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Review

Analytical Method Validation for Related Substances of Dicycloverine Tablets 10 mg, 20 mg by HPLC

R. Linga Reddy Mallampati[*], **Prof. D. Ramsachandran**

Acharya Nagarjuna University, Nagarjuna Nagar, Guntur District, Andhra Pradesh, India

*Author for Correspondence: R Linga Reddy Mallampati
Email: ram_reddy14311@yahoo.com

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Abstract

The objective of this study is to validate the HPLC method to be used for the determination of related substances of Dicycloverine Tablets 10 mg and 20 mg by HPLC.

Introduction: A dicycloverine tablet is an anticholinergic antispasmodic medication used to relieve cramps, pain, and spasms in the stomach and intestines, commonly for conditions like irritable bowel syndrome (IBS) by relaxing gut muscles.

Materials and Methods: Waters HPLC Model no. 2695, Agilent HPLC Model no.1100, HPLC Column: Inertsil ODS-3V, 250 mm x 4.6 mm, 5-µm was used with Mix mobile phases of Mobile phase A: ammonium acetate solution in water and Mobile Phase B: Methanol.

Results and Discussion: System precision and System suitability, Specificity, Limit of Detection (DL) & Limit of Quantitation (QL), Precision at QL, Method precision, Linearity, Accuracy, Range, Intermediate Precision and Robustness were evaluated.

Conclusion: The HPLC method for Related substances of Dicycloverine Tablets 10 mg and 20 mg has been validated. The test method was found to be specific, precise, linear and accurate in the range of QL (Limit of Quantitation) to 200% of Dicycloverine specification level and can be used for intended purpose.

Keywords: HPLC, dicycloverine, irritable bowel syndrome (IBS).

Introduction

IUPAC name of 2-(diethylamino)ethyl [1,1'-bi(cyclohexane)]-1-carboxylate hydrochloride, molecular structure is given in Figure 1. Dicycloverine hydrochloride (or dicyclomine HCl) is a white, crystalline powder with a bitter taste and a molecular weight of 345.95 g/mol, commonly used as a spasmolytic agent. It is freely soluble in water (approx. 77 mg/mL), alcohol, and chloroform, and is very slightly soluble in ether. melting point range of 172 to 174°C. It is used to treat gastrointestinal spasms. In this article Dicyloverine is used as synonymous to Dicycloverine Hydrochloride.

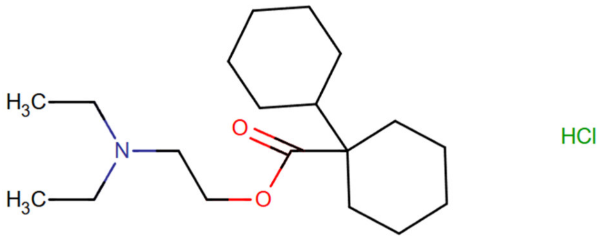

Figure.1. Molecular structure of Dicycloverine hydrochloride

Dicycloverine (or dicyclomine) hydrochloride impurities, including degradation products and synthetic intermediates, are monitored to ensure pharmaceutical quality. Key impurities include Dicycloverine Impurity A ([1'-Bicyclohexyl]-1-carboxylic acid), Dicycloverine Impurity B (1-(1-cyclohexen-1-yl)-cyclohexanecarboxylic acid 2-diethylaminoethyl ester) and Dicyclohexyl Methanol. Other identified impurities include N-Nitroso Desethyl Dicycloverine and various cyclohexyl derivatives.

Materials and Methods:

Chemical and reagents include Potassium dihydrogen phosphate, Ortho phosphoric acid and Sodium hydroxide were purchased from Rankem, Methanol, Hydrogen peroxide (30%) were purchased from Fisher scientific, Hydrochloric acid was purchased from Merck, 0.45 µm Nylon filter was purchased from Axiva, 0.45 µm PVDF filter was purchased from Simple pure.

Standards and Samples like Dicycloverine working standard, Placebo for Dicycloverine, Dicycloverine drug substance, Dicycloverine Impurity A and Dicycloverine Impurity B were purchased from Chromachemie. Dicycloverine drug product and Placebo were prepared in-house.

Waters HPLC Model no. 2695, Agilent HPLC Model no.1100, HPLC Column: Inertsil ODS-3V, 250 mm x 4.6 mm, 5-µm was used with Mix mobile phases of Mobile phase A: ammonium acetate solution in water and Mobile Phase B: Methanol.

Analytical test method:

Preparation of mobile phase-A: Weigh and Transfer 2.31g of ammonium acetate to dissolve in 500mL water and mix. Filter the solution through a 0.45micronn membrane filter, and degas by sonication for 5minutes.

