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FACULTY OF PHARMACEUTICAL SCIENCES

# QUALITY OF ESSENTIAL DRUGS ON THE TANZANIAN MARKET: INFLUENCE OF TROPICAL CLIMATE ON IN VITRO DISSOLUTION AND BIOAVAILABILITY

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*To my wife Kissah for the love, continuous support and encouragement.*

*To my daughter Siama Neema for the inspiration that in a very special way kept me going.*

*To my mother Siama for the love and care, for the efforts and sacrifices you made to single-handedly bring us up.*

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# 1. GENERAL INTRODUCTION

## 1.1 Tanzania: geographical location and social economical characteristics

Tanzania is a sub-Sahara African country located in the Great Lakes region (Fig. 1.1). It is a fairly large country with an area of about 950.000 km$^2$ and a population of about 33 million, 70% of them living in rural areas (Bureau of Statistics Tanzania, 1992). Economically it is a poor country with an annual Gross Domestic Product (GDP) of 290 US$ and a Gini coefficient (a parameter representing the distribution of the wealth of a nation amongst the population) of 0.355, indicating that the national wealth is fairly distributed amongst the population (Odedokun and Round, 2001).

*Figure 1.1: Map of Tanzania and its neighbouring countries*

Shortly after independence from Britain in 1961, the country embraced a socialistic policy whereby the economy was centrally controlled. However, after the fall of the Berlin wall a liberal market economy has been introduced. The result has been the reversal of the economic slump into an annual economic growth of around 5% as the country has attracted direct foreign investments (especially in the mining sector). Currently Tanzania is rated as the country attracting the most external investment in sub-Saharan Africa.

## 1.2 Health care services

Since gaining independence in 1961, the country has strived to improve the provision of social services to its people, especially in health care. This was in recognition of the fact that improving the health status of a population in a country has a big impact on the reduction of poverty. Some commendable achievements have been made in this respect. A survey on the geographical access to health care facilities in Tanzania has shown that there are 479 health centres and 3995 dispensaries throughout the country. Furthermore, it is estimated that about 70% of the population in rural areas are within a 5 km and 80% within a 10 km radius of a health facility (Ministry of Health Tanzania, 1997). The health status of the populations has also improved as indicated by the rise in the life expectancy from 41 years in 1961 to 52 years in 2001. Correspondingly, the infant mortality rate has been reduced from 250 to 164 per 1000 live births.

An important component for a health care program is ensuring constant availability of drugs in the health care facilities. The government has the obligation to ensure that effective, safe and good quality drugs are available at these facilities. However, the prevailing economic situation has made this task very difficult in Tanzania. In developing countries, drugs are known to consume more than 40 to 60% of the total public and private spending on health, while in the developed countries it is limited to about 15 to 20% (WHO, 2000). One of the major reasons for these differences is the increased frequency of contracting diseases in the developing countries. In Tanzania, pharmaceuticals consume about 40% of the health budget (Ministry of Health Tanzania, 1997). Against the background of the expanded and widespread health services, the Tanzanian government needed to

commit much more financial resources for the acquisition of drugs. But due to the prevailing poor economy, these resources were not always forthcoming.

In order to realise its objective of improving health services as the means of reducing poverty, the government had to find ways of ensuring a sustained access to drugs and continue with the efforts to expand its capacity in the provision of health services to the general public. One of the most rational options was to adopt the World Health Organisation's (WHO) essential drugs concept.

## 1.3 The Essential Drugs Concept (EDC)

The vicious circle of diseases and poverty (diseases leading to a decline in economical productivity, hence more poverty and more diseases) is an unfortunate situation that exists in most developing countries. The drugs to treat most of the diseases prevalent in the developing countries exist. However, most of the populations in these countries lack access to such drugs as they are too expensive or the populations are underserved by medical facilities.

In the mid 70's, the WHO reviewed the major drug problems facing the developing countries and proposed the essential drugs concept as a strategic policy that would enable developing countries to improve the availability and access to drugs by the general public. The most important component of this concept is the recognition of the fact that only a few drugs are necessary for the treatment, diagnosis and prophylaxis of diseases facing the majority of people in a community. By concentrating on few essential drugs the meagre resources available in developing countries could be well managed and wastage minimised. This policy encouraged the compiling of a list of essential drugs tailored to each country health needs. It was recommended that as far as possible the drugs included in the list should be generic drugs (drugs which are off patent) as these are usually cheaper (about 30% less) and have a proven safety record. Furthermore, the essential drugs concept emphasised the importance of encouraging the rational use of drugs as a means of minimising wastages due to the misuse or excessive use of drugs.

In 1975, The World Health Assembly (WHA) of the WHO passed resolution WHA 28.66 that marked the birth of the essential drugs concept. The WHA requested

the Director General of the WHO to call upon and assist developing countries to implement the outlined policy proposals. According to the WHO, the adoption of the concept is a means of bridging the gap between the need of medicines and the ability of the population to afford them. Essential drugs have the potential to reduce morbidity and mortality from common illnesses for millions of people in the developing countries where medicines are unaffordable, unavailable, improperly used or of poor quality. The main pillars of the essential drugs concept are: established safety and efficacy, proven quality, constant availability, affordability and rational use.

The Tanzanian government through the Ministry of Health has since then recognised the importance of the essential drugs concept and has issued measures and policies to ensure the implementation of the concept. It is the policy of the ministry that all health workers in governmental, private and non-governmental organisations should strictly adhere to the national essential drugs list, while purchasing, labelling, prescribing and dispensing of pharmaceuticals should be in generic names as much as possible (Ministry of Health Tanzania, 1998).

## 1.4 Adoption of EDC in Tanzania

Studies done in Tanzania using the International Network for Rational Drug Use (INRUD) indicators have shown that much success has been achieved in the promotion of rational drug use (Gilson et al., 1993; Massele and Mwaluko, 1993). According to the INRUD, the major indicators for irrational drug use are prescribing in brand names, prescribing too many drugs as well as unnecessary use of injections and antibiotics. These studies have shown that the average number of drugs per prescription is about 1.8 and more than 75% of all drugs are prescribed and dispensed as generics. About 80% of all prescribed drugs are from the national essential drugs list and less than 40% were injectables. In addition, Tanzania has been categorized by the WHO as a country where the essential drugs list exists and is used in all aspects of drug management (WHO, 1988). While much success has been achieved in this field, not much information is available concerning the quality of the essential drugs on the market.

## 1.5 Quality of drugs – a global concern

The importance of drugs in reducing mortality, increasing the quality of life and reducing suffering cannot be overemphasised. However, poor quality drugs are potentially dangerous. In addition to the possibility of causing death and increasing suffering, poor quality drugs increase treatment costs.

There is a worldwide concern about the quality of pharmaceuticals sold around the world. The WHO has been tracking and documenting the incidences of substandard drugs. The records show that problems of substandard and counterfeit drugs are on increase as 50% of all reported cases occurred in the period 1993 to 1997. Most of these incidences (70%) were reported in developing countries. The report identifies the causes of the poor quality of drugs: in about 50% of all the cases the formulations did not contain any drug, 20% contained the wrong active ingredient and 10% the wrong amount of the active ingredient. Only in 5% of the reported incidences did the drugs contain the right active ingredient in the correct amounts, but were judged substandard by failing other quality tests. The antibiotics were the major pharmacological class of drugs with the largest incidence (60%) of counterfeiting (WHO, 2000), posing an even greater health risk as substandard anti-infective drugs may lead to selection of resistant strains of microorganisms, reducing the achievements made so far in combating infectious diseases.

According to the International Federation of Pharmaceutical Manufacturers Association (IFPMA) about 7% of all drugs being sold around the world in 1992 were of poor quality: being counterfeit or substandard. The problem is worldwide and occurs both in rich and poor countries. Global drug expenditures were estimated at 226 billion US$ in the year 1992 (Pharma daten, 1993). According to the IFPMA, the economic impact of the presence of substandard/counterfeit drugs in the world is estimated to cost more than 10 billion US$ in trade only. The impact is even much more if consideration is given to the increased treatment costs, reduced productivity, patient suffering and possible development of resistance by susceptible microorganisms.

Substandard drugs are not found only in developing countries. In developed countries where the drug regulations are strictly enforced, some incidences about

the presence of substandard drugs on the market have been reported. Regular surveillance of the quality and bioequivalency of pharmaceuticals on market in Finland has identified amongst different brands of erythromycin tablets, one with a very low bioavailability (Venho et al., 1987). This brand had to be withdrawn from the market.

However, there is more prevalence of substandard drugs in the developing countries in general as less stringent quality control measures are in place in these countries. In a study to evaluate the bioavailability of 3 different brands of ampicillin capsules marketed in Nigeria, Ogunbona and Akanni (1985) reported that one of the analysed brands was not bio-equivalent to the innovator brand (Penbritin®). A study on different ampicillin brands on the Sudanese market had previously obtained similar findings (Ali et al., 1981). Sowunmi et al. (1994) evaluated the quality of quinine tablets marketed in Nigeria and reported the presence of fake quinine tablets.

Shakoor et al. (1997) evaluated the quality of pharmaceuticals on market in Thailand and Nigeria. The potency of 81 and 15 drug formulations from Nigeria and Thailand, respectively, was determined. The sampled drugs were antimalaria and antibiotic formulations that are the most frequently used in these countries. The study revealed that 36% (26) of the samples from Nigeria and 40% (6) from Thailand did not comply with the British Pharmacopoeia standards. The content of the active ingredient in some of the failed samples was marginally outside the official limits. Three of the substandard samples from Nigeria (2 chloroquine and 1 amoxycillin) and 3 from Thailand (all chloroquine) were fake. Since the authors could not detect impurities or degradation products (except from an ampicillin/ cloxacillin suspension), they suggested that the major reason for substandard drugs in the developing countries was poor manufacturing practices on the part of the suppliers.

Taylor et al. (2001) evaluated the quality of 581 formulations of 27 different drugs from 35 urban pharmacies in Nigeria. The assayed potency of 48% of the samples did not comply with the pharmacopoeia specifications for drug content. Most of the failed samples had a drug content that was marginally below the pharmacopoeia limits.

Some reports on the presence of substandard drugs on the market have also been made from the East African region of which Tanzania forms a part. In Kenya, the quality of metronidazole products available on market was evaluated. The assay for drug content of all products conformed to pharmacopoeia specifications. However two formulations failed in the dissolution test as they released only 46.8% and 45.8% of drug in 40 minutes (Kibwage et al., 1991) whereas a minimum of 80% drug release within 40 min is required. Kibwage et al. (1992) reported that about 45% of the drugs sampled on the Kenyan market and analysed at the Daru quality control laboratory on a routine basis were of substandard quality in terms of the drug content.

Another study done in Kenya in 1995 evaluated the bioavailability of different brands of carbamazepine tablets on market. This was done after complaints from clinicians that breakthrough seizures were reported on switching from one brand to another. Four different brands were on market at that time. The quality of one brand was found to be poor in terms of friability, content uniformity, disintegration and dissolution. Upon determination of its bioavailability, this brand was found to give lower plasma drug concentrations ($C_{max}$) and a reduction in the extent of drug absorption (AUC) compared with the other three (Oluka, 1995). Presumably this was responsible for the reported breakthrough seizures. This brand was also the cheapest on the market.

A study was done to evaluate the quality of 9 different brands of chloroquine marketed in Dar es Salaam (Tanzania). The drug content and dissolution performance of the tablets were determined. All brands complied with the USP requirement for drug content; however, one formulation (a sugar-coated brand) failed the dissolution test (Abdi et al., 1995).

The influence of storage at tropical conditions (as is found in many developing countries) on the stability and quality of essential drugs has been one of the concerns of the WHO. Nazerali and Hogerzeil (1998) conducted a study in Zimbabwe to investigate the influence of storage in a tropical climate on the quality of 13 essential drugs. Samples of the same batch of a drug were taken from the government medical stores and from district hospitals and health centres. The content of the active ingredient was assayed and the influence of storage conditions on the quality of the drugs evaluated. Only two drugs (ergometrine

injection and retinol capsules) showed a significant loss of potency, indicating that they were unstable during storage at tropical conditions. The other drugs, including those with known instability (acetylsalicylic acid tablets, amoxicillin and ampicillin capsules), remained stable during the study period. The authors concluded that with few exceptions, essential drugs have a good stability profile during storage in tropical climates.

A pilot study was carried out in Sudan to evaluate the stability of 15 essential drugs during transport and storage at tropical climatic conditions. Samples of drugs supplied by International Dispensary Association (IDA) to the Nile provinces in Sudan were collected and evaluated for potency. The assay results were compared with those from the original batch kept by the IDA in Amsterdam (The Netherlands). The authors reported that only 3 (ergometrine injection, epinephrine injection and retinol capsules) of the 15 drugs analysed had a significant loss in potency. The other drugs including those with suspected instability (acetylsalicylic acid tablets, ampicillin capsule) did not show a significant loss of potency and were considered stable on exposure to tropical climatic conditions during transport and storage (Hogerzeil et al., 1991a). The results of the pilot study were confirmed by an extensive study where drugs transported to Bangkook (Thailand), Lagos (Nigeria) and Kampala (Uganda) from Aarhus (Denmark) showed no significant drug degradation for the majority of essential drugs (except for ergometrine injection and retinol capsules), despite being exposed to temperatures of up to 42°C in some regions during the sea voyage (Hogerzeil et al., 1991b).

As can be summarized from the literature cited, the problem of substandard drugs is more pronounced in the developing countries and some factors responsible for this can be identified. One is that in these countries there are few functioning drug regulatory authorities. It is estimated that only 30% of the developing countries have an established and functioning drug regulatory authority (WHO, 2000). In addition, most of the regulatory authorities have not established quality control laboratories. Even when these are available, most of the time they lack proper equipment and resources to perform the work properly and/or to monitor the quality of the drugs on market. In some cases they have not instituted good laboratory practices, which is required for the reliability of the analytical results. In 1994, Roy reported on the existence of substandard formulations (37 out of 137) in

Bangladesh, some of which had been found to be of acceptable quality by the local Drug Regulatory Authorities. Gomez et al. (1998) reported on the differences in assay results of antimalaria drugs analysed by the quality control laboratory of the Institute of Drug Control in Vietnam. Some of the drugs that had passed the quality tests by the Institute failed when independently assessed by a World Health Organization accredited laboratory.

The other factor contributing to the prevalence of substandard drugs in developing countries is inadequate financing. In more than 36 developing countries, Tanzania being one of them, the average annual per capita expenditure on drugs by the population is less than one US dollar. This compares unfavourably to other countries in the developed world such as the USA, Germany and Belgium where the expenditure is estimated at 110, 98 and 70 US $, respectively (WHO, 1988). This low per-capita consumption is a good indicator for the inability of the people to afford basic drugs.

As the purchasing power for pharmaceuticals in these countries is very low, most importers and distributors of pharmaceuticals would tend to pay more attention to low prices rather than to the quality. They will source their imports from the cheapest suppliers and may circumvent regulatory and quality assurance systems. The general population will be attracted to lower priced drugs that are available even outside pharmacies (Pecoul et al., 1999).

## 1.6 Situation in Tanzania

Tanzania is a net and multisource importer of pharmaceuticals. The country has a tropical climate and is classified by the International Conference on Harmonization (ICH) as a country having a Class IV tropical climate (Grimm, 1998). If imported drug formulations have not been optmised for stability in tropical climates, their effectiveness may be compromised on exposure to conditions of high humidity/high temperature during transport and storage.

During the 1980's the country was enduring an economic slump and had to introduce structural adjustment programmes and reforms recommended by the International Monetary Fund (IMF). In the health sector, one of the results of implementing the reforms was increased participation of the private sector in

importation of pharmaceuticals (previously it was a public sector domain), without a parallel increase in the capacity of the regulatory authority to institute effective quality assurance measures. Tanzania has an established drug regulatory authority since 1968. The Pharmacy Board is the authority that has been empowered by law to control and regulate the manufacture, import, distribution and sale of pharmaceuticals in the country. However, its capacity to control the quality of drugs on market has been limited by the lack of facilities. For more than 30 years of its existence it had no quality control laboratory.

There is a wide price difference of the formulations containing the same amount of active ingredient(s). In some cases the difference in price between an innovator brand and a generic equivalent is as high as 1000%. Suppliers of pharmaceuticals to a market characterised by such a low purchasing power as in Tanzania would go for cheap brands, as low prices would be most favourable to the general population. With such a large difference in prices one would be interested to know if those brands are pharmaceutically equivalent. In addition, the free substitution of one brand for another is practised on the assumption that those dosage forms containing the same amount of active ingredient are equivalent. It is a known fact that the bioavailability of generically identical drugs might vary (Hendels et al., 1993; Maddock, 1986). The outcome of such variations may have serious therapeutic implications, especially for drugs with a narrow therapeutic index and a steep dose response curve. It is reasonable to discourage substitution between different brands/generics, unless the necessary measures have been taken to show that the products are equally effective in delivering the medicament to the systemic circulation.

The Pharmacy Board established a quality control laboratory in the year 2000 as part of its efforts to monitor the quality of drugs on market in the country. This is an important achievement. However, for a country as large as Tanzania, a lot of resources are required to enable the Board to conduct post-marketing quality surveillance of the drugs. A single laboratory in the whole country will have a limited effectiveness in evaluating the quality of the imported drugs and monitoring the quality of those already on the market.

In an environment having the inadequacies highlighted above (multi source importation, market preference for cheaper brands and lack of quality control

facilities) the trade in substandard/counterfeit drugs is likely to flourish and make the assurance of quality of drugs on market a challenging problem. Moreover, the confidence of health workers and patients in the health system is easly eroded when there are reports (even when they are unsubstantiated) of substandard drugs in circulation. Prescribers overwhelmed by presence of many brands, some of which are perceived to be of poor quality may prefer the use of expensive brands as they could associate good quality with high price. This may negatively influence the achievements made through the efforts by the government to increase access to safe, effective and affordable medicinal drugs to the majority of its people.

Currently there is no information available on the quality of drugs marketed in Tanzania. This study is aimed at evaluating the in vitro and in vivo quality parameters and the stability of the most commonly used essential drugs on market. The results will be useful to the Ministry of Health and especially to the Pharmacy Board in developing appropriate intervention strategies to ensure that only effective drugs are allowed on the Tanzanian market and to promote the public confidence in the quality of the medicinal drugs. This will contribute towards the implementation of the Essential Drugs Concept as envisaged in the WHO guidelines.

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# 2. OBJECTIVES OF THE STUDY

## 2.1 Objectives

*Main objective*

The evaluation of the quality of different brands of oral solid dosage forms of essential drugs available on the Tanzanian market.

*Specific objectives*

1. Determination of the drug potency.
2. Determination of the in vitro dissolution as a function of storage time at tropical conditions.
3. Determination of the human bioavailability as a function of storage time at tropical conditions of some selected brands.

## 2.2 Selection of the drugs

The drugs evaluated during this study have been chosen on the basis of two criteria. The first criterion was the drug's relevance to public health: included in the National Essential Drugs List (NEDL) and commonly used. The other criterion was known or suspected quality problems in adverse storage conditions.

A drug list was obtained from Médecins sans Frontières, an organization of health professionals working in many developing countries. The 15 most commonly used tablet/capsules by this organization were compared with a list of the ten most commonly prescribed drugs at Muhimbili Medical Centre, the biggest University Teaching Hospital in Tanzania. Four drugs listed by both organisations and all in the National Essential Drugs List (NEDL) of Tanzania were then chosen. The drugs are: chloroquine, metronidazole, paracetamol and the sulfamethoxazole/ trimethoprim combination. During the course of this study the Ministry of Health (Tanzania) withdrew chloroquine as the primary antimalarial drug and replaced it with a sulfadoxine/pyrimethamine combination, so it was decided to include this combination in the study.

Diclofenac sodium, formulated as an enteric-coated tablet, is also in the NEDL of Tanzania and was included in the study as a modified release formulation.

Ciprofloxacin, a life saving drug and also in the NEDL of Tanzania, is included in the study for economical reasons. It is among drugs whose current market price differential between the innovator brand and the generics is more than 2000%.

Acetylsalicylic acid tablets and amoxycillin capsules, both being in the NEDL of Tanzania, have been included in the study because of their reported instability in a tropical climate (high humidity and high temperature).

## 2.3 Selection of sampling points

The study aimed to establish the quality of the drugs at the user level. The drugs were sampled in Dar es Salaam from different sources: the Tanzanian Medical Stores Department (MSD) and 10 registered pharmaceutical wholesalers.

The MSD is an autonomous body that imports and distributes drugs to all governmental health facilities in the country. The pharmaceutical wholesalers are among the major importers and were selected based on the pharmaceuticals' import data from the Pharmacy Board (Tanzania). Both the MSD and the wholesalers are multi-source importers of pharmaceuticals. These pharmacies distribute their products to both the public and private health facilities in the country.

The drugs were anonymously purchased in their original package as supplied by the manufacturers. Furthermore only one package of a particular brand was purchased from one wholesaler. Samples from the MSD (2 chloroquine, 2 ciprofloxacin, 2 paracetamol and 2 metronidazole formulations) were donated for the study.

# 3. IN VITRO EVALUATION OF POTENCY AND INFLUENCE OF TROPICAL CLIMATIC CONDITIONS ON DISSOLUTION

## 3.1 Introduction

A number of in vitro tests are recommended to evaluate the quality of pharmaceutical solid dosage forms. Some tests (such as friability and hardness) are considered to be more of in process tests performed by manufacturers to check batch to batch uniformity. Other tests (recommended in pharmacopoeia monographs) might be useful in predicting the in vivo efficacy of formulations. The assay for drug content in a formulation is one of such tests as the efficacy and safety of a dosage form depends on the amount of the drug contained in.

For an oral solid dosage form, the ability to release the drug in the gastrointestinal (GI) fluids is an important quality attribute. Even when a formulation contains the required amount of drug, the manufacturing process and/or formulation components may affect the quality and result in a failure to release the drug into solution at the site of absorption in the gastrointestinal tract. The disintegration test is recommended in pharmacopoeia (Eur. Ph.; BP; USP) as an in vitro quality control test that determines the potential of a solid dosage form to disintegrate in the GI fluids. The test measures the time required for the dosage form to disintegrate into its primary particles. However, there is a possibility (especially for poorly soluble drugs) that a dosage form may disintegrate into primary particles and yet fail to dissolve in the GI fluids.

Dissolution is another in vitro test that determines the ability of the drug to dissolve in the GI fluids. The in vitro dissolution test (as recommended by the USP) measures the amount of drug dissolved in the dissolution medium (usually aqueous solutions with modified pH) after a specified time interval. The test is predictive of the absorption potential of a dosage form since when the drug is in solution in the GI tract, its absorption is no longer influenced by factors related to the drug formulation.

