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Method Development Validation and Estimation of Impurities in Dicyclomine Hydrochloride Capsule by RP HPLC

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ABSTRACT:

This study's objective was to Developed and validated a new Reverse Phase- High-Performance Liquid Chromatography (RP-HPLC) method for the determination of Impurities in Dicyclomine HCl capsules. The method was developed by adapting the USP API monograph and checked for feasibility study and applied to capsule formulation, In this API methods methods not suitable for estimation of impurities in capsule formulation, hence study was made in changing the column for Known impurity, diluent composition, pH of the mobile phase, Flow rate change and different mobile phases for unknown impurities. By trailed with above explained aspects developed new method for determination of impurities in capsule formulation. This method was developed with an emphasis on specific, linear, accurate andreproducible and compliant with International Council for Harmonization (ICH) guidelines for methodvalidation.Theobtainedresultsofvalidationpa rameterwithintheAcceptance criteria. This implies high reliability of % impurities present in determination of Dicyclomine Hcl capsules with accuracy in method precision samples. In conclusion, the newly developed RP-HPLC methods provides an efficient and precise tool for estimation of impurities in capsule formulations. This facilitates accurate impurity contentassessmentsinroutinequalitycontroltestsforp harmaceuticalcompanies.This study makes a significant contribution to the evolution of pharmaceutical analytical techniques, offering valuable insights into the use of validated RP-HPLCmethod.

KEYWORDS: Dicyclomine Hcl, RP-HPLC Method Development & Method Validation.

I. INTRODUCTION :

Present work focusing in developing and validating a new high performance liquid chromatography method for estimation of Dicyclomine Hcl in capsule dosage form. The method was performed on Waters HPLC instrument using C8 (150 mm x 4.6 mm, 3.5 µm) Xbridge Column and di-Potassium hydrogen Phosphate Buffer (pH 7.50): Acetonitrile (30:70% v/v) as mobile phase at ambient temperature. Detection was carried out at 215 nm. Concentration range LOQ -150% level (0.74 - 6µg/ml) for Dicyclomine Hydrochloride. The Percentage recovery of Dicyclomine Hydrochloride was found to be 107.7% to 101.0%. Correlation coefficient for Dicyclomine Hydrochloride was found 0.999. The Rt values for Dicyclomine Hydrochloride were found to be 10.0 min respectively. The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully employed in the estimation of commercial formulations.

Along with focusing in developing and validating a new high performance liquid chromatography method for estimation of **Dicyclomine Related Compound-A (Known impurity)** in Dicyclomine Hcl capsule dosage form. The method was performedonAgilentHPLCinstrumentusingSymmet ryC8,150mmx4.6mm,3.5mandMonobasicPotassiu mhydrogenPhosphateBuffer(pH3.50):Acetonitrile(4 5: 55% v/v) as mobile phase-A and Monobasic Potassium hydrogen Phosphate Buffer (pH 3.50): Acetonitrile (20:80% v/v) as mobile phase-B with gradient method at ambient temperature. Detection was carried out at 215 nm. Concentration range LOQ
-200%level(0.423–
8.453µg/ml)forDicyclomineHydrochlorideRelatedc

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ompound-A.

The Percentage recovery of Dicyclomine Hydrochloride Related compound-A was found to be 105.7% to 98.3%. Correlation coefficient for Dicyclomine Hydrochloride Related compound-A was found 1.000. The Rt values for Dicyclomine Hydrochloride Related compound-A were found to be 16.0min. The method was validated according to the guidelines of International Conference on harmonization (ICH) and was successfully employed in the estimation of commercial

II. AIM AND OBJECTIVES :

The number of drug formulations introduced into the market has been increasing atanalarmingrate.Standardanalyticalproceduresforth esedrugsofformulationsmay not be available and if available may not suit to our actual conditions of use. So it is require to develop newer analytical methods which are accurate, precise, specific and linear. The developed methods is validated for parameters such as system suitability, precision, accuracy, linearity, LOD and LOQ and evaluation of analytical method validation report generated for the developed methods as per ICHguidelines.

III. MATERIALS AND METHODS :

The materials involve in the progress are, Volumetric flasks, Beakers , Measuring cylinder, Pipettes, Balance, HPLC system, Column

The ReagentsUsed in the method are,Monobasic potassiumPhosphate,Di potassiumhydrogen phosphate, anhydrous, Triethylamine, Orthophosphoric acid, Acetonitrile, Hydrochloric acid,

Sodium hydroxide pellets, Hydrogen peroxide, Water

METHOD DEVELOPMENT STUDIES

Related compound-A Impurity:

TRAIL-I:

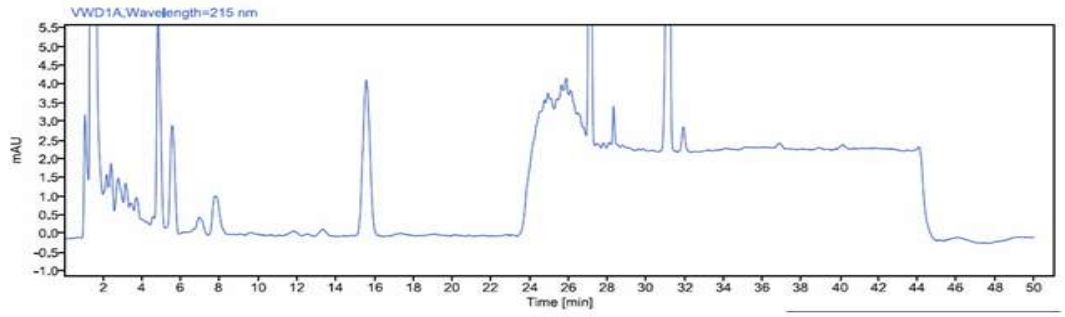
Chromatographic Conditions:

Column : XBridgeC8, 150 mm x 4.6 mm, 3.5□ m or equivalent. Wavelength: 215 nm.

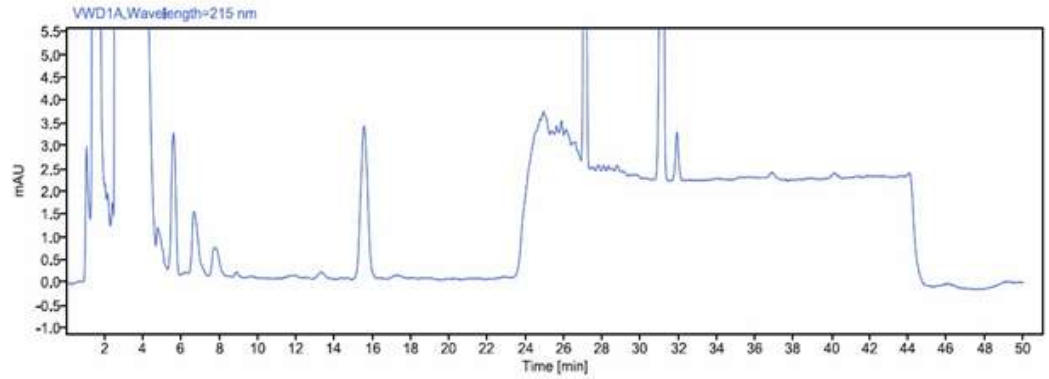
Flowrate: 1 mL / min. Column Temp: Ambient Samplercooler : Ambient Injection volume : 100 µL

Run time: 50min

Reference sample chromatogram:



Reference Placebo chromatogram:



