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Validated estimation procedure of Amoxycillin Trihydrate, Cloxacillin sodium in Pharmaceutical Formulation by RP-HPLC Method

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ABSTRACT

A rapid and sensitive high performance liquid chromatography method for determination of Amoxycillin Trihydrate and Cloxacillin sodium has been developed. The chromatography system used a reverse phase C_{18} Column with UV-Vis detector at 225nm. Mobile phase consisted of 0.02(M) potassium dihydrogen phosphate: Acetonitrile (80:20 v/v) (pH adjusted to 6.8 using 10% Sodium hydroxide) at a flow rate of 2.0 ml/min. The calibration curve was linear in the concentration range of 100mcg/ml to 5 mcg/ml for Amoxycillin Trihydrate, and 100mcg/ml to 5 mcg/ml for Cloxacillin sodium respectively.

Keywords: Amoxycillin Trihydrate, Cloxacillin sodium, HPLC analysis.

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INTRODUCTION

Amoxycillin Trihydrate is a broad spectrum antibiotic [1,2],chemically (2S,5R,6R)-6-{[(2R)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptanes -2- carboxylic acid [3],Which acts on both gram positive and gram-negative bacteria. Amoxicillin acts by inhibiting the synthesis of bacterial cell wall. It inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell walls of both Gram-positive and Gram negetive bacteria [4, 5].

Cloxacillin, chemically monosodium (2*S*, 5R, 6R)-6-{[3-(2-chlorophenyl)-5-methyl-oxazole-4-carbonyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylicacid is a semisynthetic antibiotic in the same class as penicillin [6]. Cloxacillin is a semisynthetic antibiotic in the same class as penicillin. Cloxacillin is used against staphylococci that produce b-lactamase. This drug has a weaker antibacterial activity than benzylpencillin, and is devoid of serious toxicity except for allergic reactions. It used against staphylococci that produce *beta*-lactamase. From literature survey it was found that various methods have been reported for both the drugs individually but no HPLC methods were reported for such a combination in any type of pharmaceutical dosage form so far [7,8]. The present work describes a simple, precise, and accurate reverse phase HPLC method for simultaneous estimation of amoxycillin and Cloxacillin in combination of dosage form.

The objective of the present work was to develop and validate the rapid and sensitive high-performance liquid chromatography (HPLC) method for estimation of Amoxycillin Trihydrate, and Cloxacillin sodium in capsules.

MATERIAL AND METHODS

Drugs used

Amoxycillin Trihydrate, and Cloxacillin sodium (In-house reference standards).

Chemicals and solvents

Potassium dihydrogen phosphate, Sodium Hydroxide, MilliQ water. All chemicals and solvents used were of AR/HPLC grade.

HPLC System

The HPLC system consisted of a solvent delivery module Agilent 1100 Series Isocratic pump equipped with 20 µl loop and G1365B Multi Wavelength Detector. Integration was achieved by using the software Chemstation. Separation was carried out on a Zorbax, (150×4.6mm) 5µm C-18 Column.

Chromatographic conditions

The mobile phase consisted of Potassium dihydrogen phosphate buffer and acetonitrile in (80:20)v/v ratio, pH adjusted to 6.8±0.2 using 10% NaOH. The solution was filtered through a Millipore 0.45 µm membrane filter and ultrasonically degassed prior to use. Throughout the experiment the Potassium dihydrogen phosphate Buffer pH 6.8 is used as diluent. The eluent was monitored with a UV-Vis detector set at 225 nm with a flow rate of 2ml/min under ambient condition.

Standard solution and calibration curve

A standard stock solution of Amoxycillin Trihydrate, (1000 µg/ml) and Cloxacillin sodium (500µg/ml) were prepared in Potassium dihydrogen phosphate Buffer pH 6.8. Subsequent dilutions were made in mobile phase to give the concentrations 100,80,50,40,25,10 and 5 µg/ml for Amoxycillin Trihydrate; 80,50,40,25,20,10 and 5 µg/ml for Cloxacillin sodium. The calibration curve was obtained by plotting the ratio of peak area of drugs versus concentration.

Assay

Net content for twenty capsules was weighted accurately by the electronic balance (Model Metler Toledo AG285). The powder equivalent to 50 mg Amoxycillin Trihydrate and 50 mg of Cloxacillin sodium was weighted accurately and dissolved in 100 ml diluent. The solution was filtered through 0.45 µm membrane filter paper to get a solution having a concentration of 0.5 mg/ml of Amoxycillin Trihydrate, and 0.5 mg/ml of Cloxacillin sodium. Reference standard 25 mg of Amoxycillin Trihydrate (moisture content 12.86 %w/w) and 25 mg of Cloxacillin sodium (moisture content 4.21% w/w) was weighted accurately and dissolved in 50ml diluent. Then 20 µl of sample and reference standard solution was injected in triplicate under the specified conditions. The peak areas obtained related to slopes and intercepts from the calibration data to calculate concentration of the drugs (Table 1).

