WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.041
Volume 5, Issue 4, 499-508 Research Article ISSN 2278 – 4357

A SIMPLE VALIDATED RP- HPLC METHOD FOR THE ANALYSIS OF FLUCLOXACILLIN SODIUM IN CAPSULE DOSAGE FORM

Michael Worlako Klu*, Bright Selorm Addy and David Ntinagyei Mintah

Department of Pharmaceutical Sciences, School of Applied Sciences, Central University, P.O. Box 2305, Tema, Ghana.

Article Received on
10 Feb. 2016,
Revised on 01 March 2016,
Accepted on 22 March 2016
DOI: 10.20959/wjpps20164-6522

***Correspondence for Author**
Michael Worlako Klu
Department of Pharmaceutical Sciences, School of Applied Sciences, Central University, P.O. Box 2305, Tema, Ghana.

ABSTRACT

A simple, cheap, fast and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the assay of flucloxacillin sodium in capsule dosage form. The method was developed using a Phenomenex ® Bondclone 10 C18 column (300×3.9 mm, 5µm) and a mobile phase system made of 60% Methanol and 40% KH_2PO_4 buffer adjusted to a pH of 5 with 1M sodium hydroxide solution. The method was validated and it was found to be linear, selective, accurate, robust and precise. The limit of detection (LOD) and limit of quantification (LOQ) respectively were 0.00437% w/v and 0.0132% w/v. The method is suitable for routine assay of flucloxacillin sodium in capsule dosage form because it is simple, cheap and accurate.

KEYWORDS: Flucloxacillin sodium, RP- HPLC, assay.

INTRODUCTION

Flucloxacillin sodium is an isoxazolyl penicillin that has a bulky side chain to make it resistant to beta lactamases produced by bacteria that are killed by it.[1]

It can be used in the treatment of some skin infections like boils, meningitis among other bacterial infections. It causes muscle pains as one of its side effects and it is not used in persons who react to penicillins.[2,3]

Flucloxacillin, chemically known as (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, is normally synthesized for use pharmaceutically usually as the sodium salt and less so, the magnesium salt.[4, 5]

Several methods of analysis have been reported in literature for the determination of flucloxacillin sodium in formulations. Acid-base titrimetric methods are available for assaying flucloxacillin sodium as well as iodimetry which is also used successfully to assay flucloxacillin sodium.[6-8] These methods of titration are tedious and time consuming although they have the advantage of being cheap to use.

Ultraviolet-visible spectroscopic methods are also available for the assay of flucloxacillin. These employ the use of compounds like imidazole mercury reagent or 1,2,4-triazole with mercury (II) chloride for derivatizing flucloxacillin to make the analysis possible and accurate.[9,10] The derivatization compounds used are expensive and the procedure involves heating which can be time consuming.

In more recent times, most assays of flucloxacillin, either alone or in combination with other drugs in pharmaceutical formulations and body fluids have been done with the use of HPLC. Flucloxacillin have been assayed in blood plasma using dicloxacillin as internal standard and acetonitrile as a component of the mobile phase.[11] Several other validated and accepted HPLC methods employ acetonitrile as a component of their mobile phases.[4,5,12] Acetonitrile compared to most organic solvents is more expensive and may not facilitate routine analysis in a poor facility.

It is therefore, very important that a method that is very accurate, fast, reliable and cheap be developed and validated to allow for very frequent analysis of flucloxacillin sodium in solid dosage forms.

MATERIALS AND METHODS

Materials

Disodium hydrogen orthophosphate and Potassium dihydrogen orthophosphate (both from BDH, Poole, England), Methanol (Philip Harris plc, Shaneson, England) and distilled water. All reagents and chemicals used were of analytical grade. The pure powders of amoxicillin trihydrate (assay: 99.60%) and flucloxacillin sodium (99.80%) were obtained from Letap Pharmaceutical Company, Accra, Ghana.

Instrumentation

Hanna instruments pH 211 microprocessor pH meter, Adam-analytical weighing balance, WA 210; 210/ 0.0001g, Kontron instruments HPLC pump 422, Power Chrom integrator, Perkin Elmer UV/visible detector.

