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Simultaneous estimation of amoxicillin trihydrate and dicloxacillin sodium in capsule

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Amoxicillin trihydrate,
Dicloxacillin sodium,
Capsule.

Abstract

Amoxicillin trihydrate and dicloxacillin sodium were simultaneously determined using RP-HPLC in bulk and formulations using a simple, fast, precise, selective and accurate method. Water and potassium dihydrogenortho phosphate buffer were used to achieve isocratic separation on a C18 column. The flow rate was 1.2ml\minute and effluent were detected at 225nm.The retention time of amoxillintrihydrate and dicloxacillin sodium were. 943 mins and 11. 678 mins respectively. There was linearity between 0. 999 and 0. 999, with correlation coefficients of 0. 9998 and 0. 9995. Percent recoveries obtained for both the drugs were 98. 45-100.65% and 98.79 - 100.01% respectively. linearity, accuracy, robustness, specificity and precision of the method were validated according to ICH guidelines. Analyzing amoxicillin trihydrate&dicloxacillin sodium in their combined dosage form can be routinely done using the method developed

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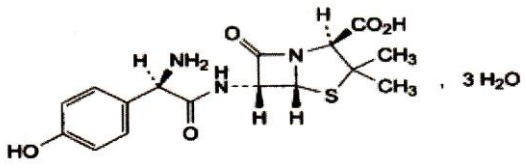
INTRODUCTION

With HPLC, most drugs in multicomponent dosage forms can be analysed because of its advantages such as rapidity, specificity, accuracy, precision, and ease of automation. [1] USP guidelines (2), ICH guidelines (3), and FDA guidelines (4) provide frameworks for the validation of pharmaceutical methods. [2] As well as being cumbersome, these procedures may not be reliable. [3]

Structure: [4]

Amoxicillin Trihydrate:



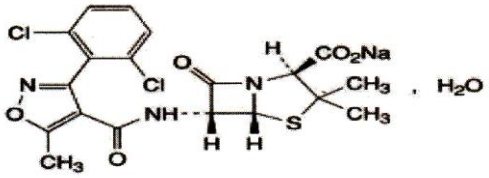
The chemical name of amoxicillin trihydrate is (2s, 5R, 6R)-6- [[c2R)- 2- amino -2-(4-hydroxy phenyl) acetyl] amino]3,3-dimethyl -7-oxo- 4-thia 1- azabicyclo [3.2.0] heptane-2- carboxylic acid, trihydrate with molecular weight 419.4

Molecular formula: - c16 H19 N3 o5 S 3H20

Structure:[5]

Dicloxacillin sodium

DICLOXICILLIN SODIUM:



The chemical name of Dicloxacillin sodium is 4-Thia 1- azabicyclo [3.2.0] heptane 2-carboxylic acid monohydrate with molecular weight 510

Molecular formula: - c19 H16 N3 Na O5.H20

MATERIALS & METHODS

Amoxicillin trihydrate&Dicloxacillin sodium reference standards were obtained from Dr. Reddy's laboratories, Hyderabad, along with potassium dihydrogen orthophosphate AR grade & potassium hydroxide AR grade. [6]

INSTRUMENTATION

The HPLC system consists of waters 2487 Dual\ absorbance detector, waters 2695 separations module which was connected with TDT-08L & Dissolution tester USP &Labindia u\controlled pH Analyserphan&Enertech fast clean ultra-sonic cleaner. [7]

HPLC CONDITION

The content of the mobile phase

Mobile phase A: -A mixture of 50 volumes of diluent, 50 volumes of water

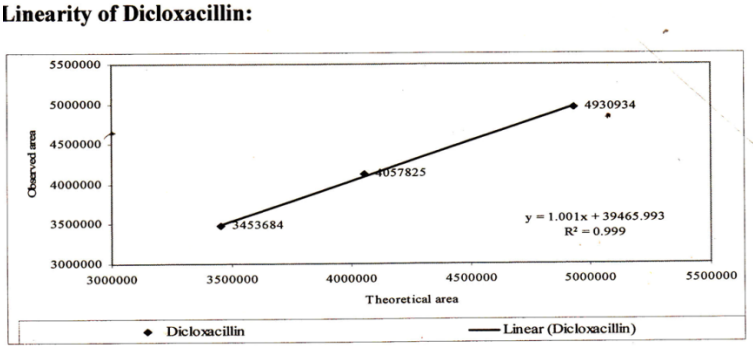
Mobile phase B: -A mixture of 50 volumes of diluent, 10 volumes of water & 40 volumes of acetonitrile.

