

Accuracy (Recovery)

Known amount of impurities spiked with sample at about LOQ, 100% and 150% of specification limit. The percentage recovery was calculated from the amount found and actual amount added. Known amount of Diltiazem Hydrochloride spiked with placebo at about LOQ, 100% and 150% of specification limit. The percentage recovery was calculated from the amount found and actual amount added. The results are tabulated in Table - 8a and 8b.

Acceptance criteria

Percentage recovery at LOQ level is between 75.0 and 125.0.

Percentage recovery at 100,150 level is between 90.0 and 110.0.

Percentage relative standard deviation (% RSD) is not more than 10.0 at each level.

Solution Stability

Stability of analytical solution was verified by analyzing the standard and sample solution of 360 mg capsules spiked with known impurity at specification level initially and also at different time intervals as mentioned below by storing in sample compartment of HPLC instrument at 25°C (ambient). Calculated the cumulative percentage relative standard deviation (% RSD) for peak areas of standard and percentage of known impurity and total impurities in sample. The results are tabulated in Table - 9a and 9b.

Acceptance criteria

Cumulative percentage relative standard deviation (% RSD) is not more than 5.0 for peak areas of standard.

Cumulative percentage relative standard deviation (% RSD) is not more than 5.0 for percentage of known, total impurities in sample.

Table 1.System Precision

Injection No.	Peak area
1	250230
2	253579
3	246799
4	251182
5	252208
6	251494
Mean	250915
% RSD	0.9

Table 2. Method Precision

Sample No.	Percentage of impurity (w/w)	
	Desacetyl Diltiazem	Total impurities
1	0.34	0.34
2	0.34	0.34
3	0.34	0.34
4	0.34	0.34
5	0.34	0.34
6	0.34	0.34
Average	0.34	0.34
% RSD	0.0	0.0

Table 3. Intermediate Precision (Ruggedness)

Sample No.	Percentage of impurity (w/w)			
	Desacetyl Diltiazem	Desacetyl Diltiazem	Total impurities	
	I	II	I	II
1	0.34	0.34	0.34	0.34
2	0.34	0.34	0.34	0.34
3	0.34	0.34	0.34	0.34
4	0.34	0.34	0.34	0.34
5	0.34	0.34	0.34	0.34
6	0.34	0.34	0.34	0.34
Average	0.34	0.34	0.34	0.34
% RSD	0.0	0.0	0.0	0.0
Overall mean	0.34		0.34	
Overall % RSD	0.0		0.0	
I - Analyst-1 II - Analyst-2				

Table 4a. Specificity-Peak purity

	Peak Name	Purity Angle	Purity threshold	Purity flag
Impurity spiked sample	Desacetyl Diltiazem	2.693	2.892	No
	Diltiazem hydrochloride	0.038	0.254	No

Table 4b. Specificity-Retention time

	Peak Name	RT	RRT
Impurity spiked sample	Desacetyl Diltiazem	14.23	0.71
	Diltiazem hydrochloride	20.07	1.00
RT - Retention time in minutes RRT - Relative Retention Time			

Table 5. Forced degradation

S. No.	Condition	% Purity	% Degradation	Purity Angle	Purity Threshold	Purity flag
1	Unstressed Sample	99.96	0.04	0.046	0.245	No
2	Acid stressed sample	90.58	9.42	0.069	0.251	No
3	Alkali stressed sample	85.23	14.77	0.068	0.268	No
4	Peroxide stressed sample	91.91	9.09	0.041	0.236	No
5	Thermal stressed sample	99.96	0.04	0.041	0.243	No
6	Photo light stressed sample Controlled	99.95	0.06	0.057	0.249	No
7	Photo light treated sample Uncontrolled	99.96	0.04	0.051	0.245	No

Table 6a. LOQ Peak areas

Inj. No.	Desacetyl Diltiazem	Diltiazem Hydrochloride
1	8800	10947
2	8694	10131
3	7579	10063
4	8916	10582
5	8314	8811
6	8868	10408
Mean	8529	10157
% RSD	6.0	7.2

Table 6b. LOD Peak areas

Inj. No.	Desacetyl Diltiazem	Diltiazem hydrochloride
1	2684	2765
2	2300	3060
3	2492	2373
4	3017	2656
5	3086	2817
6	2926	3315
Mean	2751	2831
% RSD	11.4	11.5

Table 6c. LOD and LOQ values

Name	LOD			LOQ		
	Con. in µg/mL	%	S/N	Con. in µg/mL	%	S/N
Diltiazem hydrochloride	0.1690	0.01	13	0.5122	0.04	25
Desacetyl Diltiazem	0.1357	0.01	12	0.4113	0.03	27
S/N: Signal to noise ratio. %: Percentage calculated with respect to test concentration.						

