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Influence of storage condition on the shelf life of amoxicillin tablets

S.S. Kiron[1,*], Arun Shirwaikar[2], M Saritha[3], P.L.Rajagopal[4] and Sreejith.K.R[5]

[1]Department of Pharmacy Practice, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala- 670503.

[2]College of Pharmacy, Gulf Medical University, Ajman, UAE.

[3]Department of Pharmacy Practice, Crescent B.Pharm College, Payangadi, Kannur, Kerala.

[4]Department of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala- 670503

[5]Department of Pharmaceutical Chemistry, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala- 670503.

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ABSTRACT

Quality of pharmaceutical is of vital importance for patient safety. The loss of potency may influence the efficacy and safety of pharmaceuticals. This study determines the influence of storage conditions on the shelf life and potency of selected antibiotics. A significant statistical difference was observed across the potency data selected antibiotics brands. The data point out that maximum reduction in shelf life was observed at room temperature as compared with cool temperature. These results authenticated that improper storage conditions resulted in failure to meet product characteristic and specification during shelf life. Optimum storage conditions and procedures ensure that the potency and integrity of medicinal products are maintained throughout their shelf life.

Introduction

Pharmaceuticals have myriads of uses for both humans and animals, including therapy, disease prevention, diagnosis, cosmetics and lifestyle.[1] Pharmaceutical product quality is of vital importance for patient safety. The loss of potency may influence the efficacy and safety of pharmaceuticals. Impurities and potential degradation products can cause changing of chemical, pharmacological and toxicological properties of drugs having significant impact on product quality and safety. [2] Stability of a pharmaceutical preparation can be defined as the capability of a particular formulation (dosage form or drug product) in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf life. [3, 4]. Physical, chemical, and microbiological properties of drugs were generated as a function of time and storage conditions (e.g., temperature and relative humidity [RH]). Strict storage conditions in pharmacies were necessary for the maintenance of drug integrity and activity. Potency testing of an active substance or finished product provides evidence on quality of a drug substance or drug product which was influenced by a variety of environmental factors such as temperature, humidity and light. Knowledge from these studies enables understanding of the long-term effects of the environment on the drugs. Stability testing studies provide the information about degradation mechanisms, potential degradation products, possible degradation pathways of drug as well as interaction between the drug and the excipients in drug product. [5]

According to the Drugs and cosmetic rules, 1945, only a refrigerator is one of the mandatory requirements to operate a community pharmacy. Therefore, a dilemma remains on how to meet the storage conditions prescribed on the labels of drug product. In different parts of India, the temperature variations at various times of the year are so varied, and many times, at extremes (from -15 to + 40^{0}C). As per the Act, currently air conditioning of community pharmacies is not mandatory. In different parts of India, in addition to temperature, humidity conditions also vary widely. Temperature and humidity may fluctuate to both extremes, therefore majority of drugs may not be able to withstand these fluctuations. [6]

Improper storage of the pharmaceutical products is one the fundamental concerns in patient care. This study emphasizes the importance of proper storage of pharmaceuticals from the time of their movement from manufacturing premises till it reaches the consumers. [7] The last leg of its journey at wholesale dealer's premises and retail outlets occupies a sufficiently long period of storage. Pharmaceuticals get exposed to varying temperature and humidity conditions during this part of movement, and with the prevailing conditions of the pharmacy outlets in India. Kerala is a tropical region and several places in the state are very hot and/or humid and at times with an intense light. [8] Pharmaceutical products sometimes cannot remain stable in such conditions unless the labeled storage conditions on the products are adhered to. Accordingly, it becomes the duty of everybody in the distribution chain to value the storage conditions. The pharmacist in a pharmacy has the specific responsibility in this regards as the retention time of the product in the establishment is fairly long. This work will act as a guidance document for helping the community pharmacist and hospital pharmacist to recognize the importance of proper storage practices.

