In vitro Activity and Evaluation of Quality of Some Selected Penicillins on the Ghanaian Market using Developed HPLC Methods

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Abstract

The use of antibiotics in health delivery is inevitable since it is one of the most prescribed medications. The quality and efficacy of these medications are crucial in health systems since they can affect the quality of healthcare delivery. The study was designed to determine the quality and activity of some penicillins on the Ghanaian market. A total of 54 samples (29 capsules and 25 suspensions) of different brands and batches were collected from different pharmacies in Accra and Kumasi, Ghana, from October 2011 to May 2012. The activity (zones of inhibition) and minimum inhibitory concentration (MIC) of the samples were determined by the agar-well diffusion and micro-dilution methods respectively against two types strains of Gram-negative and Gram-positive bacteria. Quality of the samples was determined quantitatively by developed and validated HPLC methods. The MICs of flucloxacillin and cloxacillin samples were ≥ 1400 µg/mL, whiles that of amoxicillin samples were ≥ 200 µg/mL with reference to the standard antibiotics which gave MICs of 200 to 800 µg/mL against all the test bacteria with the suspensions exhibiting higher antimicrobial activity. Specificity, linearity, precision and accuracy of the developed HPLC method were determined. HPLC analysis of the samples revealed that 75% of amoxicillin capsule samples and 92.3% of amoxicillin suspension samples contained the right amount of active pharmaceutical ingredient (API) with percentages ranging from 93.2 to 104.3% and 81.0 to 104.1% respectively. For samples of flucloxacillin capsules, 62.5% of the samples showed API content from 96 to 120.5%. All the suspension samples have their API within BP and USP specification of 114.4 to 120.0%. Capsules (58.6%) of all the samples contained the right API whereas 64% of them were recorded for suspensions. Out of the 54 samples evaluated, 61.1% were within the BP and USP specifications. The biological assay revealed higher MIC values for all the penicillin samples evaluated compared with the reference samples. Among the samples evaluated, amoxicillin showed better quality of 82.8% as compared to flucloxacillin (31.3%) and cloxacillin (44.4%) samples. Efforts should therefore be made to improve the quality and storage conditions of these antibiotics and also constant monitoring and surveillance of activity and potency of these antibiotics should be done.

Keywords. Penicillins; HPLC; Minimum inhibitory concentration (MIC); Active pharmaceutical ingredient (API)

Introduction

The World Health Organization (WHO) defines counterfeit products as those which are deliberately and fraudulently mislabeled with respect to identity or source [1,2]. Substandard medicines, on the other hand, are medicines that do not meet official standards and specification for strength, purity, quality, packaging, and labeling and their presence are one of the latest threats facing the pharmaceutical industry and healthcare delivery system globally. As a result of weak or no regulatory systems in many low and middle income countries [3,4], most of the medicines in circulation in these countries do not meet internationally accepted quality and specification and may be detrimental to patients. The total worldwide trade in counterfeit medicines is estimated to be 5 to 7% of the pharmaceutical market [5]. The problem is more severe in developing countries. More than 30% of all medicines sold in Africa are counterfeit medicines [6]. Counterfeit and substandard medicines are not only available in the developing countries but also in the developed world [7]. In 1999, 22% of the 771 reports of counterfeit medicines received by WHO came from the developed countries, the remaining 78% were from the developing countries [3].

Prevalence of counterfeit and substandard medicines has a major effect on the health delivery system. They can result in treatment failure, toxicity, adverse reaction or severe side effects thereby increasing mortality rate [8]. Counterfeit and substandard medicines may be found in all classes of medicines. The two major classes most counterfeited in the developing countries are anti-parasitic and anti-infective medicines [2]. Exposure of microorganisms to counterfeit and substandard anti-infectives leads to antimicrobial resistance, thereby putting health of patients at risk [9]. Antimicrobial resistance contributes to high cost of healthcare as patients using these counterfeit and substandard medicines do not respond to treatment and have to resort to higher doses and newer medicines. Additionally, patients remain ill for longer period leading to the loss of productivity [1,10]. Infectious diseases are taking lives of people and believed to be the world’s leading cause of death. It is estimated that 50,000 people die a day out of infectious diseases [11].

Medicines need to be of acceptable quality, safety and efficacy, especially antibiotics [12]. The appropriate active pharmaceutical ingredients (API) quantity and its efficacy to effect treatment must be ascertained. This is achieved through analysis and comparison to the manufacturer’s specifications or standard specification in the pharmacopoeias. Consequently, there is the need to sample and evaluate some of the antibiotics on the Ghanaian market to ensure that they meet the required specifications as spelt out in the United States Pharmacopoeia (USP) and British Pharmacopoeia (BP) to avoid all the problems associated with counterfeit and substandard medicines.

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Antibiotics are natural or synthetic chemical agents that can inhibit the growth or kill microorganisms [13]. Antibiotics are one class of antimicrobials and they are either referred to as bactericidal or bacteriostatic when they kill or inhibit growth or bacteria respectively [14]. They are heterogeneous and the only common property is that they are all organic in nature. A required feature of any antibiotic is its effect on bacteria at low concentration since that differentiate antibiotics from other compounds which have antimicrobial effect at higher concentrations e.g. ethanol. The discovery of antibiotics have significantly reduced mortality resulting from infectious diseases and also facilitated the success rates of many medical procedures such as surgery [15,16]. They are also employed extensively to prevent and treat infectious diseases in humans and animals [17]. These agents are mostly directed against some targets that are peculiar to bacteria, interfering with the growth of sensitive structures or processes that are critical to the survival and growth of the bacteria. Antibiotics inhibit sensitive bacteria by blocking important macromolecules like enzymes and nucleic acid activity which are very important in cell multiplication or division [18]. In effect, they are able to bind to specific site on the macromolecule to form a complex, different from the original entity and are unable to perform its function. The main targets are bacterial cell wall synthesis (peptidoglycan), bacterial protein synthesis (bacterial ribosome), bacterial DNA replication (bacterial enzymes involved in DNA supercoiling) and cytoplasmic membrane function [19]. The aim of this study was to determine the antibacterial activity and develop HPLC methods to analyze API content of various samples of amoxicillin, flucloxacillin and cloxacillin on the Ghanaian market.

## Materials

### Chemicals and reference drugs

All chemicals used for the HPLC analysis including reference compounds such as amoxicillin trihydrate (96% HPLC), flucloxacillin (98% HPLC), cloxacillin (98% HPLC), caffeine anhydrous (98% HPLC) and acetaminophen (98% HPLC), solvents etc. were of analytical and chromatographic grade purchased from Sigma-Aldrich, Darmstadt, Germany unless otherwise stated and they were available in the Forensic Laboratory of Ghana Standard Authority, Accra, Ghana. All materials and equipment used in the microbiological evaluation are available in the Microbiology Section, Department of Pharmaceutics, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.