Table 7a. Linearity of Desacetyl Diltiazem

Desacetyl Diltiazem		
Level (%)	**Concentration in µg/mL**	**Peak area**
LOQ	0.4224	9614
20	0.7100	16862
40	1.4200	31892
50	1.7749	41305
70	2.4849	56530
80	2.8399	65188
100	3.5499	81374
120	4.2599	95706
140	4.9698	109264
150	5.3248	114651
Correlation coefficient	0.99910	
Slope	21707.2320	
Y-intercept	1986.9216	
Residual sum of squares	22802350.0429	

Table 7b. Linearity of Diltiazem Hydrochloride

Diltiazem Hydrochloride		
Level (%)	**Concentration in µg/mL**	**Peak area**
LOQ	0.5042	9912
20	2.3729	48336
40	4.7457	99278
50	5.9322	123609
70	8.3050	171442
80	9.4915	197100
100	11.8643	247505
120	14.2372	287981
140	16.6100	332878
150	17.7965	354697
Correlation coefficient	0.99954	
Slope	19986.1301	
Y-intercept	3682.2079	
Residual sum of squares	116345545.4887	

Table 7c. Relative Response Factor

Name of impurity	Relative response factor (RRF)
Desacetyl Diltiazem	1.14

Table 8a. Accuracy - Desacetyl Diltiazem

Desacetyl Diltiazem					
Level	**Amount found**	**Actual amount**	**% Recovery**		
Level - 1 (LOQ)	0.4329	0.4338	99.8	98.9	0.8
	0.4278	0.4338	98.6		
	0.4269	0.4338	98.4		
Level - 2 (100%)	3.6573	3.6452	100.3	100.5	0.4
	3.6799	3.6452	101.0		
	3.6534	3.6452	100.2		
Level - 3 (150%)	5.5431	5.4678	101.4	100.9	0.4
	5.5042	5.4678	100.7		
	5.5062	5.4678	100.7		

Table 8b. Accuracy - Diltiazem Hydrochloride

Diltiazem Hydrochloride					
Level	**Amount**	**Actual amount**	**%**		
Level - 1 (LOQ)	0.5170	0.5133	100.7	99.8	1.9
	0.5017	0.5133	97.7		
	0.5187	0.5133	101.1		
Level - 2 (100%)	12.1292	12.0776	100.4	100.0	0.4
	12.0459	12.0776	99.7		
	12.0505	12.0776	99.8		
Level - 3 (150%)	18.0739	18.1164	99.8	99.9	0.4
	18.0276	18.1164	99.5		
	18.1694	18.1164	100.3		

Table 9a. Solution stability-Standard

STANDARD		
Time in Hrs.	**Peak area**	**Cum. % RSD**
Initial	250230	-
7	253000	0.8
12	252043	0.6
16	249873	0.6
20	252885	0.6
24	253056	0.6
32	253939	0.6
40	243417	1.4
48	253945	1.3

Table 9b. percentage of known impurity and total impurities in sample

Sample		
Time (Hrs)	**Desacetyl Diltiazem**	
	%	**Cum. % RSD**
Initial	0.32	-
7	0.33	2.2
12	0.34	3.0
16	0.35	3.9
20	0.35	3.9
24	0.36	4.3
32	0.38	5.7
40	0.41	8.1
48	0.41	9.0

