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ROBUST AND PRECISE RP-HPLC METHOD DEVELOPMENT AND VALIDATED OF SELECTED ANTI-BACTERICAL DRUGS IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A Simple and Robust method was developed for simultaneous estimation of Cloxacillin and Ampicillin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ampicillin and Cloxacillin by using C_{18} column (4.0×125mm) 5µ and the mobile phaseis Acetonitrile: Methanol: Buffer Solution (0.01% 1-Heptane Sulfonic Acid Sodium Saltand the pH3.0 was adjusted with OPA), flow rate at a 1.0ml/min, detection was carried out with UV detector and the isobestic point is 252nm. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Cloxacillin and Ampicillin was found in concentration range of 5µg-25µg and 50µg-250µg and r^2 was found to be 0.999 and 0.999% respectively. Recovery was found to be 98.47% and 98.53%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.65 and 0.36 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17, 5.68 and LOQ value was 0.0172 and 0.2125 respectively. In this improved method has been applied in active pharmaceutical ingredient and real marketed samples.

KEYWORDS

C18 column, Cloxacillin and Ampicillin, RP-HPLC, Validation and ICH guidelines.

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INTRODUCTON

Cloxacillin is chemically (2S, 5R, 6R)-6-[3-(2-chlorophenyl)-5-methyl-1, 2-oxazol-4-amido]-3, 3-dimethyl-7-oxo- known as 4.-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid, chemical formula for C19H18ClN3O5S. It is not recommended for those with a previous penicillin allergy. It appears to be relatively safe for use during pregnancy. It is semi-synthetic and is in the same class as penicillin[1]. Cloxacillin is used against β-lactamase-

producing staphylococci because of its long R-chain, which interferes with the binding of β-lactamase. This drug has weaker antibacterial activity than benzylpenicillin and has no significant toxicity other than allergic reactions. Cloxacillin he patented in 1960 and was approved for medical use in 1965. It is on the World Health Organization's list of essential medicines. Not commercially available in the US[2].

Ampicillin is chemically (2S, bb5R, b6R)-6-[(2R)-2-amino-2-phenylacetamido]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2 0] Heptane-2-carboxylic acid, chemical formula C16H19N3O4S. It belongs to the category of amides, aminopenicillins and ampicillins. Ampicillin binds to specific penicillin-binding proteins (PBPs) in bacterial cell walls, ampicillin inhibits the third and final step of bacterial cell wall synthesis and cell lysis is mediated by bacterial cell wall autolytic enzymes such as autolysins. Increase Ampicillin may interfere with autolysine inhibitors[3]. Ampicillin is an antibiotic used to prevent and treat many bacterial infections such as respiratory infections, urinary tract infections, meningitis, salmonellosis, and endocarditis. It can also be used to prevent group B streptococcal infections in newborns. It is given orally, intramuscularly, or intravenously. Common side effects include rash, nausea, and diarrhea. Do not use if you are allergic to penicillin. Serious side effects may include Clostridium difficile colitis or anaphylaxis. It can also be used in patients with kidney problems, although dose reduction may be required. Use during pregnancy and lactation is generally considered harmless[4,5].

From various literature searches, it is known about the analysis of cloxacillin and ampicillin by simultaneous evaluation using RP-HPLC, spectrophotometric, HPLC and HPTLC compound analysis methods individually or in combination with other dosage forms. There appear to be only a few analytical methods available. Therefore, we found a need to develop a new analytical method for the simultaneous determination of cloxacillin and ampicillin in pharmaceutical dosage forms. This study aims to develop a robust, simple, fast, accurate, efficient and reproducible RP-HPLC method for the simultaneous determination of cloxacillin and ampicillin. The developed method is validated according to ICH guidelines for various parameters specified in ICH guideline Q2 (R1).

The primary objective of this study was to develop an analytical method for the simultaneous determination of cloxacillin and ampicillin using an RP-HPLC method for the analysis of active pharmaceutical ingredients (APIs) and marketed drug formulations.

MATERIAL AND METHODS

Chemicals and Instruments used

Methodology

To develop a new analytical method for the simultaneous estimation of Cloxacillin and Ampicillin by RP-HPLC.

RESULTS AND DISCUSSION

The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Ampicillin and Cloxacillinby RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Ampicillin and Cloxacillinby RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Ampicillin and Cloxacillin in pharmaceutical dosage form.

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Ampicillin and Cloxacillin was obtained and the isobestic point of Ampicillin and Cloxacillin showed absorbance's maxima at 252 nm. The chromatographic method development for the simultaneous estimation of Ampicillin and Cloxacillin were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Ampicillin and

Cloxacillin in API and pharmaceutical dosage form by RP-HPLC method. Optimized chromatographic conditions given below Figure No.1.