In this chapter, the quality of selected essential drug formulations available on the Tanzania market is evaluated by performing drug assay and in vitro dissolution

tests. In addition, the influence of tropical storage conditions on the in vitro dissolution of these formulations is investigated.

## 3.2 General methods

Drug assay

The assay for the active ingredient(s) in the formulations was performed using high performance liquid chromatographic (HPLC) methods described in the USP 24 monographs. For chloroquine tablets an HPLC method cited in literature (Pussard et al., 1986) was used instead of the USP 24 recommended method which is based on chloroform extraction and UV detection. For metronidazole tablets, an HPLC method that had been developed in the Laboratory of Drug Analysis (Faculty of Pharmaceutical Sciences, Ghent University, Belgium) for the analysis of metronidazole and its degradation products (Baeyens et al., 1998) was used. All analyses (HPLC assays as well as dissolution tests) were performed using validated techniques and were done at the Laboratory of Pharmaceutical Technology (Faculty of Pharmaceutical Sciences, Ghent University, Belgium). The assays were repeated three times and the results are presented as the mean of 3 determinations (± standard deviation).

In vitro dissolution

The stability of the in vitro drug dissolution at tropical storage conditions was investigated by performing an accelerated stability test under Class IV climatic conditions as recommended for Tanzania by the International Convention on Harmonization (Grimm, 1998). The samples in their original package were stored in a sealed chamber containing a saturated sodium chloride solution (relative humidity (RH): 75 ± 5%) and in an oven (Memmert, Namen, Belgium) maintained at 40 ± 2°C. The dissolution profile (n = 6) of each formulation was determined immediately after purchase (0 months) and after a storage period of 3 and 6 months at the above-mentioned conditions.

Evaluation of the dissolution data

The dissolution profiles were evaluated using the USP 24 tolerance limits for dissolution: a minimum percentage of drug dissolved after a specified time interval.

Futhermore, the profiles of formulations that met these requirements were further evaluated using the similarity factor ($f_2$) as recommended by the FDA for the comparison of the dissolution profiles of immediate release dosage forms (FDA, Guidance for industry, 1997). In this study, the dissolution profile of the reference formulation (innovator brand) obtained before storage at simulated tropical conditions was compared with dissolution characteristics of the formulations containing the same drug. To evaluate the influence of storage conditons on the in vitro drug release, the profiles obtained after 3 and 6 months storage at the test conditions were compared with those obtained immediately after purchase (0 months).

The similarity factor was computed using the equation by Moore and Flanner (1996) recommended by the FDA (1997).

$$f_2 = 50 \times \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} w_t (R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

Where $R_t$ and $T_t$ are the percentages of drug dissolved at time *t* (for t = 1, 2,…..n), of the reference and test formulation, respectively. The measurements at each time point can be weighed according to its importance in the dissolution curve using $w_t$ as an optional weight factor. If all points are considered equal $w_t$ is assigned a value 1.

As recommended in the FDA SUPAC guidelines, the profile of two batches is considered similar and pharmaceutically equivalent when the average difference at any sample time point is less than 10% which corresponds to a similarity factor ($f_2$) greater or equal to 50.

Equipment

The HPLC apparatus consisted of a LaChrom L-7100 isocratic pump, a L-7400 UV/VIS detector and a L-7000 integrator (Merck, Darmstadt, Germany). A Lichrospher® 100RP-C18 endcapped (5 µm) column (Merck, Darmstadt, Germany) was used for chromatographic separations. The dissolution tests were carried out using a Vankel VK 7010 dissolution apparatus that was linked to a VK 8000 automatic sampler (Vankel Technology, Cary, NC, USA). The drug

concentration of the dissolution samples was measured spectrophotometrically using a Lambda 12 UV/VIS spectrophotometer (Perkin Elmer, Norwalk, USA).

## 3.3 Amoxicillin formulations

Four amoxicillin formulations, all of them capsules, were analysed. Three formulations were obtained from the Tanzanian market, while the fourth one from Eurogenerics (used as a reference) was purchased in a Belgian pharmacy. Table 3.1 gives the details of the formulations evaluated.

*Table 3.1: Amoxicillin 250 mg capsule formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| Eurogenerics[a] (Belgium) | Blister, 100 capsules | 00C04D | N/A |
| Medochemie (Cyprus) | Blister, 100 capsules | P4551 | 27.0 |
| Medopharm (India) | Blister, 100 capsules | 006 | 24.5 |
| Shelys Pharmaceuticals (Tanzania) | Blister, 100 capsules | 435 | 25.0 |

[a]Reference formulation containing 500mg amoxicillin per capsule.
N/A Not available on the Tanzanian market

### *3.3.1 Materials*

Amoxicillin trihydrate (Eur. Ph.) was obtained from Sigma Aldrich (Steinheim, Germany) and acetonitrile from Biosolve (Valkenswaard, The Netherlands). Monobasic potassium phosphate and potassium hydroxide were obtained from Vel (Leuven, Belgium).

### *3.3.2 Assay for amoxicillin*

#### *3.3.2.1 Methods*

The amount of amoxicillin in each formulation was determined by the method described in the USP 24 monograph for amoxicillin.

Sample preparation

The content of 20 capsules was removed as completely as possible. The combined contents were properly mixed and an amount equivalent to 200 mg of anhydrous amoxicillin was transferred into a 200 ml volumetric flask. A sufficient quantity of diluent (0.05M monobasic potassium phosphate pH 5.0) was added, the resulting suspension was mixed and sonicated to dissolve the drug. A portion of the solution was filtered through a 0.5 µm cellulose acetate filter (Minisart®, Sartorius, Goettingen, Germany) and the filtered solution was used as the assay preparation.

Calibration curve

A calibration curve (peak area vs. concentration) y = 2096.2x (± 46.8) + 186198 (± 38507) with a coefficient of determination ($R^2$) of 0.9996 ± 0.0004 (n = 5) was constructed using standard solutions with amoxicillin concentrations of 400, 600, 800, 1000 and 1200 mg/l. The unknown concentration of amoxicillin in the assay preparation was determined from the calibration curve.

The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after repeated injections (n = 5) of an amoxicillin standard solution (1000 mg/l).

Mobile phase

The mobile phase consisted of a mixture of a 0.05M monobasic potassium phosphate (pH adjusted to 5.0 with 0.2N KOH) and acetonitrile in a ratio of 96:4(v/v). Aliquots of the assay preparation and standard solutions (20 µl) were injected into the chromatographic system and the peaks were monitored by UV absorbance at 272 nm. The analyses were performed within six hours of preparing the solutions.

### *3.3.2.2 Results*

The relative standard deviation (RSD) for replicate injections of the standard preparation (1000mg/l) was 0.6% and 1.9% for the within day and between day analyses, respectively. According to the USP 24, the RSD should be less than 2%. The results of the assay (Table 3.2) shows that all formulations complied with the USP 24 specifications for amoxicillin content (90 - 120% of the labelled content).

*Table 3.2: Mean amoxicillin content of different formulations expressed as a percentage of the labelled amount*

| Manufacturer | Mean amoxicillin content per capsule (mg) | % of the labelled amount per capsule |
|---|---|---|
| Eurogenerics[a] | 495.7 ± 9.4 | 99.1 |
| Medochemie | 248.5 ± 4.7 | 99.4 |
| Medopharm | 244.2 ± 4.6 | 97.7 |
| Shelys | 230.8 ± 4.3 | 92.3 |

[a]Reference formulation containing 500 mg amoxicillin per capsule

## *3 3.3 In vitro dissolution*

### *3.3.3.1 Methods*

Dissolution testing

Dissolution tests were performed using the USP basket method (Method 1) at a rotational speed of 100 rpm for formulations containing 250 mg amoxicillin, and using the USP paddle method (Method 2) at a rotational speed of 75 rpm for the Eurogeneric formulation which contained 500 mg amoxicillin. The volume of the dissolution medium (distilled water) was 900 ml per vessel maintained at a temperature of 37 $\pm$ 0.5°C. Dissolution samples (5 ml) were taken after 10, 20, 30, 40, 50 and 60 min. All samples were analysed spectrophotometrically at 272 nm. Dissolution samples from the Eurogenerics formulation were diluted twice with distilled water before UV analysis.

### 3.3.3.2 Results

Table 3.3 shows the percentage amoxicillin dissolved within 1 hr of dissolution testing and Figure 3.1 the dissolution profiles of the four amoxicillin formulations before and after storage at simulated tropical conditions. Before storage all formulations had a drug release that complied with the USP 24 dissolution requirements, the amount of drug released within 60 min ranging from 94.7 to 102.9%.

*Table 3.3: Percentage of drug released after 1 hour of dissolution testing on amoxicillin capsules stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirement: more than 80% is released within 1 hour*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| Eurogenerics[a] | 94.7 | 94.4 | 94.2 |
| Medochemie | 102.9 | 93.4 | 93.9 |
| Medopharm | 96.3 | 96.8 | 94.3 |
| Shelys | 96.0 | 92.1 | 91.2 |

[a]Reference formulation

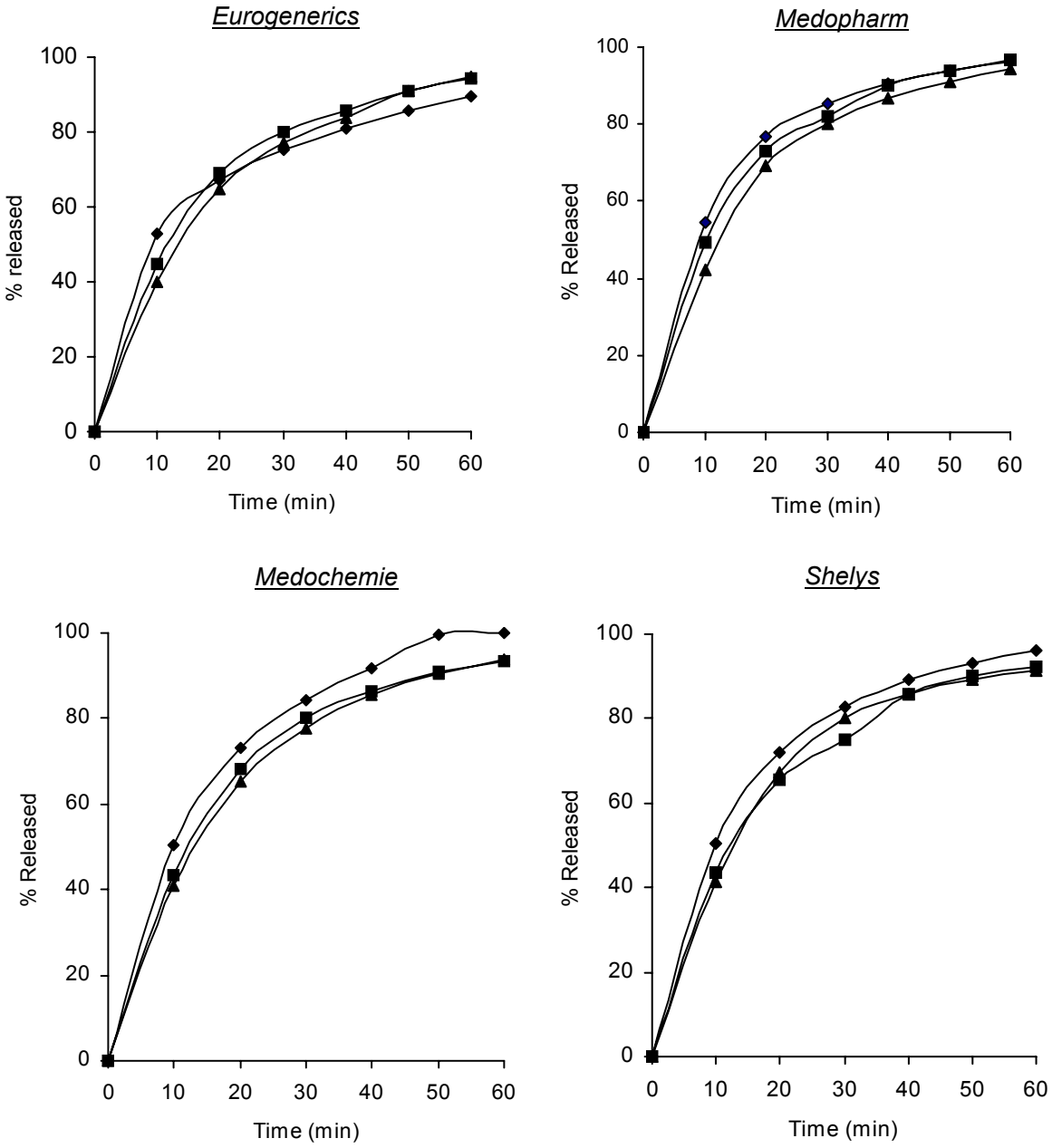
The dissolution profile of the Eurogenerics formulation obtained before storage at simulated tropical conditions (0 months) was compared with those of the other three formulations. The $f_2$ factors for the Medochemie, Medopharm and Shelys formulations were 50, 54 and 60, respectively, indicating that the drug dissolution profiles from these formulations were pharmaceutically similar to that of the Eurogenerics capsules.

Upon storage at the specified conditions for 3 and 6 months, the percent drug released within 60 min of dissolution testing from all formulations did not show any appreciable change. The percent drug release remained above 90% and all formulations met the USP 24 requirements for dissolution.

The dissolution profile of each formulation obtained after 3 and 6 months of storage at simulated tropical conditions was compared with those obtained at 0

months for the same formulation. The $f_2$ values for the profiles obtained after 3 months storage were 63, 58, 64 and 76 for the Eurogenerics, Medochemie, Shelys and Medopharm formulations, respectively. The respective values for the profiles obtained after 6 months storage were 59, 55, 58 and 59. This shows that the dissolution profiles of the four formulations obtained after storage at simulated tropical conditions were pharmaceutically equivalent to the ones obtained immediately after purchase and that the storage conditions did not influence the in vitro dissolution profiles of these formulations.

*Figure 3.1: Dissolution profiles of amoxicillin capsule formulations after 0 (♦), 3 (■) and 6 (▲) months of storage at simulated tropical conditions (40°C, 75% RH)*



## 3.4 Acetylsalicylic acid formulations

Four formulations were evaluated, three were sampled from the Tanzanian market, while the Bayer formulation (the innovator brand used as a reference) was obtained from a retail pharmacy in Belgium. Table 3.4 gives the detailed description of the analysed formulations.

*Table 3.4: Acetylsalicylic acid (300 mg) tablet formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| Bayer[a] (Belgium) | Blister, 100 tablets | 98A05 | 37.5[b] |
| Shelys Pharmaceuticals (Tanzania) | Bulk, 1000 tablets | 5497 | 1.3 |
| Betahealth (Kenya) | Bulk, 1000 tablets | 1700 | 1.3 |
| Mansoor Daya (Tanzania) | Bulk, 1000 tablets | 214372 | 1.3 |

[a]Reference formulation, containing 500 mg acetylsalicylic acid per tablet

[b]Market price if purchased in Dar es Salaam

### *3.4.1 Materials*

Acetylsalicylic acid, 1-heptanesulfonic acid and formic acid were purchased from Sigma Aldrich (Steinheim, Germany), while salicylic acid was obtained from Ludeco (Brussel, Belgium). Sodium acetate anhydrous and glacial acetic acid were purchased from Vel (Leuven, Belgium). Acetonitrile was obtained from Biosolve (Valkenswaard, The Netherlands).

### *3.4.2 Assay for acetylsalicylic acid and salicylic acid*

#### *3.4.2.1 Methods*

The amount of acetylsalicylic acid and salicylic acid in each formulation was determined by the method described in the USP 24 monograph for acetylsalicylic acid tablets.

Sample preparation

20 tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 100 mg of acetylsalicylic acid, was transferred into a 20 ml volumetric flask, and 20 ml of diluting solution (a mixture of acetonitrile and formic acid in a ratio of 99:1 v/v) was added. The resulting mixture was vigorously shaken for 10 min and allowed to equilibrate to room temperature. A portion of the mixture was filtered through a 0.5 µm cellulose acetate filter (Minisart®, Sartorius, Goettingen, Germany). 1 ml of the clear filtrate was transferred into a 10 ml volumetric flask, diluted to volume with the diluting solution and homogenised. This assay solution was used to determine the acetylsalycilic acid and salicylic acid content.

Calibration curves

A calibration curves (peak area vs. concentration) $y = 4034.3x$ (± 55.7) - 186198 (± 656) with a coefficient of determination ($R^2$) of 0.9997 ± 0.0002 (n = 5) for acetylsalicylic acid was constructed using standard solutions at acetylsalicylic acid concentrations of 200, 300, 400, 500 and 600 mg/l. Salicylic acid standard solutions with concentrations of 0.8, 1.0, 1.2, 1.5 and 2.0 mg/l were made and a calibration curve $y = 9689.4x$ (± 695.3) – 2895 (± 115.8), with a coefficient of determination ($R^2$) of 0.9996 ± 0.0005 (n = 5) was obtained. The unknown concentration of acetylsalicylic acid and salicylic acid in the samples were determined from the respective calibration line.

The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after repeated injections (n = 5) of acetylsalicylic acid (500 mg/l) and salicylic acid (1.5 mg/l) standard solutions.

Mobile phase

The mobile phase consisted of a mixture of a 0.35% (w/v) aqueous heptanesulfonic acid solution and acetonitrile in a ratio of 85:15 (v/v). Aliquots of the assay and standard solutions (50 µl) were injected into the HPLC system and the peaks were monitored by UV absorbance at 280 nm.

#### 3.4.2.2 Results

The relative standard deviation (RSD) for replicate injections of the standard preparation of acetylsalicylic acid was 0.75% and 1.25% for the within day and between days analysis, respectively. The respective values for salicylic acid were 5.0 and 7.5%. According to the USP 24 specifications the RSD for acetylsalicylic acid should be less than 2%.

The drug content in all formulations ranged from 95.9 to 105.4% (Table 3.5) and was within the 90 to 110% range specified in the USP 24. The content of salicylic acid in all formulations was below the limit of detection of the analytical method (0.3 mg/l) and below the maximum tolerable level of 0.3% specified in the USP 24.

*Table 3.5: Mean acetylsalicylic acid (ASA) content of the different formulations expressed as a percentage of the labelled amount*

| Manufacturer | Mean ASA content per tablet (mg) | % of the labelled amount per tablet |
|---|---|---|
| Bayer[a] | 494.1 ± 6.1 | 98.8 |
| Betahealth | 287.8 ± 3.6 | 95.9 |
| Mansoor Daya | 316.2 ± 3.9 | 105.4 |
| Shelys | 295.6 ± 3.7 | 98.5 |

[a]Reference formulation, containing 500 mg acetylsalicylic acid per tablet

### 3.4.3 *In vitro dissolution*

#### 3.4.3.1 Methods

Dissolution testing

Dissolution profiles were determined using the USP basket method (Method 1) at a rotational speed of 50 rpm. The volume of the dissolution medium (0.05M acetate buffer pH 4.5) was 900 ml per vessel maintained at a temperature of 37 ± 0.5°C. Samples (5 ml) were automatically withdrawn from each dissolution vessel after 5, 10, 15, 20, 25 and 30 min. The samples were diluted (2:1) with the dissolution medium before being spectrophotometrically analysed at 265 nm.

### 3.4.3.2 Results

The dissolution profiles obtained for each formulation before and after stability testing are shown in Figure 3.2 and the percent drug dissolved in Table 3.6. Only one formulation (Bayer) complied with the USP 24 requirements. This formulation released 98.1% of the labelled amount, while the others released less than 40% of their acetylsalicylic acid content.

*Table 3.6: Percentage of drug released after 30 minutes of dissolution testing on acetylsalicylic acid tablets stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirement: more than 80% is released within 30 minutes*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| Bayer[a] | 98.1 | 94.3 | 90.2 |
| Betahealth | 33.1 | 32.2 | 24.8 |
| Mansoor Daya | 38.3 | 37.2 | 36.8 |
| Shelys | 24.7 | 17.5 | 12.1 |

[a]Reference formulation

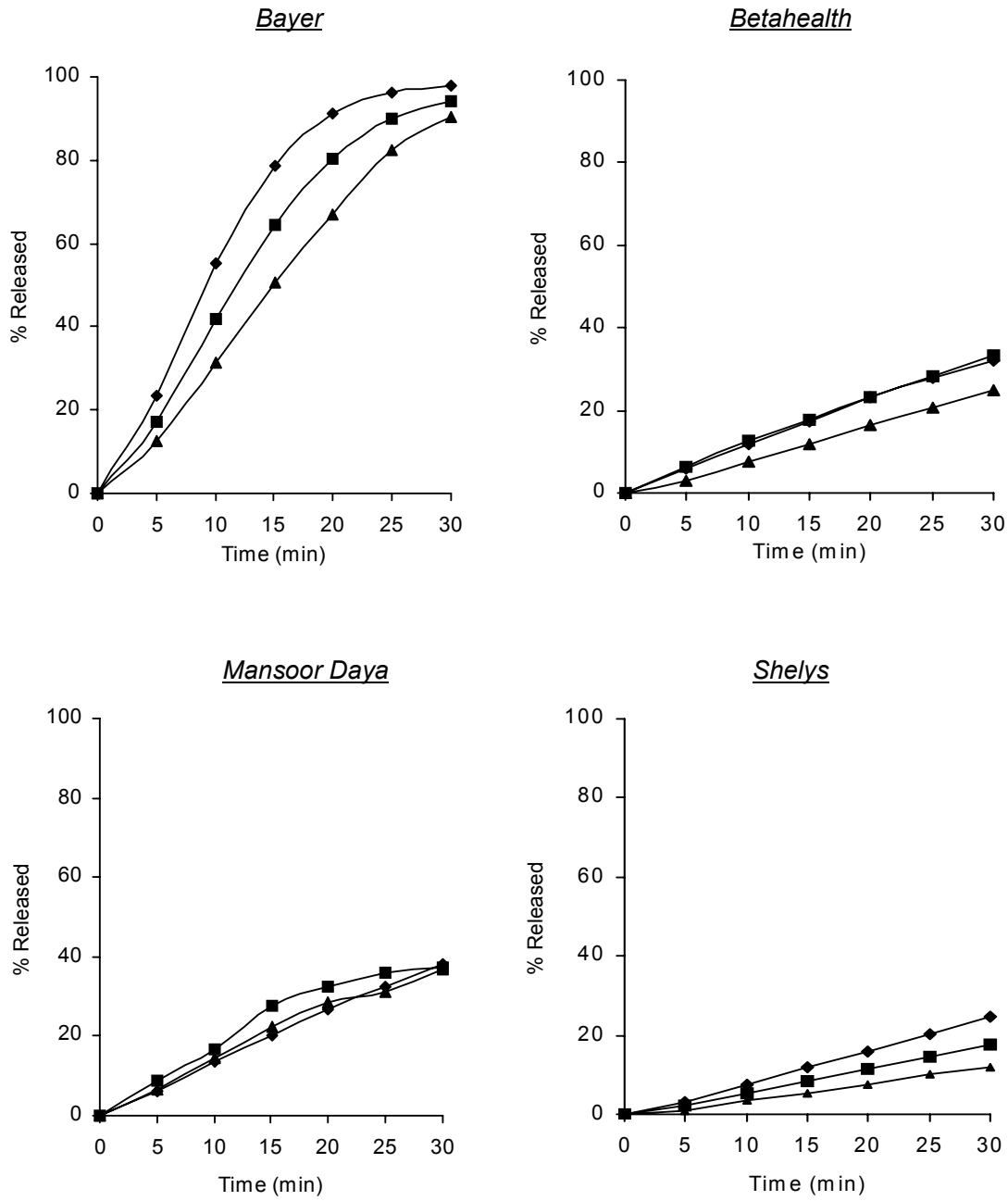
Upon storage at simulated tropical conditions all formulations that failed the dissolution test had as expected a drug release that was below the USP 24 recommended values. The drug release from the Shelys formulation was halved (from 24.7 to 12.1%) after 6 months storage, while that from the Betahealth formulation decreased by about 10%. It was observed that the tablets from the Betahealth, Shelys and Mansoor Daya formulations failed to disintegrate during the dissolution test after storage at simulated tropical conditions.

