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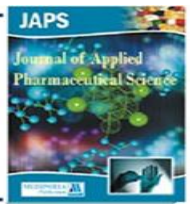

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Accelerated stability and antimicrobial sensitivity studies of amoxicillin dry suspensions marketed in Bangladesh

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ABSTRACT

Since its introduction, amoxicillin dry suspension has been the mainstay for the antibacterial therapy for paediatric patients. But use of substandard preparation of antibiotic is one of the most important causes of microbial resistance. The present study has been carried out to evaluate the quality and stability status of 10 marketed amoxicillin dry suspensions of Bangladesh. All the brands were analyzed for their potency using chemical and microbiological methods described in the United States Pharmacopoeia and British Pharmacopoeia. Potency determination was done at three controlled temperatures - refrigerated, room and elevated (40°C) showed that two samples were over potent but one sample was substandard out of the 10 samples. The initial potencies of the two samples were within USP range when freshly reconstituted but after 7 days, at room temperature, potencies deteriorated and came down to 90%. In refrigerated condition, all the samples remained in good condition and at 40°C, a considerable loss of potencies in all the samples were observed. Results of microbiological assay also support the results of chemical assay. The study emphasizes the necessity of routine inspection, monitoring and evaluation of quality of formulations containing amoxicillin dry syrup.

Keywords: Amoxicillin, dry suspension, paediatric patients, substandard, reconstituted.

INTRODUCTION

Bangladesh is a developing country where a significant proportion of the total population lives down the poverty line and cannot afford for appropriate medical care, adequate nutrition and proper sanitation (Walsh, 1979). Due to poor sanitation and unhygienic environment microorganisms can grow favorably. Infectious diseases are therefore the most prevalent diseases here (Chowdhury, 1995; Das, 1994). Among the infectious diseases, bacterial infections contribute to most of the mortality specially among the young children (Riley et al., 1983). It has been reported that in every year, 5 million children die from infectious diseases (WHO, 1997). In order to combat the bacterial infections, antibacterials are used. In most of the cases, the physicians start antibiotic therapy with penicillin group, for example ampicillin and amoxicillin. Amoxicillin is available in liquid form such as dry syrups or powder for suspension. Amoxicillin dry syrup, a paediatric preparation, ranks the first position in the antibiotic therapeutic class in Bangladesh. Approximately 35.1% of the prescriptions were found to contain amoxicillin dry syrups (Islam, 1998). Antibiotic resistance has now-a-days become a global health problem (Kunin, 1983). One of the causes of microbial resistance includes the use of substandard preparations. The availability and consumption of antimicrobial agents without prescription facilitates the development of

resistance and this seriously limits the therapeutic options in the treatment of life-threatening infections (Lampiris, 2001). But the use of substandard antibiotic preparations also contributes to wastage of resources and complication of diseases (Das, 1994). Therefore, it is very important to ensure the quality of antibiotic preparations, especially paediatric preparations to combat childhood mortality. It is necessary to evaluate the quality and stability of the marketed antibiotic preparations. Although there are several studies regarding the quality of different marketed drugs and hazards associated with the use of drugs (Roy, 1994; Haque, et al., 1997 and Roy et al., 1997), there is no published data till date on the marketed paediatric preparations. The present study has been performed to evaluate the quality and stability of 10 marketed dry syrups of amoxicillin.

MATERIALS AND METHODS

Materials

Collection of samples

The samples of amoxicillin dry suspension were collected randomly from the retail medicine shops located at different areas of Bangladesh.

Bacterial strains

Six bacterial strains were collected for the analytical purposes. The pathogenic strains included *Streptococcus pyogenes, Streptococcus viridans, Haemophilus influenzae, Bacillus cereus, Escherichia coli, Shigella and Klebsiella Sp.*

Analytical methods

Potency determination

Amoxicillin contents in dry syrups were determined by iodometric titration method described in the United States Pharmacopoeia (USP, XXIII, NF, XVIII, 1995a).

Moisture content determination

At first 0.5 gm sample was weighed and then put into a drying oven for 4 hours at 105°C. The bottle was taken out and weighed again. The difference of the two values gave the amount of water in the sample. Thus, the percentage of loss on drying (LOD) was calculated by taking the ratio of weight of water in the sample and total weight of the wet sample and by multiplying with 100. % LOD gave the measure of the moisture content in the sample (Rankell AS, et al., 1989).

pH determination

The pH values of the freshly reconstituted amoxicillin dry syrups were measured by using a pH meter.

