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Analytical method development and validation for amoxicillin capsules

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Abstract
Aim: Aim of the study was to establish analytical method development and validation for Amoxicillin capsules according to ICH guidelines. The chromatographic separation was achieved by using Syncrosis C8 (100x4.6mm, 3µ) column by applying isocratic elution using the mobile phase consist of 0.1% formic acid in water and acetonitrile (90:10), detection was carried at the 230nm by employing 0.8ml/min flow rate. The retention time was found to be 2.897 min, 2.808min for generic samples and 2.897 min, 2.798 min for branded samples respectively. For the developed method linearity range was obtained by UPLC method in between 10-1000µg/ml. Robustness, accuracy and precision were evaluated for the developed method and results were found to be within the limit and the result were reproducible. Based on these results, it was concluded that developed method was precise and accurate for the analytical method development and validation for Amoxicillin capsules. This method can be helpful for *in-vitro* studies in future days.

Keywords: amoxicillin, validation, ICH guidelines, UPLC

Introduction

UPLC is a modern technique which gives a new direction for liquid chromatography. UPLC refers to ultra-performance liquid chromatography, which enhance mainly in three areas: "speed, resolution and sensitivity. In twenty first centenary pharmaceutical industries are focusing for new ways to in economy and shorten time for development of drugs In UPLC main advantage is better efficiency with speedy analysis and this achieved by only smaller particle size.

UPLC analysis improves in three areas of

1. Produced Chromatogram with resolved peak.
2. Fast analysis
3. Sensitive analysis it uses fine particles and saves time and reduces solvent consumption.

Instrumentation

- Sample Injection
- UPLC Columns
- Detector

Amoxicillin

Amoxicillin is one of the most commonly used antibiotics in the primary care setting. It is an amino-penicillin, created by adding an extra amino group to penicillin to battle antibiotic resistance. Amoxicillin covers a wide variety of gram-positive bacteria, with some added gram-negative coverage compared to penicillin.

Mechanism of action

Amoxicillin is in the class of beta-lactam antimicrobials. Beta-lactams act by binding to penicillin-binding proteins that inhibit a process called transpeptidation (the cross-linking process in cell wall synthesis), leading to activation of autolytic enzymes in the bacterial cell wall. This process leads to lysis of the cell wall, thus destroying the bacterial cell. This type of activity is referred to as bactericidal killing, Amoxicillin administration can also be in combination with a beta-lactamase inhibitor. Some examples of these are clavulanic acid and sulbactam. These beta-lactamase inhibitors work by binding irreversibly to the catalytic site of an organism's beta-lactamase enzyme, which causes resistance to the original beta-lactam ring of amoxicillin. These drugs do not have inherent bactericidal activity; however, they may broaden amoxicillin's spectrum to organisms that produce the beta-lactamase enzyme when combined with amoxicillin

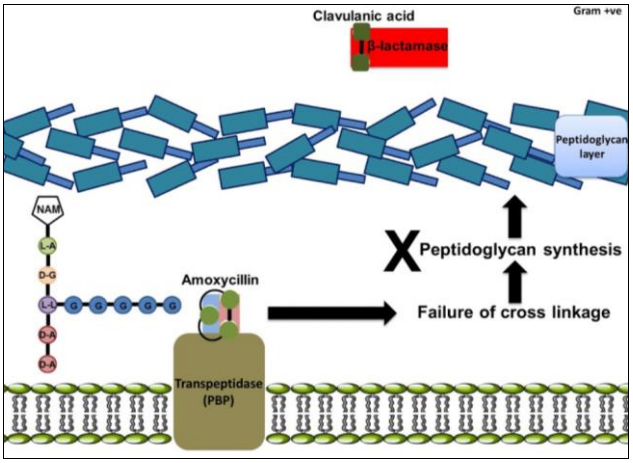

Fig 1: Mechanism of action of amoxicillin

Validation

It is a process involving confirmation by laboratory studies that a method, developed, system, analysis give accurate and reproducible results intended for analytical application in a proven and established range.

To establish that performance characteristics of the method (accuracy, prediction, sensitivity, ruggedness etc) meet the requirements of intended analytical application. Process of providing documented evidence that the system or the procedure does what it is intended to do precisely and reliably.

