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A STUDY OF ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IMPURITIES IN DICYCLOMINE HYDROCHLORIDE CAPSULE DOSAGE FORM BY RP-HPLC METHOD

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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 and reproducible and compliant with International Council for Harmonization (ICH) guidelines for method validation. The obtained results of validation parameter within the Acceptance 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 content assessments in routine quality control

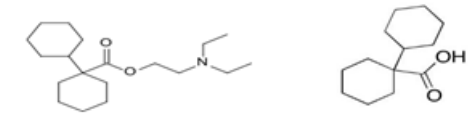
tests for pharmaceutical companies. This study makes a significant contribution to the evolution of pharmaceutical analytical techniques, offering valuable insights into the use of validated RP-HPLC method.

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

INTRODUCTION

Dicyclomine is a muscarinic M1, M3, and M2 receptor antagonist as well as a non-competitive inhibitor of histamine and bradykinin used to treat spasms of the intestines seen in functional bowel disorder and irritable bowel syndrome. Dicyclomine chemical name as 2-(Diethyl amino) ethyl 1-cyclohexylcyclohexane-1-carboxylate and Dicyclomine Releated compound-A chemical name as ([1, 1'-Bi(cyclohexane)]-1-carboxylic acid). Dicycloverine blocks the action of acetylcholine on cholinergic receptors on smooth muscles in the GI tract, relaxing the smooth muscle. Dicyclomine is used to treat a certain type of intestinal problem called irritable bowel syndrome. It helps to reduce the symptoms of stomach and intestinal cramping. Dicyclomine is used to treat the symptoms of irritable bowel syndrome, specifically hyper motility, in adults. This medication works by slowing the natural movements of the gut and by relaxing the muscles in the stomach and intestines. Common side effects include dry mouth, blurry vision, weakness, sleepiness, and lightheadedness. Serious side effects may include psychosis and breathing problems in babies.

Structure of Dicyclomine and Releated compound-A



No published work has been reported to describe the estimation of impurities of Dicyclomine Hydrochloride capsule dosage form with HPLC. This paper describes the development and validation of new precise, simple and reliable methods with gradient elution of Known impurity and Isocratic elution of Unknown impurities for the estimation of Dicyclomine Hydrochloride Capsules. The method has been proven to be suitable for bulk, final product release and stability testing in oral pharmaceuticals formulations.

Materials and Reagents

Working standard (Dicyclomine hydrochloride), Dicyclomine Releated compound-A from

pharmaffiliates Analytical and Synthetics (P) LTD, ISO Accredited Company. Dipotassium phosphate anhydrous, Triethylamine, Orthophosphoric acid, Acetonitrile, Monobasic potassium phosphate, Sodium hydroxide, Hydrochloric acid and Sodium hydroxide pellets. These all the above chemicals and solvent are from merck. Water-Milli Q.

HPLC SYSTEM

For Unknown impurities

The analysis was conducted using a high performance liquid chromatography system (waters) which was furnished with an auto sampler (e2695) and Detector should be PDA, Data were captured with the aid of Empower software. The standard and sample were analysed using an analytical X Bridge C8 column, 150x4.6mm, 3.5µm. The initial mobile phase was (K2HPO4+1ml triethylamine) Buffer: Acetonitrile in the ratio of 30:70, after mixing filtered through 0.45µm memberane filter and the diluent (K2HPO4+2ml trietylamine) Buffer: Acetonitrile(50:50). The flow rate was set at 1.0ml/min, the column and sampler temperature set at Ambient and injection volume was 50µl, wavelength 215nm and Run time 50minutes. Pump mode: Isocratic.

For known impurities

The analysis was conducted using a high performance liquid chromatography system (Agilent system) and Detector should be DAD, Data were captured with the aid of open lab software. The standard and sample were analysed using an analytical Symmetry C8, 150 mm x 4.6 mm, 3.5 µm column. The initial mobile phase-A was (buffer (KH2PO4) PH 3.5), Buffer: Acetonitrile in the ratio of (55:45), and mobile phase-B was Acetonitrile: buffer in the ratio of (80:20), after mixing filtered through 0.45µm memberane filter and the diluent was Acetonitrile: water (70:30). The flow rate was set at 1.0ml/min, the column and sampler temperature set at Ambient and injection volume was 50µl, wavelength 215nm and Run time 50minutes. Pump mode: Gradient.