We filtered these through 0.45 u membrane filters and degassed them with sonication before using them. We optimized the mobile's flow rate to 1.2 ml/minute. We ran the experiment for 10 minutes at ambient temperature & set the run time to 10 minutes. Injection volume was 20 ul, eluent was detected at 225 nm, and each standard & test preparation was injected into the column, and the results were recorded. [8]

PREPARATION OF STOCK SOLUTION

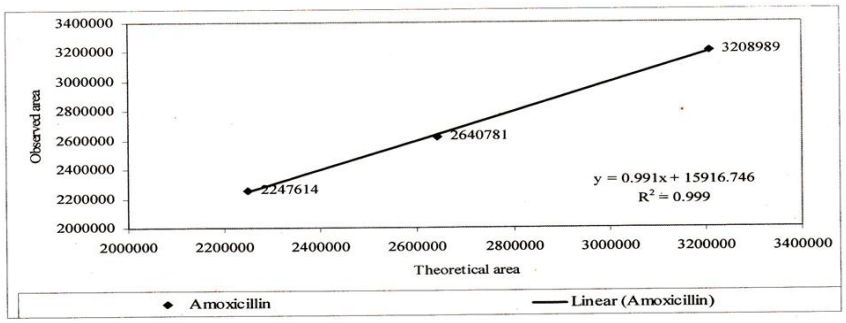
Figure 1:

Linearity of Dicloxacillin:



S.No	Parameters	Calculated values	Limits
1	Correlation coefficient (R^2)	0.999	Not less than 0.999

Figure 2:



S.No	Parameters	Calculated values	Limits
1	Correlation coefficient (R^2)	0.999	Not less than 0.999

STANDARD PREPARATION

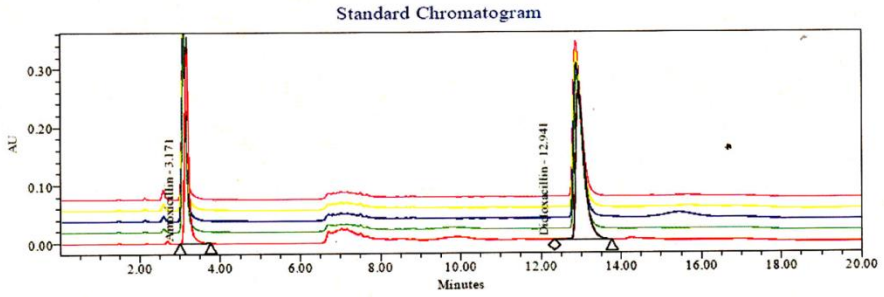
Weighed accurately about 57mg of Amoxicillin trihydrate WS & about 55mg 0f Dicloxacillin sodium WS in a 50ml volumetric flask. In order to make up the volume with the same, dissolve the powder in water and mix well. Pipetted out 5ml of the above solution in a 50ml volumetric flask, add 25ml of diluent & made up to the volume with water. [9]

SYSTEM SUITABILITY STUDIES

For the working standard solutions, the resolution, number of theoretical plates, and peak symmetry are shown in the table below

It shows system suitability parameters

Figure 3:



Peak: Amoxicillin

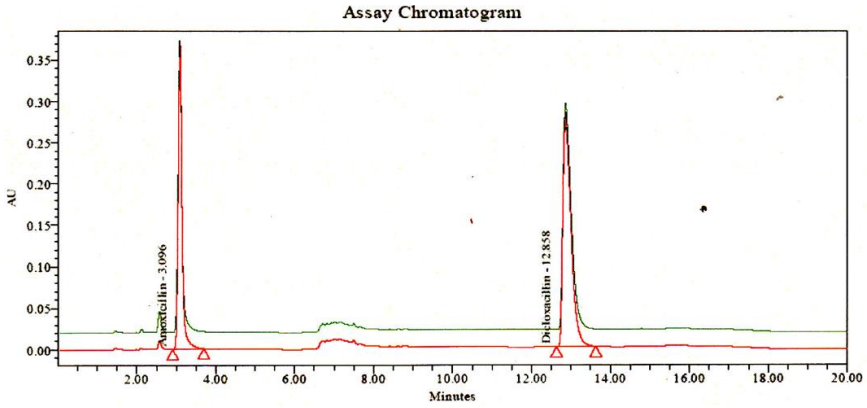
SampleName	Inj	Peak	RT	Area	Reso	TPlates	Tailing	K Prime
Rug std	1	Amoxicillin	3.171	2334050		5807	1.31	1.54
Rug std	2	Amoxicillin	3.110	2326222		5446	1.31	1.49
Rug std	3	Amoxicillin	3.102	2308380		5635	1.31	1.48
Rug std	4	Amoxicillin	3.100	2293355		5599	1.31	1.48
Rug std	5	Amoxicillin	3.100	2262114		5617	1.31	1.48
			3.117	2304824		5621	1.31	1.49
			0.99	1.24				