Linearity and range

Linearity: Ability of the method to elicit test results that are directly proportional to the concentration of analyte.

Range: Lowest and highest level of analyte that the method can determine with reasonable accuracy and precision in the range of 80/100/120% of the claim.

Methodology

Preparation of mobile phase

0.1% formic acid in water and acetonitrile were mixed in the proportion of 90:10 v/v considered as mobile phase. It was then filtered through 0.45µm membrane filter. Finally, the mobile phase was sonicated for 20 min to degas it.

Preparations of standard solutions

Weighed 10 capsules and average weight was calculated. Approximately 100mg of amoxicillin powder was taken from a marketed formulation and was dissolved in 100ml of HPLC grade water (1mg/ml), and sonicated for 20 mins and filtered through Whatmann's filter paper. From this stock solution series of dilutions were prepared by using HPLC grade waterasdiluent. the various dilutions were 10µg/ml,20µg/ml,50µg/ml,100µg/ml,200µg/ml,500µg/ml,1000µg/ml.

Preparation of sample solutions

Weighed 10 capsules and average weight was calculated. Approximately 100mg of amoxicillin powder were taken from generic and branded formulation and were dissolved in 100ml of HPLC grade water (1mg/ml), and sonicated for 20 mins and filtered through Whatmann's filter paper. From this solution a dilution of 200µg/ml was prepared by using HPLC grade water.

Results and discussion

Analytical method development and validation

Development and optimization of chromatographic condition

The Amoxicillin Samples were eluted on a C 8column using Mobile phase 0.1% formic acid in water and acetonitrile in the ratio 90: 10 (v/v) at the flow rate 0.8ml/min. The eluents were monitored at 230 nm, the retention time was found to be 2.897 min, 2.808min for generic samples and 2.897 min, 2.798 min for branded samples respectively showed in the below

Table 1: Standard Chromatographic Conditions for amoxicillin

Instrument	Waters (acquity UPLC)
Column	C8 Syncrosis (100x4.6mm, 3µ)
Mobile phase	0.1% formic acid in water and acetonitrile
Wavelength	230nm
Flow rate	0.8ml/min
Retention time	2.897 min, 2.808min for generic samples and 2.897 min, 2.798 min for branded samples
Run Time	10 min