Figure 1. Linearity plot for Desacetyl Diltiazem

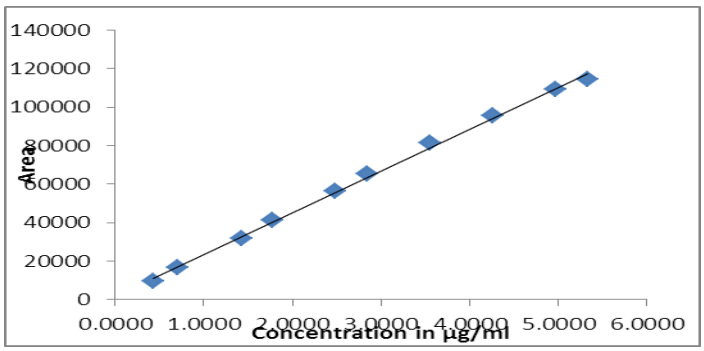


Figure 2. Linearity plot for Diltiazem Hydrochloride

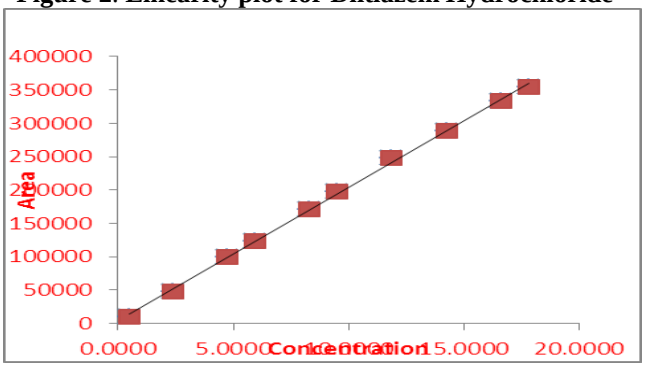


Figure 3. Chromatogram of blank

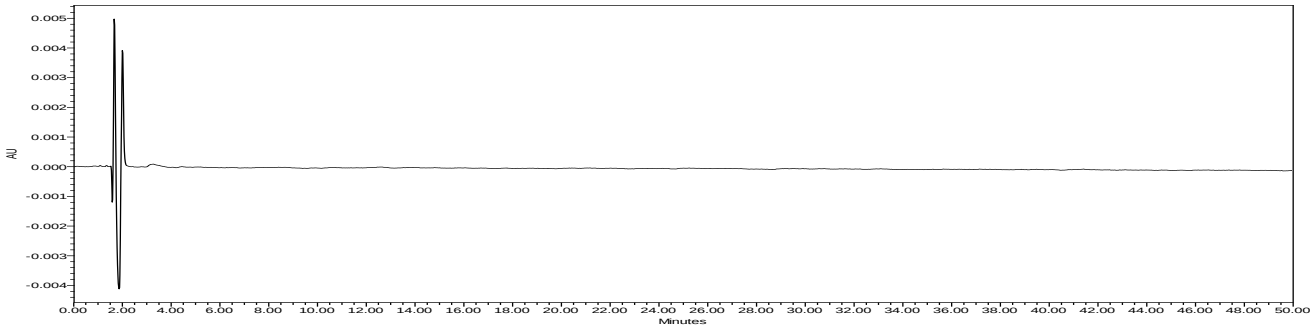


Figure 4. Chromatogram of Placebo

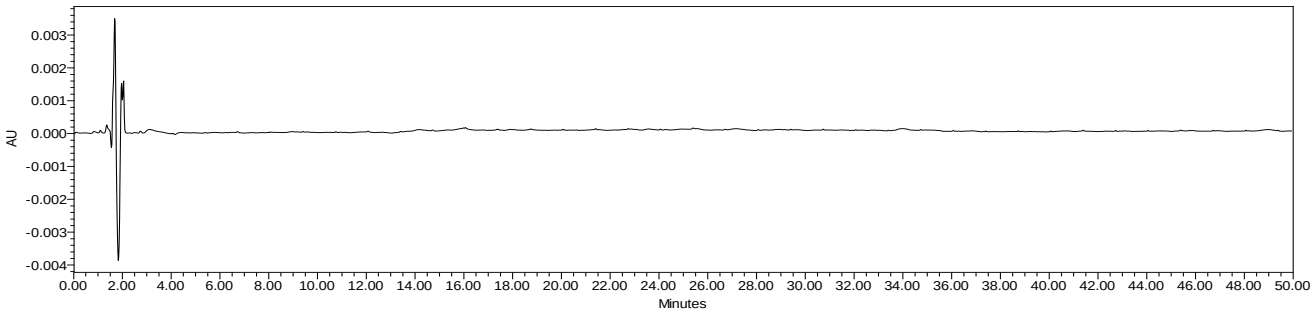
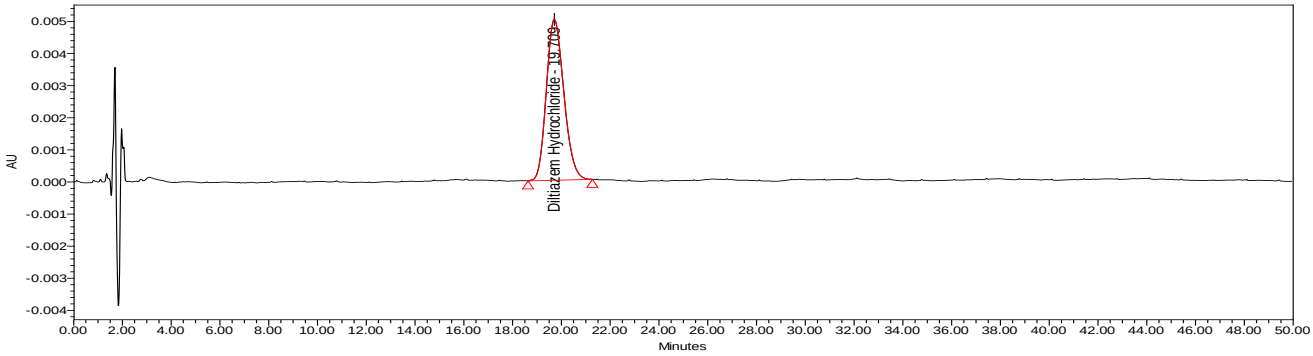
