

## Stability and Comparative Dissolution Studies of Five Brands of Norfloxacin Tablets Marketed in Addis Ababa, Ethiopia

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The dissolution profiles of five different brands of norfloxacin (400 mg) tablets designated as A, B, C, D, and E, marketed in Addis Ababa were compared with those of an innovator product (F). The stability of these tablets was evaluated under the influence of accelerated conditions ( $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and  $75\% \pm 5\% \text{ RH}$ ). The  $t_{50\%}$  and  $t_{90\%}$  (time required for releasing 50% and 90% of the drug, respectively) were used as dissolution parameters to compare the dissolution profiles of the tablets. The  $t_{50\%}$  results indicated that except for Product C, all the others released 50% of the drug below 10.2 min (the time taken by the innovator product to release 50%). However, the  $t_{90\%}$ s for three products (B, C, and E) were longer (42.1, 37.4 and 29.0 min, respectively) than that of the innovator product (17.6 min) showing slower dissolution rates for the brand products relative to the innovator product. Product D showed a faster dissolution rate than the innovator product with 90% release at 9.8 min. The stability testing revealed that during the six months storage under the accelerated conditions, physical changes like film cracking, decrease in hardness, increase in moisture content and changes in dissolution profiles have occurred. The highest change in drug content was 3.6% at six-months. Accordingly, no significant change in drug content has occurred in any of the investigated norfloxacin tablets stored under stressed conditions for six months.

**Keywords:** norfloxacin, film coated tablet, dissolution profile, stability, accelerated conditions

### INTRODUCTION

Pharmaceutical products usually undergo series of changes in the course of storage and this is highly influenced by the nature of the material and the conditions under which they are stored (WHO, 1996). The primary environmental factors that can affect stability include temperature, relative humidity, and light (USP, 2008). Stability of a pharmaceutical formulation can be considered a major factor in ensuring the quality of the drug product and consequently, the efficacy of the treatment (Lima *et al.*, 2008). A drug product with no sufficient stability, can result in changes in its physical and chemical characteristics (ASEAN, 2005).

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature,

humidity, and light, to establish a retest period for the drug substance or a shelf-life for the drug product and recommend storage conditions (EMEA, 2006).

In addition, product-related factors influence stability, e.g., the chemical and physical properties of the active substance and the pharmaceutical excipients, the dosage form and its composition, the manufacturing process, the nature of the container-closure system, and the properties of the packaging materials. The interactions of all these features affect the eventual stability of the product (Haywood *et al.*, 2006; WHO, 2006). Thus, stability testing is the only way to demonstrate that a pharmaceutical product would meet the laid-down specifications within acceptance criteria throughout its shelf-life (Singh and Kumar, 2006). The criteria should include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence the quality, safety and/or efficacy (ASEAN, 2005).

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The first stage in ascertaining the therapeutic equivalence of any drug product involves determining the pharmaceutical and biopharmaceutical equivalency of such drug products (Adegbolagun *et al.*, 2007). The drug release rate is one of the most important *in vitro* parameters for establishing such equivalency in solid oral drug delivery systems (Lima *et al.*, 2008). Differences in solid-state properties, formulations and/or manufacturing processes of tablets can lead to disparities in the bioavailability between brands of the same drug.

In addition to the one locally produced, Ethiopia is importing norfloxacin tablets sourced from many countries. Therefore, it is imperative to compare the *in vitro* dissolution profiles of the various brand/generic products of norfloxacin tablets with that of the innovator product for quality control purpose. Furthermore, for tablets containing a hygroscopic substance as norfloxacin, distributed to and stored in regions where the climate is hot and humid (e.g., Gambella, western Ethiopia), the fate of the physical and chemical stabilities of the tablets might be of concern to the government, the regulatory body, distributors, the private sectors and the community at large. Hence, it was deemed necessary to study the stability of different brands of norfloxacin tablets imported into Ethiopia under the influence of simulated tropical conditions.

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## MATERIALS AND METHODS

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### Materials:

**Test samples.** Six different brands of norfloxacin 400 mg film coated tablets were purchased from retail outlets in Addis Ababa (Table 1). All the samples used for the study were within their shelf-life during the time of investigation.

**Chemicals and reagents.** Phosphoric acid 85% (Riedel-de Haen, Germany), HPLC grade acetonitrile and analytical reagent grade sodium hydroxide pellets (Fisher Scientific Inter. Co, UK), glacial acetic acid and triethylamine (Fluka Chemie, GmbH, Switzerland) were used as received. USP norfloxacin reference standard (RS) was obtained from Addis Pharmaceuticals Factory (APF) (Adigrat, Tigray, Ethiopia).

### Methods:

#### Physical properties of norfloxacin tablets:

**Weight variation.** Twenty tablets from each product were weighed individually using analytical balance (Sartorius AG, CP124S, Germany) and the mean of the weights was calculated (BP, 2000) and the standard deviation determined.

**Measurement of physical dimensions and crushing strength.** The diameter (for round shape tablets), the width and the length (for oblong shape tablets) and the thickness (for both shapes), were measured by taking ten randomly selected tablets from each product using Hardness Tester (Pharma Test, CE type PTB311E, Germany), which simultaneously measures all the parameters. The crushing strength of ten randomly selected tablets (BP, 2000) were measured using Hardness Tester (Pharma Test, CE type PTB311E, Germany).

**Moisture content.** Moisture content was determined as per USP (2008). Immediately after sampling, tablets were powdered and 1 g of the powder was dried in a vacuum oven (Javac, DS40, Australia) at a pressure not exceeding 5 mm of mercury at 100 °C to a constant weight. The percent reduction in weight was taken as the moisture content of the tablets.

**Table 1.** List of norfloxacin 400 mg film coated tablets investigated.

Brand name	Manufacturer	Country	Batch No.	MFD	EXD	Mode of packaging
Norcin (A)	APF	Ethiopia	2220	03/2007	03/2010	10 tabs/blister, 20 blisters/box
Norfen (B)	Cadila	India	E7015	06/2007	07/2010	10 tabs/blister, 10 blisters/box
Norbek (C)	Houns	Korea	7009	06/2007	06/2010	10 tabs/blister, 10 blisters/box
Trizolin (D)	Remedica	Cyprus	36500	03/2008	03/2011	10 tabs/blister, 10 blisters/box
Gyrablock (E)	Medochemie	Cyprus	A2G015	07/2008	03/2011	10 tabs/blister, 10 blisters/box
<sup>a</sup> Noroxin (F)	Merck Sharp & Dohme	Netherlands	NK09620	06/2008	12/2010	10 tabs/blister, 10 blisters/box

MFD = Manufacturing date; EXD = expiry date; <sup>a</sup>Utilized for *in vitro* dissolution comparison only

### Dissolution profiles:

Dissolution profiles of the five brands of norfloxacin tablets and the innovator product were determined at zero-month immediately after collection from market for the *in vitro* dissolution comparison. The innovator product (Noroxin<sup>®</sup>) was studied for this purpose only. To examine the stability of the tablets, the dissolution profiles of the five generic brands were performed after storing for three months and six months in a stability chamber (Weiss, PHARMA 600, Germany) set at 40 °C ± 2 °C and 75% ± 5% RH. The tests were performed on six tablets of each brand using dissolution tester (Pharma Test, DISS TYPE PTWS610, Germany) equipped with rotary paddles (USP Apparatus 2) maintained at 50 rpm (USP, 2008). The dissolution medium was 750 ml acetate buffer pH 4.0, which was maintained at 37 ± 0.5 °C. A sample of 5 ml was withdrawn from each vessel at 5, 10, 15, 20, 25, 30, 35, 40 and 60 min, and kept in amber coloured bottles. After each sampling, the medium was replenished with 5 ml of fresh medium maintained at 37 ± 0.5 °C. Then, 1 ml of each filtered sample was diluted to 100 ml with the dissolution medium using volumetric flask wrapped with aluminum

foil. The corresponding absorbance reading was taken at 278 nm using UV-VIS spectrophotometer (Shimadzu, PHARMA SPEC UV-1700CE, Japan), concomitantly with norfloxacin RS of fixed concentration. The percent of drug released at each time point was calculated in comparison with norfloxacin RS using the formula:  $A_s/A_r \times 100$ , in which  $A_s$  and  $A_r$  are the absorbance readings of the sample and the reference standard, respectively. According to USP monograph, not less than 80% of the labeled amount should be dissolved in 30 min.

### Assay:

High performance liquid chromatography (HPLC) method developed and validated elsewhere (Kassab *et al.*, 2005) was employed for the assay analyses of the five different brands of norfloxacin tablets.

**Chromatographic conditions.** HPLC equipped with UV-VIS detector (SPD-20A), degasser (DGU-20A5), pump (LC-20AT), and auto-sampler (SIL-20A) (Shimadzu, 20A, Japan) was used. The analytical column was a reversed phase Lichrospher<sup>®</sup> 100 RP-18 (125 x 4 mm, 5 µm) (CS-Chromatographie Service GmbH, Germany).

The mobile phase consisted of a volumetric mixture of water:acetonitrile:triethylamine (80:20:0.3). The pH of the final mixture was adjusted to 3.3 with phosphoric acid using pH meter (Mettler-Toledo, GmbH 8603 Schwerzenbach, Switzerland). The flow rate was 1.0 ml/min and the volume of injection was 10  $\mu$ l. The UV detection was carried out at 279 nm. All analyses were done at ambient temperature ( $24 \pm 2$  °C) under isocratic conditions.

#### Accelerated stability study:

The five different brands of norfloxacin tablets were assessed for physical and chemical parameters immediately after purchase. Concurrently, tablets were placed in accelerated stability chamber (Weiss,

PHARMA 600, Germany) set at  $40 \pm 2$  °C and  $75\% \pm 5\%$  RH. Samples of the tablets were tested for physical and chemical stability parameters at three months and six months period as per the ICH guidelines (ICH, 2003).

## RESULTS AND DISCUSSION

### Physical stability

The results of the physical stability tests of the five different brands of norfloxacin tablets at zero, three and six months storage under  $40 \pm 2$  °C and  $75\% \pm 5\%$  RH are shown in Table 2. Some changes in physical properties of the products were observed during the course of storage for six months.

**Table 2.** The physical properties of five different brands of norfloxacin tablets at zero-, three- and six-month storage under  $40 \pm 2$  °C and  $75\% \pm 5\%$  RH.

Physical property	Time (month)	Product				
		*A	**B	**C	*D	**E
Colour	Zero	White	White	White	White	Yellow
	Three	White	White	White	White	Yellow
	Six	White	White	White	White	Yellow
Diameter (mm)	Zero	$13.0 \pm 0.01$	-	-	$12.1 \pm 0.05$	-
	Three	$13.4 \pm 0.03$	-	-	$12.1 \pm 0.01$	-
	Six	$13.5 \pm 0.02$	-	-	$12.2 \pm 0.04$	-
Length (mm)	Zero	-	$15.6 \pm 0.06$	$14.4 \pm 0.02$	-	$14.2 \pm 0.03$
	Three	-	$15.8 \pm 0.02$	$14.5 \pm 0.03$	-	$14.5 \pm 0.01$
	Six	-	$16.0 \pm 0.06$	$14.5 \pm 0.02$	-	$14.7 \pm 0.02$
Width (mm)	Zero	-	$7.9 \pm 0.09$	$8.3 \pm 0.02$	-	$8.2 \pm 0.02$
	Three	-	$8.1 \pm 0.03$	$8.4 \pm 0.02$	-	$8.4 \pm 0.01$
	Six	-	$8.1 \pm 0.05$	$8.3 \pm 0.02$	-	$8.4 \pm 0.01$
Thickness (mm)	Zero	$5.1 \pm 0.05$	$5.2 \pm 0.11$	$6.6 \pm 0.01$	$5.1 \pm 0.05$	$5.4 \pm 0.04$
	Three	$5.6 \pm 0.05$	$5.3 \pm 0.19$	$6.7 \pm 0.03$	$5.1 \pm 0.04$	$5.5 \pm 0.02$
	Six	$5.3 \pm 0.04$	$5.3 \pm 0.12$	$6.7 \pm 0.03$	$5.1 \pm 0.02$	$5.5 \pm 0.01$
Tablet weight (mg)	Zero	$635.9 \pm 5.08$	$569.8 \pm 12.78$	$731.6 \pm 4.65$	$571.6 \pm 5.85$	$523.5 \pm 3.03$
	Three	$665.0 \pm 4.86$	$592.8 \pm 11.32$	$746.1 \pm 3.52$	$584.2 \pm 4.48$	$557.7 \pm 3.19$
	Six	$671.8 \pm 3.16$	$595.1 \pm 10.96$	$755.7 \pm 7.63$	$594.9 \pm 3.30$	$568.9 \pm 3.07$
Crushing strength (N)	Zero	$145.4 \pm 7.33$	$154.0 \pm 19.27$	$176.0 \pm 16.80$	$170.6 \pm 10.18$	$138.1 \pm 8.97$
	Three	$33.7 \pm 3.11$	$113.0 \pm 7.89$	$126.1 \pm 8.95$	$125.1 \pm 14.17$	$110.3 \pm 9.37$
	Six	$23.2 \pm 3.40$	$759.9 \pm 8.77$	$117.2 \pm 8.30$	$106.3 \pm 10.37$	$48.9 \pm 1.85$

\* Round shape tablets; \*\* Oblong shape tablets; The results are indicated as mean  $\pm$  SD with n = 10 for diameter, length, width, thickness and crushing strength and n = 20 for tablet weight.

Obviously, this is due to the extreme environmental conditions. Hence, formulation and process variables of products meant for tropical areas need to be optimized to withstand the harsh storage conditions. In addition to careful selection of the coating polymer(s) as well as the amount and type of plasticizer(s); polymer(s) concentration of the coating solution, the spraying rate of the coating solution, the drying temperature and rate, and the thickness of the film deposited thereof need to be optimized. For example, it has been shown that increasing the molecular weight of hydroxypropyl methylcellulose (HPMC) as a coating material was shown to produce a marked reduction in the incidence of cracking of HPMC aqueous film coated tablets (Cole *et al.*, 2002). It has also been reported elsewhere (Laksmana *et al.*, 2008) that the stress-relaxation of HPMC films increase at increasing moisture content resulting in film cracking.

In addition, all the products showed - increase in mean weights with time. This is due to the moisture gained by each product upon storage under the accelerated conditions. On the other hand, all the products showed acceptable uniformity of weights as none had deviation greater than 5% from their respective mean weights as stipulated in the British Pharmacopoeia (BP, 2000).

Before storage under accelerated conditions (at zero-month), the tablets had mean crushing strength ranging from 138.1 to 176.0 N. These differences might be mainly due to variations in compression force and the quantity and/or type of binder used in the formulations. The hardness of all the five different brands of norfloxacin tablets decreased upon storage under the accelerated conditions. Among the five products, Product A showed the highest change in hardness at three-month (from 145.4 N at zero-month to 33.7 N at three-

month). Product B (154.0 N at zero month to 113 at 3 month), Product C (176.0 N at zero-month and 126.1 N at three-month), and Product D (170.6 N at zero-month and 125.1 N at three-month) are the other products that showed significant reduction in tablet strength. Product A not only showed the highest change but it is also the product with the least crushing strength (33.7 N) at three-month, which is far below the minimum acceptable crushing strength, 50 N.

At six-month, Product E showed the highest reduction in hardness, from 110.3 N at three-month to 48.9 N at six-month, followed by Product B (from 113.0 N at three-month to 75.9 N at six-month). The reason for the general decrease in hardness for all the five different brands of norfloxacin tablets is due to the moisture gained upon storage under high RH (75%). Similar results have been reported elsewhere (Marias *et al.*, 2003). The highest change in hardness shown by Product A at three-month and Product E at six-month is attributed to high amount of moisture absorbed by the two products (Table 3). The results indicate that norfloxacin tablets stored under high temperature and high RH conditions soften adversely; and this may also be attributed to the integrity of the packaging materials.

### Moisture content

Table 3 shows moisture contents of the five different brands of norfloxacin tablets at zero, three and six month storage period under accelerated conditions. Product B had the highest moisture content (6.5%) of all the products followed by Product E (5.4%) at zero-month storage. The moisture could be residual moisture and/or moisture absorbed during the products' storage since their manufacture. Product D had the least moisture content (3.6%) at zero-month, before storage under the accelerated condition.

**Table 3.** Moisture contents of five different brands of norfloxacin tablets (mean  $\pm$  SD, n = 3) at zero-, three- and six-month storage under 40 °C  $\pm$  2 °C and 75%  $\pm$  5% RH.

Product	Moisture content (%)		
	Zero-month	Three-month	Six-month
A	5.0 $\pm$ 0.04	9.1 $\pm$ 0.11	10.6 $\pm$ 0.10
B	6.5 $\pm$ 0.08	8.1 $\pm$ 0.10	10.4 $\pm$ 0.22
C	4.3 $\pm$ 0.08	6.4 $\pm$ 0.05	8.4 $\pm$ 0.02
D	3.6 $\pm$ 0.04	5.8 $\pm$ 0.06	7.8 $\pm$ 0.05
E	5.4 $\pm$ 0.05	11.1 $\pm$ 0.21	13.4 $\pm$ 0.15

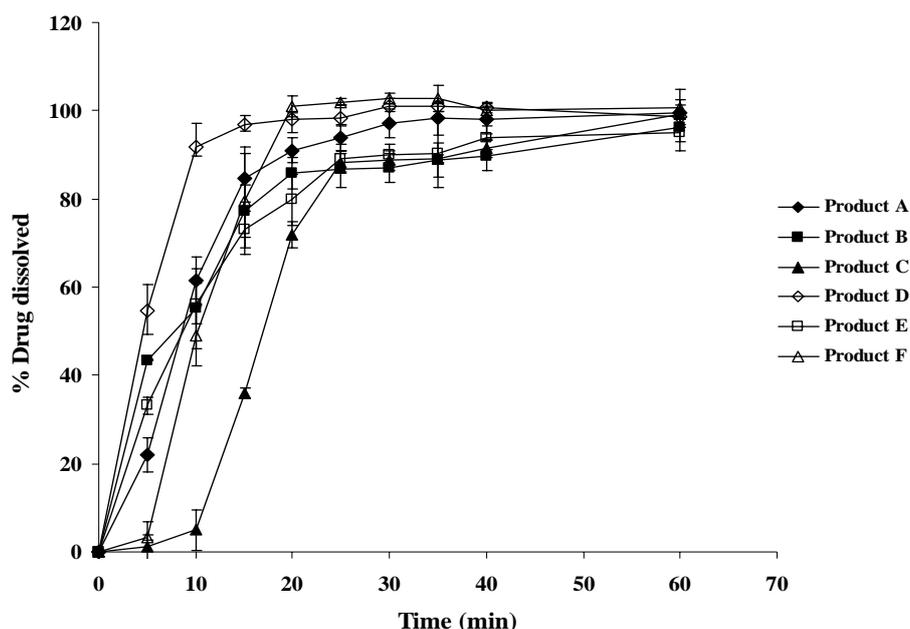
Product E gained the highest amount of moisture (105.6% increment) after three months storage under simulated tropical conditions (40 °C  $\pm$  2 °C and 75%  $\pm$  5% RH) followed by product A (82.0% increment). Product B gained the least moisture (24.6% increment). Also at six-month, the moisture uptake of Product E was the highest.

The variation in the extent of moisture uptake could have resulted from the difference in the effectiveness of film coating. This can be affected, among others, by the polymer used as film forming material to prevent moisture penetration into the core tablet. For example, methacrylates can provide better protection against moisture sorption than HPMC (Nada *et al.*, 2006). The excipients used for different purposes could also impact the degree of moisture uptake. For instance, superdisintegrants such as croscarmellose sodium (Ac-Di-Sol<sup>®</sup>) and sodium starch glycolate (Primojel<sup>®</sup>) could facilitate water uptake into the tablet once the moisture penetrates the film coating. In addition, polyvinylpyrrolidone (PVP) used as a binder, absorbs a significant quantity of water on exposure to elevated humidity (Fitzpatrick *et al.*, 2002). Furthermore, the thickness and type of the packaging material (e.g. polyvinylchloride) may vary with the different brands of norfloxacin tablets resulting in variation in moisture uptake (Ahmad and Shaikh, 2003).

### Comparison of *in vitro* dissolution profiles

Dissolution profiles of the six different brands of norfloxacin tablets at zero-month are depicted in Fig. 1. The USP specifies that the amount of norfloxacin released should not be less than 80% of the labeled amount at 30<sup>th</sup> min. At 20<sup>th</sup> min, except for Product C, all the products released more than 80% of the drug. Furthermore, Product D and Product F (the innovator product) released 100% at 30<sup>th</sup> min. However, three Products, B, C and E attained the maximum release at 60 min; indicating slower dissolution rates than that of Product F, the innovator product.

During the first 10 min, Product C showed the slowest dissolution rate probably due to factors such as the properties of excipients used in the formulation, coating material formulation, and manufacturing process variables including coating process variables and plasticity of the coating material. The plastic nature of the coating film is governed by the type of the coating polymer used, the concentration of the polymer in the coating solution, the amount and type of plasticizers used and the thickness of the film formed. Cao *et al.* (2004) showed that as the mixing time of HPMC-based coating solution increased from 1 h to 5 h, the release rate was decreased due to the plasticization of the



**Figure 1.** Dissolution profiles of five brands of norfloxacin tablets and the innovator product (Product F) at zero-month.

polymer. Likewise, the release profile of drug coated with different HPMC grades provided lag phases of varying duration (Zema *et al.*, 2007). In addition, it has been shown that an increase in the level of coating, measured as percent increase of the product weight, reduced drug release rate due to increased physical barrier between the drug and the medium (Cao *et al.*, 2004). Similarly, variation in drug release among different formulations of norfloxacin tablets has been reported and this was due to different polymer concentrations in the formulations (Bomma *et al.*, 2009). Another factor which may contribute to slow dissolution rate is the amount of hydrophobic lubricant (e.g. magnesium stearate) used and the duration of its mixing with other components of the tablet mixture which results in retarding the penetration of water into the tablet, and hence dissolution (Proost *et al.*, 1983). In addition, of the five brands, Product C had the highest crushing strength of 176.0 N (Table 2). This can also contribute to the observed slow drug release rate from this product.

Product D released the highest (54.7%)

amount of the drug at 5 min and attained the pharmacopoeial specification at 10 min. This fast rate of dissolution could also be attributed to formulation and manufacturing process variables. It has been reported that increasing the proportion of PVP, a water soluble polymer, into ethylcellulose (EC), a water insoluble polymer, increased the rate of release of norfloxacin (Venkateshwar *et al.*, 2004). Here, PVP, on the one hand, reduces the resistance offered by the EC film alone, and, on the other, increases the pores and/or their diameter for the drug to diffuse with less resistance. The amount of diluent used can also play a part in causing the observed variation in drug release behaviour. It has been reported that increasing starch content from 5% to 20% resulted in a three-fold increase in the dissolution rate of tablets (Ranjha *et al.*, 2001).

Some disintegrants have good water uptake and effective swelling properties and they can bring about fast drug dissolution (Marias *et al.*, 2003). Thus, variation in the type and the amount of disintegrants used may also contribute to the differences in drug release rate observed in this study. Size

and moisture content of the granules, compression force and other processing factors as well may affect the dissolution behaviour of the different brands (Ranjha *et al.*, 2001). Particle size of norfloxacin could also influence dissolution as tablets containing micronized drug have been shown to exhibit faster *in vitro* dissolution rates and improved bioavailability (Katdare *et al.*, 1987).

The  $t_{50\%}$  and  $t_{90\%}$  are used as dissolution parameters to compare dissolution profiles of different brands (Dahiya, 2006). The  $t_{50\%}$  and  $t_{90\%}$  of the different brands of norfloxacin tablets are presented in Table 4. As can be observed from the  $t_{50\%}$  values, except for Product C, all the products released 50% of their drug content below the time taken by the innovator product (10.2 min). However, the time taken by three products (B, C, and E) to release 90% of the drug is much longer than that taken by the innovator product indicating slower dissolution rates of these products than the innovator product. On the other hand, Product D released 90% of the drug in a shorter period of time (9.8 min) than that taken by the innovator product (17.6 min). Product A released 90% of the drug at a time comparable to that taken by the innovator product.

### ***In vitro* dissolution stability**

#### ***In vitro* dissolution stability at three-month**

The dissolution profiles of the five different brands of norfloxacin tablets after three months storage under 40 °C 2 °C and 75% ± 5% RH are shown in Fig. 2. Like at zero-month, at the 5<sup>th</sup> min, the highest

percent drug was released by Product D, whereas the lowest was released by Product C at three-month. As the dissolution profiles show, except for Product A, all the products released greater than the lower limit of the pharmacopoeial specification (80% of the labeled amount) at the 15<sup>th</sup> min. At the 30<sup>th</sup> min single point pharmacopoeial dissolution time, all the products released above 90% of the labeled amount and hence met the pharmacopoeial specification. However, slight increase and decrease in the dissolution rates of Products B and D, respectively, were observed when compared to the zero-month dissolution rates.

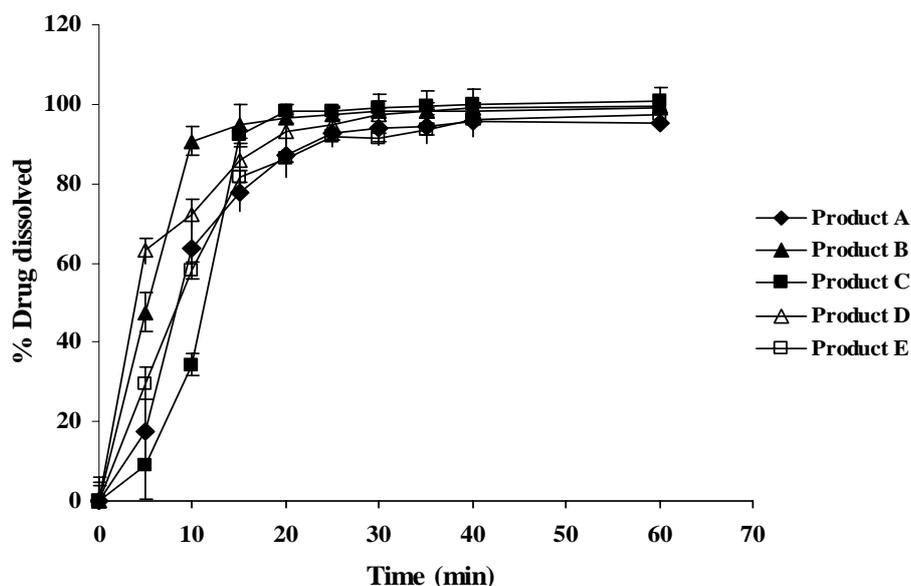
#### ***In vitro* dissolution stability at six-month**

The dissolution profiles for five different brands of norfloxacin tablets after six months storage under the same storage condition are shown in Fig. 3. The rate and extent of dissolution increased as the storage time increased from zero-month to six-month for Product B. For instance, the amount of norfloxacin dissolved at 10 min was 55.1%, 90.6% and 93.3% at the storage time zero-, three- and six-month, respectively. Moreover, 100% dissolution was obtained at 15 min at the sixth month, whereas it was only 77.2% at zero-month. Similarly, a slight increase in the dissolution profile of Product E was observed. It is known that the water solubility of a drug hydrate is less than its anhydrous form because of its greater thermodynamic stabilization by the interaction of water molecules (Khankari and Grant, 1995). However, it has been indicated that norfloxacin in its hydrate form seems to be

**Table 4.** Dissolution parameters ( $t_{50\%}$  and  $t_{90\%}$ ) for five different brands of norfloxacin tablets and the innovator product (F).

Product		A	B	C	D	E	F
Parameter	$t_{50\%}$	8.6	7.9	17.0	4.7	8.8	10.2
	$t_{90\%}$	19.4	42.1	37.4	9.8	29.0	17.6

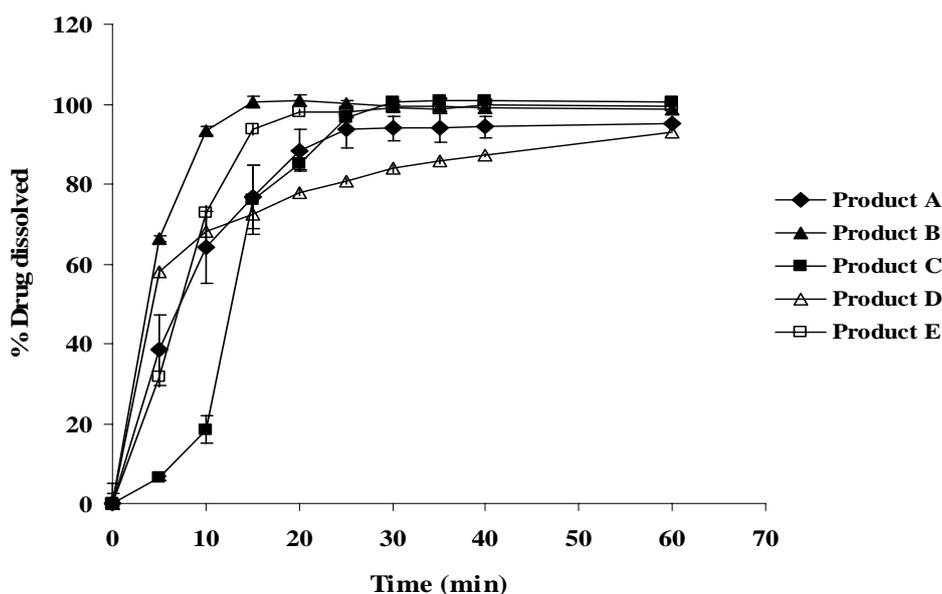
$t_{50\%}$  = Time taken to release 50% of the drug;  $t_{90\%}$  = Time taken to release 90% of the drug.



**Figure 2.** Dissolution profiles of five different brands of norfloxacin tablets at three-month storage under  $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and  $75\% \pm 5\% \text{ RH}$ .

more soluble in water than in the anhydrous form (Ting-Chou *et al.*, 2002). When norfloxacin anhydrous is transformed to its hydrate form, the main functional groups change from  $\text{COOH}$  to  $\text{COO}^-$  and  $\text{NH}$  to  $\text{NH}_2^+$  because of proton transfer from  $\text{COOH}$  group to  $\text{NH}$  group (Ting-Chou *et al.*, 2002). Thus, upon hydration, norfloxacin changes

from non-ionized form to ionized form thereby increasing its solubility. As a result, the dissolution profiles showed increase in the amount of norfloxacin dissolved and the rate at which it dissolved as the tablet product stored for six months under conditions of high temperature and high relative humidity.



**Figure 3.** Dissolution profiles of five different brands of norfloxacin tablets at six-month storage under  $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and  $75\% \pm 5\% \text{ RH}$ .

Product D showed a decrease in the rate and extent of dissolution with time. For example, the amount of norfloxacin dissolved at 30 min was 100.9%, 97.4% and 83.9 at zero-, three- and six-month, respectively. This phenomenon was also exhibited by Product A. The amount of moisture absorbed by the products increased from zero-month to six-month (Table 3). As a result, there may be prior saturation of water which might have hampered disintegration mechanism which relies on water uptake and subsequent swelling. The water absorbed might also act as a binding agent thereby increasing the bonding between the particles; and consequently decreasing the rate of dissolution and drug release. Therefore, storing norfloxacin tablets under simulated tropical conditions may bring about increase or decrease in dissolution rate depending upon the various formulation ingredients and process parameters by manufacturers.

### Chemical assay

Table 5 presents the assay results of the five different brands of norfloxacin tablets obtained at zero-, three- and six-month storage under  $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and  $75\% \pm 5\% \text{ RH}$ . The assay values range between 90% and 110% of the labeled amount specified for the 400 mg norfloxacin tablets in the USP at all the three storage time points.

The highest decrease in assay value as compared with the initial value is 3.6% (product D). The WHO stability guideline (WHO, 1996) specifies that a significant change is considered to have occurred if the

**Table 5.** Percent of drug contents (mean  $\pm$  SD, n = 3) of five different brands of norfloxacin tablets at zero-, three- and six-month storage under  $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and  $75\% \pm 5\% \text{ RH}$ .

Prod.	Drug content (%)		
	Zero-month	Three-month	Six-month
A	102.0 $\pm$ 0.30	101.7 $\pm$ 0.68	99.8 $\pm$ 0.56
B	100.1 $\pm$ 1.31	99.8 $\pm$ 0.94	99.1 $\pm$ 0.72
C	99.2 $\pm$ 1.04	99.1 $\pm$ 0.91	99.0 $\pm$ 0.86
D	105.7 $\pm$ 0.30	103.5 $\pm$ 1.08	102.4 $\pm$ 0.40
E	99.6 $\pm$ 3.46	99.6 $\pm$ 0.73	99.4 $\pm$ 0.73

assay value shows a 5% decrease as compared with the initial assay value of a product. Hence, the contents of all norfloxacin tablets studied were not significantly decreased upon storage under accelerated conditions.

### CONCLUSION

From the foregoing, it is apparent that tropical climatic conditions can bring about physical instability but not significant chemical instability on norfloxacin tablets. Despite minor differences, the five brands of norfloxacin tablets investigated released the amount of norfloxacin within the USP specification before storage and also after storage under simulated tropical climate conditions for six months.

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### REFERENCES

- Adebolagun OA, Olalade OA, Osumah SE (2007). Comparative evaluation of the biopharmaceutical and chemical equivalence of some commercially available brands of ciprofloxacin hydrochloride tablets. *Trop J Pharm Res* 6: 737-745.
- Ahmad I, Shaikh RH (2003). Effect of moisture on the stability of packaged paracetamol tablet formulations. *Pak J Pharm Sci* 16: 13-16.
- ASEAN (Association of South East Asian Nations) (2005). Asean guideline on stability study of drug product. 9<sup>th</sup> ACCSQ-PPWG Meeting, Philippines, 21-24 February 2005.

- Bomma R, Naidu RAS, Yamsani MR, Veerabrahma K (2009). Development and evaluation of gastroretentive norfloxacin floating tablets. *Acta Pharm* **59**: 211-221.
- BP (British Pharmacopoeia) (2000). The Pharmaceutical Press, Her Majesty's Stationery Office, London, Vol. I, II.
- Cao QR, Choi HG, Kim DC, Lee BJ (2004). Release behavior and photo-image of nifedipine tablet coated with high viscosity grade hydroxypropyl methylcellulose: effect of coating conditions. *Int J Pharm* **15**: 107-117.
- Cole G, Hagon J, Aulton M (2002). *Pharmaceutical Coating Technology*, 5<sup>th</sup> edn, Taylor and Francis Publisher, pp 3320-3335.
- Dahiya S (2006). Performance evaluation of marketed brands of sustained release diclofenac sodium tablets using statistical approaches. *Ethiop Pharm J* **24**: 91-97.
- EMA (2006). Stability testing of new drug substances and products: European Medicines Agency. CPMP/ICH/2736/99.
- Fitzpatrick S, McCabe JF, Petts CR, Booth SW (2002). Effect of moisture on polyvinylpyrrolidone in accelerated stability testing. *Int J Pharm* **246**: 143-151.
- Haywood A, Mangan M, Glass B (2006). Stability implications of repackaging paracetamol tablets into dose administration aids. *J Pharm Pract Res* **36**: 25-28.
- ICH (International Conference on Harmonization) (2003). Stability testing of new drug substance and product. Guidance for industry, Q1A (R2).
- Kassab NM, Singh AK, Kedor-Hackmam ERM, Santoro MIRM (2005). Quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography. *Rev Bras Cienc Farm* **41**: 507-513.
- Katdare AV, Oddoye DD, Bavitz JF (1987). Effect of micronization of norfloxacin on tablet properties. *Drug Dev Ind Pharm* **13**: 281-288.
- Khankari RK, Grant DJW (1995). Pharmaceutical hydrates. *Thermochim Acta* **248**: 61-79.
- Lakshmana FL, Paul JA, Kok H, Frijlink HW, Vromans H, Maarschalk KVV (2008). Using the internal stress concept to assess the importance of moisture sorption-induced swelling on the moisture transport through the glassy HPMC films. *AAPS PharmSciTech* **9**: 891-898.
- Lima DM, Dos Santos LD, Lima EM (2008). Stability and *in vitro* release profile of enalapril maleate from different commercially available tablets: possible therapeutic implications. *J Pharm Biomed Anal* **47**: 934-937.
- Marias AF, Song M, de Villiers MM (2003). Effect of compression force, humidity and disintegrant concentration on the disintegration and dissolution of directly compressed furosemide tablets using croscarmellose sodium as disintegrant. *Trop J Pharm Res* **2**: 125-135.
- Nada A, Sharaf MA, El Gholmy ZA, Khalafallah NM (2006). Effect of raw materials on the formulation of norfloxacin tablets. *Pharm Technol Eur* **18**: 36-45.
- Proost JH, Bolhuis GK, Lerk CF (1983). The effect of the swelling capacity of disintegrants on the *in vitro* and *in vivo* availability of diazepam tablets, containing magnesium stearate as a lubricant. *Int J Pharm* **13**: 287-296.
- Ranjha NM, Mufti AUR, Mahmood W (2001). Effect of pH on dissolution behaviour of commercially available diclofenac sodium tablets. *J Res (Sci)* **12**: 78-84.
- Rao V, Shyale S (2004). Preparation and evaluation of ocular inserts containing norfloxacin. *Turk J Med Sci* **34**: 239-246.
- Singh S, Kumar V (2006). Recent developments on long-term stability test conditions. *The Pharma Review*, December Issue.
- Ting-Chou HU, Shun-Li W, Ting-Fang C, Shan-Yang L (2002). Hydration-induced proton transfer in the solid state of norfloxacin. *J Pharm Sci* **91**: 1351-1357.
- USP (The United States Pharmacopoeia) (2008). USP31/NF26, Rockville, Vol.1, MD, USA.
- WHO (World Health Organization) (2006). Draft regional guidelines on stability testing of active substances and pharmaceutical products: Regional Committee for the Eastern Mediterranean, Fifty-third Session, EM/RC53/12.
- WHO (World Health Organization) (1996). Guidelines for stability testing of pharmaceutical products containing well established drug substances in conventional dosage forms. WHO Technical Report Series, No. 863.

Zema L, Maroni A, Foppoli A, Palugan L, Sangalli ME, Gazzaniga A (2007). Different HPMC viscosity grades as coating agents for an oral time and/or site-controlled delivery system: an investigation into the mechanisms governing drug release. *J Pharm Sci* **96**: 1527-1536.