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EVALUATION AND VALIDATION OF A UPLC METHOD FOR ESTIMATION OF AMOXYCLAV IN ORAL DOSAGE FORM

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ABSTRACT

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of amoxicillin and potassium clavulanate in market dosage form. The method employed, with Xterra RP-8 (150mm x 4.6 mm i.d., particle size 5 µm) in a gradient mode, with mobile phase of KH2PO4: Methanol (80:20). The flow rate was 0.5 ml/min and effluent was monitored at 248 nm. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 250-750 µg/ml amoxicillin and 62.5-187.5 µg/ml for potassium clavulanate respectively. The LOD and LOQ values for were found to be 0.0029 (µg/ml) and 0.0091 (µg/ml) for amoxicillin and 0.0052 (µg/ml) and 0.0160 (µg/ml) for potassium clavulanate respectively. The developed & validated RP-UPLC methods employed here proved to be specific, fast, precise and accurate for the simultaneous estimation and stability indicating assays as well as related substance quantifications of AMOXYCLAV in combine dosage form.

KEYWORDS: Amoxyclav, oral dosage form, UPLC method.

INTRODUCTION

Amoxicillin (AMX), (2*S*,5*R*,6*R*)-6-{[(2*R*)-2-amino- 2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7- oxo-4-thia-1-azabicyclo[3.2.0]heptane-24-carboxylic acid, is a β-lactam semisynthetic penicillin from the aminopenicillin class with a broad antibacterial spectrum, used to treat a large number of infections with susceptible Gram-positive and Gram-negative bacteria. It is one of the most frequently prescribed penicillin derivatives within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics (Block, Beale, 2011).

AMX is susceptible to degradation by β-lactamase producing bacteria, which are resistant to a narrow spectrum of β-lactam antibiotics, such as natural penicillins. For this reason, it is often combined with clavulanic acid, a β-lactamase inhibitor.

Clavulanic acid (C L A), (2*R*, 5*R*,*S*) - 3 - (2 - hydroxyethylidene)-7-oxo-4-oxa-1-aza-bicyclo[3.2.0] heptane-2-carboxylic acid, is an oxapenam derivative lacking the 6-acylamino side chain characteristic for penicillin derivatives, which exhibits very weak antibacterial activity, and, therefore, is not useful as an antibiotic. It is used combined with penicillin group antibiotics to overcome resistance to bacteria that secrete β-lactamase. CLA can be described as a "suicide inhibitor", covalently bonding to a serine residue in the active site of the β-lactamase (Block, Beale, 2011).

Combining these two drugs increases effectiveness by reducing susceptibility to β-lactamase resistance. Combinations of AMX trihydrate and the potassium salt of CLA are available in various fixed-doses of oral and injectable dosage forms intended for the treatment of skin, respiratory, ear, and urinary tract infections caused by β-lactamase producing bacterial strains (Block, Beale, 2011; Todd, Benfield, 1990).

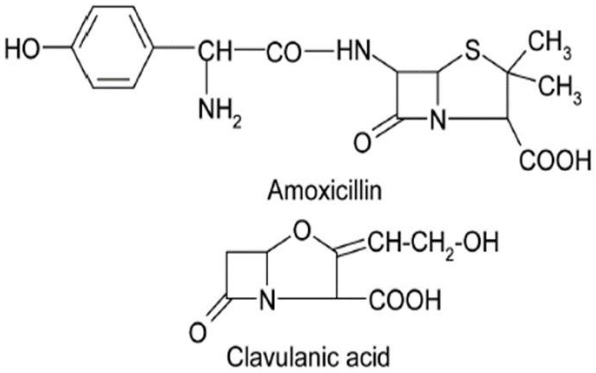


Fig. 1: Chemical structure of amoxicillin and potassium clavulanate.

Validation of Analytical Methods (USP/ICH)

Method validation, according to the United States Pharmacopeia (USP), is performed to ensure that an analytical methodology is accurate, specific, reproducible, and rugged over the specified range that an analyte will be analyzed. Regulated laboratories must perform method validation in order to be in compliance with FDA regulations. In a 1987 guideline (Guideline for Submitting Samples and Analytical Data for Methods Validation), the FDA designated the specifications in the current edition of the USP as those legally recognized when determining compliance with the Federal Food, Drug and Cosmetic Act can be referred to as the "eight steps of method validation".