The dissolution profiles obtained from the Bayer formulation after 3 and 6 months of storage at simulated tropical conditions were compared with that obtained at 0 months. The $f_2$ value obtained after 3 months was 52, while that of the 6 months profile was 35. The $f_2$ analysis shows that in the first 3 months of storage the drug release profile was pharmaceutically equivalent to that obtained at 0 months. However, after 6 months the dissolution pattern of this formulation had changed, indicating that the storage conditions affected the in vitro dissolution behaviour.

Despite the observed changes in drug release profile, the percent drug dissolved remained within the USP 24 tolerance limits and the changes may not be pharmaceutically significant.

The drug content of all formulations was determined after the 6 months storage period at simulated tropical conditions. The assay results showed that compared with the values obtained immediately after purchase, the acetylsalicylic acid content (ranged from 95.5 to 101.3%) remained relatively unchanged and was within the USP 24 specifications. The salicylic acid content remained below the maximum tolerance limit (0.3%).

*Figure 3.2: Dissolution profiles of acetylsalicylic acid formulations after 0 (♦), 3 (■) and 6 (▲) months of storage at simulated tropical conditions (40°C, 75% RH)*



## 3.5 Ciprofloxacin formulations

Nine formulations, all available on the Tanzanian market were evaluated. Table 3.7 shows the detailed information of the ciprofloxacin formulations sampled.

*Table 3.7: Ciprofloxacin (500 mg) film coated tablet formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| Ajanta Pharmaceuticals (India) | Blister, 100 tablets | P44418A | 48.0[b] |
| Aurobindo (India) | Blister, 100 tablets | E9002 | 58.0 |
| Bayer[a] (Belgium) | Blister, 10 tablets | 98L16 | 2000.0 |
| Cadila Pharmaceuticals (India) | Blister, 100 tablets | E902 | 87.5 |
| Flamingo Pharmaceuticals (India) | Blister, 100 tablets | SL272 | 60.0 |
| Freudun Pharmaceuticals (India) | Blister, 100 tablets | T9002 | 58.8 |
| Medopharm (India) | Blister, 100 tablets | 980234 | 48.0[b] |
| Intas Pharmaceuticals (India) | Blister, 100 tablets | 9012 | 58.0 |
| S Kant Health Care (India) | Blister, 100 tablets | SK 105 | 58.0 |

[a]Reference formulation
[b]Sample obtained from the Medical Stores Department

### *3.5.1 Materials*

Ciprofloxacin hydrochloride (Ph. Eur.) was purchased from Alpha Pharma (Zwevegem, Belgium), triethylamine was from Sigma Aldrich (Steinheim, Germany), orthophosphoric acid was obtained from Vel (Leuven, Belgium) and acetonitrile from Biosolve (Valkenswaard, The Netherlands).

### *3.5.2 Assay for ciprofloxacin*

#### *3.5.2.1 Method*

The amount of ciprofloxacin in each formulation was determined by the method described in the USP 24.

Sample preparation

5 tablets were put into a 500 ml flask. About 400 ml of distilled water was added and the mixture was sonicated for 20 min. The volume was adjusted with distilled water, mixed and left to settle for 30 min. An aliquot (50 µl) of this mixture was transferred into a 20 ml volumetric flask and diluted to volume with distilled water, a portion (10 ml) was filtered through a 0.5 µm cellulose acetate filter (Minisart®, Sartorius, Goettingen, Germany) and used as the assay preparation.

Calibration curve

A calibration curve (peak area vs. concentrations) y = 195415.5x (± 1563.3) + 220761.9 (± 1766.1) with a coefficient of determination ($R^2$) of 0.9999 ± 0.0004 (n = 5) was constructed using standard solutions at ciprofloxacin hydrochloride concentrations of 6.0, 8.0, 10.0, 12.0 and 15.0 mg/l. The unknown concentration of ciprofloxacin (calculated on the anhydrous basis and taking into account the molecular weights of ciprofloxacin hydrochloride and anhydrous ciprofloxacin) in the assay preparation was determined from the calibration curve.

The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after repeated injections (n = 5) of a 12.0 mg/l ciprofloxacin hydrochloride standard solution.

Mobile phase

The mobile phase was a mixture of a 0.025M phosphoric acid buffer (pH 3.0 with triethylamine) and acetonitrile in a ratio of 3:1 (v/v). Aliquots (20 µl) of the assay and standard solutions were injected into the HPLC system and the peaks were monitored by UV absorbance at 277 nm.

#### 3.5.2.2 *Results*

The relative standard deviation (RSD) was 0.6% and 0.8% for the within day and between days analysis, respectively. According to the USP 24 specification the RSD should be less than 1.5%.

The assayed drug content of each formulation is shown in Table 3.8. All formulations passed the assay test as the drug content ranged from 93.2 to 100.8%. This is within the USP 24 acceptance range: 90 to 110% of the labelled content.

*Table 3.8: Mean ciprofloxacin content of the different formulations expressed as a percentage of the labelled amount per tablet*

| Manufacturer | Mean ciprofloxacin content per tablet (mg) | % of the labelled amount per tablet |
|---|---|---|
| Ajanta | 466.1 ± 3.7 | 93.2 |
| Aurobindo | 496.3 ± 3.9 | 99.3 |
| Bayer[a] | 503.9 ± 4.0 | 100.8 |
| Cadila | 500.7 ± 4.0 | 100.1 |
| Flamingo | 474.2 ± 3.8 | 94.8 |
| Freudun | 493.8 ± 3.9 | 98.8 |
| Medopharm | 474.3 ± 3.8 | 94.9 |
| Intas | 470.9 ± 3.7 | 94.2 |
| S Kant | 479.8 ± 3.8 | 96.0 |

[a]Reference formulation

### 3.5.3 *In vitro dissolution*

#### 3.5.3.1 *Methods*

Dissolution testing

Dissolution tests were performed using the USP paddle method (Method 2) at a speed of 50 rpm. The volume of the dissolution medium (distilled water) in each vessel was 900 ml, maintained at a temperature of 37.0 ± 0.5°C. Samples (5 ml) were withdrawn from each vessel every 5 min (5, 10, 15, 20, 25 and 30 min). The

samples were diluted with the dissolution medium (1:50) and the drug concentration was spectrophotometrically measured at 276 nm.

### *3.5.3.2 Results*

Table 3.9 shows the percent drug released from the formulations. Before storage at simulated tropical conditions all formulations met the USP 24 specifications for dissolution.

*Table 3.9: Percentage of drug released after 30 min of dissolution testing on ciprofloxacin tablets stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirements: more than 80% is released within 30 minutes*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| Ajanta | 95.3 | 94.0 | 95.0 |
| Aurobindo | 96.8 | 97.1 | 96.2 |
| Bayer[a] | 100.5 | 100.7 | 99.9 |
| Cadila | 98.4 | 98.8 | 98.0 |
| Flamingo | 97.3 | 95.0 | 94.9 |
| Freudun | 92.9 | 93.3 | 90.2 |
| Medopharm | 96.5 | 93.8 | 90.8 |
| Intas | 94.2 | 93.2 | 91.7 |
| S Kant | 97.0 | 97.1 | 96.3 |

[a]Reference formulation

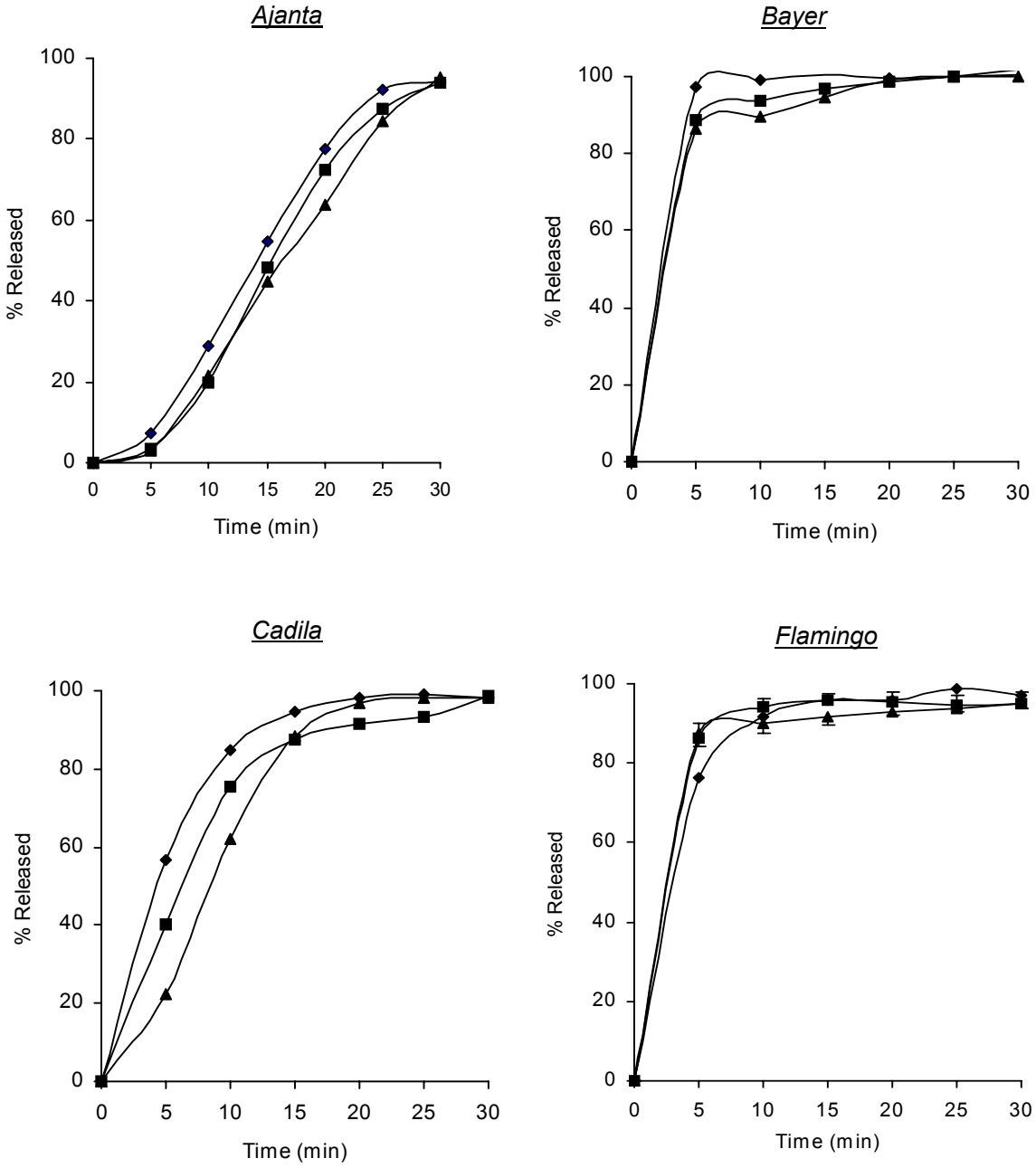
The dissolution profile of the Bayer formulation obtained at 0 months was compared with the profiles of the other formulations. The calculated $f_2$ values are shown in Table 3.10. Only the formulation from Flamingo had an $f_2$ value above 50 ($f_2$ = 52). The other formulations had $f_2$ values that ranged from 14 to 37. Compared with the other formulations, the Bayer and Flamingo tablets had initially a higher drug release as more than 90% ciprofloxacin was dissolved within the first 5 min. The drug release rate from the other formulations during the same interval was slower. The Ajanta formulation had the lowest $f_2$ factor ($f_2$ = 14) and also the slowest drug release (less than 5%) during the same time interval (Fig.3.3).

*Table 3.10: The $f_2$ values of the dissolution profiles obtained from the different ciprofloxacin formulations before storage at simulated tropical conditions using the Bayer formulation as the reference formulation*

| Formulation | $f_2$ value |
|---|---|
| Ajanta | 14 |
| Aurobindo | 24 |
| Cadila | 37 |
| Flamingo | 52 |
| Freudun | 34 |
| Medopharm | 23 |
| Intas | 35 |
| S Kant | 31 |

After storage at simulated tropical conditions for 6 months, all formulations passed the USP 24 dissolution requirements. Calculation of the $f_2$ factors from the dissolution data obtained after 3 and 6 months of storage showed that, with the exception of the Cadila formulation, the profiles were similar to those obtained before the storage. The $f_2$ values of the profiles from the Cadila formulation obtained after 3 and 6 months storage were 45 and 38, respectively, indicating that the drug release pattern was different and was influenced by the storage conditions.

*Figure 3.3: Dissolution profiles of ciprofloxacin formulations after 0 (♦), 3 (■) and 6 (▲) months of storage at simulated tropical conditions (40°C, 75% RH)*



## 3.6 Sulphamethoxazole / trimethoprim formulations

Five sulphamethoxazole (400 mg) / trimethoprim (80 mg) formulations were analysed. All formulations are available on the Tanzanian market under the generic name cotrimoxazole. The innovator brand from Roche was obtained from a retail pharmacy in Ghent (Belgium), while the others were obtained in Tanzania. Table 3.11 gives the detailed information of the sampled formulations.

*Table 3.11: Sulphamethoxazole (400 mg) / trimethoprim (80 mg) tablet formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| ACE laboratories (India) | Blister,100 tablets | TE446 | 10.0 |
| Shalina laboratories (India) | Blister,100 tablets | SL272 | 9.5 |
| S Kant Health care (India) | Blister,100 tablets | SK 503 | 10.0 |
| Roche[a] (Belgium) | Blister, 20 tablets | B143 98DO6 | 65.0 |
| TPI (Tanzania) | Bulk, 1000 tablets | NE 15 | 8.75 |

[a]Reference formulation

### *3.6.1 Materials*

Sulfamethoxazole and trimethoprim (Ph. Eur.) were obtained from Alpha Pharma (Zwevegem, Belgium). Hydrochloric acid and glacial acetic acid were purchased from Merck (Darmstadt, Germany). Triethylamine was obtained from Sigma Aldrich (Steinheim, Germany) and acetonitrile was from Biosolve (Valkenswaard, The Netherlands).

### *3.6.2 Assay for sulfamethoxazole and trimethoprim*

#### *3.6.2.1 Methods*

The content of sulfamethoxazole and trimethoprim in each formulation was determined by the method described in the USP 24.

Sample preparation

20 tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to 160 mg of sulfamethoxazole, was transferred into a 100 ml volumetric flask and about 50 ml of the mobile phase was added. The mixture was sonicated for 5 min, allowed to equilibrate to room temperature, then adjusted to volume with the mobile phase. The resulting mixture was filtered through a 0.5 µm cellulose acetate filter (Minisart®, Sartorius, Goettingen, Germany). 5 ml of the clear filtrate was diluted 10 times with the mobile phase to obtain the assay solution.

Calibration curves

A calibration curve (peak area vs. concentrations) $y = 13135x$ (± 26.3) + 32372 (± 2366) with a coefficient of determination ($R^2$) of 0.9995 ± 0.0003 (n = 5) was constructed using standard solutions with sulfamethoxazole concentrations of 40, 80, 120, 160 and 180 mg/l. Similarly, a calibration curve $y = 7156.3x$ (± 100.2) + 2247.8 (± 29.5) with a coefficient of determination ($R^2$) of 0.9997 ± 0.0006 (n = 5) was obtained for trimethoprim using standard solutions with concentrations of 16, 20, 24, 32 and 36 mg/l. The unknown concentration of sulfamethoxazole and trimethoprim in the assay solution was determined from the respective calibration curve.

The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after repeated injections (n = 5) of sulfamethoxazole (160 mg/l) and trimethoprim (32 mg/l) standard solutions.

The resolution factor (R) between sulfamethoxazole and trimethoprim was determined from the peaks obtained from the sulfamethoxazole and trimethoprim standard solutions. Based on the retention times and the baseline widths of the

peaks R was calculated as

$$R = \quad 2(t_1 - t_2)/(W_1 + W_2)$$

with $t_1$ and $W_1$ being the retention time and baseline width, respectively, of the sulfamethoxazole peak and $t_2$ and $W_2$, the respective values for the trimethoprim peak.

Mobile phase

The mobile phase consisted of a water, acetonitrile and triethlylamine mixture in a 650:250:1 (v/v) ratio. The pH was adjusted to 5.9 with diluted acetic acid (0.1N). Aliquots (20 µl) of the assay and standard solutions were injected into the HPLC system and the peaks monitored by UV absorbance at 254 nm.

#### *3.6.2.2 Results*

The relative standard deviation (RSD) for replicate injections of the sulfamethoxazole standard preparation was 0.2% and 0.4% for the within day and between day analysis, respectively. The respective values for trimethoprim were 0.1% and 1.4%. According to the USP 24, the RSD should be not more than 2.0%. The resolution factor R between the sulfamethoxazole and trimethoprim peaks was 6.5.

The mean sulfamethoxazole and trimethoprim contents of each formulation expressed as a percentage of the labelled amount are shown in Table 3.12. All formulations passed the assay requirements specified in the USP 24 for both drugs (93 to 107% for sulfamethoxazole and trimethoprim). The drug content in the formulations ranged from 96.2 to 103.0% and from 96.6 to 101.0% for sulfamethoxazole and trimethoprim, respectively.

*Table 3.12: Mean sulfamethoxazole and trimethoprim content of the different formulations expressed as percentage of the labelled amount*

| Manufacturer | Mean drug content per tablet (mg) | % of the labelled amount per tablet |
|---|---|---|
| **Sulfamethoxazole** | | |
| ACE Laboratories | 397.6 ± 1.6 | 99.4 |
| Shalina Laboratories | 392.8 ± 1.5 | 98.2 |
| S Kant Health care | 408.4 ± 1.4 | 102.1 |
| Roche[a] | 412.0 ± 1.6 | 103.0 |
| TPI | 385.0 ± 1.5 | 96.2 |
| **Trimethoprim** | | |
| ACE Laboratories | 80.1 ± 1.0 | 100.1 |
| Shalina Labolatories | 78.3 ± 1.0 | 97.9 |
| S Kant Health care | 79.8 ± 1.1 | 99.8 |
| Roche[a] | 80.8 ± 1.1 | 101.0 |
| TPI | 77.3 ± 1.2 | 96.6 |

[a]Reference formulation

### *3.6.3 In vitro dissolution*

#### *3.6.3.1 Methods*

Dissolution testing

The in vitro dissolution profiles were determined using the USP paddle method (Method 2) at 75 rpm. The volume of the dissolution medium (0.1N HCl) in each vessel was 900 ml maintained at 37 ± 0.5°C during the entire duration of the test. Samples (5 ml) were withdrawn at 10 min intervals (10, 20, 30, 40, 50 and 60 min) and diluted 5 times with the mobile phase before HPLC analysis. The concentration of trimethoprim and sulfamethoxazole in the samples were analysed by UV after chromatographic separation by HPLC as described in the assay procedure (section 3.6.2).

#### *3.6.3.2 Results*

Table 3.13 shows the percent drug released after 1 hr dissolution testing on the tablets from the different manufacuters. Before being subjected to the accelerated

stability test conditions, two out of five formulations failed the USP 24 drug release specifications: the sulfamethoxazole release from the ACE and Shalina formulations being 63.3 and 69.2%, respectively. It was observed that tablets from these formulations did not completely disintegrate during the dissolution test.

*Table 3.13: Percentage of sulfamethoxazole and trimethoprim released after 1 hr of dissolution testing on sulfamethoxazole/trimethoprim tablets stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirements: more than 70% of both sulfamethoxazole and trimethoprim is released within 1 hr*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| **Sulfamethoxazole** | | | |
| ACE | 63.3 | 60.9 | 55.2 |
| Shalina | 69.2 | 64.5 | 63.6 |
| S Kant | 79.4 | 76.6 | 71.1 |
| Roche[a] | 100.3 | 98.8 | 98.1 |
| TPI | 100.4 | 93.5 | 88.6 |
| **Trimethoprim** | | | |
| ACE | 82.9 | 78.7 | 76.0 |
| Shalina | 94.9 | 88.2 | 79.8 |
| S Kant | 96.3 | 95.2 | 94.2 |
| Roche[a] | 98.4 | 97.6 | 97.3 |
| TPI | 97.8 | 95.5 | 90.5 |

[a]Reference formulation

The $f_2$ values were calculated for the formulations that satisfied the USP 24 requirements for dissolution, taking the Roche profile as a reference. The $f_2$ values for the TPI formulation were 76 and 72 for sulfamethoxazole and trimethoprim, respectively. The respective values for the S Kant formulation were 26 and 44. The Roche and TPI formulation had released more than 90% of sulfamethoxazole during the first 10 min, while the S Kant formulation released only 70% sulfamethoxazole during the same time interval (Fig. 3.4). Although the S Kant

formulation released 96.3% trimethoprim within 1 hr, it had a slower drug release rate during the first 10 min.