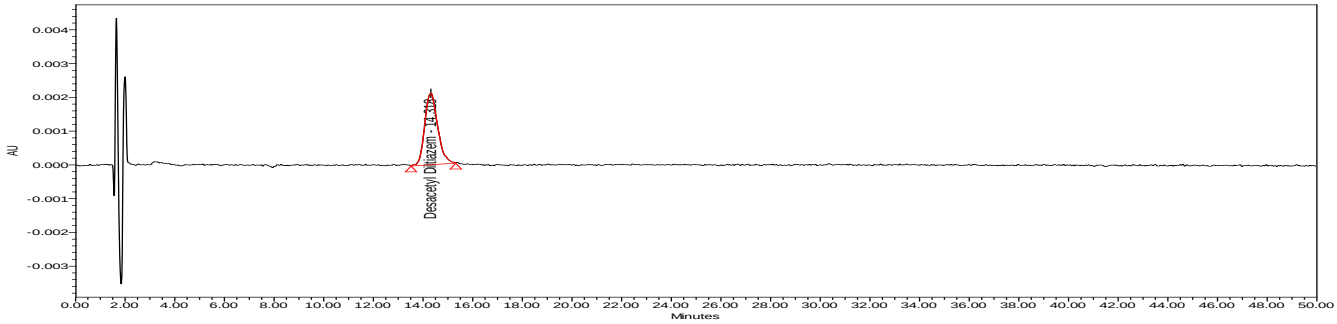


Figure 5. Chromatogram of Standard



S.No	Name	Retention Time	Area	% Area	USP Tailing	USP Plate Count
1	Diltiazem Hydrochloride	19.709	250230	100.00	1.18	3521.90

Figure 6. Chromatogram of Desacetyl Diltiazem hydrochloride



S.No	Name	Retention Time	Purity1 Angle	Purity1 Threshold	Area	% Area	Purity1 Flag
1	Desacetyl Diltiazem	14.318	4.209	4.687	77128	100.00	No