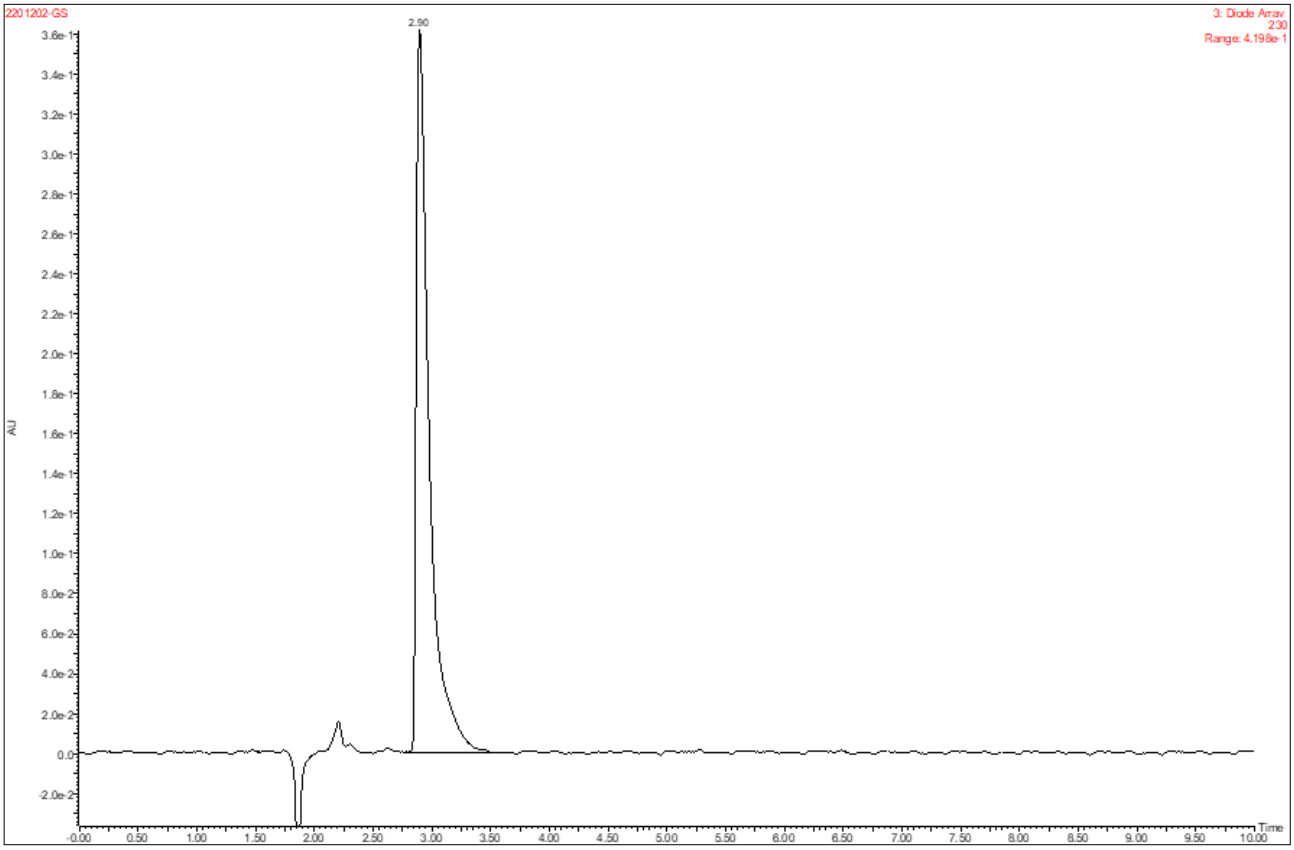

Fig 2: Chromatogram of GENERIC SAMPLE 200) 1µg/ml)

