

Research Article <http://dx.doi.org/10.21477/ijapsr.v2i03.8255>

RP-HPLC Method for the Simultaneous Estimation of Ampicillin and Cloxacillin in Capsule Dosage Form

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Article History:

Received: 17 May 2017
Accepted: 20 June 2017
Available online: 1 July 2017

Keywords: RP-HPLC; estimation; ampicillin; cloxacillin; simultaneous; capsule dosage form;

Abstract:

Combination formulation of ampicillin and cloxacillin sodium is β -lactam antibiotic. They are amino penicillin-isoxazol penicillin combination drugs. In HPLC method development was carried out with acetonitrile: phosphate buffer pH 5 (35:65), hypersil C18, 250X4.6mm, 5 μ m column, UV detector at 254nm and flow rate of 1ml/min. Ampicillin retention time is 3.130min and cloxacillin retention time is 7.907min. In capsule brand no.:1. ampicillin content is found to be 250.29mg i.e. 100.1% and cloxacillin content is found to be 252.01mg. i.e. 102.75%. Validation results obtained are satisfactory for accuracy, precision, limit of detection, limit of quantification, linearity, system suitability, ruggedness and robustness parameters.

1. Introduction

Analysis of drugs present in combined pharmaceutical dosage forms is a quite challenging problem and hence attempts were made to develop analytical methods for relative substances of drugs containing ampicillin and cloxacillin in capsule dosage forms.

Chemically ampicillin is a glucocorticoid and its IUPAC name is (2S, 5R, 6R)-6-[(2R)-2-amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2 carboxylic acid. Chemical structure of ampicillin trihydrate is depicted in Figure 1 (AudumbarDigambar Mali et al., 2015). Ampicillin is a β -lactam antibiotic. It can sometimes result in allergic reactions that range in severity from a rash to potentially lethal anaphylaxis. Ampicillin is able to penetrate gram-positive and some gram-negative bacteria (A Ashnagar et al., 2007).

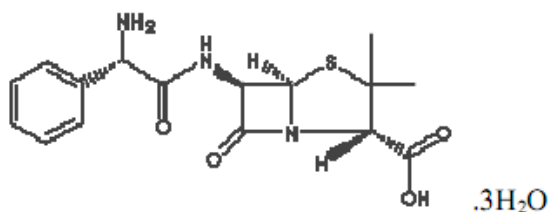


Figure 1: Structure of Ampicillin Trihydrate

Cloxacillin, chemically known as monosodium(2S,5R,6R)-6-[o-(2-chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]-heptane-2-carboxylate monohydrate, is a semi-synthetic antibiotic in the same class as penicillin. It used against staphylococci that produce β -lactamase. The chemical structure of cloxacillin sodium is depicted in Figure 2 (Nikita Patel et al., 2015).

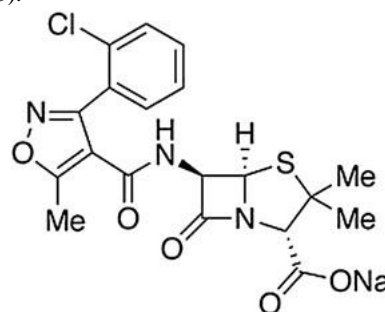


Figure 2: Chemical structure of Cloxacillin Sodium

Analysis of drugs present in combined pharmaceutical dosage forms is a quite challenging problem and hence attempts were made to develop analytical methods for relative substances of drugs in ampicillin and cloxacillin dosage forms.

All the proposed methods are simple, selective, reproducible, sensitive and accurate with good precision.

Some of the methods were proved to be superior to most of the reported methods.

2. Materials and methods

The API of ampicillin and cloxacillin was received from KP labs Hyderabad. Capsules of combination of ampicillin and cloxacillin (label claim ampicillin 250mg and cloxacillin 250mg) were obtained from local pharmacy.