### Test bacteria

Four typed strains of bacteria consisting of two Gram-negative and two Gram-positive bacteria were used for the microbiological evaluation. All organisms were typed cultures stored at the Microbiological Research Laboratory, Department of Pharmaceutics, KNUST, Kumasi, Ghana with the following identities: \textit{Escherichia coli} ATCC 25922, \textit{Pseudomonas aeruginosa} ATCC 4853, \textit{Staphylococcus aureus} ATCC 25923 and \textit{Bacillus subtilis} NTCC 10073.

### Test penicillin samples

Imported and locally manufactured penicillin samples were randomly purchased from different Pharmacies in Accra and Kumasi, Ghana. The reasons for the choice of samples were to compare different brands and different batches within a brand. Sampling of antibiotics was done from October, 2011 to May, 2012.

### Methods

#### Determination of antibacterial activity

The antimicrobial activity was determined using modified method described by Agyare et al. [20] and Girish and Satish [21]. Twenty (20) milliliters stabilized agar at 45°C was seeded with 100 µL of 10¹ colony forming units (cfu)/mL of 18 to 24 h culture of \textit{S. aureus} and rolled in the palm for uniform distribution and was aseptically poured into sterilized Petri dish and allowed to set. Four wells were bored with diameter of 10 mm. The wells were filled with 200 µL each of respective concentrations and allowed to stand for 1 h on the bench to allow diffusion of antibiotic. The plate was then incubated at 37°C for 24 h and zones of growth inhibition recorded in millimeter (mm). The method used was performed in triplicate for all test samples using \textit{B. subtilis}, \textit{E. coli} and \textit{P. aeruginosa}. Concentrations used were 0.125 to 1.0 µg/mL for amoxicillin samples and 1.25 to 10.0 mg/mL for flucloxacillin and cloxacillin samples.

#### Determination of minimum inhibitory concentration

Minimum inhibitory concentrations (MIC) of the various antibiotic samples were determined using the method described by Agyare et al. [20]. Sterile 96-well microtitre plates were labeled appropriately for \textit{S. aureus}. Total volume of 200 µL were prepared by dispensing a fixed volume of 100 µL sterile double strength nutrient broth and 20 µL (10⁵ cfu/mL) of 18 h culture was aseptically added to the medium. Amoxicillin samples were evaluated within concentration range of 0.1 to 0.5 mg/mL. The MIC of flucloxacillin and cloxacillin samples was determined within a concentration range of 0.5 to 2.2 mg/mL. Experiments were performed in triplicate under the same conditions for all samples. Reference samples were prepared and the MIC determined under the same conditions as described above.

The plates were incubated at 37°C for 24 h. Microbial growth was determined by addition of 30 µL 3-(4,5-dimethylthiazole -2-yl)-2,5- diphenyltetrazolium bromide (MTT) after incubation and as growth of organism was indicated by purple to blue coloration and yellow coloration indicated no growth of organism. The well with least concentration of test sample without bacterial growth recorded as the MIC. The procedure above was repeated for all test samples using \textit{E. coli}, \textit{B. subtilis} and \textit{P. aeruginosa} respectively.

#### HPLC analysis of reference and test samples

Reference amoxicillin trihydrate samples were dissolved in 0.1 M hydrochloric acid. Samples were analyzed at concentrations of 5.26, 10.52, 15.78, 21.04 and 26.3 µg/mL with an injection volume of 100 µL. Reference flucloxacillin and cloxacillin samples were dissolved in sterile distilled millipore water. They were analyzed at concentrations of 25.35, 50.7, 101.4, 152.1 µg/mL and 11.72, 23.44, 35.16, 58.6 µg/mL for reference standard and the sample respectively, with an injection volume of 1 mL. All samples were analyzed under isocratic conditions with Shim-Pac CLS ODS (M) C18 column for amoxicillin and analysis of amoxicillin trihydrate samples. Concentrations of 1.4156 µM and 1.3296 µM of acetaminophen (paracetamol) were used for the HPLC method development for flucloxacillin and cloxacillin respectively. The same concentrations were used for the analysis of flucloxacillin and cloxacillin samples.

#### Preparation of test sample solutions

Concentrations of amoxicillin trihydrate equivalent to 15.78 µg/mL were prepared. They were dissolved in 0.1M hydrochloric acid and mobile phase consisting of methanol/ 0.01M potassium dihydrogen phosphate (65:35, v/v). Equivalent of 50.7 and 11.72 µg/mL of flucloxacillin and cloxacillin were prepared. Samples were dissolved in sterile distilled water and mobile phase.
Statistical analysis

All graphs were plotted with Excel version 2010 and graph pad prism (Graph Pad Prism 5 Software, San Diego, CA, USA) for all the statistical analysis. Data analysis was by one-way analysis of variance (ANOVA). There is not enough evidence at alpha=0.05 and the model for the method development is not significant since F-value > F-crit and P>0.05 (alpha). ChromQuest and Endnote X6 (Bld 6348) were used to generate HPLC analysis data and references respectively.

Results

Antibacterial activities of samples

The MICs of capsules were within the range of 200 to 800 µg/mL for amoxicillin trihydrate samples and ≥ 800 to 1900 for flucloxacillin and cloxacillin test samples. Reference amoxicillin samples showed lower MICs of 200 µg/mL against E. coli, 500 µg/mL against P. aeruginosa, 300 µg/mL against B. subtilis and 200 µg/mL against S. aureus. MICs of reference flucloxacillin sample were 800 µg/mL against E. coli, 1500 µg/mL for P. aeruginosa, 1400 µg/mL for B. subtilis and 1400 µg/mL for S. aureus. MICs for reference cloxacillin sample were 800 µg/mL against E. coli, 1500 µg/mL against P. aeruginosa, 1500 µg/mL against B. subtilis and 1500 µg/mL for S. aureus (Table 1).

Antibacterial activity of sampled antibiotic suspensions of amoxicillin, flucloxacillin and cloxacillin samples. Evaluation of samples gave MICs within the range of 200 to 700 µg/mL for amoxicillin test samples, 800 to 1600 for flucloxacillin and 500 to 1700 cloxacillin samples (Table 2).

Antibacterial activity of sampled antibiotic capsules of amoxicillin, flucloxacillin and cloxacillin samples. Evaluation of samples at test concentrations gave mean zones of inhibition within the range of 0.0 to 30.0 mm for amoxicillin test samples, 0.0 to 31.67 mm for flucloxacillin and 0.0 to 29.83 mm for cloxacillin samples (Table 3).

