# Simultaneous quantification of five penicillins and detection of penicilloic acid in single and/or multi– component flucloxacillin pharmaceutical formulations

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**ABSTRACT**

**Background:** The role of β-lactams among antibacterial agents in the treatment and management of infectious diseases cannot be under-estimated. Swift actions regarding quality assessment of penicillins (most frequently prescribed β-lactam) supplied to patients especially in circumstances where the products do not conform to specifications remain a challenge, i.e. inability to detect quality products from counterfeit/substandard ones.

**Objectives:** The goals of this study were to develop a simple RP-HPLC method for the simultaneous quantification of amoxicillin trihydrate (AXT), ampicillin trihydrate (APT), benzylpenicillin sodium (BPS), flucloxacillin sodium (FXS) and phenoxymethyl penicillin sodium (PMS) in single and combination dosage forms, detect substandard penicillin antibacterial agents and also detect penicilloic acid, a major breakdown product of penicillins and a major semi-synthetic precursor of penicillins.

Methods: Separation was achieved on a Phenomenex® LICHROSORB 10 RP-1 $C_{18}$ column (250 × 4.60 mm I.D., 5 µm particle size) with methanol: 0.02M $KH_2PO_4$(pH = 3.70) (4:6v/v) in isocratic mode as mobile phase. A flow rate of 1.00 mL/min with UV detection at 225 nm was optimized, validated, and employed to assay samples from the Ghanaian market.

**Results:** The calibration plots for the determination of the penicillins showed correlation values ($r^2$) between 0.9984 - 0.9995 in the concentration range of 0.005 - 0.1%w/v. Accuracy for the analytical method was confirmed with mean recoveries (n=3) in the range of 99.88 - 101.33%, 100.01 - 100.66% and 99.10 - 100.79% at concentration levels of 80, 100 and 120% respectively. Method precision with relative standard deviation (RSD) obtained for intra-day precision were 1.62 - 4.03% and the inter-day precision were 1.22 – 3.97%. The developed method was found suitable for the detection of penicilloic acid and could distinguish between good quality and substandard penicillin antibacterial products sampled from Kumasi metropolis.

**Conclusion:** The method is suitable for the simultaneous assay of AXT, APT, BPS, PMS and FXS in either single/multi – component dosage forms. It is also applicable for routine screening of penicillin antibiotics

for adulteration and counterfeiting and the detection of penicilloic acid, a major decomposition product in penicillins.

**Key words:** Penicillins, Impurity profiling, RP-HPLC, Isocratic mode, Validation

**La quantification simultanée de cinq pénicillines et la détection de l'acide pénicillinique dans des formulations pharmaceutiques simples et/ou multiples des composants de la flucloxacilline**

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**RESUME**

**Contexte**: Le rôle des β-lactames parmi les agents antibactériens dans le traitement et la gestion des maladies infectieuses ne peut pas être sous-estimé. Des actions rapides concernant l'évaluation de la qualité des pénicillines (plus souvent β-lactame prescrit) fournies aux patients surtout dans des circonstances où les produits n'obéissent pas aux normes demeure un défi, c'est-à-dire l'incapacité de détecter les produits de qualité des faux/de qualité inférieure.

**Objectifs:** Les objectifs de cette étude étaient de développer une méthode RP-HPLC simple pour la quantification simultanée de la tri-hydrate d'amoxicilline (AXT), tri-hydrate d'ampicilline (APT), du sodium de benzyl-pénicilline (BPS), du sodium de flucloxacilline (FXS) et du sodium de pénicilline phénoxyméthyl (PMS) en formes de dosage unique et combiné, détecter les agents antibactériens de la pénicilline de qualité inférieure et également détecter l'acide pénicillinique, un produit de décomposition majeure des pénicillines et un précurseur semi-synthétique majeur des pénicillines.

**Méthodes:** Une séparation a été réalisée sur un Phenomenex® LICHROSORB 10 RP-1 $C_{18}$ colonne (250 × 4.60 mm I.D., 5 µm taille du particule) avec le méthanol: 0.02M $KH_2PO_4$(pH = 3.70) (4:6v/v) en mode Isocrate comme phase mobile. Un faible taux de 1.00 mL/min avec détection UV à 225 nm fut optimisé, validé, et employé pour essayer des échantillons du marché ghanéen.

**Résultats:** Les graphes de calibration pour la détermination des pénicillines ont montré des valeurs de corrélation ($r^2$) entre 0.9984 - 0.9995 dans l'échelle de concentration de 0.005 - 0.1%w/v. La précision pour la méthode analytique a été confirmée avec des récupérations moyennes (n=3) à l'échelle de 99.88 - 101.33%, 100.01 - 100.66% et 99.10 - 100.79% à des niveaux de concentration de 80, 100 et 120% respectivement. La précision de méthode avec écart type relatif (RSD) obtenu pour la précision intra-jour était 1.62 - 4.03% et la précision inter-jour était 1.22 – 3.97%.
La méthode développée a été jugée adéquate pour la détection de l'acide pénicillinique et pourrait établir une distinction entre la bonne qualité et la qualité inférieure des produits antibactériens de la pénicilline enquêtés dans la métropole de Kumasi.

**Conclusion:** La méthode est adéquate pour l'essai simultané des AXT, APT, BPS, PMS et FXS l'une ou l'autre forme de dosage unique ou multiple des composants. Cela s'applique au test de routine des antibiotiques à la pénicilline pour le dépistage de la dénaturation et la contrefaction et la détection de l'acide pénicillinique, un produit majeur dans la décomposition dans les pénicillines.

**Mots-clés:** Pénicillines, profilage d'impureté, RP-HPLC, mode Isocrate, validation

**INTRODUCTION**

Antibiotics are compounds of natural, semi-synthetic or synthetic origin which either kill or inhibit growth of microorganisms without significant toxicity to the human or animal host. Penicillins are a class of antibiotics originally produced by some members of the genus Penicillium. Penicillins can be classified in several ways. These include classification as natural or synthetic, acid stable or acid sensitive, penicillinase stable or sensitive and finally narrow or broad spectrum penicillins.[1] Penicillins continue to find use in the treatment and management of a large number of bacterial infections in humans and animals. The primary mechanism of action is inhibition of the bacterial cell wall synthesis.[2]

Despite the similarities among the penicillins physicochemically, they differ in their range or spectrum of activity owing to their different side chains.[2] They are therefore not interchangeable implying one cannot completely substitute one for the other. Substituting one penicillin for the other during manufacture constitutes drug counterfeiting.[3] Price variations among the different penicillin products available on the market make the penicillin industry a potential lucrative target of drug counterfeiting by drug counterfeiters.[4]

The mass production and marketing different brands of the same generic products once the patent has expired is increasing. Manufacturing errors resulting in legitimate drugs with no or wrong or sub-lethal amounts of active ingredients continue to exist. These activities continue to hinder efforts put up by national regulatory authorities aimed at reducing the availability and use of these products especially in sub-Sahara Africa.