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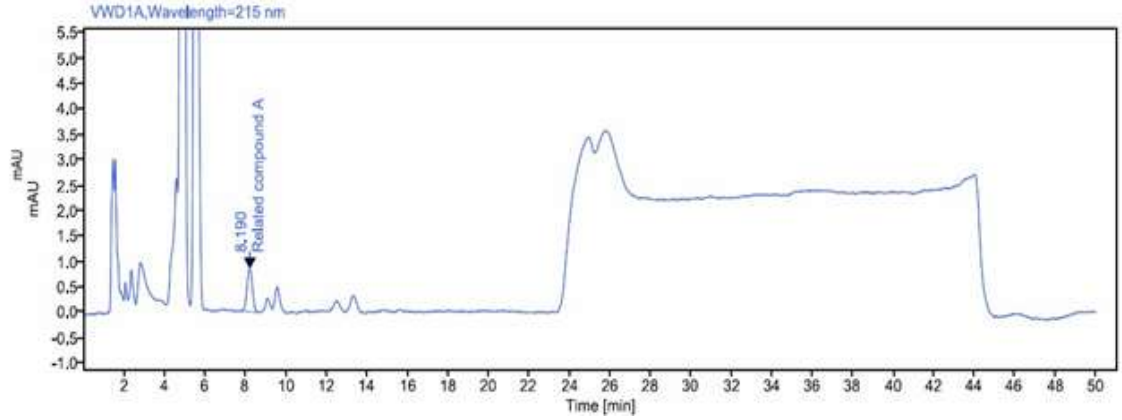
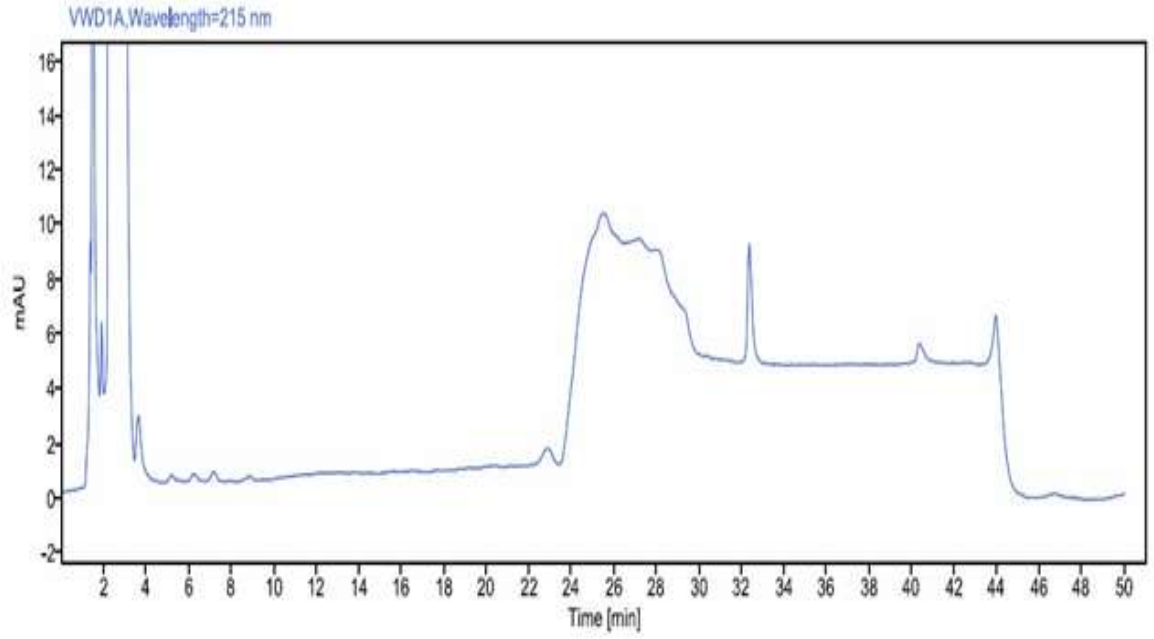
Reference Standard chromatogram:



OBSERVATION:
In this method Trail, interference observed at the retention time of related compound-A (RT-8.19min) Peak in placebo.

TRAIL-2:
Chromatographic Conditions:
Column : Symmetry C8, 150 mm x 4.6 mm, 3.5 µm or equivalent.
Wavelength : 215 nm.
Flowrate : 1 mL / min.
Column Temp: Ambient
Samplercooler : Ambient
Injection volume : 100 µL
Run time : 50min

Reference sample chromatogram:



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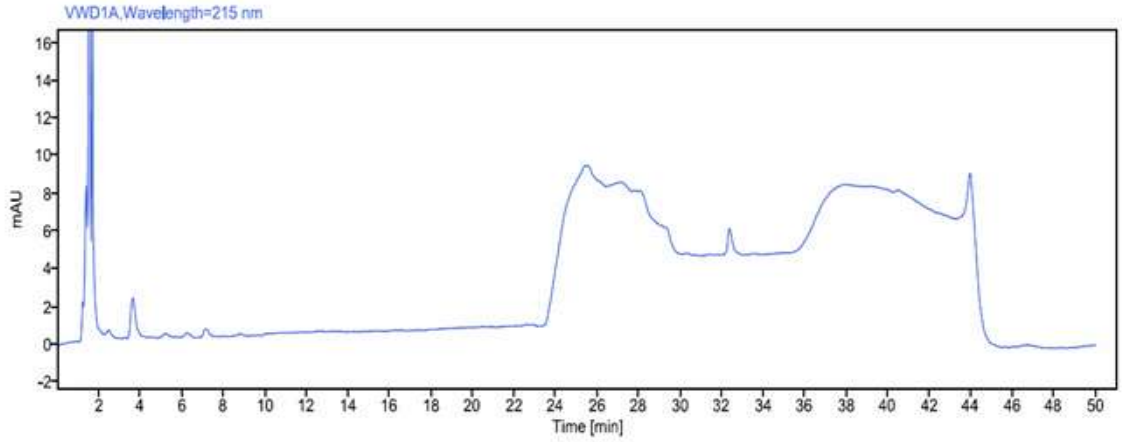
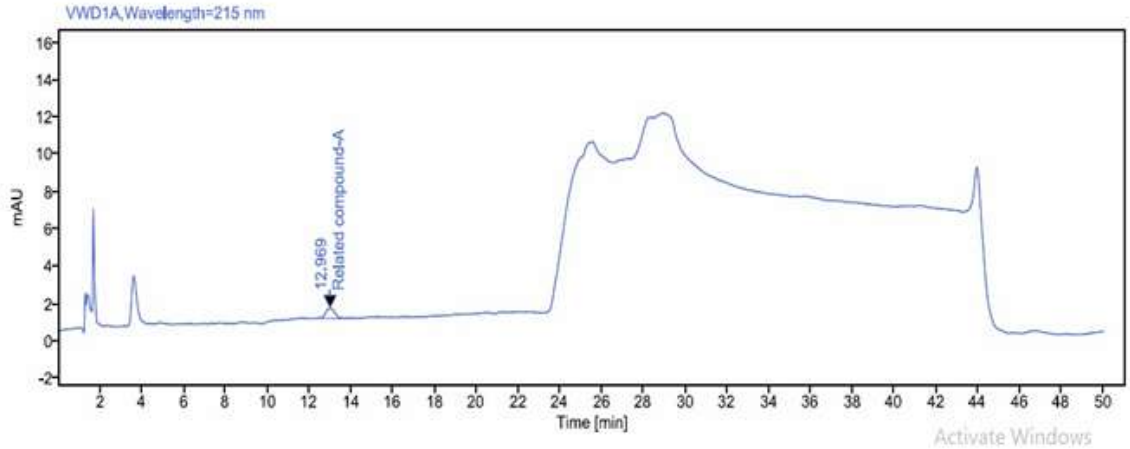
Reference Placebo chromatogram:



Reference Standard chromatogram:



Observation:

In this trail different column used no interference was observed at the retention time at Dicyclomine Related compound-A and also eluted at 12.9min.

Conclusion:

Based on the observation this method was selected for estimation of Dicyclomine Related compound-A impurity and need to validation.

METHOD VALIDATION PARAMETERS OF RELATED COMPOUND-A

SYSTEM SUITABILITY:

Toverifythattheanalyticalsystemisworking properlyandcangiveaccurateand precise results, the system suitability parameters are to be set. Injected blank (diluent) (1 injection), Standard solution (6 injections) and checked the following system suitability. The Results were tabulated inTable-1.

S. No	Acceptance criteria	Result
1	Signal to noise ratio should be NLT 10 in sensitivity solution.	28.07
2	The RSD for 6 replicate injections of standard solution should be not more than 5.0%.	1.97%

Table-1: Results of System Suitability

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PRECISION:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogenous test. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements.

SYSTEM PRECISION:

The system precision is checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area response of six determinations should be measured and % relative standard deviation should be calculated.