Antibacterial activity of sampled antibiotic suspensions of amoxicillin, flucloxacillin and cloxacillin samples. Evaluation of samples at test concentrations gave mean zones of inhibition within the range of 0.0 to 28.67 mm for amoxicillin test samples, 0.0 to 37.83 mm for flucloxacillin and 0.0 to 33.83 mm for cloxacillin samples (Table 4).

Antibacterial activity of reference antibiotic of amoxicillin, flucloxacillin and cloxacillin samples. Evaluation of samples at test concentrations gave mean zones of inhibition within the range of 0.00 to 30.83 mm for amoxicillin test samples, 0.00 to 38.00 mm for flucloxacillin and 0.00 to 30.00 mm for cloxacillin samples (Table 5).

HPLC analysis of amoxicillin samples

The active pharmaceutical ingredients (APIs) in the samples were determined using the developed and validated HPLC method. The chromatographic conditions for the analysis of amoxicillin trihydrate were made up of mobile phase consisting of methanol:0.01M potassium dihydrogen phosphate (65:35, v/v) yielded maximum sensitivity and separation. Flow rates between 0.5 and 1.2 mL/min on a Shim-pack CLS-ODS C18 (M) 250 x 4.6 mm, 5 microns column were studied and a flow rate of 1.0 mL/min gave an optimal signal to noise ratio with a reasonable separation time of 1.42 min for amoxicillin when injected alone.

HPLC chromatogram of amoxicillin (Figure 1) as reference sample alone and reference amoxicillin and caffeine as internal standard (Figure 2). The running time of the reference sample and the internal standard was less than 3 min. The major peak at 1.421 min is for amoxicillin whereas that for caffeine is 2.974 min (Figure 1).

A five-point calibration curve was generated for amoxicillin in the concentration range of 5.26 to 263.0 µg/mL (Figure 3). The calibration curve provided a linear relationship between the peak area (y-axis) and the concentrations of amoxicillin trihydrate with the regression equation of y=194.41x + 0.004, R²=0.9995 (Figure 3). The residual points of the calibration curve were well distributed within acceptable limits (Figure 4).

Regression analysis cannot minimize the distance for all points simultaneously but does it for most of the points. The residual plot of points shows maximum points closer to line for amoxicillin (Figure 4).

The developed HPLC methods were validated using the International Conference on Harmonization guidelines and the parameters therein. It was performed using a well-designed experiment and statistically relevant methods in accordance with International Conference on Harmonization (ICH) guidelines on validation of analytical procedures [22,23].

The linearity of the detector response for amoxicillin was confirmed from 5.26 to263.0 µg/mL. The calibration curve (Figure 3) and the residuals (Figure 4) were inspected to assess linearity (Table 6).

### Table 1: MICs of capsule samples of amoxicillin, flucloxacillin and cloxacillin.

| Sample | Organisms/MIC (µg/mL) | E. coli | P. aeruginosa | B. subtilis | S. aureus |
|--------|-----------------------|--------|---------------|-------------|-----------|
| Reference sample | AMOXICILLIN | 200 | 500 | 300 | 200 |
| 01A | 200 | 500 | 400 | 400 |
| 01B | 300 | 700 | 500 | 500 |
| 02A | 300 | 700 | 400 | 300 |
| 03A | 200 | 600 | 300 | 300 |
| 03B | 200 | 700 | 300 | 300 |
| 03C | 200 | 600 | 300 | 300 |
| 04A | 200 | 700 | 300 | 300 |
| 05A | 300 | 600 | 300 | 300 |
| 06A | 400 | 800 | 400 | 400 |
| 06B | 400 | 700 | 500 | 300 |
| 06C | 300 | 700 | 500 | 400 |
| 07A | 200 | 500 | 300 | 200 |
| 07B | 400 | 800 | 400 | 400 |
| 08A | 300 | 700 | 400 | 400 |
| 09A | 300 | 500 | 300 | 200 |
| FLUCLOXACILLIN | Reference | 800 | 1500 | 1400 | 1400 |
| FLMG01 | 1300 | 1900 | 1500 | 1500 |
| FLMG02 | 1200 | 1700 | 1400 | 1500 |
| FLMG02 | 800 | 1500 | 1500 | 1500 |
| FLLP04 | 1300 | 1800 | 1500 | 1500 |
| FLLP05 | 1200 | 1600 | 1500 | 1500 |
| FLLP06 | 1300 | 1700 | 1500 | 1500 |
| FLAR07 | 800 | 1600 | 1500 | 1500 |
| FLAR08 | 800 | 1600 | 1500 | 1500 |
| CLOXACILLIN | Reference | 800 | 1500 | 1500 | 1500 |
| CLLP01 | 800 | 1500 | 1500 | 1500 |
| CLLP02 | 900 | 1600 | 1500 | 1500 |
| CLLP03 | 800 | 1500 | 1500 | 1500 |
| CLAR04 | 900 | 1600 | 1500 | 1500 |
| CLAR05 | 800 | 1500 | 1500 | 1500 |
| CLMG06 | 800 | 1400 | 1500 | 1400 |

MIC = minimum inhibitory concentration (µg/mL)
### Table 2: MICs of suspension of amoxicillin, flucloxacillin and cloxacinil samples

| Sample | Organisms/MIC (µg/mL) | E. coli | P. aeruginosa | B. subtilis | S. aureus |
|--------|-----------------------|---------|---------------|-------------|-----------|
|        | AMOXICILLIN           |         |               |             |           |
| S01    | 300                   | 600     | 300           | 200         |           |
| S02A   | 200                   | 500     | 300           | 200         |           |
| S02B   | 300                   | 500     | 300           | 200         |           |
| S02C   | 300                   | 600     | 300           | 200         |           |
| S03A   | 200                   | 500     | 300           | 200         |           |
| S04A   | 300                   | 600     | 300           | 200         |           |
| S05A   | 300                   | 500     | 300           | 200         |           |
| S06A   | 200                   | 500     | 300           | 200         |           |
| S06B   | 300                   | 700     | 400           | 300         |           |
| S06C   | 300                   | 600     | 300           | 200         |           |
| S07A   | 200                   | 500     | 300           | 200         |           |
| S08A   | 200                   | 500     | 300           | 200         |           |
| S08B   | 200                   | 500     | 300           | 200         |           |
|        | FLUCLOXACILLIN        |         |               |             |           |
| FLSMG01| 800                   | 1500    | 1400          | 1400        |           |
| FLSMG02| 800                   | 1600    | 1400          | 1400        |           |
| FLSMG03| 800                   | 1500    | 1400          | 1500        |           |
| FLSLP04| 800                   | 1600    | 1400          | 1600        |           |
| FLSLP05| 800                   | 1600    | 1500          | 1600        |           |
| FLSLP06| 800                   | 1500    | 1500          | 1400        |           |
| FLSAR07| 800                   | 1500    | 1400          | 1400        |           |
| FLSAR08| 800                   | 1500    | 1600          | 1400        |           |
|        | CLOXACILLIN           |         |               |             |           |
| CLSLP01| 800                   | 1500    | 1500          | 1600        |           |
| CLSLP02| 800                   | 1700    | 1500          | 1500        |           |
| CLSLP03| 800                   | 1600    | 500           | 1500        |           |
| CLSMG04| 800                   | 1500    | 1600          | 1600        |           |
| CLSMG05| 800                   | 1600    | 1600          | 1600        |           |

