Post-marketing stability surveillance: Amoxicillin

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Abstract

Background

To ensure the successful treatment of infectious disease using antimicrobial therapy, a sufficient concentration of the stable, active drug is required at the site of infection. For the achievement of this with respect to the ß-lactam group of antibiotics, of which amoxicillin is a member, the presence of an intact ß-lactam ring is essential. Destruction of this sensitive ring can lead to the ingestion of an inactive drug. This can contribute to treatment failure and antibiotic resistance. Thus, the aims of this study were to determine whether the types of packaging in which amoxicillin preparations are dispensed and the temperature and humidity conditions under which they are stored by patients are adequate and appropriate to ensure drug stability.

Methods

A mini-survey of pharmacies and patients was conducted in order to determine the types of packaging in which amoxicillin preparations are dispensed and the temperature and humidity conditions under which they are stored by patients. The amoxicillin preparations in the identified types of packaging were subjected to simulated conditions that represented the identified temperature and humidity stresses that occurred under patient storage conditions for a duration of 14 days. The extent of breakage of the ß-lactam ring was then chemically determined on day 1, day 7 and day 14, using an iodometric titration method.

Results

The mini-survey identified four types of packaging in which amoxicillin capsules are dispensed – plastic packets, flip-top amber bottles, flip-top amber bottles with cotton wool and flip-top transparent bottles with cotton wool. The laboratory analyses showed that only those amoxicillin capsules stored between 20 and 25 ^{0}C and protected from moisture were stable in all four identified types of packaging for 14 days. The mini-survey also indicated that 47% of the patient sample did not store their antibiotic suspensions in the refrigerator. The laboratory analyses showed that only amoxicillin suspensions stored between 2 and 8 ^{0}C for seven days showed the lowest level of degradation.

Conclusion

The results of this study indicate that reconstituted amoxicillin suspensions should be stored in the temperature range 2 to 8 ^{0}C, and that the reconstitution and dispensing of a 14-day supply of amoxicillin suspensions should be discouraged, even if the drug is stored in this temperature range. For amoxicillin capsules, the results obtained in this study indicate that significant breakage of the ß-lactam ring of amoxicillin capsules can occur in hot and humid climatic conditions if inadequate types of packaging are used and storage occurs under inappropriate conditions. The results of the study point to the importance of drug stability knowledge as a prerequisite for the dispensing of medicines, the importance of the provision of patient counselling with regard to drug storage requirements, as well as a requirement for amoxicillin capsules to be dispensed in the original manufacturers' containers in geographical areas that are hot and humid.

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Introduction

Some of the major concerns expressed by healthcare practitioners with regard to drug stability are the effects of the environmental stresses to which drug products are exposed throughout a product's lifetime and the effects of such exposure on a product's integrity.[1] The packaging of a dispensed medicine is an integral part of the product.[2] It thus is important to ensure that containers are not only convenient for use by the patient, but also that they are sufficiently robust to protect the contents from the mechanical hazards of handling, transport and environmental stress.[2]

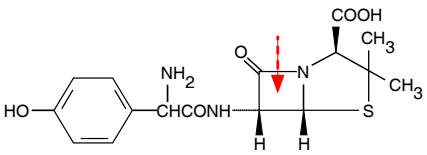
Stability assessment starts with stress testing – the application of a suitable stressor or challenge to the medicine – and the measurement of the effects of such a stressor on the physical and chemical properties of the medicine in the dispensed packages at appropriate time intervals.[2] The principal tests used as part of stability studies are storage tests on the product, which are carried out under controlled stresses that represent the conditions expected during storage.[3] The purpose of stability testing is thus to provide evidence of how the quality of the drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity and light, enabling the recommendation of storage conditions, re-test periods, beyond use dates and shelf life.[2]

The chemical degradation of drugs can lead to the loss of potency of the product, therefore the person preparing and dispensing medicines must have full knowledge of the chemical nature of the substance being handled so that all the possible types and causes of degradation can be determined and suitable measures can be taken to retard these reactions. Drugs can degrade by various chemical reactions; the most common of which are oxidation, hydrolysis and racemisation. The rate of these reactions is affected by a variety of factors, such as pH, temperature, carbon dioxide, oxygen, light and humidity. Thus the stability of a medicine relates to the various changes that can occur in the medicine during preparation and storage, and to the effects of these changes on its fitness for use. Since drugs degrade by various chemical reactions, their degradation can often be retarded by the judicious selection of containers and closures and by controlling storage conditions.[2]