Time	Height	Area	Area%
2.897	361075	48254.238	100.00

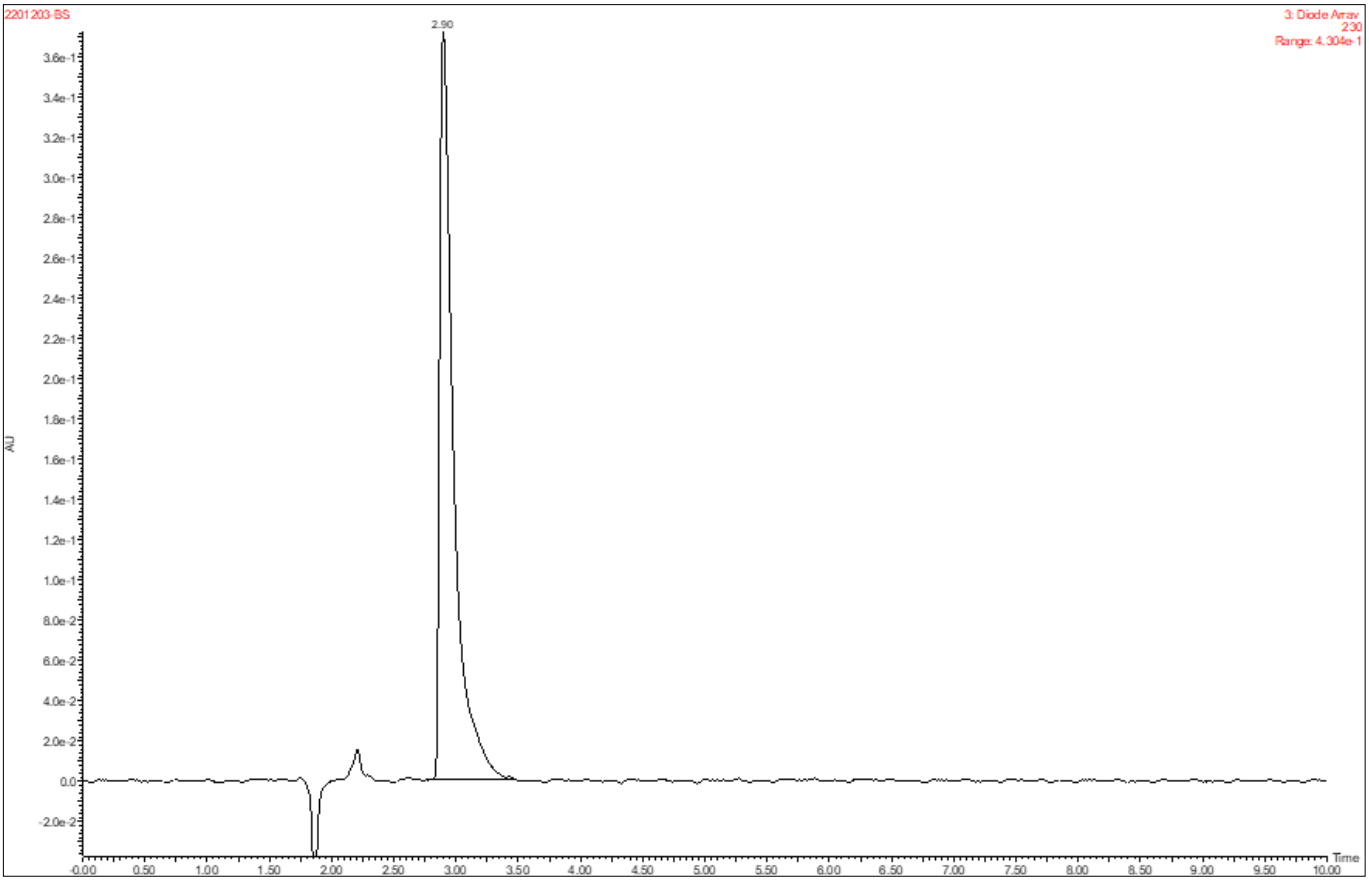

Fig 3: Chromatogram of BRANDED SAMPLE 200) 1µg/ml)

Time	Height	Area	Area%
2.897	371936	48557.770	100.00

Table 2: Peak area and concentrations of generic and branded samples

Samples		
Name	**Area**	**Conc**
GS	48254.24	190.98
BS	48557.77	192.15
GS-2	48419.62	191.62
BS-2	48922.16	193.56

Validation of developed UPLC method according to ICH guidelines

Linearity and Calibration Curve

Form the standard stock solutions, suitably mixed standard solutions were prepared to contain 10µg/ml1000-µg/ml AMOXICILLIN. The solutions were injected into the chromatographic system and peak area of each peak at each concentration was noted. The calibration curve was plotted using peak area versus concentration of the standard solution. The linearity was tested in the concentration range of 10-1000µg/ml and the calibration curve constructed was evaluated by its correlation coefficient. The correlation coefficient (r2) for all the calibration curves was consistently greater than 0.999 represented in graph.

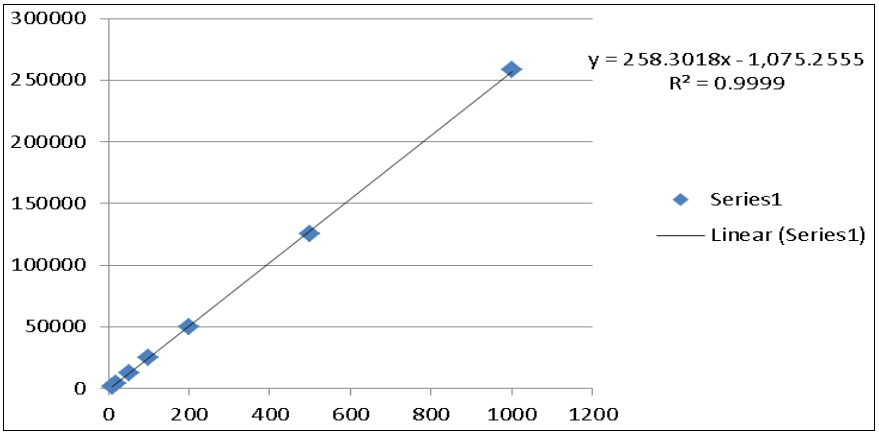

Fig 4: Standard Calibration Graph of Amoxicillin.

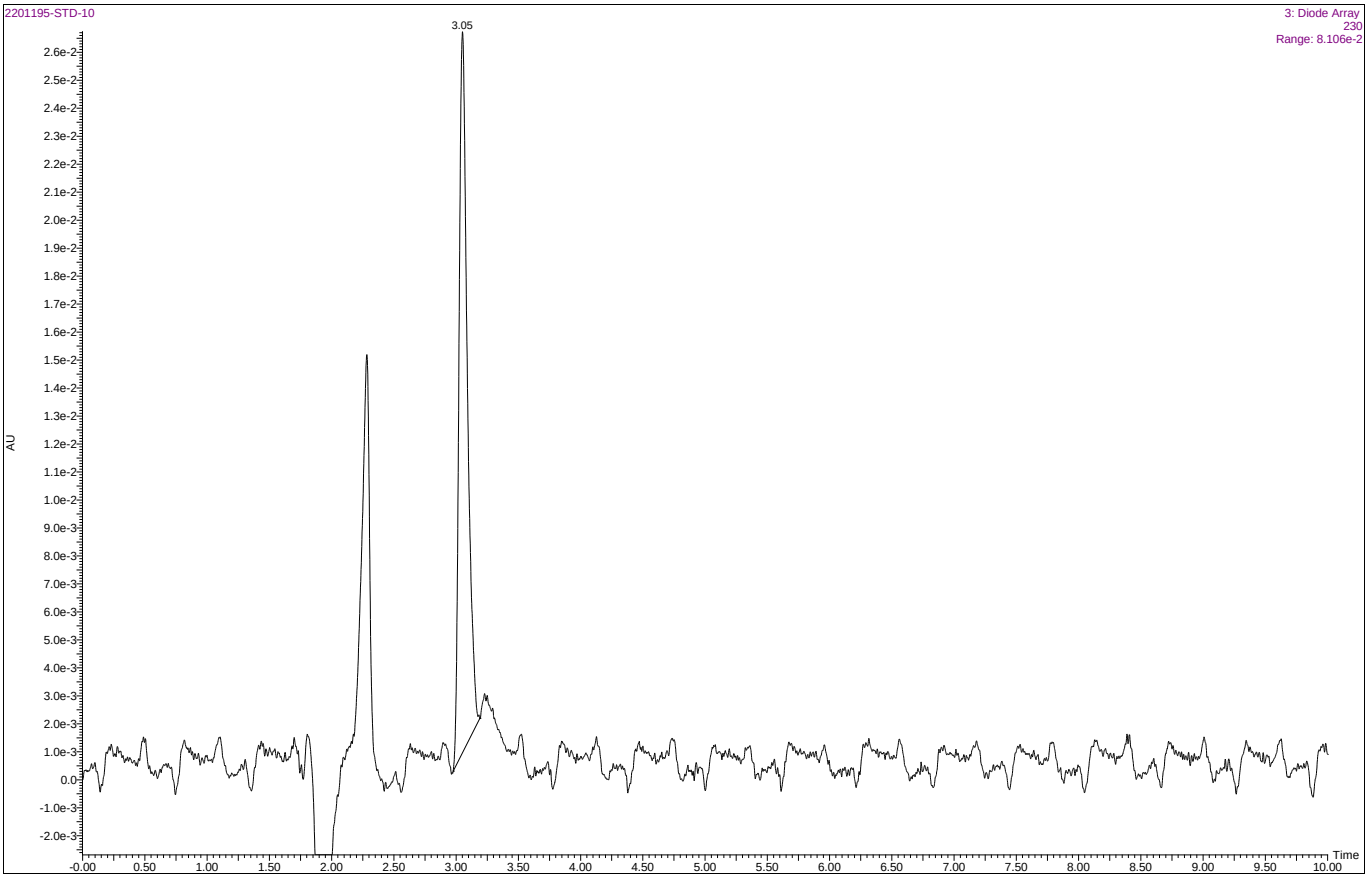

Fig 5: Chromatogram of standard drug (10µg/ml)

Time	Height	Area	Area%
3.052	25760	2002.681	100.00

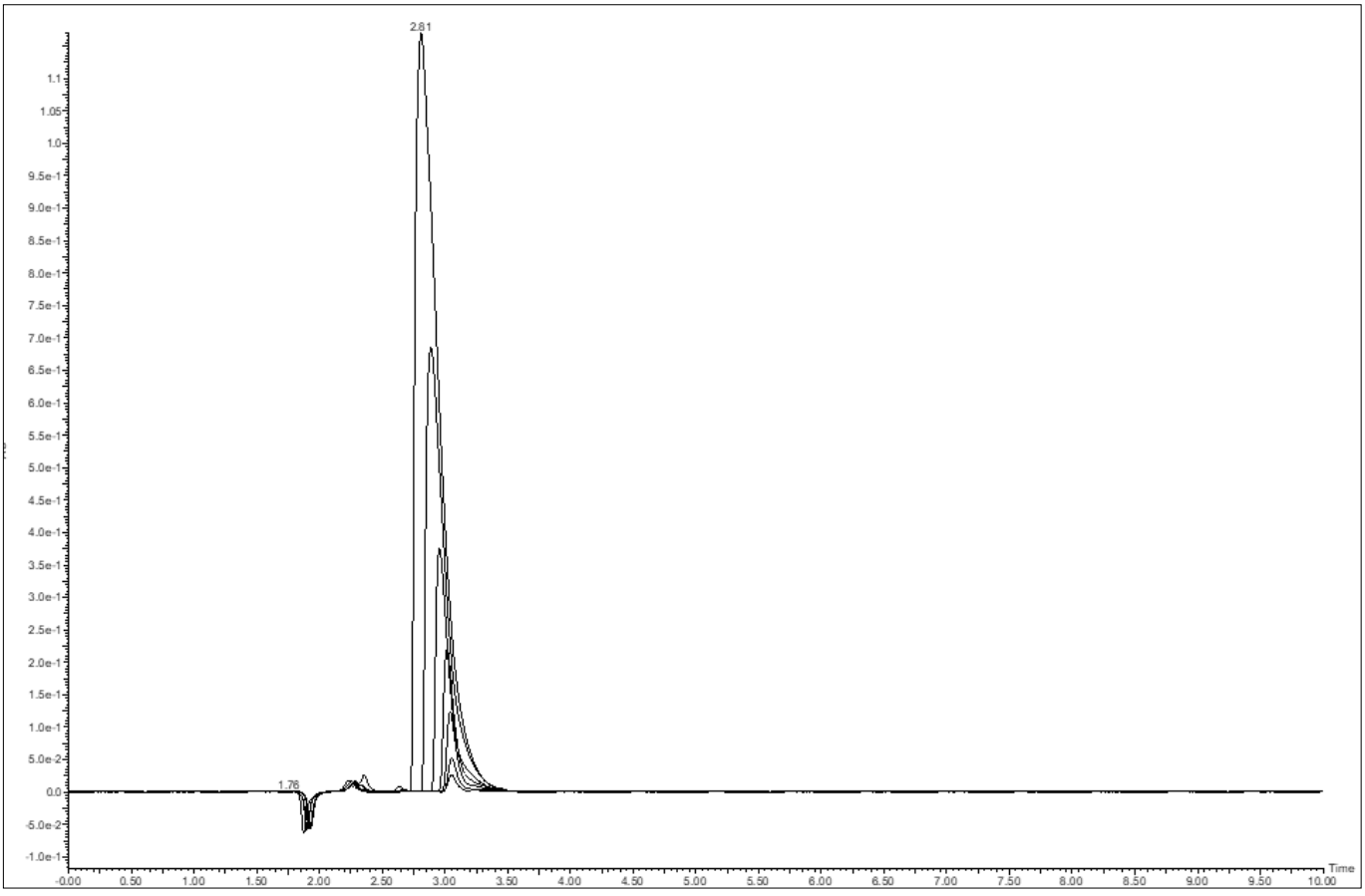

Fig 6: Overlay of Standard Solutions

Table 3: Standard linearity

Standard Linearity	
Conc	**Area**
10	2002.681
20	4299.524
50	12395.118
100	24950.512
200	50299.945
500	125735.688
1000	258397.063

LOD and LOQ

The LOD and LOQ were determined based on the signal to noise ratio of 3.3 and 10, respectively. The standard deviation (σ) was found to be 593.9228, and the slope (S) of the calibration curve (obtained from the linearity) was observed to be 258.3018 herefore, the LOD was calculated as 7.58µg/mL, and the LOQ was 22.99µg/mL.