Injected Blank (diluent) (1 injection), standard solution (6 injections), and check the following parameters, The Results were tabulated in Table-2.

S.NO	RT	Area
1	12.97	16.23
2	12.96	15.96
3	12.98	15.97
4	12.97	15.35
5	12.93	16.17
6	13.02	15.87
Average	12.97	15.93
StandardDeviation	0.0293	0.313
%RSD	0.2	1.97

Table-2: Results of System Precision

Specificity:

Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. Performedthespecificityparameterofthemethodbyinjectingblank,placebo,standard solution, sample solution, sample spiked with Relatedcompound-A.The Results were tabulated in Table-3.

S. No	Name	RT IMP-A (in min)
1	Blank solution	ND
2	Placebo solution	ND
3	Standard solution	13.02
4	Spiked sample	13.06

Table-3: Results of Specificity

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Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement betweenthevalue,whichisacceptedaseitheraconventi onaltruevalueoranaccepted reference value and the foundvalue.

Accuracy samples were prepared ranging from LOQ, 50% level, 100% level and 150% level of the test preparation and the results are tabulated below.

The accuracy of the method was assessed by spiking Related compound-A drug substance to the Sample in triplicates in each level, and 100% is 6 levels.

Accuracy level	mg added	mg found	% Recovery	%Mean recovery	%RSD
Accuracy-50%-1	5.04	4.89	97.1	99.1	1.8
Accuracy-50%-2	5.04	5.02	99.7		
Accuracy-50%-3	5.04	5.07	100.6		
Accuracy-100% -1	5.04	5.05	100.2	100.1	2.0
Accuracy-100% -2	5.04	5.03	99.9		
Accuracy-100% -3	5.04	5.15	102.2		
Accuracy-100% -4	5.04	4.86	96.4		
Accuracy-100% -5	5.04	5.04	100.0		
Accuracy-100% -6	5.04	5.12	101.6		
Accuracy-150% -1	5.04	4.90	97.2	98.3	1.0
Accuracy-150% -2	5.04	4.97	98.6		
Accuracy-150% -3	5.04	4.99	99.0		

Table-4: Accuracy Results for Related compound-A Solution stability of analytical solutions:

The stability of the standard solution was determined by making a series of injections over a period at RT (Room temperature).

The % Difference between initial Area to after specified time Areaof Related compound-A in standard stability and sample Spiked stability was performed at 25°C.The Results were tabulated in Table-5.

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Standard		Spiked Sample	
Interval (Hrs.)	**%Difference at 25°C**	**Interval (Hrs.)**	**%Difference at 25°C**
Initial	Not Applicable	Initial	Not Applicable
15.7	-1.0	11.3	-1.0
26.2	1.9	20.1	6.2
36.6	3.0	30.5	4.0

Table-5: Results Solution Stability At25°C

Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly or by a well- defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Performed the Linearity of Related compound-A.Recorded the area response at each level and calculated slope, intercept, correlation coefficient and regression coefficient (R square). Test the intercept for statistical equivalence to zero.

Weighed 1.1007mg of Related compound-A standard into a 50.0 mL volumetric flask. Dissolved and diluted to volume with diluents (Stock-I).

Linearity levels (%)	**Concentration (ppm)**	**Area Response**
Linearity level 1-2.5%	0.106	0
Linearity level 2-5%	0.211	0
Linearity level 3-10%	0.423	0.97
Linearity level 4-20%	0.845	2.89
Linearity level 5-50%	2.113	7.85
Linearity level 6-100%	4.227	14.84
Linearity level 7-150%	6.340	23.66
Linearity level 8-200%	8.453	31.51
Slope		**3.784**
Intercept		**-0.531**
Regression coefficient (r^2)		**0.999**

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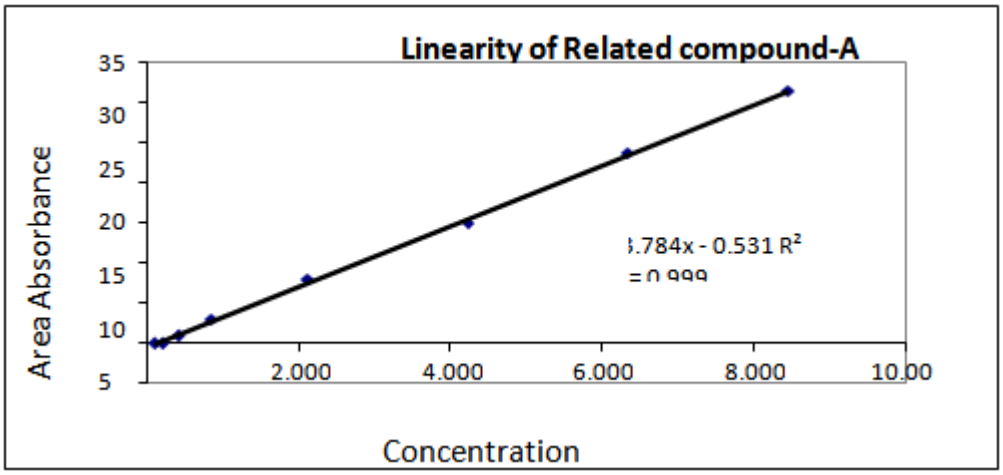
Correlation coefficient	**1.000**
% y intercept	**-3.58**
LOQ In ppm	**0.92**
LOD In ppm	**0.30**

Table-6: Linearity results For Related compound A Linearity plot

Linearity plot



LIMIT OF QUANTIFICATION (LOQ):

A solution containing Dicyclomine HCl Related compound A at Weight of 1.1007mg of Related compound-A standard into a 50.0 mL volumetric flask. Dissolved and diluted to volume with diluents (Stock-I).

	Related compound A	
Samples	**Retention time**	**Area**
LOQ-1	13.21	3.87
LOQ-2	13.18	3.74
LOQ-3	13.18	3.78
LOQ-4	13.16	3.91
LOQ-5	13.16	3.62
LOQ-6	13.16	3.50
Mean	**13.18**	**3.74**
SD	**0.0197**	**0.154**
% RSD	**0.1**	**4.13**

Table-7: Related compound A LOQ Precision results

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LIMIT OF DETECTION (LOD):

A solution containing Dicyclomine HCl Related compound A at Weight of 1.1007mg of Related compound-A standard into a 50.0 mL volumetric flask. Dissolved and diluted to volume with diluents (Stock-I).

Samples	Peak RT	Peak Area
LOD	13.23	1.68

Table-8: Related compound A LOD results

Method description (final method)

ChromatographicConditions:

Column : Symmetry C8, 150 mm x 4.6 mm, 3.5 µm or equivalent. Wavelength : 215 nm. Flowrate: 1.0 mL / min. Column Temp : Ambient Samplercooler : Ambient Injection volume : 100µL

Run time : 50 min.
Pump mode :Gradient

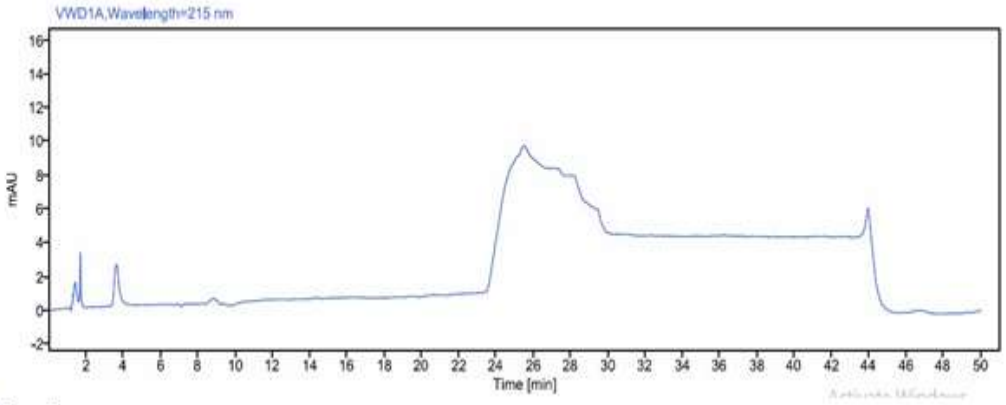
Procedure:

EquilibratetheHPLCinstrumentunderspecifiedmethodconditionsandproceedasper belowtable.