UNKNOWN IMPURIIES

Preparation of Standard and sample solution

A standard solution consisting of Dicyclomine hydrochloride (4ppm) was prepared with solvent. Also sample solution was prepared with solvent (2000ppm).

Standard preparation

Weigh accurately about 10mg of Dicyclomine hydrochloride working standard to a 100ml

volumetric flask. Add about 50ml of diluent sonicate to dissolve it completely and make up the volume with diluent. Further dilute 4.0ml this solution to 100ml with diluent. For sensitivity from that solution 5.0ml into 10ml with diluent.

Sample preparation

Weigh accurately 20 capsules of average weight, transfer a portion of powder is equivalent to 200mg of Dicyclomine Hydrochloride to a 100ml volumetric flask. Add about 70ml of diluent to dissolve it completely and sonicate for 30 minutes with intermediated shaking, Make up the volume with diluent and centrifuge the portion of solution (4000RPM), after centrifuge filter the sample through 0.45µm Nylon filter.

VALIDATION (Unknown impurities)

System suitability

A sample solution was injected five times in order to obtain the retention times of the Dicyclomine Hydrochloride and all the important parameters of system suitability testing were calculated and the parameters like tailing and plate count were determined. (RSD of area of Dicyclomine hydrochloride peak and should be less than 5.).

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 homogeneous test.

System precision (repeatability)

The system precision was examined by analysing the area response ratio of six determination should be measured and % Releative standard deviation should be calculated for Dicyclomine hydrochloride standard.

Method precision

In method precision, a homogenous test of a single batch should be analysed six times. This indicates whether a method is giving consistent results for a single batch.

Specificity/selectivity

Checking of the interference in the optimized method. We should not find any interfering peaks in blank and placebo at the retention times of the drugs in this method. So this method to be specific. Furthermore, forced degradation studies were conducted in order to prove the selectivity of the method. The sample solution was subjected to acidic and basic hydrolysis

(using 1N Hcl and 0.05N NaoH, respectively for 60°C 30minutes, to oxidative hydrolysis (using 5% H202 for 30minutes) and for heat degradation (using 105°C for 1 day).

LINEARITY, LIMLT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Nine standard solutions were prepared for the linearity test in the range of 5-200% of working concentration for Dicyclomine hydrochloride and in the range of LOQ-150% of working concentration. Each solution was injected and linear regression analysis for each ingredient was performed.

ACCURACY

Recovery of three sample preparation at four concentration levels (LOQ, 50%, 100%, 150%) of working concentration of Dicyclomine hydrochloride was examined.

RESULTS (Unknown impurities)

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.

The Results were tabulated in Table-1.

Table 1: Results of System Suitability.

S.No.	Acceptance criteria	Result
1.	The %RSD for Six replicate injections of Dicyclomine HCl standard solution is not more than 5.0%.	3.6

SYSTEM PRECISION

The area response ratio of Six determinations should be measured and % Relative standard deviation should be calculated for Dicyclomine Hcl standard.

The Results were tabulated in Table-2

Table 2: Results of System Precision for Dicyclomine HCL Capsules.

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	1125.282
%RSD	0.2	3.6

METHOD PRECISION

In method precision, a homogenous test of a single batch should be analyzed six times. This indicates whether a method is giving consistent results for a single batch. The Results were tabulated in Table-3.

Table 3: Results of Method Precision for Dicyclomine HCL Capsules.

Sample	% Impurity
preparation_1	0.07
preparation_2	0.06
preparation_3	0.07
preparation_4	0.07
preparation_5	0.07
preparation_6	0.07
Mean	0.07
STD.DEV	0.0041
% RSD	6.0

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.

The Results were tabulated in Table-4.

TABLE 4: RESULTS OF SPECIFICITYFOR DICYCLOMINE HCL CAPSULES.