Chromatographic conditions

Column: Phenomenex ® Bondclone 10 C18 (300×3.9 mm, 5µm)

Flow rate: 1.00 ml/min

Mobile phase: 60% Methanol: 40% KH_2PO_4 buffer (pH=5 adjusted with 1M sodium hydroxide solution)

Wavelength of detection: 225nm

Retention time: 5.36 ± 0.07 mins (n=3)

Temperature: ambient (about 25ºC)

Injection volume: 100µl

Column conditioning and equilibration

Before samples were injected onto the column, a water-methanol mixture of a ratio of 50:50 was pumped through the column for about an hour followed by the mobile phase for about 30 minutes to ensure that good results were obtained.

Method validation

The procedure was validated using the parameters in the ICH guidelines.[13]

Linearity

An initial concentration of 0.10% w/v of flucloxacillin sodium was prepared with the mobile phase. From this concentration, serial dilutions were made using the mobile phase to obtain concentrations of 0.08% w/v, 0.05% w/v, 0.03% w/v and 0.01% w/v. Each concentration was injected three times through a filter onto the column to obtain chromatograms with peak areas. The mean peak area for each concentration was plotted against their respective concentrations to get the calibration curve. The equation of the regression line and the coefficient of correlation were obtained from the calibration curve.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

These were calculated using the residual standard deviation of the regression line and slope of the calibration curve.

Selectivity

Very dilute concentrations each of flucloxacillin sodium and amoxicillin tri-hydrate were made with the mobile phase. The solution of flucloxacillin sodium was spiked with a small solution of amoxicillin trihydrate and mixed thoroughly. A sample of the resulting solution was injected three times and the retention times noted. All sample concentrations were prepared using the mobile phase and were injected through filters onto the column.

Precision

For the intra-day precision, 0.08% w/v of flucloxacillin sodium was analysed at six different times on a particular day and for each time, a triplicate determination was made. The relative standard deviation was then calculated.

For inter-day precision, 0.08% w/v of flucloxacillin sodium was analysed on three consecutive days to obtain six different determinations for each of the three days. Each determination was done three times. The relative standard deviation was thus calculated. All sample concentrations were prepared using the mobile phase and were injected through filters onto the column.

Robustness

Using a concentration of 0.08% w/v of flucloxacillin sodium, injections were made at varying flow rates while holding other parameters constant. The detection wavelength was also varied while keeping other parameters constant. All sample concentrations were prepared using the mobile phase and were injected through filters onto the column.

System suitability tests

A chromatogram was obtained for 0.08% w/v flucloxacillin sodium and compared with a standard chromatogram obtained for the same concentration for peak width, shape and baseline resolution.[14]

Statistical Analysis: A T-test was applied to data (% contents of flucloxacillin sodium) for robustness to test for statistical significance at 99% confidence level ($p < 0.01$).[15]

RESULTS AND DISCUSSION

The chromatographic conditions used were considered optimum since they gave sharp peaks with very resolved baselines for chromatograms of flucloxacillin sodium (Figure 1). These peaks had a mean retention time of 5.36 ± 0.07 mins (n=3).

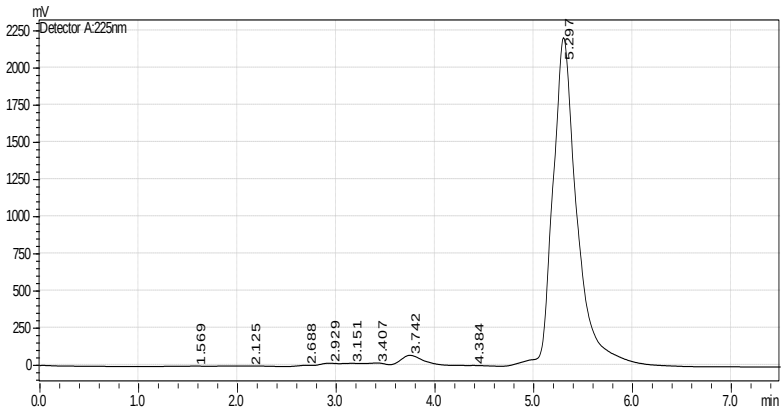

Figure 1: A typical chromatogram of flucloxacillin sodium.

The method was linear since the calibration curve (Figure 2) from the plot of peak area against concentration gave a correlation coefficient, R^2, of 0.9988 (Table 2). The data for plotting the calibration curve are found in table 1. This high value for R^2 is indicative of a very strong degree of correlation or direct proportionality between concentration and peak area.

