

**Research Article**

# DESIGN, DEVELOPMENT AND VALIDATION OF AMOXICILLIN AND CLOXACILLIN FORMULATION BY HPLC

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**Abstract**

**Background:** A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of amoxicillin and cloxacillin in pure and tablet forms. The method was validated with respect to linearity, precision, accuracy, and selectivity. The mean values of slope, intercept and correlation coefficient were 0.9991(r2) respectively. The % COV values for repeatability and intermediate precision studies were < 2 indicates good precision of the method. The recovery of the drug ranged from 99.95-100.72% from a mixture of degradation products. The method was specific to drug and also selective to degradation products. The method showed a linear response for concentrations in the range of 300-1500 µg/ml using 0.01 M potassium dihydrogen phosphate (pH 5.0) buffer: acetonitrile [15:85] as the mobile phase with detection at 225.0 nm and a flow rate of 5 ml/min and retention time 15-20 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, forced degradation, solution stability and selectivity. Quantitative and recovery studies of the dosage form

were also carried out and analyzed; the % RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.

**Keywords:** Amoxicillin, cloxacillin, stability, HPLC, Validation

**Introduction**

Amoxicillin is an strong antibacterial agent & chemically it is 4-thia-1-azabicyclo (3.2.0) heptane-2-carboxylic acid, 6-(amino 4-hydroxyphenyl acetyl) amino-3,3-dimethyl-7-oxo. OR Hydroxyl-ampicillin. Cloxacillin is also from same class as an antibacterial agent & chemically it is Sodium (6R)-6-(3-(2-chlorophenyl)-5-methyl isoxazole-4-carbomido penicillate monohydrate. The combination of antibiotic in the infection of the otitis media (s.pyrogenes) bronchitis, pneumonia, gonorrhea and urinary tract infection. Empirical therapy of infection is probably the most common reason using a combination of antibiotic. The combining use of amoxicillin and cloxacillin vastly improve curve rate by preventing development of resistance. They act by mechanism inhibition of synthesis of the bacterial peptidoglycan cell wall.<sup>13</sup> The combination of antibiotic amoxicillin and cloxacillin is used in the infection of the otitis media (s.pyrogenes) bronchitis, pneumonia, gonorrhea and urinary tract infection.<sup>14</sup> The combination containing amoxicillin and cloxacillin shows synergistic effect for oral absorption of drug and extensively used for the treatment of certain systemic and urinary tract infections for which oral administration is desirable, leading to higher plasma level. The combined drugs have the same or identical bacterial spectrum as active against the same gram-positive organisms that are susceptible to other penicillin's & more active against some gram-negative bacteria & enterococci. It is particularly useful in the treatment of acute urinary tract infections caused by E.coli or proteus mirabilis it is the agent of choice against H.influenzae infections.<sup>15,16</sup>

**Material and Methods:**

Pure amoxicillin and cloxacillin were obtained as gift samples from kiwi laboratories Ltd., vadodara. HPLC grade acetonitrile and buffer were purchased from Rajesh chemicals. All other chemicals were of analytical reagent grade.

The HPLC system consisted of an on-line degasser (DGU-14A), low pressure gradient flow control valve (FCV-10ALvp), Solvent delivery module (LC-10ATvp), auto injector (SIL-10ADvp), Column oven (CTO-10ASvp), uv-visible dual wavelength detector (SPD-10Avp), system controller (SCL-10Avp), CLASS-vp software, version-6.13. The separations were achieved on an intersil ODS<sub>2</sub> column grace vydac C-18 column.

#### PREPARATION OF STANDERD&SAMPLE SOLUTION:

##### Preparation of standard stock solution:

The std. stock solution 1000 µgm/ml of each were prepared separately by dissolving working in small proportion of methanol and later diluted to volume with mobile phase.

##### Preparation of std.calibration solution:

The std.calibration solution of amoxicillin and cloxacillin having the conc. in the range of 50-1000 µg/ml were prepared by diluting stock solution with mobile phase.

##### Preparation of sample stock solution:

Ten capsules were weighted; their mean weight determined and crushed in mortar. An amount of powdered mass equivalent to one capsule was transferred into 50ml volumetric flask containing 10ml of methanol. Mechanically shaken for 10min., ultrasonicated for 5min. and then diluted volume with mobile phase.

##### Preparation of sample solution:

About 10ml of sample stock solution was centrifuged at 10,000rpm and 5ml of aliquot dilute to 50ml with mobile phase. Small portion of sample solution was filtered through 0.45 µ nylon filter and used for injection on HPLC.

##### Procedure for analysis of dosage form:

Ten capsules were weighed and their mean weight determined and crushed in mortar. An amount of powdered mass equivalent to one capsule content was transferred into 50 ml volumetric flask containing 10 ml of methanol, mechanically shaken for 10 min., ultrasonicated for 5 min. and then diluted to volume with mobile phase (sample stock solution). About 10 ml of sample stock solution was

centrifuged at 10000 rpm and 5 ml aliquots diluted to 50 ml with mobile phase (Sample solution). A small portion of sample solution was filtered through 0.45µm nylon filter and used for injection on HPLC.