Upon storage at simulated tropical conditions the sulfamethoxazole release from the Roche and S Kant formulations remained stable and met the USP 24 dissolution criteria. The $f_2$ factors for the S Kant dissolution profiles after 3 and 6 months storage at simulated tropical conditions were 69 and 63, respectively. The respective values for the Roche formulation were 78 and 75. The TPI formulation showed a decrease in the percent sulfamethoxazole released during dissolution testing after storage at simulated tropical conditions. This was reflected in the $f_2$ values being 40 and 35 for the 3 and 6 months profiles, respectively. Although the sulfamethoxazole release from the TPI tablets remained within the USP 24 limits, the dissolution profiles obtained before and after storage were not equivalent and the in vitro drug release profile was not stable during storage at simulated tropical conditions (Fig. 3.4).

Similar to sulfamethoxazole, the percent trimethoprim released from the TPI formulation during 1 hr dissolution testing decreased after storage at simulated tropical conditions. The $f_2$ values for the 3 and 6 months profiles were 39 and 32, respectively, and this shows that the dissolution profiles obtained before and after the stability test were not equivalent (Fig 3.5). Similar observations were made for the ACE formulation ($f_2$ values of 44 and 37, respectively). The dissolution profile obtained after 3 months storage of the Shalina formulation was similar to that obtained before storage ($f_2$ = 65), but after 6 months storage the drug release profile had changed ($f_2$ = 48). The percent trimethoprim released from the Roche and S Kant formulations remained stable during 6 months of storage at simulated tropical conditions. The $f_2$ values obtained for the Roche and S Kant profiles after 6 months were 71 and 69, respectively, indicating that the in vitro drug release from these formulations was not influenced by storage at simulated tropical conditions.

*Figure 3.4: Dissolution profiles of sulfamethoxazole from sulfamethoxazole/ trimethoprim (cotrimoxazole) formulations after 0 (♦), 3 (■) and 6 (▲) months of storage at simulated tropical conditions (40°C, 75% RH)*

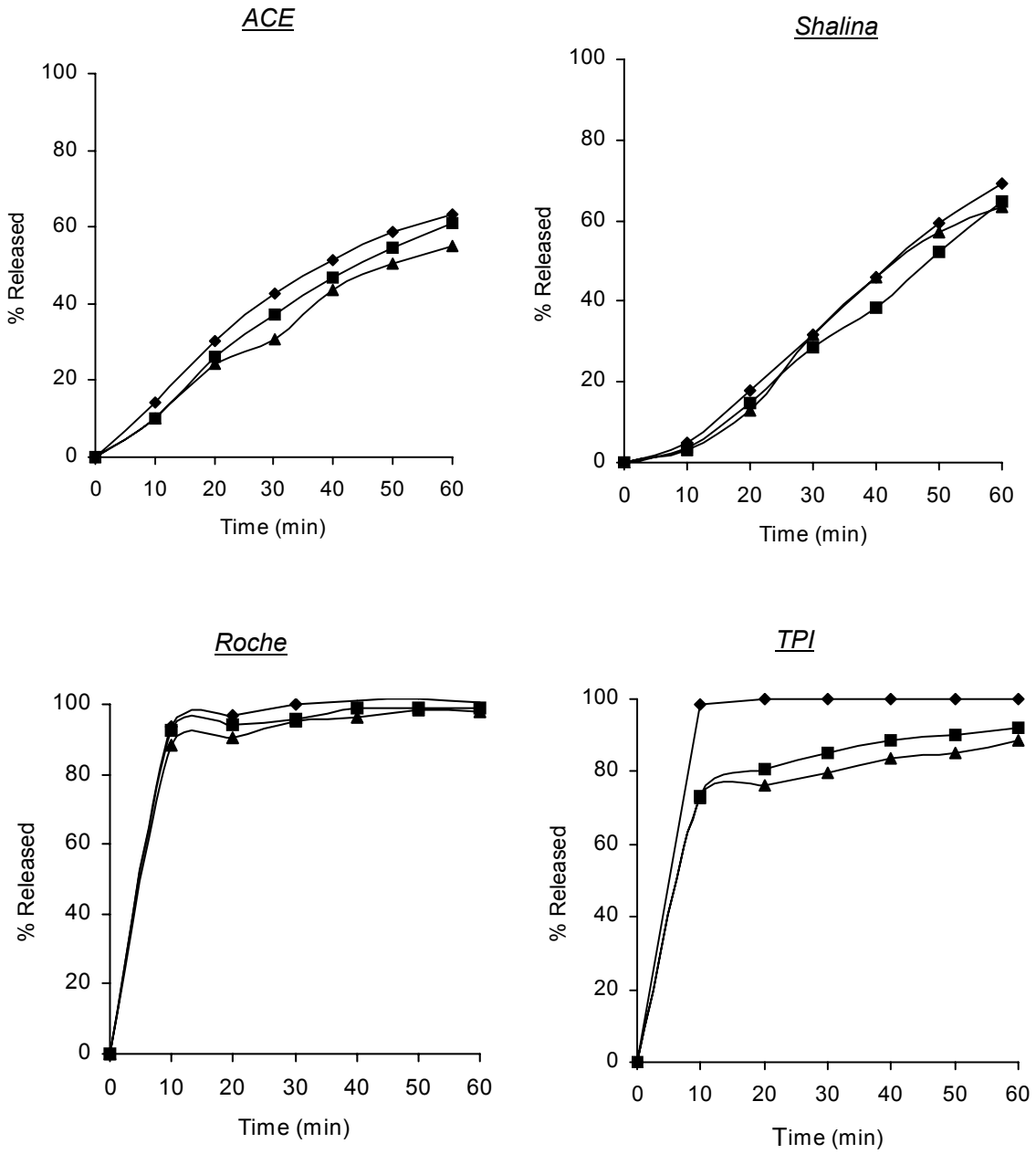
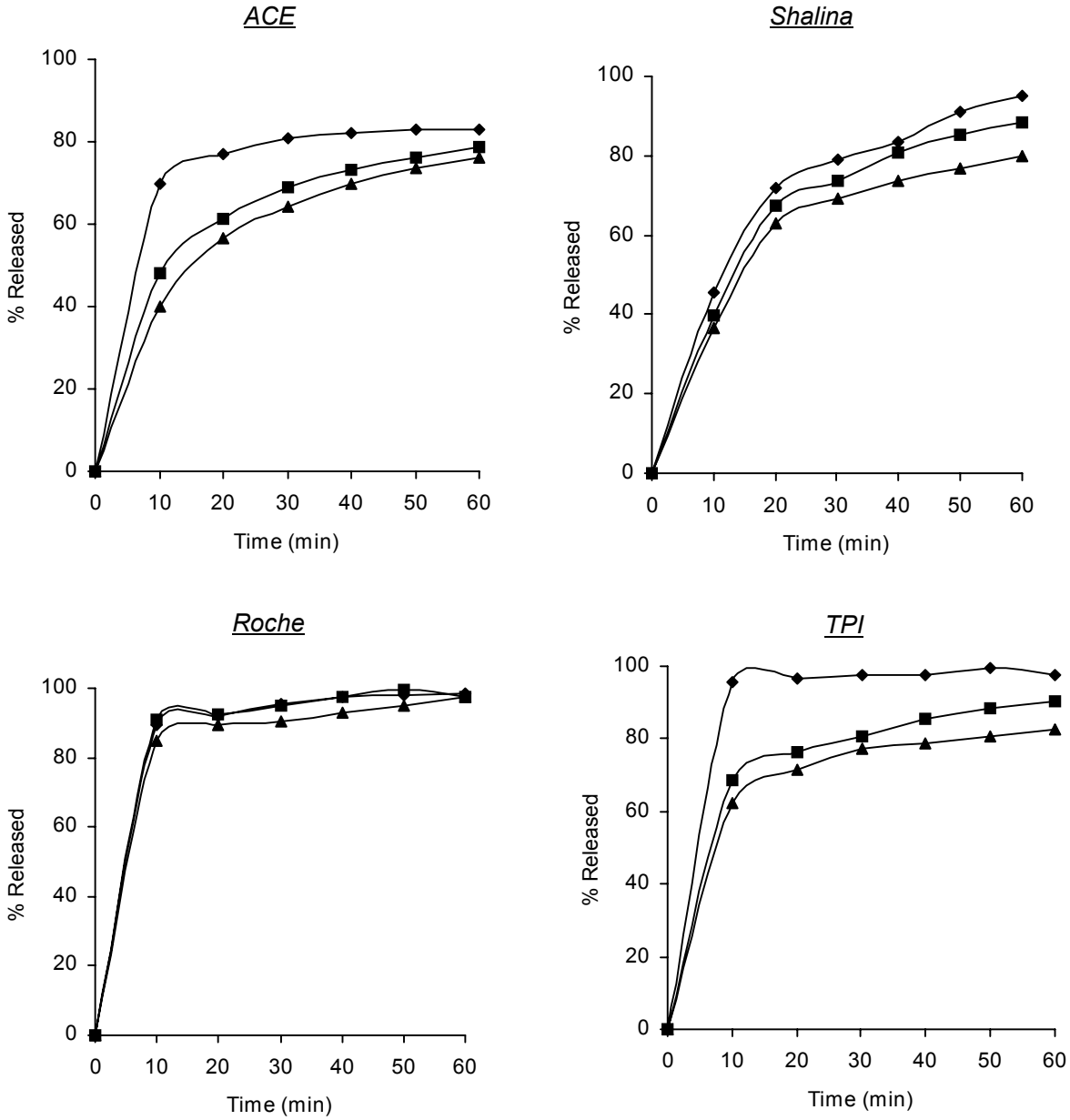


*Figure 3.5: Dissolution profiles of trimethoprim from sulfamethoxazole/trimethoprim (cotrimoxazole) formulations after 0 (♦), 3 (■) and 6 (▲) months of storage at simulated tropical conditions (40°C, 75 % RH)*



## 3.7 Chloroquine formulations

Seven formulations were analysed: 6 formulations contained chloroquine phosphate and were available on the Tanzanian market, while the reference formulation (purchased in a Belgian pharmacy) contained chloroquine sulphate. Table 3.14 gives the detailed information of the samples.

*Table 3.14: Chloroquine phosphate (250 mg) tablet formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| ACE laboratories (India) | Bulk, 1000 tablets | T70120 | 8.5 |
| Mepro Pharmaceuticals [b] (India) | Bulk, 1000 tablets | 9048 | 8.5 |
| Rhône Poulenc Rorer[a] (Belgium) | Bulk, 100 tablets | 96J17 | N/A |
| Shanghai Simplex (China) | Bulk, 1000 tablets | F610080 | 8.3 |
| Shelys Pharmaceuticals (Tanzania) | Bulk, 1000 tablets | 573 | 6.4[c] |
| TPI (Tanzania) | Bulk, 1000 tablets | LK 72 | 8.3 |
| Taiyuan Yangling Shanxi (China) | Bulk, 1000 tablets | 971001 | 8.8 |

[a]Reference sample, contained chloroquine sulphate equivalent to 100 mg chloroquine base per tablet

[b]Chloroquine phosphate sugar coated tablets

[c]Sample obtained from the Medical Stores Department

N/A Not available on the Tanzanian market

### *3.7.1 Materials*

Chloroquine phosphate (Ph. Eur.) was obtained from Alpha Pharma (Zwevegem, Belgium), potassium dihydrogen phosphate from Merck (Darmstadt, Germany) and acetonitrile from Biosolve (Valkenswaard, The Netherlands). Orthophosphoric acid was obtained from Vel (Leuven, Belgium).

### *3.7.2 Assay for chloroquine*

#### *3.7.2.1 Methods*

The amount of chloroquine in each formulation was determined by the method described by Pussard et al. (1986).

Sample preparation

20 tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to 100 mg chloroquine phosphate was transferred into a 100 ml volumetric flask and about 50 ml of distilled water was added. The mixture was sonicated for 20 min after which it was adjusted to volume with the mobile phase. The suspension was allowed to settle for 1 hr, after which 10 ml of the clear supernatant was transferred into a 100 ml volumetric flask and diluted to volume with the mobile phase. The solution was filtered through a 0.5 µm cellulose acetate filter (Minisart®, Sartorius, Goettingen, Germany) and used as the assay solution.

Calibration curve

A calibration curve (peak area vs. concentrations) $y = 56384x$ (± 1691.5) – 2975 (± 89.3) with a coefficient of determination ($R^2$) of 0.998 (± 0.0005) was constructed using standard solutions with chloroquine phosphate concentrations of 60, 80, 100, 120 and 140 mg/l. The unknown concentration of chloroquine phosphate in the assay solution was determined from the calibration curve.

The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after repeated injections (n = 6) of a chloroquine phosphate standard solution (100 mg/l).

Mobile phase

The mobile phase was a mixture of a 0.2M monobasic sodium phosphate buffer (pH 3.0 with phosphoric acid) and acetonitrile in a ratio of 10:3 (v/v). Aliquots (50 µl) of the assay and standard solutions were injected into the HPLC system and the peaks monitored by UV at 340 nm.

### *3.7.2.2 Results*

The relative standard deviation (RSD) for replicate injections of the standard preparation was 2.4% and 3.1% for the within day and between day analysis, respectively. The assay values for chloroquine sulphate obtained from the Rhône Poulenc Rorer formulation were converted to an equivalent amount of chloroquine phosphate. The assayed chloroquine phosphate content of each formulation is shown in Table 3.15. The assay results show that all formulations contained chloroquine in an amount that was within the range specified by the USP 24, being 93.0 to 107.0% of the labelled amount.

*Table 3.15: Mean chloroquine phosphate content of the different formulations expressed as a percentage of labelled amount*

| Manufacturer | Mean chloroquine phosphate content per tablet (mg) | % of the labelled amount per tablet |
|---|---|---|
| ACE Laboratories | 242.5 ± 7.6 | 97.0 |
| Mepro Pharmaceuticals | 247.1 ± 7.5 | 98.8 |
| Rhône Poulenc Rorer[a] | 167.1 ± 5.2[b] | 103.8 |
| Shanghai Simplex | 249.7 ± 7.7 | 99.9 |
| Shelys Pharmaceuticals | 249.2 ± 7.6 | 99.7 |
| TPI | 246.6 ± 7.7 | 98.6 |
| Taiyuan Yangling Shanxi | 242.9 ± 7.5 | 97.2 |

[a]Reference formulation, contained 100 mg chloroquine base per tablet
[b]converted into an equivalent amount of chloroquine phosphate

## *3.7.3 In vitro dissolution*

### *3.7.3.1 Methods*

Dissolution testing

Dissolution profiles were determined using the USP paddle method (Method 2) at 100 rpm. The volume of dissolution medium (distilled water) in each vessel was 900 ml, maintained at 37 ± 0.5°C. At different time intervals (10, 20, 30, 40 and 45 min), 5 ml samples were withdrawn from the vessels, diluted (1:10) with the dissolution medium and the absorbances measured at 343 nm.

### 3.7.3.2 Results

Table 3.16 shows the percentage drug released after 45 minutes of dissolution testing on all formulations. Before storage at simulated tropical conditions, all formulations complied with the USP 24 requirements for drug release: the percentage drug dissolved ranged from 90.8 to 98.5%.

*Table 3.16: Percentage of chloroquine phosphate released after 45 min of dissolution testing on chloroquine tablets stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirement: more than 75% of chloroquine phosphate is released within 45 min*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| ACE | 90.8 | 89.9 | 89.5 |
| Mepro | 95.3 | 92.5 | 85.1 |
| Rhône Polenc Rorer[a] | 96.0 | 83.4 | 50.3 |
| Shanghai Simplex | 97.3 | 97.6 | 96.3 |
| Shelys | 95.2 | 94.7 | 88.8 |
| TPI | 94.1 | 54.9 | 42.2 |
| Taiyuan Yangling Shanxi | 98.5 | 98.0 | 96.2 |

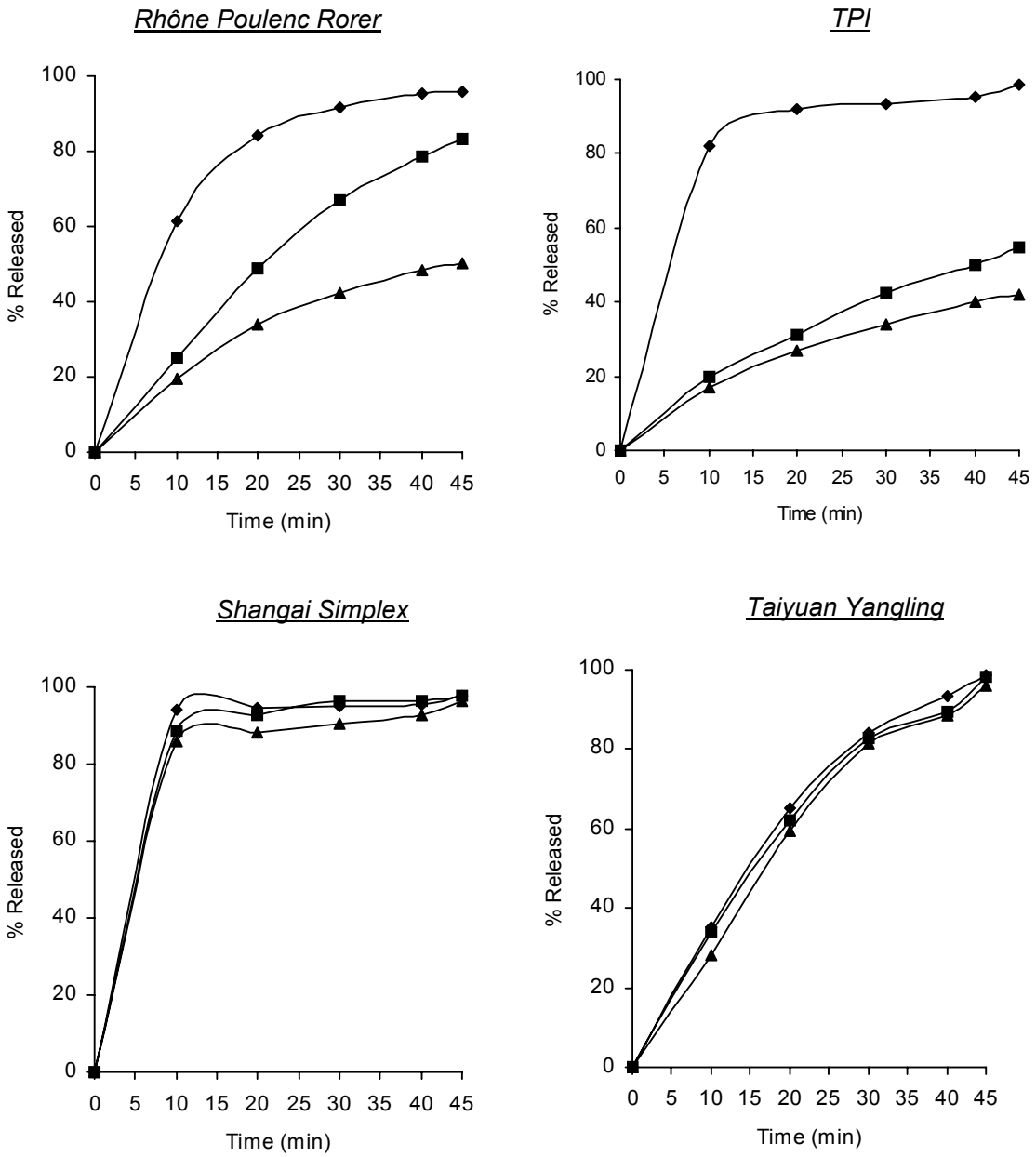
[a]Reference formulation

The dissolution profile of Rhône Poulenc Rorer was compared with the profiles from the other formulations. The $f_2$ values obtained show that only the Shelys profile was similar to that of Rhône Poulenc Rorer ($f_2$ = 57). Both formulations had a slower drug release in the first 10 min (67 and 76%, respectively) compared with the TPI ($f_2$ = 49) and Shanghai Simplex ($f_2$ = 42) formulations (82 and 90% drug released, respectively). The other three formulations had a much slower drug release (less than 35%) in the same interval and their $f_2$ values were 35, 41 and 43 for the Mepro, ACE and Taiyuan Yangling Shanxi formulations, respectively (Fig. 3.6).

Upon storage at the test conditions it was observed that the percentage drug release from the TPI formulation decreased to 42.2% after 6 months of storage. The Rhône Poulenc Rorer formulation also had a decrease in drug release from 96.0% to 83.4

and 50.3% after 3 and 6 months, respectively (Fig. 3.6). The percentage drug released from the other formulations remained within the USP 24 recommended values. However, only the Taiyuan Yangling Shanxi and the Shangai Simplex formulations had dissolution profiles that were stable upon exposure to the test conditions as the $f_2$ values at 6 months were 70 and 59, respectively (Fig. 3.6).

*Figure 3.6: Dissolution profiles of chloroquine formulations after 0 (♦), 3 (■) and 6 (▲) months of storage at simulated tropical conditions (40°C, 75% RH)*



## *3.8 Enteric coated diclofenac sodium formulations*

Four enteric coated formulations available on the Tanzanian market were analysed. Table 3.17 gives the detailed information of the sampled formulations.

*Table 3.17: Diclofenac sodium (50 mg) enteric coated tablet formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| Camden (Malyasia) | Blister, 100 tablets | 00523 | 10.0 |
| Intas Pharmaceuticals (India) | Blister, 100 tablets | A005 | 12.0 |
| Novartis[a] (Belgium) | Blister, 20 tablets | 00D03BT | 125.0 |
| Remedica (Cyprus) | Blister, 100 tablets | 13915 | 40.5 |

[a]Reference formulation

### *3.8.1 Materials*

Diclofenac sodium (Ph. Eur.) was obtained from Alpha Pharma (Zwevegem, Belgium), while methanol was purchased from Biosolve (Valkenswaard, The Netherlands). Ortho-phosphoric acid, monobasic sodium phosphate, tribasic sodium phosphate, hydrochloric acid and sodium hydroxide were obtained from Vel (Leuven, Belgium).

### *3.8.2 Assay for diclofenac sodium*

#### *3.8.2.1 Methods*

The amount of sodium diclofenac in each formulation was determined by HPLC using the method described in the USP 24.

Sample preparation

20 tablets were transferred into a 1000 ml volumetric flask and about 800 ml of diluent (methanol:water, 7:3 v/v) was added and the mixture was sonicated for 20

min to allow the tablets to disintegrate. After all tablets had disintegrated, the mixture was stirred for 5 min and the volume adjusted with the diluent. A portion (15 ml) of the resulting mixture was filtered through a 0.5 µm cellulose acetate filter (Minisart®, Sartorius, Goettingen, Germany). 7.5 ml of the filtrate was diluted to 10 ml and used as the assay solution.

Calibration curve

A calibration curve (peak area vs. concentration) y = 21603.6x (± 118.2) + 342034 (± 1894) with a coefficient of determination of 0.9998 (± 0.0003) (n = 5) was constructed using standard solutions with diclofenac sodium concentrations of 250, 500, 750, 1000 and 1250 mg/l. The unknown concentration of diclofenac sodium in the assay solution was determined from the calibration curve.
The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after repeated injections (n = 5) of a diclofenac sodium standard solution (750 mg/l).