Peak: Dicloxacillin

SampleName	Inj	Peak	RT	Area	Reso	TPlates	Tailing	K Prime
Rug std	1	Dicloxacillin	12.941	3902273	35.78	17548	2.05	9.35
Rug std	2	Dicloxacillin	12.901	3877030	36.29	18106	1.94	9.32
Rug std	3	Dicloxacillin	12.887	3853095	36.66	18219	1.89	9.31
Rug std	4	Dicloxacillin	12.879	3873305	36.58	18463	1.94	9.30
Rug std	5	Dicloxacillin	12.875	3869711	36.54	18397	1.93	9.30
			12.896	3875082	36.37	18147	1.95	9.32
			0.21	0.46				

Sample Name: Rugg sample 1, Rugg sample 2
Sample Type: Unknown
Vial: 43, 44
Injection #: 1
Injection Volume: 20.00 ul

Figure 4:



Peak Results

Sample Name	Vial	Name	RT	Area	TPlates	Reso	Tailing	K Prime
Rugg sample 1	43	Amoxicillin	3.096	2486826	5473		1.31	1.48
Rugg sample 1	43	Dicloxacillin	12.858	3974803	18144	36.32	1.93	9.29
Rugg sample 2	44	Amoxicillin	3.091	2384684	5413		1.33	1.47
Rugg sample 2	44	Dicloxacillin	12.861	3961746	18302	36.26	1.94	9.29

ASSAY

Sample solutions-Preparation

20 capsules were taken. The content of the capsules was weighed &mixed well. Weighed accurately the powderequivalent to 250m g of Amoxicillin trihydrate&transferred to a 250ml volumetric flask, added 200ml of water, shaken for 10 minutes &sonicated for 5 minutes & made up to the volume with water. Pipetted out 5ml of the above solution &transferred in a 50ml of volumetric flask, added 25ml of diluent & made up to the volume with water. Filtered through the 0.45u membrane filter [10]

METHOD

By means of an isocratic elution technique with a UV detector, Amoxicillin trihydrate&Dicloxacillin sodium were separated using RP-HPLC. With a concentration range of 225nm for Amoxicillin trihydrate&Dicloxacillin sodium, we examined capsule powder equivalent to 57mg Amoxicillin trihydrate& 55mg Dicloxacillin sodium, dissolved in 50ml volumetric flasks, and made up to the volume with water. Using Whatman filter paper

no. 41, we filtered the solution after sonicating it for 15 minutes at 100 rpm, centrifuged it for 15 minutes, and centrifuged it again for 15 minutes. Add 25 ml of diluent and water to fill the volumetric flask to volume with 5 ml of the above solution. [11, 12]

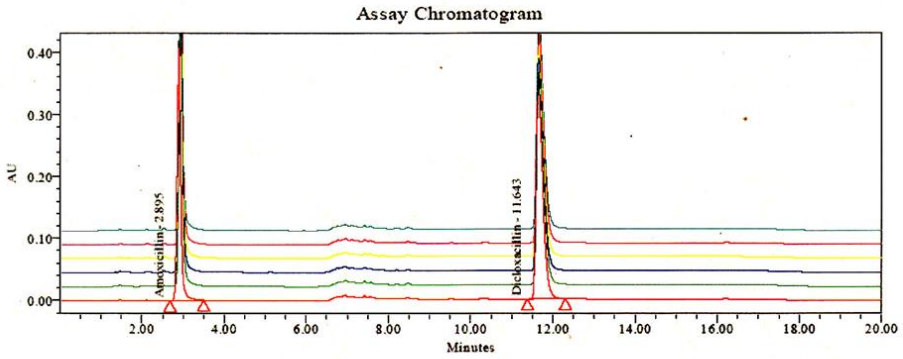
Figure 5:

Peak	RT	Area	Reso	T Plates	Tailing	K Prime
Amoxicillin	2.948	2437031		5495	1.25	1.36
Amoxicillin	2.948	2434230		5489	1.25	1.36
Amoxicillin	2.941	2428057		5561	1.28	1.35
Amoxicillin	2.939	2425271		5582	1.28	1.35
Amoxicillin	2.940	2415827		5596	1.28	1.35
	2.943	2428083		5545	1.27	1.35
	0.15	0.34				