Experimental

Materials

EQUIPMENTS	SOURCE
Ultra Pressure Liquid Chromatography (UPLC)	Acquity UPLC Systems, Waters Laboratories
Chromatographic data software	Empower
Column	Xterra RP-8 (150mm x 4.6 mm i.d., particle size 5 µm)
Detector	PDA
Injector	Automated
Electronic Balance	Eagle
Sonicator	Band Line Sonerex
p^H Meter	Lab India p^H meter

METHODOLOGY

Method Validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. The described method extensively validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

RESULTS

Preparation of Standard Stock Solution

The pure drug of Amoxicillin trihydrate and Potassium Clavulanate were injected into the UPLC system and run in different solvent systems. Different mobile phases like acetonitrile and water; methanol and water; methanol and buffer were tried in order to find the best conditions for the separation of Amoxicillin trihydrate and Potassium Clavulanate. It was found that Methanol and Potassium dihydrogen phosphate gives satisfactory results as compared to other mobile phases. This mobile phase system was tried with different proportions and using different flow rates. A mixture of Buffer and Methanol in the ratio of 80:20 was prepared and pH = 3.0 was maintained, at which they showed better separation. Hence 80:20 v/v ratio of mobile phase was considered to be the optimal composition and pH of mobile phase is maintained to 3.0.

1. Preparation of mobile phase:

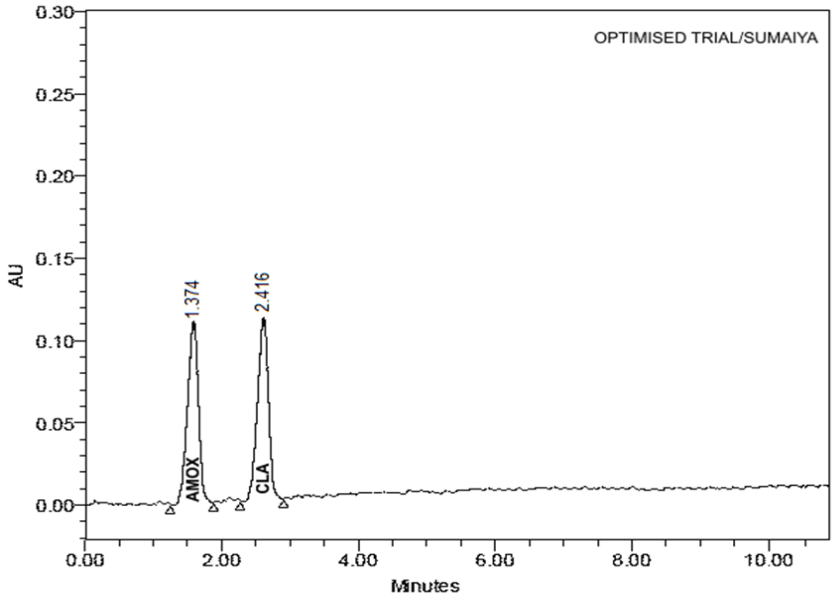
Mobile phase: KH2PO4: Methanol (80:20).

2. Preparation of standard stock solution

A standard stock solution was prepared by accurately weighing about 100 mg of Amoxicillin trihydrate and 25 mg of Potassium Clavulanate standard and is transferred into 20 ml volumetric flask; add 5ml of methanol and sonicate for 10min, make up the volume to 20 with methanol (stock solution-I). From this, transfer 5ml of above solution to 50ml volumetric flask and make up the volume with methanol labeled as stock solution-II.

A. Analysis of the marketed formulation.

Twenty tablets (AMOXYCLAV) were weighed and crushed to fine powder. The tablet powder equivalent to 50 mg of Amoxicillin trihydrate and 12.5 mg of Potassium Clavulanate was transferred to a 10 ml volumetric flask and dissolved in 5ml of methanol. The suspension was sonicated for 15minutes. Finally, the volume was made up to the mark with methanol. The solution was filtered through 0.4 µm membrane filter paper. Transfer 5ml of the above solution into 50ml volumetric flask and make up the volume with methanol. The clear solution obtained was diluted with the same solvent systems as used for calibration graphs to give a final concentration within the range of linearity. The solution was analyzed for the estimation of drug by proposed method.



Chromatogram of standard preparation of AMOXYCLAV (KH2PO4: Methanol (80:20))

Accuracy study

Recovery level	Set No.	Amoxicillin		Clavulanate	
		Wt. Taken (µg/ml)	Amount found (µg/ml)	Wt. Taken (µg/ml)	Amount Found (µg/ml)
50%	**Set 1**	5.27	5.22	1.25	1.24
	Set 2	5.26	5.21	1.24	1.23
	Set 3	5.27	5.21	1.25	1.24
100%	**Set 1**	10.02	9.87	2.5	2.48
	Set 2	10.01	9.85	2.4	2.47
	Set 3	10.02	9.86	2.5	2.47
150%	**Set 1**	15.12	14.90	3.78	3.76
	Set 2	15.12	14.89	3.78	3.75
	Set 3	15.11	14.89	3.76	3.74

System Precision

Procedure

"The parameters, retention time (RT), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated at a concentration of 10 µg/mL (*AMOXYCLAV*)."