Table -1. Results of HPLC assay

Amoxycillin Trihydrate		Cloxacillin sodium	
Amt. claimed (mg/tablet)	Amt. found (mg/tablet)	Amt. (mg/tablet) Claimed	Amt. found (mg/tablet)
250	249.97	250	250.55
	250.41		250.12
	249.87		250.06
Mean	250.0833		250.2433
RSD	0.0011		0.001

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Validation of the assay

To study the accuracy, reproducibility and precision, recovery experiments were carried out. The recovery of the added standard was studied at three levels. To an aliquot of the analyzed formulation a known concentration of standard solution was added. The content of Amoxycillin Trihydrate and Cloxacillin sodium was determined (table 2). The linearity of the standard curve was confirmed by plotting the peak area ratio of drug/RS. versus concentration. Linear regression analysis was performed to calculate the slope, the intercept and correlation coefficient (R^2) of the calibration curve (table 3).

Table 2. Results of recovery studies

	Amoxycillin Trihydrate			Cloxacillin sodium		
Amount added (mg)	5	10	15	5	10	15
Amount found (mg)	254.12	258.99	263.77	252.33	257.89	264.15
Percentage recovery	99.65	99.61	99.53	98.95	99.18	99.67
Mean	99.59			99.26		

Table 3. Linear regression data for calibration curve

Drugs	AmoxycillinTrihydrate	Cloxacillin sodium
Concentration range (mcg/ml)	100mcg/ml-5 mcg/ml	80 mcg/ml-5 mcg/ml
Slope	11.598	34.004
Intercept	-4.64	3.07
R^2	0.9998	1

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms of two drugs with internal standard. As per USP-XXIII system suitability tests were carried out on freshly prepared standard stock solutions of drugs (table4). The calibration curve was linear in the range of 100-5 µg/ml for AmoxycillinTrihydrate, 80-5 µg/ml for Cloxacillin sodium. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.2295 µg, 0.6950µg/ml for Amoxycillin Trihydrate; 0.0773 µg and 0.2343 µg/ml for Cloxacillin sodium.

Table 4. System suitability parameters

Parameters	AmoxycillinTrihydrate	Cloxacillin sodium
Calibration range (µg/ml)	100mcg/ml-5 mcg/ml	80mcg/ml-5 mcg/ml
Theoretical plates	2245	2546
Tailing factor	1	1
LOD	0.2295 mcg/ml	0.0773 mcg/ml
LOQ	0.6950 mcg/ml	0.2343 mcg/ml

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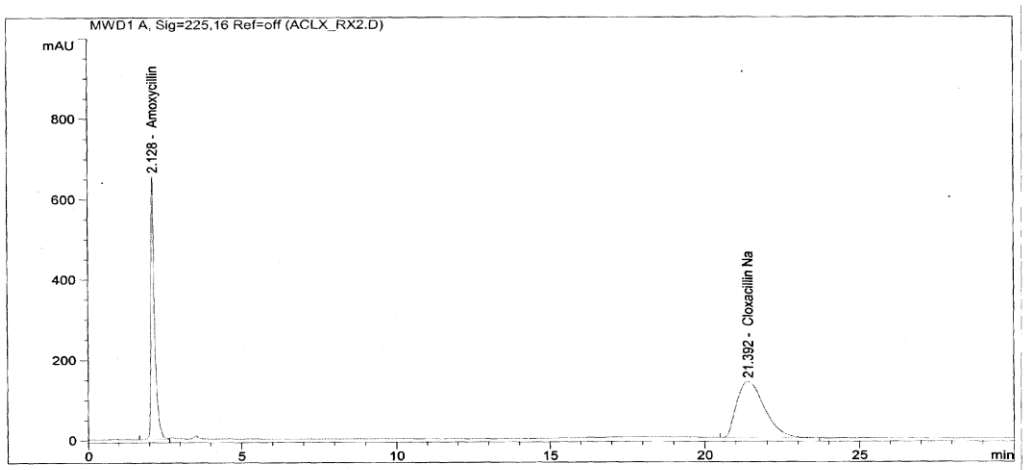

Area Percent Report

Sorted By : Signal
Calib. Data Modified : 4/8/2010 3:29:40 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=225,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	2.128	VV	0.1234	5577.11670	39.2683	Amoxycillin
2	21.392	PB	0.9838	8625.47461	60.7317	Cloxacillin Na
Totals :				1.42026e4		

Figure 1: Chromatogram of Amoxycillin and Cloxacillin Sodium

CONCLUSION

In conclusion our method is rapid, sensitive, reproducible and well suited to the simultaneous determination of Amoxycillin Trihydrate, Cloxacillin sodium and by reference standard method.

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