Mobile phase

The mobile phase consisted of a mixture of methanol and a phosphate buffer in a ratio 7:3 (v:v). The buffer was a mixture of equal volumes of 0.01M phosphoric acid and 0.01M monobasic sodium phosphate adjusted to pH 2.5 with phosphoric acid. 20 µl aliquots of the assay and standard solutions were injected into the HPLC system and the peaks were monitored by UV absorbances at 276 nm.

### *3.8.2.2 Results*

The relative standard deviation (RSD) for replicate injections of the standard preparation was 0.3% and 0.6% for the within day and between day analysis, respectively. According to the USP 24 the RSD should be less than 2%. The mean sodium diclofenac content of each formulation expressed as a percentage of the labelled amount is shown in Table 3.18.

*Table 3.18: Mean diclofenac sodium content of the different formulations expressed as a percentage of the labelled amount*

| Manufacturer | Mean diclofenac sodium content per tablet (mg) | % of the labelled amount per tablet |
|---|---|---|
| Camden | 48.9 ± 0.3 | 97.8 |
| Intas Pharmaceuticals | 49.9 ± 0.3 | 99.7 |
| Novartis[a] | 49.6 ± 0.2 | 99.2 |
| Remedica | 50.2 ± 0.2 | 100.4 |

[a]Reference formulation

All formulations meet the USP 24 requirements for drug content. The drug content in the formulations ranged from 97.8 to 100.4%, which is within the specified range of 90 - 110%.

### *3.8.3 In vitro dissolution*

#### *3.8.3.1 Methods*

Dissolution testing

**Acid stage**

Dissolution profiles were determined using the USP paddle method (Method 2) at a rotational speed of 50 rpm. The dissolution vessels were filled with 900 ml of 0.1N HCl, maintained at 37 ± 0.5°C. After 2 hr of the test, the tablets were transferred into another vessel which contained 900 ml phosphate buffer (pH 6.8) also maintained at 37 ± 0.5°C. To the 0.1N HCl remaining in the vessel 20 ml of a 5.0N NaOH solution was added, stirred for 5 min and a 5 ml sample withdrawn for spectrophotometrical analysis at 276 nm.

**Buffer stage**

The tablets from the acid stage were tested in the phosphate buffer media for 50 min using the USP paddle method (Method 2) at a speed of 50 rpm. Samples (5 ml) were automatically withdrawn after 10, 20, 30, 40, 45 and 50 min. The samples were diluted (1:1) and spectrophotometrically analysed at 276 nm.

### 3.8.3.2 *Results*

Table 3.19 shows the percent drug released from the formulations before and after storage at simulated tropical conditions. Immediately after purchase, all formulations met the USP 24 specifications for dissolution (not less than 75% of the labelled amount should be dissolved within 45 min and no drug release during the acid stage). The in vitro drug release ranged from 87.3 to 100.1% of the labelled amount and the enteric coat of all formulations remained intact during the acid stage test.

*Table 3.19: Percentage of drug released after 45 min of dissolution testing (in buffer medium pH 6.8) on enteric coated diclofenac sodium tablets stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirement: more than 75% is released within 45 min*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| Camden | 87.3 | 12.1 | 10.4 |
| Intas | 94.7 | 51.4 | 41.9 |
| Novartis[a] | 100.1 | 99.8 | 100.2 |
| Remedica | 99.3 | 98.2 | 98.6 |

[a]Reference formulation

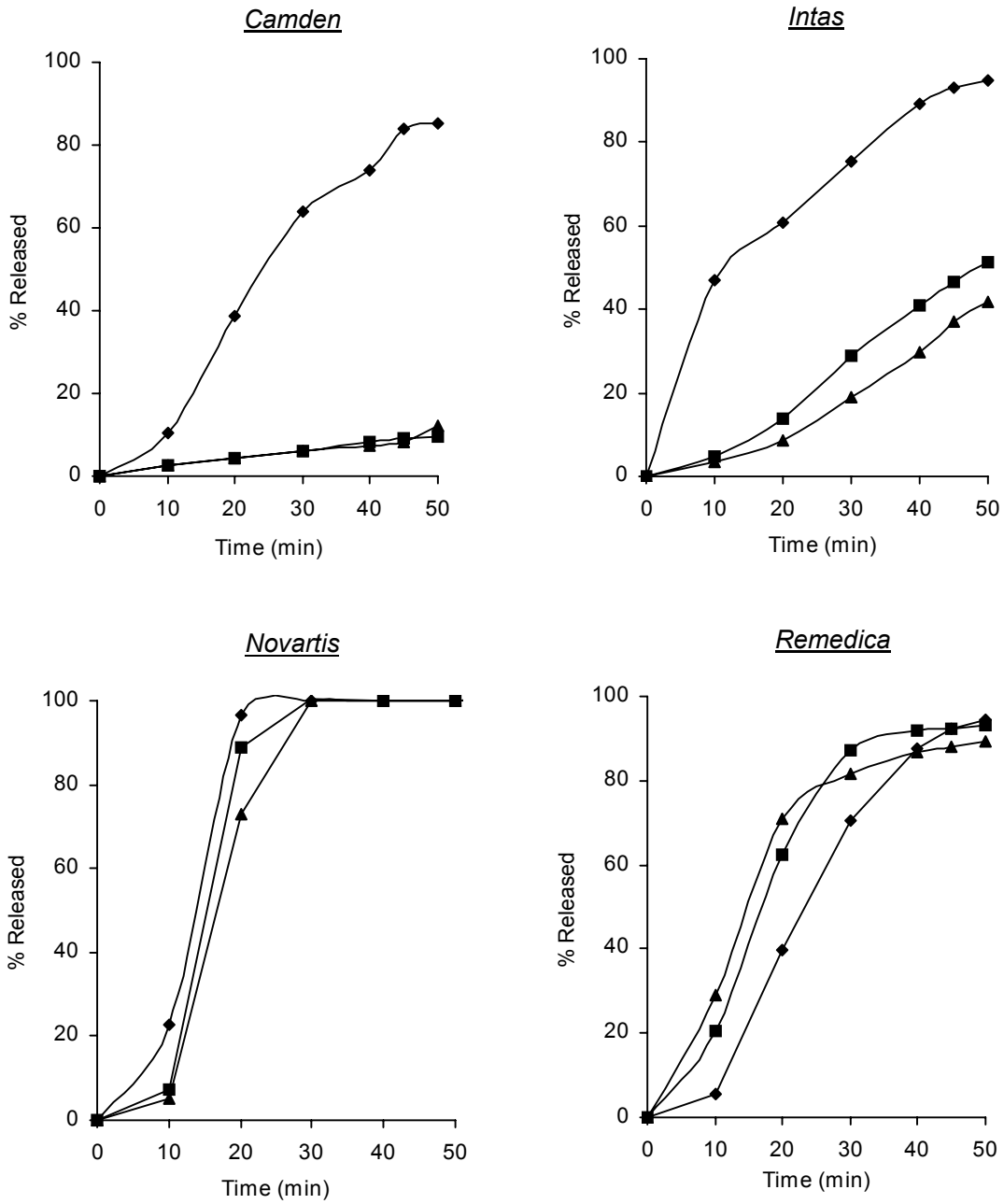
Fig. 3.7 shows the dissolution profiles obtained before and after storage at simulated tropical conditions. The dissolution profile of the Novartis formulation (the reference) was compared with the profiles of the other formulations. The $f_2$ values for the Camden, Intas, and Remedica formulations were 23, 34 and 46, respectively, indicating that despite meeting the USP 24 drug release requirements, their dissolution profiles were not pharmaceutically equivalent to that of the Novartis formulation. This formulation had a higher drug release during the initial 10 min in the phosphate buffer compared with the Camden and Intas formulations. Although the drug release of the Remedica and Novartis formulations were similar during the first 10 min, after 20 min the percentage drug

released from Novartis formulation was 100%, while that of Remedica was only about 70%.

After exposure to simulated tropical conditions the dissolution characteristics of the formulations from Intas and Camden changed dramatically. After 3 and 6 months of storage, it was visually observed that the enteric coat from the Intas formulation disintegrated during the acid stage testing. However, the release of diclofenac sodium in this medium remained relatively low (1.7% after 3 months and 3.2% after 6 months) due to the low solubility of diclofenac sodium in acidic media. The drug release during the buffer stage from the Camden and Intas formulations decreased dramatically after storage at simulated tropical conditions. After 6 months storage, the formulation from Camden released 10.4% of the drug, while that from Intas released 41.9% of the labelled drug content (Fig. 3.7). Although the in vitro drug release of these two formulations decreased dramatically, the drug content in both formulations was still within the USP 24 specifications as assays performed after 6 months storage showed that the drug content was 98 and 97% for the Camden and Intas formulations, respectively. The respective values for the Novartis and Remedica formulations were 99.9 and 100.0%.

The dissolution profiles from the Novartis and Remedica obtained after 3 and 6 months were compared with those obtained before stability testing. The $f_2$ values for the Novartis formulation were 57 and 46 for the profiles obtained after 3 and 6 months, respectively. The respective values for the Remedica formulation were 44 and 38. The dissolution profiles of the Novartis formulation obtained after 6 months of storage at the test conditions showed a decrease in the drug release during the first 10 min. Before exposure to the storage conditions, the drug released in this time interval was more than 20%, wheras it was only 5% after 6 months. For the Remedica formulation, the drug release in the first 10 min was 5%, but after the storage it increased to 28%, indicating that the enteric coat disintegrated more rapidly (in the phosphate buffer) upon exposure to conditons of high temperature and high humidity. However, these detailed differences between the profiles of the two formulations as explained by $f_2$ analysis probably have no pharmaceutical significance.

*Figure 3.7: Dissolution profiles of diclofenac sodium formulations after 0 (♦), 3 (■) and 6 (▲) months at simulated tropical conditions (40°C, 75% RH)*



## 3.9 Metronidazole formulations

A total of 11 metronidazole formulations from 10 manufacturers were evaluated. 10 of these formulations were available on the Tanzanian market, the reference formulation was purchased in a retail pharmacy in Ghent (Belgium). Table 3.20 gives the detailed information of the samples.

*Table 3.20: Metronidazole (200 mg) tablet formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| ACE Laboratories (India) | Bulk, 1000 tablets | TE565 | 4.4 |
| Flamingo Pharmaceuticals (India) | Bulk, 1000 tablets | 3956 | 4.5 |
| Intas Pharmaceuticals (India) | Blister, 100 tablets | 3792 | 6.0 |
| Medopharm (India) | Bulk, 1000 tablets | M0019 | 4.4 |
| Pharmamed[b] (Malta) | Bulk, 100 tablets | 1865 0107 | 3.0[c] |
| Rhône Poulenc Rorer[a] (Belgium) | Blister, 20 tablets | 97A28 | 45.0 |
| Shelys Pharmaceuticals[b] (Tanzania) | Bulk, 1000 tablets | 415 | 3.0[c] |
| S Kant Health Care (India) | Blister, 100 tablets | SK 101 | 6.3 |
| TPI (Tanzania) | Bulk, 1000 tablets | LG 27 | 4.3 |
| TPI (Tanzania) | Bulk, 1000 tablets | ND 34 | 4. 3 |
| Vinpa Exports (India) | Bulk, 1000 tablets | 2E107 | 4.5 |

[a]Reference formulation, contained 500 mg of metronidazole per tablet
[b]Contained 250 mg of metronidazole per tablet
[c]Sample obtained from the Medical Stores Department

### *3.9.1 Materials*

Metronidazole and sulfamethoxazole (Ph. Eur.) were obtained from Alpha Pharma (Zwevegem, Belgium). Orthophosphoric acid and hydrochloric acid were obtained from Vel (Leuven, Belgium), sodium dodecyl sulphate (SDS) from Fluka Chemie (Buchs, Switzerland) and acetonitrile from Biosolve (Valkenswaard, The Netherlands).

### *3.9.2 Assay for metronidazole*

#### *3.9.2.1 Methods*

The amount of metronidazole in each formulation was determined by the method described by Baeyens et al. (1998).

Sample preparation

20 tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to 160 mg metronidazole, was transferred into a 100 ml volumetric flask and the mobile phase was added to volume. The mixture was sonicated for 15 min and the suspension left to settle for 1 hr. From the clear supernatant, 10 ml was transferred into a 100 ml flask and 10 ml of the internal standard solution (1400 mg/l sulfamethoxazole) was added. The volume was adjusted with the mobile phase to obtain the assay solution.

Calibration curve

A calibration curve defined by the equation (metronidazole/sulfamethoxazole peak area ratio vs. concentration) $y = 0.0195x$ (± 0.0004) – 0.0515 (± 0.0001) with a coefficient of determination ($R^2$) of 0.9998 (± 0.0001) (n = 5) was made. The peak area ratios were determined from standard solutions with metronidazole concentrations of 80, 120, 140, 160 and 180 mg/ml spiked with sulfamethoxazole (to obtain a concentration 140 mg/l). The unknown concentration of metronidazole in the assay solution was determined from the calibration curve.

The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after

repeated injections (n = 5) of a standard solution with 160 mg/l metronidazole and 140 mg/l sulfamethoxazole.

The resolution factor (R) between metronidazole and sulfamethoxazole was determined from the peaks obtained from the standard solutions. Based on the retention times and baseline widths R was calculated as

$$R = 2(t_1 - t_2)/(W_1 + W_2)$$

with $t_1$ and $W_1$ being the retention time and baseline width, respectively, of the sulfamethoxazole peak, while $t_2$ and $W_2$ are the respective values for the metronidazole peak.

Mobile phase

492 µl of ortho-phosphoric acid (85%) was transferred into a 2 l flask and 1.4 l of distilled water was added. 3.46 g of sodium dodecyl sulphate and 200 ml of acetonitrile were added and mixed. The pH of the solution was adjusted to 3.5 with 0.2M sodium hydroxide, carefully added to avoid precipitation of the sodium salt. The volume was adjusted with distilled water and the solution was homogenized. The buffer was mixed with acetonitrile at a ratio of 3:2 (v/v) to obtain the mobile phase. 20 µl of the assay preparation and a standard solutions were separately injected into the HPLC system and the peaks were monitored by UV absorbance at 278 nm.

### *3.9.2.2* Results

The relative standard deviation (RSD) for replicate injections of the standard preparations was 0.17% and 0.62% for the within day and between day analysis, respectively. The resolution between the metronidazole and the internal standard (sulfamethoxazole) peak was 6.4.

The metronidazole content of each product expressed as a percentage of the labelled amount is shown in Table 3.21. The content of metronidazole in the formulations ranged from 92.7 to 99.7%. This is within the 90 - 110% range specified in the USP 24.

*Table 3.21: Mean metronidazole content of the different formulations expressed as a percentage of the labelled amount*

| Manufacturer | Mean metronidazole content (mg) per tablet | % of the labelled content per tablet |
|---|---|---|
| ACE | 185.4 ± 1.2 | 92.7 |
| Flamingo | 198.6 ± 1.2 | 99.3 |
| Intas | 195.0 ± 1.3 | 97.5 |
| Medopharm | 199.4 ± 1.1 | 99.7 |
| Shelys [b] | 247.9 ± 1.5 | 99.2 |
| S Kant | 196.2 ± 1.1 | 98.1 |
| Pharmamed[b] | 245.8 ± 1.6 | 98.3 |
| RhônePoulenc Rorer[a] | 497.8 ± 3.1 | 99.6 |
| TPI (batch LG 27) | 187.9 ± 1.3 | 94.0 |
| TPI (batch ND 34) | 193.5 ± 1.1 | 96.8 |
| Vinpa | 190.8 ± 1.2 | 95.4 |

[a]Reference formulation, contained 500 mg metronidazole per tablet.
[b]Contained 250 mg metronidazole per tablet.

### *3.9.3 In vitro dissolution*

#### *3.9.3.1 Methods*

Dissolution testing

Dissolution tests were performed using the basket method (USP 1 method) at a rotational speed of 100 rpm. The volume of the dissolution medium (0.1N HCl) in each vessel was 900 ml, maintained at 37 ± 0.5°C. 5 ml samples were withdrawn after 10, 20, 30, 40, 50 and 60 min, diluted (1:20) with the dissolution medium before being spectrophotometrically analysed at 278 nm.

#### *3.9.3.2 Results*

The percentage drug released from the formulations before and after storage at the stability test conditions are presented in Table 3.22. Before being stored at simulated tropical conditions, all formulations met the USP 24 requirements for dissolution.

*Table 3.22: Percentage of metronidazole released after 1 hr of dissolution testing on metronidazole tablets stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirement: more than 80% is released within 1hr*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| ACE | 97.3 | 94.1 | 93.7 |
| Flamingo | 100.0 | 96.5 | 93.9 |
| Intas | 97.8 | 97.6 | 93.9 |
| Medopharm | 100.9 | 100.3 | 99.8 |
| Pharmamed | 98.3 | 98.1 | 98.0 |
| Rhône Poulenc Rorer[a] | 97.5 | 97.2 | 96.1 |
| Shelys | 97.2 | 97.0 | 94.3 |
| S Kant | 95.7 | 94.7 | 93.7 |
| TPI (batch LG 27) | 86.5 | 64.7 | 52.6 |
| TPI (batch ND 34) | 97.1 | 52.6 | 52.7 |
| Vinpa | 95.2 | 94.1 | 93.7 |

[a]Reference formulation

The dissolution profile of the Rhône Poulenc Rorer formulation before exposure to simulated tropical conditions was compared with those from the other formulations for similarity. The $f_2$ factors (Table 3.23) indicate that although all formulations met the USP 24 specifications for drug release, the dissolution profiles of all but the TPI formulation (batch ND 34) were not similar to that of the Rhône Poulenc Rorer tablets ($f_2 < 50$). The TPI and the Rhône Poulenc Rorer formulations had a slower drug release during the first 10 min compared with the others. While the other formulations had released more than 90% drug within 10 min, these two formulations had released only 75% (Fig. 3.8).

Upon storage at simulated tropical conditions, the percentage drug released after 60 min of dissolution test from all formulations (except TPI) remained fairly constant during the entire storage period at the test conditions. The drug release from these formulations ranged from 93.7 to 99.8% and remained within the USP 24 recommended values for dissolution.

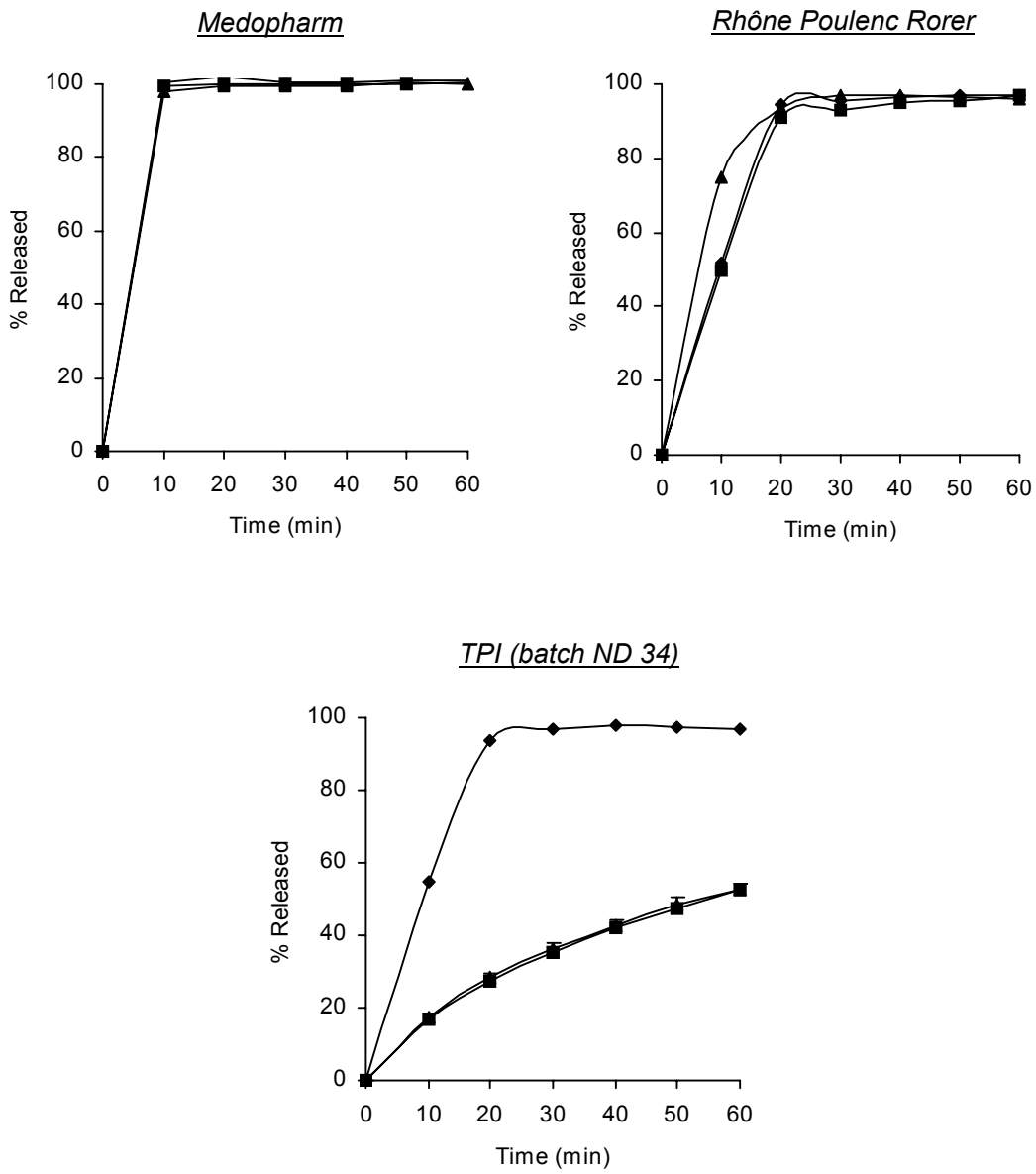
*Table 3.23: The $f_2$ values of the dissolution profiles obtained from the different metronidazole formulations before storage at simulated tropical conditions, using the Rhône Polenc Rorer formulation as a reference*

| Formulation | $f_2$ value |
|---|---|
| ACE | 37 |
| Flamingo | 37 |
| Intas | 35 |
| Medopharm | 34 |
| Pharmamed | 35 |
| Shelys | 38 |
| S Kant | 37 |
| TPI (batch LG 27) | 37 |
| TPI (batch ND 34) | 87 |
| Vinpa | 42 |

The in vitro drug release of both TPI batches was considerably reduced after storage at simulated tropical conditions. After 3 months the drug release decreased from 86.5 and 97.1% to 64.7 and 52.6% for batch LG 27 and ND 34, respectively. The tablets of both batches failed to disintegrate during the dissolution test. Assays performed on the tablets from both batches after the 6 months storage period revealed that the metronidazole content (96.2% batch ND 34; 93.5% batch LG 27) remained within USP 24 recommended range.

