STABILITY AND COMPARATIVE DISSOLUTION STUDIES OF FIVE BRANDS OF NORFLOXACIN TABLETS MARKETED IN ADDIS ABABA

By

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements of the Degree of Master of Science in Pharmaceutics in the Department of Pharmaceutics, School of Pharmacy

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</tr>
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<td>APF</td>
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<td></td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the concentration time curve</td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>British Pharmacopoeia</td>
<td></td>
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<tr>
<td>C_{max}</td>
<td>Maximum drug concentration in the plasma</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>Ethylcellulose</td>
<td></td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
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</tr>
<tr>
<td>HPMC</td>
<td>Hydroxylpropyl methylcellulose</td>
<td></td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization</td>
<td></td>
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<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>N</td>
<td>Newton</td>
<td></td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinylchloride</td>
<td></td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
<td></td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
<td></td>
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<tr>
<td>RS</td>
<td>Reference standard</td>
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<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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ABSTRACT

Norfloxacin is hygroscopic and can undergo polymorphic change upon exposure to high relative humidity. Currently, many different brands of norfloxacin tablets are available in the local market, Addis Ababa, Ethiopia. The quality of these brands may vary with time under the influence of a variety of environmental factors such as temperature and humidity, and also a brand with poor quality may exist. The availability of many brands and the susceptibility of the active ingredient of norfloxacin tablets to environmental factors are the major reasons for evaluating the quality and stability of the tablets. Dissolution profile is a very important quality parameter for solid oral dosage forms. Furthermore, the purpose of stability testing is to provide evidence on how the quality of a drug substance or a drug product varies with time under the influence of a variety of environmental factors. Accordingly, the objective of the present study is to compare the dissolution profiles of five different brands of norfloxacin tablets with that of the innovator and study the stability of the five brands of norfloxacin tablets under the influence of accelerated conditions (40°C and 75% RH).

Norfloxacin 400 mg film coated tablets of six different brands, A, B, C, D, E and F (codes given to the brands) were purchased from Addis Ababa, Ethiopia. The tablets were assessed for physical and chemical parameters immediately after purchase. At the same time, these samples were stored in an accelerated stability cabinet maintained at 40°C ± 2°C and 75% ± 5% RH. Then, the physical and chemical stability parameters were tested at three and six months according to International Conference on Harmonization (ICH) Guidelines. The t50% and t90% (time required for releasing 50% and 90% of the drug, respectively) were used as dissolution parameters to compare dissolution profiles.

The t50% results indicated that except for Product C all the products released 50% of the drug below 10.2 min (the time taken by the innovator product to release 50%). However, the values of t90% 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 relative to the latter. On the other hand, product D released 90% at 9.8 min showing faster rate of dissolution than the innovator product. The stability testing indicated that during the six months storage physical changes like film cracking, decrease in hardness, increase in moisture content and changes in
dissolution profiles were observed. These were mainly due to moisture sorbed by the products. The highest change in drug content was 3.6% at six-month, less than 5%. Accordingly, no significant change in drug content occurred in any of the investigated norfloxacin tablet product stored under conditions of 40 °C ± 2 °C and 75% ± 5% RH for six months.

Key words: Norfloxacin, Film coated tablet, Dissolution profile, Stability, Accelerated conditions
1. INTRODUCTION

The stability of pharmaceutical preparations under tropical conditions is not normally investigated during manufacturers' stability studies; and if it is, the results are not readily available. In third world countries, it is often practically and/or economically impossible to protect pharmaceutical preparations from the harmful effects of high temperatures and high relative humidity during transportation, storage and use (Groot et al., 1994). Consequently, the quality of drugs imported into developing countries having a tropical climate may be adversely affected if their formulations have not been optimized for stability under these conditions. The influence of climate conditions in tropical countries on the quality of essential drugs has been of concern to the World Health Organization (WHO) and regulatory bodies. The WHO has conducted studies on the stability of essential drugs during distribution and storage in tropical climates and has recommended testing the stability of drugs manufactured for the global market under class IV conditions (40 °C and 75 % relative humidity (RH)) (Risha et al., 2003).

The introduction of generic drug products from multiple sources into the health care delivery system of many developing countries was aimed at improving the overall healthcare delivery systems in such countries. However, this has been accompanied by a variety of problems of which the most critical is the widespread distribution of counterfeit drug products. The production of counterfeit drug products is a vast and underreported problem, particularly affecting poorer countries. It is an important cause of unnecessary morbidity, mortality, and loss of public confidence in medicines and health structures. In 2004, it has been estimated that up to 15% of all sold drugs were counterfeit, and in parts of Africa and Asia this figure exceeded 50% (Adegbolagun et al., 2007; Cockburn et al., 2005).

Consequently, the need to select one product from among several generic drug products of the same active ingredient during the course of therapy is a cause of concern to a healthcare practitioner. The first stage in ascertaining the therapeutic equivalence of any drug product involves ascertaining the pharmaceutical and biopharmaceutical equivalency of such drug products (Adegbolagun et al., 2007).
The evaluation of dissolution profiles is a very important quality parameter for solid oral dosage forms. The absorption of drugs from solid pharmaceutical forms after oral administration depends, among other factors, on the release of the drug from the pharmaceutical form, its dissolution or solubility in physiological conditions, and its permeability through the gastrointestinal tract. Due to the critical nature of the two initial stages, dissolution tests \textit{in vitro} can be relevant to predict the performance of the drug \textit{in vivo}. Based on these considerations, dissolution tests are largely used to assure the quality of the pharmaceutical product (Ferraz \textit{et al.}, 2007). Furthermore, dissolution tests can be used for comparing new or generic formulations with an existing product. Thus, \textit{in vitro} dissolution testing can be a valuable predictor of the \textit{in vivo} bioavailability and bioequivalence of oral solid dosage forms (Patel \textit{et al.}, 2005; Eryol \textit{et al.}, 2004).

1.1. Stability

Stability is defined as the extent to which a product retains the same properties and characteristics that it possessed at the time of manufacture within a specified limit throughout its storage period and use (USP, 2008). Stability of a pharmaceutical product refers to the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods (Lucas \textit{et al.}, 2004). The expiration dating period is defined as the time interval that a drug is expected to remain within the approved specifications after manufacture. To determine the length of time that a drug product remains within specification, a stability study is conducted (Chen \textit{et al.}, 1997).

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 \textit{et al.}, 2008). A drug product, which is not of a sufficient stability, can result in changes in physical (like hardness, dissolution rate, phase separation, etc.) as well as in chemical characteristics (formation of high risk decomposition substances). Microbiological instability of a sterile drug product could also be hazardous (ASEAN, 2003). Moreover, if the pharmaceutical product is unstable, then quality of commercial supplies becomes unreliable. Possibly sub-potent product
or potentially toxic unknown impurities may pose risk to patients. In such cases, the drug potentially becomes unsafe and/or ineffective (Malinowski and Johnson, 2006).

In general, there are five types of stability: chemical, physical, microbiological, therapeutic and toxicological (USP, 2008). Chemical stability is aimed at testing whether each active ingredient retains its chemical integrity and labeled potency, within the specified limits. Physical stability is used to evaluate whether the original physical properties, including appearance, palatability, uniformity, dissolution, and suspendability are retained or not. Microbiological stability test proves sterility or resistance to microbial growth and effectiveness of antimicrobial agents. Therapeutic effect and any significant increase in toxicity are dealt under the therapeutic and toxicological stability types, respectively.