Peak: Dicloxacillin

Peak	RT	Area	Reso	T Plates	Tailing	K Prime
Dicloxacillin	11.675	3778449	37.85	22533	1.70	8.34
Dicloxacillin	11.672	3772442	38.11	22932	1.69	8.34
Dicloxacillin	11.676	3770567	37.88	22676	1.70	8.34
Dicloxacillin	11.679	3770487	38.06	22735	1.69	8.34
Dicloxacillin	11.685	3771450	38.16	23104	1.70	8.35
	11.678	3772679	38.01	22796	1.70	8.34
	0.04	0.09				

Sample Name: Precision 1, Precision 2, Precision 3,
Sample Type: Unknown
Vial: 4, 5, 6, 7, 8, 9
Injection #: 1
Injection Volume: 20.00 ul



Peak Results

Peak	RT	Area	Reso	TPlates	Tailing	K Prime
Amoxicillin	2.948	2437031		5495	1.25	1.36
Amoxicillin	2.948	2434230		5489	1.25	1.36
Amoxicillin	2.941	2428057		5561	1.28	1.35
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Amoxicillin	2.940	2415827		5596	1.28	1.35
	2.943	2428083		5545	1.27	1.35
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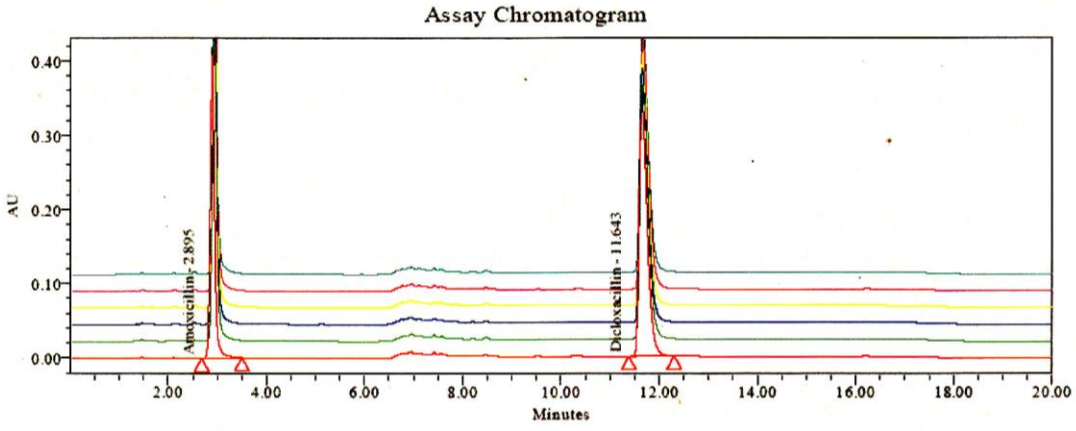
Dicloxacillin	11.685	3771450	38.16	23104	1.70	8.35
	11.678	3772679	38.01	22796	1.70	8.34
	0.04	0.09				

Sample Name: Precision 1, Precision 2, Precision 3,
Sample Type: Unknown
Vial: 4, 5, 6, 7, 8, 9
Injection #: 1
Injection Volume: 20.00 ul



Peak Results

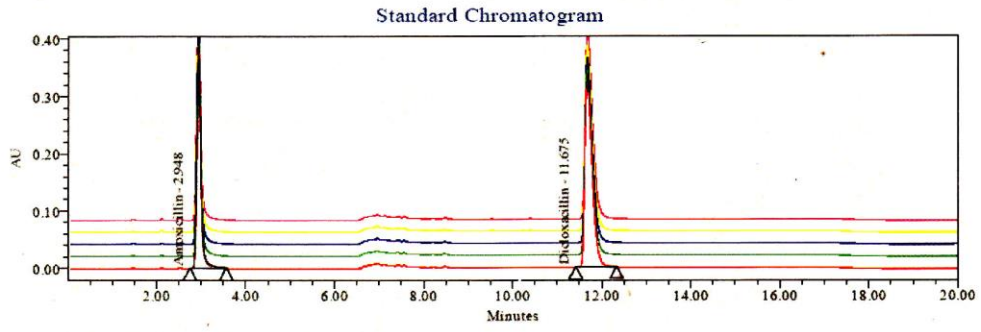
Amoxicillin:

Standard No	Retention time	Area	Sample No	Area
Injection 1	2.948	2437031	Sample 1	2607269
Injection 2	2.948	2434230	Sample 2	2605148
Injection 3	2.941	2428057	Sample 3	2594895
Injection 4	2.939	2425271	Sample 4	2593512
Injection 5	2.940	2415827	Sample 5	2599169
			Sample 6	2580758
Mean value	2.943	2428083		
Percent RSD	0.15%	0.34%	Limit: Not more than 2%	