The $f_2$ factors computed for each formulation (except for TPI) using the dissolution data obtained at 0 months as a reference show that the dissolution profiles of all formulations did not change ($f_2$>50) during storage at simulated tropical conditions. The Medopharm formulation (Fig. 3.8) had the highest $f_2$ factor ($f_2$ = 85).

*Figure 3.8: Dissolution profiles of metronidazole tablets after 0 (♦), 3 (■) and 6 (▲) months at simulated tropical conditions (40°C, 75% RH)*



## 3.10 Paracetamol formulations

A total of 9 paracetamol formulations were sampled, 8 of them from the Tanzanian market. Table 3.24 gives the detailed information of the sampled formulations.

*Table 3.24: Paracetamol (500 mg) tablet formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| Alferez (India) | Bulk, 1000 tablets | CTC –378 | 3.0 |
| Granules (India) | Blister, 100 tablets | 084 | 5.0 |
| Interchem Pharma (Tanzania) | Bulk, 1000 tablets | 951203 | 3.0 |
| Jansen-Cilag[a] (Belgium) | Blister, 100 tablets | 99G05B110 | N/A |
| Medopharm (India) | Bulk, 1000 tablets | M6018 | 2.5[b] |
| Medopharm (India) | Bulk, 1000 tablets | M0173 | 3.2 |
| Panacea Biotec (India) | Blister, 100 tablets | 101569 | 5.0 |
| TPI (Tanzania) | Bulk, 1000 tablets | LK 72 | 2.5[b] |
| TPI (Tanzania) | Bulk, 1000 tablets | NH 231 | 3.0 |

[a]Reference sample
[b]Sample obtained from the Medical Stores Department
N/A Not available on the Tanzanian market

### *3.10.1 Materials*

Paracetamol (Ph. Eur.) was supplied by Ludeco (Brussel, Belgium), methanol was obtained from Biosolve (Valkenswaard, The Netherlands), while potassium dihydrogen phosphate and sodium hydroxide were from Vel (Leuven, Belgium).

### *3.10.2 Assay for paracetamol*

#### *3.10.2.1 Methods*

The amount of paracetamol in each formulation was determined by the method described in the USP 24.

Sample preparation

20 tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to 100 mg of paracetamol, was transferred into a 100 ml volumetric flask and about 50 ml of the mobile phase was added. The mixture was sonicated for 5 min, then the volume was adjusted with the mobile phase and homogenised. The suspension was filtered through a 0.5 µm cellulose acetate filter (Minisart®, Sartorius, Goettingen, Germany). 1 ml of the filtrate was transferred into a 100 ml volumetric flask, diluted to volume with the mobile phase and homogenised to make up the assay solution.

Calibration curve

A calibration curve (peak area vs. concentration) $y = 108338.4x$ (± 1697.6) – 9746.7 (± 1043.1) with a coefficient of determination ($R^2$) of 0.9997 (± 0.0002) (n = 5) was constructed using standard solutions with paracetamol concentrations of 3, 6, 10, 15 and 20 mg/l. The unknown concentration of paracetamol in the assay solution was determined from the calibration curve.

The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after repeated injections (n = 5) of a 10 mg/l paracetamol standard solution.

Mobile phase

The mobile phase consisted of a mixture of methanol and water in a ratio of 25:75 (v/v). 20 µl aliquots of the assay and standard solutions were injected into the HPLC system and the peaks monitored by UV absorbance at 243 nm.

#### *3.10.2.2 Results*

The relative standard deviation (RSD) for replicate injections of the standard preparation was 0.2% and 1.2% for the within day and between day analysis,

respectively. According to the USP 24 the RSD should be less than 2%.

The assayed paracetamol content of each formulation is shown in Table 3.25. All were in compliance with the USP 24 specifications (90-110% of the labelled amount). The amount of paracetamol found in the formulations ranged from 92.9 to 99.5%.

*Table 3.25: Mean paracetamol content of the different formulations expressed as percentage of the labelled amount*

| Manufacturer | Mean paracetamol content per tablet (mg) | % of the labelled amount per tablet |
|---|---|---|
| Alferez | 486.7 ± 5.8 | 97.3 |
| Granules | 484.1 ± 5.8 | 96.8 |
| Interchem Pharma | 489.2 ± 5.9 | 97.8 |
| Jansen-Cilag[a] | 497.3 ± 5.9 | 99.5 |
| Medopharm (batch M6018) | 486.9 ± 5.8 | 97.4 |
| Medopharm (batch M0173) | 483.5 ± 5.8 | 96.7 |
| Panacea Biotec | 481.6 ± 5.8 | 96.3 |
| TPI (batch LK 72) | 464.6 ± 5.6 | 92.9 |
| TPI (batch NH 231) | 479.9 ± 5.7 | 96.0 |

[a]Reference sample

### *3.10.3 In vitro dissolution*

#### *3.10.3.1 Methods*

Dissolution testing

Dissolution tests were conducted using the USP paddle method (Method 2) at a rotational speed of 50 rpm. The volume of the dissolution medium (0.2M phosphate buffer pH 5.8) in each vessel was 900 ml, maintained at a temperature 37 ± 0.5°C. Dissolution samples (5 ml) were automatically withdrawn after 5, 10, 15, 20, 25 and 30 min and diluted with the dissolution medium (1:40) before measuring their absorbances at 243 nm.

### *3.10.3.2 Results*

The percentage drug released from the formulations is shown in Table 3.26. Before the stability test at simulated tropical conditions, only two formulations failed the USP 24 requirements for dissolution. The formulation from Interchem failed the dissolution test marginally as it released 78.1% of the labelled claim. The Medopharm formulation batch M6018 (sampled in 1998) released only 20% of labelled amount of paracetamol (the tablets failed to disintegrate). On the contrary, another batch from the same manufacturer (sampled in 2000) had a 100% drug release (Fig. 3.9).

*Table 3.26: Percentage of paracetamol released after 30 min of dissolution testing on paracetamol tablets stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirement: more than 80% is released within 30 min*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| Alferez | 92.0 | 56.3 | 50.0 |
| Granules | 100.7 | 92.9 | 88.3 |
| Interchem | 78.1 | 78.3 | 76.8 |
| Jansen Cilag[a] | 94.3 | 94.3 | 94.5 |
| Medopharm (batch M6018) | 20.0 | 19.2 | 18.0 |
| Medopharm (batch M0173) | 101.2 | 100.8 | 97.8 |
| Panacea Biotec | 101.6 | 100.1 | 99.9 |
| TPI (batch LK 72) | 88.8 | 21.5 | 20.0 |
| TPI (batch NH 231) | 100.1 | 20.8 | 16.2 |

[a]Reference sample

The dissolution profiles of the 6 formulations that complied with the USP 24 specifications were compared with that from Jansen-Cilag (the reference formulation). All $f_2$ factors were less than 50 (Table 3.27), indicating that there were differences between the profiles. The reference formulation had released more than 90% drug within 5 min, while the others had a lower drug release (46 - 70%) within this interval.

*Table 3.27: The $f_2$ values for the dissolution profiles obtained from the different paracetamol formulations before storage at simulated tropical conditions, using the Jansen Cilag formulation as a reference*

| Formulation | $f_2$ value |
|---|---|
| Alferez | 41 |
| Granules | 35 |
| Medopharm (batch M0173) | 44 |
| Panacea Biotec | 44 |
| TPI (batch LK72) | 40 |
| TPI (batch NH231) | 41 |

Upon storage at simulated tropical conditions, the drug release of two formulations decreased dramatically. The drug release from the Alferez formulation decreased to 56.3% after 3 months of storage, then to 50.0% after 6 months. Similar observations were made for both batches of the TPI formulation. After 3 months, the drug release of this formulation had already decreased to about 20% (Fig. 3.9). It was observed that the tablets of both batches failed to disintegrate during the dissolution test.

The drug content of the formulations that failed the in vitro dissolution after storage at simulated tropical conditions was evaluated. The assay results showed that there was no appreciable change in paracetamol content in these formulations during the 6 month test duration. This indicates that the decreased drug release occurred as a result of factors other than chemical instability.

The percentage drug released from the other four formulations remained within the USP 24 tolerance limits for dissolution during the 6 months storage period at simulated tropical conditions. The $f_2$ values of the dissolution profiles obtained after 6 months (compared with that obtained before stability testing) were 82, 78, 61 and 50 for the Jansen-Cilag, Panacea Biotec, Medopharm and Granules formulations, respectively, indicating that the storage conditions did not infuence the in vitro drug release profiles of these formulations.

*Figure 3.9: Dissolution profiles of paracetamol formulations after 0 (♦), 3 (■) and 6 (▲) months of storage at simulated tropical conditions (40°C, 75% RH)*

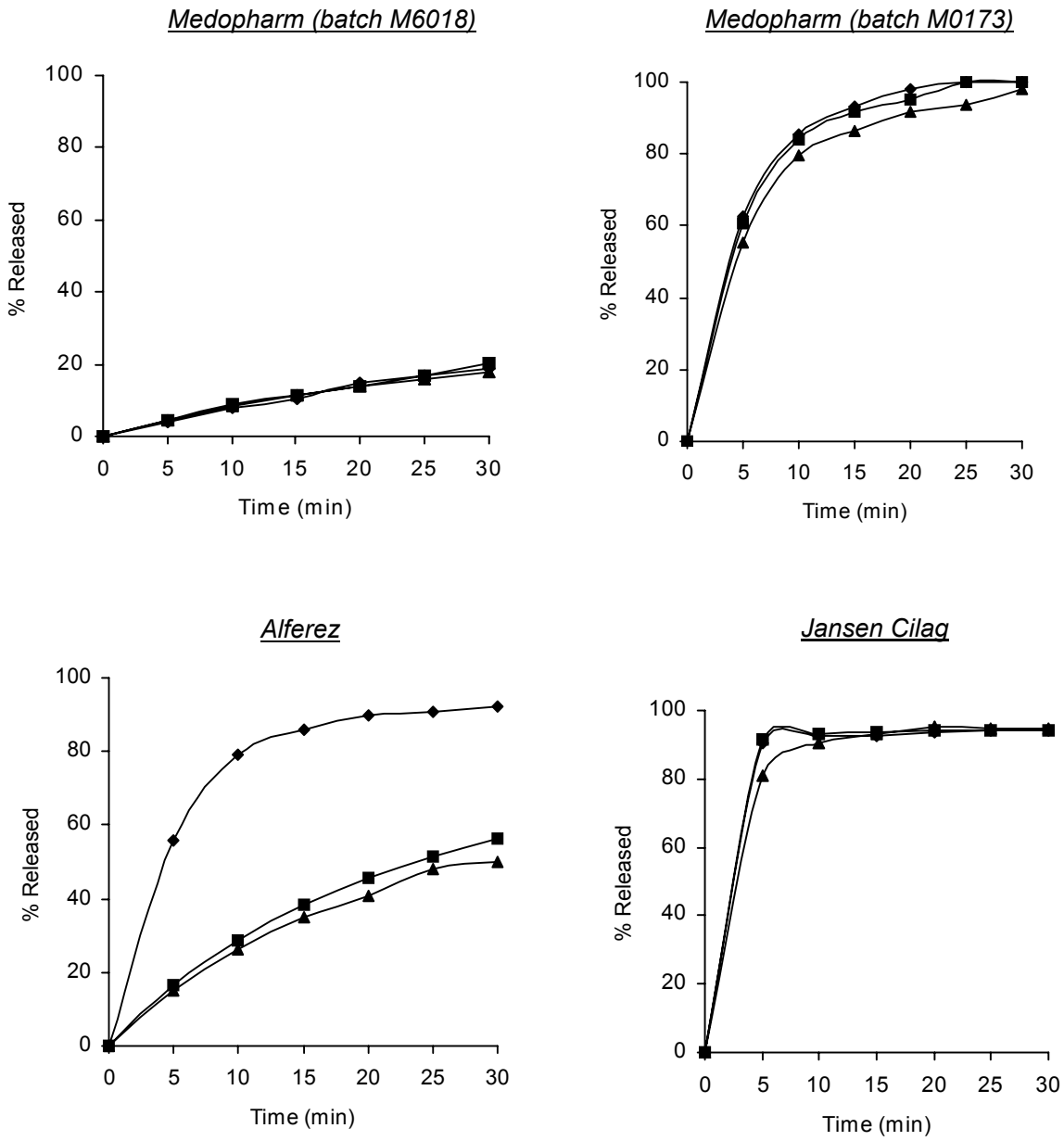
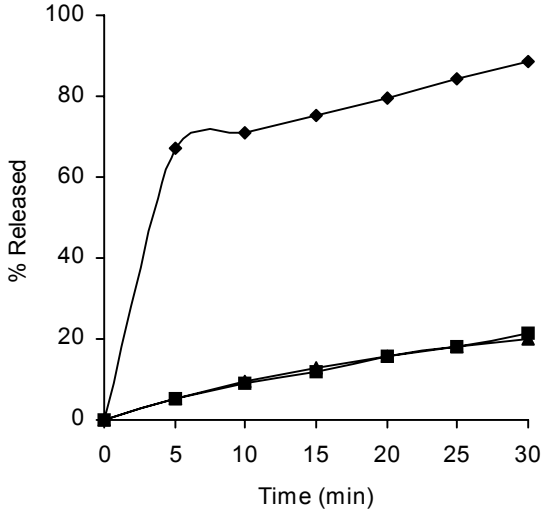
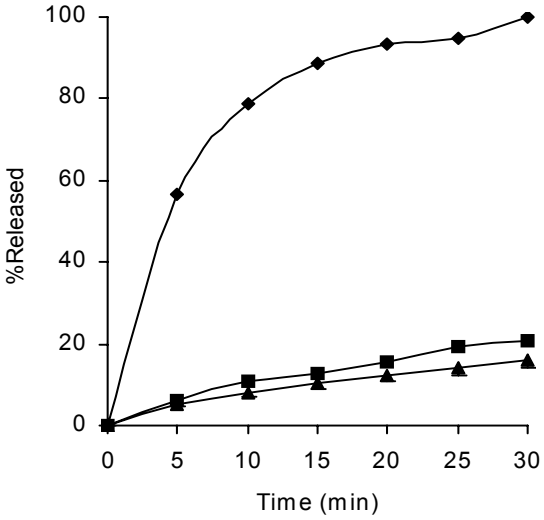


*Figure 3.9 (continued)*

*TPI (batch LK 72)*




*TPI (batch NH 231)*



## 3.11 Sulfadoxine/pyrimethamine formulations

Four formulations were evaluated, all of them available on the Tanzanian market. The detailed information of the sampled formulations is shown in Table 3.28.

*Table 3.28: Sulfadoxine (500 mg) / pyrimethamine (25 mg) tablet formulations*

| Manufacturer | Package | Batch number | Price per 1000 units (US$) |
|---|---|---|---|
| Ellys Chemical Industries (Kenya) | Blister, 100 tablets | OE84 | 125 |
| Flamingo Pharmaceuticals (India) | Blister, 100 tablets | 5560 | 130 |
| Roche[a] (Switzerland) | Blister, 250 tablets | B3015 | 500 |
| Shelys Pharmaceuticals (Tanzania) | Blister, 100 tablets | 068 | 120 |

[a]Reference sample

### *3.11.1 Materials*

Sulfadoxine (USP) was obtained from Indis (Aartselaar, Belgium), while pyrimethamine (USP) and phenacetin were from Sigma Aldrich (Steinheim, Germany). Acetonitrile was obtained from Biosolve (Valkenswaard, The Netherlands), glacial acetic acid from Merck (Darmstadt, Germany) and perchloric acid from UCB (Brussel, Belgium). Potassium dihydrogen phosphate and sodium hydroxide were obtained from Vel (Leuven, Belgium).

### *3.11.2 Assay for sulfadoxine and pyrimethamine*

#### *3.11.2.1 Methods*

The amount of sulfadoxine and pyrimethamine in each formulation was determined using the method described in the USP 24.

Sample preparation.

10 tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to 550 mg sulfadoxine and 27.5 mg pyrimethamine was transferred into a 100 ml volumetric flask and 35 ml of acetonitrile were added. The mixture was sonicated for 25 min, adjusted to volume with the mobile phase and then homogenised. The resulting mixture was filtered through a 0.5 µm cellulose acetate filter (Minisart®, Sartorius, Goettingen, Germany). 10 ml of the filtrate were transferred into a 100 ml volumetric flask and diluted to volume with the mobile phase. From this solution, 5 ml was transferred into a 10 ml volumetric flask, 1 ml of phenacetin solution (120.0 mg/l) was added and the volume was adjusted with the mobile phase to make the assay solution.

Calibration curves

For the sulfadoxine assay, a calibration curve $y = 0.0409x$ (± 0.0006) + 0.3499 (± 0.0052) with a coefficient of determination ($R^2$) of 0.9993 (± 0.0004) (n = 5) was constructed using the peak area ratio (sulfadoxine/phenacetin) vs. concentration obtained from standard solutions with sulfadoxine concentrations of 50, 100. 150, 275 and 350 mg/l. The concentration of phenacetin in each standard solution was 12 mg/l. Similarly a calibration curve $y = 0.04400x$ (± 0.0007) – 0.00613 (± 0.0001) with a $R^2$ of 0.9998 (± 0.0007) (n = 5) was obtained for pyrimethamine using standard solutions with concentrations of 6, 8, 12.5, 16 and 25 mg/l. The unknown concentrations of sulfadoxine and pyrimethamine in the assay solution were determined from the respective calibration line.

The precision of the assay method was determined by calculating the relative standard deviation (within day and between days) of the peak areas obtained after repeated injections (n = 6) of a standard solution having concentration of 275 mg/l sulfadoxine, 12.5 mg/l pyrimethamine and 12 mg/l phenacetin.

The resolution factor (R) between sulfadoxine and phenacetin and between pyrimethamine and phenacetin was determined from the peaks obtained from the standard solutions. Based on the retention times and baseline widths R was calculated as:

$$R = 2(t_1 - t_2)/(W_1 + W_2)$$

with $t_1$ and $W_1$ being the retention time and baseline width, respectively, of the phenacetin peak, while $t_2$ and $W_2$ are the respective values of the sulfadoxine or pyrimethamine peak.

Mobile phase

The mobile phase was a mixture of a dilute acetic acid solution (1:100), acetonitrile and perchloric acid (70%) in the following propotions: 800:200:8 (v/v). Samples (20 µl) were injected into the HPLC system and the peaks were monitored by UV absorbance at 254 nm.

#### *3.11 2.2 Results*

The relative standard deviation (RSD) for replicate injections was 0.26% and 0.73% for the within day and 0.86% and 1.44% for the between day analysis for sulfadoxine and pyrimethamine, respectively. According to the USP 24 the RSD should be below 2.5%. The resolution factor R between sulfadoxine and phenacetin and between pyrimethamine and phenacetin was 2.2 and 2.0, respectively. According to the USP 24, R should be not less than 1 for both drugs.

The sulfadoxine and pyrimethamine contents of each formulation, expressed as a percentage of the stated amount, are shown in Table 3.29. All formulations passed the assay requirements specified in the USP 24 (90 –110% of the labelled content for both sulfadoxine and pyrimethamine). The assayed sulfadoxine in the formulations ranged from 95.5 to 101.1% and that of pyrimethamine from 99.2 to 101.6%.

### *3.11.3 In vitro dissolution*

#### *3.11.3.1 Methods*

Dissolution testing

Dissolution tests were performed using the USP paddle method (Method 2) at a rotational speed of 75 rpm. The dissolution vessels were filled with 900 ml of dissolution medium (phosphate buffer pH 6.8), maintained at 37 $\pm$ 0.5°C. Samples (5 ml) were withdrawn at regular intervals (5, 10, 15, 20, 25 and 30 min). The samples were diluted (1:1) with the mobile phase before being analyzed by HPLC as is described in the assay (section 3.11.2).

*Table 3.29: Mean sulfadoxine and pyrimethamine content of the different formulations expressed as a percentage of the labelled amount*

| Manufacturer | Mean drug content per tablet (mg) | % of the labelled amount per tablet |
|---|---|---|
| **Sulfadoxine** | | |
| Ellys | 490.3 ± 4.2 | 98.1 |
| Flamingo | 477.5 ± 4.1 | 95.5 |
| Roche[a] | 498.6 ± 4.3 | 99.7 |
| Shelys | 505.5 ± 4.6 | 101.1 |
| **Pyrimethamine** | | |
| Ellys | 25.4 ± 0.4 | 101.6 |
| Flamingo | 24.8 ± 0.4 | 99.2 |
| Roche[a] | 25.2 ± 0.4 | 100.8 |
| Shelys | 25.0 ± 0.4 | 100.0 |

[a]Reference sample

### *3.11.3.2 Results*

The percent drug sulfadoxine and pyrimethamine released before and after storage are shown in Table 3.30 and the dissolution profiles in Fig. 3.10 and Fig. 3.11.

Before storage at simulated tropical conditions, only two of the four formulations complied with the USP 24 requirements for drug release. The formulation from Ellys released 100.2% sulfadoxine and 71.3% pyrimethamine, while that from Roche was 97.8 and 66.4% for sulfadoxine and pyrimethamine, respectively.

The dissolution profiles obtained from the Ellys formulation before storage at simulated tropical conditions (0 months) were compared with those of the Roche formulation. The $f_2$ values (calculated using the Roche formulation data as the reference) were 53 and 51 for sulfadoxine and pyrimethamine, respectively, showing that the two formulations had pharmaceutically equivalent drug release profiles.

*Table 3.30: Percentage of sulfadoxine and pyrimethamine released after 30 minutes of dissolution testing on sulfadoxine/pyrimethamine tablets stored at simulated tropical conditions (40°C, 75% RH). USP 24 requirements: more than 60% of both sulfadoxine and pyrimethamine is released within 30 min*

| Manufacturer | Storage time | | |
|---|---|---|---|
| | 0 months | 3 months | 6 months |
| **Sulfadoxine** | | | |
| Ellys | 100.2 | 96.1 | 84.2 |
| Flamingo | 53.3 | 51.7 | 51.3 |
| Roche[a] | 97.8 | 92.0 | 89.3 |
| Shelys | 60.9 | 41.6 | 30.0 |
| **Pyrimethamine** | | | |
| Ellys | 71.3 | 72.0 | 60.8 |
| Flamingo | 17.4 | 17.2 | 15.6 |
| Roche[a] | 66.4 | 63.2 | 60.4 |
| Shelys | 26.5 | 20.0 | 13.6 |

[a]Reference formulation

Upon storage at simulated tropical conditions, the release of both sulfadoxine and pyrimethamine from the Roche and Ellys formulations remained within the USP 24 recommended values for in vitro drug release. The Ellys formulation however, showed a marked decrease in vitro release of both drugs during storage at simulated tropical conditions. The sulfadoxine release decreased to 96.1 and 84.2% after 3 and 6 months storage, respectively. The respective values for pyrimethamine during the same intervals were 72.0% and 60.8%.

