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RP-HPLC method for simultaneous estimation of amoxicillin and carbocisteine in dosage forms

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ABSTRACT

A rapid, sensitive, precise and economical along with excessive resolution RP-High Performance Liquid Chromatographic method for determination of Amoxicillin and Carbocisteine has been developed. The chromatography system contain reverse phase C_{18} Column (250×4.6mm, 5µm) with a mixture of Acetonitrile: Water in ratio of 10:90 as a mobile phase. The pH 3 of mobile phase was adjusted by OPA, flow rate of mobile phase was 1.0 ml min^{-1}. UV detection was performed at 220 nm and 20µl sample was injected. The method was validated as per ICH guideline. The retention time of Carbocisteine was 2.3 min and Amoxicillin was 5.1 min. The calibration cure was linear in the range of 10-50 and 5-25 µg/ml for Amoxicillin and Carbocisteine respectively. The percentage RSD for precision and accuracy of method was found to be less than 2 %. The lower limit of detection and limit of quantification was 0.003, 0.002 and 0.01, 0.007 ppm for Carbocisteine and Amoxicillin Respectively. Recovery from tablet was between 100.32, 101.26, and 101.35 %. The reported method is cost effective as compared to earlier method[8] because a) Use of acetonitrile is much lesser which ultimately makes it cost effective method, b) Use of water, pH adjusted to 3 instead of buffer reduces the backpressure of system. c) Buffer is hazardous to Column life, d) Resolution is more, better for quantification of Amoxicillin and Carbocisteine from bulk as well dosage forms.

Key Words: Amoxicillin, Carbocisteine, RP-HPLC, UV Detection.

INTRODUCTION

Amoxicillin trihydrate (AMOX) [[2S-[2α, 5α, 6β(S)]]-6-[[amino(4-hydroxyphenyl) acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3,2,0]heptane-2-carboxylic acid] is antibacterial agent used in urinary tract infection, respiratory tract infection[1,4]. Carbocisteine chemical name is (2R)-2-amino-3-[(carboxymethyl) sulphanyl] propanoic acid used as mucolytic agent which reduces the viscocity of sputum. In chronic obstructive pulmonary disease (COPD) symptoms can be reduced with Carbocisteine. Additional characteristics of COPD include airflow limitation, oxidative stress, and airway inflammation [5,6].

High Performance Liquid Chromatography is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with second solid or liquid phase, the different solutes interact with the other phase to differing degrees due to differences in adsorption, ion exchange partitioning or size. Liquid chromatography is separation technique based on the difference in a

distribution of the component between two non-miscible phases in which liquid mobile phase elute through a stationary phase in column. The three forms of HPLC most often used are based on mechanism of partition, adsorption and ion exchange[7].

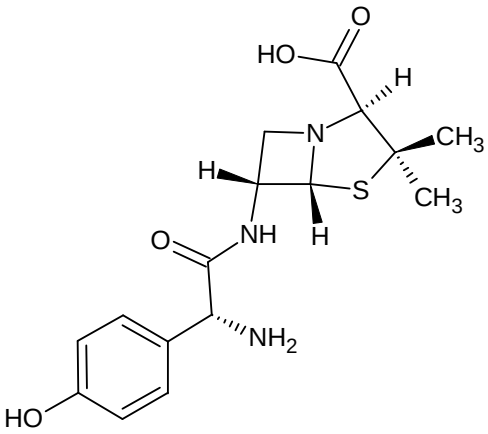

Figure.1- Chemical structureAmoxicillin

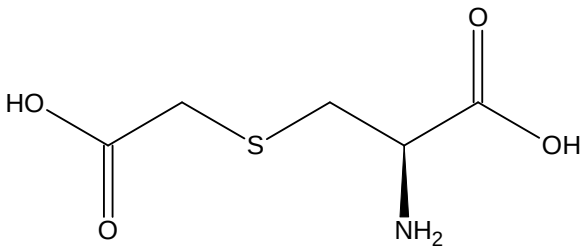

Figure.2- Chemical structure Carbocisteine

MATERIALS AND METHODS

Chemicals and Reagents

Working standard of Amoxicillin and Carbocisteine was obtained from well reputed research laboratories. Fixed dose of Amoxicillin (250 mg) + Carbocisteine (150mg) containing tablet Mucobron-250 was purchased from localmarket. All the chemicals were of HPLC grade, purchasedfrom Merck Chemicals, India. Water used was doubledistilled and filtered through a 0.45µ filter disc. HPLCsystem consisted of Agilent Technology 1200 seriesquaternary gradient with Agilent SPD-M20A prominence diode array detector and a 515 auto injector. Data wereacquired and processed by use of EZ Chrome Elite software.The chromatographic separations were carried out on a C_{18}Column (250×4.6mm, 5µm).