Determination of sedimentation volume

Sedimentation volume was determined by taking a definite volume of the reconstituted sample into a graduated cylinder and then keeping it undisturbed for 7 days. After 7 days, sedimentation volume (F) was calculated from the ratio of the final volume (V_f) of the sediment to the original or initial volume (V_i) of the suspension before settling (Martin A, 1993). Sedimentation volumes were determined for both freshly reconstituted samples and also for samples undergone storage tests for two months.

Stability testing

In order to test the stability of reconstituted suspensions, the samples were kept at three controlled temperatures, namely room, refrigerated and elevated (40ºC) temperatures for 7 days. The intact samples were studied after keeping at 30ºC and 40ºC for two months. All the samples were assayed chemically by the iodometric method as described in the USP (USP, XXIII, NF XVIII, 1995b). In each sample, the decline in concentration appeared to follow the first order reaction. Following the Arrhenius equation ($\log K = \log A - E_a/2.303\ R.\ 1/T$), log K values at 30 and 40°C were plotted against the respective reciprocal values of absolute temperatures , 1/T, when a straight line was obtained. This when extrapolated to give log K_{25}, and from that shelf-life (t_{90}) of each samples were determined.

Microbiological assay

In order to mesure the biopotency, agar diffusion method was followed as per the British Pharmacopeia (BP, 1995).

RESULTS AND DISCUSSIONS

% Potency of fresh Amoxycillin Dry Syrups

Determination of potency was divided into two segments: i) Determination of initial potencies of the samples immediately after reconstitution and ii) Study of the stability-time profiles of the active ingredient of the samples kept at three controlled temperature conditions for 7 days. The temperatures were refrigerated, room and elevated (40°C) temperatures. All the samples were assayed chemically by iodometric method as described in the USP (USP, XXIII, NF XVIII, 1995b).

Table 1: Initial and final potency of fresh Amoxycillin Dry Syrups.

Sample Code	Initial Potency (%)	Final Potency (%)		
		Condition of temperature		
		Room temperature	Refrigeration temperature	Elevated temperature
SH1	119.45	116.32	118.10	76.05
SH2	104.7	102.89	103.79	80.47
SH3	94.84	89.26	91.05	70.53
SH4	120.80	117.21	118.10	77.84
SH5	111.84	90.37	110.05	65.32
SH6	80.52	79.21	88.40	54.3
SH7	121.68	112.73	116.31	73.36
SH8	105.75	98.42	100.20	62.63
SH9	105.58	95.74	101.11	80.53
SH10	94.68	88.26	90.21	67.11

USP specification: (90-120)%

The results of percentage potency of ten samples (Table 1) show that there is striking differences in the initial concentrations

of the amoxicillin among the various samples. Sample SH6 did not comply with the lower limit to the USP specification and thus can be regarded as sub-standard or non-compliant in respect of potency. It is also evident (Table 1) that two samples SH3 and SH10, although were within the USP range, did not comply with the range after 7 days at room temperature. The low potencies in these samples might be attributed to the inadequate amount of amoxicillin initially, hydrolytic degradation of the drugs due to improper drying, lack of appropriate desiccants in the preparations, lack of maintaining proper storage condition, etc. The hot and humid weather conditions that prevail in many districts of Bangladesh may cause problems in keeping reconstituted preparations at room temperature. On the other hand, two other samples SH4 and SH7 contained excess amount of amoxicillin compared to the USP specification. This might be due to the awareness of the manufacturers regarding declining potencies for which they gave some overage in their products. Overages are added to the pharmaceutical formulations to keep the content of the active ingredient within the limits compatible with therapeutic requirements for over a predetermined period of time. The amount of overage depends upon the specific ingredient and Galenical dosage form. The International Pharmaceutical Federation (Gennaro AR , 1985) has recommended that overages be limited to a maximum of 30% over the labeled potency of an ingredient. It is also evident that the potencies of two samples (sample SH3 and SH10), although were within the USP range, after 7 days following reconstitution and at room temperature, did not comply with the range. At the refrigerated condition, these samples managed to contain the 90% range of the specification. At 40°C, there was a considerable loss of potencies in all samples.