### Table 3: Antibacterial activity (mean zones of inhibition ± SEM) of test samples (capsules).

| Samples | Concentrations (µg/mL) | Organisms | E. coli | P. aeruginosa | B. subtilis | S. aureus |
|---------|------------------------|-----------|---------|---------------|-------------|-----------|
|        | AMOXICILLIN            |           |         |               |             |           |
| 01A     | 1000                   | 22.33±0.82| 16.00±0.63| 20.50±0.55    | 21.67±0.52  |           |
|         | 500                    | 20.83±0.75| 12.67±0.52| 18.50±0.55    | 19.33±0.52  |           |
|         | 250                    | 25.00±0.0 | 12.00±0.0 | 18.17±0.41    | 17.83±0.75  |           |
|         | 125                    | 0.0       | 0.0       | 0.0           | 0.0         |           |
| 01B     | 1000                   | 25.83±0.41| 26.66±0.52| 24.67±0.82    | 21.67±0.52  |           |
|         | 500                    | 25.00±0.63| 24.67±0.82| 23.00±0.63    | 19.87±0.62  |           |
|         | 250                    | 22.67±0.52| 22.67±0.52| 21.00±0.89    | 18.33±1.37  |           |
|         | 125                    | 0.0       | 0.0       | 0.0           | 0.0         |           |
| 02A     | 1000                   | 25.67±1.03| 24.00±0.9  | 19.00±0.0    | 23.50±0.55  |           |
|         | 500                    | 23.33±1.03| 17.50±0.55| 14.17±0.75    | 22.50±0.84  |           |
|         | 250                    | 22.17±0.41| 16.17±0.75| 17.00±0.0    | 21.50±0.55  |           |
|         | 125                    | 0.0       | 0.0       | 0.0           | 0.0         |           |
| 02B     | 1000                   | 25.67±0.52| 23.33±1.21| 25.53±0.51    | 23.00±0.89  |           |
|         | 500                    | 24.50±1.38| 22.50±0.55| 24.83±0.98    | 20.83±1.17  |           |
|         | 250                    | 22.50±1.05| 18.50±0.15| 22.67±0.52    | 18.50±0.84  |           |
|         | 125                    | 0.0       | 0.0       | 0.0           | 0.0         |           |
| 03A     | 1000                   | 24.8±0.41 | 20.83±0.52| 24.50±0.84    | 0.0         |           |
|         | 500                    | 21.83±0.41| 23.83±0.75| 20.00±0.0     | 0.0         |           |
|         | 250                    | 20.83±0.41| 18.33±0.75| 22.50±0.84    | 0.0         |           |
|         | 125                    | 0.0       | 0.0       | 0.0           | 0.0         |           |
| 03B     | 1000                   | 25.83±0.98| 20.83±0.75| 24.83±0.75    | 20.67±1.03  |           |
|         | 500                    | 22.67±1.21| 18.00±0.03| 23.83±0.41    | 17.83±0.75  |           |
|         | 250                    | 21.17±0.98| 12.67±0.52| 20.67±0.82    | 16.33±0.82  |           |
|         | 125                    | 0.0       | 0.0       | 0.0           | 0.0         |           |
| 03C     | 1000                   | 24.67±1.00| 18.67±0.52| 23.50±0.55    | 0.0         |           |

### Note
- E. coli: *Escherichia coli*
- P. aeruginosa: *Pseudomonas aeruginosa*
- B. subtilis: *Bacillus subtilis*
- S. aureus: *Staphylococcus aureus*
Table 4: Antibacterial activity (mean zones of inhibition ± SEM) of suspension samples.

| Sample | Concentration (µg/mL) | S. aureus | E. coli | B. subtilis | P. aeruginosa |
|--------|-----------------------|-----------|---------|-------------|--------------|
|        |                       | AMOXICILLIN |         |             |              |
| S01A   | 1000                  | 21.83±1.22 | 18.00±0.68 | 22.50±0.81 | 0.0          |
|        | 500                   | 19.67±0.91 | 15.83±0.31 | 21.33±0.76 | 0.0          |
|        | 250                   | 18.67±0.91 | 15.00±0.00 | 18.33±0.42 | 0.0          |
|        | 125                   | 0.0        | 0.0       | 0.0         | 0.0          |
| S02A   | 1000                  | 14.00±0.22 | 19.83±0.14 | 16.17±0.14 | 13.00±0.31  |
|        | 500                   | 15.50±0.19 | 19.50±0.29 | 15.00±0.22 | 11.50±0.19  |
|        | 250                   | 13.50±0.19 | 18.00±0.00 | 13.33±0.18 | 0.0          |
|        | 125                   | 0.0        | 0.0       | 0.0         | 0.0          |
| S02B   | 1000                  | 19.33±0.18 | 19.50±0.29 | 19.00±0.26 | 0.0          |
|        | 500                   | 15.67±0.16 | 18.33±0.26 | 18.33±0.60 | 0.0          |
|        | 250                   | 15.17±0.14 | 15.67±0.28 | 15.38±0.34 | 0.0          |
|        | 125                   | 0.0        | 0.0       | 0.0         | 0.0          |
| S02C   | 1000                  | 18.33±0.17 | 18.00±0.22 | 18.17±0.14 | 0.0          |
|        | 500                   | 15.80±0.29 | 16.33±0.18 | 15.17±0.14 | 0.0          |
|        | 250                   | 12.50±0.19 | 12.00±0.00 | 12.17±0.14 | 0.0          |
| S03A   | 1000                  | 20.00±0.00 | 17.67±0.28 | 20.33±0.56 | 22.67±0.28  |
|        | 500                   | 18.67±0.28 | 17.00±0.00 | 18.50±0.57 | 20.33±0.36  |
|        | 250                   | 19.17±0.45 | 14.67±0.28 | 18.00±0.38 | 19.67±0.18  |
|        | 125                   | 0.0        | 0.0       | 0.0         | 0.0          |
| S04A   | 1000                  | 17.33±0.18 | 18.83±0.14 | 21.17±0.28 | 18.33±0.18  |
|        | 500                   | 15.83±0.14 | 14.67±0.17 | 20.17±0.14 | 17.33±0.28  |
|        | 250                   | 15.00±0.00 | 13.00±0.00 | 19.00±0.00 | 14.67±0.18  |
|        | 125                   | 0.0        | 0.0       | 0.0         | 0.0          |

SEM = standard error mean, Diameter of well = 10 mm
HPLC analysis of flucloxacillin and cloxacillin samples

HPLC method was developed and validated for the evaluation of flucloxacillin and cloxacillin samples. Analysis was carried out in an ambient temperature (25°C) with Shim pack CLC-NH2 C18 column 150 × 4.6 mm, 5 microns column and a Finnigan Spectra System HPLC. A mobile phase consisting of acetonitrile: 0.01M potassium dihydrogen phosphate, KH₂PO₄, with a ratio of 60:40 (v/v) yielded maximum sensitivity and separation with sample detection at UV wavelength of 225 nm.

HPLC analysis of reference flucloxacillin

HPLC chromatograms of flucloxacillin as reference sample (Figure 5) and with acetaminophen (paracetamol) as an internal standard (Figure 6) were developed. The running time for the reference sample and the internal standard were within four (4) min. The peak at 3.146 min is for flucloxicillin whereas that for acetaminophen is 1.953 min.

A four-point calibration curve was generated for flucloxacillin in the concentrations range of 25.35 to 152.10 µg/mL (Figure 7). The calibration curve provided a linear relationship between the area under curve (y) and the concentrations of flucloxacillin with the regression equation of y=156.94x + 0.0699 (R²=0.995) (Figure 7). The residual points of the calibration curve were well distributed within acceptable limits (Figure 8).

The methods were validated using the International Conference on Harmonization guideline and the parameters therein. It was performed using a well-designed experiment and statistically relevant methods in accordance with International Conference on Harmonization (ICH) guidelines on validation of analytical procedures [22, 23]. The linearity of the detector response for flucloxacillin was confirmed within 25.35 to 152.10 µg/mL (Figure 7).

Calibration curves were analyzed using a linear regression model.
Citation: Boadu RF, Agyare C, Yiadom MA, Adu F, Boamah VE, et al. (2015) In vitro Activity and Evaluation of Quality of Some Selected Penicillins on the Ghanaian Market using Developed HPLC Methods. Med chem 5: 001-014. doi:10.4172/2161-0444.1000235

and linear co-efficients (Table 8). The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the signal–to–noise ratio ICH-Q2B, 1996 and were found to be $1.2837 \times 10^{-4}$ and $3.89 \times 10^{-4} \mu g/mL [23]$. Areas under curve ratios were directly proportional to concentrations as increase or decrease in peak area of analyte also affected area of internal standard (Table 9).

Accuracy for flucloxacillin was determined by the mean and SDV of the percentage recovery studies (Table 10).

### Table 6: Statistical validation of the calibration data for quantitative determination of amoxicillin.

| Parameter                        | Amoxicillin trihydrate |
|----------------------------------|------------------------|
| Concentration range              | 5.26 to 263.0 µg/mL    |
| Number                           | 5                      |
| Average values                   | 0.001315               |
| Correlation coefficient          | 0.9995                 |
| Relative standard deviation (%)  | 0.7483                 |
| Calibration equation             | $y=194.41x + 0.004$    |
| Limit of detection (LOD)         | $1.6703 \times 10^{-1}$ |
| Limit of quantification (LOQ)    | $5.0817 \times 10^{-1}$ |
| System suitability               | 0.002                  |
| Method precision                 | 0.58%                  |

LOD=3.3 × e/S, where e= SDEV of the responses, S= slope of the regression line

LOQ=10 × e/S, where e= SDEV of the responses, S= slope of the regression line

### HPLC analysis of cloxacillin

HPLC chromatograms of cloxacillin as reference sample (Figure 9) and acetaminophen (paracetamol BP) as internal standard (Figure 10). The cloxacillin peak is at 2.874 min and that of acetaminophen is 1.933 min.

A four-point calibration curve was generated for cloxacillin in the concentration range of 11.72 to 58.6 µg/mL. The calibration curve provided a linear relationship between the peak area ($y$) and the concentrations of amoxicillin injected ($x$) with the regression equation of $y=787.78x + 0.0839$ ($R^2=0.9986$) (Figure 11). The residual points of the calibration curve were well distributed within acceptable limits (Figure 12).

The methods were validated using the International Conference on Harmonization guidelines and the parameters therein. It was performed using a well-designed experiment and statistically relevant methods in accordance with International Conference on Harmonization (ICH) guidelines on validation of analytical procedures (Q2A and Q2B). The linearity of the detector response for cloxacillin was from 11.72 to 58.6 µg/mL. The calibration curve (Figure 11) and the residuals (Figure 12) were inspected to assess linearity.

Calibration curves were analyzed using a linear regression model and linear coefficients (Table 11). The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the signal–to–noise ratio and were found to be $9.5246 \times 10^{-6}$ µg/mL and $2.8861 \times 10^{-5}$ µg/mL respectively.

### Table 7: Analysis of homogenous reference amoxicillin solution for system suitability and precision analysis.

HPLC analysis of cloxacillin

HPLC chromatogram of flucloxacillin as reference sample (Figure 9) and acetaminophen (paracetamol BP) as internal standard (Figure 10). The cloxacillin peak is at 2.874 min and that of acetaminophen is 1.933 min.

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### Table 7: Analysis of homogenous reference amoxicillin solution for system suitability and precision analysis.

| IS (AUC) | RS (AUC) | IS:RS (AUC ratio) |
|----------|----------|-------------------|
| 165429   | 478918   | 0.3454            |
| 164384   | 472481   | 0.3478            |
| 166734   | 479600   | 0.3477            |
| 165828   | 474066   | 0.3498            |
| 166732   | 474678   | 0.3513            |
| 172047   | 493711   | 0.3484            |

Mean=0.3484
SDEV=0.00201
%RSD=0.58%

### Table 8: Analysis of homogenous reference amoxicillin solution for system suitability and precision analysis.

| Parameter                        | Amoxicillin trihydrate |
|----------------------------------|------------------------|
| Concentration range              | 5.26 to 263.0 µg/mL    |
| Number                           | 5                      |
| Average values                   | 0.001315               |
| Correlation coefficient          | 0.9995                 |
| Relative standard deviation (%)  | 0.7483                 |
| Calibration equation             | $y=194.41x + 0.004$    |
| Limit of detection (LOD)         | $1.6703 \times 10^{-1}$ |
| Limit of quantification (LOQ)    | $5.0817 \times 10^{-1}$ |
| System suitability               | 0.002                  |
| Method precision                 | 0.58%                  |

LOD=3.3 × e/S, where e= SDEV of the responses, S= slope of the regression line

LOQ=10 × e/S, where e= SDEV of the responses, S= slope of the regression line

### Table 9: Analysis of homogenous reference amoxicillin solution for system suitability and precision analysis.

| Parameter                        | Amoxicillin trihydrate |
|----------------------------------|------------------------|
| Concentration range              | 5.26 to 263.0 µg/mL    |
| Number                           | 5                      |
| Average values                   | 0.001315               |
| Correlation coefficient          | 0.9995                 |
| Relative standard deviation (%)  | 0.7483                 |
| Calibration equation             | $y=194.41x + 0.004$    |
| Limit of detection (LOD)         | $1.6703 \times 10^{-1}$ |
| Limit of quantification (LOQ)    | $5.0817 \times 10^{-1}$ |
| System suitability               | 0.002                  |
| Method precision                 | 0.58%                  |

LOD=3.3 × e/S, where e= SDEV of the responses, S= slope of the regression line

LOQ=10 × e/S, where e= SDEV of the responses, S= slope of the regression line

### Table 10: Analysis of homogenous reference cloxacillin solution for system suitability and precision analysis.

| Parameter                        | Cloxacillin |
|----------------------------------|-------------|
| Concentration range              | 11.72 to 58.6 µg/mL |
| Number                           | 5           |
| Average values                   | 0.001315    |
| Correlation coefficient          | 0.9995      |
| Relative standard deviation (%)  | 0.7483      |
| Calibration equation             | $y=787.78x + 0.0839$ |
| Limit of detection (LOD)         | $1.6703 \times 10^{-1}$ |
| Limit of quantification (LOQ)    | $5.0817 \times 10^{-1}$ |
| System suitability               | 0.002       |
| Method precision                 | 0.58%       |

LOD=3.3 × e/S, where e= SDEV of the responses, S= slope of the regression line

LOQ=10 × e/S, where e= SDEV of the responses, S= slope of the regression line
Peak ratios were directly proportional to concentrations as increase or decrease in peak area of analyte also affected area of internal standard (Table 12).

Accuracy for cloxacillin was determined by the mean and SDEV of the percentage recovery studies (Table 13).

HPLC analysis show that 75% amoxicillin capsules and 92.3% of suspension were within USP specification of 93.2 to 104.3% and 81.0 to 104.1% respectively. Sample of flucloxacillin capsules had 62.5% of the samples within specification of 96 to 120.5%. All suspension samples were below the required USP specification. None of cloxacillin capsule samples were within the USP specification. All the suspension samples,
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Discussion

The samples of the three different penicillins evaluated varied slightly from the standard reference samples in the microbiological evaluation. Suspensions had lower MICs as compared to the capsule samples. All samples in general showed higher MIC compared to the reference standards. The developed and validated HPLC methods were suitable for the intended purpose. HPLC analysis of the samples showed some of the samples contained the right amount of active pharmaceutical ingredients as stated in the USP [24] and BP [25] but they had higher MICs against the test bacteria.

Antibacterial activities of penicillin samples

Most of the penicillin samples were active against all the organisms but the mean zones of inhibition varied with different bacteria and sample as well as different concentrations. The pattern of zones of inhibition were not consistent as, in some cases, lower concentrations of the same sample had bigger or same sizes of zones of inhibition as compared to higher concentrations. This could be attributed to the fact that the antibiotic had to diffuse through the solid medium and the more concentrated they are, the higher the viscosity, hence, less diffusion rate. Consequently, the micro-dilution method was selected and used in the determination of the MIC as the test organisms are in direct contact with the antibiotic [26].

Helegbe et al. [27] reported that some selected antibiotics were active against some bacteria and recommended further studies on a larger scale. The current study, however, revealed higher MIC for the samples and this may be due to insufficient amount in the penicillin samples analyzed. A typical example is the report by Rahman et al. [28] which showed that zones of inhibition of amoxicillin samples against selected bacteria at 100 µg/mL were 19.5 mm for E. coli, 15.3 mm for B. subtilis and 17.0 mm for S. aureus. The current study on the other hand had no zones of inhibition at concentration below 250 µg/mL. The amoxicillin samples had MIC of 125, 180 and 220 µg/mL against E. coli, S. aureus and B. subtilis respectively and the current study, amoxicillin had MICs of 200, 200 and 300 µg/mL against E. coli, S. aureus and B. subtilis respectively.

There are differences between the literature values and that obtained from this study, but samples showed some level of sensitivity towards the test bacteria. Generally, there were differences in the sensitivity of Gram-negative and Gram-positive bacteria which could be due to the composition of the cell wall of two types of bacteria [29-31].

Some samples exhibited variations in the MIC. The antibacterial activity and MIC of samples varied from bacteria to bacteria which

Table 8: Statistical validation of the calibration data for quantitative determination of flucloxacillin.

| Parameter          | Flucloxacillin |
|--------------------|----------------|
| Concentration range| 25.35 – 152.10 µg/mL |
| Number             | 4 |
| Average values     | 0.0066 |
| Correlation coefficient (R²) | 0.995 |
| Relative standard deviation (%) | 0.9262 |
| Calibration equation | y=156.94x + 0.0699 |
| Limit of Detection | 1.2837 × 10¹ µg/mL |
| Limit of Quantification | 3.89 × 10⁻¹ µg/mL |
| System suitability  | 0.00253 |
| Method precision   | 0.25% |

LOD = Limit of detection, LOQ = Limit of quantification
LOD=3.3 × ϭ/S, where ϭ= SDEV of the responses, S= slope of the regression line
LOQ=10 × ϭ/S, where ϭ= SDEV of the responses, S= slope of the regression line

Table 9: System suitability and precision parameters for reference flucloxacillin.

| IS (AUC) | RS (AUC) | IS:RS (AUC ratio) |
|----------|----------|-------------------|
| 780955   | 799289   | 1.0235            |
| 812336   | 830814   | 1.0227            |
| 801131   | 823499   | 1.0279            |
| 822182   | 843224   | 1.0256            |
| 797503   | 814643   | 1.0215            |

Mean = 1.02424
SDEV = 0.00253
% RSD = 0.25%

AUC = Area under curve, IS = Internal standard, RS = Reference standard, SDEV= Standard deviation, %RSD = Percent relative standard deviation

Table 10: Standard and internal standard recovery studies of reference flucloxacillin.

| Number (n) | % Recovery |
|------------|------------|
| 1          | 92.36      |
| 2          | 99.02      |
| 3          | 107.87     |
| 4          | 94.71      |
| Mean       | 98.49      |
| SDEV       | 6.834486   |

SDEV= Standard deviation, n=4

Figure 9: HPLC chromatogram of cloxacillin as reference at λ 225 nm.
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Figure 10: HPLC chromatogram of cloxacillin as reference and acetaminophen as internal standard at wavelength 225 nm.