Preparation of Standard Stock and Sample Solution-

A 10mg amount Amoxicillin and Carbocisteineof reference substance was accurately weighed and dissolved in mobile phase in volumetric flask to obtain 100 ppm concentration of stock solution. From stock solution by the serial dilution we prepared required concentrations of 10, 20, 30, 40 and50 ppm for Amoxicillin and 5, 10, 15, 20 and 25 ppmfor Carbocisteine.

A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Amoxicillin and Carbocisteine was accurately weighted and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase was added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of solution was diluted with mobile phase to a concentration of 15µg/ml for Carbocisteine and Amoxicillin 30 µg/ml.

Chromatographic Conditions
The mobile phase used Acetonitrile: Water (10:90 v/v) of pH 3.0 was maintained by OPA as buffer. Injections were carried out using a 20µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 220 nm with 15.0 min runtime.

Method Validation-
Method validation was performed following ICH specifications for, range of linearity, precision, robustness, Recovery, LOD and LOQ, Tablet Assay.

RESULTS AND DISCUSSION

System Suitability
Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table1.

Table.1- System suitability parameter

Mobile phase	Acetonitrile : Water 10 : 90
Pump mode	Isocratic
pH	3.0 adjusted OPA
Diluents	Mobile phase
Column	C_{18} column (250 X 4.6 mm, 5µm)
Column Temp	Ambient
Wavelength	220nm
Injection volume	20 µl
Flow rate	1ml/min
Run time	15 minutes
Retention time	Carbocisteine-2.4 Amoxicillin- 5.2

Range of linearity
Amoxicillin and Carbocisteine standard curves were constructed using five different standard concentrations in a range of 10, 20, 30, 40, 50µg/ml and 5, 10, 15, 20, 25µg/ml respectively. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation of Amoxicillin; y = 31.455x - 109.2 (R^2 = 0.995)and Carbocisteine; y = 9.0686x-10.119 (R^2 = 0.998). Linearity valuesare shown in Table 2 and 3

Table.2- Range of linearity of Amoxicillin

Concentration of Amoxicillin in ppm	Peak Area
10	230.68
20	463.32
30	861.7
40	1164.8
50	1425.71

