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RP-HPLC Method for Estimation of Flucloxacillin Magnesium and Sodium Benzoate in Oral Suspension

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A simple, sensitive and precise reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed for the estimation of flucloxacillin magnesium and sodium benzoate (preservative) in oral suspension. The quantification was carried out using a Kromasil 100, C-18 column, 150 mm × 4.6 mm i.d. and 5 µm particle size in gradient mode with mobile phase comprising pH 5.0 phosphate buffer and acetonitrile at a flow rate of 1.5 mL/min. The eluent was monitered at 225 nm. The retention time of the drug was 10.9 min and of the sodium benzoate (preservative) was 2.02 min. The calibration curve was linear in the concentration range of 10-150 µg/mL. The proposed method was statistically evaluated and can be applied for routine quality control analysis of flucloxacillin magnesium and sodium benzoate in oral suspension.

Key Words: Flucloxacillin magnesium, Sodium benzoate, RP-HPLC, Oral Suspension.

INTRODUCTION

Flucloxacillin[1] is chemically 6-((S)-3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-4-carboxamido)-3,3-dimethyl-7-oxo-4-thia-l-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is a narrow spectrum β-lactam antibiotic of penicillin class used to treat infections caused by susceptible Gram-positive bacteria notably against β-lactamase producing organisms such as *Staphylococcus aureus* which would otherwise be resistant to most penicillin's. Literature survey reveals that few HPLC[2-4] methods for estimation of flucloxacillin were reported. The proposed method is simple, fast, accurate and precise for estimation of flucloxacillin in oral suspension.

EXPERIMENTAL

An isocratic high performance liquid chromatograph (Waters 2695) variable wavelength programmable UV detector Waters 2695, EMPOWER software and RP C-18 column (150 mm × 4.6 mm i.d., 5 µm particle size) was used.

Chromatographic conditions: The chromatographic column used was a 150 mm × 4.6 mm i.d., Kromasil 100, C-18 column with 5 µm particles. The mobile phase consists of pH 5.0 phosphate buffer (prepared by dissolving 2.72 g of potassium dihydrogen phosphate in 1000 mL milli-Q water and adjusted the pH to 5.0 with

dilute sodium hydroxide solution) and acetonitrile. The mobile phase was filtered through 0.45 µm membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1.5 mL/min. The column was maintained at ambient temperature and the detection was carried out by UV detector at 225 nm. The injection volume was 10 µL.

Gradient programme:

Time (min)	pH 5.0 phosphate buffer (%)	Acetonitrile (%)
0.01	80	20
5	74	26
14	74	26
15	80	20
20	80	20

Procedure

Standard stock solution for flucloxacillin magnesium: About 55 mg of pure sample was weighed accurately and transferred to 50 mL volumetric flask and dissolved in 25 mL of diluent. The solution was sonicated for 5 min and then made up the volume to 50 mL with diluent (It is a mixture of pH 5.0 buffer and acetonitrile in the ratio 75:25).

Standard stock solution for sodium benzoate: About 50 mg of sodium benzoate was weighed accurately and transferred to 50 mL volumetric flask and dissolved in 25 mL of diluent. The solution was sonicated for 5 min and then made up the volume to 50 mL with diluent.

Standard solution for assay: From sodium benzoate standard stock solution, 5 mL was taken and diluted to 50 mL and from this again 10 mL was taken in 50 mL volumetric flask. To this 5 mL of standard stock solution of flucloxacillin magnesium was added to get 100 µg/mL. Subsequent dilutions of this solution ranging from 10-150 µg/mL were made in 10 mL volumetric flasks. The solutions were filtered through 0.45 µm membrane filter and then 10 µL of filtrate was injected each time into the column at flow rate of 1.5 mL/min. Evaluation of the drug was performed with UV detector at 225 nm. Peak area was recorded for all peaks. A plot of peak area *versus* the respective concentration gives the calibration curve. The regression of drug concentration over the peak area was computed. The regression equation was used to estimate the amount of flucloxacillin magnesium in oral suspension.