2.1 Chemicals and reagents used

All the chemicals and reagents were supplied by S.D. Fine Chemicals Ltd., India; Qualigens Fine Chemicals Ltd., Mumbai, India

2.2 Instruments used

Method development was carried out using Shimadzu HPLC (model SPD20A).

2.3 Selection of mobile phase

2.3.1 Preparation of Phosphate Buffer pH 5

Dissolve 6.8gms of potassium dihydrogen phosphate in 100ml of water and adjust the PH to 5.0 with 10M potassium hydroxide.

Mobile Phase: Acetonitrile: Phosphate buffer in the ratio of 35:65

2.4 Evaluation of system suitability

Inject 10 μ l of the diluted standard solution in five replicate injections, into the chromatograph and record the chromatograms.

The column efficiency as determined from ampicillin and cloxacillin peaks is not less than 3000 USP plate count and the tailing factor for ampicillin and cloxacillin peaks is not more than 2.0.

The relative standard deviation for the peak areas of the five replicate injections is not more than 2.0%.

Standard Preparation :25mg of ampicillin and 25mg of cloxacillin dissolved in 25ml of distilled water.

Sample Preparation :51mg of sample is dissolved in 25ml of distilled water.

Procedure: Separately inject 10 μ l of the blank, Standard (five injections) and sample solution in duplicate into the liquid chromatograph, record the chromatographs and measure the peak areas.

2.6 Validation Parameters

The method was validated according to ICH guideline for LOD and LOQ accuracy, precision, limit of detection, limit of quantification, linearity, system suitability, ruggedness and robustness parameters (ICH guideline, 1996).

3. Results & discussion

3.1 Chromatographic conditions

Column :Hypersil, C18 250 X 4.6mm, 5 μ m
 Detection wavelength : 254nm
 Flow rate : 1.0 ml/min
 Injection volume : 20 μ l
 Column Temperature : 300C
 Instrument Compan : Simadzu

Capsule Brand Name No.1

Label Claim	
Ampicillin	250mg
Cloxacillin	250mg
Total weight of capsule	591mg

In HPLC method the ampicillin content is calculated by following formula.

For Ampicillin Content

$$\frac{\text{sample area}}{\text{standard area}} \times \frac{\text{standard weight}}{\text{standard dilution}} \times \frac{\text{sample dilution}}{\text{sample weight}} \times \frac{\text{standard purity}}{100} \times \frac{(100-w)}{100} \times \text{Avg. fill wt.}$$

$$= \text{mg of Ampicillin / Capsule}$$

Where W is water content of standard Ampicillin Trihydrate.

Cloxacillin content is Calculated by the following formula.

$$\frac{\text{sample area}}{\text{standard area}} \times \frac{\text{standard weight}}{\text{standard dilution}} \times \frac{\text{sample dilution}}{\text{sample weight}} \times \frac{\text{standard purity}}{100} \times \frac{(100-w)}{100} \times \text{Avg. fill wt.} \times 0.95$$

$$= \text{mg of Cloxacillin / capsule}$$

Where W is water content of Standard Cloxacillin Sodium

Calculation for capsule brand No.1

Calculations for Ampicillin content:

$$\frac{533.67}{530.04} \times \frac{25}{25} \times \frac{25}{51} \times \frac{99.2}{100} \times \frac{100-13.5}{100} \times 591$$

$$= 250.29\text{mg} \quad \text{i.e. } 100.1\%$$

Calculations for Cloxacillin content

$$\frac{2003.28}{2058.72} \times \frac{25}{25} \times \frac{25}{51} \times \frac{99.5}{100} \times \frac{100-3.6}{100} \times 591 \times 0.95$$

$$= 256.87\text{mg} \quad \text{i.e. } 102.75\%$$

3.2 Accuracy (Recovery)

A study of accuracy was conducted. Drug assay was performed in triplicate as per test method with equivalent amount of ampicillin and cloxacillin into each volumetric flask for each spike level to get the concentration of

ampicillin and cloxacillin equivalent to 80%, 100% and 120% of the labeled amount as per the test method. The average % recovery of ampicillin and cloxacillin was calculated.