Column: Phenomenex Luna 5µ C18 (250*4.6mm)
Column temperature : Ambient
Wavelength : 252nm
Mobile phase ratio : Acetonitrile: Methanol: 0.01% 1-Heptane Sulfonic Acid Sodium Salt Buffer (Buffer pH 3.0 was adjusted with OPA) (50: 20:30 % v/v/v)
Flow rate : 1.0ml/min
Auto sampler temperature : Ambient
Injection volume : 10µl
Run time : 8.0 minutes

Figure No.1 Optimized Final Chromatogram in mobile phase of Acetonitrile: Methanol: 0.01% 1-Heptane Sulfonic Acid Sodium Salt Buffer (50: 20:30 % v/v/v) and flow rate of 1.0ml^{-1}. [A) Blank B) Ampicillin and Cloxacillin Drug Substance (API) C) Ampicillin and Cloxacillin marked sample].

METHOD VALIDATION REPORT

The finalized chromatographic condition was validated as per the ICH Guideline. The assay method is specific in relation to the placebo used in this study because there was no excipients peak co-eluted with the analytes (Figure No.1). The linearity study was performed for concentration range of 5.µg-25µg and 10µg-50µg of Ampicillin and Cloxacillin and the correlation coefficient was found to be 0.999 and 0.999 (NLT 0.999) and it's showed in Table No.3, Table No.4, Figure No.2 and Figure No.3. LOD value was 3.17 and 5.68 and LOQ value was 0.0172 and 0.2125 respectively. Robustness study reveals that small changes did not alter the retention times, retention factor and resolutions more than 2.0 % and therefore it would be concluded that the method conditions are robust.

Table No.1: List of chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
1	Water	Rankem	HPLC grade
2	Methanol	Rankem	HPLC grade
3	Acetonitrile	Rankem	HPLC grade
4	Ortho phosphoric acid	Rankem	G.R
5	KH_2PO_4	Rankem	G.R
6	K_2HPO_4	Rankem	G.R
7	0.45µ filter paper	Millipore	HPLC grade
8	Ampicillin and Cloxacillin	Gifted Sample from Hetro Drugs Lab	

Table No.2: Name of the instruments used

S.No	Instrument name	Model number	Soft ware	Manufacturers Name
1	HPLC –UV detector	LC-10AT and SPD 10 A (Detector)	LC Solution	Shimadzhu Ltd
2	U.V double beam spectrometer	Double Beam Spectrophotometer-2202	LC Solution	Systronics
3	Digital weighing balance (sensitivity 5mg)	Meritor	-	Lab india
4	pH meter	LI 120	-	Elico India
5	Sonicator	SOLTEC	-	Spincotech Pvt Ltd

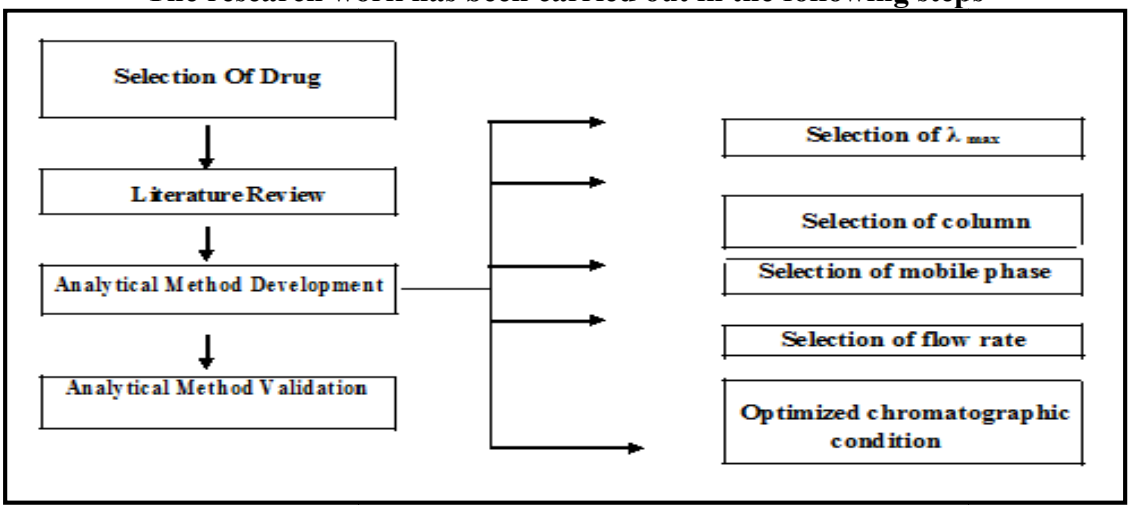
Table No.3: Linearity results for ampicillin