Preparation of mobile phase-B: Methanol (HPLC Grade)

Preparation of Mobile Phase: Mix Mobile Phase_A and Mobile phase _B in the ratio of 100:900 V/V and mix and sonicate for 2 minutes.

Chromatographic conditions:

Column	: Inertsil ODS-3V, 250 mm x 4.6 mm, 5-µm.
Flow rate	: 1.0 mL /min.
Wavelength	: 215 nm
Column temperature	: 25°C
Injection Volume	: 100 µL
Run Time	: 35 minutes
Retention time	: Dicycloverine hydrochloride, RT about 14.5min
Diluent	: Mobile phase use as diluent.

Standard and check standard stock Preparation: Weigh and transfer accurately 25mg of Dicycloverine working standard or reference standard into a 50 mL volumetric flask add 30 ml of diluent sonicate 5 minutes and make up to volume with diluents.

Diluted standard and check standard preparation: Pipette out 2 mL of above solution into 100mL volumetric flask and make up to mark with diluent.

Test preparation: Weigh and transfer powder tablet containing 50mg of Dicycloverine in 10 ml volumetric flask add 5 ml of diluent sonicate 10 minutes then make up to volume with diluent and centrifuge the solution 10 minutes at 3000 rpm and filter the Surprenant liquid through 0.45-micron syringe filter.

Procedure:

Table.1. HPLC Sequence of injections

S. No.	Sample ID	No of Injections
1	Blank	1
2	Standard preparation	2
3	Check Standard preparation	1
4	Test Placebo Preparation for RS	1
5	Test Preparation for RS	1
6	Bracketing standard	1

System suitability:

Standard preparation:

I. Tailing factor for Dicycloverine peak should be no more than 2.0
II. Theoretical plates should not be less than 2000.
III. %RSD of two replicates of standard solution should not be more than 10.0
IV. Bracketing standard %RSD should not be more than 10.0

Check standard preparation: Percentage of the recovery should be 95.0 to 105. 0.

Calculations:

$$\text{Unknown impurity} = \frac{T}{S} \times \frac{Ws}{50} \times \frac{2}{100} \times \frac{10}{Wt} \times \frac{AW}{L} \times \frac{P}{100} \times 100$$

Where,
T = Area of impurity in test preparation.
S = Area of standard preparation.
Ws = Weight of standard taken, in mg, for standard preparation.
Wt = Weight of sample taken, in mg.
P = Potency of Dicycloverine standard
L= Labelled amount of Dicycloverine

Results and Discussion

Method Validation Summary:

System precision and system suitability: The standard, check standard and sample solution (six times) at specification level were prepared as per test method and injected into HPLC system. The system suitability parameters, % RSD for peak areas of Dicycloverine and Unknown impurities and RRTs for Dicycloverinewere evaluated as per the test method and found to be within the acceptable limits. The results are summarized in Table 2 and Table 3. Typical chromatogram of standard solution is given in Figure 2.

Table.2. System suitability data for Dicycloverine

System suitability parameters for Dicycloverine	Method Precision	Intermediate precision	Accuracy	Acceptance criteria
%RSD	2.8	0.6	2.2	Not more than 5.0
Tailing factor	1.3	1.1	1.1	Not more than 5.0
Theoretical plates	3243	101.9	9634	Not less than 2000
Check standard recovery (%)	99.1	10151	97.9	Between 95.0 to 105.0

Table.3.System precision data for Dicycloverine

S.No.	Type of compound	% RSD	Acceptance criteria
1.	Dicycloverine	1.0	Not more than 5.0

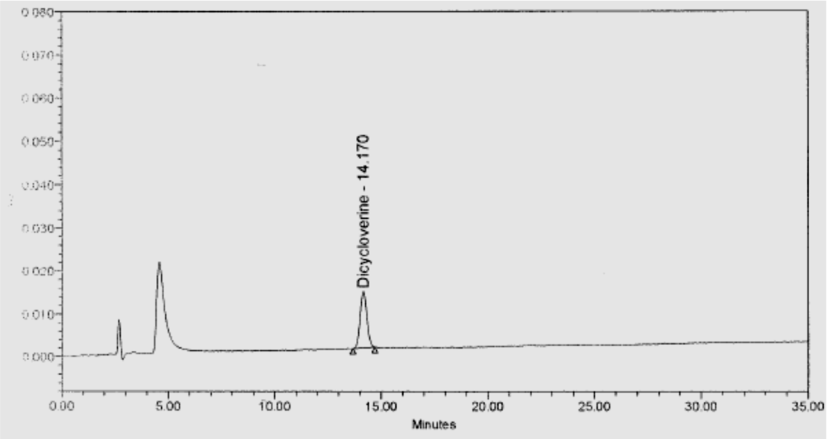

Figure.2.Typical chromatogram of standard solution

Specificity

Blank interference: Blank solution was prepared and injected as per test method. It was observed that no blank peaks were eluting at the retention time of Dicycloverine and known impurity peaks.

