Co – amoxiclav for paediatric use comes in the form of an oral powder, which has to be reconstituted before administration. Concerns have been raised regarding the appropriateness of using certain types of water for the reconstitution of oral powders such as that of co – amoxiclav into suspensions. A mini-survey was conducted to ascertain the variations in the types of water used for the reconstitution of co-amoxiclav as well as the existing storage conditions for the oral powder. Distilled, treated tap water and mineral water were used for the study. The conductance and pHs of these types of water were recorded, before they were used for reconstitution. A stability study was carried out on different brands of co – amoxiclav suspensions which were reconstituted with distilled water (the recommended choice), treated tap water and commercial mineral water and kept under the standard storage condition of 2 - 8ºC. Both compounds (amoxicillin and clavulanic acid) were considered stable if they retained ≥90% of their initial concentrations. From the study, it was found that the three kinds of water, irrespective of their mineral or ionic content, had no significant detrimental effect on the stability of amoxicillin and clavulanic acid, throughout the duration of therapy (7 days). Thus, the above mentioned types of water can be conveniently used for the reconstitution of co – amoxiclav in our part of the world. Further analysis was carried out to ascertain the stability of amoxicillin and clavulanic acid in their oral suspension (reconstituted with distilled water) when there were inconsistencies in the standard storage condition of 2 - 8ºC. Both amoxicillin and clavulanic acid remained stable although oral suspension was kept in (2 - 8ºC) and out of the fridge (25ºC) throughout the 7 day period of therapy. However, the standard storage temperature should be adhered to stringently to guarantee maximum therapeutic benefit. A simulation was carried out where co – amoxiclav oral suspension was kept in a bowl of water under normal
room temperature and analysed. This also revealed that amoxicillin remained stable throughout the duration of therapy but clavulanic acid did not. Physical compatibility was assessed by visual observation for discoloration and precipitation throughout the duration of therapy. The chemical stability of the drugs was analysed by a simple and cost effective HPLC method.

INTRODUCTION
The stability of a drug or any product is the time from manufacture and packaging of the product to the time when its chemical activity is not lower than a predetermined level of labelled potency. Its physical characteristics should also be intact. Generally, 90% of labelled potency is generally regarded as the minimum acceptable potency level[I]. A stable drug should also be able to guard against microbial contamination. The parameters that are peculiar to stability include; environmental conditions of storage such as temperature, light, air, humidity and the type of packaging. Pharmacopeial articles must have the requisite storage conditions on their labelling. These are the conditions within which the expiration date is valid. Storage conditions specified on the labelling of the article must be adhered to, all through the supply of the article. It is however very uneasy to monitor and control storage conditions once product ends up with the consumer. Patient may not adhere stringently to instructions pertaining to the handling of drug (especially when it comes to oral powder for reconstitution) even though labelling might clearly spell out the appropriate storage conditions [II]. All penicillins including A moxicillin have the beta-lactam ring as part of their structure. This β-lactam ring is very reactive but can be split open in either a neutral or basic medium, resulting in its inactivity[III]. Although amoxicillin is still susceptible to destruction by Staphylococcal enzymes, it does have a much broader spectrum against the gram negative cell wall[IV]. Antibiotic susceptibility testing is essential to enable the identification of organisms that are resistant to penicillins such as amoxicillin and ampicillin [V]. Amoxicillin is usually prescribed with clavulanic acid as the potassium salt. Clavulanic acid is a naturally occurring, β-lactamase inhibitor, produced by fermentation of Streptomyces clavuligerus for treatment of infection caused by β-lactamase producing bacteria that are resistant to amoxicillin alone[VI]. The stability of potassium clavulanate is very low, as such their formulation as an oral powder for reconstitution[VII]. Amoxicillin and clavulanic acid (Co – amoxiclav) oral powder for reconstitution can be stored under normal room temperature (25ºC), for as long as the expiry date will allow. After, reconstitution however, the suspension has to be strictly stored in a refrigerator (2 – 8ºC) to ensure stability during dosing regimen (5 or 7 days). Studies have indicated different ‘drug in – home’ storage practices such as the keeping of drugs on dining tables, on top of refrigerators, inside first aid boxes, in bags, in the car, within closed cabinets, suit cases and the like as well as in the kitchen and bathroom. These practices may result in degradation[VIII]. Will non-adherence to the standard storage condition of 2 - 8ºC affect stability? Should reconstitution of the powder be done with a particular type of water, or the type of water can be varied? Will there be any significant effect on the stability of amoxicillin – clavulanic acid oral suspension?
This study aimed at conducting a stability assessment of different brands of reconstituted co-amoxiclav suspension by ascertaining whether or not there is variation in the type of water used for the reconstitution of amoxicillin – clavulanic acid oral powder for suspension as well as developing an HPLC method of assay for amoxicillin – clavulanic acid combination therapy. The study also analyzed the effect of different qualities of water on the stability of reconstituted amoxicillin – clavulanic acid oral suspension. The stability of reconstituted amoxicillin – clavulanic acid oral suspension under different storage conditions including patient simulated conditions was assessed as well.