The dissolution profiles obtained for both sulfadoxine and pyrimethamine before and after stability test from the Roche and Ellys formulations were compared for similarity. For the Roche formulation the $f_2$ value of the 6 months profiles were 53 and 50, for sulfadoxine and pyrimethamine, respectively. The respective values for the Ellys formulation were 37 and 48. The in vitro drug dissolution from the Roche formulation was not influenced by storage at simulated tropical conditions (Fig 3.10 and 3.11). The $f_2$ values for sulfadoxine and pyrimethamine dissolution

profiles from the Ellys formulation after 3 months were 53 and 79, respectively. This shows that the drug release profile after 3 months storage was similar to the one immediately after purchase. However, after 6 months of storage at the test conditions, the drug release profiles were not similar to the ones obtained before the storage. Although this formulation had acceptable drug release characteristics before the stability test, the $f_2$ analysis of the profiles indicates that the dissolution pattern was influenced by storage at simulated tropical conditions.

In addition to the failure to meet the USP 24 dissolution specifications immediately after purchase, the drug release from the Shelys formulation also decreased during storage at simulated tropical conditions. The sulfadoxine release from this formulation decreased to 41.6% and 30.0%, after 3 and 6 months, respectively. The respective pyrimethamine release was 20.0% and 13.6%. The drug release from the Flamingo formulation did not decrease appreciably during the accelerated stability test and the in vitro drug dissolution remained well below the USP 24 specifications.

*Figure 3.10: Dissolution profiles of sulfadoxine from sulfadoxine/pyrimethamine tablets after 0 (♦), 3 (■) and 6 (▲) months of storage of storage at simulated tropical conditions (40°C, 75%RH)*

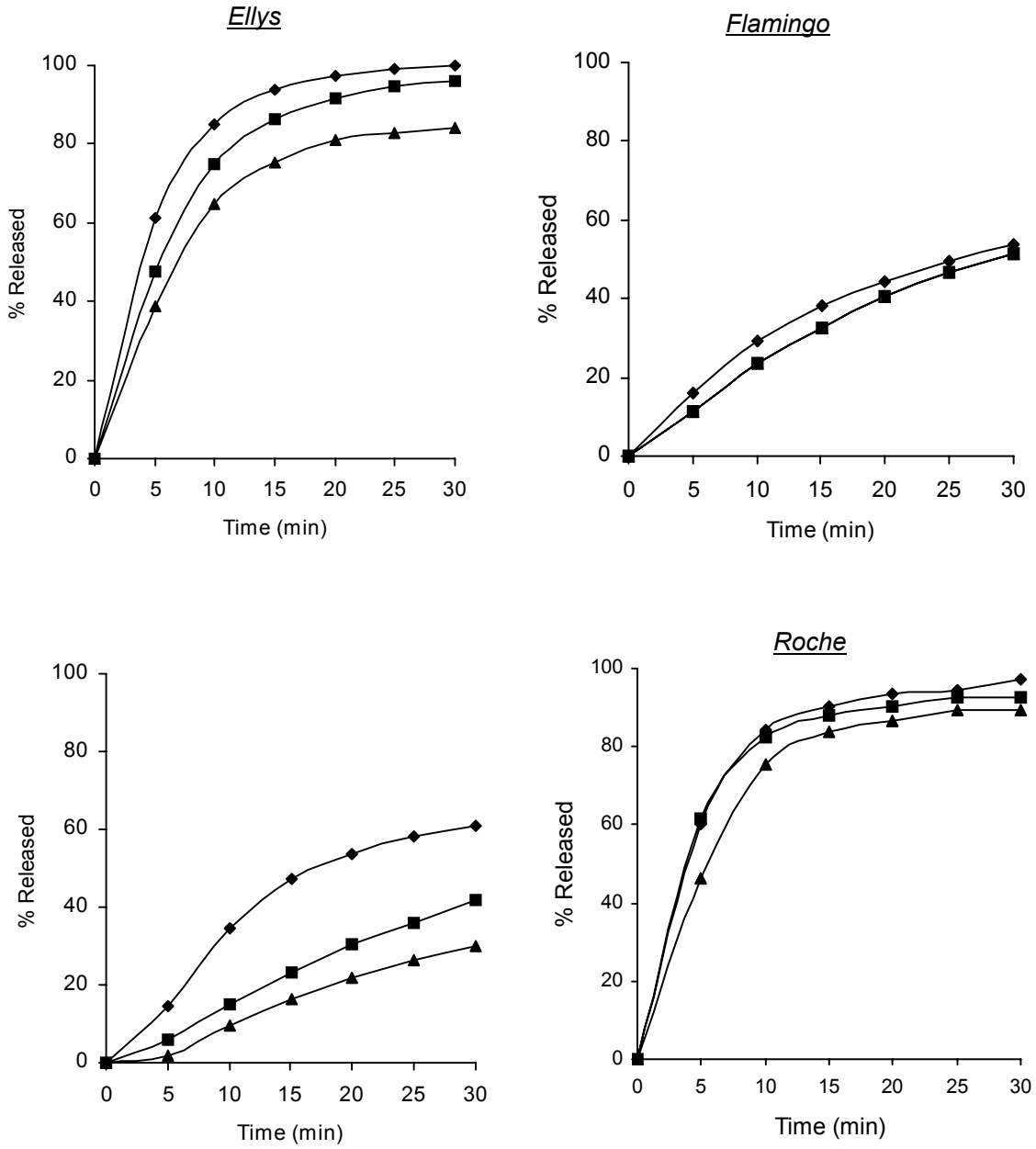
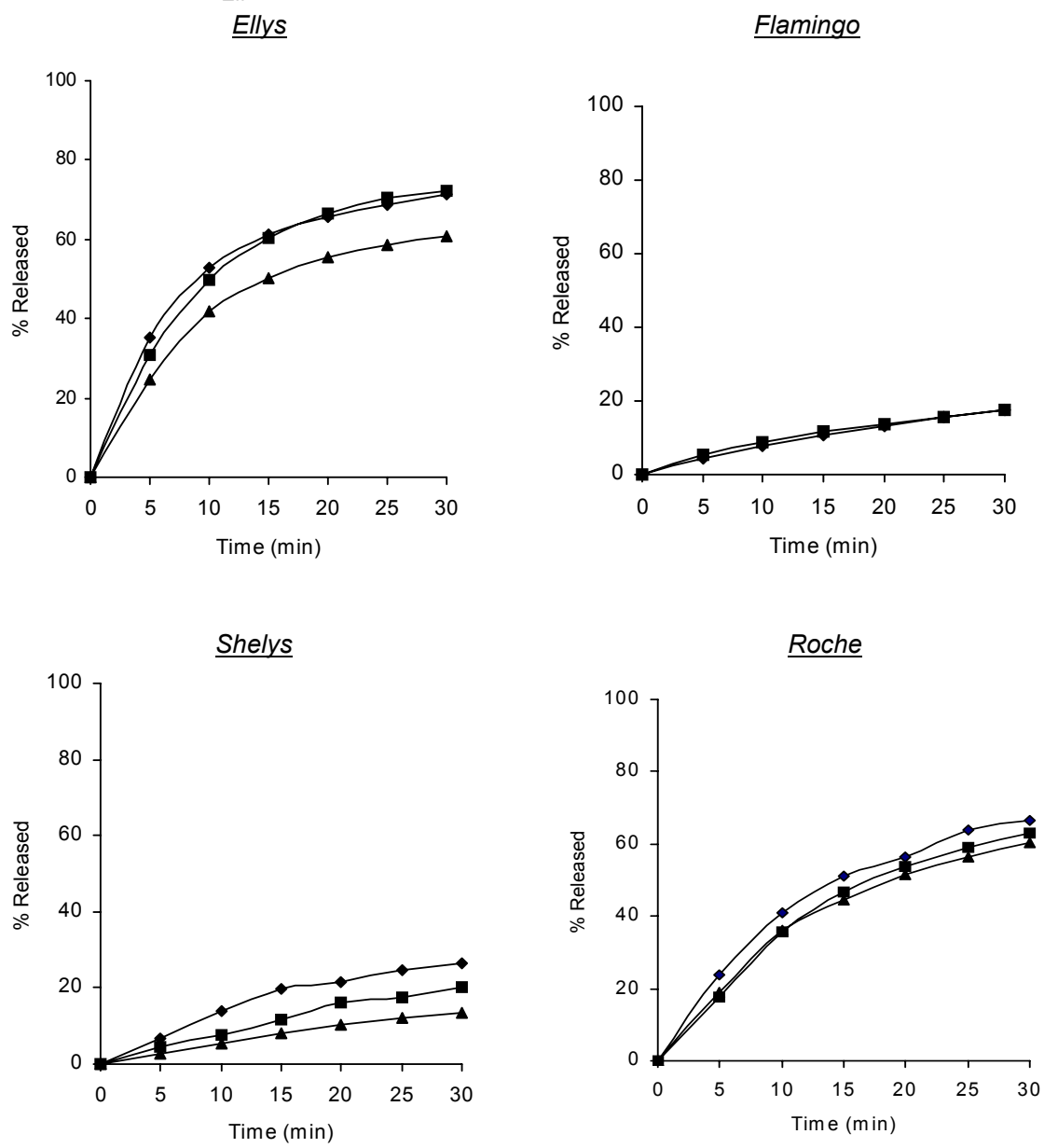


*Figure 3.11: Dissolution profiles of pyrimethamine from sulfadoxine/pyrimethamine tablets after 0 (♦), 3 (■) and 6 (▲) months of storage at simulated tropical conditions (40°C, 75% RH)*



## 3.12 Discussion

During the acquisition of the samples it was evident that there are large price differences between the innovator brands and their generic equivalents. The availability of low priced generics on the market may be considered as a welcome development resulting from the implementation of the Essential Drugs Concept. It is imperative that measures are put in place to control and monitor the quality of the drugs on the market. As has been observed by Pecol et al. (1999) the quality of drugs becomes less certain especially for poor populations who are attracted to lower priced drugs in an unregulated environment. In this case it is important that the drug regulatory authority in Tanzania put in place the necessary controls to ensure that the drugs on market are consistently of good quality.

The assay results on the content of the active substance showed that this parameter was within the specifications recommended by the USP 24 for all formulations. The findings are similar to those obtained by Abdi et al. (1995) on the quality of chloroquine tablets on market in Tanzania, where all samples from 10 different manufacturers passed the assay test. However, Kibwage et al. (1992) reported that about 45% of drugs sampled on the Kenyan market and analysed at the Daru quality control laboratory on a routine basis were of substandard quality in terms of the content of the active ingredient. Shakoor et al. (1997) reported on the presence of both fake and substandard drugs on Thai and Nigerian markets where 32% of 89 samples failed the assay determination. These differences in findings cannot be explained on the basis of the existence of an effective drug control and monitoring system in Tanzania. On the contrary such a system was not in place during the period when the samples were taken. For example, it was only until July 2000 when the Pharmacy Board of Tanzania established the only drug control laboratory in the country and the drug registration exercise has taken place recently. As has been noted by Shakoor et al. (1997) in many reports concerning the quality of drugs in developing countries the terms counterfeit and substandard drugs have been used interchangeably. Counterfeiting is a criminal activity driven by the motive for quick profits. Consequently, counterfeit drugs exists on market sporadically thus the absence of counterfeit drugs amongst the analysed formulations could possibly be due the sampling window or sample size. On the other hand, the presence of cheap generic brands on market may have

been deterrent to introducing counterfeit drugs, as it makes counterfeiting less profitable. A systematic quality assurance system is required as a further deterrent to the introduction of counterfeit / substandard drugs into the country.

Several studies have been reported in the literature on the stability of essential drugs under real storage conditions in the tropics (Hogerzeil et al., 1991; Ballereau et al., 1997; Nazerali and Hogerzeil, 1998). All these studies have dwelt on the chemical stability of the drug. It is known that under conditions of high temperature and humidity the drug may undergo polymorphic or crystal changes that may decrease its inherent solubility. In addition, excipient - excipient and/or excipient - drug interactions may occur under the influence of high temperature and high relative humidity conditions, reducing the dissolution rate of a formulation containing a chemically stable drug (Saville, 2001). In the present study dramatic changes in the dissolution behaviour of some formulations have been observed. The drug formulations (paracetamol, acetlysalicylic acid, diclofenac sodium, metronidazole, sulfadoxine/pyrimethamine and chloroquine) that failed the stability test had a more than 40% reduction in the amount of drug released after 3 and 6 months of stability testing. It was not possible to identify the cause of the failure in dissolution of the formulations, as the exact composition of the formulations was not available. It is known that the interactions that may occur for a drug formulation stored at high temperature and humidity conditions are complex and formulation dependent (Murthy and Ghebre-Sellassie, 1993). The formulations may have for example contained disintegrants such as maize starch, which can loose its capacity to swell on ageing or on exposure to high humidity/temperature (Pandit et al., 1997).

For the formulations that failed the dissolution test, no firm conclusion may be drawn on their bioavailability. Murthy and Gherbe-Sellasie (1993) have reported on cases of experimental drug formulations with a largerly reduced in vitro drug release on ageing, but with a similar bioavailability profiles to fresh formulations. Similar cases have been reported for aged nitrofurantoin capsules (Vila-Jato et al., 1987). On the other hand other authors have reported cases where nitrofurantoin tablet formulations exposed to stress conditions had a reduced in vitro drug release and also a significant reduction in their rate of absorption (Gouda et al., 1987). The failure to meet the USP dissolution specifications may be taken as an

indication of a potential bioavailability problem. Further in vivo work needs to be done to determine the effect of the changed dissolution characteristics on the bioavailability of the failed formulations.

Due considerations should be given to the formulation and manufacturing process to ensure a reproducible and stable in vitro and in vivo drug availability. The in vitro performance of a solid dosage form depends on the use of ingredients with predetermined properties as well as on controlled and validated manufacturing procedures. These are achieved if the manufacturer adheres to the Good Manufacturing Practices (GMP) principles as is advocated by the WHO. In this study it has been observed that different batches of the same drug from the same manufacturer had different in vitro drug dissolution characterisitics (eg. paracetamol from Medopharm). Furthermore, samples of different drugs from the same manufacturer were found to have a different in vitro dissolution stability (eg. amoxcicillin and metronidazole from Shelys had a satisfactory drug release, but acetylsalicylic acid and sulfadoxine/pyrimethamine tablets were not stable). It may be considered that the manufacturer of these formulations did not use appropriate ingredients in the formulation and/or applied an inappropriate manufacturing process and that the products were not manufactured in accordance to the WHO GMP guidelines for the manufacture of pharmaceuticals.

The dissolution tests were able to discriminate poor quality formulations amongst the samples that had passed the assay tests. 16% (9/57) of the analysed formulations failed the initial dissolution test: 3 acetylsalicylic acid, 2 sulfadoxine/pyrimethamine, 2 paracetamol and 2 sulfamethoxazole/trimethoprim formulations. From the 48 samples that passed the initial dissolution test, 19% (9/48) of them (3 paracetamol, 2 chloroquine, 2 enteric coated diclofenac sodium and 2 metronidazole formulations) failed the dissolution test after being subjected to a stability test at simulated tropical conditions

As regards the regulatory aspects, the WHO recommends an accelerated stability test under zone IV climatic conditions to be performed on all drugs intended for the global market (Matthews, 1999). A stability test is recommended as a quality control tool that may be used to verify if a formulation and the manufacturing process do not affect the efficacy and safety of the product under the distribution and storage conditions. The failure of some formulations to satisfy dissolution

requirements after being subjected to a stability test (at simulated tropical conditions) infers that the drug formulations are unsuitable for marketing in countries with tropical climatic conditions such as Tanzania. Regular monitoring of the quality and stability in tropical conditions of the drugs on market by the regulatory authority of Tanzania (The Pharmacy Board) should be encouraged as means of preventing the access to the market of drugs with inferior quality.

## 3.13 Conclusion

The in vitro evaluation of 54 formulations of 9 essential drugs available on the Tanzanian market has shown that all of them meet the USP 24 potency specifications. However, the initial in vitro dissolution characteristics as well as the dissolution behaviour after storage at simulated tropical conditions of 18 of these formulations were not satisfactory. The evaluation of the bioavailability of these formulations is recommended to shed light on the effect of poor dissolution on their bioavailability.

## 3.14 References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# 4. EVALUATION OF DISSOLUTION PROFILES: INTER LABORATORY COMPARISON OF DISSOLUTION DATA

## 4.1 Introduction

The potential for variation between results of an analytical test performed in different laboratories is well known. Analyses performed in developing countries are especially prone to this problem as in many of these countries quality control laboratories are not equipped with the appropriate equipment and training of personnel to man the laboratories is not routinely done and good laboratory practices (GLP) are not in place. In such laboratories the analytical results are usually treated with reservations. Gomes et al. (1998) has reported on the differences in assay results of antimalaria drugs analyzed by the Institute of Drug Quality Control of Vietnam and those from a WHO accredited laboratory. The results from the Institute showed a failure of 3.2% of all sampled drugs. Ten percent of the samples originally analyzed at the Institute were reanalyzed in a WHO accredited laboratory where it was found that 70% of them failed to meet specifications for drug content. Roy (1994) has also reported on the existence of substandard drugs in Bangladesh that had been affirmed to be of satisfactory quality by the local drug authority.

In the execution of this research project (a sandwich program), in vitro dissolution tests on the same batches of ciprofloxacin and metronidazole tablet formulations were performed in two laboratories. The tests were done at the Laboratory of Pharmaceutical Technology, Ghent University (Ghent - Belgium) and at the laboratory of Tanzania Pharmaceutical Industries (Arusha - Tanzania).

## 4.2 Comparison of dissolution data

Various methods have been described to compare dissolution data. The United States Pharmacopoeia (USP 24) recommended method for the evaluation of the dissolution data involves a single point determination of the percentage drug dissolved at a predefined time. The FDA guidance for industry on dissolution testing has three categories of dissolution test specifications: (1) single point

specification, (2) two point specification and (3) dissolution profile comparison (FDA Guidance for industry, 1997). The point (single and 2 point) estimate is suitable for high solubility – high permeability drugs, but may not be adequate for drugs with low solubility. For poorly soluble drugs, the dissolution curves of two batches can differ significantly before reaching the same value at the predefined time point and this can result in different plasma concentration – time profiles.

In the literature, other methods have been described to compare dissolution profiles. They may be classified into model independent and model dependent methods. In the model independent methods, the data are analysed in their native form without transformation. Analysis of variance (ANOVA) may be applied on the dissolution data in the native form to compare the level and shape of dissolution profiles (Mauger et al., 1986). Similarities and differences between profiles can be determined using the fit factors: $f_1$, difference factor and $f_2$, similarity factor (Moore and Flanner, 1996). In the model dependent methods, the dissolution data are fitted into a mathematical model such as the Weibull distribution (Langenbucher, 1972) and the model parameters are employed for pairwise statistical comparison of the dissolution data.

The fit factors compare the two mean curves and do not consider the within curves variability. However, they are simple to apply and interpret. The ANOVA based and model dependent methods are more complicated, but describe better the relationship between the percent dissolved and time variables. The ANOVA methods provide detailed information on the level and shape of the dissolution profile, which is useful in formulation development. In comparison to the fit factors, the ANOVA and model dependent methods are considered to be more discriminative.

Principal Component Analysis (PCA) is an important visualization tool for multivariate data (Vandeginste et al., 1998). PCA can reduce a large number of original variables to a few principal components (PCs) that still contain the most important information. PCA has been shown to be a useful method for visualizing differences within a set and between sets of dissolution profiles (Adams et al., 2001). PCA can also be used to compare dissolution data where measurements have been made at different sample time points (Adams et al., 2002).

In this chapter the inter-laboratory dissolution data is compared using the FDA similarity factor and Principal Component Analysis (PCA).

### 4.2.1 Similarity factor

The FDA recommends the use of the similarity factor ($f_2$), which is calculated using the equation proposed by Moore and Flanner (1996)

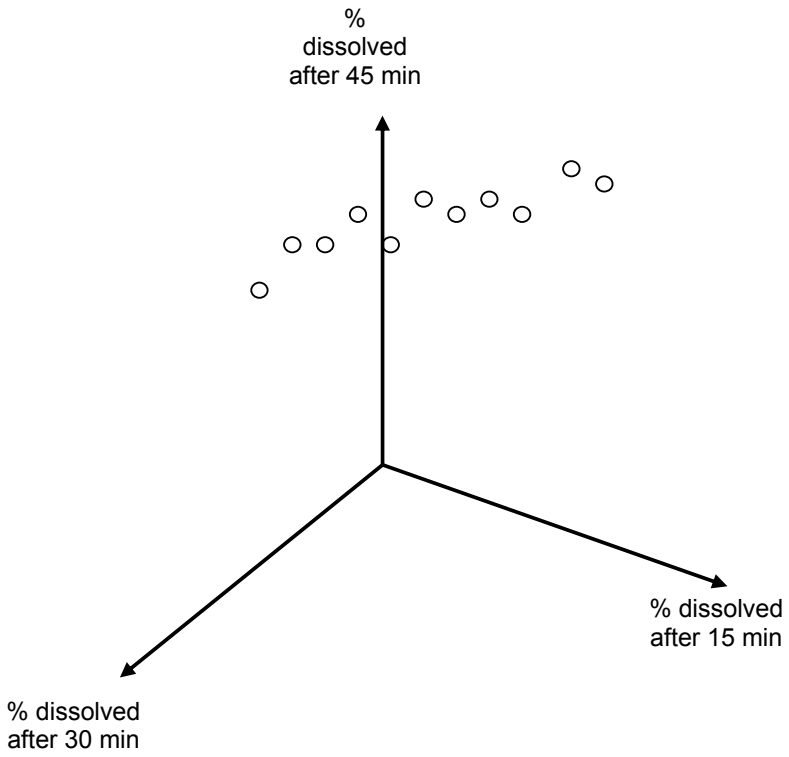
$$f_2 = 50 \times \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} w_t (R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

with $R_t$ and $T_t$ the average percentage dissolved at time *t* (for t = 1, 2, ..., *n*) for the reference and the test batch, respectively, and $w_t$ the optional weight factor (mostly $w_t$ = 1). Two sets are considered equivalent when the $f_2$ factor is between 50 and 100. A value of 100 is obtained when both batches are identical. The lower limit of 50 was determined empirically by permitting a 10 % average difference at any sample time point. The FDA considers the dissolution profiles of two batches to be equivalent when the $f_2$ factor is greater or equal to 50 and allows the use of such in vitro data to ensure the product quality in case of scale-up and post approval changes (SUPAC) like manufacturing site changes, increase or decrease of batch size and changes in excipients (FDA Guidance for Industry, 1995, 1997).