Accuracy

Table 4: Accuracy data for AMOXICILLIN by UPLC method (% recovery data)

Sl. No	% of drug added	Amount of drug taken (µg /ml) (STD)	Amount of drug added (µg /ml) (sample)	Total amount of drug(n=3)	Total amount of drug found	% Recovery	Mean	% SD	% RSD
1.	50%	50	25	75	75.0	100	99.86	0.1154	0.1155
					74.9	99.8			
					74.9	99.8			
2.	100%	50	50	100	100.1	100.2	99.86	0.3055	0.3059
					99.8	99.6			
					99.9	99.8			
3.	150%	50	75	125	125.0	100	99.93	0.1154	0.1154
					125..0	100			
					124.9	99.8			

STD- Standard, SD- Standard Deviation, RSD- Relative Standard Deviation

Precision

Table 5: Precision Data of AMOXICILLINby UPLCMethod

Sl. No	Conc. In µg /ml	Peak area	Conc. Obtained in µg/ml	Average	STD DEV	% CV
1.	50	11839.834	50	49.96	0.1527	0.305
		11865.6646	50.1			
		11788.1746	49.8			
2.	100	24950.512	100.75	100.35	0.35	0.348
		24806.584	100.2			
		24780.754	100.1			
3.	200	50299.945	198.89	199.49	0.6050	0.303
		50455.953	199.5			
		50610.935	200.1			

STD- Standard, SD- Standard Deviation, RSD- Relative Standard Deviation % CV coefficient of variation

Robustness

The robustness for this method was successfully validated by adjusting the pH of the mobile phase. The results are shown in Figure 16, 17.

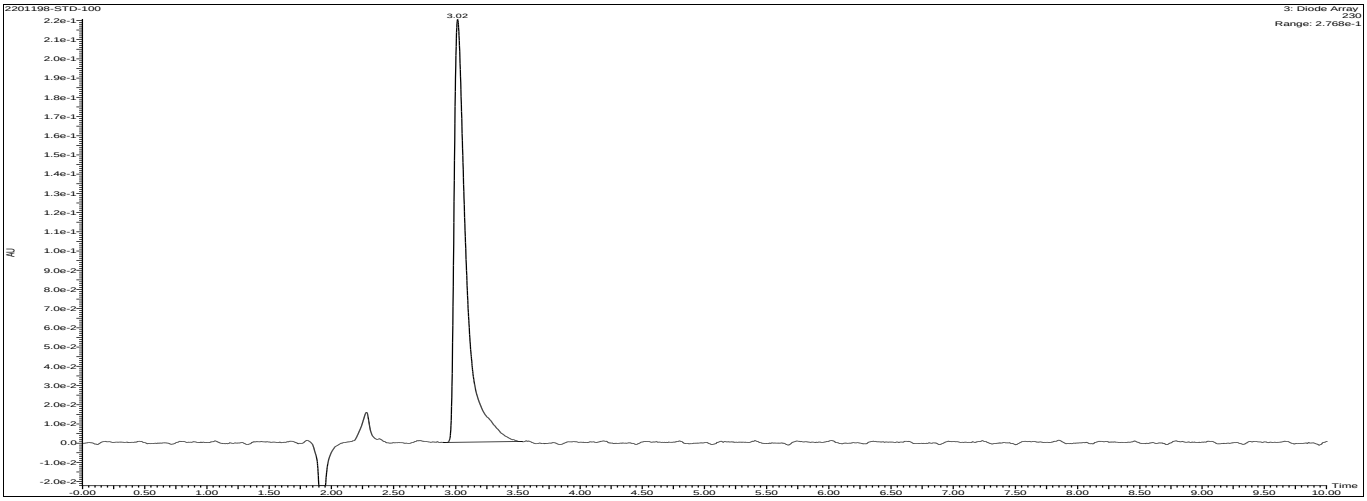

Fig 5: Robustness for Mobile phase - A: 0.05% Formic acid in Water

Time	Height	Area	Area%
3.015	220088	24950.512	100.00

Conclusion

A simple, precise and accurate analytical method for generic and branded formulations of amoxicillin has been developed and validated in compliance with ICH guidelines. Amoxicillin with its comparable clinical efficacy to other antibacterial and favorable dosage, pharmacokinetic profile and tolerability is an excellent candidate to treat various infectious diseases. As it is less effective against gram negative organisms and bacterial resistance develop to the drug candidate, it is the one area where the major development is required. Sensitive, specific and reproducible analytical method was developed & validated.

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