RJC
http://www.rasayanjournal.com

RASĀYAN *J. Chem.*
Vol.2, No.1 (2009), 57-60
ISSN: 0974-1496
CODEN: RJCABP

SIMULTANEOUS ESTIMATION OF AMOXICILLIN AND FLUCLOXACILLIN IN ITS COMBINED CAPSULE DOSAGE FORM BY HPLC

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ABSTRACT

A simple, precise, fast and accurate HPLC method has been developed for the simultaneous estimation Amoxicillin and Flucloxacillin in capsules. The analytes were resolved, by using a mobile phase mixture of buffer (prepared from 0.001m Diammonium hydrogen orthophosphate and 0.04m tetra butyl ammonium bromide pH adjusted to 7.0±0.1 with ortho phosphoric acid) and acetonitrile in the ratio (99:10v/v), on a strong cation exchange column, (LUNA SCX, 250mm x 4.6mm I.D. 5 µm particles).The retention times for amoxicillin and flucloxacillin were found to be 3.828 and 5.89, respectively. The validation of the method was performed, and specificity, precision, accuracy, linearity, range, robustness were confirmed.

Key words: HPLC, Amoxicillin, Flucloxacillin.

INTRODUCTION

Amoxycillin is a moderate-spectrum β-lactam antibiotic used to treat the bacterial infections. It is the usual drug of choice within the class because it is better absorbed, by oral administration. Amoxicillin is susceptible to degradation by β-lactamase producing bacteria, and so may be given with clavulanic acid to decrease its susceptibility. Flucloxacillin or floxacillin is a narrow spectrum β -lactam antibiotic of the penicillin class, used to treat infections caused by susceptible Gram-positive bacteria. It is active against β lactamase producing organisms such as Staphylococcus aureus, which would otherwise be resistant to most Penicillins[1]. The chemical name of Amoxicillin[2] is (2S, 5R, 6R) -6- [[(2R) -2 -amino-2-(4 hydroxyphenyl) acetyl] amino]-3, 3-dimethyl7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. And the chemical name of Flucloxacillin[2]is (2S,5R,6R,)-6-[[[3-(2-chloro-6-fluorophenyl)-5-methyl isoxazole -4- yl] carbonyl] amino] -3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane -2-carboxylate. Literature survey [3,4,5] indicates that some spectrophotometric methods have been reported for estimation of Amoxicillin and Flucloxacillin both individually and in combination with other drugs. And a HPLC method [6] was also reported for the estimation of two combination drugs in injection formulation, but with an acidic mobile phase. Since antibiotics are very sensitive and labile in nature, in the present investigation an attempt was made to develop a HPLC method for simultaneous estimation of the two compounds with a neutral mobile phase which will not interfere with the stability of the drug.

EXPERIMENTAL

Instrumentation

The chromatographic separation was performed on a Shimazdu liquid chromatographic system equipped with a LC-10ATV$_P$ solvent delivery system , SPD-10AV$_P$ Schimazdu UV-Vis detector, Rheodyne (7725i) injector , 20 µl external loop, and luna SCX strong cation exchange column, 250mm x 4.6mm as the stationary phase.

Chromatographic conditions

Column : LUNA, SCX, 250 x 4.6mm, 5µ.
Wavelength : 254nm
Temperature : Ambient
Flow rate : 1ml / min
Injection volume : 20 µl

Label claim

Amoxicillin trihydrate equivalent to Amoxicillin - 250mg
Flucloxacillin sodium equivalent to Flucloxacillin - 250mg.

Preparation of standard solution

About 57 mg of Amoxicillin trihydrate and 55mg of Flucloxacillin sodium were accurately weighed, transferred into a 100 ml volumetric flask, dissolved with 50 ml of diluent and made up to the mark with the diluent.

Preparation of sample solution

20 capsules content were weighed and powdered. About 50 mg equivalent weight of the sample was accurately weighed, transferred to a 100ml volumetric flask, dissolved with 50 ml of diluent, sonicated for 15 mins, made up to the mark with diluent and filtered through 0.45µ membrane before injection.

Diluent

Highly Purified water B.P. obtained form Milli Q, water filter.

Preparation of mobile phase

A mixture of buffer and acetonitrile mixed in the ratio of 90:10 (v/v) was used as the mobile phase.

Preparation of buffer

Weighed 1.32 gm Of Diammonium hydrogen ortho phosphate and 12.90 gm of tetra butyl ammonium bromide, dissolved in 1000 ml of water and pH adjusted to 7.0 ± 0.1 with ortho phosphoric acid.

Estimation method

The sample solution obtained from the formulation was injected and the chromatogram was developed. The amount of drug present is calculated by using the following formula.

$$\frac{T_a}{S_a} \times \frac{S_w}{T_w} \times D.F \times \frac{\text{Purity of standard}}{100} \times T_N = \text{Amount present}$$

Where, T_a = Peak area of sample solution, S_a = Peak area of standard solution, S_w = Standard weight, T_w = Test weight , D.F - Dilution factor, T_N – Average net content of capsule.

Validation of the method[7]

The developed method[7] was validated in terms of precision, accuracy, and linearity.

1.Precision

The precision of the test method is determined by carrying out six determinations at 100% of the text concentration from a homogenous sample and estimating the % RSD for the assay value, **Table-1.** And for system precision standard solutions prepared as per the method are injected 5 times in to the HPLC system and the % RSD for the peak areas is calculated, Table-2.

2. Accuracy

The accuracy of the method was studied by performing recovery studies at various concentrations. The standard drugs at the concentration level of 40%, 60% and 80% were added to the known amount of sample and the analysis was carried out as per the assay method. The results were expressed in terms of percentage recovery. The results are given in **Table-3.**

3. Linearity

Linearity is determined by the statistical method of regression analysis, with standard preparations in the range of 50% to 150% concentration. The response to Amoxicillin was linear over the range of 0.26 mg / ml to 0.78 mg/ ml and 0.25 mg/ml to 0.76 mg / ml for Flucloxacillin. The results are given in Table-4.

4. System Suitability

System suitability studies were carried out to determine the resolution factor, retention time (R_t), tailing factor, theoretical plates. The values obtained demonstrated the suitability of the system for the analysis of this drug combination. The system suitability was assessed by 6 replicate analysis of the standard solution prepared in the same concentration as that of the target assay method. The results are given in Table -5.

RESULTS AND DISCUSSION

The mobile phase mixture; buffer and acetonitrile in the ratio (90: 10% v/v), pH 7.0, gave a good resolution and sensitivity for Amoxicillin and Flucloxacillin. Under these conditions the analyte peaks were well defined and resolved. The elution order was Amoxicillin (t_1 = around 4 mins) and Flucloxacillin (t_2 = 6 around mins) at a flow rate of 1.0ml/min. The optimum wavelength of detection was 254 nm. The validation of the method was performed. The accuracy of the method was determined by recovery studies. From the data obtained, recoveries for the drugs were considered sufficiently accurate. The precision data shows that the repeatability of the assay procedure was satisfactory. The calibration curve shows linear response over the range of concentration used in the assay procedure.

Table-1: Method Precision

Parameter	Amoxcillin	Flucloxacillin
Amount present*	248.47	251.30
%R.S.D	0.68	0.66

*Mean value of six readings

Table-2: System Precision

Parameter	Amoxicillin	Flucloxacillin
Peak Area*	3053439	4735833
%R.S.D	0.06	0.12

*Mean value of five readings

Table-3: Recovery Studies

Drug	Amount of Drug added	Amount of Drug recovered	% Recovery
Amoxicillin	45.33 mg	45.58 mg*	100.55%*
	64.82 mg	64.52 mg*	99.54%*
	84.88 mg	83.85 mg*	98.79%*
Flucloxacillin	37.55 mg	37.38 mg*	99.55%*
	55.14 mg	54.71 mg*	99.22%*
	73.16 mg	72.50 mg*	99.10%*

*Mean value of three readings

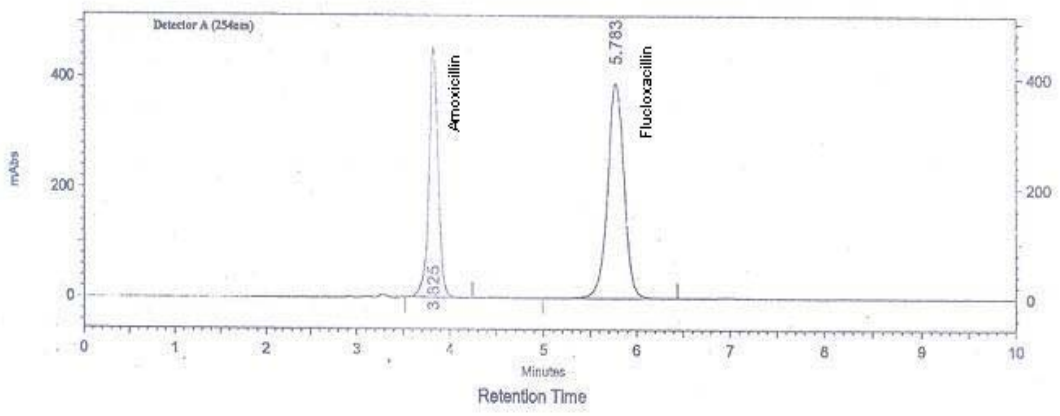
Table-4: Linearity

Parameter	Amoxicillin	Flucloxacillin
Concentration (of std)	0.26 to 0.78 mg/ml	0.25 to 0.76 mg/ml
Regression (R^2)	0.9998	0.9997
Slope (m)	31328.48	46097.18
Intercept (C)	123571.00	174637.60

Table-5: system Suitability

Parameters	**Amoxicillin**	**Flucloxacillin**
Tailing factor	0.98	0.97
Theoretical plates	7677	5200
% RSD for R_t	0.12	1.06
% RSD for Area	0.06	0.12
Resolution	8.22	

The robustness of the method showed that there were no marked changes in the chromatographic parameters which demonstrate that the method was robust. Further there were no interferences due to exciepients. The system suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry.



Chromatogram of mixed standard

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(Received: 15 October 2008 Accepted: 14 November 2008 RJC-259)

"A man's character may be learned from the adjectives which he habitually uses in conversation"

-Mark Twain