Materials and Methods

The formulations in their final marketed packs were procured from wholesale dealers in adequate quantities. The samples in their final packs were stored in cool and room temperature. All the samples were analyzed initially before placing in the respective storage conditions. These samples were withdrawn at different time intervals for estimation of potency. Time interval was selected based on comparison with ICH Q1A (R2) stability guidelines. [9] Accordingly sampling time selected

Tele:

E-mail addresses: sskiron@yahoo.com

was every 3 months in the first year; every 6 months from the second year, then annually through proposed re-test period: e.g. 0, 3, 6, 9, 12, 18, 24, 36 months. During these time intervals samples from both storage conditions were sent to analytical testing (Table 1). All the analytical evaluations were done using UV spectrophotometric to Indian Pharmacopoeia (1996)[10] and Quantitative analysis of Drugs in Pharmaceutical formulations by P.D. Sethi (2004).[11, 12] Human ethical clearance was obtained from Ethical Committee of Academy of Medical Sciences, Pariyaram Medical College, Kannur (*Order no: B2.9799/04/ACME dated on 19/04/2006*).

Estimation of Amoxycillin:

Standard Preparation: 50 mg of Amoxycillin was weighed accurately and diluted to 100 ml in a 100ml volumetric flask with distilled water (500µg/ml). From the above stock solution, 2 ml was pipetted out into a 50 ml volumetric flask and the volume was made up with distilled water (20µg/ml).

Sample preparation: Tablet powder equalent to 50mg of Amoxicillin was taken and to this added 70ml of distilled water. Sonicated for 5 minutes and diluted to 100ml. 2ml of above solution diluted to 50ml with distilled water.

Procedure: 2ml of the final standard and sample solution of amoxicillin was taken and diluted to 50ml with Formaldehyde reagent. Then heat the sample and standard solution at 90^0C ($\pm 0.5^0$C) on constant temperature bath for 60 minutes. Cool and measure the absorbance at 397nm against reagent blank (Formaldehyde reagent) using UV/Visible spectrophotometer. [10, 11, 12]

Dissolution testing of selected antibiotics:

Dissolution studies were carried out for collected sample using the dissolution apparatus USP type II (paddle type) LABINDIA DISSO 2000. Suitable buffer solutions were prepared and used as the dissolution media for particular study. Speed and duration of rotation, pH of the buffer were fixed as per the IP standard. 5ml of dissolution medium was withdrawn and filtered, from this 1 ml of sample was taken and volume made up and assayed spectrometrically. [13, 14, 15]

Antibiotics sensitivity studies:

E.Coli (MTCC 443) was used for the microbiological sensitivity studies. Minimum inhibitory concentrations of Amoxicillin, Norfloxacin and Cephalexin were performed by Nutrient agar method. Weight of each tablet equivalent to 250mg was diluted to 100ml (2.5mg/ml). 1ml was taken from stock solution and diluted to 100ml (0.025mg/ml or 25µg/ml), again diluted to 10µg/ml. Test concentration used for the studies were10µg/ml. [16, 17, 18, 19]

Results and discussion

Strict storage conditions are necessary for the maintenance of integrity and product activity. Storage simply means keeping things at a place, till it is used, so as to preserve the same property.[20] Five different brands of Amoxicillin was procured from wholesale dealers and selected brands of each drugs were coded as sample A, B, C, D and E. The procured samples were divided into two portions. One portion was kept in cool condition and another portion was kept at room temperature. According to I.P cool temperature is the temperature between 8^0C to 25^0C and room temperature is temperature prevailing in working area. The definition of controlled temperature conditions indicates a particular range but also allows variations in the form of excursions over a certain period of time. This is based on Mean Kinetic Temperature.

All the selected brands were analyzed at the beginning of the study, which was taken as the initial reading. As indicated by product label, Amoxicillin DT have expiry period of 24 months. The samples were withdrawn according to the expiry date. Accordingly, amoxicillin samples were withdrawn at 3, 6, 12, 18 & 24 months. The percentage drug content of Amoxicillin at Cool and room storage conditions are shown in Table 3 respectively. At these storage conditions the Amoxicillin samples showed a remarkable variation in the percentage drug content. The sample stored at cool temperature showed comparatively high percentage of drug content than samples stored at room temperature. The data (Table 3) of samples withdrawn at 24 months study period indicated that the Amoxicillin samples stored at cool temperature have a better shelf life as compared with Amoxicillin stored at room temperature.

The data obtained from these periods of time were subjected to statistical analysis using magastat software. A two way ANOVA with replication was carried out to assess whether there was any significant effect on the shelf life of Amoxicillin between the storage condition and the sampling time intervals. A significant statistical difference (p= 0.0000) was observed across the potency data of five selected amoxicillin brands. The data point out that maximum reduction in shelf life was observed at room temperature as compared with cool temperature. The statistical interaction effect was also performed between storage conditions of antibiotics and sampling time intervals. The statistical interaction value (p= 0.0000) indicated that increase in antibiotics storage time in hospital and community pharmacy will decrease the shelf life, if it is not stored properly.