Chemical stability is important for selecting storage conditions (temperature, light, humidity), selecting the proper container for dispensing (glass versus plastic, clear versus amber or opaque) and for anticipating interactions when mixing drugs and dosage forms. Stability and expiration dating are based on reaction kinetics, i.e., the study of the rate of chemical change and the way this rate is influenced by conditions of concentration of reactants, products, and other chemical species that may be present, and by factors such as solvent, pressure, and temperature (USP, 2008).

1.1.1. The effect of environmental conditions on stability
The physical and chemical properties of pharmaceutical products may be altered during storage leading to deterioration and decrease in their therapeutic usefulness. In certain cases, such deterioration may result in toxicity of the drug substance (Ibezim, 2005). The primary environmental factors that can affect stability include temperature, relative humidity, and light (USP, 2008).

Elevated temperature, especially if coupled with high relative humidity, is known to cause and accelerate physical deterioration and chemical degradation (Croce et al., 1986). Humidity can have a significant effect on solid drug substances or drug products; even for reactions which themselves do not involve water. Among the effects are changes in the drug form (such as hydrate formation) and plasticization of drug or excipients. Plasticization, where water acts to
lower the glass transition of a material, can lead to a significant increase in mobility and corresponding reactivity in solid dosage forms (Waterman and Adami, 2005). Use of humidity resistant packaging, which could protect the drug until the moment of consumption, is too expensive for general use in developing countries (up to one third of the price of the unpacked drug). Thus, as soon as a sealed container is opened, humidity can penetrate and accelerate physical, chemical, as well as microbiological deterioration and affect the stability of the drug (Groot et al., 1994).

Pharmaceutical products are exposed to light while being manufactured as a solid or solution, packaged, held in pharmacy shops or hospitals pending use, or held by the consumer pending use. Light exposure can induce chemical degradation in susceptible molecules (Waterman and Adami, 2005; Row, 2002). In susceptible compounds, photochemical energy creates free radical intermediates, which can perpetuate chain reactions. Most compounds will degrade as solutions when exposed to high energy ultraviolet (UV) exposure (Row, 2002).

As a result, 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. Thus, the storage conditions recommended by manufacturers on the basis of stability studies should guarantee the maintenance of quality, safety, and efficacy throughout the shelf life of a product (WHO, 1996).

1.1.2. Stability testing

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, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions (EMEA, 2006). In addition, product-related factors influence the 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 stability of excipients that may contain or form reactive degradation products has to be considered as well. The interactions of all these features affect the eventual stability of the product (WHO, 2006; Haywood et al., 2006).
No drug product in a container-closure system is indefinitely stable, and the manufacturer or packer of a drug product is responsible for determining the stability characteristics for each of the product (Croce et al., 1986). Stability testing is the only way to demonstrate that the pharmaceutical product would meet the laid-down specifications within acceptance criteria throughout its lifetime (Singh and Kumar, 2006). It should include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety and/or efficacy (ASEAN, 2003).

1.1.2.1. Types of stability testing

**Accelerated testing**

Accelerated testing refers to the study designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as part of the formal stability studies. Formal stability study includes long term and accelerated (and intermediate) studies undertaken on primary batches according to a prescribed stability protocol to establish or confirm the re-test period of a drug substance or the shelf life of a drug product. Data from the accelerated stability studies, in addition to long term stability studies, can be used to assess longer term chemical effects at non-accelerated conditions and to evaluate the effect of short term excursions outside the label storage conditions such as might occur during shipping (ICH, 2003; Fizpatrick et al., 2002).

At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., zero, three, and six months), from a six-month study is recommended. Where an expectation exists that results from accelerated testing are likely to approach significant change criteria, increased testing should be conducted by including a fourth time point in the study design (ICH, 2003).

**Stress testing**

Stress testing for drug substance is the study undertaken to elucidate the intrinsic stability of the drug substance while stress testing for drug product is the study undertaken to assess the effect of severe conditions on the drug product. It should include the effect of temperatures (in 10°C increments (e.g., 50°C, 60°C, etc.) above that for accelerated testing), humidity (e.g., 75% RH
or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension (ICH, 2003).

**Long-term testing**

Long-term testing is the stability study undertaken under the recommended storage condition for the re-test period of a drug substance or the shelf life of a drug product proposed (or approved) for labeling (ICH, 2003). The testing includes experiments conducted on the physical, chemical, and microbiological characteristics of a drug or a drug product under the storage conditions expected in the intended market (WHO, 1996).

For long-term studies, frequency of testing should be sufficient to establish the stability profile of the drug product. For products with a proposed shelf life of at least twelve months, the frequency of testing at the long-term storage condition should normally be every three months over the first year, every six months over the second year, and annually thereafter throughout the proposed shelf life (WHO, 1996).

**Climatic zones**

For convenience in planning for packaging, storage and stability studies, international practice identifies four climatic zones, which are described in Table 1.2. The values are based on observed temperatures and relative humidity both outside and in rooms, from which mean annual temperature and relative humidity values are calculated (USP, 2008).

<table>
<thead>
<tr>
<th>Climatic zone</th>
<th>Climatic definition</th>
<th>Mean annual temperature</th>
<th>Mean annual relative humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Temperate</td>
<td>20 °C</td>
<td>45%</td>
</tr>
<tr>
<td>II</td>
<td>Subtropical</td>
<td>21.6 °C</td>
<td>60%</td>
</tr>
<tr>
<td>III</td>
<td>Hot and dry</td>
<td>26.4 °C</td>
<td>40%</td>
</tr>
<tr>
<td>IV</td>
<td>Hot and humid</td>
<td>26.7 °C</td>
<td>70%</td>
</tr>
</tbody>
</table>

Table 1.2: International climatic zones and climatic conditions (USP, 2008)
Furthermore, at the fortieth meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations, Geneva, October 2005, it was recommended to split the current climatic zone IV (hot and humid) into climatic zone IVA – for which 30 °C and 65% RH will remain the standard long term testing condition – and climatic zone IVB, for which, if justified, 30 °C and 75% RH will become the long term testing condition (WHO, 2006).

1.1.2.2. Stability testing conditions

In general, a pharmaceutical product should be evaluated under storage conditions that test its thermal stability and, if applicable, its sensitivity to moisture or potential for solvent loss. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use with due regard to the climatic zone(s) in which the product is intended to be marketed (ICH, 2003). There are general case storage conditions recommended for long-term and accelerated testing of drug products proposed to be marketed in climatic zones III and IV (Table 1.1).

<table>
<thead>
<tr>
<th>Study</th>
<th>Storage conditions</th>
<th>Minimum time period (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long term</td>
<td>30 °C ± 2 °C and 65% ± 5% RH</td>
<td>12</td>
</tr>
<tr>
<td>Accelerated</td>
<td>40 °C ± 2 °C and 75% ± 5% RH</td>
<td>6</td>
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</table>

The accelerated stability study test conditions for climatic zones I and II are the same as that for climatic zones III and IV. However, for the product registration purpose, it is up to the applicant to decide whether long-term stability studies are performed at 25 ± 2°C and 60% RH ± 5% RH or 30°C ± 2°C and 65% RH ± 5% RH in these climatic zones (ICH, 2004; WHO, 2009).