Figure 11: HPLC calibration curve of cloxacillin (reference standard).

Figure 12: Residual plot of the HPLC calibration curve of cloxacillin (reference standard).
amoxicillin are required for the treatment of infections due to these bacteria. Amoxicillin has enantiomers with its mirror image having the same chemical structure. A compound and its enantiomer show different activity with only one of its enantiomers usually biologically active [34].

Antibacterial activities of samples were similar but not the same as those of the reference standard. In general, flucloxacillin and cloxacillin samples were much active against S. aureus and B. subtilis compared to E. coli and P. aeruginosa. This could be due to the simple reason that isoxazolyl antibiotics are not very active against Gram-negative bacteria [27]. Samples in suspension forms showed higher activity as compared to the capsules against Gram-negative and Gram-positive bacteria. The possible reason could be due to the nature of formulation and the type of experimental design (In vitro) used. Capsules are to be swallowed and an acidic environment is required to enhance dissolution and release of API.

The isoxazolyl antibiotics such as flucloxacillin are not sensitive to penicillinase enzymes secreted by many penicillin-resistant bacteria, but able to bind to penicillin-binding proteins (PBPs) and inhibit peptidoglycan cross-linkage. This is made possible due to the presence of the isoxazolyl group on the side-chain of the penicillin nucleus which facilitates the β-lactamase resistance, since they are relatively intolerant of side-chain steric hindrance but it is not inactivated by β-lactamases. They are acid stable and have proven to be effective against S. aureus [35,36].

There are some antibiotics that have been found to be substandard and counterfeited [37,38]. Substandard and counterfeit antibiotics are also noted to be one of the main causes of bacterial resistance to antibiotics [39]. Reports on substandard and/or counterfeit antibiotics on various markets have triggered investigations into their quality and activity. Different approaches, both biological and chemical analysis are used in the evaluations. The unavailability of specific materials such as the type of column and solvent systems to be used in chemical analysis in some laboratories in some developing countries and comparison of the results with specifications in standard reference books such as United State Pharmacopoeia (USP) and the British pharmacopoeia (BP) have made it necessary for the modification and validation of the existing methods with materials readily available to suit the type of analysis being performed especially in resource restrain areas or settings.

**HPLC analysis of penicillin samples**

The internal standard (IS), caffeine, was selected based on the fact that caffeine did not interact with the sample and absorbs at the same wavelength as the sample but it did not have the same retention time as the sample.

HPLC method with a good linearity depicts the direct proportionality between concentration of analytes and the area under curve of the peaks. With correlation coefficient (r) of 0.9997 and R² of 0.9995 from the regression analysis of the calibration curve shows the direct proportional relationship between concentrations and peak area ratios. This represents an excellent linearity between them and how precise the HPLC method is. The method was shown to be linear. Observation of the calibration curve also confirms the linearity of the method developed (Figure 3).

The ability for the analyte of interest as far as this study is concerned, to elute in the presence of other compounds was ensured. A specific method is able to distinguish analyte even in the presence of other similar compounds. The ability of the amoxicillin to elute at the same retention time when spiked with the internal standard (Figure

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### Table 11: Statistical validation of the calibration data for quantitative determination of reference cloxacillin.

| Parameter | Cloxacillin |
|-----------|-------------|
| Concentration range | µg/mL |
| Number | 4 |
| Average values | 0.0025784 |
| Correlation coefficient | 0.9996 |
| Relative standard deviation (%) | 1.1340 |
| Calibration equation | y=787.78x + 0.0839 |
| Limit of detection (LOD) | 9.524×10⁻³ µg/mL |
| Limit of quantification (LOQ) | 2.886×10⁻² µg/mL |
| System suitability | 0.00275 |
| Method precision | 0.0336% |

LOD=3.3 × s/S, where s=SDEV of the responses, S=slope of the regression line

LOQ=10 × s/S, where s=SDEV of the responses, S=slope of the regression line

### Table 12: Internal standard, system suitability and precision parameters for reference cloxacillin.

| Number | % Recovery |
|--------|------------|
| 1 | 91.17 |
| 2 | 91.51 |
| 3 | 96.46 |
| 4 | 113.41 |
| Mean | 98.1375 |
| SDV | 10.46475 |

SDEV= Standard deviation, %RSD=Percent relative standard deviation, IS=Internal standard, AUC=Area under curve

### Table 13: Standard and internal standard recovery studies of reference cloxacillin (n=4).

| Concentration range | µg/mL |
|---------------------|-------|
| 0.0025784 | |

Other reason that could account for differences in literature values and that of present study is the inoculum size of test organisms. Gbedema et al. [32] reported MIC of 0.46, 640, 0.29 and 0.26 mg/mL against E. coli, P. aeruginosa S. aureus and B. subtilis 10⁸ cfu/mL using the agar diffusion method. The inoculum size used in the present study was 10⁶ cfu/mL and it is higher than the inoculum size used by Gbedema et al. [32]. This might have resulted in the higher MICs recorded for the samples compared to the values reported by earlier workers [28,32]. Besides that, the micro-dilution method used in the determination of the MIC is reported to be a better approach than the agar diffusion technique [20,21].

Beta-lactams are inhibited by the beta lactamases produced by bacteria and the size of inoculum will have direct influence on the performance of the antibacterial agent. The inoculum size will determine the amount of beta-lactamase present to deactivate the beta lactam ring [33].

Comparison results from the biological and chemical method revealed that some of the samples passed the chemical assay but had higher MIC values. For this reason higher doses of these samples of

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different concentrations of the same samples and they gave distinctive peaks of the two compounds at their respective retention times (Figure 2).

Analysis of the samples revealed that the content of all 16 different samples of the capsules were in the range of 81.53 to 104.34% (Tables 14 and 15). Twelve samples had their content within the USP [24] specification of 92.5 to 110.0%. The sample with API of 93.2% was analyzed just 2 years before its expiry and few months after manufacturing and this means that the probability of the product failing later analysis before its expiry may be high.

The amount of API in suspension samples was 92.3% and these values are below the acceptable limit [24]. Percentages of active ingredient range of the suspension samples were from 81.03 to 104.1%. Two batches were found to contain 81.0 and 81.33% active ingredient respectively and these samples have their API fall below the USP [24] specification. The fact that they were analyzed few months after their manufacture may indicate the samples may breakdown before expiry or did not contain the right amount of API. Almost 8% of the samples had their APIs below the USP [24] range.

After observing flow rates between 0.5 and 1 mL/min, the later was found to give an optimal signal-to-noise ratio with a reasonable separation and retention. In the quest of finding internal standard, various reference standards were used including amoxicillin cloxacin and flucloxacillin. Injection of flucloxacillin and cloxacin gave peaks with almost the same retention time and hence could not be used as the internal standard. Acetaminophen gave a retention time different from that of cloxacin and flucloxacillin. Hence, it was used as internal standard for the analysis of cloxacin and flucloxacillin samples. Environmental changes that could not be or difficult to control such as variations from run to run, temperature, pressure and power fluctuations during the run time were also monitored by the use of the internal standard in the analysis of the samples (Tables 9 and 12).