As per I.P 1996, amoxicillin DT should be stored in tightly closed containers in a cool, dry place. The monograph for potency was not less than 90% and not more than 120% of stated amount of anhydrous Amoxicillin. The data of Amoxicillin tablets showed (Table 3) that the samples stored at cool temperature keep-up its shelf life near to expiry date whereas samples kept at room temperature disastrous in maintaining its shelf life near to expiry date. These results authenticated that improper storage conditions resulted in failure to meet product characteristic and specification during shelf life. These results call attention to the importance of storage conditions of antibiotics in hospital and community pharmacies. Hence it is the duty of pharmacist to ensure that antibiotics are kept in an environment that maintains their efficacy.

Different environmental factors that may affect the shelf life of drugs were temperature, light, humidity, storage condition, time of storage and type of dosage form. According to Pharmapedia [21] storage of medicine in a cool place (below 15^0C) will prolong the shelf life. High humidity brings about the deterioration of tablets and solid preparations. High temperatures will accelerate oxidation, reduction and hydrolysis reaction which leads to drug degradation. Due to environmental factors the physical changes can have deleterious effect on tablets. That leads to hardening of tablets and may show very slow dissolution time which may result in the decreased bioavailability of drugs. Patrica Griffin kellicker (2006) [22] stressed that the medications will work only when they are handled properly.

Amoxicillin is a semi-synthetic aminopenicillin containing β lactam ring, with a broad-spectrum bactericidal activity, which is used as trihydrate, and it is well absorbed when given orally. [14] A notable property of the β lactam ring is its ease to undergo hydrolysis. The greatest single cause of antibiotic resistance to β lactam antibiotics arises from the irreversible hydrolysis of the

amide bond in the β lactam ring. Destruction of sensitive β lactam ring can lead to the ingestion of an inactive drug. Naidoo KK.et.al [23] carried out a study on post-marketing stability surveillance of amoxicillin at Durban. The study found that some of the major concerns expressed by healthcare practitioners with regard to drug stability were the effects of the environmental stresses to which drug products are exposed throughout product's lifetime and the effects of such exposure on a product's integrity. Regarding Amoxicillin capsules, the results obtained from this study indicate that significant breakage of the β -lactam ring of amoxicillin capsules can occur in hot and humid climatic conditions. This might have resulted in decreased therapeutic efficacy and increased antibiotic resistance of these drugs, if inadequate types of packaging are used and storage takes place under inappropriate conditions.

The microbial data of Amoxicillin at room and cool temperature were given in Figure 1 and 2. *E.Coli* (MTCC 443) was used for the microbial sensitivity studies. The microbial data of Amoxicillin samples (Figure: 1 & 2) stored at cool temperature were found under the sensitive range. But samples at room temperature were in the sensitive and moderately resistance range. The sensitivity data were analyzed based on standard criteria. [16, 17] A. Kheirolomoom.et.al [24] expressed that potency of Penicillin G decreased by increases in temperature conditions. This might be the reason for decreased sensitivity of amoxicillin samples kept at room temperature during the study. Margaret Planta [25] pointed out that improper storage of antimicrobials may affect their potency and that may contribute to antibiotic resistance.

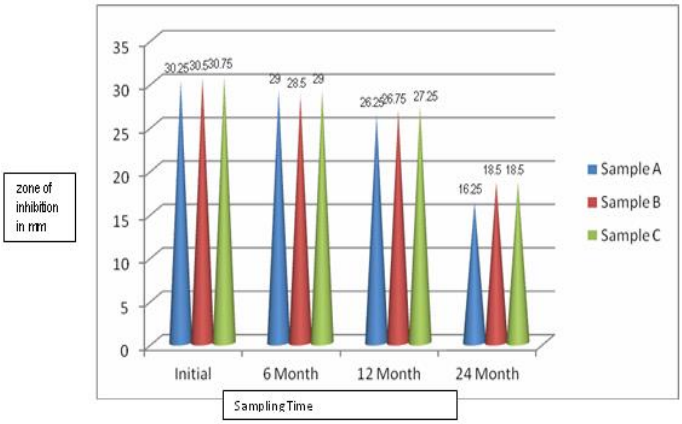

Figure 1: Microbial Data Of Amoxicillin 250mg Based On Zone Of Inhibition In Mm (Room)