MATERIALS AND METHODS
The amoxicillin trihydrate reference powder was obtained from Ernest chemist laboratory, whilst that of clavulanic acid potassium was obtained from Shandong New Time Pharmaceutical Co. Ltd. These reference standards were assayed by iodimetry and acid-base titration respectively. Distilled water, treated tap water and commercial mineral water were used for the reconstitution of three brands of co-amoxiclav suspension including the innovator brand. These were obtained commercially. A mini-survey was first conducted by the distribution of questionnaires to health personnel in selected health facilities (both hospital and community pharmacies) in the Kumasi metropolis to find out whether or not there are variations in the type of water used in the reconstitution of co-amoxiclav oral powder and the consistency of usage of a particular type of water. The health personnel included medical counter assistants, dispensing technologists and the pharmacists. The sample size was over 100 with 86 respondents. The data obtained was analysed by Microsoft Excel. For the laboratory analyses, all chemicals used were of analytical grade.

Experimental Procedure
The pHs and conductance of distilled water, treated tap water and commercial mineral water were measured before their use for the reconstitution of three samples each of the three brands (A, B, C). The pHs of the three brands of co-amoxiclav oral suspensions were monitored throughout the 7 day period of therapy. A sample each from the three brands (A, B, C) was reconstituted with distilled water and kept in and out of the fridge. Thus, samples were kept in the fridge only on alternate days throughout the 7 day duration of therapy. For the patient simulated storage condition, two samples of brand B were each reconstituted with a randomly selected commercial water sample (sachet water). One sample, after reconstitution was kept in the refrigerator whilst the other was kept in bowl of water and in a cupboard. All reconstituted samples were analysed each day by a validated HPLC method.

Materials and chromatographic conditions
HPLC, aCecil CE 2041 2000 Series-UV spectrophotometer and a pH meter (Eutech instruments pH 510) were used for analyses. Chromatographic analyses were carried out with an HPLC system (Kontron instruments) equipped with a pump and a UV-Visible detector with detection at 220nm. Solutions of samples to be analysed were
prepared by pipetting 1.00ml of each of the reconstituted suspension was pipetted and carefully transferred quantitatively into a 100.00ml volumetric flask respectively. About 50.00ml of distilled water was added and vigorously shaken to ensure dissolution of the active ingredients. More distilled water was added and made up to the 100.00ml mark. The solutions were then filtered by means of a No. 1 Whatman filter paper as well as a membrane filter before injection. The concentration of final solution expected was 0.0800%w/v and 0.0114%w/v for amoxicillin and clavulanic acid potassium respectively.

Concentrations of amoxicillin and clavulanic acid were determined by a stability indicating HPLC assay. Separation of the compounds was based on an isocratic method of elution. A mobile phase system of water, Na acetate buffer of pH 4.4 and methanol in the ratio of 65:20:15 was found out to be an effective and efficient mobile phase composition. The mobile phase was pumped through the column at a flow rate of 1ml/minute. The volume of sample injected was 100µl in each case.

RESULTS AND DISCUSSION
TLC results of reference samples.
Mobile phase system (Amoxycillin) comprised of ethyl acetate, glacial acetic acid and water in the ratio 3:1:1 respectively, whereas that of Clavulanic acid comprised of ethylacetate, methanol and water in the ratio 3:1:4 respectively.

![Fig. 1 TLC for amoxicillin](image1)  ![Fig. 2 TLC for clavulanic acid](image2)

The average Rₜ values for Amoxicillin and Clavulanic acid were found to be 0.77 and 0.56 respectively.

Results for survey conducted in health facilities in the Kumasi Metropolis, Ghana.
Fig. 3 Total number of health personnel from survey.