Separately inject the blank, placebo ampicillin and cloxacillin into the chromatograph.

Acceptance Criteria: The mean % recovery of the ampicillin and cloxacillin at each level should be not less than 95.0% and not more than 105.0%

Observation: The recovery results indicating that the test method has an acceptable level of accuracy.

3.3 Precision

System precision: Standard solution prepared as per test method and injected five times.

Method precision: Prepared five sample preparations individually using single batch of Ampicillin and cloxacillin capsules (500mg) as per test method and injected each solution.

Acceptance Criteria: The % relative standard deviation of individual ampicillin and cloxacillin from the five units should be not more than 2.0%.

The assay of ampicillin and cloxacillin should be not less than 95.0% and not more than 105.0%

Observation: Test results for ampicillin and cloxacillin are showing that the test method is precise.

3.4 Linearity of test method

Prepare serial dilutions of 20, 40, 60, 80, 100mcg of ampicillin and cloxacillin, calculate peak area of each dilution plot a calibration curve of above serial dilute solutions.

Acceptance Criteria: Correlation Coefficient should be not less than 0.9990.

% of y-Intercept should be ± 2.0 .

Observation: The correlation coefficient was found to be 0.9972.

From the above study it was established that the linearity of test method is from 20, 40, 60, 80, 100 mcg concentration solutions of ampicillin and cloxacillin.

3.5 system suitability

A standard solution was prepared by using ampicillin and cloxacillin per test method and was injected ten times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from ten replicate injections for ampicillin and cloxacillin retention times and peak areas.

Acceptance criteria:

The % RSD for the retention times of principal peak from 10 replicate injections of each standard solution should be not more than 2.0%.

The number of theoretical plates (N) for the ampicillin and cloxacillin peaks is NLT 3000.

The Tailing factor (T_j) for the ampicillin and cloxacillin peaks is NMT 2.0.

Observation:

The % RSD for retention times and peak areas were found to be within the limits.

3.6 Limit of detection

Limit of detection can be calculated by following formula

$$\frac{3.3 \times \% RSD}{slope}$$

For Ampicillin

$$\frac{3.3 \times 0.541}{108.06} = 0.0165 \text{mcg/ml}$$

The limit of detection of ampicillin is found to be 0.0165mcg/ml

For Cloxacillin

$$\frac{3.3 \times 0.4359}{421.41} = 0.00341 \text{mcg/ml}$$

The limit of detection of cloxacillin is found to be 0.00341 mcg/ml

3.7 Limit of quantification

The limit of quantification can be calculated by following formula

$$\frac{10 \times \% RSD}{slope}$$

For Ampicillin

$$\frac{10 \times 0.541}{108.06} = 0.05 \text{mg/ml}$$

The limit of detection of ampicillin is found to be 0.05mg/ml

For Cloxacillin

$$\frac{10 \times 0.4359}{421.41} = 0.010 \text{mg/ml}$$

The limit of detection of cloxacillin is found to be 0.010 mcg/ml

3.8 Ruggedness of test method

System to system / analyst to analyst / column to column variability study was conducted on different HPLC systems, different columns and different analysts under similar conditions at different times. Six samples were prepared and each were analysed as per test method. The relative standard deviation for ampicillin and cloxacillin were found to be below 2% on the columns, systems and analysts. Comparison of both the results obtained on two different HPLC systems, different column and different analysts shows that the assay test method is rugged for system to system / analyst to analyst / column variability. Acceptance Criteria: The % relative standard deviation of ampicillin and cloxacillin from the six sample preparations should be not more than 2.0%.

The % of ampicillin and cloxacillin should be between 95.0% - 105.0%.

Observation: The %RSD was found within the limits.

3.9 Bench top stability of standard and test preparation

A study to establish stability of ampicillin and cloxacillin standards and test preparations on bench top was conducted over period of two days. Ampicillin and cloxacillin test preparation spiked to target concentration are injected initial, 6.0Hr, 12Hr and 18Hr. The difference in % of ampicillin and cloxacillin from initial to 18 hours is within the limits. In the similar way standard preparations were injected initial, 6.0Hr, 12Hr and 18Hr.