Stability testing and determination of shelf-lives

Table 2 shows the values of log K at 30, 40 and 25°C (log K_{25}). It can be observed from this table that three samples SH_3, SH_6 and SH_{10} appeared to be inferior products. Their predicted shelf-lives were 1 year for SH3 and 1.5 years for SH_6 and SH_{10}, whereas, their shelf-lives indicated on the container were 2 years for SH3 and 3 years for SH6 and SH10. Only two samples SH2 and SH5 maintained their shelf-lives of 2 and 3 years, respectively in accordance with their indicated shelf-lives. The rest of the samples examined were found to have shorter shelf-lives than that were indicated on their containers.

Table 2: Accelerated stability of intact samples at 30ºC and 40ºC.

Sample Code	30ºC			40ºC			log K_{25}	K_{25}	t_{90} (yr)
	C_0	C_{60}	Log K	C_0	C_{60}	Log K			
SH1	119.45	116.63	-3.40	119.45	102.89	-2.60	-3.88	$1.32X10^{-4}$	2
SH2	104.70	103.00	-3.54	104.70	98.42	-3.00	-3.88	$1.32X10^{-4}$	2
SH3	94.84	91.47	-3.22	94.84	80.52	-2.60	-3.60	$2.51X10^{-4}$	1
SH4	120.80	116.50	-3.22	120.80	96.63	-2.40	-3.70	$1.99X10^{-4}$	1.5
SH5	111.84	110.51	-3.70	111.84	107.34	-3.20	-4.00	$1.00X10^{-4}$	3
SH6	80.52	78.34	-3.34	80.52	61.58	-2.40	-3.70	$1.99X10^{-4}$	1.5
SH7	121.68	119.18	-3.46	121.68	104.68	-2.60	-3.88	$1.32X10^{-4}$	2
SH8	105.75	102.89	-3.34	105.75	90.37	-2.60	-3.70	$1.99X10^{-4}$	1.5
SH9	105.58	102.72	-3.34	105.58	89.47	-2.60	-3.70	$1.99X10^{-4}$	1.5
SH10	94.68	91.60	-3.26	94.68	74.11	-2.40	-3.70	$1.99X10^{-4}$	1.5

Initial Concentration = C_0, Concentration after 60 days = C_{60}
log K at 25°C after extrapolation = log K_{25}, Shelf-life = t_{90}

Except two samples (SH2 and SH5), all other samples failed to maintain their indicated shelf-life period (Table 2). This is alarming, especially for such products (*i.e.* antibiotic formulations) as this may lead to undesired consequences including treatment failure and antibiotic resistance. In order to maintain adequate drug stability, stringent control over storage conditions for antibiotic preparations should be provided bearing in mind the tropical nature of the climate.

Determination of sedimentation volume

The highest and the lowest initial sedimentation volumes were found to be 0.98 ml (sample SH1) and 0.13 ml (sample SH7), respectively (Table 3). The sedimentation volumes of the reconstituted suspensions also varied accordingly, the highest being 0.88 ml (sample SH1) and the lowest being 0.15 ml (sample SH7). Between flocculated and deflocculated systems, the flocculated system is considered to be the most preferable one. Careful observation of the sedimentation behaviors show that 9 of the tested samples were flocculated suspensions except sample SH7, which behaved as a deflocculated suspension. The observed values (Table 3) also indicate that unfavorable storage condition has considerable effects on the sedimentation volumes of the products.

Table 3: Sedimentation volumes products when freshly reconstituted and when kept at 40C for 60 days.

Sample Code	Sedimentation Volumes					
	when freshly reconstituted		F (%)	when kept at 40°C for 60 days		F (%)
	V_i	V_f		V_i	V_f	
SH1	0.98	0.88	89.80	0.78	0.56	71.79
SH2	0.39	0.26	66.67	0.23	0.2	86.96
SH3	0.4	0.45	112.50	0.28	0.29	103.57
SH4	0.75	0.63	84.00	0.45	0.44	97.78
SH5	0.26	0.25	96.15	0.15	0.15	100.00
SH6	0.48	0.44	91.67	0.25	0.21	84.00
SH7	0.13	0.15	115.38	0.05	0.11	220.00
SH8	0.53	0.49	92.45	0.44	0.34	77.27
SH9	0.25	0.25	100.00	0.21	0.18	85.71
SH10	0.69	0.63	91.30	0.49	0.47	95.92

Initial volume of sediment after few minutes = Vi, Final volume of sediment = Vf, F = sedimentation volume = 100Vf/Vi

The sedimentation volumes that were recorded for the samples stored at 40°C for 60 days were smaller than that of the fresh samples (Table 3).