The data from dissolution studies of Amoxicillin (Figure- 3 and 4; and Table- 4 and 5) reiterated the results from microbial and potency studies. All the samples stored at room temperature illustrated decline in dissolution rate as compared with cool temperature, which could affect the bioavailability of drugs. These data evidently showed that the potency and efficacy of selected antibiotics declined in case of room temperature as compared with cool temperature. Similar results were observed during a study conducted by Molokhia. AM [26] where the capsules of certain brands of Cephalexin degraded more rapidly in hot conditions and this caused serious fluctuations in absorption.

It was pointed out by WHO Conference of Experts on the Rational Use of Drugs (Nairobi, 1985) that "No tests or certification can prevent the gradual deterioration of products passing though a storage and distribution system, subjected to prolonged heat and humidity, rough handling and careless dispensing". The findings of the present study suggest a need for temperature quality control in primary care and community pharmacies. Medications last only as long if their storage conditions are favorable. This research highlights some important areas in the medicine management. Drugs can lose their potency long before the expiration date if exposed to oxygen, heat, light or humidity. To rectify this, the concerned regulatory authorities should implement etiquette for the arrangement of proper storage conditions within the hospital and community pharmacies.

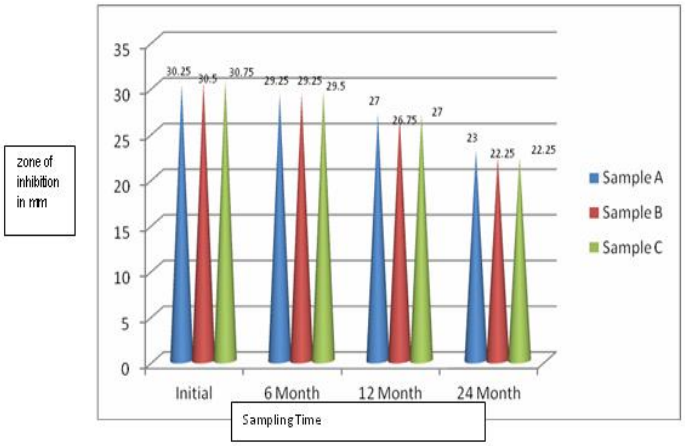

Figure 2: Microbial Data Of Amoxicillin 250mg Based On Zone Of Inhibition In Mm (Cool)

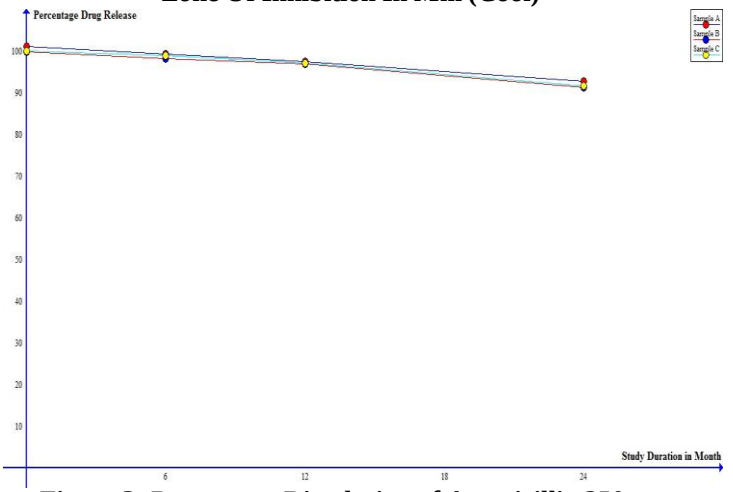

Figure 3: Percentage Dissolution of Amoxicillin 250mg (Cool)