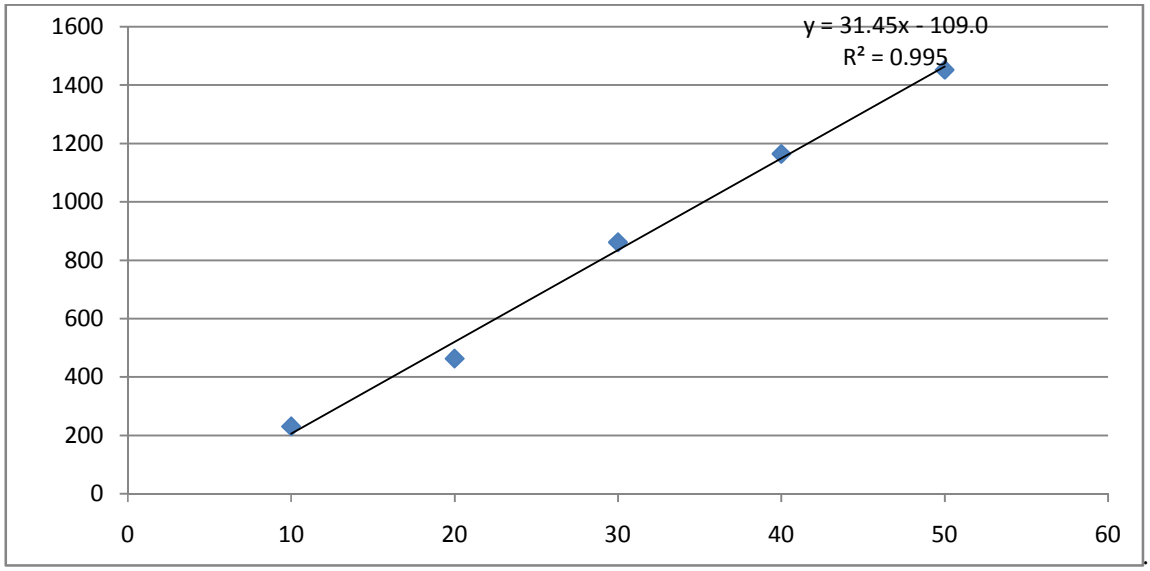

Figure.3- Linearity of Amoxicillin

Table.3 - Range of linearity (carbocisteine)

Concentration of Carbocisteine in ppm	Peak Area
5	37
10	76.55
15	128.44
20	171.14
25	216.42

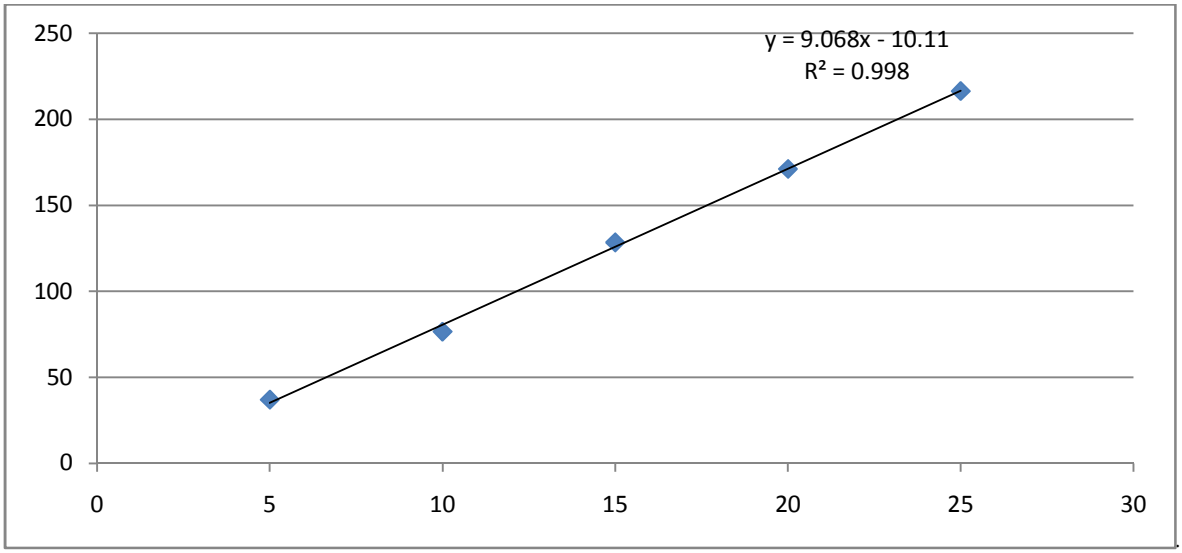

Figure.4- Linearity Carbocisteine

3 Precision -

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. To study precision, six replicate standard solutions of Amoxicillin (30ppm) and Carbocisteine (15ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD)

for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table 4 and 5.

Table 4.Result of Intraday Precision

Injection No.	Peak Areas	
	Amoxicillin	Carbocisteine
1	861.70	128.4408
2	861.65	128.4601
3	861.69	128.4403
4	861.72	128.4410
5	861.71	128.4399
6	861.68	128.4591
	Mean - 861.6917 SD - 0.024 RSD- 0.002	Mean- 128.45 SD- 0.010 RSD-0.008

Table 5. Result of Interday precision

Injection No.	Peak Areas	
	Amoxicillin	Carbocisteine
1	861.97	128.49
2	861.40	128.87
3	861.45	128.78
4	861.92	128.54
5	860.99	128.39
6	861.25	128.98
	Mean - 861.4917 SD - 0.382 RSD- 0.04	Mean- 128.675 SD- 0.23 RSD-0.182

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.003 ppm of CarbocisteineAnd 0.002 ppm of Amoxicillindilution Peak was not clearly observed, based on which 0.003 ppm and 0.002 ppm is considered as Limit of Detection and Limit of Quantification is 0.01(Carbocisteine) And 0.007(Amoxicillin).