Placebo interference: Placebo solution was prepared in duplicate and injected as per test method. It was observed that no placebo peaks were interfering at the retention time of Dicycloverine and known impurity peaks.

Impurity interference: Individual impurity solutions were prepared at specification level of test concentration and analyzed as per test method. It was observed that impurities are not co eluting with each other and with Dicycloverine peak.

Forced degradation: Performed the forced degradation studies on the placebo and the drug product for Acid, Alkali, Peroxide, Water, Thermal, Sunlight and Humidity degradations and injected as per test method. Evaluated the Dicycloverine peak purity in each stressed condition using Empower software. The peak purity of Dicycloverine peak in the entire diluted stressed sample was found to be within the acceptable limit. The results are summarized in Table 4 to Table 6. Typical chromatograms are summarized in Figure 3 and Figure 4.

Table.4.Blank and Placebo interference

Sample No.	Peak found at RT of Dicycloverinepeak (Yes / No)	
	Blank	Placebo
1	No	No
2	No	No

Acceptance criteria: Chromatogram of blank and placebo should not show any peak at the retention time of Dicycloverine peak and known impurities peaks.

Table.5.Impurity interference

Peak Name	RT of impurities & main analyte		
	Spiked sample RT	Individual impurities	Acceptance criteria
Dicycloverine	8.84min	N/A	Known impurities should not co-elute with each other and with Dicycloverine peak.
DicycloverineLactam	5.98	5.91	
DicycloverineSulfoxide	11.39	11.49	

Table.6.Peak purity table

S. No.	Condition	Procedure	Purity angle	Purity threshold	Peak Purity
1.	Sample Acid degradation	1N HClat 60°C for 30 mts	0.917	1.291	Pass
2.	Sample Alkali degradation	1N NaOH at 60°C for30 mts	1.058	1.283	Pass
3.	Sample Peroxide degradation	1% H_2O_2at 60°C for30 mts	0.928	1.306	Pass

4.	Sample Water degradation	Waterat 60°C for 30 mts	0.982	1.320	Pass
5.	Sample Thermal degradation	Sample kept at 60°C for 24Hrs	1.063	1.558	Pass
6.	Sample Sunlight degradation	Sample kept on sunlight for 24Hrs	1.029	1.334	Pass
7.	Sample Humidity degradation	Sample kept in humidity chamber 48Hrs	1.116	1.370	Pass

Acceptance criteria: The peak purity of Dicycloverine peak in stressed sample should pass. Purity angle should be lessthan purity threshold in waters empower soft water.