The limit of detection and limit of quantitative of the analysis indicate the sensitivity of the method. The direct proportional relationship between concentrations and peak area ratios with correlation coefficient R² of 0.995 for flucloxacillin and 0.9986 for cloxacin from the regression analysis of the calibration curves

| Sample / Amount / % API | 92.5 to 110% (USP, 2011) | 92.5 to 110% (USP, 2011) | 90-120% (USP, 2011) |
|------------------------|--------------------------|--------------------------|---------------------|
| **Amoxicillin capsules 250 mg** | | | |
| Sample code | Amount (mg) | % API | Sample code | Amount (mg) | % API | Sample code | Amount (mg) | % API |
| 01A | 260.85 | 104.34 | FLMG01 | 276.10 | 110.44 | CLLP01 | 156.00 | 62.40 |
| 01B | 227.80 | 91.12 | FLMG02 | 161.63 | 64.65 | CLLP02 | 177.75 | 71.10 |
| 02A | 255.95 | 102.38 | FLMG03 | 111.85 | 44.74 | CLLP03 | 145.18 | 58.07 |
| 02B | 244.83 | 97.93 | FLLP04 | 269.08 | 107.63 | CLAR04 | 139.60 | 55.84 |
| 03A | 203.83 | 81.53 | FLLP05 | 250.98 | 100.39 | CLAR05 | 201.95 | 80.78 |
| 03B | 240.15 | 96.06 | FLLP06 | 239.90 | 95.96 | CLAR06 | | |
| 03C | 244.53 | 97.81 | FLAR07 | 301.13 | 120.45 | CLMG | | |
| 04A | 230.07 | 92.03 | FLAR08 | 147.65 | 59.06 | | | |
| 05A | 237.45 | 94.98 | | | | | | |
| 06A | 217.20 | 86.88 | | | | | | |
| 06B | 253.48 | 101.39 | | | | | | |
| 06C | 238.58 | 95.43 | | | | | | |
| 08A | 232.97 | 93.19 | | | | | | |
| Amoxicillin capsules 500mg | | | |
| 07A | 480.00 | 96.00 | | | | | | |
| 07B | 481.85 | 96.37 | | | | | | |
| 09A | 493.15 | 98.63 | | | | | | |

Table 14: HPLC analysis of amoxicillin, flucloxacillin and cloxacin capsule samples.
In vitro Activity and Evaluation of Quality of Some Selected Penicillins on the Ghanaian Market using Developed HPLC Methods.

Citation: Boadu RF, Agyare C, Yiadom MA, Adu F, Boamah VE, et al. (2015) In vitro Activity and Evaluation of Quality of Some Selected Penicillins on the Ghanaian Market using Developed HPLC Methods. Med chem 5: 001-014. doi:10.4172/2161-0444.1000235

Table 15: HPLC analysis of amoxicillin, flucloxacillin and cloxacillin suspension samples.

| Sample code | Amount | % API |
|-------------|--------|-------|
| S01         | 117.56 | 94.05 |
| S02A        | 101.29 | 81.03 |
| S02B        | 114.15 | 91.32 |
| S02C        | 101.66 | 81.33 |
| S03A        | 120.56 | 96.45 |
| S04A        | 117.30 | 93.84 |
| S05A        | 98.38  | 78.70 |
| S06A        | 125.53 | 100.42|
| S06B        | 126.75 | 101.40|
| S06C        | 127.23 | 101.79|
| S07A        | 130.14 | 104.11|
| S08A        | 121.20 | 96.96 |
| S08B        | 110.53 | 88.42 |

API: active pharmaceutical ingredient

and these indicate the level of linearity. For five runs of the same homogenous reference solution (Tables 9 and 12) the suitability and precision of the method were in the acceptable limit as stated in USP [24] with SDEV of 0.0025 and %RSD of 0.25 for flucloxacillin and standard deviation of 0.028 and %RSD of 0.034 for cloxacillin. All these values were less than 2% in the USP [24]. The range of recovery for flucloxacillin and cloxacillin were 92.4 to 107.9% and 91.2 to 113.4% respectively with an average percentage recovery of 98.5% for flucloxacillin and 98.1% for cloxacillin. These represent a high level of accuracy of the methods.

In the evaluation of flucloxacillin samples (capsules) using the acceptance limit of 92.5 to 110 % as stated in USP [24], 5 out of 8 samples evaluated were within the specification of USP [24] with percentage of 95.96 to 120.45 representing 62.5% of samples. The remaining samples had API of 44.7 to 64.7% which did not meet the specification in USP [24].

All the samples of flucloxacillin suspension analyzed were in the range of 36.0 to 50.1%. These content are outside the USP [24] and BP [25] range of acceptance limit of 80 to 120%. These low amounts of APIs may be due to insufficient active ingredients or poor storage conditions of the samples leading to the degradation of the API.

Antibiotics of this quality are threat to patients, the nation, and the world at large. Patients receiving such antibiotics would obviously not respond to minimum doses and would have to resort to higher doses. The activity of these antibiotic samples that failed the various evaluations may lead to antibiotic resistance in previously susceptible organisms.

Ensuring the quality, efficacy and safety of antibiotics would go a long way to prevent the problems associated with substandard and counterfeit antibiotics. The regulatory authorities that are mandated to regulate medicines must intensify their effort to monitor the quality and conditions of storage conditions of these antibiotics in especially developing countries.

Conclusion

All the penicillin samples (amoxicillin, flucloxacillin and cloxacillin) evaluated showed activity against test bacteria (E. coli, P. aeruginosa, S. aureus and B. subtilis). The level of activity and concentrations of penicillin samples gave different zones of inhibitions against these bacteria. Amoxicillin was observed to have broad spectrum activity showing activity against all bacteria used in the evaluation. Flucloxacillin and cloxacillin samples were observed to have higher activity against Gram-positive bacteria as compared to Gram-negative bacteria. P. aeruginosa was found to be most resistant bacteria to the penicillin samples. Suspension samples exhibited higher activity compared to capsule formulations. The MICs of 200 to 800 µg/mL were recorded for amoxicillin samples whereas flucloxacillin and cloxacillin samples had MIC of 500 to 1900 µg/mL. All samples of flucloxacillin suspensions and cloxacillin capsules had their API below the USP specification. Almost 83% of amoxicillin samples contained the right amount of API compared to 32.1% of cloxacillin and 44.4% of cloxacillin samples having the right amount of API.

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