Figure 7. Chromatogram of unspiked sample

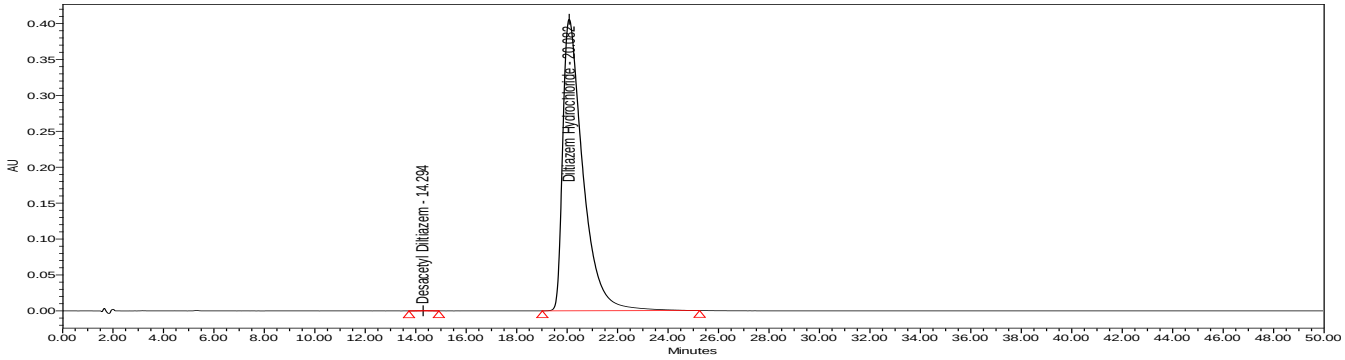
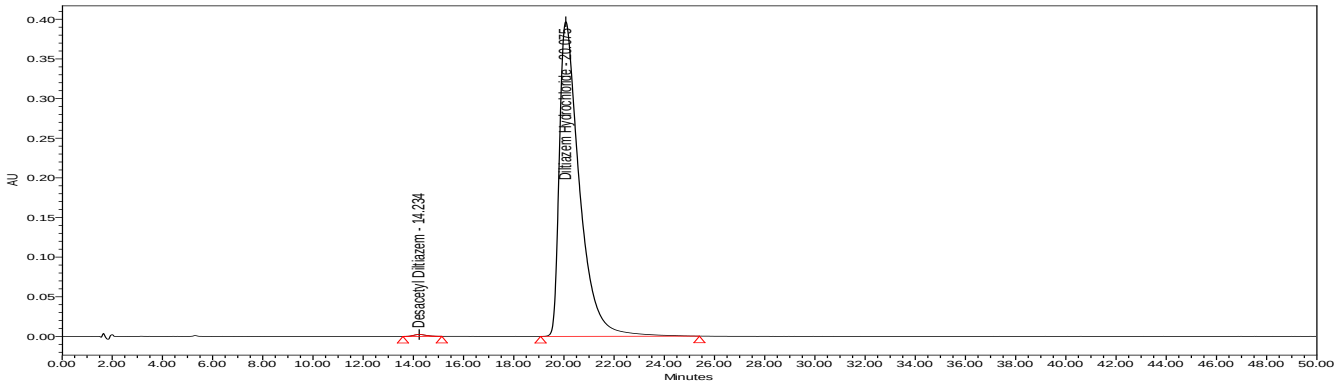
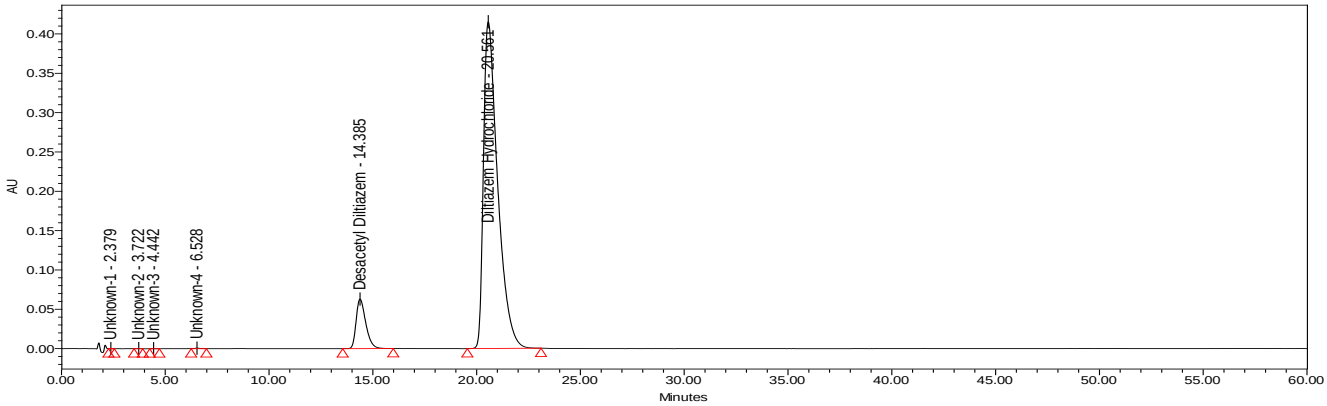