For climatic zones I and II, if long-term studies are conducted at 25°C ± 2°C and 60% RH ± 5% RH and “significant change” occurs at any time during six months’ testing at the accelerated storage condition, additional testing at the intermediate storage condition (30 °C ± 2 °C and 65% ± 5% RH) should be conducted and evaluated against significant change criteria. Where a “significant change” for a drug product is defined as (WHO, 1996, ICH, 2004):

- A 5% change in assay from its initial value;
- Any degradation product’s exceeding its acceptance criterion;
- Failure to meet the acceptance criteria for physical attributes (e.g., color, phase separation, resuspendability, caking, hardness);
- Failure to meet the acceptance criterion for pH; or
- Failure to meet the acceptance criteria for dissolution for 12 dosage units

Stability testing at a high humidity condition, e.g., 25°C and 80% RH is recommended for solid dosage forms in water-vapour permeable packaging, e.g., tablets in PVC/aluminum blisters, intended to be marketed in territories with extremely high humidity conditions in zone IV. However, for solid dosage forms in primary containers designed to provide a barrier to water vapour, e.g. aluminum/aluminum blisters, stability testing at a storage condition of extremely high humidity is not considered necessary (ICH, 2004; WHO, 1996).

1.1.3. Stability test parameters

Test parameters to be measured in a stability study are determined by the dosage form (APVMA, 2006). In general, appearance, assay and degradation products should be evaluated for all dosage forms, as well as preservative and antioxidant content if applicable (DDA, 2007). The list of parameters for each dosage form is presented in WHO guidelines on “stability testing of active substances and pharmaceutical products” (WHO, 2006). Besides the above parameters, tablets are tested for dissolution (or disintegration, if justified), water content and hardness/friability. The drug release rate is one of the most important parameters for solid oral drug delivery systems (Lima et al., 2008).

1.1.3.1. Drug release/In vitro dissolution

The most important property of formulation is the capacity to allow the active ingredient to arrive at its site of action in an amount sufficient to produce the desired pharmacological effect. Dissolution is crucial to the process of drug release from the formulation (Hernandez and Antonio, 2006). Drug dissolution or release for most pharmaceutical products containing a drug in the solid state is an essential step in delivering drug molecules to their site(s) of action (FDA, 2005). It is positively correlated with absorption, and hence is a determinant of the bioavailability of an orally administered drug. In vitro drug release studies are also required for the development and quality assurance of oral pharmaceutical formulations (Hernandez and
The purpose of the *in vitro* dissolution studies in the early stage of drug development is to select the optimum formulation, evaluate the active ingredient and excipient, and assess any minor changes for drug products (Sirisuth and Eddington, 1999). Dissolution tests can also be used as quality control and stability indicating tests during the formulation development stage (Eryol *et al.*, 2004).

Furthermore, characterization of the dissolution against time profile is essential to establish a correlation between the amount of drug dissolved and bioavailability *in vivo*, as well as to compare a test with a reference formulation (Hernandez and Antonio, 2006). Generally, the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is the plasma drug concentration or amount of drug absorbed (Sirisuth and Eddington, 1999). The United States Pharmacopoeia (USP) also defines *in vitro-in vivo* correlation (IVIVC) as “the establishment of a relationship between a biological property, or a parameter derived from a biological property produced from a dosage form, and a physicochemical property of the same dosage form” (USP, 2008). Typically, the parameter derived from the biological property is the area under the concentration time curve (AUC) or the maximum drug concentration in the plasma (C$_{\text{max}}$), while the physicochemical property is the *in vitro* dissolution profile. A linear relationship with slope of unity, if possible, is preferred, as the dissolution profile is a representative of the absorption profile (Sirisuth and Eddington, 1999).

As a result, drug dissolution *in vitro* can be considered as a surrogate marker of oral bioavailability, although the results should be confirmed by an *in vivo* assay. Hence, dissolution tests represent a rapid and economical alternative for the comparison of a test with a reference drug product, yielding useful information on bioequivalence or non-bioequivalence (Hernandez and Antonio, 2006).

The simplest way to compare dissolution profiles of test and reference formulations is to check the percentage of the dissolved active compound in the dissolution medium after a certain period of time. For rapidly dissolving drug products, the use of single point comparison of the dissolution profiles may be sufficient. However especially in the case of slowly dissolving or poorly water-soluble drugs, comparison of the multiple time points is recommended by the
Food and Drug Administration (FDA). Comparison of multiple time points or of complete dissolution profiles is more complex than with a single point test (Eryol et al., 2004).

*In vitro* dissolution data are also supportive in the evaluation and interpretation of possible risks, especially in the case of controlled/modified-release dosage forms - e.g. as regards dose dumping, food effects on bioavailability or interaction with other drugs, which influence gastrointestinal environmental conditions. Last but not least, *in vitro* dissolution data will be of great importance when assessing changes in production site, manufacturing process or formulation and assist in decisions concerning the need for bioavailability studies (FIP, 1995). The FDA guidelines also advise the use of *in vitro* dissolution testing to ensure product quality in case of certain scale-up and post approval changes (SUPAC) such as manufacturing site changes, increase or decrease in batch size and small quantitative changes in excipients (FDA 1995; FDA, 1997).

### 1.2. Overview of quinolones and fluoroquinolones

Quinolones are a group of synthetic antibacterial agents known to have broad-spectrum antibacterial activity and rapid bactericidal action (Ugbogu et al., 2007). The original quinolone antibiotics included nalidixic acid, cinoxacin and oxolinic acid (Okumura et al., 2009). The essential structure of quinolones (Koga et al., 1980; Wikipedia, the free encyclopedia, 2009) is shown in Figure 1.1.

![Figure 1.1: Essential structure of all quinolones](image)

The addition of fluorine to the original quinolone antibacterial compounds yielded a new class of drugs, the fluoroquinolones, which have a broader antimicrobial spectrum and improved
pharmacokinetic properties (Wise and Honeybourne, 1999). Because of their excellent safety and tolerability, they have become popular alternatives to penicillin and cephalosporin derivatives in the treatment of various infections (Lode and Allewelt, 2002).

Fluoroquinolones have gained stupendous importance during the last two decades because of their potent anti-bacterial activity against wide varieties of Gram-positive and Gram-negative pathogenic bacteria with minimum toxic side-effects, and some what different mechanism of action than other available antibacterial drugs (Rishipathak et al., 2009). These agents target bacterial type II topoisomerases, deoxyribonucleic acid (DNA) gyrase and topoisomerase IV and prevent their replication, making them active against a wide range of Gram-positive and Gram-negative bacteria (Drlica and Zhao, 1997). Nowadays, they are one of the frequently prescribed classes of antibacterial agents. They are the fastest growing antibacterial class in terms of global revenue as well, increasingly being used in both the hospital and community sectors to treat broad range of infections (Bhanot et al., 2001; Velissariau, 2006; Arash et al., 2008).

1.2.1. Norfloxacin

Norfloxacin, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, is a synthetic broad antibacterial compound belonging to the group of fluoroquinolones (Barbas et al., 2007). Figure1.2 shows its structural formula.

![Figure1.2: Structural formula of norfloxacin](image)

The addition of a fluorine atom at the carbon-6 and a piperazine ring at the carbon-7 of the quinolones’ structure has increased its potency in relation to other quinolones (Tillotson, 1996; Mansilla et al., 2006). It is mainly used in the treatment of urinary, respiratory and
gastrointestinal tracts infections (Rao et al., 2004). It is also used for sexually transmitted
diseases, sinuses, prostate infections, typhoid fever, and pneumonia (Lode and Allewelt, 2002;
Bedor et al., 2007).