REFERENCE CHROMATOGRAMS:

S.No	Chromatogram
1.0	Blank Chromatogram
2.0	Control Placebo Chromatogram
3.0	Standard solution Chromatogram
4.0	Sensitivity Solution Chromatogram
5.0	Control sample Chromatogram
6.0	Control API Chromatogram
7.0	Spiked sample Chromatogram

Blank



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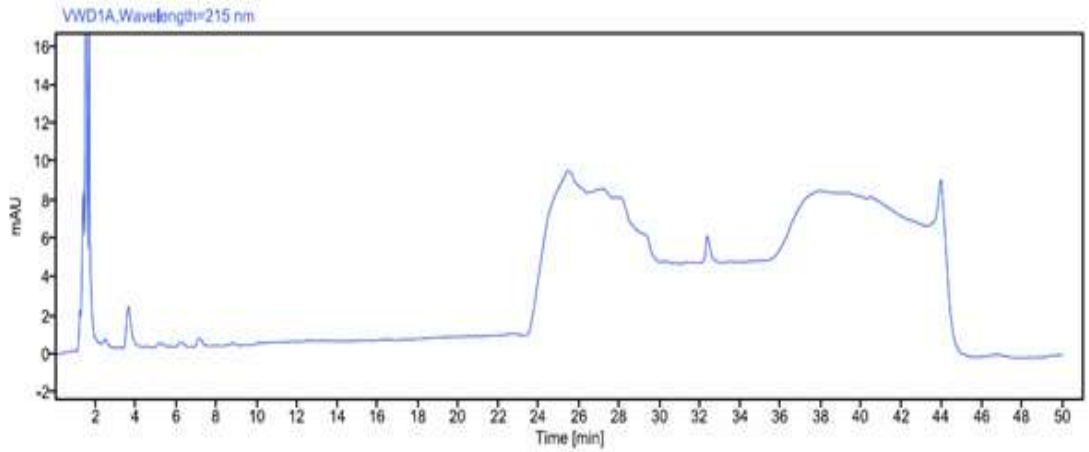
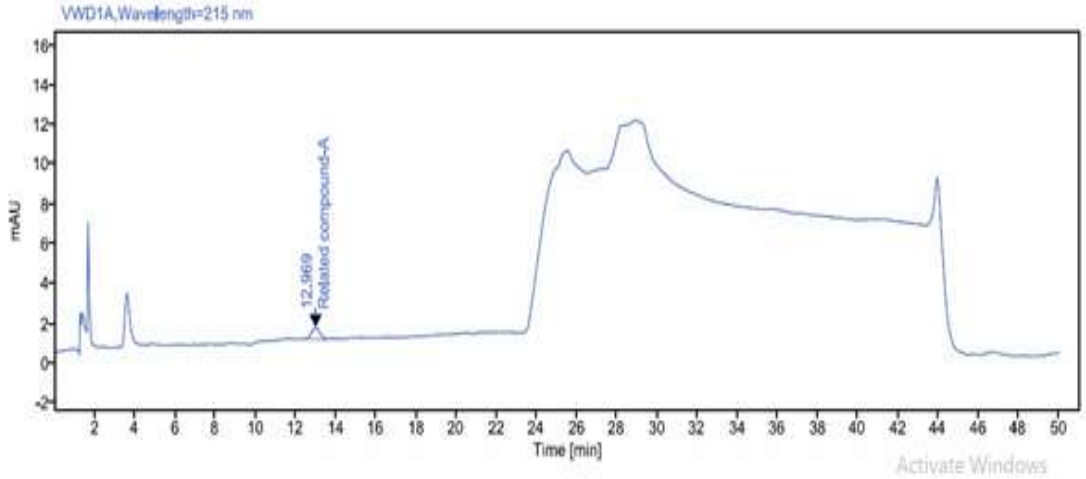
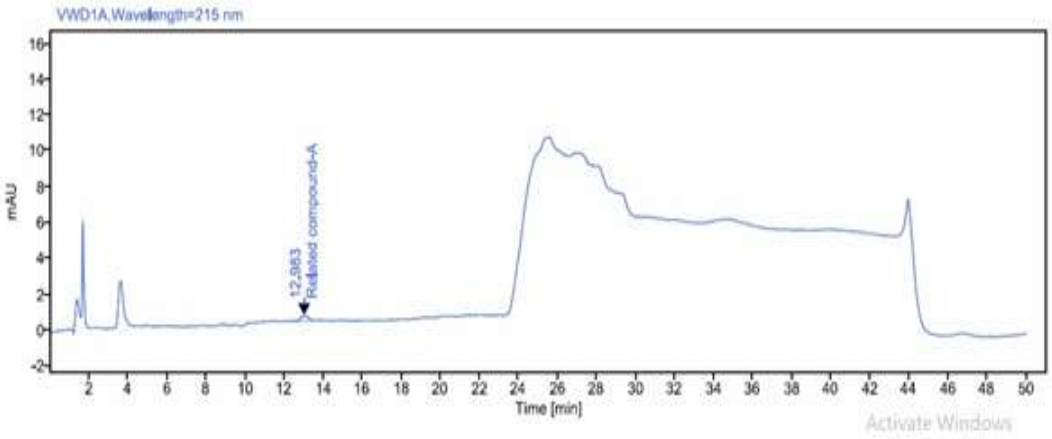
Control Placebo



Standard solution



Sensitivity solution



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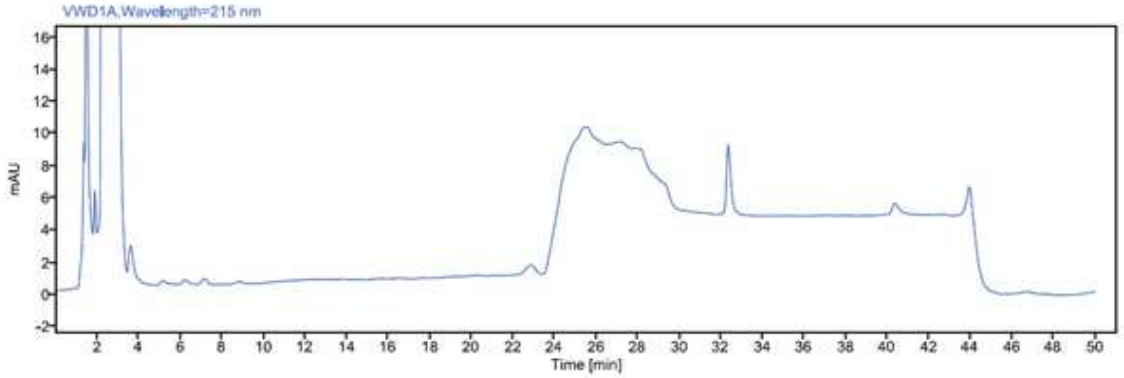
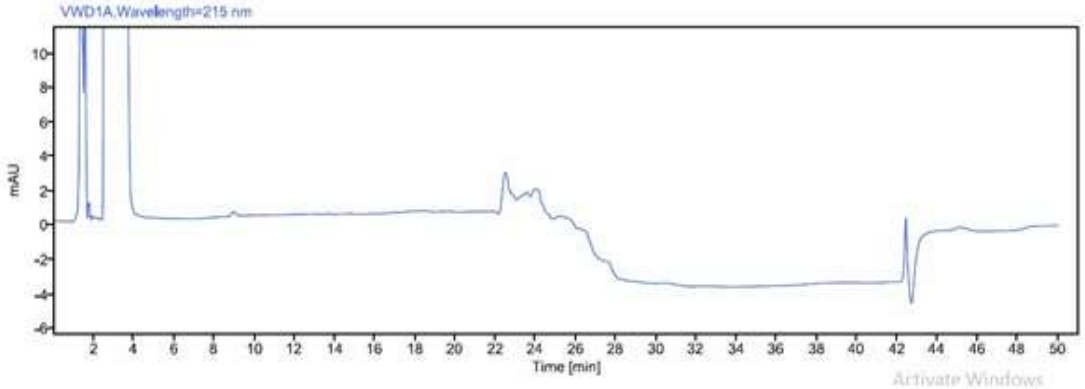
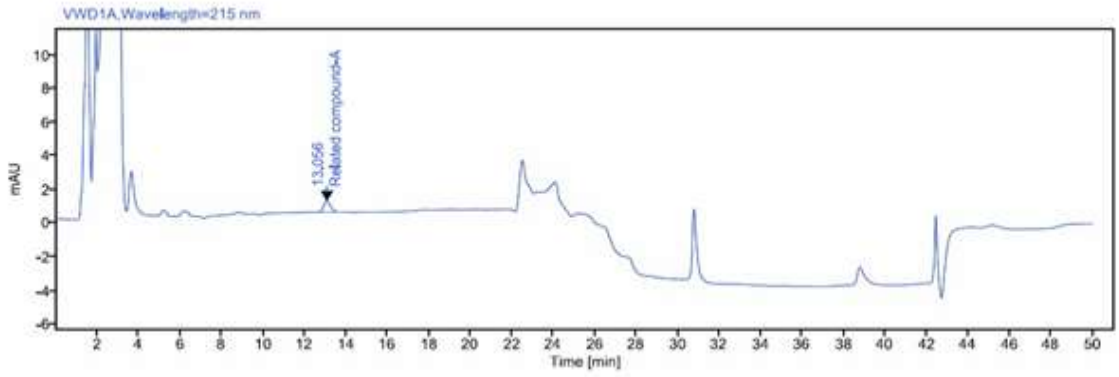
Control Sample