Figure 8. Chromatogram of spiked sample



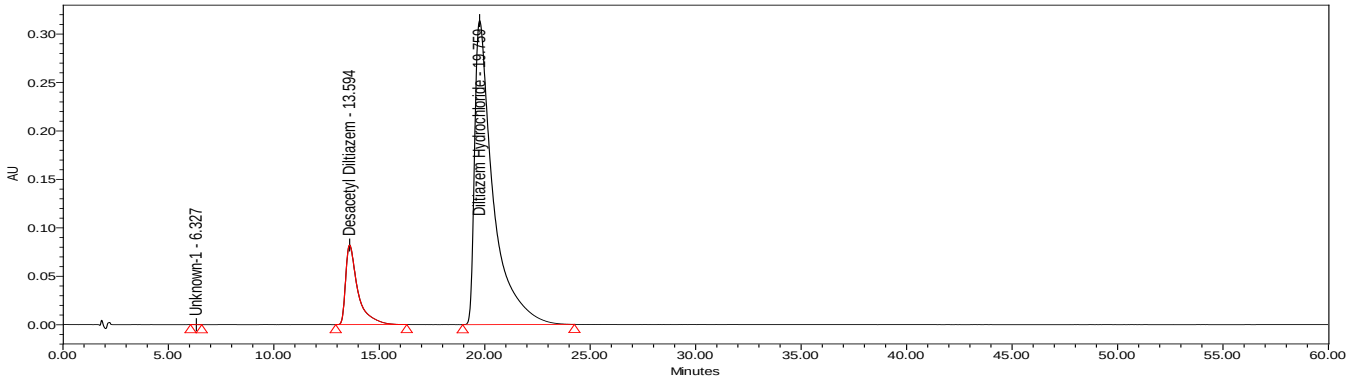
S.no	Name	Retention Time	Purity1 Angle	Purity1 Threshold	Area	% Area	RT Ratio	Purity1 Flag
1	Desacetyl Diltiazem	14.234	2.693	2.892	83583	0.38	0.71	No
2	Diltiazem Hydrochloride	20.075	0.038	0.251	21909526	99.62	1.00	No

Figure 9. Chromatogram of acid stressed sample



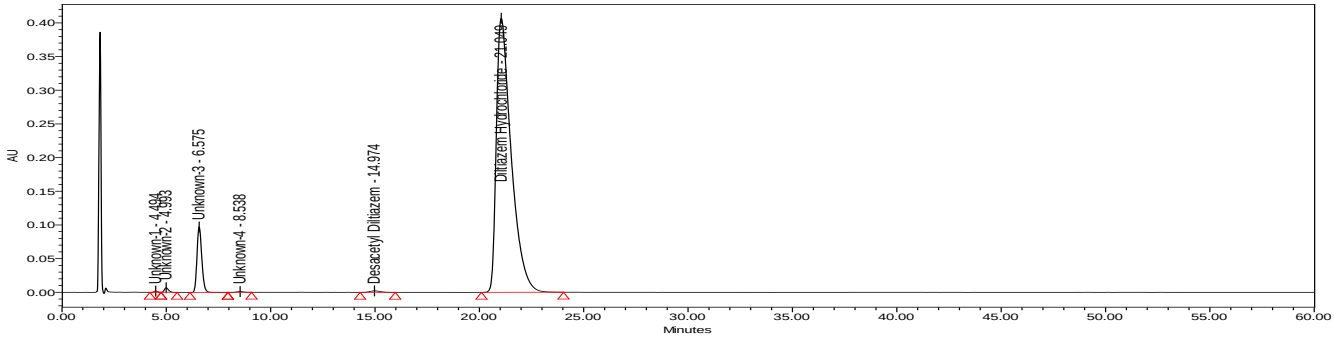
S.no	Name	Retention Time	Area	Purity1 Angle	Purity1 Threshold	RT Ratio	% Area	Purity1 Flag
1	Unknown-1	2.379	1197	14.223	20.355	0.12	0.01	No
2	Unknown-2	3.722	2171	9.157	11.660	0.18	0.01	No
3	Unknown-3	4.442	2085	41.840	36.593	0.22	0.01	Yes
4	Unknown-4	6.528	10968	8.042	10.402	0.32	0.05	No
5	Desacetyl Diltiazem	14.385	2028979	0.162	0.340	0.70	9.34	No
6	Diltiazem Hydrochloride	20.561	19675088	0.069	0.251	1.00	90.58	No