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

The Results of forced degradation were tabulated in Table-5.

TABLE 5: RESULTS OF FORCED DEGRADATION DICYCLOMINE HCL CAPSULES.

Table 5: DEGRADATION SAMPLES RESULTS.

Unknown Impurity@RRT	Control sample	Acid Sample (1.0N HCl)	Base Sample (0.05N NaOH)	Peroxide Sample (50%H2O2)	Thermal Sample (105°C, 1D)
0.229	ND	ND	ND	4.10	ND
0.271	ND	ND	ND	ND	ND
0.388	ND	ND	ND	ND	0.05
0.448	ND	ND	ND	ND	0.09
0.458	ND	ND	ND	ND	0.07
0.468	ND	ND	ND	0.04	ND
0.490	ND	ND	0.02	ND	0.01
0.517	ND	ND	ND	ND	0.01
0.531	ND	ND	0.01	ND	ND
0.801	0.08	0.07	0.07	0.04	0.11
1.310	ND	ND	ND	ND	ND
2.184	ND	ND	ND	ND	0.13
Unknown Maximum	0.08	0.07	0.07	4.10	0.13
Total Unknowns	0.08	0.07	0.10	4.18	0.47

LINEARITY, LIMLT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Performed the Linearity of Dicyclomine Hcl. Recorded the area response at each level and calculate slope, intercept, correlation coefficient and regression coefficient (R square). Test the intercept for statistical equivalence to zero. From the linearity graph estimated LOQ and LOD concentration. LOQ = 10 x STYEX/SLOPE, LOD = 3.3 x STYEX/SLOPE.

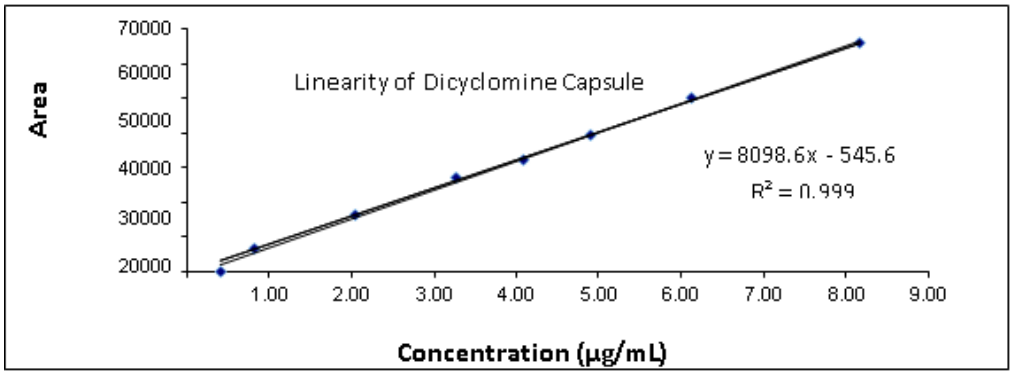
The Results were tabulated in Table-6.

Table 6: Linearity results.

Level (%)	Dicyclomine HCl	
	Concentration (µg)	Area
5	0.2	0
10	0.41	0
20	0.82	6415
50	2.04	16062
80	3.27	26968
100	4.08	32253
120	4.9	39110
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

Linearity plot



The LOQ & LOD Results were tabulated in Table-7.

Table 7: LOQ & LOD TABLE.

Name	LOQ		LOD	
	Concentration		Concentration	
	µg/mL	% w/w	µg/mL	% w/w
Dicyclomine Hcl	0.67	0.034	0.22	0.011

ACCURACY

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

The Results were tabulated in Table-8.

Table number 8: Accuracy results.

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		
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	101	4.2
150% level_Prep-2	6.13	6	98		
150% level_Prep-3	6.13	6.27	102.3		

KNOWN IMPURIIES

Preparation of Standard and sample solution

A standard solution consisting of Dicyclomine hydrochloride (100ppm) was prepared with solvent. Also sample solution was prepared with solvent (2000ppm).