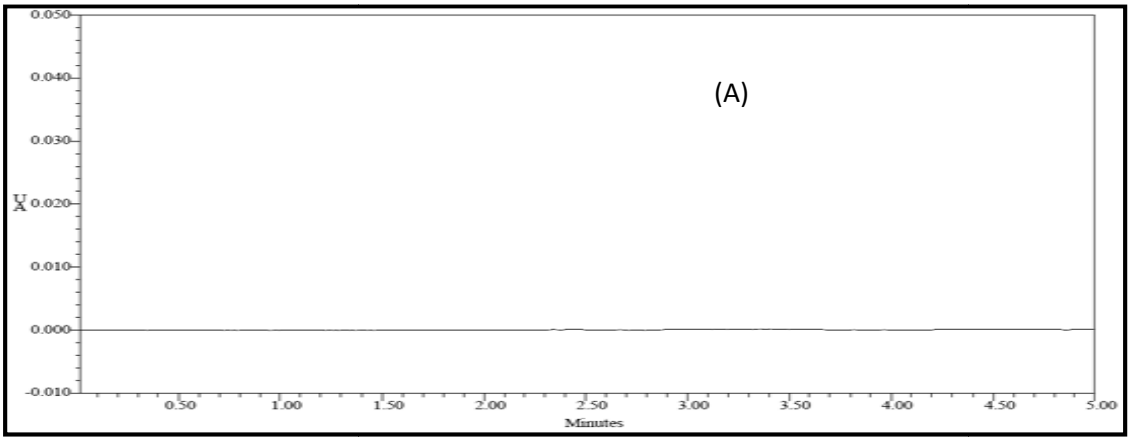
Linearity level	Concentration	Peak Area
I	5ppm	281543
II	10ppm	536277
III	15ppm	794999
IV	20ppm	1059124
V	25ppm	1320213
Correlation Coefficient		0.999

Table No.4: Linearity results for cloxacillin

Linearity Level	Concentration	Peak Area
I	10ppm	534722
II	20ppm	738417
III	30ppm	926552
IV	40ppm	1115416
V	50ppm	1305670
Correlation Coefficient		0.999