Fig. 4 Reconstitution done by health personnel in health facilities

Fig. 5 Types of water for reconstitution
Fig. 6 Variation in water for reconstitution

Table 1. Conductivity results for types of water used for reconstitution of amoxicillin-Clavulanic acid oral powder for suspension

<table>
<thead>
<tr>
<th>Type of water</th>
<th>pH</th>
<th>Measured Conductance (µs)</th>
<th>Conductivity(µs/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral water (volitc)</td>
<td>6.30</td>
<td>01.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Distilled water</td>
<td>6.00</td>
<td>02.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Treated Tap water</td>
<td>6.00</td>
<td>495.00</td>
<td>495.00</td>
</tr>
</tbody>
</table>

HPLC results of analysis

Fig. 7 chromatogram of pure Amoxycillin.  
Fig. 8 chromatogram of Clavulanic acid
Table 2 Retention times

<table>
<thead>
<tr>
<th>Retention time (clavulanic acid)</th>
<th>2.96 ± 0.01 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (amoxicillin)</td>
<td>4.09 ± 0.02 (min)</td>
</tr>
</tbody>
</table>

Calibration curves for amoxicillin and clavulanic acid were drawn and were linear for concentration ranges of 0.0100 - 0.1000%w/v and 0.01425%w/v to 0.00015%w/v respectively. The Coefficient of correlation $R^2$ for amoxicillin was 0.9979 and the equation of the line was $y = 66.262x + 0.0984$ whilst clavulanic acid had its coefficient of correlation $R^2$ being 0.9969 and its equation of line as $y = 61.166x + 0.0824$. The LOD and LOQ for amoxicillin were 0.00614%w/v and 0.0186%w/v respectively and the LOD and LOQ for clavulanic acid were 0.00126%w/v and 0.003818%w/v for clavulanic acid respectively.

The method proved to be precise with an RSD of 0.76% (intraday) and 1.03% (interday) for amoxicillin and 0.2% (intraday) for clavulanic acid. The percentage recoveries ranged from 99.2% - 99.83% with RSD ≤ 2% indicating accuracy.

Stability studies on co-amoxiclav suspension

Stability profiles of amoxicillin and clavulanic acid in brand A reconstituted with different types of water (2 - 8°C).

Table 3 pHs of Brand B (sachet water) and stored in a fridge and bowl of water.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand B, Fridge</td>
<td>4.60</td>
<td>4.70</td>
<td>5.50</td>
<td>5.50</td>
<td>5.50</td>
<td>5.50</td>
<td>5.50</td>
</tr>
<tr>
<td>Brand B, in bowl of water</td>
<td>4.60</td>
<td>5.50</td>
<td>6.00</td>
<td>6.30</td>
<td>6.40</td>
<td>6.60</td>
<td>6.60</td>
</tr>
</tbody>
</table>

![Stability profile of clavulanic acid in brand A (2-8°C)](image)

Fig.9 Clavulanic acid in brand A (2-8°C)
pH of sampled sachet water = 5.50 – 5.70
Results for assay of oral suspension B in sachet water (simulation in bowl of water) (25°C)

Chromatographs for Brand B suspension in sampled sachet water

Table 4 HPLC simulation results of brand B (sachet water, in bowl of water)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area(C.A)</td>
<td>0.7660</td>
<td>0.7500</td>
<td>0.7260</td>
<td>0.6520</td>
<td>0.5060</td>
<td>0.4480</td>
<td>0.3300</td>
</tr>
<tr>
<td>%w/v(C.A)</td>
<td>0.0112</td>
<td>0.0109</td>
<td>0.0105</td>
<td>0.0093</td>
<td>0.0069</td>
<td>0.0060</td>
<td>0.0041</td>
</tr>
<tr>
<td>%content(C.A)</td>
<td>98.3</td>
<td>95.6</td>
<td>92.1</td>
<td>81.6</td>
<td>60.5</td>
<td>42.6</td>
<td>36</td>
</tr>
<tr>
<td>Area (Amox)</td>
<td>5.6100</td>
<td>5.6000</td>
<td>5.5900</td>
<td>5.5400</td>
<td>5.5320</td>
<td>5.5280</td>
<td>5.2780</td>
</tr>
<tr>
<td>%w/v (Amox)</td>
<td>0.0832</td>
<td>0.0830</td>
<td>0.0829</td>
<td>0.0821</td>
<td>0.0820</td>
<td>0.0819</td>
<td>0.0782</td>
</tr>
<tr>
<td>%content(Amox)</td>
<td>104</td>
<td>103.7</td>
<td>102.6</td>
<td>101.6</td>
<td>100.3</td>
<td>99</td>
<td>97.8</td>
</tr>
</tbody>
</table>
Fig. 13: Stability profile of amoxicillin and clavulanic acid (brand B) kept at 25°C