Table 1: Concentrations of flucloxacillin sodium and their corresponding peak and nominal peak areas.

Concentration of flucloxacillin sodium (%w/v)	Peak area (y)	Nominal peak area (y_i)	Residual peak area ($y-y_i$)
0.10	6.250 ± 0.07	6.231	0.019
0.08	5.240 ± 0.07	5.208	0.032
0.05	3.610 ± 0.03	3.673	-0.063
0.03	2.580 ± 0.05	2.650	-0.070
0.01	1.710 ± 0.06	1.620	0.090

Data for the peak areas are provided as mean± SD (n=3). The nominal peak areas were obtained with the equation of the regression line from the calibration curve (Figure 2) using the mean peak areas.

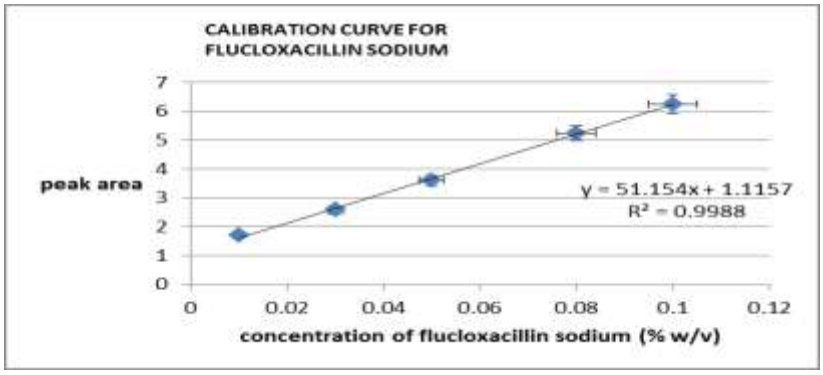

Figure 2: Calibration curve for flucloxacillin sodium.

Table 2: Data from calibration curve.

Parameter	Value
Equation of the regression line	y = 51.154x + 1.1157
slope	51.154
Correlation coefficient (R^2)	0.9988
Y- intercept	1.1157
Range	0.01%w/v – 0.10%w/v

To further confirm linearity, a residual plot was drawn (Figure 3). For a five point calibration graph, a distribution between three positive values and two negative values as seen from the residual plot (or vice versa) confirms linearity. Data for obtaining the residual plot (Figure 3) are found in table 1. The method was linear within the concentration range of 0.01% w/v – 0.10% w/v.

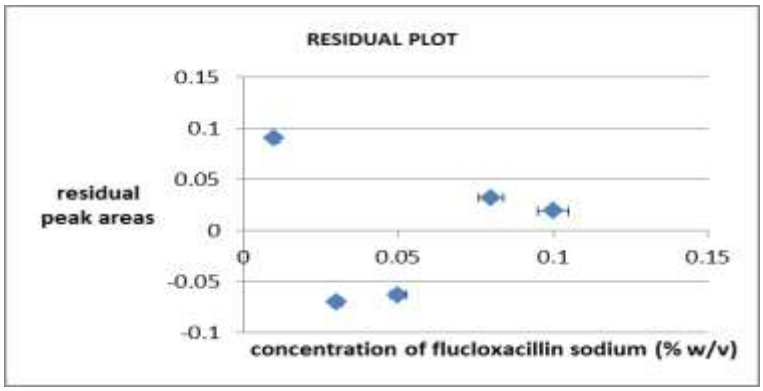

Figure 3: Residual plot from the calibration curve for flucloxacillin sodium.

The limit of detection (LOD) and the limit of quantification (LOQ) calculated from the calibration curve were 0.00437% w/v and 0.0132% w/v respectively. In capsule formulations of flucloxacillin sodium, the drug concentrations are normally very high as such the values obtained for the LOD and LOQ make the method adequate to be used.

The relative standard deviation (RSD) for the intra-day precision was 0.77 (Table 3) and the RSD calculated for inter-day precision was 0.91 (Table 4). Both RSDs were less than 2 hence the method can be said to be precise.[12]

Table 3: Intra-day Precision.