The research work has been carried out in the following steps





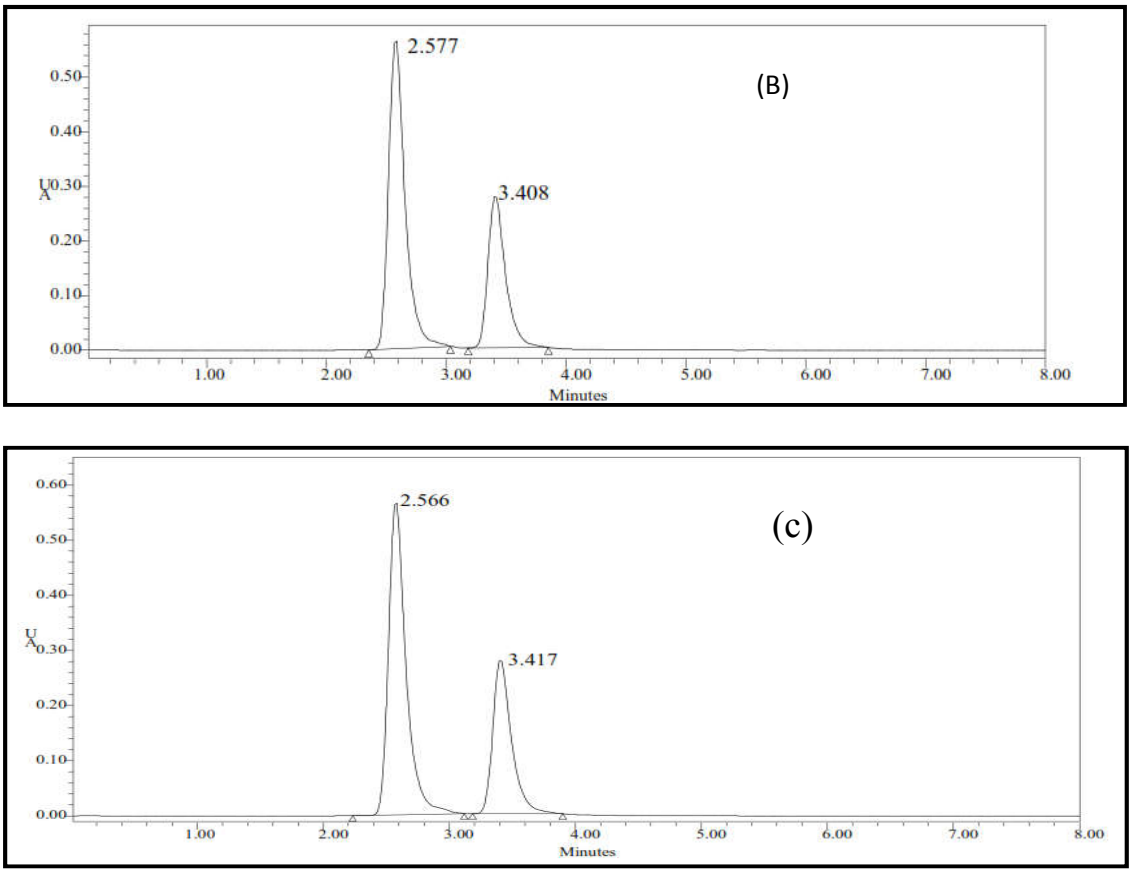


Figure No.1: Optimized Final Chromatogram in mobile phase

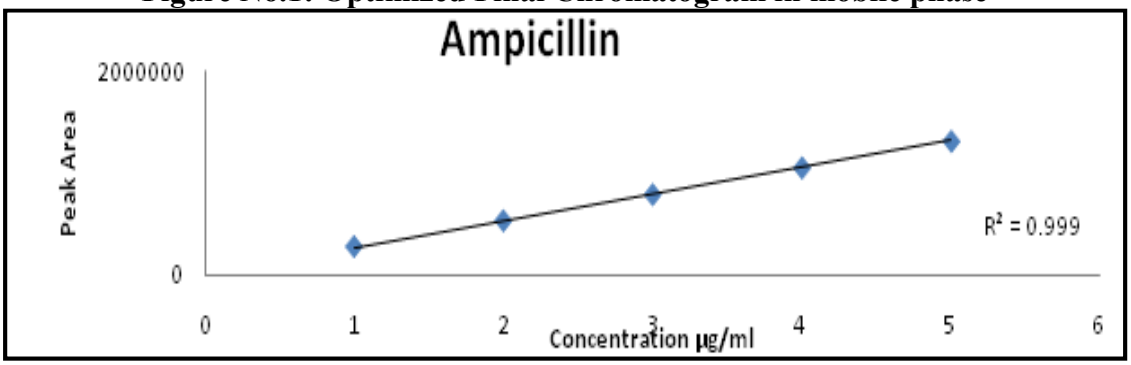


Figure No.2: Showing calibration graph for Ampicillin

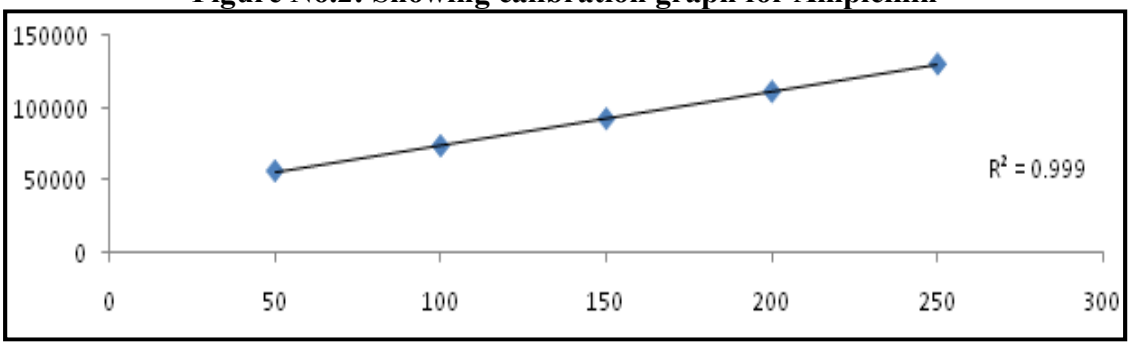


Figure No.3: Showing calibration graph for cloxacillin

SUMMARY AND CONCLUSION

A simple and robust method was established for simultaneous estimation of Cloxacillin and Ampicillin by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Cloxacillin and Ampicillin by using stationary phase Phenomenex Luna 5µ C18 (250*4.6mm) and mobile phase ratio is Acetonitrile: Methanol: 0.01% 1-Heptane Sulfonic Acid Sodium Salt Buffer (Buffer pH 3.0 was adjusted with OPA) (50: 20:30 % v/v/v). For detection carried out with UV detector at 252nm with ambient temperature and flow rate of 1.0ml/min. The overall run time is 8.0 minutes. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Cloxacillin and Ampicillin was found to be 101.27% and 99.97% respectively. The system suitability parameters for Cloxacillin and Ampicillin such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines [ICH, Q2 (R1)]. The linearity study Cloxacillin and Ampicillin was found in concentration range of 5.0 µg-25µg and 10µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 98.47% and 98.53%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.65 and 0.36 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68 and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Cloxacillin and Ampicillin in API and Pharmaceutical dosage form.

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CONFLICT OF INTEREST

The entire author's declared as no conflict of interests.

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