Standard preparation

Weigh accurately about 5mg of Dicyclomine related compound A to a 50ml volumetric flask. Add about 25ml of diluent sonicate to dissolve it completely and make up the volume with diluent. Further dilute 4.0ml this solution to 100ml with diluent. For sensitivity from that solution 5.0ml into 10ml with diluent.

Sample preparation

Weigh accurately 20 capsules of average weight, transfer a portion of powder is equivalent to 200mg of Dicyclomine Hydrochloride to a 100ml volumetric flask. Add about 10ml of water to dissolve it completely and sonicate for 2 minutes, add about 70ml of diluent, sonicate for 5 minutes and shake for mechanical shaker for 30minutes, after add 10ml of water, equilibrate at room temperature and make up the volume with diluent. Filter through the sample 0.45µm nylon filter.

VALIDATION (Known impurity)

SYSTEM SUITABILITY

The system suitability parameters were determined by preparing standard solution of Dicyclomine hydrochloride and the solution was injected six times and the parameters like retention times, peak tailing and USP plate count were determined.

SYSTEM PRECISION

The system precision evaluates the reliability of the analytical system to precisely measure the component while the method precision takes into account also the variability of the sample preparation.

METHOD PRECISION

A homogenous test of a single batch should be analysed six times. The degree of agreement

among individual test results when the procedure is applied repeatedly to multiple samplings.

SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix. Specificity ensures the identity of the analyte of interest.

SOLUTION STABILITY

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.

ACCURACY

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.

LINEARITY

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

LIMIT OF QUANTIFICATION (LOQ)

A solution containing Dicyclomine HCl Related compound A at concentration listed below was prepared and injected six consecutive times. The lowest analyte concentration that can be quantitatively detected with state of accuracy and precision.

LIMIT OF DETECTION (LOD)

A solution containing Dicyclomine HCl Related compound A concentration listed below were prepared and injected. The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value.

RESULTS (Known impurity)

SYSTEM SUITABILITY

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

The Results were tabulated in Table-1.

TABLE 1: RESULTS OF SYSTEM SUITABILITY.

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%

PRECISION

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 Results were tabulated in Table-2.

TABLE 2: RESULTS OF SYSTEM PRECISION.

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
Standard Deviation	0.0293	0.313
%RSD	0.2	1.97

METHOD PRECISION

In method precision, a homogenous test of a single batch should be analyzed six times. Analyze the sample as per analytical procedure. Inject separately each of the following solutions into the chromatograph. The Results were tabulated in Table-3.

TABLE 3: RESULTS OF METHOD PRECISION (Spiked).

S. No	Sample ID	Related compound A
1	Sample Prep_1	0.20
2	Sample Prep_2	0.20
3	Sample Prep_3	0.20
4	Sample Prep_4	0.19
5	Sample Prep_5	0.19
6	Sample Prep_6	0.20
Average		**0.20**
Standard Deviation		**0.0055**
%RSD		**2.6**

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.

The Results were tabulated in Table-4.

TABLE 4: RESULTS OF SPECIFICITY.

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

ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted as either a conventional true value or an accepted reference value and the found value. The Results were tabulated in Table-5.

TABLE 5: Accuracy Results for Related compound-A.

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		

Related compound-A Stock solution for LOQ Accuracy

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

The Results were tabulated in Table-6.

TABLE 6: LOQ Accuracy Results for Related Compound-A.

Accuracy level	mg added	mg found	% Recovery	% Mean recovery	%RSD
Accuracy-LOQ-1	1.10	1.16	105.1	105.7	0.6
Accuracy-LOQ-2	1.10	1.17	106.3		
Accuracy-LOQ-3	1.10	1.16	105.7		

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 standardstability and sample Spiked stability was performed at 25°C.

The Results were tabulated in Table-7.

TABLE 7: Results of Solution Stability at 25°C.