Moisture content and pH

The moisture content of the samples (Figure 1) complied the USP specification (not more than 3%) (USP, XXIII, NF XVIII, 1995a), the highest being 2.64% (sample SH6) and the lowest being 1.21 (sample SH7). The pH of the tested samples also complied the USP specification (5.0-7.5) (USP, XXIII, NF XVIII, 1995a) with exception of the sample SH6 (pH = 4.83) which was out of the compendial range. The maximum stability of aqueous preparations of penicillin is within pH range 6 – 6.8 (Martin, 1998). The low pH value can be correlated with the high amount of moisture that facilitates degradation and formation of more acidic hydrolytic products. This type of problem could be eliminated by

properly drying the preparations, limiting the temperature and humidity variations, protecting the finished batch from moisture, storing in lined containers with silica desiccant bags and above all adding suitable buffer. Buffer system especially phosphates and citrates exert a favorable effect on penicillin stability independent of pH effect.

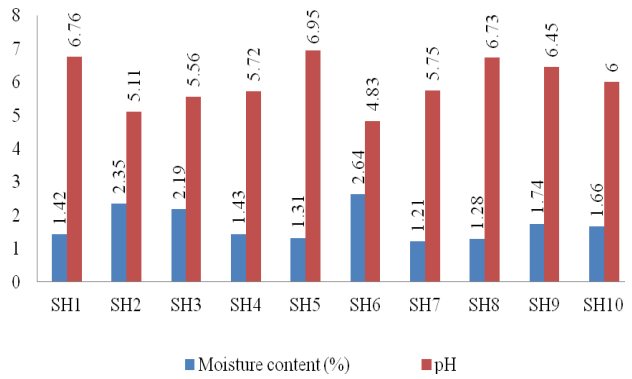

Fig 1: Moisture content (MC) and pH (USP specification: Moisture content – NMT 3.0%, and pH – 5.0-7.5).

Microbiological Assay

The zones of inhibition (Table 4) reveal that three out of the selected microorganisms were sensitive to the standard as well as the tested samples (*Streptococcus pyogenes*, *Streptococcus viridans* and *Escherichia coli*). The rest four strains (*Haemophilus influenzae*, *Bacillus cereus*, *Shigella* and *Klebsiella*) were found to be resistant. Factors such as inactivation by bacterial enzymes (Martin, 1998), presence of permeability barrier preventing penetration of the antibiotic to the target (Hugo, 1984), alteration in PBPs (Mandel, 1996) and spread of antibiotic resistance (Madigan et al., 1997), may contribute to the resistance to amoxicillin. Table 4 illustrates that the samples showed acceptable performance with respect to antimicrobial activities with a few exception (particularly SH6 and SH10).

Table 4: Zone of Inhibition produced by Amoxicillin against three sensitive strains.

Sample Code	Amount given on the plate	Zone of Inhibition in mm		
		Streptococcus pyogenes	*Streptococcus viridians*	*Escherichia coli*
Amoxicillin Standard	20 µg	24.0	21.0	25.5
SH1	20 µg	23.0	20.0	25.0
SH2	20 µg	20.5	18.0	23.5
SH3	20 µg	19.0	18.0	22.5
SH4	20 µg	22.7	20.0	24.5
SH5	20 µg	21.1	20.5	24.0
SH6	20 µg	17.5	17.0	21.0
SH7	20 µg	23.1	19.0	24.7
SH8	20 µg	20.0	18.1	23.0
SH9	20 µg	20.1	18.5	23.0
SH10	20 µg	19.5	18.1	22.7

CONCLUSION

In order to minimize the problem of resistance against antimicrobial agents, some rational steps and proper responsibilities should be taken by the appropriate authorities. Manufacturers should perform routine quality control of the finished products, add proper labels and instructions regarding use and storage of the dry and reconstituted suspensions, etc. Consumers have also some role to play such as they should not buy the product that is out of date and should best use the suspension as per the instructions from the label and the physicians. The respective authorities should take necessary measures to identify and stop the production of substandard preparations. The outcome of the present study, beyond any doubt, will help to increase the awareness regarding the use of quality products and discarding the substandard ones.

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