Gastro-intestinal absorption of norfloxacin is 40 to 50% (Bedor et al., 2007; Norrbey, 1983). This
drug has a repetitive dose schedule (400 mg twice daily) (Gerald, 2004). Following a
single oral dose of 400 mg, serum concentrations of about 1.4 mg/l are achieved approximately
one hour after administration (End, 1983). Elimination of norfloxacin is mainly by renal route
and 30 to 40 % of the dose administered is recovered in the urine as antibacterially active drug.
The half-life in serum is about 3 to 4 h in healthy subjects and seems to increase with
decreasing renal function (Venkateshwar, 2004; End, 1983; Ruiz et al., 1997). The urine
concentrations achieved exceed the minimum inhibitory concentrations (MICs) of most
pathogens for at least 12 h after a 400 mg dose (Norrbey, 1983).

1.2.1.1. Properties of norfloxacin

Norfloxacin is a white or pale-yellow, hygroscopic, photosensitive, crystalline powder, very
slightly soluble in water, slightly soluble in acetone and in alcohol (BP, 2000). It exists in
several solid forms: two anhydrous polymorphs (form A and form B) and several hydrated
forms (Chongharoen et al., 2008). Different polymorphs exhibit different physicochemical
properties such as solubility, dissolution rate, bioavailability and chemical and physical
stabilities. Many commercial samples of norfloxacin are provided as the metastable form at
room temperature and then, undesirable transformations could occur (Barbas et al., 2007).

Norfloxacin is most stable at acidic and basic pH, in darkness and at low temperature. Two
photodecomposition products of norfloxacin analogs, formyl piperazine analog 1-ethyl-6-
fluoro-4-oxo-7-(1-formyl-4-piperazinyl)-1,4-dihydroquinoline-3-carboxylic acid and
ethylenediamine analog 7[(2-aminoethyl)-amino]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-
3-carboxylic acid have, so far, been identified (Nangia et al., 1991).

1.3. Film coated tablets

A significant fraction of commercially available tablets are film-coated tablets, wherein the
tablet is coated with a thin layer of a polymeric material. In the pharmaceutical industry, film
coating has been applied for various purposes, such as appearance, taste masking, protection from environmental conditions and sustained or controlled release purposes. The presence of the coating film can pose problems. In the literature, it has often been demonstrated that the coating functionality is correlated with coating properties such as coating thickness and porosity (Laksmana et al., 2009).

In the aqueous film coating process, tablets are exposed to wide temperature range and humidity variations that may promote undesired water penetration into the tablet core during coating or storage. Penetrated water can cause changes in the structure of the film core interface, core expansion and increase the risk of degradation of moisture-labile drugs (Patel et al., 2009).

1.4. Rationale of the study
Differences in solid-state properties, formulations and/or manufacturing processes of tablets can lead to disparities in bioavailability between brands of the same drug (Schuebel et al., 2007). In addition to one locally produced, Ethiopia is importing norfloxacin tablets sourced from many different manufacturers. Therefore, it is important to compare the in vitro dissolution profiles of the local product and the imported norfloxacin tablets with that of the innovator product for quality purpose. Furthermore, for a hygroscopic norfloxacin, which absorbs moisture from the environment, 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 a concern to the government, the regulatory body, the distributors, the private sectors and the community. 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.

1.5. Objectives

1.5.1. General objective
The aim of the present study is to compare the in vitro dissolution profiles of five different brands of norfloxacin tablets with that of the innovator brand and to study the stability of the
five brands of norfloxacin tablets, under the influence of accelerated conditions (40 °C and 75% RH), marketed in Addis Ababa.

1.5.2. Specific objectives

• To assess the influence of tropical climate conditions (40 °C and 75% RH) on drug content, *in vitro* dissolution and other physical properties of norfloxacin tablets
• To compare the stability of norfloxacin tablets from different manufacturers under the effect of high temperature and high relative humidity (40 °C and 75% RH),
• To compare the quality of norfloxacin tablets from different manufacturers
2. EXPERIMENTAL

2.1. Materials

2.1.1. Test samples

Norfloxacin 400 mg film coated tablets of six different brands were purchased from Addis Ababa local market pharmacies (Table 2.1). All the samples used for the study were within their shelf life during the time of investigation.

Table 2.1: List of norfloxacin 400 mg film coated tablets investigated

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

MFD = manufacturing date; EXD = expiry date; autilized for in vitro dissolution comparison only

2.1.2. Chemicals and reagents

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

2.2. Methods

2.2.1. Physical properties

2.2.1.1. Weight variation

Twenty tablets from each product were weighed individually using analytical balance (Sartorius AG, CP124S, Germany) and the mean of the weights were calculated (BP, 2000). The percentage deviation of each tablet from the mean weight and the standard deviations were determined.

2.2.1.2. Diameter/ width, length, and thickness

The diameter (for the round shape tablets), the width and the length (for the 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 these parameters and hardness. Then, the corresponding mean and standard deviations were calculated.

2.2.1.3. Hardness

Hardness was done for each product by measuring the crushing strength of ten randomly selected tablets (BP, 2000) using Hardness tester (Pharma test, CE type PTB311E, Germany). Then the mean and standard deviations were calculated.

2.2.1.4. 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 vacuum oven (Javac, DS40, Australia) at a pressure not exceeding 5 mm of mercury at 100 °C to constant weight. The percent mass lost after drying was calculated with respect to the mass before drying.
2.2.2. Dissolution test

2.2.2.1. Calibration curve for dissolution test method

A stock solution was prepared by dissolving 10 mg of norfloxacin USP RS in 100 ml of acetate buffer, pH 4.0. Six concentration levels were prepared by diluting 1, 2, 3, 4, 5 and 6 ml of the stock solution to 100 ml with acetate buffer to get 1, 2, 3, 4, 5 and 6 µg/ml, respectively. All the flasks used were covered with aluminum foil to protect norfloxacin from light. The respective absorbance readings of the six concentration levels were taken at 278 nm. Then, the concentration versus the absorbance was plotted to obtain the Beer-Lambert calibration curve shown in Figure 2.1. The linear regression equation is: Y= 0.1264X + 0.0021, where Y is the absorbance and X is the concentration in µg/ml; r² = 0.9998. The result reveals that there is a strong linear relationship between the concentration of the tested samples and the absorbance values over the concentration range 1 to 6 µg/ml.

![Beer-Lambert calibration curve for norfloxacin USP RS in acetate buffer of pH 4.0 and maximum wave length of 278 nm over the range of 1 to 6 µg/ml](image)

Figure 2.1: Beer-Lambert calibration curve for norfloxacin USP RS in acetate buffer of pH 4.0 and maximum wave length of 278 nm over the range of 1 to 6 µg/ml

2.2.2.2. Dissolution profiles

Dissolution profiles of six brands of norfloxacin tablets were determined at zero-month immediately after collection from market for the in vitro dissolution comparison. The innovator
product (Noroxin) was studied for this purpose only. And the dissolution profiles of five brands were performed for stability study after storing for three and six months in stability cabinet (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 revolutions per min (USP, 2008). The dissolution medium was 750 ml acetate buffer pH 4.0, which was maintained at 37 ± 0.5 °C in six vessels. A sample of 5 ml was withdrawn from each vessel at 5, 10, 15, 20, 25, 30, 35, 40 and 60 min, and was kept in amber color 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 the same concentration. The percentage of drug released at each time point was calculated in comparison with norfloxacin RS using the formula:

\[ \frac{A_s}{A_r} \times 100 \]

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.