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

LINEARITY

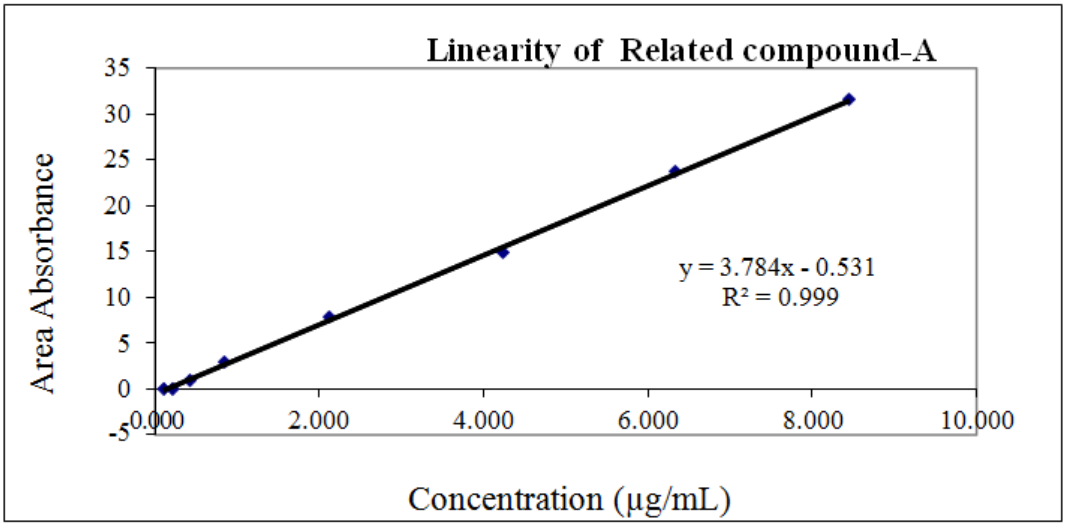
The linearity of an analytical method is its ability to elicit test results that are directly or by a well- defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Performed the Linearity of Related Compound-A.

The Results were tabulated in Table-8.

Table 8: Linearity results for Related Compound A.

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

Linearity plot



LIMIT OF QUANTIFICATION (LOQ):

A solution containing Dicyclomine HCl Related compound A at concentration listed below was prepared and injected six consecutive times.

The Results were tabulated in Table-9.

Table 9: Related compound A LOQ Precision results.

Samples	Related compound A	
	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**

LIMIT OF DETECTION (LOD)

A solution containing Dicyclomine HCl Related Compound A concentration listed below were prepared and injected. The Results were tabulated in Table-10.

Table 10: Related compound A LOD results.

Name	Peak RT	Peak Area
LOD	13.23	1.68

SUMMARY AND CONCLUSION

From the reported literature, there were few methods established for the determination of impurities in Dicyclomine Hydrochloride in 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. The scope and objective of the present work is to develop and validate a new RP-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 optimization was Acetonitrile: Dipotassium Phosphate buffer pH 7.5 (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 Dicyclomine Hcl.

Along with for **Dicyclomine Related compound-A**, Using Agilent HPLC with DAD detector and column used is Symmetry C8; 4.6mm X 150 mm; 3.5microns particle size for known impurity. Injection volume of 100µL is injected and eluted with 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 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 Related Compound-A was found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for Dicyclomine Hcl Related Compound-A.

Summary Table for Unknown Impurity and Related Compound-A

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%)

CONCLUSION

A RP-HPLC method for Dicyclomine Hcl capsule was developed and Validated 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) was 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 in the relationship between concentration and

peak area. So the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

The developed method was validated for various parameters as per ICH guidelines like system suitability, linearity, system precision, method precision and accuracy.

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.

DISCUSSION

In this wat, a novel and reliable approach for the determination of impurities in Dicylomine Hydrochloride in capsule dosage form was created. We rigorously validated it while adhering to the ICH, USP, Food and Drug Administration (FDA) standards. The procedure was primarily intended for the measurement of dicyclomine hydrochloride capsules. Additionally, this HPLC approach were used to detect and quantitatively analyse the amount of Dicyclomine hydrochloride that degraded in the variety of environments like acidic, alkaline, oxidative and thermal conditions.

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CONTRIBUTIONS

1. CHITRA. M[1] – Contributed for the conceptual work in schemes of research work.
2. GOKULAKANNAN. M[*1]- Contributed for the laboratory works in research and literature works.
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