Figure 10. Chromatogram of alkali stressed sample



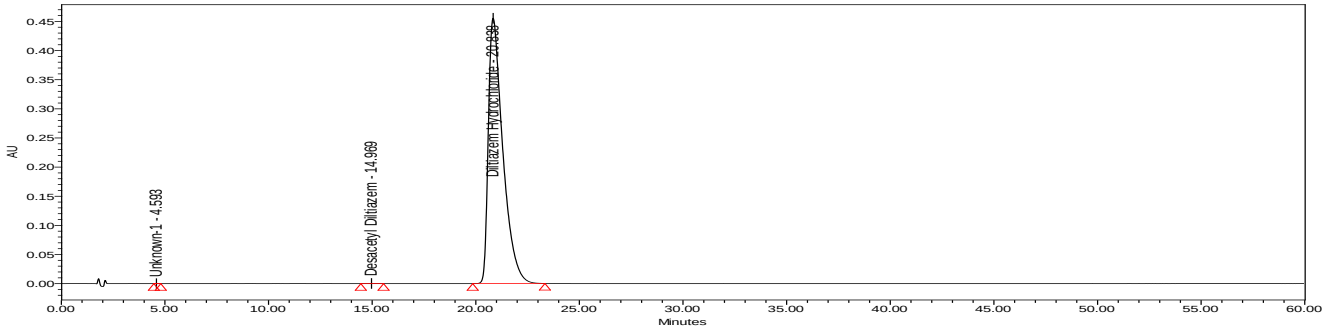
	Name	Retention Time	Area	Purity1 Angle	Purity1 Threshold	RT Ratio	% Area	Purity1 Flag
1	Unknown-1	6.327	643	61.141	90.000	0.32	0.00	No
2	Desacetyl Diltiazem	13.594	3305923	0.215	0.380	0.69	14.77	No
3	Diltiazem Hydrochloride	19.759	19081207	0.068	0.268	1.00	85.23	No

Figure 11. Chromatogram of peroxide stressed sample



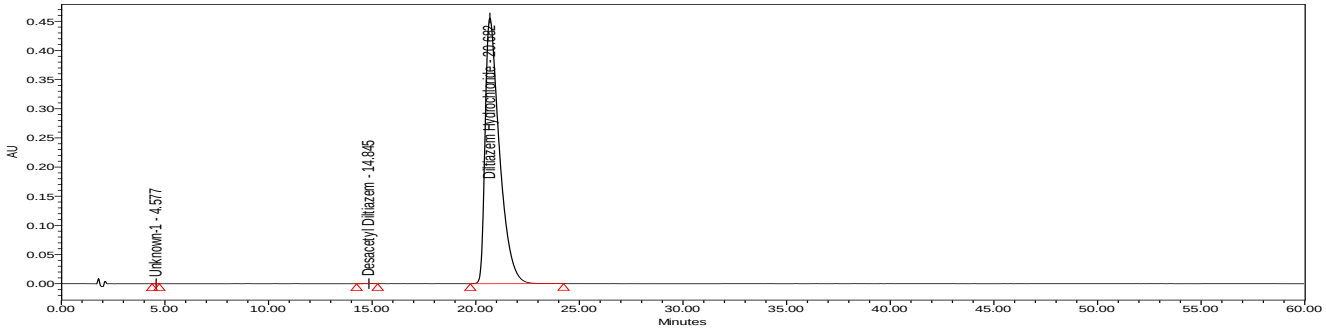
S.no	Name	Retention Time	Purity1 Angle	Purity1 Threshold	Area	% Area	Purity1 Flag
1	Unknown-1	4.494	7.389	1.719	20302	0.09	Yes
2	Unknown-2	4.993	0.843	0.577	93299	0.43	Yes
3	Unknown-3	6.575	0.049	0.235	1556326	7.12	No
4	Unknown-4	8.538	2.420	3.005	24301	0.11	No
5	Desacetyl Diltiazem	14.974	1.331	1.519	76181	0.35	No
6	Diltiazem Hydrochloride	21.049	0.041	0.236	20100602	91.91	No