It is of particular importance that, during patient counselling, the patient is given advice with respect to the storage of medication, for example: store away from heat and direct light; do not store the capsules or tablet forms of amoxicillin in the bathroom, near the kitchen sink, or in other damp places; and store liquid forms of amoxicillin in the refrigerator because heat will cause the medicine to break down.[4] However, while proper storage conditions should be reinforced with the patient during patient counselling, it is also recognised that proper control beyond the dispenser can be difficult.[5]

Amoxicillin is a ß-lactam antibiotic. It contains a ß-lactam ring and has the following structure:

Figure 1: Structure of amoxicillin



The dashed arrow, ⇢, indicates the bond in the ß-lactam ring that is susceptible to breakage

The common name of amoxicillin is p-hydroxyampicillin and its chemical name is 6-< [amino- (4-hydroxyphenyl) acetyl] amino>-3, 3-dimethyl-7-oxo-4thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid.[6] Amoxicillin is a semi-synthetic penicillin antibiotic. Semi-synthetic refers to the chemical modification of a naturally occurring substance.[7] A notable property of the ß-lactam ring is its ease of hydrolysis by aqueous acid, aqueous base and enzymatic conditions.[8] The greatest single cause of antibiotic resistance to ß-lactam antibiotics arises from the irreversible hydrolysis of the amide bond in the ß-lactam ring by ß-lactamase enzymes.[9] The activity of the ß-lactam antibiotic is thus dependent on the integrity of the ring. Breakage of this essential ring, as a result of the enzyme ß-lactamase that catalyses the hydrolysis of the amide bond of the lactam ring or the breakage of this bond brought about by external influences, can result in antibiotic resistance.

Amoxicillin is used to treat an array of infections from the Gram negative to the Gram positive,[7] and it is used widely in both the public and private sectors.[10] The aim of antibiotic treatment is to maximise antibacterial activity to prevent a recurrence of infection and the creation of resistance pathogens. For ß-lactam antibiotics, the most important determinant of the antimicrobial efficacy, and hence predictor of therapeutic efficacy, is the length of time in which serum concentrations exceed the minimum inhibitory concentration (MIC). Dosing schedules for ß-lactam antibiotics should maintain serum concentrations above the MIC for the bacterial pathogen for at least 50% of the dosing interval to achieve therapeutic efficacy and prevent the development of resistance. This is a basic criterion for the clinical efficacy of the ß-lactams.[11] For this to occur, a certain defined quantity of a chosen antibiotic is given over a period of time to enable the attainment of levels higher than the MIC.

Therefore, the use of ß-lactam antibiotics that have experienced ring breakage due to fluctuations in the external environment can result in reduced antibacterial activity and the same drug given over a standard period may thus only attain levels lower than the MIC. This may not be in a concentration high enough to completely eradicate the

organism of infection, leading to an increase in the number of recurrent infections and the development of antibiotic resistance.

The aims of this study were to determine whether the types of packaging in which amoxicillin preparations are dispensed and the temperature and humidity conditions under which they are stored by patients are adequate and appropriate to ensure drug stability.

2 Method

Prior to the commencement of the study, ethical approval was granted by the Ethics Committee of the Faculty of Health Sciences, University of KwaZulu-Natal. The research method incorporated two stages, the mini-survey (Part A) and the laboratory analyses (Part B).

2.1 Part A: the mini-survey

A mini-survey of pharmacies and patients was conducted in order to determine the types of packaging in which amoxicillin preparations are dispensed and the temperature and humidity conditions under which they are stored by patients. Questionnaires were distributed to pharmacists and patients. The choice of the five community pharmacies used in the study was based on the inclusion in the sample of at least two urban and two suburban pharmacies. The patient sample consisted of 100 pharmacy patients who had antibiotics dispensed to them. The data obtained was analysed using Microsoft Excel®. The results obtained formed the basis for Part B, the laboratory analyses.