Weak regulation of pharmaceuticals is a major contribution to poor drug quality and its related public health consequences including poor quality of healthcare, drug resistance, increase in the cost of healthcare and ultimately death of patients.[5]

Significant attempts have been made over the years in designing analytical methods that can detect and quantify different types of penicillins. The British Pharmacopoeia (BP) 1968 and 1973 described an acidbase method [6, 7], British Pharmacopoeia 1980 described an iodimetric method [8], British Pharmacopoeia 1993, Prakash et al, Dey et al and Gujral et al described UV spectrophotometric methods. [9,10,11,12] Nikam et al, Lieu et al and the British Pharmacopeia 2007, 2009 and 2103 currently propose high performance liquid chromatographic (HPLC) methods for the assay of these penicillins in their single dosage forms. [13,14,15,16,17] One analytical method for one penicillin usually proposed in the official pharmacopoeias such as the British Pharmacopoeia come with their unique challenges of requiring different specific reagents, solvents and conditions. The cost of these solvents and reagents makes the cost of analyses higher especially for developing countries such as Ghana.

Furthermore, such methods are suitable for the analysis of the individual penicillins in their single component products/formulations. They however lack selectivity when they are employed in the analyses of multicomponent formulations. This implies that differentiating between these different types of penicillins in an adulterated/counterfeited formulation still remains a challenge.

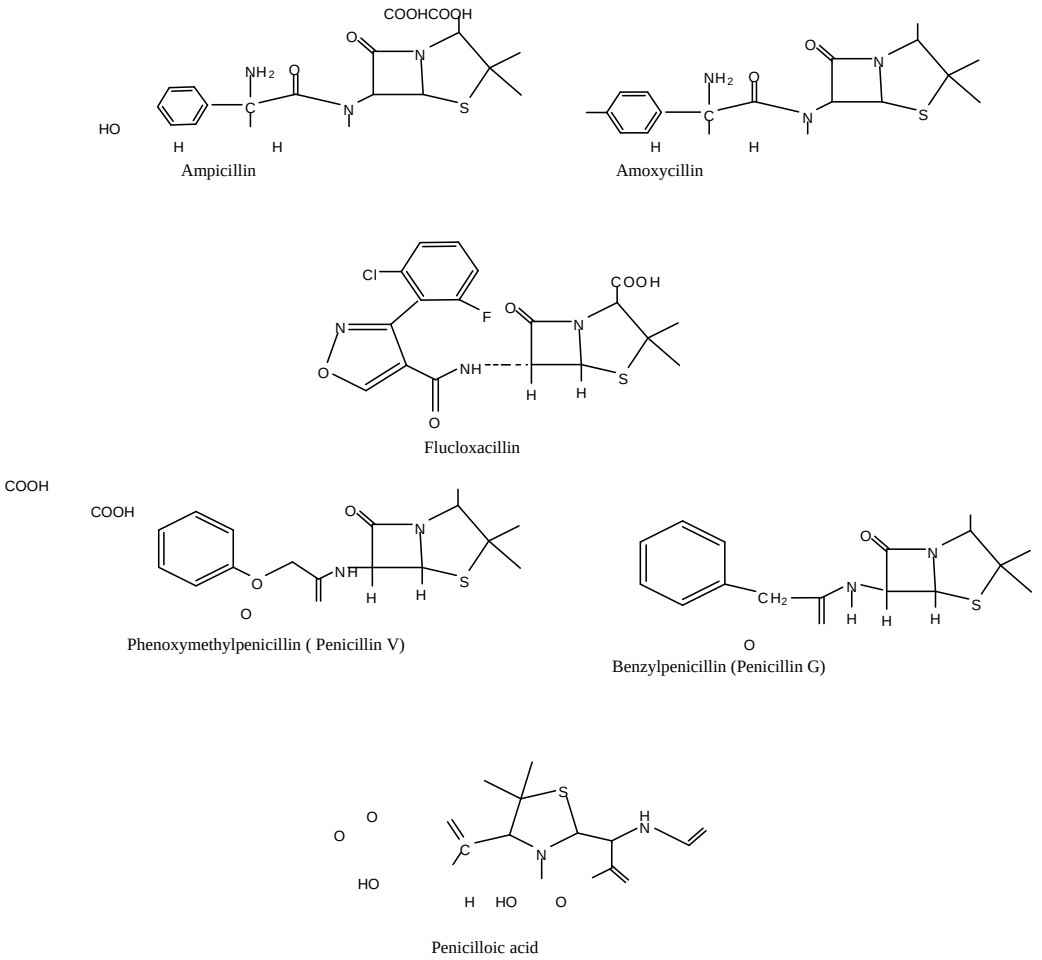



**Figure 1: Chemical structures and names of the five penicillins and penicilloic acid**

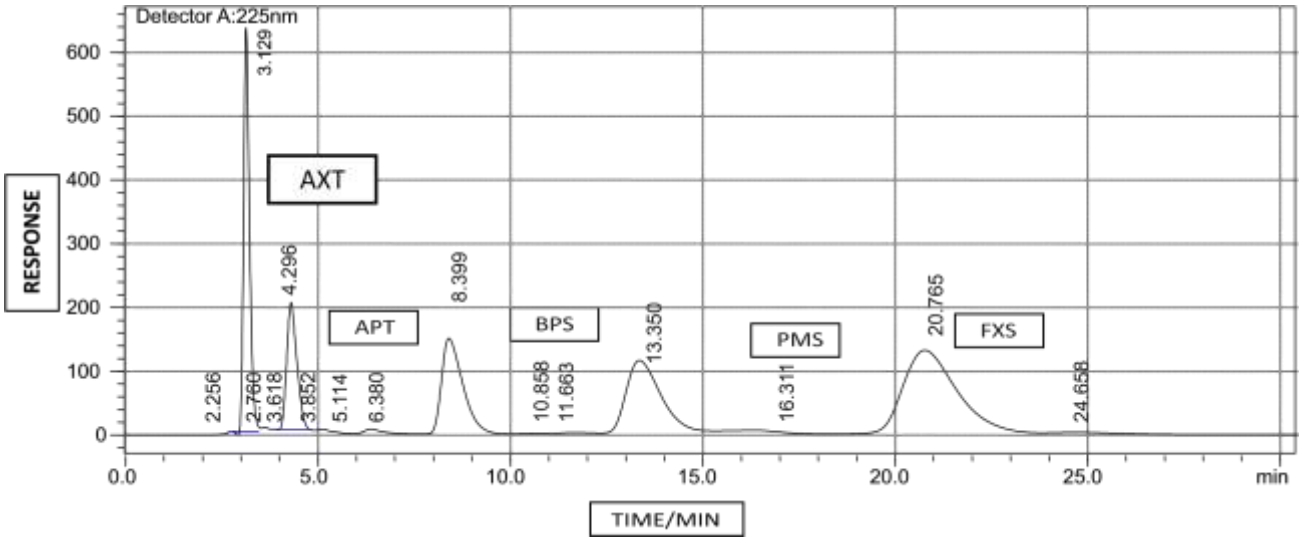
One major problem associated with the use of penicillin antibiotics is the hypersensitivity reactions some susceptible individuals have when they use them. Breakdown products such as penicilloic acids have been implicated in these reactions.[18] Penicillins on long storage degrade/breakdown to give penicilloic acid which can cause allergy in susceptible individuals. This means that penicillins during their entire shelf life should not contain above the specified limits (5%) of penicilloic acid.[19] Simple and inexpensive analytical methods that simultaneously detect penicilloic acid and assay these penicillins are therefore necessary for the routine monitoring of the products.