From the above study, it was established that the standard and test preparations were stable for a period of 18 hours on bench top.

Acceptance Criteria: The difference between initial and bench top stability sample for % ampicillin and cloxacillin should be not more than 3.0. The assay of standard kept on bench top should not differ from initial value by more than 2.0.

Observation: The % assay was found within the limits.

3.10 Robustness

Robustness of the method was studied by deliberate change in the experimental conditions. No significant changes in the chromatographic parameter were observed when small changes in mobile phase composition was done. This indication the method is robust.

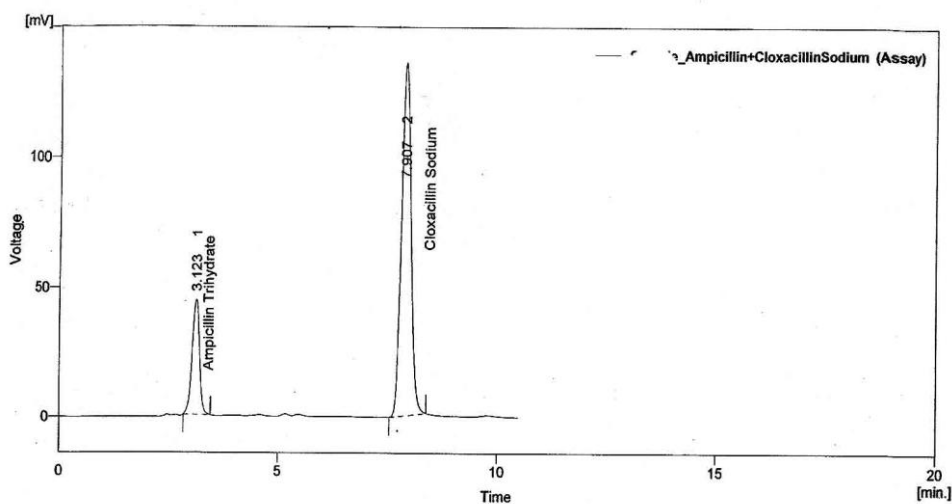


Figure 3: Typical chromatogram of Ampicillin and Cloxacillin (Standard)

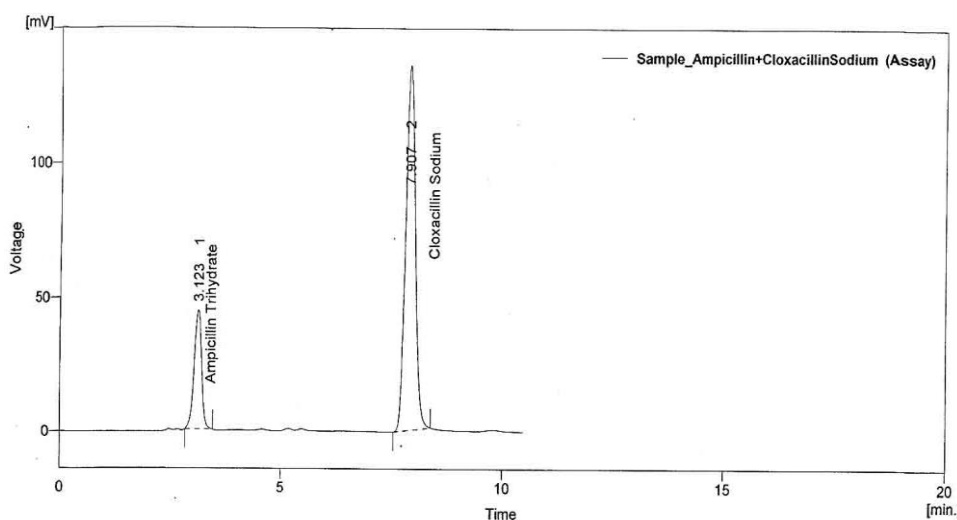


Figure 4: Typical chromatogram of Ampicillin and Cloxacillin (Sample)