2.2 Part B: the laboratory analyses

All chemicals used were of analytical grade and distilled water was used throughout. Pharmaceutical preparations of amoxicillin capsules and suspensions were obtained commercially. These preparations contained only one drug and did not exist in combination with other drugs.

The amoxicillin preparations in the identified types of packaging were subjected for 14 days to simulated conditions in the laboratory that represented the identified temperature and humidity stresses that occurred under patient storage conditions . The extent of breakage of the lactam ring was determined on day 1, day 7 and day 14 using an iodometric titration method. The percentage of degradation of each sample was calculated and compared to a 5% limit of degradation.[10] Any value above 5% was considered to be significant degradation and the sample was thus considered unstable.

Iodometric titrations are particularly suitable for the assay of penicillin antibiotics and their dosage forms. Penicillins with an open lactam ring are inactive as antibiotics, since it is the reactive lactam ring that kills the bacteria. When the lactam ring is opened it will react with iodine. One mole of the ring-open form of penicillin will react with eight equivalents of iodine; the intact lactam ring will not react. In this type of titration, excess iodine solution is added to a sample of the penicillin and the iodine that is not consumed in the reaction is calculated by a titration with sodium thiosulphate. The value obtained for the amount of hydrolysed penicillin in the sample should be no more than 5% of that obtained when all the penicillin in the same amount of sample is completely hydrolysed to the ring-opened form and then reacted with iodine. Most of the pharmacopoeial monographs for penicillins indicate that this test should be carried out.[12] A pharmacopoeial monograph is a description of a drug in an authoritative publication, for example The United States Pharmacopoeia (USP).[13]

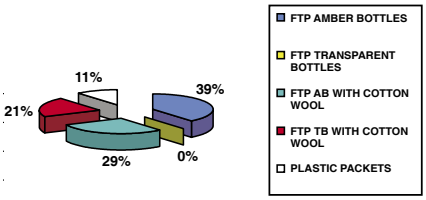
3 Results

3.1 Part A: the mini-survey

The results of the mini-survey indicate that:

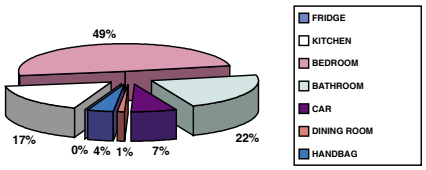
1. Of the patient sample, 89% received their antibiotic capsules in flip-top plastic (FTP) containers, while the remaining 11% received their antibiotic capsules in plastic packets. The types of containers in which the antibiotic capsules were dispensed are shown in Figure 1.

Figure 1: Types of containers in which antibiotic capsules are dispensed (TP - transparent bottle; AB - amber bottle)



2. Of the patient sample, 49% stored their capsules in the bedroom, 44% stored their medication in common areas of the house and 7% stored them in the car. The distribution of storage areas is shown in Figure 2.

Figure 2: Patient storage areas (capsules)



3. Of the patient sample, 53% stored their antibiotic suspensions in the fridge, while the remaining 47% stored their suspensions in other common areas of the house. The storage areas of antibiotic suspensions are illustrated in Figure 3.

Figure 3: Patient storage areas (Suspensions).

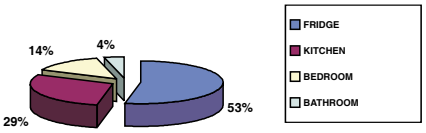


Table I: A comparative analysis of the percentage of ring breakage of amoxicillin suspension using iodometric titrations at varying temperatures on day 1, day 7 and day 14 [limit < 5 %]

Temp	Day 1 % ring breakage	Day 7 % ring breakage	Day 14 % ring breakage
2-8 °C	2.10%	5.30%	6.80%
8-15 °C	2.30%	7%	8%
15-20 °C	2.40%	7.70%	10%
20-25 °C	2.50%	11.00%	14.00%
30-35 °C	11.30%	25%	31%

Table II: A comparative analysis of the percentage of ring breakage of amoxicillin capsules using iodometric titrations at temperatures of 20-25 ^{0}C [moisture protected] on day 1, day 7 and day 14 [limit < 5 %]