2.2.3. Assay

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

2.2.3.1. HPLC 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® 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 v/v/v). The pH of the final mixture was adjusted to 3.3 with phosphoric acid using pH meter (mettler-toledo, GmbH 8603 Schwerzenbach, China). The flow rate was 1.0 ml/min and volume of injection was 10 µl. The UV detection was carried
out at 279 nm. All analyses were done at ambient temperature (24 ± 2 °C) under isocratic conditions.

2.2.3.2. System suitability

**Calibration curve**

10 mg of USP norfloxacin RS was dissolved in 40 ml aqueous phosphoric acid (1 in 1000) in a 100 ml volumetric flask. It was diluted to volume with the mobile phase to get a 100 µg/ml stock solution. Five concentration levels were prepared by diluting 8, 9, 10, 11 and 12 ml of the stock solution to 50 ml with the mobile phase to obtain 16.0, 18.0, 20.0, 22.0 and 24.0 µg/ml, respectively. All the flasks used were covered with aluminum foil to protect norfloxacin from light. Each solution was injected into the chromatographic system and the concentrations were plotted against peak areas together with 95% confidence interval (Figure 2.2). The regression equation is: \( Y = 71043X - 1466 \), where \( Y \) is the peak area and \( X \) is the concentration in µg/ml; \( r^2 = 0.9978 \). The value of correlation coefficient (\( r \)) is 0.9989, indicating good linear correlation between the concentration of the test sample and the response (peak area).

![Figure 2.2: Calibration curve of norfloxacin USP RS: Lichrospher® 100 RP-18 (125 x 4 mm, 5 µm) column and mobile phase water:acetonitrile:triethylamine (80:20:0.3 v/v/v) mixture (pH 3.3) were used at a flow rate of 1.0 ml/min and volume of injection of 10 µl and UV detection at 279 nm](image-url)
Precision
The precision of the method was evaluated through intra-day repeatability of responses after replicate (n = 6) injections of RS solution (20.0 µg/ml). The precision is expressed as relative standard deviation (RSD) amongst responses (USP, 2008). Accordingly, the RSD value was found to be 1.15%, being within an acceptable range. It should be less than 2%.

Standard preparation
10 mg of USP norfloxacin RS was taken and dissolved in 40 ml aqueous phosphoric acid (1 in 1000) in a 100 ml volumetric flask. Then, it was diluted to volume with the mobile phase to get a 100 µg/ml stock solution. The stock solution was filtered through a 0.45 µm membrane filter (Whatman GmbH, Germany) and 10 ml of the filtrate was transferred to a 50 ml volumetric flask and diluted to volume with the mobile phase in order to obtain solution with final concentration of 20 µg/ml. All the flasks used were covered with aluminum foil to protect norfloxacin from light.

Sample preparation
20 tablets from each brand of norfloxacin tablet were individually weighed, combined and ground into a fine powder using mortar and pestle. A portion of the powder equivalent to 10 mg of norfloxacin was accurately weighed using analytical balance (Sartorius AG, CP124S, Germany) and transferred to a 100 ml volumetric flask. It was dissolved in 40 ml aqueous phosphoric acid and diluted to volume with the mobile phase. Then, it was filtered through a 0.45 µm membrane filter (Whatman GmbH, Germany) and 10 ml of the filtrate was transferred to 50 ml volumetric flask and diluted to volume with the mobile phase. All the flasks used were covered with aluminum foil to protect norfloxacin from light.

2.2.3.3. Assay procedure
Equal volumes (10 µl) of the standard preparation and the assay preparation were separately injected into the HPLC system, the chromatograms were recorded and the peak areas were obtained. Then, the percentage of norfloxacin in the portion of the tablets was calculated by the formula:
PA_u/PA_s × 100

where PA_u and PA_s are the norfloxacin peak areas obtained from the assay preparation and the standard preparation, respectively. The USP specifies that each tablet should contain not less than 90% and not more than 110% of the labeled amount.

2.2.4. Identification test
Identification test was done as per USP, 2008. The retention times of the peaks in the chromatogram of the assay preparation and in the chromatogram of the standard preparation were compared. The retention times in the chromatograms of all the five brands of norfloxacin tablets correspond to that of the USP norfloxacin RS (Figures 3.5, 3.6 & 3.7).

2.2.5. Accelerated stability study
The five different brands of norfloxacin tablets were assessed for physical and chemical parameters immediately after purchase. Concurrently, the tablets were placed in accelerated stability cabinet (Weiss, PHARMA 600, Germany) set at 40 °C ± 2 °C and 75% ± 5% RH. Then, samples of the tablets were taken out of the cabinet and the physical and chemical stability parameters were tested at three and six months as per ICH guidelines (ICH, 2003).
3. RESULTS AND DISCUSSION

3.1. Physical stability tests

The physical stability results of the five different brands of norfloxacin tablets after zero, three and six months storage under 40 °C ± 2 °C and 75% ± 5% RH are shown below (Table 3.1).

Table 3.1: The physical properties of five different brands of norfloxacin tablets at zero-, three- and six-month storage under 40 °C ± 2 °C and 75% ± 5% RH

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

* Oblong shape tablets; ** Round shape tablets; the results are indicated as mean ± SD with n = 10 for diameter, length, width, thickness and crushing strength and n = 20 for tablet weight.
The physical properties of tablets and proper control of these properties are as important as it is in any oral dosage forms. Some of the fundamental physical characteristics of tablets, such as porosity, hardness, and moisture content are subject to change on aging and upon exposure to harsh climatic conditions. Therefore, when formulators design a solid dosage form, particular attention must be paid to maintenance of not only chemical potency on aging but also the physical stability.

Some changes in physical properties were observed on the products investigated during the course of storage under 40 °C ± 2 °C and 75% ± 5% RH (accelerated conditions) for six months. First, cracking of coating film was observed on the outer surface of Product A, after three months of storage under these conditions.

One possible reason for this phenomenon may be the coating film was not effective enough to resist the exaggerated environmental conditions such as 75% relative humidity. This, in turn, may be due to the type of polymer used and the thickness of the film formed. In the formulation of Product A, hydroxyl propyl methyl cellulose (HPMC) was used as coating material and it is available in a variety of grades, which depends on the molecular weight of the polymer. Increasing the molecular weight was shown to produce a marked reduction in the incidence of cracking of HPMC aqueous film coated tablets (Cole et al., 2002). Thus, the use of HPMC with low molecular weight may be one possible cause for the film cracking observed in this study. It was also demonstrated that the stress-relaxation of HPMC films increased at increasing moisture content (Laksmana et al., 2008) which may result in film cracking. Moreover, the amount and type of plasticizer used in the coating material formulation can also affect the quality of film formed. In the formulation of Product A, propylene glycol was used as a plasticizer. Accordingly, the film cracking observed under this study may be due to the less plasticizing efficiency or less amount of propylene glycol used.