Table 1: Ampicillin trihydrate accuracy results

S.No	80%	100%	120%
1.	430.62	534.30	642.14
2.	426.43	539.12	647.98
3.	433.79	542.17	640.98
Average	430.28	538.53	643.7
	79.898984 mcg	100mcg	119.5291mcg

Table.2 Cloxacillin Sodium Accuracy Results

S.No	80%	100%	120%
1.	1685.67	2073.99	2509.34
2.	1681.23	2089.39	2497.97
3.	1684.50	2088.33	2489.93
Average	1683.80	2083.903	2499.08
	80.800293mcg	100mcg	119.923mcg

Table 3: Ampicillin Trihydrate precision results

S.No.	Retention time	Area
1	3.123	455.58
2	3.127	461.74
3	3.120	456.39
4	3.133	459.32
5	3.123	459.19
Average	3.1252	458.444
	0.160628	0.541041 %RSD

Table 4: Cloxacillin Sodium precision results

S.No.	Retention time	Area
1	8.057	1752.49
2	8.080	1759.16
3	8.123	1747.06
4	8.133	1751.02
5	8.103	1766.49
Average	8.0992	1755.244
	0.384185	0.435951 % RSD

Table 5:Linearity table for ampicillin

Concentration	Ampicillin Trihydrate (Peak Area)
20mcg	105.37
40mcg	216.25
60mcg	312.84
80mcg	449.66
100mcg	533.70

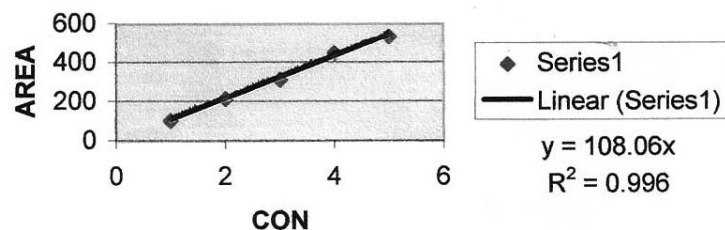


Figure 5: Calibration curve of Ampicillin

Table 6: Linearity table for cloxacillin

Concentration	Cloxacillin Sodium (Peak Area)
20mcg	436.57
40mcg	863.65
60mcg	1254.03
80mcg	1732.49
100mcg	2064.38

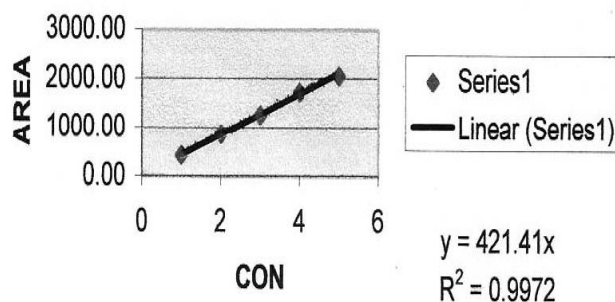


Figure 6: Calibration curve of Cloxacillin

4. Conclusion

A simple, rapid and accurate method was developed for the simultaneous estimation of ampicillin and cloxacillin in capsule dosage form. In the proposed method symmetrical peaks with good resolution were obtained and this method was validated according to ICH guidelines.

Conflict of interest

None declared

5. References:

1. AAshnaga, N GharibNaseri (2007). Analysis of three penicillin antibiotics (ampicillin, amoxicillin and cloxacillin) of several Iranian pharmaceutical

companies by HPLC. E-Journal of Chemistry. 4(4):536-545.

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How to cite this article:

Shireesha Battula, Anusha Shivaraj (2017). RP-HPLC method for the simultaneous estimation of ampicillin and cloxacillin in capsule dosage form. *Int J App Pharm Sci Res.* 2(3):93-98. <http://dx.doi.org/10.21477/ijapsr.v2i03.8255>.