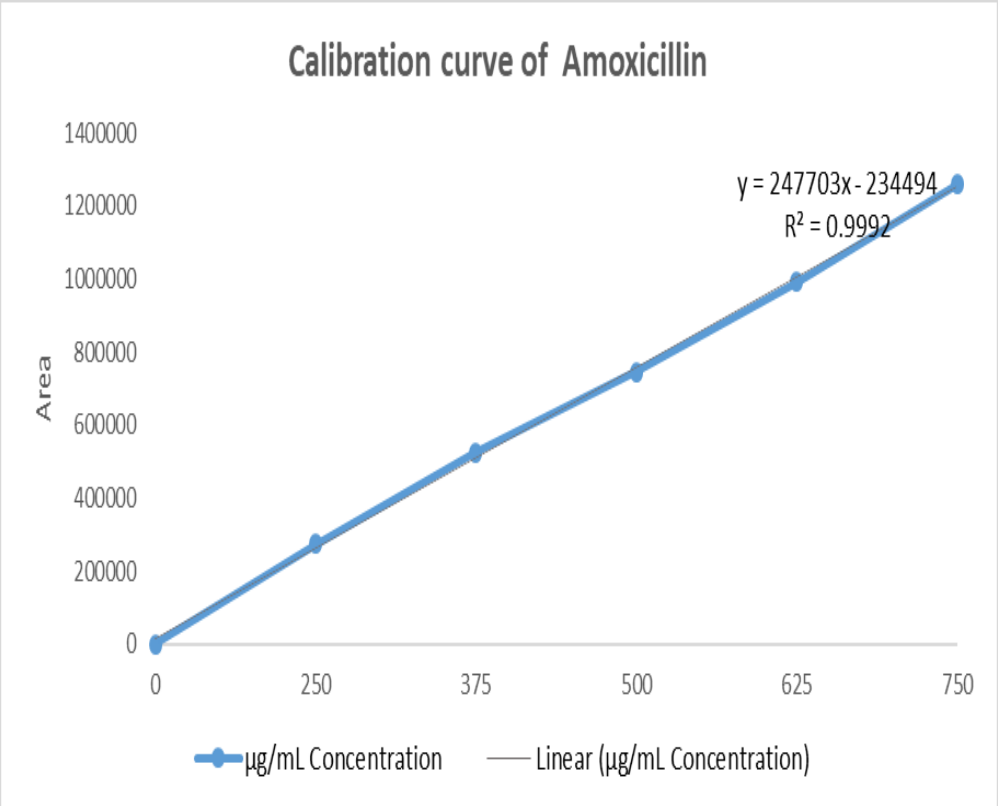
Parameters	Amoxicillin	Potassium Clavulanate
Retention time (min) ± % RSD	1.315 ± 0.13	2.493 ± 0.19
Theoretical plates ± % RSD	5827.36 ± 0.50	5958.87 ± 0.50
Asymmetry ± % RSD	1.08 ± 0.05	1.25 ± 0.05
Repeatability (% RSD)	0.02%	0.02%

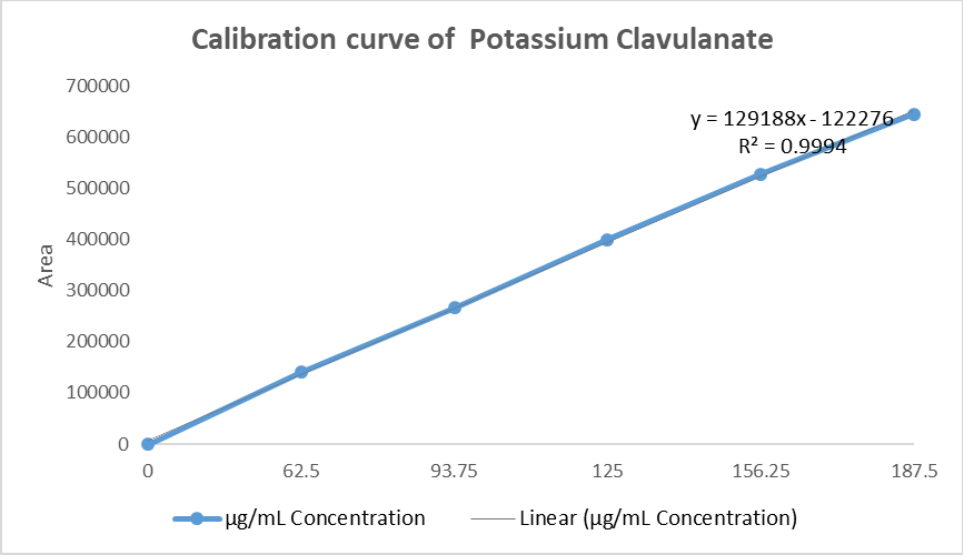
Precision

Replicate		***AMOXYCLAV***
S.No.	**Injection volume (µl)**	**Area**
1	10 ul	13,34,544
2		13,34,305
3		13,34,303
4		13,34,416
5		13,34,328
6		13,33,984
Average	13,34,313	
Std.Dev	98.89	
% RSD	0.02%	
Standard weight	625 mg	
Standard potency	99.98%	