Control API



Spiked Sample Chromatogram



DETERMNATION OF UNKNOWN IMPUIRITIES
TRAIL-1
ChromatographicConditions:
Column : X BridgeC8, 150 mm x 4.6 mm, 3.5 □ m or equivalent.
Wavelength: 215 nm.
Flowrate: 1 mL / min.
Column Temp: Ambient
Samplercooler: Ambient
Injection volume : 50µL
Run time: 50 min
Pump mode:Isocratic

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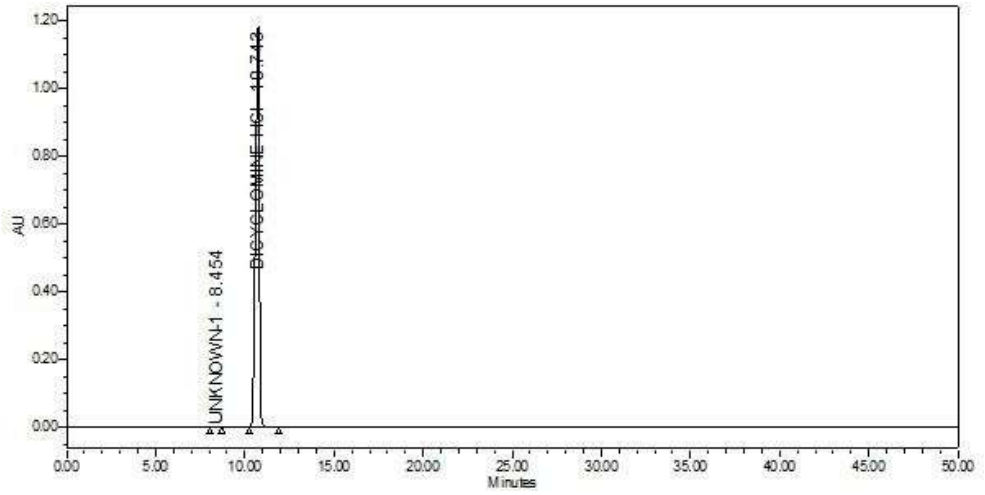
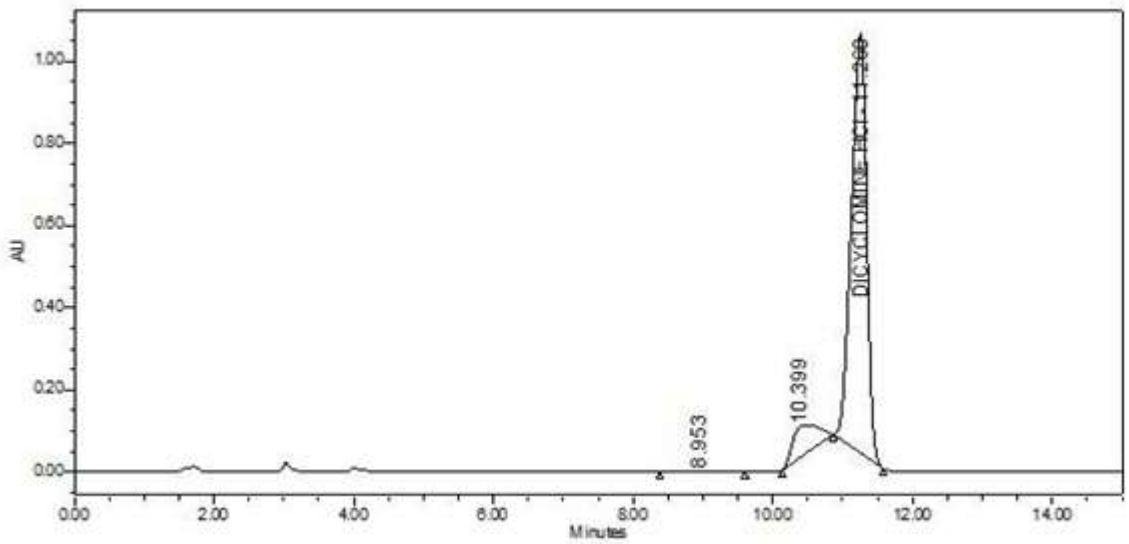
Observation:
In this trail impurities separated from main peak and also analyte peak shape was good. Using this method will be application to capsule dosage form.



Trial chromatogram 1

TRAIL-2
Observation:
In this trail impurities separated from main peak but analyte peak shape was not good.
Based on the analyte peak elution to be trail with chromatographic conditions.



Trial chromatogram 2

TRAIL-3
Chromatographic conditions:
Column : X BridgeC8, 150 mm x 4.6 mm, 3.5 □ m or equivalent. Wavelength : 215 nm.
Flowrate: 0.9 mL / min and 1.1mL /min Column Temp: Ambient and30°C
Samplercooler: Ambient Injection volume : 40, 50 µL Run time: 50 min
Pump mode:Isocratic

Observation:
In this trail impurities separated from main peak but analyte peak shape was not good. Based on the observation analyte peak need to trail with diluent.In this trail peak splitting was observed same way of trail-02.

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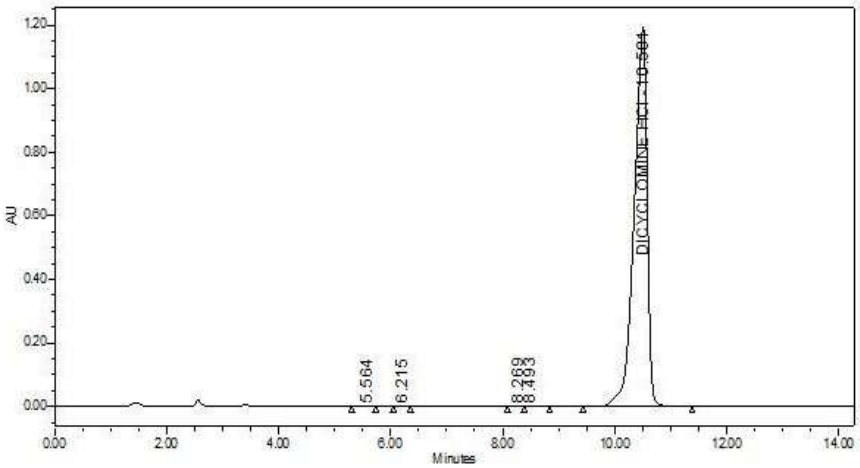
TRAIL-4

Chromatographic conditions:

Column : X BridgeC8, 150 mm x 4.6 mm, 3.5 □ m or equivalent. Wavelength: 215 nm.
Flowrate: 1.0 mL / min Column Temp: Ambient Samplercooler: Ambient Injection volume : 50µL
Run time: 50 min
Pump mode:Isocratic

Observation:

Based on the observation of API samples with different ratio shows precipitation.
Hence diluent ratio of 50:50%v/v selected for preparation of samples. But need to optimizing the mobile phase due to little variation in peak symmetry so will be trailed with dipotassium hydrogen phosphate buffer.