This study was focussed at developing a more rapid, simple, inexpensive and robust analytical technique for the simultaneous assay of five different types of penicillins and detecting penicilloic acid a common breakdown product. The chemical structures of the penicillins are shown in figure 1.

## MATERIALS AND METHODS

### Standards and Reagents

Flucloxacillin (96.0%), benzylpenicillin sodium powder and phenoxymethylpenicillin sodium reference standards were obtained from Letap Pharmaceuticals (Ghana), amoxicillin (98.80%) and ampicillin trihydrate (98.20%) reference standards were obtained from Ernest Chemist Ltd (Ghana). Potassium dihydrogen orthophosphate was of analytical grade and was purchased from BDH, Poole, England. Redistilled methanol was a general purpose grade from Fisher Scientific UK Limited, which has been freshly distilled




repeatedly to rid it of any impurities. All stock and working solutions were freshly prepared with distilled water for HPLC analyses.

**Instrumentation**

A Shimadzu Ultra-Fast Liquid Chromatograph with LC20AB Prominence solvent delivery system, DGU-20A3 Prominence degasser fitted with SDA-20A Prominence UV/VIS detector from Shimadzu Scientific Instruments, Germany was employed in the study. A mobile phase composed of redistilled methanol: 0.02M $KH_2PO_4$ (pH = 3.70) (4:6 v/v) at a flow rate of 1.00 mL/min. Chromatographic separation was achieved on a Phenomenex® LICHROSORB 10 RP-1 $C_{18}$ column (250 × 4.60mm I.D., 5µm particle size) with isocratic mode of elution with UV detection at 225 nm. Injection volume was 20 µL. All analyses were done at ambient temperature.

**Sample preparation**

Standard mixtures containing AXT, APT, BPS, FXS and PMS were prepared in distilled water by weighing accurately 60mg each of AXT and APT and 100mg each of BPS, FXS and PMS and dissolving to make 10mL of solution (stock solution). Then 1 in 10 dilutions was made to get a working solution such that concentration of 0.60 mg/mL was obtained for (AXT and APT) and 1mg/mL was obtained for BPS, FXS and PMS. Separate solutions containing the stated concentrations of the above penicillins were injected prior to the mixture to determine the retention times for the individual penicillins.

Method Development and optimization of analytical parameters

The investigation commenced with a 50:50 mixture of redistilled methanol and 0.02M $KH_2PO_4$ (pH = 3.70) at a flow rate of 1.00 mL/min which could not resolve completely all the five penicillins. The inorganic component (0.02M $KH_2PO_4$ (pH = 3.70) of the mobile phase system was increased steadily until no overlaps of the mixture of penicillins were observed and all five penicillins were neatly resolved. The phosphate component was increased to 60% and the methanol component reduced to 40% which gave well resolved

mV

peaks for the penicillins within a retention time of 22 minutes. A further increase in the phosphate component resulted in broadening of the peaks with an undue increase in the retention times. Change in flow rate from 1.00 mL/min to 0.50 mL/min resulted in peak broadening and longer retention times. Therefore, 4:6 (v/v) combination of redistilled methanol: 0.02M $KH_2PO_4$ (pH = 3.70) as mobile phase and 1.00 mL/min as flow rate was considered optimum for the HPLC analytical method. Chromatograms of individual penicillins and separated penicillins are as shown in Figures 2 - 6.

**Figure 2: Chromatogram of a mixture of five penicillins**

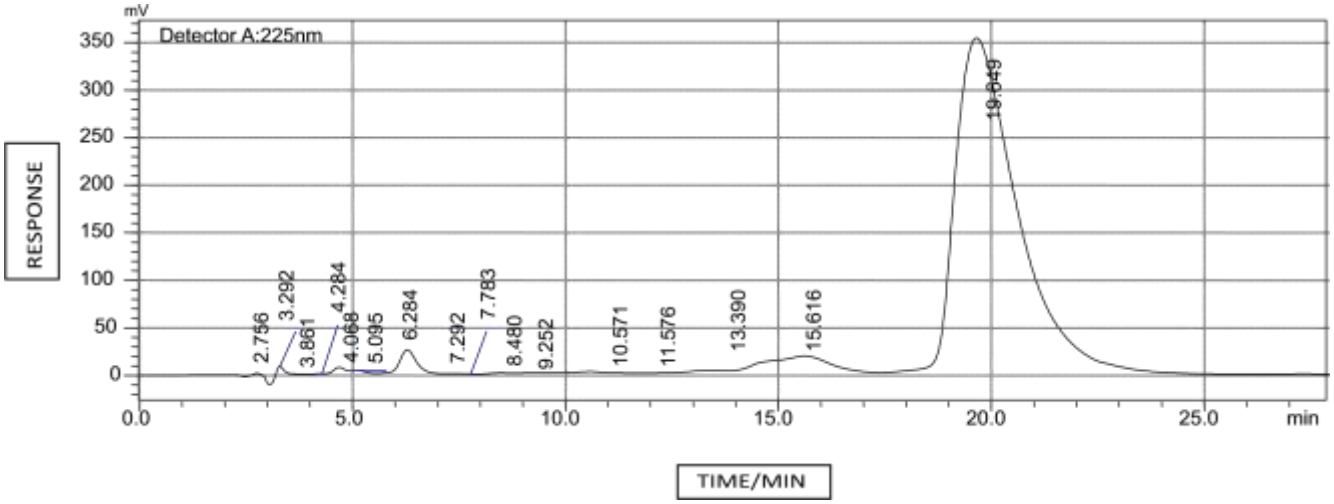



**Figure 3: Chromatogram of Flucloxacillin sodium (FXS)**



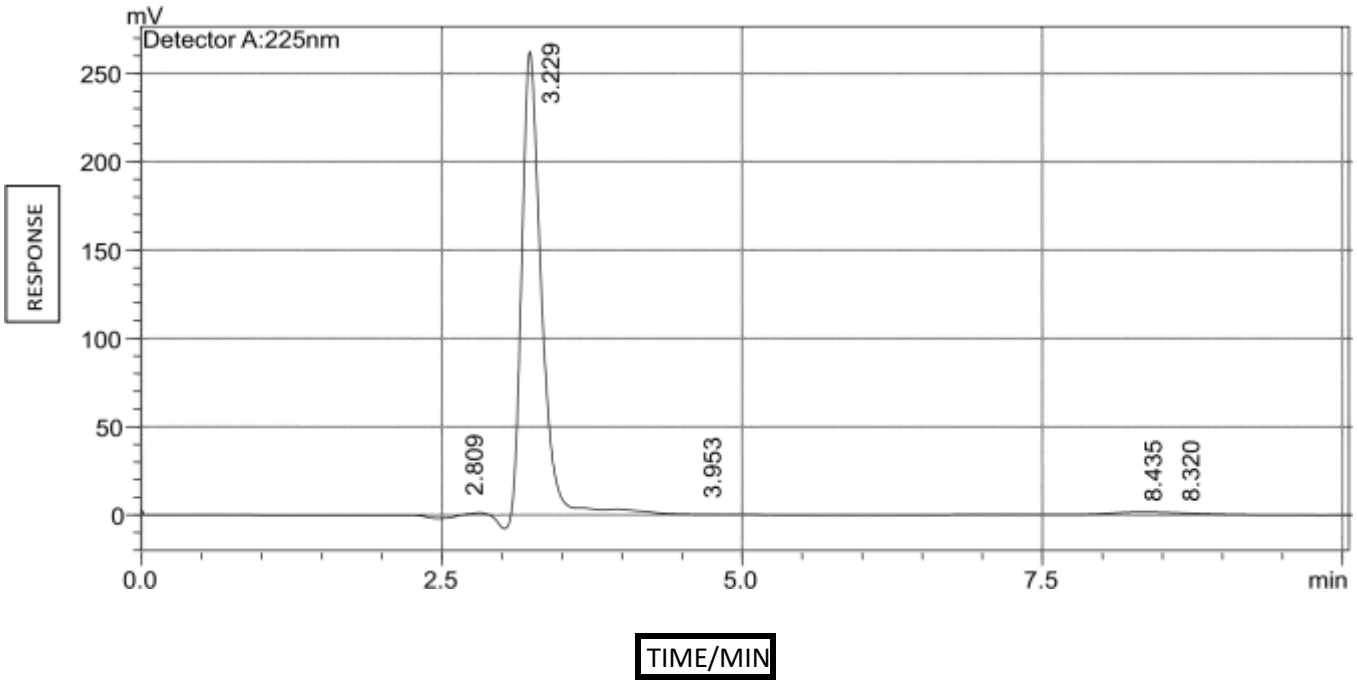


**Figure 4: Chromatogram of Amoxicillin trihydrate (AXT)**

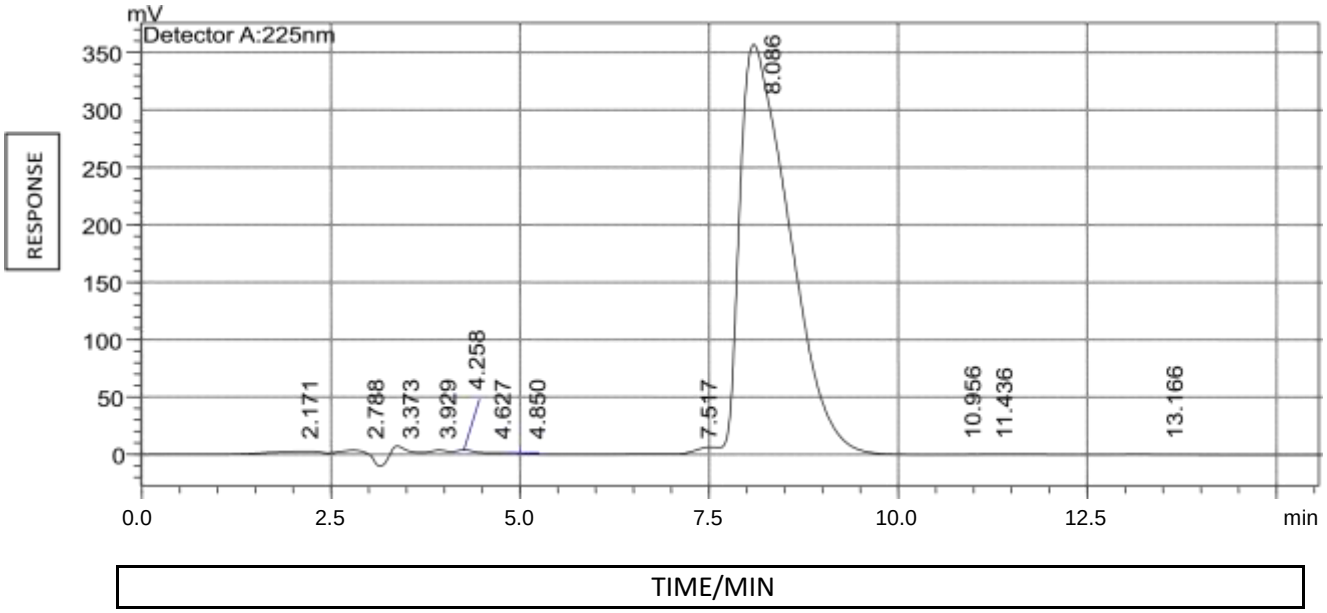



**Figure 5: Chromatogram of Benzyl penicillin sodium (BPS)**

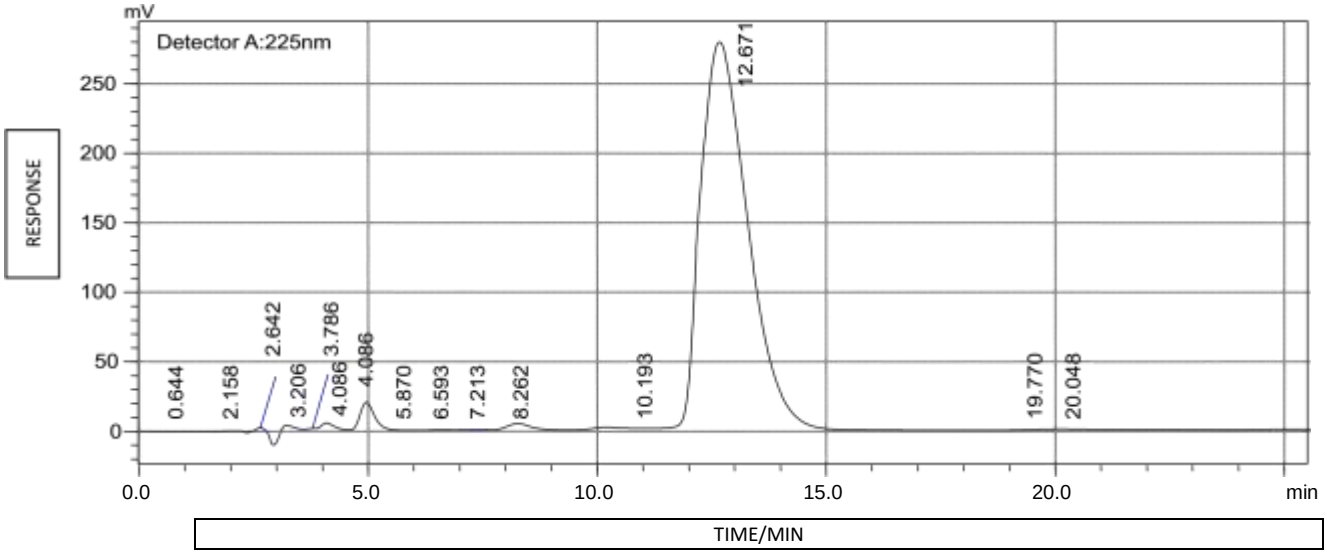



**Figure 6: Chromatogram of Phenoxymethyl penicillin sodium (PMS)**

**Validation of Developed Analytical method Linearity**

From the standard stock solution for AXT and APT, serial dilutions were made to obtain working solutions with concentrations of 0.03 mg/mL, 0.045 mg/mL, 0.06 mg/mL, 0.12 mg/mL, 0.24 mg/mL, 0.036 mg/mL, 0.48 mg/mL and 0.6 mg/mL. From the standard stock solution for BPS, FXS and PMS, serial dilutions were made to obtain working solutions with concentrations of 0.05 mg/mL, 0.075 mg/mL, 0.01 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1 mg/mL.

**Precision**

A solution made of 1 mg/mL of BPS, FXS, PMS and 0.6 mg/mL of AXT and APT was prepared. The intra-day precision was determined by analysis of replicate (n= 6) injections of the solution within the same day of preparation. The inter-day precision was determined by analysis of replicate (n= 18) injections of the solution: six injections each on three different days.

**Accuracy**

500mg each of BPS, PMS, FXS, magnesium stearate, plain carboxymethyl cellulose, starch and lactose and 300mg each of AXT and APT were accurately weighed into a clean glass mortar and triturated to ensure a uniform and homogeneous mixture. An amount of mixture equivalent to 40mg, 50mg and 60mg of BPS, PMS and FXS weighed accurately and transferred into a 50.00ml volumetric flask. Distilled water was added, shaken and sonicated for 3 minutes, then topped to the mark with more distilled water to give working solutions of 0.8 mg/mL (80% concentration level), 1 mg/mL (100% concentration level) and 1.2 mg/mL (120% concentration level). An amount of mixture equivalent to 24mg, 30mg and 36mg of AXT and APT were accurately weighed and taken through procedure described for BPS, PMS and FXS. The resulting working solutions had concentrations of 0.48 mg/mL (80% concentration level), 0.6 mg/mL (100% concentration level) and 0.72 mg/mL (120% concentration level). Each of these solutions were filtered and injected into the HPLC chromatograph three times and their mean percentage contents calculated as their mean recoveries.

**Limits of detection and quantification**

The limits of detection and quantification were estimated from the calibration curves obtained from the linearity of the respective analytes (AXT, APT, BPS, FXS and PMS) using their slopes and standard deviations of their responses. The formulae for the calculation of the LOD and LOQ are given below; LOD = $3.3\delta/S$ LOD = $10\delta/S$

Where $\delta$ = standard deviation of the response

S = slope of the calibration curve [20]

**Robustness**

The robustness of the method was determined by varying independently the flow rate and the wavelength of detection. A solution made of 1 mg/mL of BPS, FXS, PMS and 0.6 mg/mL of AXT and APT was

prepared. For flow rate variation, the same column, mobile phase system and detection wavelength were employed and the flow rate changed from 1.00 mL/min to 0.80 and 1.20 mL/min. For detection wavelength variation, the same column, mobile phase system and flow rate (1.00 mL/min) were employed and the detection wavelength changed from 225 nm to 223 and 227 nm. Injections were made into the HPLC chromatograph in triplicate. Graph pad prism version 5 was used to statistically analyse all the data obtained.

**Specificity**

The specificity of the analytical method was confirmed by injection only the mobile phase (blank solution) into the HPLC chromatograph. A solution made of 1 mg/mL of BPS, FXS, PMS and 0.6 mg/mL of AXT and APT was and analysed using chromatographic conditions described above

**Sampling of Products**

Products were sampled from retail pharmacies within Kumasi metropolis of Ghana. Different dosage forms of the products (capsule, suspension and parenteral) were obtained.

Analysis of various dosage forms of Penicillin drug products sampled

The developed analytical method was applied to samples of penicillin under study available in various pharmaceutical formulations. Drug solutions were prepared in distilled water to obtain an equivalent concentrations of 0.60 mg/mL were obtained for AXT and APT, and 1 mg/mL for BPS, FXS and PMS respectively in distilled water. For the capsules and suspensions drug solutions were sonicated for 3 minutes and filtered before analysis. Parenteral dissolved completely. Triplicate injections were made for each dosage form and their percentage contents calculated.

**Detection of penicilloic acid in stressed Flucloxacillin samples**

Flucloxacillin sodium solution of concentration 1 mg/mL was prepared. Sample solution was injected and remaining kept for 4 weeks at room temperature (25±2 °C). Samples were drawn from it and injected into the HPLC chromatograph weekly till the fourth week. For penicilloic acid detection, 5.00mL of 1M NaOH was added to 10.00ml of 1 mg/mL flucloxacillin sodium to induce the decomposition of flucloxacillin to penicilloic acid. Solution was allowed to stand for 20 minutes followed by addition of 5.00ml of 1M HCl to neutralize the NaOH initially added. Samples from this solution were injected into the HPLC chromatograph and chromatogram noted.

**RESULTS Linearity**

The calibration plots gave a straight line with correlation coefficient values ($r^2$) between the range of 0.9984 – 0.9995 for AXT, APT, BPS, FXS and PMS.

**Precision**

The relative standard deviation (RSD) obtained for the intra-day precision (repeatability) determinations were 1.62 – 4.03 for all penicillin analytes studied. The interday (intermediate precision) determinations obtained as relative standard deviation (RSD) were 1.22 – 3.97 for all penicillin analytes.

**Accuracy**

The accuracy was then calculated as the percentage of analyte recovery by the assay. Mean recoveries are shown in Table 1:

**Table 1: Mean percentage recovery for the penicillins at their various concentration levels**

| Sample | Mean Percentage Recovery (n=3) | | |
|---|---|---|---|
| | 0.48 mg/mL | 0.60 mg/mL | 0.72 mg/Ml |
| **AXT** | 99.88 | 100.57 | 99.10 |
| **APT** | 100.66 | 100.01 | 99.48 |
| | **0.80 mg/mL** | **1.00 mg/mL** | **1.20 mg/mL** |
| **BPS** | 100.30 | 100.66 | 100.79 |
| **PMS** | 100.48 | 100.48 | 100.41 |
| **FXS** | 101.33 | 100.51 | 100.40 |

**Limit of detection and quantitation**

The LOD's and LOQ's were calculated as percentage weight in volume (%w/v). Data obtained is as shown in Table 2.

**Table 2: LOQ's and LOD's for the various penicillins**

| Sample | Slope Of Calibration Curve | Standard Deviation Of Response | Limit Of Detection (LOD)/$\%^{w}/_{v}$ | Limit Of Quantification (LOQ)/$\%^{w}/_{v}$ |
|---|---|---|---|---|
| **AXT** | $2.88\times10^{8}$ | 224959.9 | 0.002578 | 0.007811 |
| **APT** | $1.79\times10^{8}$ | 153502 | 0.002830 | 0.008576 |
| **BPS** | $9.43\times10^{8}$ | 125651 | 0.004397 | 0.013325 |
| **FXS** | $3.17\times10^{8}$ | 290951 | 0.003029 | 0.009178 |
| **PMS** | $1.85\times10^{8}$ | 290595 | 0.005184 | 0.015708 |

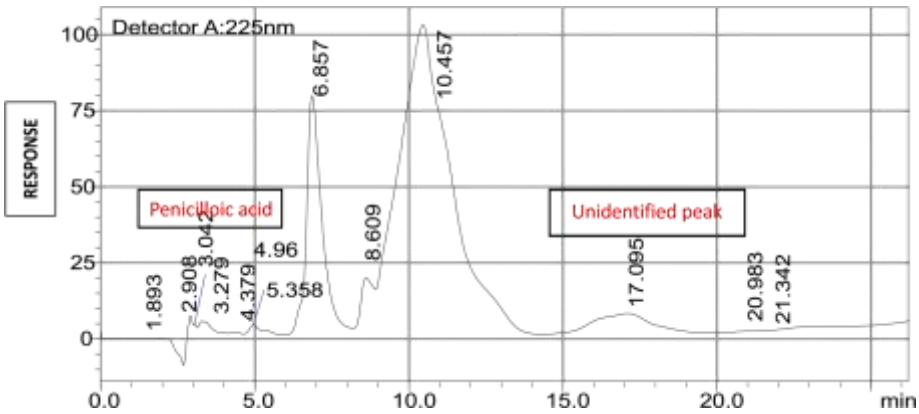
**Robustness**

For all the 5 penicillins and at all the various conditions, obtained for the mean percentage recoveries obtained the mean percentage recoveries obtained were found for flucloxacillin (as representative) are presented in not to be significantly different at 95% confidence Table 3

interval using the graph pad prism version 5. The results

**Table 3: Percentage recovery for 0.10%w/v flucloxacillin sodium investigated for a different flow rate and wavelength**

| Determination | Flow rate variation | | | Wavelength variation | | |
|---|---|---|---|---|---|---|
| | percentage recovery (%w/w) | | | percentage recovery (%w/w) | | |
| | 0.80 mL/min | 1.00 mL/min | 1.20 mL/min | 223 nm | 225 nm | 227 nm |
| **1** | 101.09 | 100.25 | 100.73 | 99.75 | 100.25 | 99.15 |
| **2** | 100.10 | 99.83 | 99.14 | 101.81 | 99.83 | 101.89 |
| **3** | 101.71 | 100.50 | 100.11 | 102.45 | 100.50 | 100.49 |




**Specificity**

No responses were obtained with the injection of the mobile phase. Well resolved peaks were obtained when a solution of 1 mg/mL of BPS, FXS, PMS and 0.6 mg/mL of AXT and APT was prepared and analysed with chromatographic conditions described under section 2.2. Chromatogram is shown in figure 2 above.

**Analysis of Different Penicillin dosage forms sampled**

A total of twenty two (22) samples were analysed. Six (6) products containing amoxicillin (3 locally manufactured/3 of imported origin), five (5) products containing benzylpenicillin (all of imported origin), four (4) products containing phenoxymethyl penicillin (all locally manufactured), three (3) products containing ampicillin (locally manufactured) and four (4) products containing flucloxacillin (3 locally manufactured and I of imported origin). Ten (10) products (4 locally manufactured and 6 imported products) representing 46% of the products analysed were substandard. The locally manufactured products were one (1) brand of amoxicillin capsules: average content of 84.25% (reference: 92.5 -110%) [17], two (2) different brands of phenoxymethyl penicillin tablets: average contents between 85.40 – 87.30% (reference: 92.5- 107.5%) [17] and one (1) brand of ampicillin injection: average content of 75.60% (reference: 95.0 – 105.0%).[17] The imported products two (2) different brands of

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amoxicillin suspension with average content between72.60 – 83.50 (reference: 80-120%) [17], three (3) different brands of benzylpenicillin injections with average content between 81.40 – 87.15% (reference: 95 -105%) [17] and one (1) brand of flucloxacillin with average content of 88.25% (reference: 92.5 -110%) [17]

Detection of penicilloic acid in stressed Flucloxacillin samples

The chromatogram of flucloxacillin after induction of decomposition by NaOH (alkaline hydrolysis to penicilloic acid) is shown in figure 7 below. Chromatograms of flucloxacillin solutions kept at room temperature (25±2°C) for 7 days and 14 days are shown in figures 8 and 9 below. Penicilloic acid, a major breakdown of penicillins was detected.

TIME/MIN

**Figure 7: Chromatogram of flucloxacillin after alkaline hydrolysis**

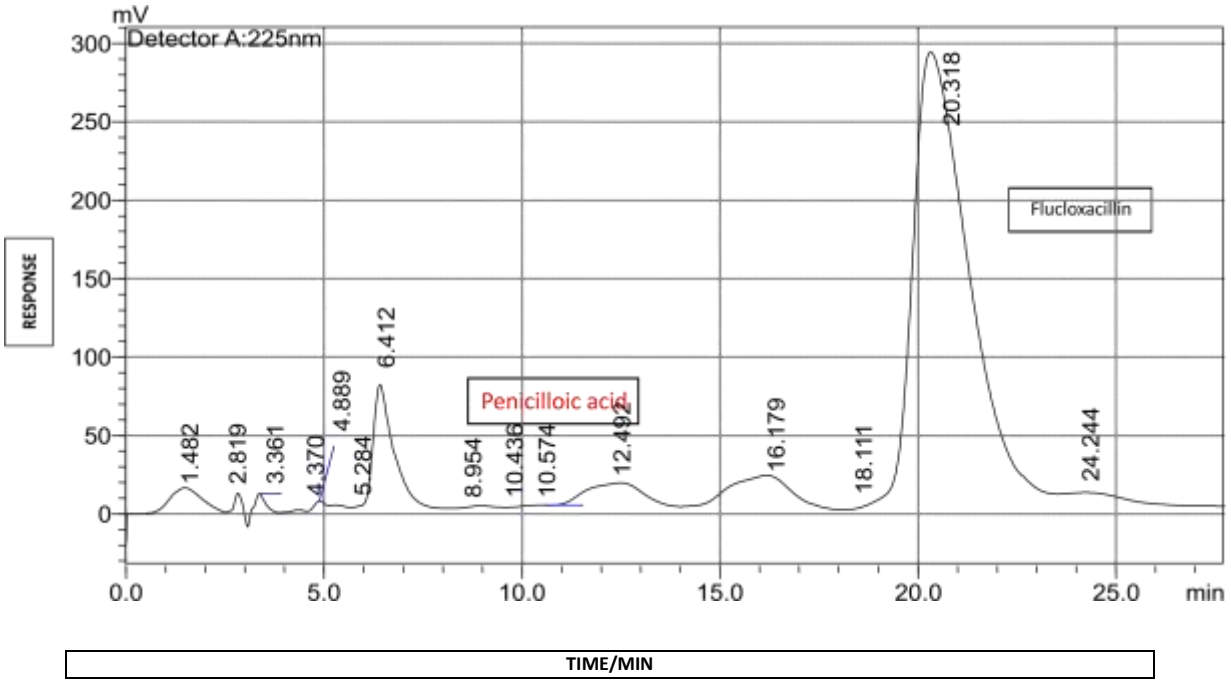


**Figure 8: Chromatogram of flucloxacillin sodium solution kept at room temperature for 7 days**

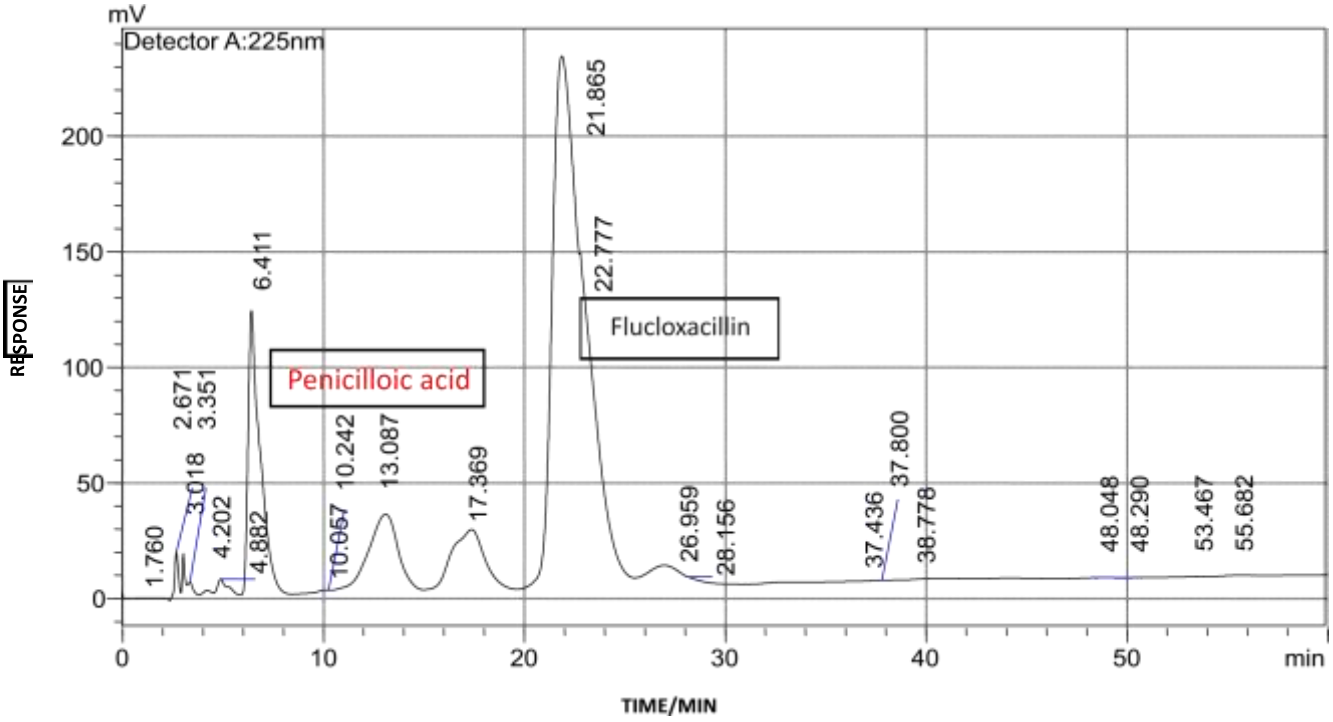


**Figure 9: Chromatogram of flucloxacillin sodium solution kept at room temperature for 14 days**

## DISCUSSION

The main goal of this study was to develop a simple RPHPLC method for the simultaneous quantification of amoxicillin, ampicillin, benzylpenicillin, flucloxacillin and phenoxymethyl penicillin in either single or multi – capsule dosage forms. The choice of methanol (redistilled) and $KH_2PO_4$ was made because they are cheap and readily obtainable. Methanol redistilled is a general purpose methanol that has been freshly distilled repeatedly to rid of any impurities. It is cheaper

than the conventional HPLC grade methanol. Potassium dihydrogen orthophosphate ($KH_2PO_4$) is also a common and readily available salt compared to other salts. A detection wavelength of 225 nm was chosen from literature.[16, 17]