Packaging	Day 1 % ring breakage	Day 7 % ring breakage	Day 14 % ring breakage
PLASTIC PACKET	2.60%	3.40%	4.90%
TB WITH WOOL	2.90%	3.50%	4.60%
AB WITHOUT WOOL	2.40%	3.0%	4.0%
AB WITH WOOL	2.30%	2.60%	3.50%

3.2 Part B: The laboratory analyses

The extent of ß-lactam ring breakage was used as an indicator of drug stability. The iodometric titration results were used to calculate the percentage of ring breakage at the varying temperatures and humidity levels on day 1, day 7 and day 14. The samples being analysed were considered unstable where values above the 5% limit of degradation were calculated. The results are reported in Table I below.

A comparative analysis of the percentage of ring breakage at different temperatures and relative humidity is provided in Tables II, III, IV and V.

Table III: A comparative analysis of the percentage of ring breakage of amoxicillin capsules using iodometric titrations at temperatures of 30-35 ^{0}C [moisture protected] on day 1, day 7 and day 14 [limit < 5 %]

Packaging	Day 1 % ring breakage	Day 7 % ring breakage	Day 14 % ring breakage
PLASTIC PACKET	5.70%	13.80%	25.0%
TB WITH WOOL	4.40%	11.50%	20.0%
AB WITHOUT WOOL	4.9%	12.70%	21.0%
AB WITH WOOL	2%	6.30%	15.0%

Table IV: A comparative analysis of the percentage of ring breakage of amoxicillin capsules using iodometric titrations at temperatures of 20-25 ^{0}C with ± 65% relative humidity on day 1, day 7 and day 14 [limit < 5 %]

Packaging	Day 1 % ring breakage	Day 7 % ring breakage	Day 14 % ring breakage
PLASTIC PACKET	2.90%	4.20%	5.40%
TB WITH WOOL	3.00%	4.00%	4.70%
AB WITHOUT WOOL	2.70%	3.40%	4.30%
AB WITH WOOL	2.40%	3.30%	4.0%

Table V: A comparative analysis of the percentage of ring breakage of amoxicillin capsules using iodometric titrations at temperatures of 30-35 ^{0}C with ± 75% relative humidity on day 1, day 7 and day 14 [Limit < 5 %]

Packaging	Day 1 % ring breakage	Day 7 % ring breakage	Day 14 % ring breakage
PLASTIC PACKET	15.70%	25.00%	41.00%
TB WITH WOOL	13.00%	19.40%	29.00%
AB WITHOUT WOOL	16.20%	23.30%	38.00%
AB WITH WOOL	11.00%	14.60%	25.00%

4 Discussion

For the amoxicillin suspensions, the lowest level of degradation was seen at storage in the temperature range 2 to 8 ^{0}C for seven days. At 14 days in the same temperature range, 6.8% degradation was observed. Thus, amoxicillin suspensions that have been reconstituted and dispensed in appropriate volumes for 14 days' treatment can have a reduced therapeutic effect by day 14, even if they are stored in the temperature range of 2 to 8 ^{0}C. At higher temperatures, significant breakage occurred from day 7 and, in the 30 to 35 ^{0}C temperature range, significant breakage occurred as early as day 1. The mini-survey revealed that a large percentage of patients do not store their antibiotic suspensions in the refrigerator. With the temperature range in Durban (KwaZulu-Natal, South Africa) being between 31 and 42 ^{0}C,[14] the results of this study point to a decrease in the therapeutic efficacy and an increase in anti-biotic resistance of these drugs if they are inadequately packaged and inappropriately stored. Furthermore, the reconstitution and dispensing of a 14-day supply of amoxicillin suspension should be discouraged due to the instability of the drug at day 14, even when stored between 2 and 8 ^{0}C.