Trial chromatogram 3

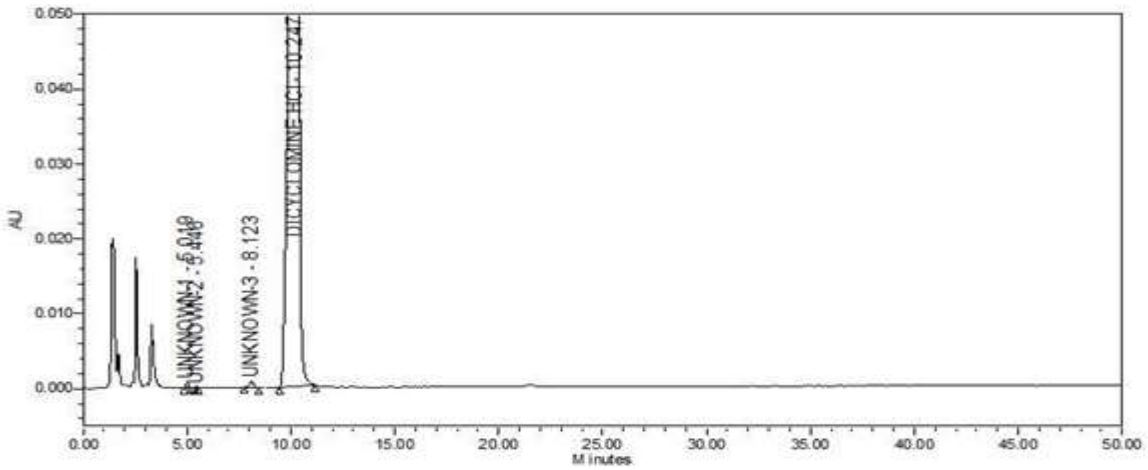
TRAIL-5

Chromatographic Conditions:

Column : X Bridge® C8, 150 mm x 4.6 mm, 3.5 µm or equivalent.
Wavelength : 215 nm.
Flowrate : 1.0 mL /min.
Column Temp :Ambient
Samplercooler :Ambient
Injectionvolume : 50 µL
Run time: 50 min
Pump mode :Isocratic

Observation:

Based on the observation after filtration samples was clear and analyte peak was not splitting and symmetrical. Hence this method was for estimation of impurities in samples and need for method validation.



Trial chromatogram 4

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METHOD VALIDATION OR DICYCLOMINE HYDROCHLORIDE

System suitability:

To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set.Injected blank (diluent) (1 injection), Standard solution (6 injections) and checked the following system suitability. The Results were Determined to be 3.6

PRECISION:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogenous test. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements.

SYSTEM PRECISION:

Thesystemprecisionischeckedbyusingstandardchemicalsubstancetoensure that the analytical system is working properly. The area response ratio of Six determinations should be measured and % Relative standard deviation should be calculated for Dicyclomine Hclstandard.

Injected Blank (diluent) (1 Injection), Standard solution (6 Injections), and check the following parameters. The Results were tabulated in Table-1

S.NO	**RT**	**Area**
1	10.624	31781
2	10.608	30698
3	10.588	32931
4	10.565	30288
5	10.558	32979
6	10.577	31268
Average	**11**	**31658**
Standard Deviation	**0.0254**	**1125.2820**
%RSD	**0.2**	**3.6**

Table-1: Results of System Precision for Dicyclomine Hcl Capsules

METHOD PRECISION:

In method precision, a homogenous test of a single batch should be analyzed sixtimes.Thisindicateswhetheramethodisgivingconsistentresultsforasinglebatch. Analyze the sample of as per analytical procedure. Inject separately each of the following solutions into the chromatograph. The Results were tabulated inTable-2.

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S. No	% Impurity
Sample preparation_1	0.07
Sample preparation_2	0.06
Sample preparation_3	0.07
Sample preparation_4	0.07
Sample preparation_5	0.07
Sample preparation_6	0.07
Mean	**0.07**
STD.DEV	**0.0041**
% RSD	**6.0**

Table-2: Results of Method Precision for Dicyclomine Hcl Capsules

SPECIFICITY:

Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components.Performed the specificity parameter of the method by injecting blank, Placebo, standard solution, and sample solution. Recorded the Retention times of all peaks.
The Results were tabulated in Table-3.

S.No	Name	Retention Time (in min)
1	Blank solution	ND
2	Placebo solution	ND
3	Standard	10.497
4	Sample	10.677

Table-3: Results of Specificity for Dicyclomine Hcl Capsules

ACCURACY :

The accuracy of the method was assessed by spiking Dicyclomine HCl standard solution to the placebo in each level.

Preparation of Standard stock solution for 50%, 100% and 150%:

Weighed and transferred 10.39 mg of Dicyclomine Hcl standard into a 100mL of volumetric flask, to this added 70mL diluent and sonicated to dissolve, diluted to volume with diluents and mix Well

Preparation of Standard solution for LOQ:

Further pipetted out 3mL of above standard stock solution into 50mL volumetric flask and diluted to volume with diluents and mixed well.

Accuracy level	**ppm added**	**ppm found**	**% Recovery**	**Mean% recovery**	**%RSD**
LOQ level-Prep-1	0.74	0.76	103.8	107.7	5.2
LOQ level-Prep-2	0.74	0.82	111.7		

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LOQ level-Prep-3	0.74	0.73	99.7		
50% level-Prep-1	2.04	2.15	105.3	103.8	2.1
50% level-Prep-2	2.04	2.09	102.3		
50% level-Prep-3	2.04	2.05	100.3		
100% level-Prep-1	4.08	4.14	101.3	100.1	1.7
100% level-Prep-2	4.08	4.04	98.9		
100% level-Prep-3	4.08	4.15	101.7		
150% level-Prep-1	6.13	6.37	104.0	101.0	4.2
150% level-Prep-2	6.13	6.00	98.0		
150% level-Prep-3	6.13	6.27	102.3		

Table-4: Accuracy results

LINEARITY:

The linearity of an analytical method is its ability to elicit test results that are directly or by a well- defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Performed the Linearity of Dicyclomine Hcl.

Recordedthearearesponseateachlevelandcalculateslope,intercept,correlation coefficient and regression coefficient (R square). Test the intercept for statistical equivalence tozero.

Linearity Standard Stock Preparation:

10.7mg of Dicyclomine hcl standard transferred into a 100 ml volumetric flask, added 50 ml diluent& sonicated to dissolve and diluted to volume with diluent.

Standard stock solution used for Linearity solutions.

Level (%)	**Concentrationof Dicyclomine(µg)**	**Standard Area**
5	0.20	0
10	0.41	0
20	0.82	6415
50	2.04	16062
80	3.27	26968
100	4.08	32253
120	4.90	39110

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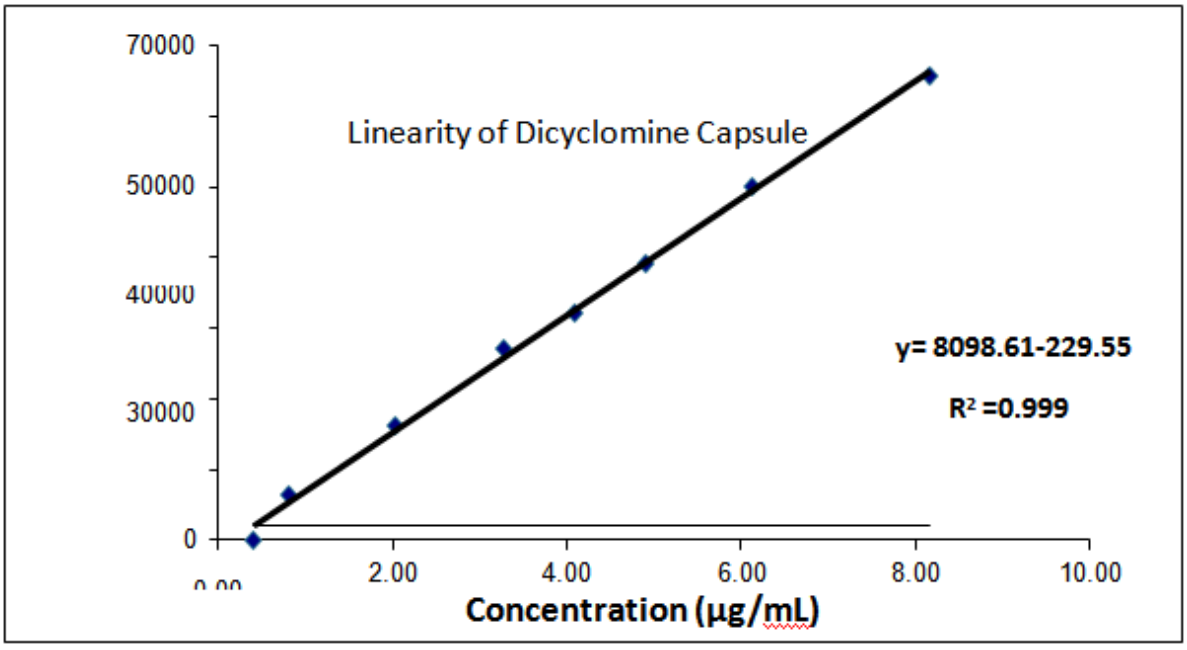
150	6.13	49992
200	8.17	65733
Slope		**8098.61765**
STYEX		**545.6505**
Intercept		**-229.5503**
r		**1.000**
r2		**0.999**
%Y-intercept		**-0.7**
LOQ		**0.67**
LOD		**0.22**

Table -5: Linearity results

Linearity plot



LOD AND LOQ :

Table -6: LOD TABLE

Product name	**Concentration**	
	µg/mL	**% w/w**
Dicyclomine Hcl	0.22	0.011

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Table -7: LOQ TABLE

Product name	Concentration	
	µg/mL	% w/w
Dicyclomine Hcl	0.67	0.034

METHOD DESCRIPTION:

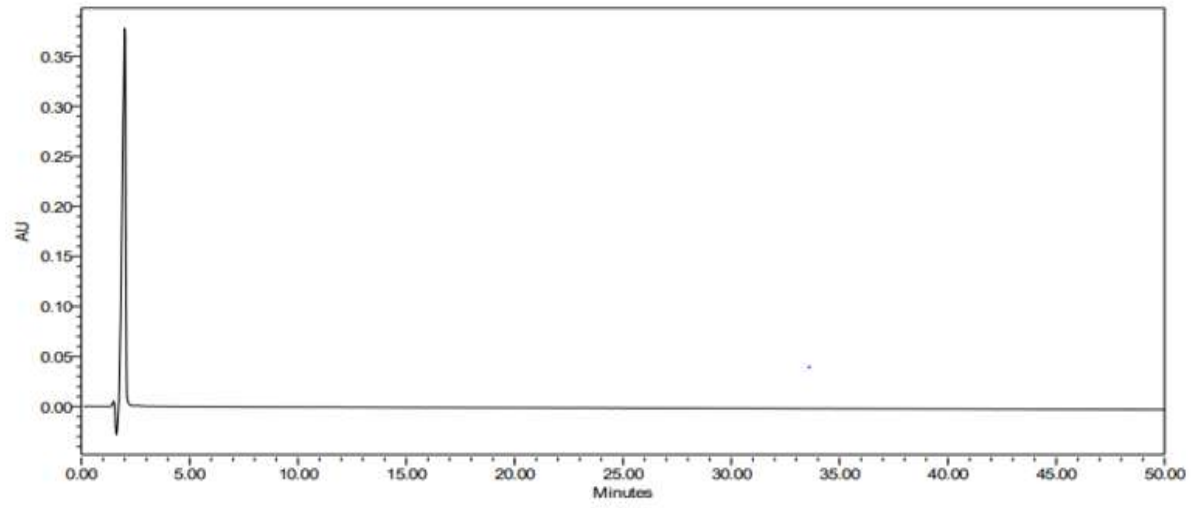
Chromatographic Conditions:

Column : X Bridge® C8, 150 mm x 4.6 mm, 3.5 µm or equivalent.
Wavelength : 215 nm.
Flowrate : 1.0 mL /min.
Column Temp :Ambient
Samplercooler:Ambient
Injectionvolume : 50 µL
Run time : 50 min
Pump mode :Isocratic

REFERENCE CHROMATOGRAMS:

S.No	Chromatogram
1.0	Blank Chromatogram
2.0	Placebo Chromatogram
3.0	Sensitivity chromatogram
4.0	Standard solution Chromatogram
5.0	Sample Solution Chromatogram

Blank chromatogram:



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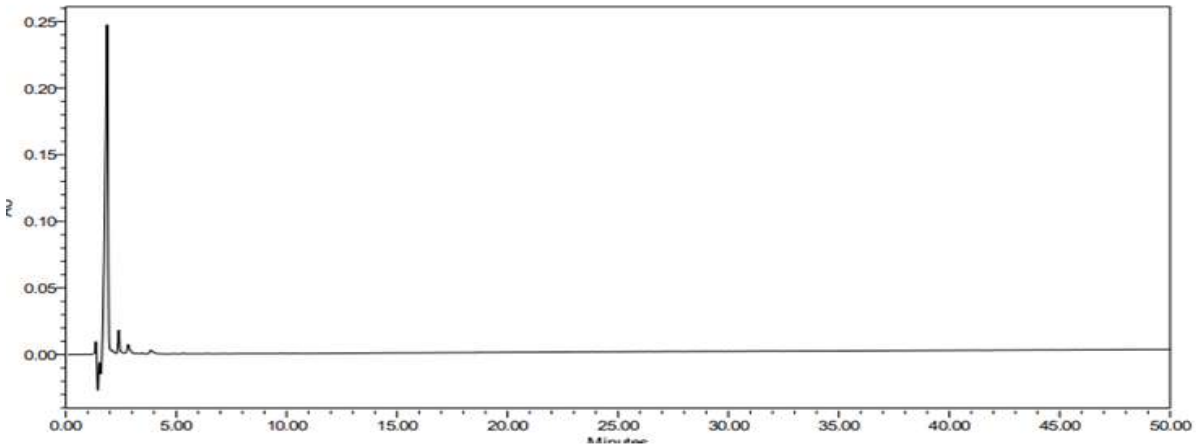
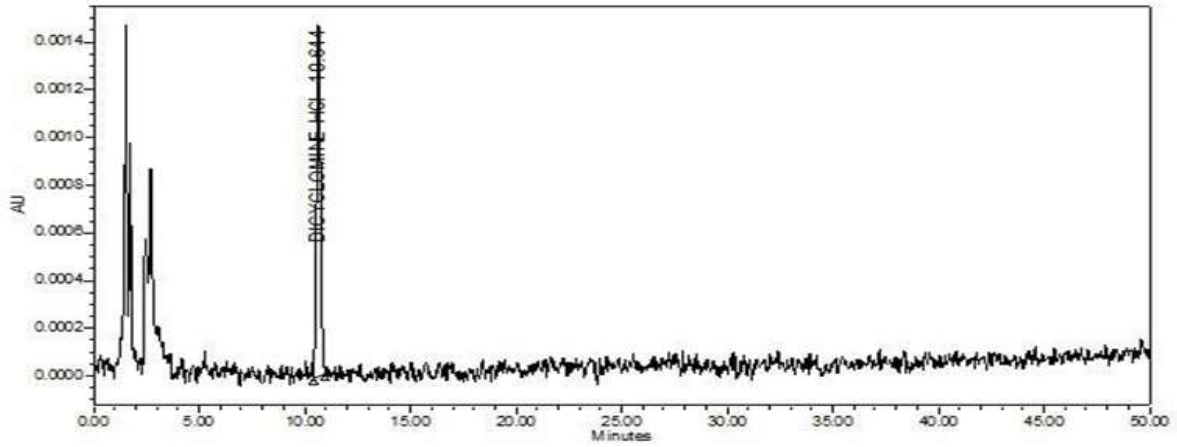
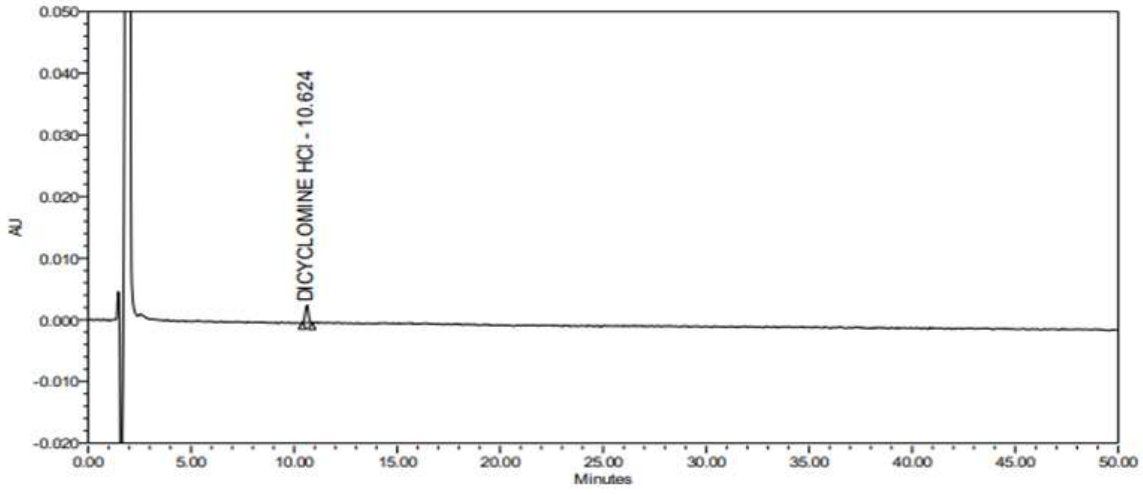
Placebo Chromatogram:



Sensitivity chromatogram:



Standard solution Chromatogram:



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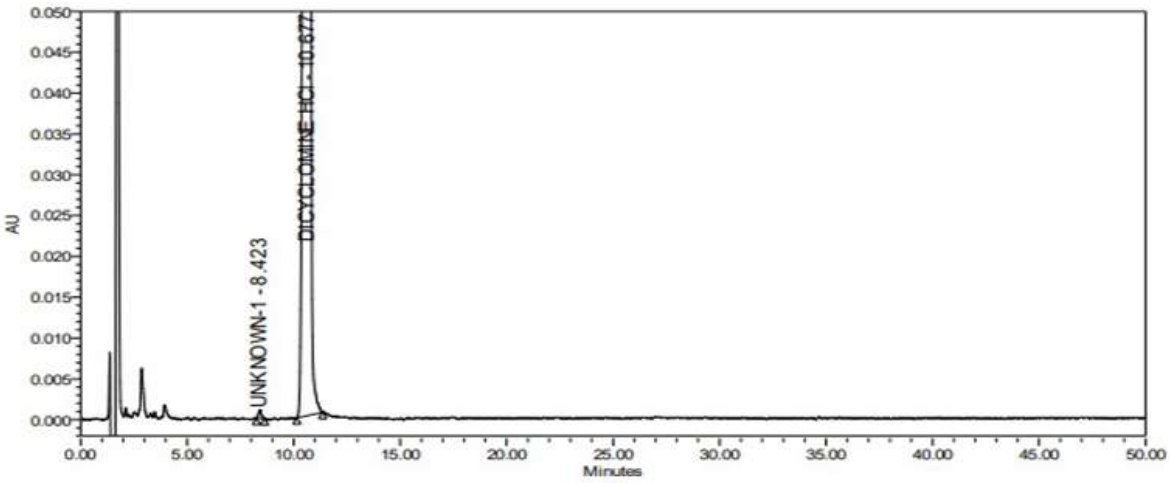
Sample Solution Chromatogram:



IV. RESULTS AND DISCUSSION

RESLUTS:

Parameters	Results		Limit
	Unknown	RC-A	
System suitability-%RSD	1.1	1.97	NMT 5.0%
System precision-%RSD	0.1	1.97	NMT 5.0%
Method Precision-%RSD	7.0	2.6	NMT 10.0%
Specificity	Specific	specific	Interference NMT ±0.5%
Accuracy %	100.8-123.8	98.3-105.7	70-130%
Linearity	r: 0.999	r: 0.999	NLT 0.995
Specification	Limit-0.2%	Limit-0.2%	Total impurities(0.4%)

DISCUSSION :

From the reported literature, there were few methods established for the determination of impurities in Dicyclomine Hydrochloride in

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capsule dosage form. It was concluded that there were only few methods reported for the estimation of impurities in Dicyclomine hydrochloride, which promote to pursue the present work. Thescopeandobjectiveofthepresentworkistodevelop andvalidateanewRP-HPLC methods for determination of impurities in capsule dosage form. In RP-HPLC method development, Waters 2695 series with 2995 PDA Detector and column used is X- Bridge C8; 4.6mm X 150 mm; 3.5microns particle size for unknown impurities. Injection volume of 40µL is injected and eluted with the mobile phase selected after optimizationwasAcetonitrile:DipotassiumPhosphat ebufferpH7.5(70:30%v/v)was found to be ideal. The flow rate was found to be optimized at 1.0 mL/min. Detection was carried out at 215 nm. This system produced symmetric peak shape, good resolution and reasonable retention times of Dicyclomine HCl were found to be 10.0 minutes. The Dicyclomine HCl showed Linearity in the range of 0.21 – 8.56 µg/mL respectively. Precision of the developed method was studied under system precision and method precision. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The %RSD value for percentage recovery of Dicyclomine HCl was found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for DicyclomineHcl.

AlongwithforDicyclomineRelatedcompou nd-A,UsingAgilentHPLCwith DAD detector and column used is Symmetry C8; 4.6mm X 150 mm; 3.5microns particlesizeforknownimpurity.Injectionvolumeof10 0µLisinjectedandelutedwith the mobile phase selected after optimization was Acetonitrile: Potassium Phosphate buffer pH 3.5 with gradient method. The flow rate was found to be at 1.0 mL/min. Detection was carried out at 215 nm. This system produced symmetric peak shape without interference at retention time of Dicyclomine HCl Related compound-A were found to be 12.5 minutes. The Dicyclomine HCl Related compound-A showed Linearity in the range of 0.42 – 8.45 µg/mL. Precision of the developed method was studied under system precision and method precision. The %RSD values for precision wasfoundtobewithintheacceptablelimit,whichreveal edthatthedevelopedmethod wasprecise.The%RSDvalueforpercentagerecoveryo fDicyclomineHClRelated compound-A was found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for Dicyclomine Hcl Related compound-A.

From the above experimental data and results, the developed RP-HPLC method is having the following advantages:

V. CONCLUSION :

ARP-HPLCmethodforDicyclomin-eHclcapsulewasdevelopedandvalidated in capsule dosage form as pre–ICH Guide lines, A Linear ,Accurate, precise methods was developed for the determination of impurities in Dicyclomine Hydrochloride in capsule dosage form. Retention time of Dicyclomine Hcl capsule were found to be 10mins for Unknown impurities and known impurity at 12.5min. The linearity results for Dicyclomine Hcl correlation coefficients (R^2) were 0.999 and Y-intercept at 100% concentration was 1.3 for unknown impurities and linearity results for Dicyclomine Hcl Related compound-A correlation coefficients (R^2) was 1.000 and Y-intercept at 100% concentration was -3.58 for known impurity, demonstrating excellent linearity intherelationshipbetweenconcentrationandpeakarea. Sothemethoddevelopedwas simpleandeconomicalthatcanbeadoptedinregularQu alitycontroltestinIndustries.

Thedevelopedmethodwasvalidatedforvario usparametersasperICHguidelineslike system suitability, linearity, system precision, method precision andaccuracy. The analytical method validation of Dicyclomine Hcl capsule by RP-HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis.

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