Estimation of flucloxacillin magnesium in oral suspension: 250 mg of flucloxacillin magnesium was accurately weighed and taken in 250 mL volumetric flask and made up to volume. From this 5 mL of the solution was taken and diluted to 50 mL with diluent and filtered through a 0.45 µm membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. The contents of the flask were made up to volume with diluent and mixed well. Each of the solutions (10 µL) was then injected 5 times into the column. From the peak areas, the drug content in oral suspension was quantified using the regression equation obtained from pure sample.

RESULTS AND DISCUSSION

A typical chromatogram of flucloxacillin magnesium and sodium benzoate was shown in Fig. 1. the retention time for flucloxacillin magnesium was 10.9 min and for sodium benzoate 2.02 min. The peak areas from such different concentrations set up above were calculated and are shown in Table-1. A good linear relationship (r = 0.9999) was observed between the concentration flucloxacillin magnesium and the respective peak area. The regression curve was constructed by linear regression fitting and its mathematical expression was y = 13780x - 5984.7 (where y is peak area and x is the concentration of flucloxacillin magnesium). The intra-day and inter-day variations of the method were determined using three replicate injections of four different concentrations, which were prepared and analyzed on the same day and three different days over a period of 2 weeks, a low coefficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise.

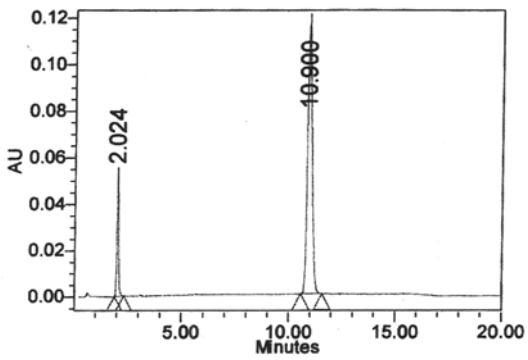

Fig. 1. Representative chromatogram of flucloxacillin magnesium and sodium benzoate

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Drug concentration (µg/mL)	Peak area	Drug concentration (µg/mL)	Peak area
10	135713	75	1035055
20	264096	100	1376014
30	397736	125	1715359
50	679890	150	2058827

Regression equation from 10-150 µg/mL
Y = 13780X – 5984.7 (r = 0.9999)

TABLE-2
PRECISION OF THE PROPOSED METHOD

Concentration of flucloxacillin (µg/mL)	Observed concentration of flucloxacillin magnesium (µg/mL)			
	Intra-day		Inter-day	
	Mean (n = 3)	CV (%)	Mean (n = 3)	CV (%)
10	10.01	0.670	9.98	0.652
20	20.05	0.435	20.03	0.576
30	30.02	0.321	30.01	0.246
50	49.99	0.167	49.99	0.099

To ensure reliability and accuracy of the method, recovery studies were carried out mixing a known quantity of drug with preanalyzed sample and the contents were reanalyzed by the proposed method. About 99.95 % of flucloxacillin magnesium could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

The drug content in oral suspensions was quantified using the proposed analytical method and the results are shown in Table-4. The oral suspensions were found to contain 99.99-100.03 % of the drug. It can be concluded that the proposed method was suitable for estimation of flucloxacilllin magnesium and sodium benzoate in routine quality control analysis.

TABLE-3
RESULTS OF THE RECOVERY STUDY

Amount of drug added (µg)	Recovery from drug solution		Recovery from suspension	
	Mean amount found (n=3)	Mean % recovery	Mean amount found (n=3)	Mean % recovery
10	10.06	100.60	10.05	100.50
20	19.99	99.95	20.05	100.25
30	29.99	99.97	30.02	100.06

TABLE-4
ASSAY OF FLUCLOXACILLIN MAGNESIUM IN ORAL SUSPENSION

S. No.	Labeled amount of drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n = 5)	Mean (±SD)% labeled amount (n=5)
Suspension-1	125	125.03 ± 0.084	100.03 ± 0.069
Suspension-2	125	125.01 ± 0.064	100.00 ± 0.048
Suspension-3	250	249.97 ± 0.058	99.99 ± 0.023
Suspension-4	250	250.01 ± 0.064	100.00 ± 0.026

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