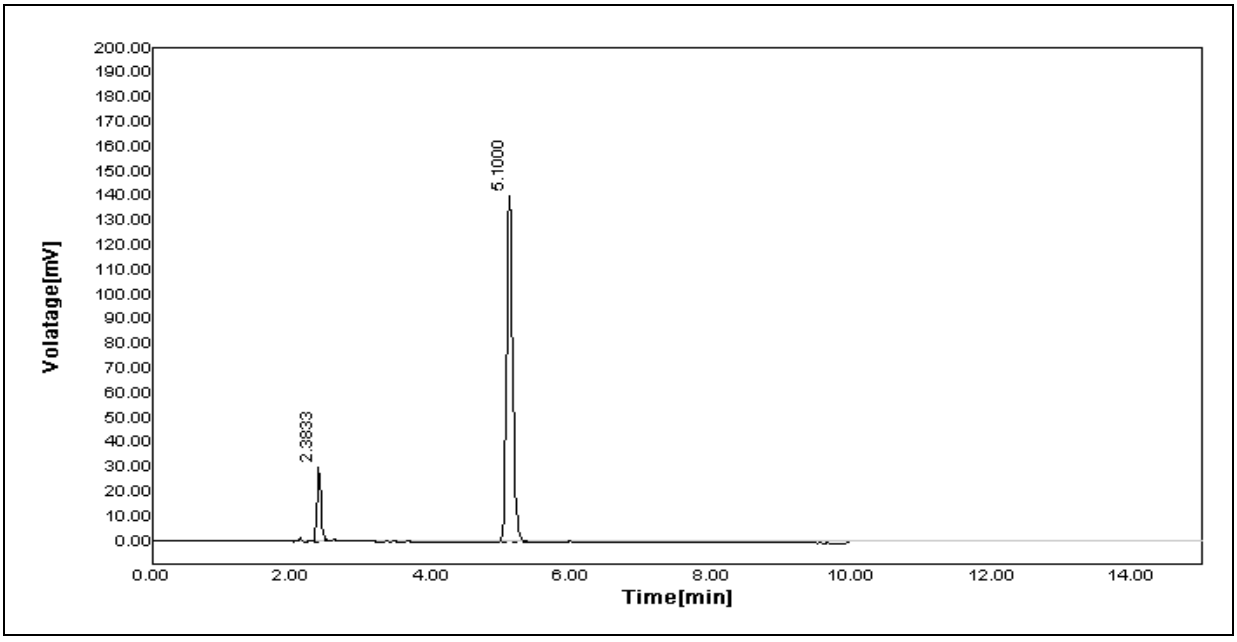

Figure.5Typical chromatogram of Amoxicillin andCarbocisteine

Table.6 Limit of Detection and Limit of Quantification

Parameter	Measured Value	
	Amoxicillin	**Carbocisteine**
Limit of Quantification	0.007	0.01
Limit of Detection	0.002	0.003

Sr. No	RT[min]	Area mv*s	Area%	TP	TF	Ressolution
1	2.383	128.4408	12.97	4535.8	1.2500	0.0000
2	5.100	861.7014	87.030	14423.1	1.1667	14.8182
Sum		990.1422				

Robustness:

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

Table.7- Result of Robustness of Amoxicillin

S. No	Parameter	Condition	Area
1	Standard	Standard conditions	861.70
2	Mobile phase	Acetonitrile : Water 20 : 80	810.93
3	Wavelength	222	892.71
4	Flow Change	0.8	945.44

Table.8- Result of Robustness of Carbocisteine

Sr. no	Parameter	Condition	Area
1	Standard	Standard conditions	128.45
2	Mobile phase	Acetonitrile : Water 20 : 80	134.44
3	Wavelength	222	125.31
4	Flow Change	0.8	160.66