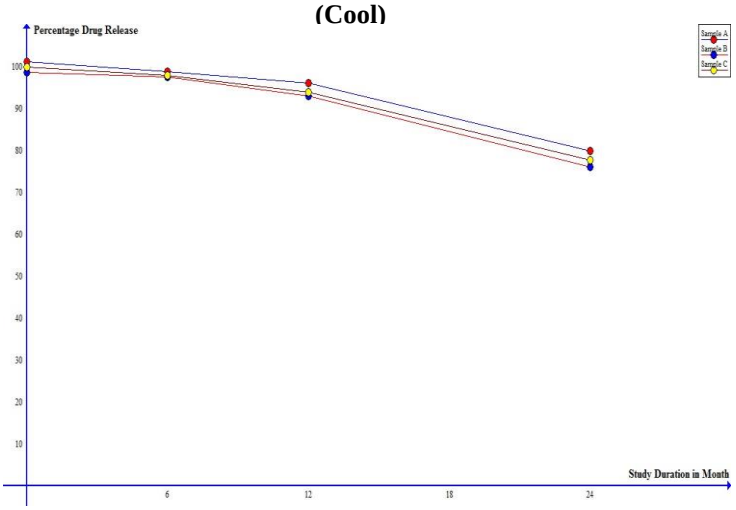

Figure 4: Percentage Dissolution Of Amoxicillin 250mg (Room)

Table 1: Analytical methods for selected sample

Sample	Materials	Instrument	Flow Rate	Wavelength of Measurement
Amoxicillin	1% v/v Formaldehyde in 0.3 HCl	UV/Visible Spectro-photometer.	NA	397 nm
Norfloxacin	0.1% v/v Phosphoric acid and Aceto nitrite in the ratio of 85:15 (Mobile Phase).	HPLC (Column: C18)	2ml/ min.	275 nm
Cephalexin	Water:Methanol:Acetonitrile: Acetic Acid in the ratio of 50: 20: 30: 0.1 v/v.	HPLC (Column: C18)	1.5 ml/min	254nm

Table 2: Dissolution parameters for sample analysis

Sample	Buffer	Speed and Duration	Wave length
Amoxicillin	900ml 0.1N Hcl	50rpm for 45 minute	228 nm
Norfloxacin	700ml Acetate Buffer	100rpm for 30 minute	278 nm
Cephalexin	900ml 0.1N Hcl	50rpm for 30 minute	261 nm

Table 3: Percentage Drug Content For Amoxicillin Dt 250mg Tablets

Sample	Storage Condition	Sample Withdrawing Time					
		Initial (% drug content)	3 Month (% drug content)	6 Month (% drug content)	12 Month (% drug content)	18 Month (% drug content)	24 Month (% drug content)
A	Room	98.57	98.57	98.56	96.04	92.56	84.93
	Cool	98.57	98.57	98.57	97.44	95.78	90.73
B	Room	100.58	100.57	100.56	97.62	92.21	81.88
	Cool	100.58	100.58	100.57	99.45	96.51	89.92
C	Room	100.49	100.48	100.48	98.89	93.08	84.18
	Cool	100.49	100.48	100.48	99.82	95.71	90.79
D	Room	99.87	99.87	99.86	96.98	91.95	82.34
	Cool	99.87	99.87	99.87	97.03	94.53	90.95
E	Room	101.07	100.07	100.99	99.42	94.26	85.32
	Cool	101.07	100.07	101.07	100.42	97.49	91.57

Table 4. Dissolution data of amoxicillin 250 mg at cool temperature

Sl. no	% Drug release at 45 minute (Cool)		
	Sample A	Sample B	Sample C
Initial	101.02	99.81	100.07
6 Month	99.31	98.12	98.89
12 Month	97.46	96.83	97.09
24 Month	92.78	91.21	91.59

Table 5. Dissolution data of amoxicillin 250 mg at room temperature

Sl. no	% Drug release at 45 minute (mm) (Room)		
	Sample A	Sample B	Sample C
Initial	101.04	98.48	99.87
6 Month	98.76	97.44	97.78
12 Month	95.93	92.84	93.82
24 Month	*79.73*	*76.04*	*77.56*

The antibiotics sometimes cannot remain stable unless the labeled storage conditions on the products are adhered to. Accordingly, it becomes the duty of the pharmacist working in hospital and community pharmacy to adhere to the prescribed storage conditions. This thesis will provide guidance for helping the hospital and community pharmacist to recognize the importance of proper storage practices. The study concludes that storage arrangement of antibiotics should be based on the labeled storage conditions. Pharmacist should take necessary steps in the storage of photosensitive drugs in suitable cartons so that they are not exposed to light.

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