With respect to the amoxicillin capsules, only those capsules that were stored in the temperature range 20 to 25 ^{0}C and that were protected from moisture were stable in all four of the identified types of packaging for 14 days. However, with an increase in the temperature range to 30 to 35 ^{0}C, only the capsules that were packaged in flip-top bottles were stable on day 1, although the capsules in all four types of packaging were protected from moisture. With the introduction of the humidity variable, the capsules contained in all four identified types of packaging were stable in the temperature range of 20 to 25 ^{0}C and at a relative humidity of +-65%, except for the capsules that were contained in the plastic packets, which showed signs of significant degradation on day 14. With a further increase in the temperature range to 30 to 35 ^{0}C and an increase in relative humidity to +-75%, the capsules that were contained in all four types of packaging showed significant degradation, even as early as day 1.

With a temperature range of 31 to 42 ^{0}C and an average relative humidity range of 61 to 80% in Durban,[14] the results of this study point to a decrease in therapeutic efficacy and an increase in antibiotic resistance of these drugs if they are inadequately packaged and inappropriately stored. Whilst the amoxicillin capsules packaged in amber flip-top containers with cotton wool displayed the most stable drug profile and those packaged in plastic packets displayed the least stable drug profile, the results obtained in the temperature range 30 to 35 ^{0}C with +-75% relative humidity point towards a requirement for amoxicillin to be dispensed in the original manufacturers' containers in geographical areas that are hot and humid.

5 Conclusion

The results of this study indicate that reconstituted amoxicillin suspensions should be stored in the temperature range 2 to 8 ^{0}C, and that the reconstitution and dispensing of a 14-day supply of amoxicillin suspension should be discouraged, even if the drug is stored in this temperature range.

Regarding amoxicillin capsules, the results obtained from this study indicate that significant breakage of the ß-lactam ring of amoxicillin capsules can occur in hot and humid climatic conditions if inadequate types of packaging are used and storage takes place under inappropriate conditions. These results point to the importance of drug stability knowledge as a prerequisite for the dispensing of medicines, the importance of the provision of patient counselling with regard to drug storage requirements, as well as the necessity for amoxicillin capsules to be dispensed in the original manufacturers' containers in geographical areas that are hot and humid.

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References

1. The United States Pharmacopoeia Online (The USP): http://www.USP.org/patientSafety/briefsArticleReports/qualityReview No.40 revised 6/94-Storage Definitions.hmtl (Accessed 19/03/2004).
2. The Pharmaceutical Handbook. 19th ed. London: The Pharmaceutical Press; 1980.
3. Glasby JS. Encyclopedia of antibiotics. New York: Wiley-Interscience Publications; 1987.
4. The United States Pharmacopoeia Drug Information (The USPDI). Advice for the patient: drug information in lay language. Rockwell, MD: The United States Pharmacopoeial, Inc.; 1994.
5. The United States Pharmacopoeia Online (The USP):
6. http:// www.USP.org/patientSafety/briefsArticleReports/qualityReview No.6 revised 03/98-Storage Stories.hmtl
7. (Accessed 19/03/2004).
8. The United States Pharmacopoeia (The USP). 24th ed. Easton: Mack Publishing Company; 2000. p. 129-131.
9. Streptococcus Pneumoniae: http://sitemaker.umich.edu/medchem6/jay (Accessed: 02/02/2006).
10. Lemke, TL. Review of organic functional groups: introduction to medicinal chemistry. 3rd ed. Philadelphia: Lea & Febiger; 1992.
11. Essack SY. The development of ß-lactam antibiotics in response to the evolution of ß-lactamases. J Pharmaceutical Research 2001;18(10):1-3.
12. Department of Health. Essential drugs programme, South Africa – standard treatment guidelines and essential drug list. Pretoria: The National Department of Health; 1998.
13. Auckenthaler R. Pharmacokinetics and pharmacodynamics of oral ß-Lactam antibiotics as a two-dimensional approach to their efficacy. J Antimicrobial Chemotherapy 2002;50:13-4.
14. Watson DG. Pharmaceutical analysis, a textbook for pharmacy students and pharmaceutical chemists. Edinburgh: Harcourt Publishers; 1999. p. 62-3.
15. Answers.com-Pharmacopoeia: http://www.answers.com/topic/pharmacopoeia (Accessed 08/02/2006).
16. BBC Weather Online: http://www.bbc.co.uk/weather/world/city_guides/city.shtml (Accessed 01/09/2004).