Determination	% content of flucloxacillin sodium (%w/v)
1	99.13 ± 1.35
2	100.21 ± 0.48
3	98.90 ± 0.98

4	100.03 ± 0.67
5	99.62 ± 0.66
6	101.01 ± 1.40

Data for % content (of flucloxacillin sodium) are given as mean± SD (n=3). The relative standard deviation (RSD) for the intra-day precision is 0.77.

Table 4: Inter-day Precision

Determination	% content of flucloxacillin sodium (%w/v)
1	99.50 ± 0.22
2	98.68 ± 0.30
3	101.40 ± 0.24
4	99.02 ± 0.35
5	100.40 ± 0.22
6	100.21 ± 0.25
7	99.60 ± 0.91
8	98.50 ± 0.81
9	99.30 ± 0.48
10	100.70 ± 1.32
11	98.67 ± 0.48
12	100.30 ± 0.65
13	100.25 ± 0.71
14	99.10 ± 0.45
15	98.43 ± 0.49
16	100.10 ± 0.45
17	101.20 ± 0.73
18	99.70 ± 0.38

Data for % content (of flucloxacillin sodium) are given as mean± SD (n=3). The relative standard deviation (RSD) for the inter-day precision is 0.91.

The method was selective. This was confirmed by introducing amoxicillin trihydrate into flucloxacillin sodium. The two drugs gave two distinct peaks at different retention times. Flucloxacillin sodium gave a retention time of 5.44 ± 0.13 mins and amoxicillin trihydrate gave 8.93 ± 0.19 mins (Table 5). This means that the method is selective for flucloxacillin sodium and therefore amoxicillin trihydrate when present in the same matrix will not interfere with the analysis of flucloxacillin sodium. The same can be said of ampicillin since it is very similar to amoxicillin chemically.

Table 5: Selectivity of method giving different retention times for flucloxacillin sodium and amoxicillin trihydrate.

Drug	Retention time (mins)
Amoxicillin trihydrate	8.93 ± 0.19
Flucloxacillin sodium	5.44 ± 0.13

Retention times are given as mean ±SD (n=3).

Accuracy was inferred once the method was linear, selective and precise.[13]

The method was robust at a flow rate of 1.20 ml/min because there was no significant difference in the % content of flucloxacillin sodium obtained at 1.20 ml/min and the % content at 1.00 ml/min when subjected to a T-test at 99% confidence level. At 0.80 ml/min, the method was not robust because the % content of flucloxacillin sodium was high and found to be significantly different from the expected 99.66± 0.99 at 1.00 ml/min as determined by the t-test at 99% confidence level (Table 6.) The content obtained at 0.80 ml/min was as a result of broadening of the peak (Figure 4). The low flow rate allowed for a much longer interaction between flucloxacillin sodium and the stationary phase in the column. This broadened the peak and gave a larger peak area which translated into high % content and also took a long time of about 13 mins to elute.

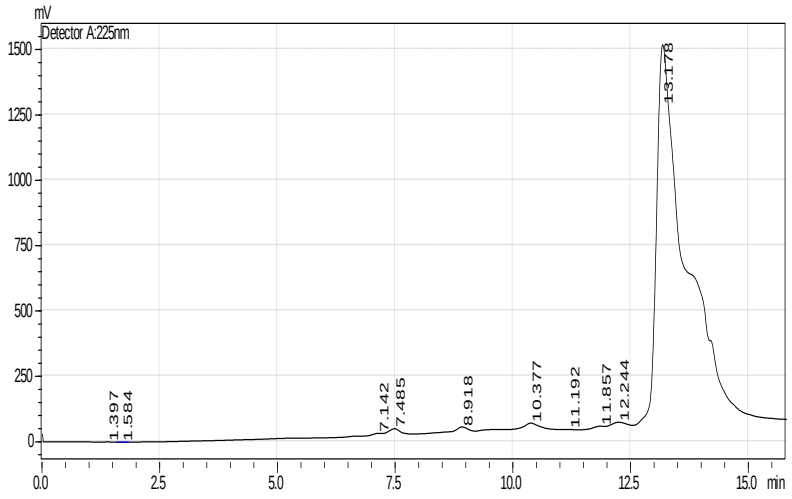

Figure 4: A chromatogram showing a broad peak of flucloxacillin sodium (0.08 %w/v) at a flow rate of 0.08 ml/min.