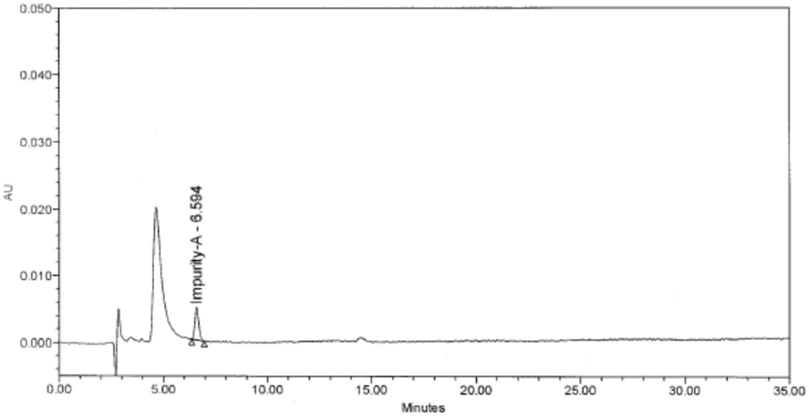

Figure.3.Typical chromatogram of DicycloverineImpurity-A

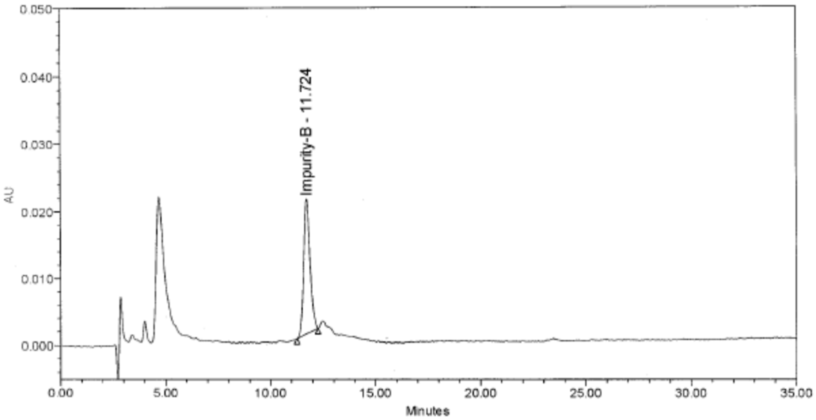

Figure.4.Typical chromatogram of Dicycloverine Impurity-B

DL, QL establishment and Precision at QL: To establish the DL and QL for Dicycloverine peak, appropriate concentrations of the Dicycloverinewas prepared and injected as per test method. Signal to noise ratio for DLof Dicycloverine were found to be 3, and for QL was found to be13. Dicycloverinesolution were spiked on the placebo at about QL concentration and injected six times in to the HPLC system. % RSD forDicycloverine of six samples were calculated and found to be within the acceptance criteria. The results are summarized in Table 7 and Table 8.

Table.7.QL and DL data of Dicycloverine with S/N ratio

Sample Name	DL Concentration (%w/w)	S/N Ratio	QL Concentration (%w/w)	S/N Ratio
Dicycloverine	0.01	3	0.03	13

Acceptance criteria: For DL signal to noise ration should be about 3. For QL signal to noise ration should be about 10.

Table.8. Precision at QL data for Dicycloverine

No. of Injection	Content at QL
	Dicycloverine
1	0.66
2	0.52
3	0.66
4	0.57
5	0.60
6	0.59
Mean	0.60
% RSD	9.0

Acceptance criteria: % RSD for % of individual Unknown impurity and Dicycloverine should be not more than 15.0

Method precision: To evaluate the precision for RS method, six samples were prepared and analyzed as per test method. The percentage RSD of six samples foreach individual Unknown impurity and the % of total impurities were calculated and found to be within the acceptance criteria.The results are summarized in Table.9.

Table.9. Method Precision data

Sample No.	Dicycloverine content(%w/w)
1	ND
2	ND
3	ND
4	ND
5	ND
6	ND
Mean	ND
% RSD	ND

Acceptance criteria: The Percentage RSD for each individual Unknown impurity and percentage of total impurities should not be more than 15.0 for replicate preparations.

Linearity: A series of Dicycloverinewas prepared in the concentration ranging from QL to 200% of specification level and injected into the HPLC system as per the test method. Linearity of detector response was established by plotting a graph between concentration and response of Dicycloverine peak. The detector response was found to be linear from about QL to 200% of specification level. The square of correlation coefficient, intercept and residual sum of squares were calculated and found to be within the acceptable limit. The linearity results and graphs are summarized in Figure 5.

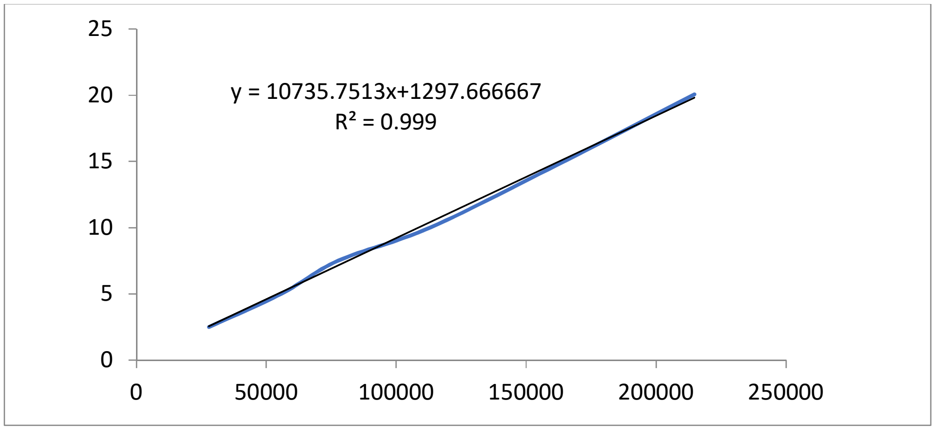

Figure.5.Linearity of detector response graph for Dicycloverine

Acceptance criteria: Square of Correlation coefficient should be not less than 0.99.