Table 5 pHs of Brands A, B and C oral suspensions kept in and out of fridge (2-8°C, 25°C)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand C</td>
<td>4.50</td>
<td>4.90</td>
<td>5.50</td>
<td>6.00</td>
<td>6.30</td>
<td>6.50</td>
<td>6.30</td>
</tr>
<tr>
<td>Brand A</td>
<td>4.40</td>
<td>4.90</td>
<td>5.20</td>
<td>5.70</td>
<td>6.30</td>
<td>6.50</td>
<td>6.30</td>
</tr>
<tr>
<td>Brand B</td>
<td>4.90</td>
<td>4.90</td>
<td>5.20</td>
<td>5.50</td>
<td>6.30</td>
<td>6.50</td>
<td>6.60</td>
</tr>
</tbody>
</table>

Stability profiles of amoxicillin and clavulanic acid in brands A, B and C reconstituted with distilled water and kept in and out of fridge (2-8°C, 25°C)

Fig. 14: Stability profile of amoxicillin and clavulanic acid in brand C (2-8°C, 25°C)
Discussion

From the survey conducted, University hospital (K.N.U.S.T hospital) had a distillation apparatus and hence used distilled water in their reconstitution of oral powders for patients whilst Suntreso government hospital used mineral water (voltic) for its reconstitution. From analysis, the type of water used for reconstitution irrespective of its ionic or mineral content had no significant detrimental effect on amoxicillin and clavulanic acid. Amoxicillin and Clavulanic acid were considered to be stable if they retained 90% or more of their baseline (initial) drug concentration\[IX\]. Changes in pH were facilitated at higher temperatures such as that of room temperature (25°C).
Amoxicillin and clavulanic acid remained stable in all three brands irrespective of the type of water used for their reconstitution. Brand B oral suspension after being kept in a bowl of water throughout the duration of therapy had its clavulanic acid content falling to 36% of the initial content by the 7th day whereas that of amoxicillin was at 97.8% by the 7th day. Oral suspensions of brands A, B and C were kept in and out of the fridge throughout the 7 day period of therapy, but breakdown products were visible only in chromatographs of brand B.

Amoxicillin and clavulanic acid can be said to have remained stable in all three brands despite the inconsistency in the standard storage condition of 2 - 8°C. From the stability profile of brand B which was kept in and out of the fridge, it was observed that there was an increase in the level of breakdown of both amoxicillin and clavulanic acid when suspension was brought out of the fridge into room temperature.

Physical observation of brands A, B and C throughout the 7 day period of intermittent storage in the refrigerator was done [X]. There was no visible evidence of precipitation, or gas formation throughout the storage period in all three samples. Brand A and B maintained their original colour of creamy white (off white) throughout the 7 day period (Fig.21 – Fig.23). Brand C, however by the 4th day had started discolouring (browning) and partitioning into two distinct phases, a lower creamy yellow portion and an upper brownish layer (Fig.24 – Fig.27). The partitioning and discolouring increased in volume by the day. From this observation, Brand C can be said to have degraded physically. This could be due to incompatibilities of the excipients used in this brand especially at higher temperatures. The above results buttresses the fact that the standard storage condition of 2 - 8°C is the ideal condition of storage for amoxicillin - clavulanic acid oral suspension and if available to patient, will have to be adhered to stringently.

Conclusion
Amoxicillin and clavulanic acid remain stable when oral powder for paediatric suspension is reconstituted with distilled water, treated tap water or mineral water and stored under a standard storage condition of 2 to 8°C over a period of seven days. Amoxicillin is however more stable under acidic conditions than clavulanic acid. Amoxicillin is stable under ambient room temperature (25°C) throughout the seven day period of therapy whilst clavulanic acid is not. The amounts of both amoxicillin and clavulanic acid are reduced when oral suspension is brought into room temperature. Depending on the frequency and length of time with which suspension is kept out of the fridge, the therapeutic value of co-amoxiclav may be affected, especially with regards to clavulanic acid.

Recommendations
Further stability studies should be carried out on the co-amoxiclavoral powder reconstituted with different types of water but kept under non standard storage conditions. Also in order to prevent antibiotic resistance and achieve maximum therapeutic effect of amoxicillin – clavulanic acid oral suspension, the standard
storage condition which is refrigeration of drug between 2 and 8°C should be emphasised and encouraged. This will ensure that both active ingredients (amoxicillin and clavulanic acid) are intact for maximum therapeutic benefit. In rural areas where the only available water is that from water bodies, boiling of the water should be mandatory before use in reconstitution of amoxicillin - clavulanic acid oral powder. This will help prevent the use of acidic water by the elimination of atmospheric carbon dioxide as well as the destruction of some microorganisms.

Acknowledgement
The authors are grateful to Navketan Industries for providing clavulanic acid reference powder.

References: