Development and Validation of RP- HPLC method for the Simultaneous estimation of Ampicillin and Cloxacillin in capsule dosage form

Dr.G. Dharmamoorthy[1]*,Dr.Anna Balaji [2.] K.Vishnu, G.Dheeraj, A.Narendrareddy & P.Vignesh,

Department of Pharmaceutical Analysis, Sree Vidyanikethan Collegeb of Pharmacy. Sree Sainath Nagar.Rangampet, Tirupathi-517502. Andhrapradesh. India.

Corresponding Author.
Dr.G.Dharmamoorthy
Associate Professor & HOD
Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy,
Tirupathi-517501. Andhrapradesh. India.
E-mail: dharmamoorthy111@gmail.com
Mobile: 9603774847

REVEIWED BY
1)Dr. P. DHARANI PRASAD
M.pharm.ph.D
Professor & HOD Department of Pharma.D,
Sree Vidyanikethan college of Pharmacy, Rangampeta. Tirupathi 517103
Mobile no; +91 9491201679 Emai id dharaniprasad@gmail.com

2)Dr.V.SHUNMUGAM
M.pharm.PhD
Professor & HOD, Department of Pharmaceutics
Sree vidyanikethan college of pharmacy, Rangampeta, Tirupathi 517103
Mobile no; +91 9052155880 Emai id; shanmugam.v@vidyanikethan.edu

ABSTRACT

In the present work development of the RP-HPLC method for the simultaneous estimation of Ampicillin and Cloxacillin in Pharmaceutical marketed products like Capsule Brand no 1 and Brand no 2.RP-HPLC Method for the simultaneous estimation of Ampicillin and Cloxacillin, Acetonitrile and Phosphate buffer pH 5 is used as mobile phase in the ratio of 35:65.Hypersil C_{18}, 250x4.6mm, 5µ is used as Column. Ampicillin Retention time was found to be 3.130min, Cloxacillin retention time was found to be 7.907min. The two peaks were well resolved with good peak shape and symmetry.RP-HPLC method validation summary satisfies all validation parameters.

Keywords: Ampicillin, Cloxacillin, Acetonitrile, Phosphate buffer

INTRODUCTION

Ampicillin is chemically (6R)-6-(α-phenyl-D-glycylamino) penicillanic acid[1,2]. Cloxacillin is chemically Sodium salt of (6R)-6-(3-(2-chlorophenyl)-5- methylisoxazole-4-carmaxamido) penicillinase monohydrate.[3,4]A combination formulation of Ampicillin and cloxacillin sodium is a β-lactam antibiotic. They are aminopenicillin-isoxazylpencillin combination drugs. They are extensively used in the therapy of penicillin-resistant infections, and also used for veterinary purposes. Ampicillin and Cloxacillin sodium is official in I.P, B.P, and U.S.P.
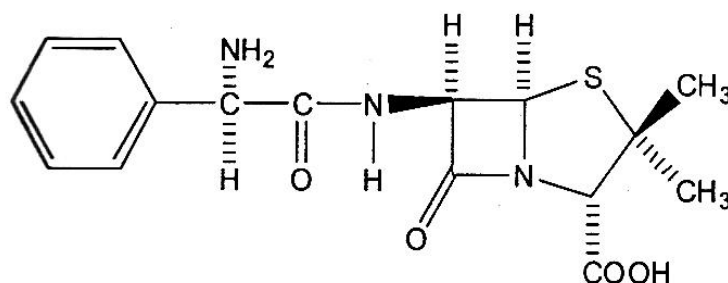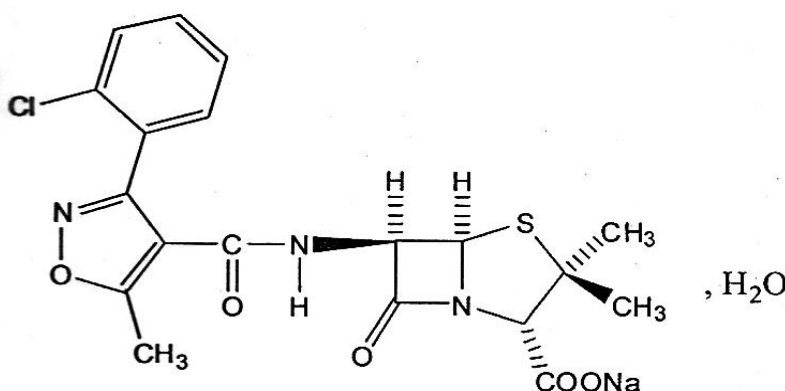
Fig.1 Structure of Ampicillin

Fig.2 Structure of Cloxacillin sodium

The survey of the literature reveals that the Spectrophotometric methods for the analysis of the drugs are individually carried out based on total penicillin[4-8.] But there is no Spectrophotometric method for the non-separative simultaneous analysis of the β-lactam antibiotic combination drugs. Due to the similarity of their structures, simple absorption spectrophotometry cannot be performed.[9,10] Hence it becomes necessary to the development of some new analytical methods for the simultaneous estimation of drugs belonging to β-lactam antibiotics. It becomes the development of the RP-HPLC method for simultaneous estimation of Ampicillin and Cloxacillin combination in marketed products (capsules). To validate the obtained results in accordance with the analytical validation parameters mentioned in the I.P. and I.C.H guidelines.[9,10]

MATERIALS
PREPARATION OF PHOSPHATE BUFFER pH 5
Dissolve 6.8gms of potassium dihydrogen phosphate in 100ml of water and adjust the P^H to 5.0 with 10M potassium hydroxide.

MOBILE PHASE: Acetonitrile: Phosphate buffer in the ratio of 35: 65

METHODS
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 10µl of the blank, Standard (five injections) and sample solution in duplicate into the liquid chromatograph, record the chromatographs and measure the peak areas.

CHROMATOGRAPHIC CONDITIONS
Column   :   Hypersil , C_{18}, 250 X 4.6mm, 5µm
Detection Wavelength: 254nm
Flow rate: 1.0 ml/min
Injection volume: 20µl
Column Temperature**:** 30^0C
Instrument Company :   Simadzu

Ampcillin and cloxacillin capsule Brand No: 1
Label claim
    Ampicillin   250mg
    Cloxacillin   250mg
Total weight of the capsule   591mg

Ampcillin and cloxacillin capsule Brand No: 2
Label claim
    Ampicillin   250mg
    Cloxacillin   250mg
Total weight of the capsule   594mg
For Ampicillin Content

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

    = mg of Ampicillin / Capsule

Where W is the water content of Standard Ampicillin Trihydrate
Cloxacillin content is Calculated by the following formula.

For Cloxacillin Content:

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

= mg of Cloxacillin / capsule

Where W is the water content of Standard Cloxacillin Sodium

The calculation for capsule brand No.1

Calculation: Calculate the amount of each drug by using the following formula

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

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

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

Calculations for Cloxacillin content

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

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

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

The calculation for capsule brand No.2

Calculation: Calculate the amount of each drug by using the following formula

For Ampicillin Content

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

= mg of Ampicillin / Capsule

Where W is the water content of Standard Ampicillin Trihydrate

$$\frac{533.61}{530.04} \times \frac{25}{25} \times \frac{25}{51} \times \frac{99.2}{100} \times \frac{100 - 13.5}{100} \times 594$$

$$= 250.01\text{mg} \quad \text{i.e. } 100.01\%$$

For Cloxacillin Content:

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

= mg of Cloxacillin / capsule

Where W is the water content of Standard Cloxacillin Sodium

$$\frac{2002.28}{2058.72} \times \frac{25}{25} \times \frac{25}{51} \times \frac{99.5}{100} \times \frac{100 - 3.6}{100} \times 594 \times 0.95$$

$$= 252.21\text{mg} \quad \text{i.e. } 101.75\%$$

Figure. 2 Typical chromatograms of Ampicillin and Cloxacillin (Standard)
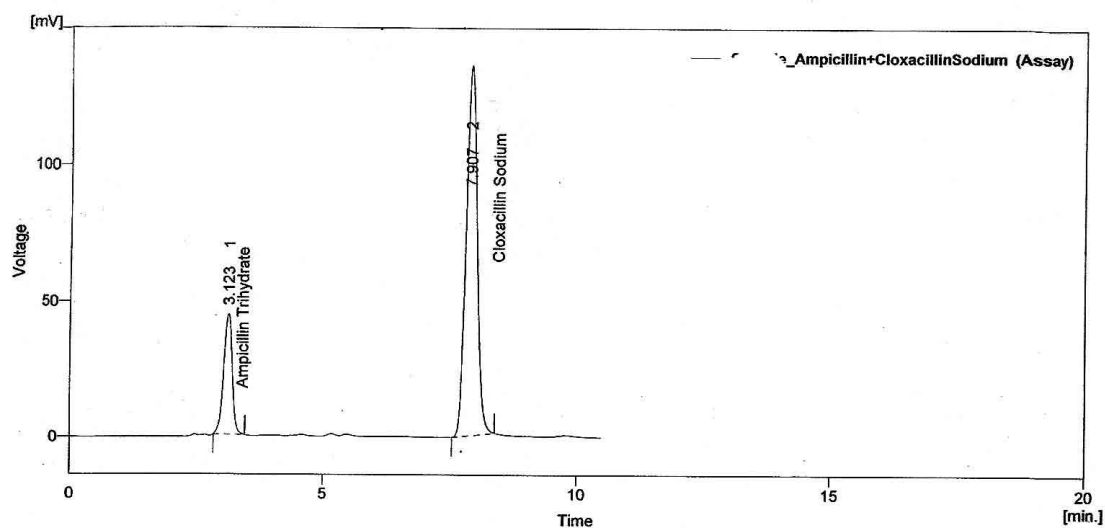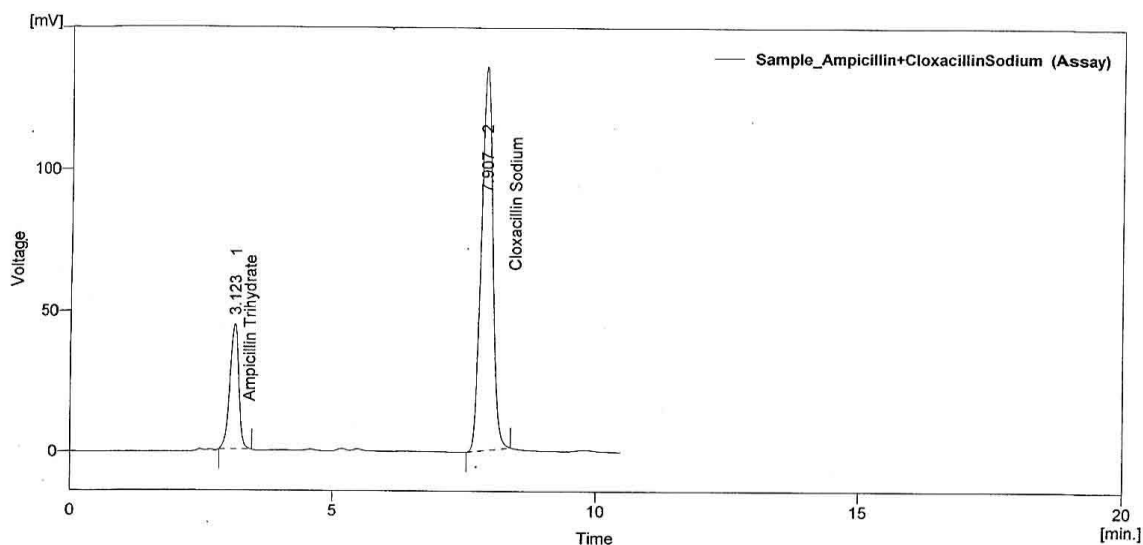

Figure. 3 Typical chromatogram of Ampicillin and Cloxacillin (Sample)

HPLC METHOD VALIDATION

1. ACCURACY (RECOVERY)

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per the test method with an 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 labelled 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.

PRECISION

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

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

LINEARITY OF TEST METHOD

Prepare serial dilutions of 20, 40, 60, 80, and 100mcg of Ampicillin and Cloxacillin calculate the peak area of each dilution plat a calibration curve of the above serial dilute solutions

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.

LIMIT OF DETECTION:

The limit of detection can be calculated by the following formula

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

For Ampicillin

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

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

For Cloxacillin

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

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

LIMIT OF QUANTIFICATION

The limit of quantification can be calculated by the following formula

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

For Ampicillin

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

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

For Cloxacillin

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

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

RUGGEDNESS OF THE 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 was analysed as per the test method. The relative standard deviation for ampicillin and cloxacillin was found to be below 2% on the columns, systems and Analysts. A comparison of the results obtained on two different HPLC systems, different columns and different analysts shows that the assay test method is rugged for a system-to-system / Analyst to Analyst/column variability.

ROBUSTNESS

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

DISCUSSION

This shows that recoveries of the Ampicillin and cloxacillin by the proposed method were satisfactory and the results are satisfied .. Ruggedness and Robustness were determined and the % RSD values calculated from precision studies were less than $\pm$ 2.0 results obtained were subjected to statistical analysis to find out standard deviation values.All validation parameters are within the limits.

CONCLUSION

Thus it can be concluded that these methods developed in the present investigation are simple, sensitive, selective, accurate, precise and economical and can be used in the simultaneous determination of Ampicillin and cloxacillin in pharmaceutical capsule dosage forms in a routine manner. Hence, the above said method can be successfully applied for the simultaneous estimation of Ampicillin and cloxacillin in capsule dosage forms.

ACKNOWLEDGMENT

Conflict of Interest; The authors declared no conflict of interest.

REFERENCES

1.   Williamson R, Hakenbeck R, Tomasz A: In vivo interaction of beta-lactam antibiotics with the penicillin-binding proteins of Streptococcus pneumoniae. Antimicrob Agents Chemother. 1980 Oct;18(4):629-37. [Article]

2.   Sala-Rabanal M, Loo DD, Hirayama BA, Turk E, Wright EM: Molecular interactions between dipeptides, drugs and the human intestinal H+ -oligopeptide cotransporter hPEPT1. J Physiol. 2006 Jul 1;574(Pt 1):149-66. Epub 2006 Apr 20. [Article]

3.   Lalanne S, Le Vee M, Lemaitre F, Le Corre P, Verdier MC, Fardel O: Differential interactions of the beta-lactam cloxacillin with human renal organic anion transporters (OATs). Fundam Clin Pharmacol. 2020 Aug;34(4):476-483. doi: 10.1111/fcp.12541. Epub 2020 Feb 25. [PMID:32100322]

4.   Thijssen HH, Mattie H: Active metabolites of isoxazolylpencillins in humans. Antimicrob Agents Chemother. 1976 Sep;10(3):441-6. doi: 10.1128/aac.10.3.441. [PMID:825029]

5.   Kumar V, Bhutani H, Singh S. Evaluation of Derivative spectrophometry for simultaneous determination of Ampicillin and Cloxacillin. Indian drugs. 43(5) May 2006; 376 –82.

6.   Straub R.F, Voyaksner R.D. Determination of penicillin G, ampicillin, amoxicillin, cloxacillin and cephapirin by high-performance liquid chromatography-electrospray mass spectrometry. J. Chromatogr. A. 1993; 647, 167-81.

7.   Qureshi S.Z, Qayoom T, Helalet M.I. Simulataneous spectorphotometric and volumetric determination of amoxicillin, ampicillin and cloxacillin in drug formulations: reaction mechanism in the base catalysed hydrolysis followed by oxidation with iodate in dilute acidic solution J. Pharm. Biomed. Anal. 1999; 21, 473-82.

8.   JehlF, Birckel P, Monteil H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography. J. Chromatogr. Biomed. Appl. 1987; 413, 109-19.

9.   Mohamed GG. Suresh K, Mohan N. Spectrophtometric determination of ampicillin, dicluxacillin, flucolxacillin and amoxicillin antibiotic drugs: ion-pair formation with molybdenum and thiocynate. J. Pharm. Biomed. Anal. 2001; 24, 561-67.

10.   Takeba K, Fujinuma K, Miyazaki T, Nakazawa H. Simultaneous determination of β-lactam antibiotics in milk by ion-pair liquid chromatography. J.Chromatogr. A. 1998; 812, 205-11.

11.   Okke I.N, Lamikanra A, Edelmant R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg. Inf. Dis. 1999; 5,p, 18-27.

12.   Ojeda C.B, Rojas F.S, Pavon J.M.C. Recent developments in derivative ultraviolet/ visible spectrophotometry. Talanta. 1995, 42,p. 1195-214.