Dicloxacillin:

Standard No	Retention time	Area	Sample No	Area
Injection 1	11.675	3778449	Sample 1	4022035
Injection 2	11.672	3772442	Sample 2	4023588
Injection 3	11.676	3770567	Sample 3	4024808
Injection 4	11.679	3770487	Sample 4	4009514
Injection 5	11.685	3771450	Sample 5	4023428
			Sample 6	4009134
Mean value	11.678	3772679		
Percent RSD	0.04%	0.09%	Limit: Not more than 2%	

Sample Name: Std
Sample Type: Unknown
Vial: 3
Injection #: 1, 2, 3, 4, 5
Injection Volume: 20.00 ul



Peak: Amoxicillin

Peak	RT	Area	Reso	TPlates	Tailing	K Prime
Amoxicillin	2.948	2437031		5495	1.25	1.36
Amoxicillin	2.948	2434230		5489	1.25	1.36
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Amoxicillin	2.940	2415827		5596	1.28	1.35
	2.943	2428083		5545	1.27	1.35
	0.15	0.34				

Peak: Dicloxacillin

Peak	RT	Area	Reso	TPlates	Tailing	K Prime
Dicloxacillin	11.675	3778449	37.85	22533	1.70	8.34
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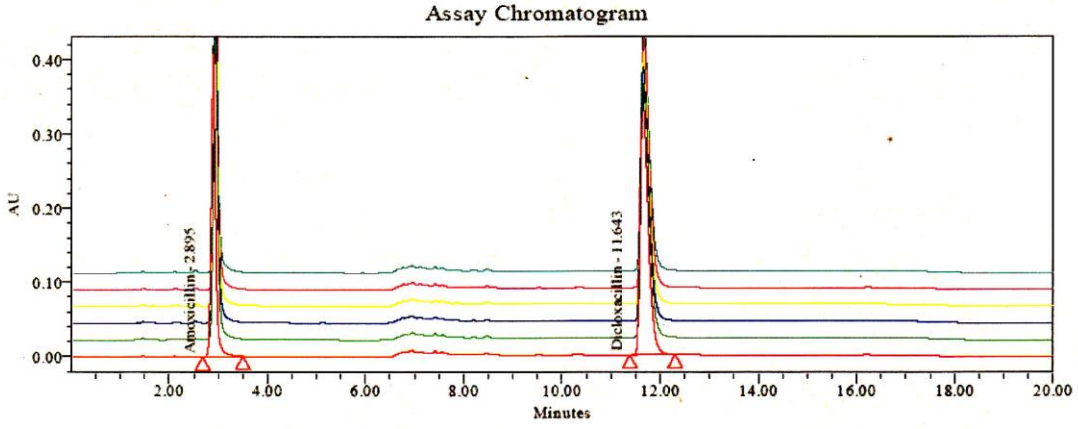
Sample Name: Precision 1, Precision 2, Precision 3,
Sample Type: Unknown
Vial: 4, 5, 6, 7, 8, 9
Injection #: 1
Injection Volume: 20.00 ul



Peak Results

We added solutions of raw material containing different concentrations to the analysed formulation fig 05 to calculate the amount of drug recovered. [13, 14] In accordance with the formulation analysis, the procedure was repeated. Using slope and intercept values from the calibration graph, the amount of drug recorded was calculated. Validation of the method was carried out according to ICH guidelines to ensure precision, accuracy, specificity, linearity, and reproducibility. [12, 15]

RESULTS & DISCUSSION

An RP-HPLC method capable of simultaneously estimating Amoxicillin trihydrate&Dicloxacillin sodium has been developed and validated. Amoxicillin trihydrate&Dicloxacillin sodium were to have correlation coefficients of 0.0 999 & 0.999, respectively. [16] For both drugs, the percent recoveries were 98.45 - 100.65% & 98.79 - 100.01%. Based on ICH guidelines, the method was validated for linearity, accuracy, robustness, specificity and precision. It is possible to use the developed method to analyze the route of AT and DS in their combined dosage form. [17]

The recovery studies range is 98.45- 100.65% & 98.79- 100.01% for Amoxicillin trihydraterespectively.The intraday & interlay analysis carried out for precision. A ruggedness study was conducted.

CONCLUSION

It was validated that the developed RP-HPLC method could be used for routine analysis of Amoxicillin trihydrate and Dicloxacillin sodium in bulk and capsule dosage formulations due to its simplicity and ease of operation.

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