Table 6: Robustness of method after flow rate variation.

Flow rate (ml/min)	% content of flucloxacillin sodium (%w/v)
0.80	166 ± 0.71
1.00	99.66 ± 0.99
1.20	98.93 ± 0.35

Data for % content (of flucloxacillin sodium) are given as mean± SD (n=3). Percentage contents at the varied flow rates were compared to the % content at 1.00 ml/min using a T-test at a 99% confidence level.[15]

The method proved to be robust at detection wavelengths of 223 nm and 227 nm (Table 7).

Table 7: Robustness of method after wavelength variation

Wavelength (nm)	% content of flucloxacillin sodium (%w/v)
223	100.65 ± 1.00
225	99.66 ± 0.99
227	99.54 ±1.66

Data for % content (of flucloxacillin sodium) are given as mean± SD (n=3). Percentage contents at the varied wavelengths were compared to the % content at 225 nm using a T-test at a 99% confidence level.[15]

The method is operational within the set parameters that were established during the validation process; it gave a chromatogram whose peak shape, peak width and baseline resolution compared to that of a standard chromatogram obtained at the same concentration of flucloxacillin sodium.[14]

CONCLUSION

The proposed RP- HPLC method is simple, cheap, fast and accurate and can be used for routine analysis of flucloxacillin sodium in capsule dosage form.

ACKNOWLEDGEMENT

The authors are very grateful to Letap Pharmaceutical Company for providing the pure powders of flucloxacillin sodium and amoxicillin trihydrate used.

CONFLICT OF INTERESTS

Authors declare that they have no competing interests.

REFERENCES

1. Sutherland R, Croydon EA, Rolinson GN. Flucloxacillin, a new isoxazolyl penicillin, compared with oxacillin, cloxacillin, and dicloxacillin. Br Med J., 1970; 4: 455–60.
2. Ritchie SR, Rupali P, Roberts SA, Thomas MG. Flucloxacillin treatment of Staphylococcus aureus meningitis. Eur J Clin Microbiol Infect Dis., 2007; 26: 501–4.
3. Cunha BA. Antibiotic Selection in the Penicillin-Allergic Patient. Med Clin North Am., 2006; 90: 1257–64.
4. Shanmugasundaram P, Raj RK, Mohanrangan J, Devdass G, Arunadevi M, Maheswari R, et al. Simultaneous estimation of amoxicillin and flucloxacillin in its combined capsule dosage form by HPLC. RASAYAN J Chem.,2009; 2: 57-60.
5. British Pharmacopoeia. Electronic. London, UK: Her Majesty's Stationery Office; 2009.
6. British Pharmacopoeia. In: Volume One. London, UK: Her Majesty's Stationery Office, 1968; 229.
7. British Pharmacopoeia. In: volume one. London, UK: Her Majesty's Stationery Office, 1973; 117.
8. British Pharmacopoeia. In: volume 1. London: Her Majesty's Stationery Office, 1980; 343.
9. Jun H, Wakai J, Yasuda H, Uno T. Spectrophotometric Assay of Penicillins by Reaction with 1,2,4- Triazole and Mercury (II) Chloride. Anal Sci., 1985; 1: 73-6.
10. British Pharmacopoeia. In: volume two. London, UK: Her Majesty's Stationery Office., 1993; 912.
11. Zhou Q, Ruan Z, Yuan H, Juan B, Xu D. RP-HPLC analysis of flucloxacillin in human plasma validation and application to a bioequivalence study. Pharmazie., 2007; 62: 101–4.
12. Nikam DS, Bonde CG, Surana SJ, Venkateshwarlu G, Dekate PG. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Amoxicillin trihydrate and Flucloxacillin sodium in capsule dosage form., 2009; 1:935-9.
13. ICH Topic Q2 (R1) Validation of Analytical Procedures : Text and Methodology. International Conference on Harmonization. 2005.
14. HPLC validation PE.pdf [Internet].[cited 2012 Jul 23]. Available from: http://www.standardbase.com/tech/HPLC validation PE.pdf.
15. Vogel AI. Vogel's Textbook of Quantitative Chemical Analysis. 5th ed. .Jeffrey.G.H, Bassett.J., Mendham J, Denney R., editors., 1989; 220-225.