Routine analyses of closely related drugs have always been a challenge in Ghana and Sub – Saharan Africa as a whole due to the lack of simple and inexpensive analytical methods that can distinguish one drug from another. Penicillin antibiotics are classical examples, all having the β-

products are desired. This method developed is decomposition of penicillins are shown below in versatile, simple, and robust and compared to figure 10. other analytical methods reported. Scheme for the

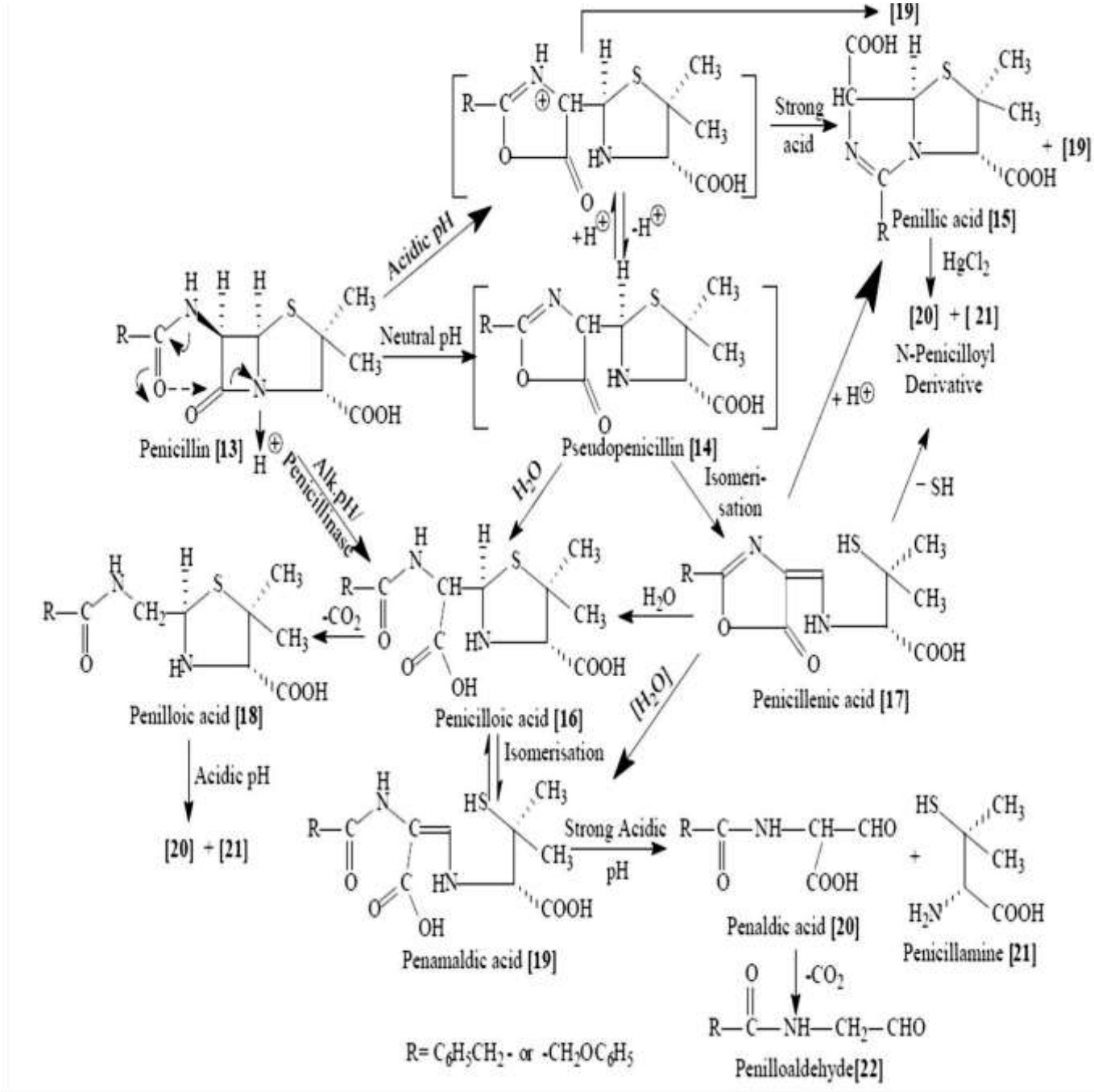


**Figure 10: Scheme for the breakdown of penicillins in different media.** [18]

lactams ring with the only difference being the side chain. This method developed presents a multi – purpose and a relatively cheaper solution to addressing the issue. The method developed in this study can detect and assay any of the five penicillins either in their respective single or in a multi – component dosage forms. It also presents a powerful tool to the regulatory bodies in respect of detecting adulteration/counterfeiting of these penicillins, which has become a routine practise of manufacturers as a means of making illegitimate gains without any regards to its consequences. One major problem associated with the use of penicillin antibiotics is the hypersensitivity reactions some susceptible individuals have when they use them. Hydrolysis of the penicillins give breakdown products such as penicilloic acid have been implicated in these reactions[18] The penicilloic acid among other breakdown products comes about when the drug is exposed to acids, bases, alcohols, penicilinases and moisture. [18] Again this method can detect penicilloic acid which previous methods mentioned earlier in the British Pharmacopeia (1968, 1973, 1980, 1993, 2007 2009 and 2013) cannot do. Critical look at our climatic conditions (warm and humid), which facilitates the decomposition of most drugs including penicillins, analytical methods that do not only detect and assay but also detects harmful breakdown

**CONCLUSION**

The proposed HPLC method is suitable for the assay of flucloxacillin sodium in flucloxacillin only dosage forms and also for the simultaneous assay of flucloxacillin sodium, amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium and phenoxymethylpenicillin sodium in combined dosage forms. The proposed method can also be used to detect breakdown products of flucloxacillin sodium and monitor its breakdown profile. It can also be used to detect any adulteration of any of the above penicillins with one another. It also has an advantage of being simple and relatively cheaper and can therefore be used for routine analysis and for regulatory purposes.

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