Second, all the products showed an increase in mean weight with time. This is due to moisture gained by each product upon storage under the accelerated conditions. On the other hand, all the products showed acceptable uniformity of weight as none had percent deviation greater than 5% from their respective mean weights as stipulated by the British Pharmacopoeia (BP, 2000). The significance of this test is to ensure content uniformity in the tablets.
The crushing strength of the tablets is an essential criterion in the determination of the ability of the tablets to resist chipping, abrasion or breakage under conditions of storage, transportation and handling before storage (Shah, 1992). Crushing strength, the measure of tablet hardness, also affects the dissolution rate of drug products. The results before storage under accelerated conditions (at zero-month) showed that the tablets examined had mean crushing strength within the range of 138.1 to 176.0 N. These differences might be mainly due to differences in compression force and the quantity and/or type of binder used in the formulation.

The hardness of all the five different brands of norfloxacin tablets showed decrement 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 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 second and third, respectively. Product A not only showed the highest change but it is also the product with the least crushing strength (33.7 N) of all the products at three-month. The minimum acceptable crushing strength for tablets is 50 N and hence the hardness of Product A was found to be below this value at three-month.

At six-month, Product E showed the highest change in hardness, from 110.3 N at three-month to 48.9 N at six-month, (i.e. 61.5 N decrement when compared to the three-month crushing strength) followed by Product B (from 113.0 N at three-month to 75.9 N at six-month; a decrement of 37.2 N). The reason for the general decrease in hardness for all the five different brands of norfloxacin tablet products is due to moisture gained upon storage under high relative humidity (75%). Moisture softens the tablet and hence hardness decreases. The same result was obtained by Chowhan and Marias et al. (Chowhan, 1979; 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 3.2). Thus, norfloxacin tablets stored under high temperature and high relative humidity conditions soften adversely.

### 3.1.2. Moisture content

The moisture content of a drug product varies with the relative humidity surrounding it and the residual moisture of the sample when packaged (Waterman and Adami, 2005). The results of
moisture contents for five different brands of norfloxacin tablets after zero, three and six months’ storage period under accelerated conditions are shown in Table 3.2.

Table 3.2: Moisture contents of five different brands of norfloxacin tablets (mean ± SD, n = 3) at zero-, three- and six-month storage under 40 °C ± 2 °C and 75% ± 5% RH

<table>
<thead>
<tr>
<th>Product</th>
<th>Zero-month (mean ± SD)</th>
<th>Three-month (mean ± SD)</th>
<th>Six-month (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.0 ± 0.04</td>
<td>9.1 ± 0.11</td>
<td>10.6 ± 0.10</td>
</tr>
<tr>
<td>B</td>
<td>6.5 ± 0.08</td>
<td>8.1 ± 0.10</td>
<td>10.4 ± 0.22</td>
</tr>
<tr>
<td>C</td>
<td>4.3 ± 0.08</td>
<td>6.4 ± 0.05</td>
<td>8.4 ± 0.02</td>
</tr>
<tr>
<td>D</td>
<td>3.6 ± 0.04</td>
<td>5.8 ± 0.06</td>
<td>7.8 ± 0.05</td>
</tr>
<tr>
<td>E</td>
<td>5.4 ± 0.05</td>
<td>11.1 ± 0.21</td>
<td>13.4 ± 0.15</td>
</tr>
</tbody>
</table>

As can be seen from the moisture content results (Table 3.2), Product B was the brand with 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 its storage time while it was in the market. The product with the least moisture content before storage under the accelerated condition (at zero-month) was Product D (3.6%).

The highest amount of moisture gained after three months storage under simulated tropical conditions (40 °C ± 2 °C and 75% ± 5 % RH) was observed by Product E (106.0% increment) followed by product A (80.9% increment). Product B gained the least moisture (24.8% increment) of all the other brands of norfloxacin tablets when compared to the zero-month moisture content. Also at six-month, Product E showed the highest rate of moisture uptake (21.4% increment when compared to the three-month moisture level).

The difference in the degree of moisture uptake could have resulted from the difference in the effectiveness of film coating and of the polymer used as film forming material to prevent moisture penetration into the core tablet. For example, polymer of methacrylate can provide better protection against moisture absorption. It has been investigated that the rank order of moisture uptake for norfloxacin tablet was: uncoated tablets > tablets coated with HPMC > tablets coated with Eudragit E-100 (poly methacrylate) (Nada et al., 2006). The excipients used
for different purposes are also another factor for the difference in the degree of moisture uptake. For instance, fast or super disintegrants such as croscarmellose sodium (Ac-Di-Sol®) and sodium starch glycolate (Primojel®) enhance disintegration by facilitating water uptake (liquid penetration) into the tablet. In addition, investigators have revealed that polyvinylpyrrolidone (PVP), used as a binder, shows absorption of a significant quantity of water on exposure to elevated humidity (Fitzpatrick et al., 2002). In this study, it was also observed that PVP, Primojel® and HPMC had been used by the manufacturer in the formulation of Product A and hence showed higher moisture content after storing under simulated tropical conditions for six months.

Furthermore, the thickness of the packaging material (e.g. polyvinylchloride, PVC) may be different for the different brands of norfloxacin tablets. It has been indicated that the rate of moisture uptake, under controlled conditions, is a function of packaging material thickness (Ahmad and Shaikh, 2003). Thus, the difference observed in the degree of moisture uptake might also be due to the difference in the thickness of packaging material.

### 3.2. Comparison of *in vitro* dissolution profiles

Dissolution profiles of six different brands of norfloxacin tablets at zero-month showing percentage of norfloxacin dissolved with time are depicted in Figure 3.1.
The USP specifies that the amount of norfloxacin released should not be less than 80% of the labeled amount at 30th min. At 20th min, except Product C all the products released amount of the drug within the USP specification. Furthermore, Product D and Product F (the innovator product) released 100% at 30th min. However, three Products, B, C and E, continued to release more amount of drug beyond this time and attained their maximum amount after 60 min. This shows that the dissolution rates of these three products are slower than that of Product F, the innovator product.

During the first 10 min, Product C showed the slowest dissolution rate. The reasons for this phenomenon may be due to several factors such as the properties of excipients used in the formulation, coating material formulation, and manufacturing process variables including coating process variables. Coating material formulation and/or coating process variables are the
most probable factors to the slowest dissolution rate observed in Product C. First, the coating film was too plastic to be ground into powder during assay. Second, it was separated from the core without being dissolved after 20 min during the dissolution process. The plastic nature of the coating film can in turn be the result of the type of the coating polymer used, the concentration of the polymer in the coating solution, the amount of plasticizers used and the thickness of the film formed. Cao et al. (2004) studied the effect of mixing time of high viscosity grade of HPMC in ethanol/water cosolvent. As the mixing time of HPMC increased from 1 h to 5 h, the release rate was decreased due to enough plasticization of the polymer (Cao et al., 2004). 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 the percent increase of the product weight, reduced drug release rate (Cao et al., 2004). This is because with increasing coating level, the thickness of the coating layer increases that creates physical barrier between the drug and the medium. 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 that during the mixing process, a hydrophobic lubricant (e.g. magnesium stearate) film might be formed on the excipient particles, which decreases the penetration of water into the tablet, and as a result, retards dissolution (Proost et al., 1983).

In addition, Product C was observed to be the brand with the highest value of crushing strength (176.0 N) of all the five brands (Table 3.1). As reported earlier this can also contribute to the cause for the observed slow drug release rate from this product. The inverse correlation between the hardness of the tablet and dissolution rate is due to the fact that the density of the tablet increased with increasing hardness and at the same time, the porosity decreased so that the dissolution medium could not penetrate the tablets and ultimately there is less dissolution of drugs (Ahmed et al., 1999).

On the other hand, Product D released the highest (54.7%) amount of the drug at 5 min and attained the pharmacopoeial specification at 10 min. It released 96.7% of the claimed amount of the active drug at 15 min. In cases where more than 85% of the drug is dissolved within 15 min, dissolution profiles may be accepted as similar without further mathematical evaluation
This fast rate of dissolution could be due to the type and the amount of coating polymer, and excipients used, and the manufacturing process variables. It was reported that increasing the proportion of PVP, a water soluble polymer, into ethyl cellulose (EC), a water insoluble polymer, increased the rate of release of norfloxacin (Venkateshwar et al., 2004). This is probably because the PVP reduces the resistance offered by the EC film alone, and by increasing pores and/or their diameter the drug diffuses with less resistance. The amount of diluent used can also play its part in causing the observed variation in drug release behaviour, as it was 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. For example, Marias et al. indicated that croscarmellose sodium (Ac-Di-Sol®) improved the rate and extent of liquid uptake and penetration into tablets (Marias et al., 2003). As a result, tablets break up quicker and thus expose the drug particles to the dissolution medium very quickly, improving the contact between drug particles and solvent molecules and hence resulting in fast dissolution. Thus, variation in the type and the amount of disintegrants used may also be the cause for the difference in drug release rate observed under this investigation.

The difference found in the dissolution profiles of these products may also be attributed to the inclusion of different amount of hydrophobic lubricants like magnesium stearate in the formulations. Size and moisture content of the granules, compression force and other processing factors as well may affect the dissolution behaviour of different brands (Ranjha et al., 2001). Particularly, the effect of particle size of norfloxacin was studied and tablets containing micronized drug showed faster in vitro dissolution rates and an improvement in bioavailability (Katdare et al., 1987).

The above discussion can be summarized by using the t50% and t90% as dissolution parameters to compare dissolution profiles of the different brands (Dahiya, 2006). The t50% and t90% of the different brands of norfloxacin tablets are presented in Table 3.3.
Table 3.3: Dissolution parameters ($t_{50\%}$ and $t_{90\%}$) for five different brands of norfloxacin tablets and the innovator product (F)

<table>
<thead>
<tr>
<th>Product</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{50%}$</td>
<td>8.6</td>
<td>7.9</td>
<td>17.0</td>
<td>4.7</td>
<td>8.8</td>
<td>10.2</td>
</tr>
<tr>
<td>$t_{90%}$</td>
<td>19.4</td>
<td>42.1</td>
<td>37.4</td>
<td>9.8</td>
<td>29.0</td>
<td>17.6</td>
</tr>
</tbody>
</table>

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

As can be observed from the $t_{50\%}$ values in Table 3.3 except for Product C all the products released 50% of the drug below the time taken by the innovator product (10.2 min) to release the same amount of the drug. Product C showed the slowest dissolution rate during the first 10 min as discussed above. 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. This indicates that the dissolution rates of these products are slower than that of the innovator product. On the other hand, Product D released 90% of the drug at time much shorter (9.8 min) than that taken by the innovator product (17.6 min). This shows that Product D has faster rate of dissolution than the innovator product. Product A released 90% of the drug at a time comparable to that taken by the innovator product.

3.3. *In vitro* dissolution stability

Bioavailability is the rate and extent of drug absorption i.e., it is the relative amount of an administered dose of the drug that reaches the systemic circulation and the rate at which this occurs. The bioavailability exhibited by a drug is thus very important in determining whether a therapeutically effective concentration will be achieved at the site of action (Ashford, 2007). *In vitro* dissolution study of a drug may have important implication for the bioavailability (Gertz *et al.*, 1995).

3.3.1. *In vitro* dissolution stability at three-month

The dissolution profiles (amount of drug dissolved versus time) for five different brands of norfloxacin tablets after three months storage under 40 °C ± 2 °C and 75% ± 5% RH are shown in Figure 3.2.
Like at zero-month, at the 5th min, the highest percent drug was released by Product D whereas the lowest was released by Product C at three-month. The possible reasons for the difference in the rate of dissolution are mentioned above under in vitro dissolution comparison. As the dissolution profiles (Figure 3.2) show except Product A all the products released greater than the lower limit of the pharmacopoeial specification (80% of the labeled amount) at 15th min. At 30th 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.

3.3.2. In vitro dissolution stability at six-month

The dissolution profiles for five different brands of norfloxacin tablets after six months storage under 40 °C ± 2 °C and 75% ± 5% RH are shown in Figure 3.3.
Figure 3.3: Dissolution profiles of five different brands of norfloxacin tablets at six-month storage under 40 °C ± 2 °C and 75% ± 5% RH

As dissolution profiles in Figure 3.3 show the amount of norfloxacin dissolved was low up to the first 10 min for Product C. As was discussed above under in vitro dissolution comparison at zero-month, the main possible reason for the slow dissolution process at the earliest time was the resistance of the coating film to the penetration of the medium into the core tablet. After 10 min, the dissolution profile rises rapidly and this is due to the removal of coating film from the core tablet during the dissolution process. At 25th min, all the products released the drug above 80% of its labeled amount and hence met the pharmacopoeial specification at this time.

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, where 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 more 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 more soluble in water than in the anhydrous form (Ting-Chou et al., 2002). This is due to the fact that when norfloxacin anhydrous is transformed to its hydrate, the main functional groups change from COOH to COO⁻ and NH to NH₂⁺ because of proton transfer from COOH group to 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 (40 °C ± 2 °C and 75% ± 5% RH).

Product D showed a decrease in the rate and extent of dissolution with time. For instance, the amount of norfloxacin dissolved at 30 min (single point pharmacopoeial dissolution time) was 100.9%, 97.4% and 83.9 at zero-, three- and six-month, respectively. This phenomenon was also slightly happened to Product A. The amount of moisture absorbed by the products increased from zero-month to six-month (Table 3.2). As a result, there may be prior saturation of water which might have hampered disintegration mechanism (i.e., which relies on water uptake and then swelling). The water absorbed might also act as a binding agent thereby increasing the bonding between the particles. Consequently, the drug release process decreases and hence dissolution rate declines.

Therefore, storing norfloxacin tablets under simulated tropical conditions may bring about increase or decrease in dissolution rate depending upon formulation of products used by manufacturers.
3.4. Chemical stability test

3.4.1. Chemical assay

According to the USP, norfloxacin tablet should contain not less than 90% and not more than 110% of the labeled claim of norfloxacin (USP, 2008). Table 3.4 depicts assay results of five different brands of norfloxacin tablets obtained at zero-, three- and six-month storage under 40°C ± 2°C and 75% ± 5% RH.

Table 3.4: Percentage of drug contents (mean ± SD, n = 3) of five different brands of norfloxacin tablets at zero-, three- and six-month storage under 40°C ± 2°C and 75% ± 5% RH

<table>
<thead>
<tr>
<th>Product</th>
<th>Zero-month</th>
<th>Three-month</th>
<th>Six-month</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>102.0 ± 0.30</td>
<td>101.7 ± 0.68</td>
<td>99.8 ±0.56)</td>
</tr>
<tr>
<td>B</td>
<td>100.1 ± 1.31</td>
<td>99.8 ± 0.94</td>
<td>99.1 ± 0.72</td>
</tr>
<tr>
<td>C</td>
<td>99.2 ± 1.04</td>
<td>99.1 ± 0.91</td>
<td>99.0 ± 0.86</td>
</tr>
<tr>
<td>D</td>
<td>105.7 ± 0.30</td>
<td>103.5 ± 1.08</td>
<td>102.4 ± 0.40</td>
</tr>
<tr>
<td>E</td>
<td>99.6 ± 3.46</td>
<td>99.6 ± 0.73</td>
<td>99.4 ± 0.73</td>
</tr>
</tbody>
</table>

The results show that the assay values lie between 90% and 110% of the labeled amount specified for norfloxacin in the USP at all the three storage time points. Thus, all the five brands of norfloxacin tablets studied at zero-, three- and six-month storage under 40°C ± 2°C and 75% ± 5% RH met the requirement for assay as specified in the pharmacopoeia.

Figure 3.4 represents the linear regression lines showing the content of norfloxacin remaining in five brands of norfloxacin tablets after zero, three and six months storage under 40°C ± 2°C and 75% ± 5% RH.
Figure 3.4: Linear regression lines showing the content of norfloxacin remaining in five brands of norfloxacin tablets stored under 40°C ± 2°C and 75% ± 5% RH for six months.

The linear regression equations and the corresponding $r^2$ values for the five products are as follow:

- **Product A**: $y = -0.355x + 102.22$, $r^2 = 0.8532$
- **Product B**: $y = -0.1733x + 100.21$, $r^2 = 0.953$
- **Product C**: $y = -0.0283x + 99.185$, $r^2 = 0.9146$
- **Product D**: $y = -0.4717x + 105.47$, $r^2 = 0.907$
- **Product E**: $y = -0.0283x + 99.612$, $r^2 = 0.9465$

Where $y$ and $x$ indicate percent drug content remaining and storage time in month, respectively.

As were calculated from the regression lines in Figure 3.4, the slopes of the lines are -0.3550, -0.1733, -0.0283, -0.4717 and -0.0283 for products A, B, C, D and E, respectively. Accordingly, the change in drug content of norfloxacin from the five products is in the order of: product D > product A > product B > product C = product E, as evidenced by the slopes. Thus, the highest and the lowest decrease in assay values as compared with the initial values are 3.6% (product D) and 0.2% (products C and E), respectively. The WHO stability guideline (WHO, 1996) specifies that a significant change is considered to have occurred if the assay value shows a 5% decrease as compared with the initial assay value of a product. The highest decrease in assay value as compared with the initial value was 3.6%. Hence, the contents of
norfloxacin tablet products were not significantly decreased upon storage under simulated tropical climate conditions for six months.

Figures 3.5, 3.6 and 3.7 indicate the HPLC chromatograms of norfloxacin RS and norfloxacin in five different brands of norfloxacin tablets at zero-, three- and six-month, respectively.

**Figure 3.5:** HPLC chromatograms of norfloxacin RS and norfloxacin in five different brands of norfloxacin tablets at zero-month: Lichrospher® 100 RP-18 (125 x 4 mm, 5 µm) column and mobile phase water:acetonitrile:triethylamine (80:20:0.3 v/v/v) mixture (pH 3.3) were used at a flow rate of 1.0 ml/min and volume of injection of 10 µl and UV detection at 279 nm.
Figure 3.6: HPLC chromatograms of norfloxacin RS and norfloxacin in five different brands of norfloxacin tablets at three-month: Lichrospher® 100 RP-18 (125 x 4 mm, 5 µm) column and mobile phase water:acetonitrile:triethylamine (80:20:0.3 v/v/v) mixture (pH 3.3) were used at a flow rate of 1.0 ml/min and volume of injection of 10 µl and UV detection at 279 nm
Figure 3.7: HPLC chromatograms of norfloxacin RS and norfloxacin in five different brands of norfloxacin tablets at six-month: Lichrospher® 100 RP-18 (125 x 4 mm, 5 µm) column and mobile phase water:acetonitrile:triethylamine (80:20:0.3 v/v/v) mixture (pH 3.3) were used at a flow rate of 1.0 ml/min and volume of injection of 10 µl and UV detection at 279 nm

The HPLC analysis indicates that the retention times of the peaks in the chromatograms of all the five brands of norfloxacin tablets obtained at zero-, three-, and six-month were similar to that of the RS. As can be seen from Figures 3.6 & 3.7, the peaks obtained after three and six months storage were not changed in all products when compared with the peaks obtained before storage (Figure 3.5). Furthermore, no new peak (i.e. secondary peak) appeared in the
chromatograms obtained at both three-month (Figure 3.6) and six-month (Figure 3.7) for all the products after exposure to 40 °C and 75% RH. These show that norfloxacin in the investigated tablets did not degrade significantly under the stated conditions. Also, in the work of Nada et al., physical instabilities were observed, but no degradation product of norfloxacin was detected in tablet formulations stored under 40 °C and 75% RH storage conditions (Nada et al., 2006). These reveal that the accelerated conditions (40 °C ± 2 °C and 75% ± 5% RH) did not bring significant chemical degradation on norfloxacin in the investigated tablets.
4. CONCLUSION

In this study, comparative in vitro dissolution profiles and stability of five brands of norfloxacin tablets were investigated under the influence of accelerated storage conditions (40 °C± 2 °C and 75% ± 5% RH). It was found that all the products released amount of the drug within the USP specification within 30 min at zero-month. However, variations in drug release profiles among different brands were obtained. Product C showed the slowest dissolution rate in the first 10 min. In addition, the dissolution rates of Products B and E were slower than that of Product F (the innovator product). On the other hand, Product D showed faster rate of dissolution than the innovator product.

The accelerated stability study revealed that simulated tropical climatic conditions brought about noticeable physical changes such as cracking of coating film on Product A, decrease in hardness and increase in moisture contents in all products. Furthermore, slight decrease for Products A and D, and increase for B and E in drug release profiles were observed indicating minor differences in dissolution stability among different brands. The assay results showed that all the brands of norfloxacin tablets contain between 90% and 110% of the labeled claim as specified in USP 2008 at zero-, three- and six-month and no significant change occurred in any of the products stored under 40 °C and 75% RH for six months.

From this study, it can be concluded that even though the dissolution rates showed minor differences, the investigated five brands of norfloxacin tablets released amount of norfloxacin within the USP specification before storage and also after storage under 40 °C and 75% RH for six months. Tropical climatic conditions can bring about physical instability but not significant chemical instability on the investigated norfloxacin tablets. In order to avoid physical instability, norfloxacin tablets should be packed with water-proof packaging materials like aluminum/aluminum films for better moisture barrier, and also should be kept under controlled temperature and relative humidity. In summary, stability study and continuous and regular quality control help the government, the private sectors and the health professionals provide the community with safe and quality drug products.
SUGGESTIONS FOR FUTURE WORK

- *In vivo* bioequivalence studies can be done for different brands of norfloxacin tablets.

- Long-term stability studies can be conducted for different brands of norfloxacin tablets at real conditions (e.g., 25 °C and 60% RH).

- Stability of different brands of norfloxacin tablets collected from areas with tropical climatic conditions can be evaluated.
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