Figure 12. Chromatogram of thermal stressed sample



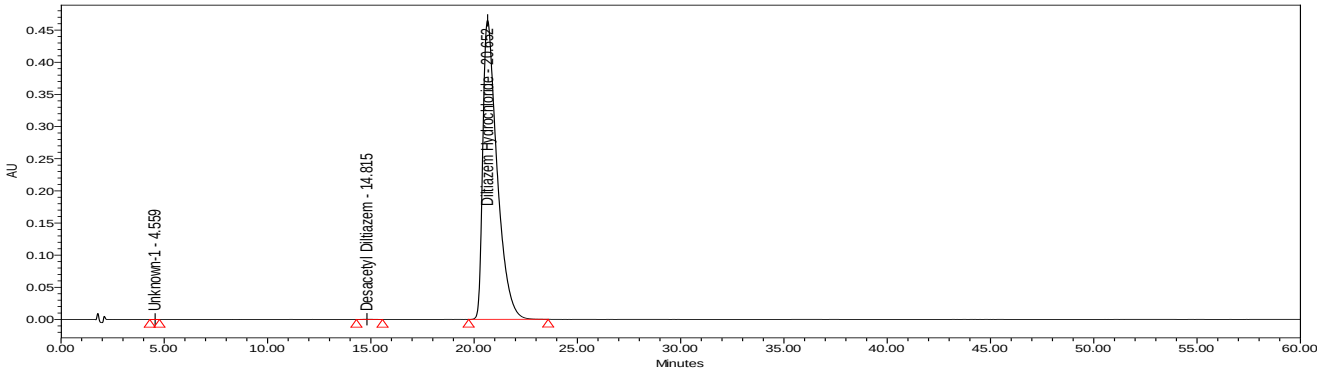
S.no	Name	Retention Time	Area	Purity1 Angle	Purity1 Threshold	RT Ratio	% Area	Purity-1 Flag
1	Unknown-1	4.593	450	21.949	27.868	0.22	0.00	No
2	Desacetyl Diltiazem	14.969	8728	12.747	12.799	0.72	0.04	No
3	Diltiazem Hydrochloride	20.838	22077534	0.041	0.243	1.00	99.96	No

Figure 13. Chromatogram of light stressed (Uncontrolled) sample



S.no	Name	Retention Time	Area	Purity1 Angle	Purity1 Threshold	RT Ratio	% Area	USP Plate Count	Purity1 Flag
1	Unknown-1	4.559	1249	13.545	16.161	0.22	0.01	4254.41	No
2	Desacetyl Diltiazem	14.815	10264	17.738	18.424	0.72	0.05	4698.14	No
3	Diltiazem Hydrochloride	20.652	22291719	0.057	0.249	1.00	99.95	4305.49	No

Figure 14. Chromatogram of light stressed (Controlled) sample



S.no	Name	Retention Time	Area	Purity1 Angle	Purity1 Threshold	RT Ratio	% Area	Purity1 Flag
1	Unknown-1	4.577	792	10.253	12.799	0.22	0.00	No
2	Desacetyl Diltiazem	14.845	8225	13.944	14.057	0.72	0.04	No
3	Diltiazem Hydrochloride	20.682	21872066	0.051	0.245	1.00	99.96	No

CONCLUSION

The proposed HPLC method for estimation of related substances for Diltiazem Hydrochloride is analyzed in Diltiazem Hydrochloride Extended Release Capsules as per ICH guidelines. The method is found to be specific for the estimation of known, unknown impurities and degradation products. The method is also stability indicating as evident from results obtained when method applied to stability samples. The assay utilized a previously unreported set of conditions, including a gradient simple mobile phase, LOD and LOQ, established by this method. The method is found to be linear in the specified range, precise and Accuracy of the method is also established for the formulation. Hence, the proposed method stands validated and may be used for routine and stability sample analysis.

ACKNOWLEDGEMENTS

The authors are grateful to Piramal Health Care Ltd, India for providing Diltiazem hydrochloride and Desacetyl diltiazem hydrochloride as a gift sample.

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