Linearity

Linearity level	***Amoxicillin***	***Potassium Clavulanate***	
Level	**Concentration (µg/ml)**	**Concentration (µg/ml)**	**Area**
1	250	62.5	4,13,908
2	375	93.75	7,90,862
3	500	125	11,45,818
4	625	156.25	15,19,772
5	750	187.5	19,03,726
Correlation co-efficient	0.9992	0.9994	
Slope	247703	129188	
Intercept	234494	122276	

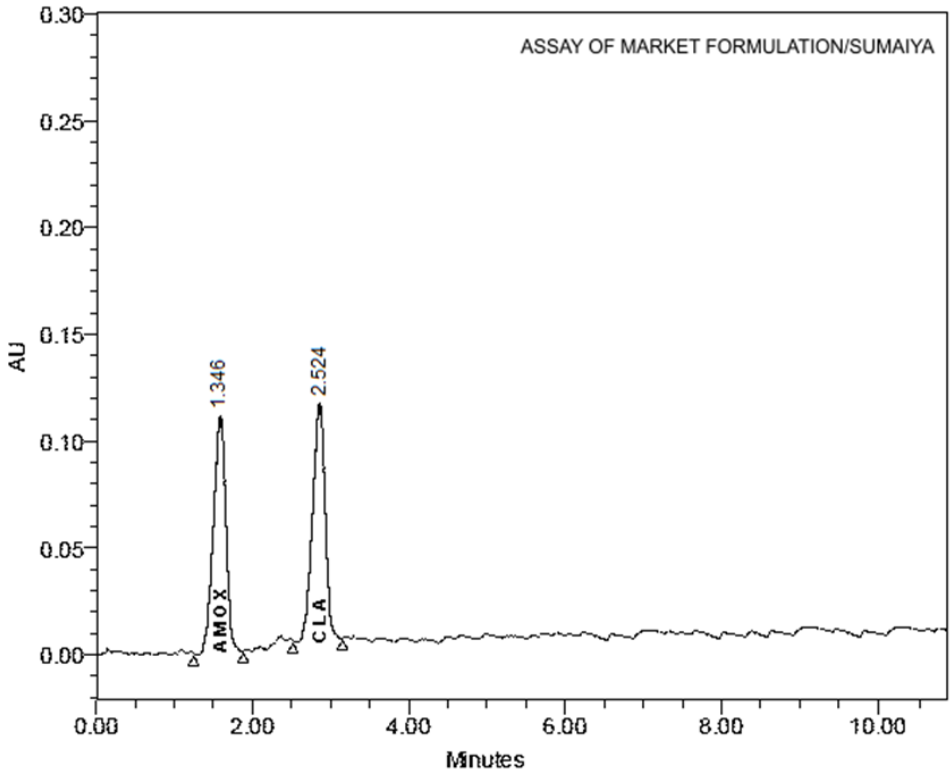




Evaluation of Method

Assay Studies

Assay studies for the analysis of formulation of Amoxicillin and Potassium Clavulanate. Fixed chromatographic conditions were made use for the analysis of formulation.



Calculation formula for AMOXYCLAV 625MG TAB

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

Whereas,"
"AT = Average area of test preparation, 1332097"
"AS = Average area of standard preparation, 1344160"
"W1 = Weight taken of reference standard (µg), 12.5"
"W2 = Weight taken of test sample (µg), 12.5"
"AW = Average weight (mg), 626.65"
"LC = Label claim (mg), 625"
"P = Potency of reference standard (%), 99.98%"

AMOXYCLAV 625MG TAB

$$\%\ Assay = \frac{1332097}{1344160} \times \frac{12.5}{100} \times \frac{1}{25} \times \frac{100}{12.5} \times \frac{25}{1} \times \frac{625.65}{625} \times 99.98$$

= 99.18%

CONCLUSION

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of AMOXYCLAV 625MG TAB in market dosage form. The method employed, with Xterra RP-8 (150mm x 4.6 mm i.d., particle size 5 µm) in a gradient mode, with mobile phase of KH2PO4: Methanol (80:20) and effluent was monitored at 248 nm. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 250-750 µg/ml amoxicillin and 62.5-187.5 µg/ml for potassium clavulanate respectively. The LOD and LOQ values for were found to be 0.0029 (µg/ml) and 0.0091 (µg/ml) for amoxicillin and 0.0052 (µg/ml) and 0.0160 (µg/ml) for potassium clavulanate respectively.

The method provides selective quantification of AMOXYCLAV 625MG TAB without interference from blank affirming precise method. The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters.

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