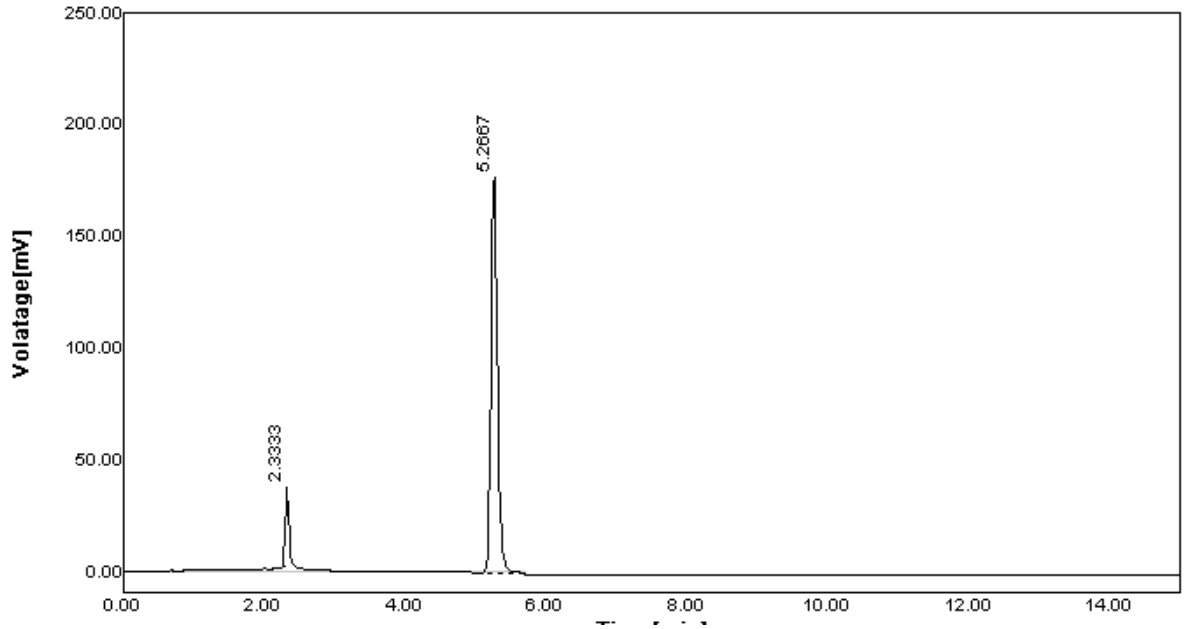

Figure.6 Chromatogram of Formulation

Sr. No	RT[min]	Area mv*s	Area%	TP	TF	Ressolution
1	2.333	138.1234	9.50	6792.9	1.0000	0.0000
2	5.2667	88.4701	90.50	11300.4	1.0714	16.0000
Sum		1019.59				

Recovery studies

Recovery test performed at 3 Different concentration of Carbocisteine 27, 30, 33 ppm and for Amoxicillin 54, 60, 66 ppm i.e. recovery perform on 80% ,100%, 120%. Results of recovery study given in table 9 and 10.

Table.9 Recovery Study of Carbocisteine

Sr.no	Conc. taken (µg/ml)	Std addition (µg/ml)	Total conc. Found (µg/ml)	% recover ± SD
1	15	12	27.10	100.08 ± 0.01
2	15	15	30.07	100.51 ±0.03
3	15	18	33.27	101.53 ± 0.008

Table.10 Recovery Study of Amoxicillin

Sr.no	Conc. taken (µg/ml)	Std addition (µg/ml)	Total conc. Found (µg/ml)	% recover ± SD
1	30	24	54.07	100.32 ± 0.02
2	30	30	60	100.00 ±0.01
3	30	36	66.27	101.53 ± 0.004

CONCLUSION

The proposed method for the analysis of Amoxicillin and Carbocisteine in Dosage form is very simple, economic and rapid. The reported method is cost effective as compared to earlier method[8] because a) Use of acetonitrile is much lesser which ultimately makes it cost effective method, b) Use of water, pH adjusted to 3 instead of buffer reduces the backpressure of system. c) Buffer is hazardous to Column life, d) Resolution is more, better for quantification. The proposed method using water in place of buffer is economical and time saving. The method was validated for specificity, linearity, precision, accuracy and robustness. The method could effectively separate the drug from its additives. Thus proposed RP-HPLC method can be successfully applied for the routine quality control analysis of Amoxicillin and Carbocisteine along with their dosage forms.

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