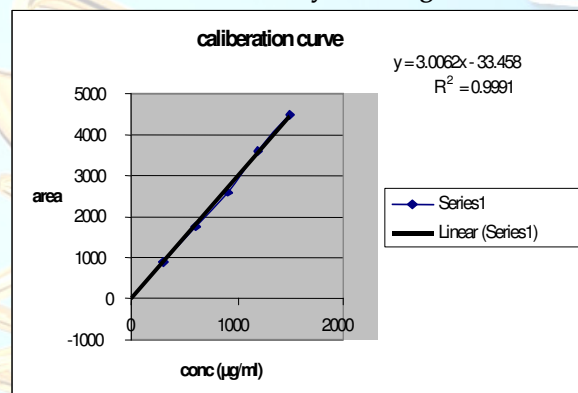
## RESULT AND DISSCUSSION

### A) Result of validation parameters:

Table: 1) Linearity data for amoxicillin and cloxacillin (n=3)

| Sr.no | Concentration of AM&CL | Average Area (n=3) | Standard Deviation | %RSD |
|-------|------------------------|--------------------|--------------------|------|
| 1     | 300                    | 894 ± 17           | 6.88               | 0.76 |
| 2     | 600                    | 1778.4 ± 14        | 6.09               | 0.34 |
| 3     | 900                    | 2598.16 ± 14       | 7.99               | 0.30 |
| 4     | 1200                   | 3595.10 ± 10       | 4.07               | 0.11 |
| 5     | 1500                   | 4494.95 ± 10       | 4.21               | 0.09 |

### Calibration curve: Linearity and range.



**2) Precision:****Table: 2) Intraday precision :( n=3)**

| Drug        | Std.Added (µg/ml) | Amount found ±(µg/ml) | ±SD  | %RSD |
|-------------|-------------------|-----------------------|------|------|
| Amoxicillin | 200               | 204.64                | 0.24 | 0.11 |
|             | 400               | 402.37                | 0.15 | 0.03 |
|             | 600               | 602.11                | 3.52 | 0.58 |
|             | 800               | 791.79                | 7.17 | 0.90 |
|             | 1000              | 1003.09               | 2.45 | 0.24 |
| Cloxacillin | 200               | 202.35                | 0.37 | 0.18 |
|             | 400               | 400.15                | 1.25 | 0.31 |
|             | 600               | 602.42                | 3.51 | 0.58 |
|             | 800               | 792.91                | 7.38 | 0.93 |
|             | 1000              | 1003.86               | 2.56 | 0.25 |

**Table: 3) Interday precision:**

| Drug        | Std.Added (µg/ml) | Amount found ±(µg/ml) | ±SD  | %RSD |
|-------------|-------------------|-----------------------|------|------|
| Amoxicillin | 200               | 207.79                | 2.01 | 0.96 |
|             | 400               | 405.29                | 0.32 | 0.08 |
|             | 600               | 600.54                | 2.37 | 0.39 |
|             | 800               | 792.31                | 2.08 | 0.26 |
|             | 1000              | 1002.58               | 8.76 | 0.87 |
| Cloxacillin | 200               | 202.57                | 2.76 | 1.36 |
|             | 400               | 403.64                | 4.85 | 1.20 |
|             | 600               | 600.09                | 5.03 | 0.83 |
|             | 800               | 786.60                | 5.03 | 0.63 |
|             | 1000              | 994.57                | 1.86 | 0.18 |

**Table: 4) Recovery studies:**

| Drug        | Added conc.(µg/ml) | Measured conc.(µg/ml) | %recovery | Mean %recovery |
|-------------|--------------------|-----------------------|-----------|----------------|
| Amoxicillin | 50                 | 50.25                 | 100.51    | 100.78±0.42 %  |
|             | 100                | 100.56                | 100.56    |                |
|             | 200                | 202.54                | 101.27    |                |
| Cloxacillin | 50                 | 49.97                 | 99.95     | 100.40±0.39 %  |
|             | 100                | 100.42                | 100.52    |                |
|             | 200                | 201.45                | 100.72    |                |

**Table: 5) Peak purity parameters:**

| Drug/degradation products | Peak purity index | Single point threshold |
|---------------------------|-------------------|------------------------|
| I                         | 0.999986          | 0.999115               |
| Amoxicillin               | 0.999999          | 0.999897               |
| II                        | 0.999991          | 0.999289               |
| III                       | 0.999987          | 0.999123               |
| IV                        | 0.999654          | 0.999883               |
| V                         | 0.999671          | 0.979359               |
| VI                        | 0.999578          | 0.958321               |
| VII                       | 0.999851          | 0.988022               |
| Cloxacillin               | 1.000000          | 0.999940               |

**Conclusion:**

The study presents a simple and validated indicating HPLC method for simultaneous estimation

of amoxicillin and cloxacillin in the presence of degradation products. The method yielded results similar to those determined by application of compendia procedures on individual drugs.

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