Accuracy:

A series of solutions were prepared in triplicate by spiking the test preparation at the specification limit in the range of about QL to 200% of test concentration and injected into HPLC system and analyzed as per the test method. Individual % recovery, mean % recovery, %RSD and linearity of the test method are calculated at eachlevel and the results were found to be within the acceptable limits. The accuracy results are summarized in Table.10.

Table.10.Accuracy data of Dicycloverine

S. No.	% spike level	Amount added (%w/w)	Amount recovered (%w/w)	% Recovery	% Mean recovery	% RSD
1.	LQ	0.050	0.0510	102.0	99.3	2.1
2.		0.050	0.0484	96.8		
3.		0.050	0.0499	99.8		
4.		0.050	0.0508	101.6		
5.		0.050	0.0494	98.8		
6.		0.050	0.0511	102.2		
1.	100	0.202	0.1925	95.30	97.8	2.4
2.		0.202	0.2018	99.90		
3.		0.202	0.1975	97.77		
1.	200	0.404	0.3842	95.10	94.2	1.1
2.		0.404	0.3753	92.90		
3.		0.404	0.3764	93.17		
4.		0.404	0.3857	95.47		
5.		0.404	0.3817	94.48		
6.		0.404	0.3780	93.56		

Acceptance criteria: Individual % recovery and mean % recovery of each known impurity should be between 70.0 to 130.0 for QL and 85.0 to 115.0 for Other Level. Percentage RSD at each level for replicate test preparations should be no more than 15.0

Range:

Based on Method precision, Linearity and Accuracy data it can be concluded that the related substances method is precise, linear and accurate in the range of QL-200%of specification level.

Intermediate precision: To evaluate intermediate precision for RS method, six samples were prepared at specification level and analyzed as per test method by using different system, different column, by different analyst on different day. Percentage RSD for each Unknown individual impurity and the % of total impurities for intermediate precision were calculated and found to be within the acceptable limits.

The overall % RSD of six samples in method precision, intermediate precision (n=6 and n=12) foreach individual Unknown impurity and percent RSD for each individual Unknown impurity and the percentage of total Unknown impurities were calculated and found to be within the acceptable limits.

Robustness

Filter Validation: A study was conducted to evaluate the filter suitability by using two different types of filters namely0.45 µm PVDF and 0.45µm Nylon filters. Standard solution was prepared in single and test solution was prepared in duplicate by spiking theknown impuritiesat specification level in as per the test method. Portion of standard and test solutions were filtered through 0.45 µm PVDF, 0.45 µm nylon filter and some portion of standard and sample solutions were centrifuged and analyzed as per test method. Similarity factors were calculated for the filtered standards against unfiltered standard (Centrifuged) and found to be within the specified limit.

The difference in the percentage of individual known impurities and the % total impurities between unfiltered (centrifuged) and filtered samples were calculated and were found to be acceptance limit. Both PVDF and Nylon filters were suitable for the intended purpose.

Flow rate variation: A study was conducted to determine the effect of variation in flow rate. Blank, Standard, and sample with at specification level were prepared as per the test method and injected into HPLC system with flow rates of 0.9mL/minute and 1.1mL/minute. System suitability parameters and

RRTs of known impurities in spiked sample were evaluated and found to be within the specified limits as per test method.

Column oven Temperature variation: A study was conducted to determine the effect of variation in Column oven Temperature. Standard and test preparations with known impurities at specification level were prepared as per the test method and injected into HPLC system with column oven temperature of 30°c. System suitability parameters and RRTs of known impurities in spiked sample were evaluated and found to be within the specified limits as per test method.

Effect of variation in mobile phase composition: A study was conducted to determine the effect of variation in mobile phase composition. Two different mobile phases of Buffer and Methanol were prepared in the ratio 90:910 v/v and 110:890% v/v as per the test method. Standard and spiked test preparations with known impurities at specification level were prepared as per the test method and injected into HPLC system suitability parameters and RRTs of known impurities in spiked sample were evaluated and found to be within the specified limits as per test method.

Effect of wavelength variation: A study was conducted to determine the effect of variation in wavelength. Method precision chromatograms were analyzed in two wavelengths 213 and 217nm as per the test method. Blank, Standard and test preparations with known impurities at specification level were prepared as per the test method and injected into HPLC. System suitability parameters and RRTs of known impurities in spiked sample were evaluated and found to be within the specified limits as per test method.

Conclusion

The HPLC method for Related substances of Dicycloverine Tablets 10 mg and 20 mg has been validated. The test method was found to be specific, precise, linear and accurate in the range of QL (Limit of Quantitation) to 200% of Dicycloverine specification level and can be used for intended purpose.

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