### 4.2.2 Principal component analysis

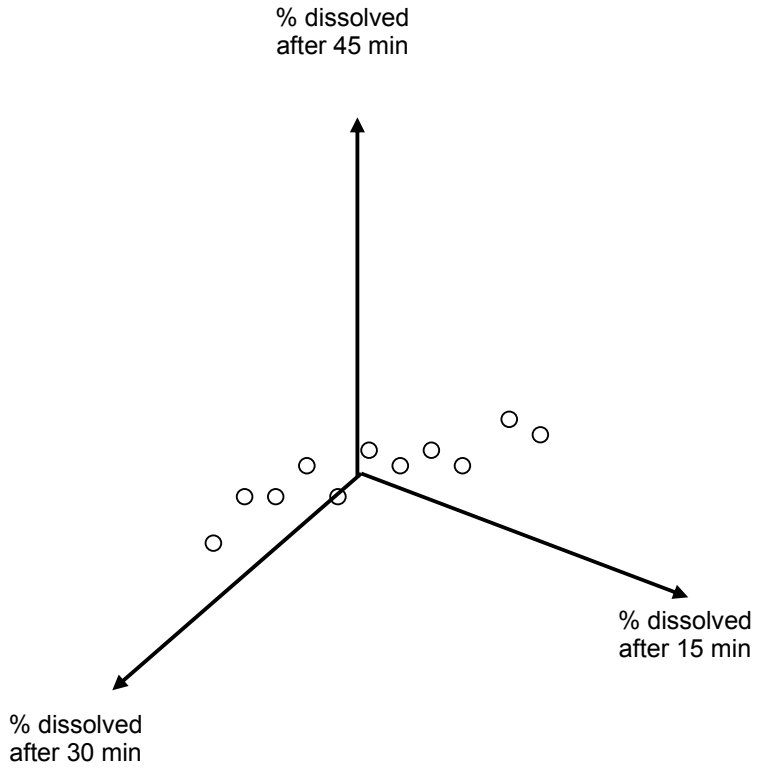
Principal Component Analysis (PCA) is a technique that allows exploring multivariate data. For example the percent drug dissolved at 3 different time points (3 variables: 15, 30 and 45 min) for a set of tablets may be compared by plotting the values (percent drug released) in a three dimensional space of their original variables (time points) as shown in Fig. 4.1. The illustration has been limited to three variables to be able to visualize PCA in a 3D-plot, but the PCA technique is not limited to 3 variables.

*Figure 4.1: Percent dissolved at the different time points plotted in 3 original variables*



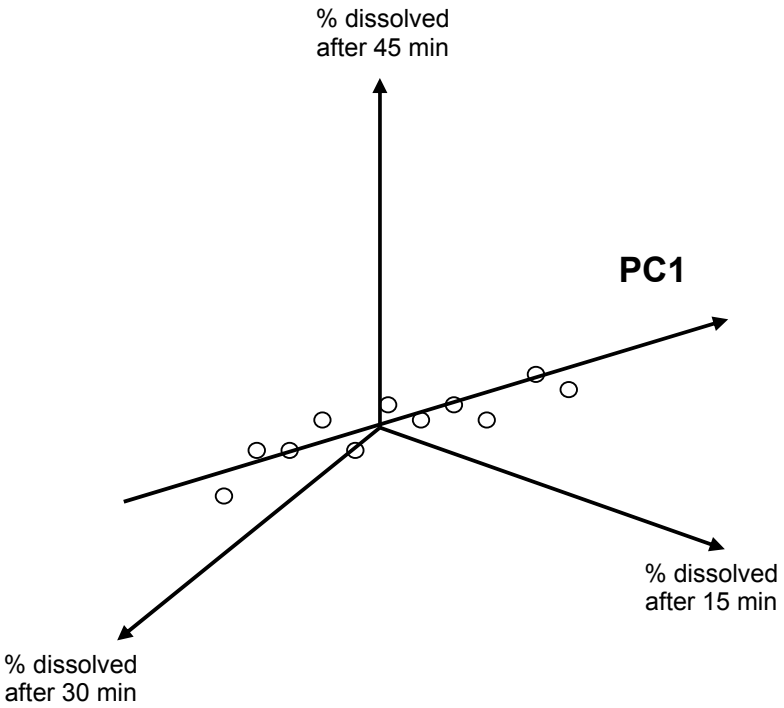

The second step in PCA is usually column centering. The central point (0,0,0) of the 3 axes is moved to the centre of the cloud of measurements (Fig. 4.2). After column centring, a line is drawn through the centre of the data points (the centre of the 3 axes) in the direction of the largest variance of the data points. This line is the first Principal Component (PC1). The direction is found by rotating the PC around the central point until a direction is found where the sum of the orthogonal distances of the data points to the PC is the smallest. The direction of the PC may point in either way since only the magnitude of variation is put into consideration (Fig. 4.3).

*Figure 4.2: Column centring of the data points*



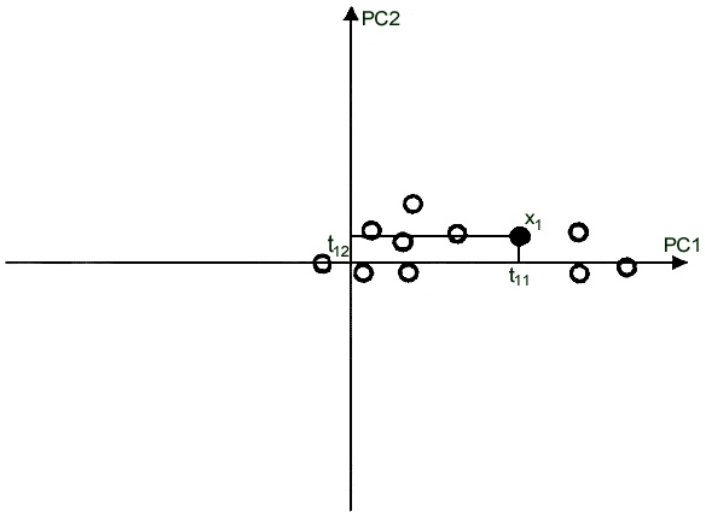

After the construction of PC1, a second PC is constructed through the centre orthogonal to PC1 and in the direction of the largest variance around PC1. As many PCs as the original variables can thus be constructed, but PC1 displays more information (variance) contained in the original variables than PC2 and the other subsequent PCs (PC1>PC2>PC3…). Usually the first few PCs displays most of the information contained in the original variables and the other PCs explain only small random variations in the data.

*Figure 4.3: The column centred measurements plotted in the 3 original variables together with the first PC*




The data measurements plotted in the 3 original variables can now be represented in a new axes system of a few latent variables or PCs as is shown in Fig 4.4.
The distance from the origin of the PC space to the perpendicular projection of an object on a PC is called the score of the object on that particular PC. In Fig. 4.4 the score of the tablet $X_1$ is $t_{11}$ on PC1 and $t_{12}$ on PC2.

*Figure 4.4: The dissolution measurements represented in the PC1/PC2 space with the scores of the tablet $X_i$ on PC1 and PC2*




Mathematically the score of an object on a PC is a linear combination of the original variables. The score of $t_{iq}$ of tablet i on $PC^q$ may be expressed as:

$$t_{iq} = \sum_{j=1}^{p} v_{jq}\,(x_{ij} - \bar{x}_j) \qquad (1)$$

Where $v_{jq}$ is the loading of tablet *i* at sample time *j* on $PC^q$, $x_{ij}$ is the percent drug dissolved from tablet *i* measured at time *j* and $\bar{x}_j$ is the mean drug dissolved at time point *j*.

In a matrix notation equation 1 can be expressed as:

$$\mathbf{T}(mxa) = \mathbf{X_c}(mxp)\mathbf{V}(pxa) \qquad (2)$$

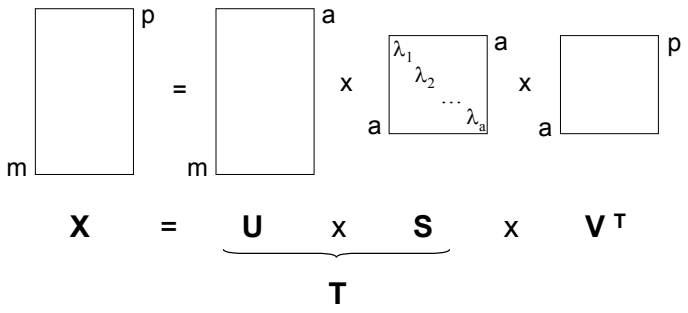
with $X_c$ being the column centred matrix ($X - \bar{X}$), V the loading matrix, m the number of tablets tested, p the number of sample time points and a the number of PCs.

To compute the scores of matrix T and loadings matrix V starting from the column centred data matrix $X_c$ the Singular Value Decomposition (SVD) algorithim is used:

$$\mathbf{X_c}(mxp) = \mathbf{X} - \bar{\mathbf{X}} = \mathbf{U}(mxa)\mathbf{S}(axa)\mathbf{V^T}(axp) = \mathbf{T}(mxa)\mathbf{V^T}(axp) \qquad (3)$$

Equation 3 may be illustrated schematically in a diagram as in Fig. 4.5.

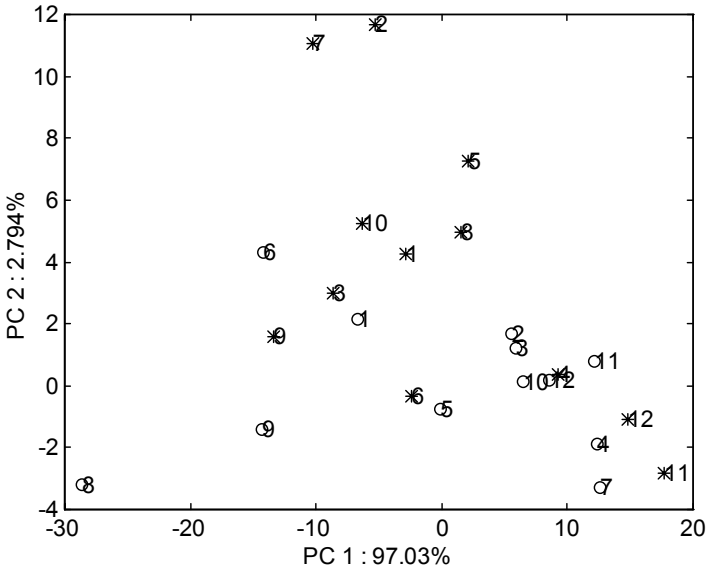
*Figure 4.5: Schematic illustration of singular value decomposition (SVD)*




with *m* the number of objects (here: the number of tablets measured in the batch), *p* the number of original variables (here: the number of time points) and *a* the number of principal components (PCs), with $a = m - 1$ if $m \leq p$ or *a* = *p* if $m > p$. U is the unweighed (normalised) and T the weighed (unnormalised) score matrix. V is the loading matrix containing the loadings of the original variables on the different PCs and S is a diagonal matrix with the singular values $\lambda_j$ (for *j* = 1, 2, … *a*) on the main diagonal. Since $\lambda_1 \geq \lambda_2 \geq ... \geq \lambda_a$, the first PCs contain the most relevant information, while the remaining PCs contain only noise.

For analysis of dissolution data the scores of the reference batch are used to construct the PC space and those of the test batch can be projected on the same PC1/PC2 space as shown in Fig. 4.6.

*Figure 4.6: An example of a PCA scores plot obtained after PCA analysis of dissolution data of the reference batch (○) and a test batch (★)*




Although PCA is a very useful technique to analyse dissolution data, it does not provide statistical criteria to decide if the dissolution behaviour of different batches is similar. This drawback has been overcome by combining PCA with the resampling with replacement (or bootstrap) method to construct confidence limits, which form the criteria for the decision on similarity.

#### *4.2.2.1 Bootstrap technique in combination with PCA*

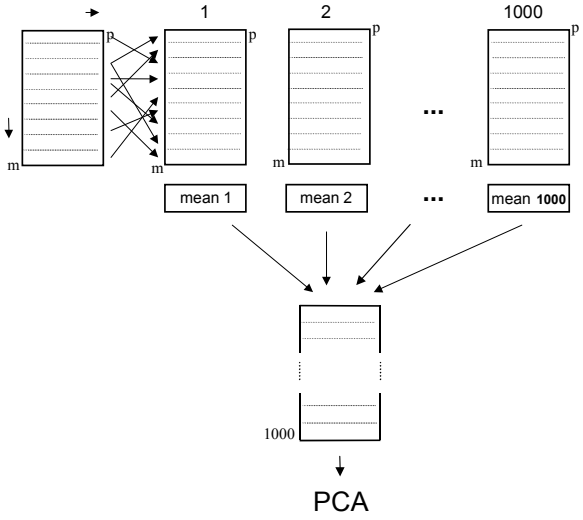
The technique of sampling with replacement or bootstrapping is used to simulate the distribution of the mean in the PC space and to construct a 95 % confidence limit for complete data matrices (Efron and Tibshirani, 1986).

The bootstrap method randomly reassigns the observations and recomputes the estimate mean. When this is repeated many times it gives an estimate of the population mean. Performing bootstrapping is similar to repeating the experiment many times over. The experimenter does with a computer what she/he would have done if circumstances would allow.

In matrix notation bootstrapping may be illustrated as follows: starting from matrix X ($m \times p$) containing the original data, a new matrix $X_1$ ($m \times p$) is generated by drawing with replacement *m* rows from the orginal matrix X. Repeating the procedure n times (usually 1000) n matrices ($X_1, X_2 \ldots\ldots, X_n$) are constructed all with size (m x p) (Fig. 4.7).

After calculation of the vector of column means for each matrix, a (n x p) matrix is formed. PCA is then performed on the (n x p) matrix so obtained.

*Figure 4.7: Schematic illustration of resampling and replacement or bootstrapping*




When combined with PCA analysis the scores of the bootstrapped values projected on a PC space will appear closely together because their mean values are similar (Fig. 4.8). A 95% confidence interval may be constructed by removing the 5% outliers. Practically this is achieved by measuring the distances towards the center of the cluster, and constructing a circle from the center as to include 95% of the values (Fig. 4.9). Similarity between the dissolution data (at 95% confidence interval) of a test and the reference formulation may be compared by performing BOOT(PCA) of the two sets and projecting the data on a PCA plot (Fig. 4.10). When there is a complete overlap between the scores of the test batch and the reference batch similarity (at 95% confidence interval) is assumed.

*Figure. 4.8: Normalised PCA scores plot after BOOT/PCA*

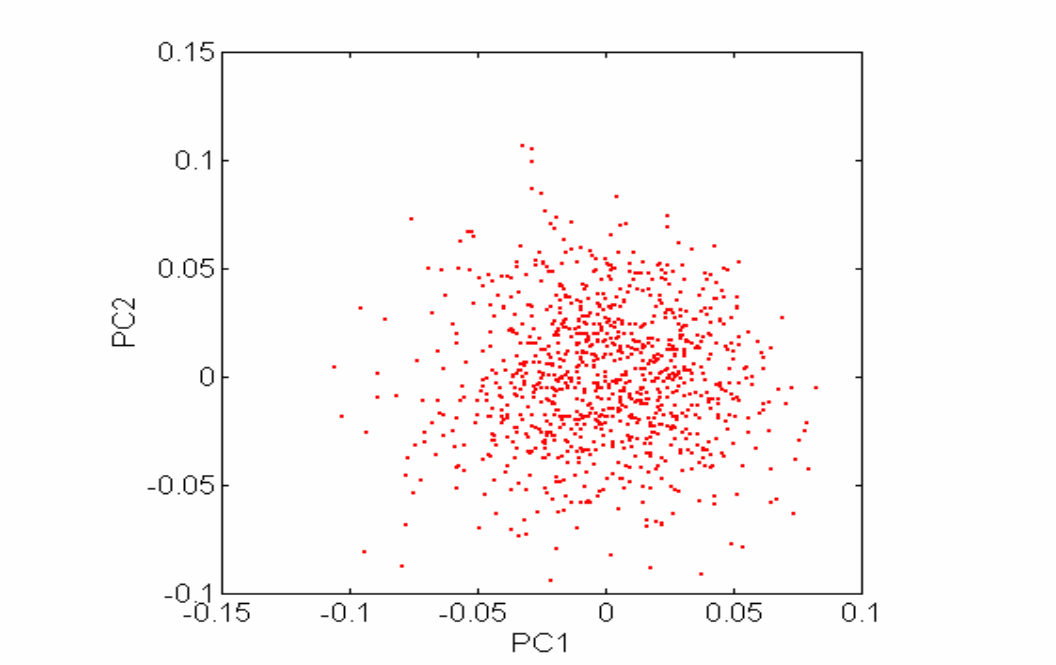



*Figure 4.9: Construction of a 95% confidence interval limit by plotting a circle around the BOOT/PCA scores to exclude 5% of the data*

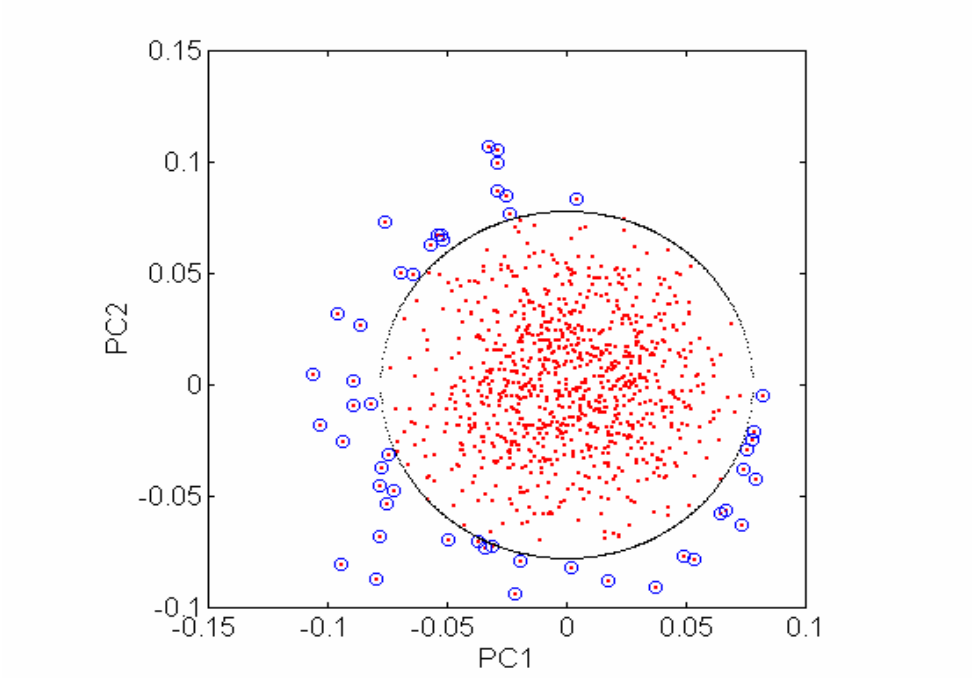
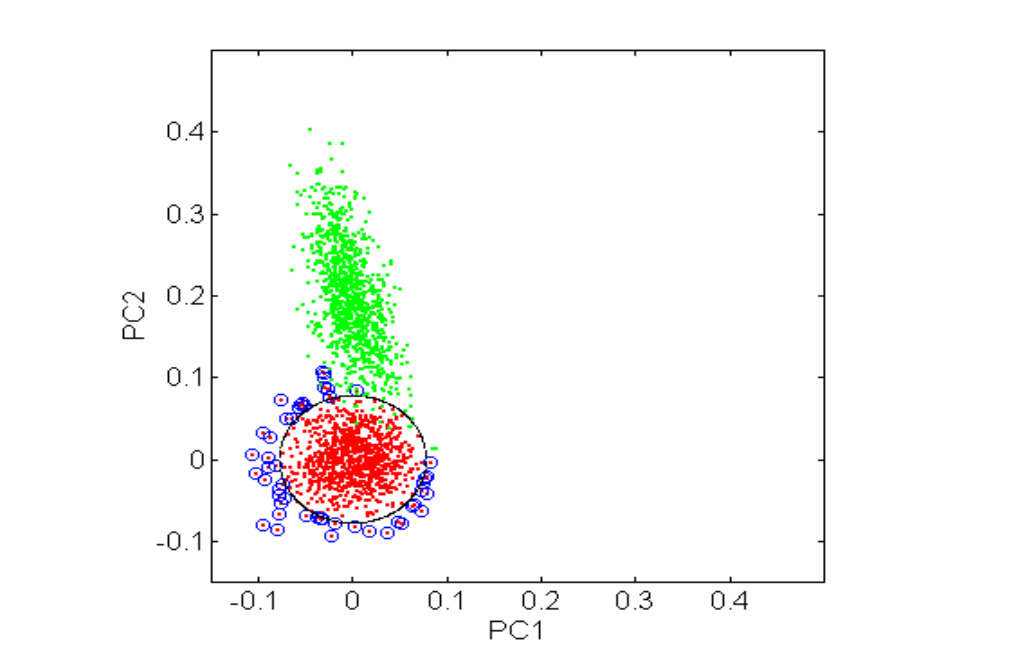


*Figure 4.10: Normalised scores plot for the reference (0) and test (0) formulation showing a partial overlap of the scores of the reference and test formulation*




#### 4.2.2.2 PCA and missing data

When many data have to be measured, the possibility arises that some values are missing. In this case, case deletion and imputation methods are frequently used to obtain a "complete" data set without missing values. In case deletion, all subjects (here: tablets) with missing values are omitted. It is clear that this approach is inefficient when only a limited number of subjects (typically 6 or 12 tablets) are measured since a substantial part of the information is discarded. Imputation methods imply that the missing data are filled in with plausible values. The easiest way is to replace the missing value(s) by the mean for that variable. However, by doing so the correlation between the data is not respected. Another possibility is the expectation-maximization (EM) algorithm. The expectation-maximization approach has been shown to be an efficient tool to deal with missing data. Furthermore, it can easily be combined with PCA (EM(PCA)) (Nelson et al., 1996; Grung and Manne, 1998).

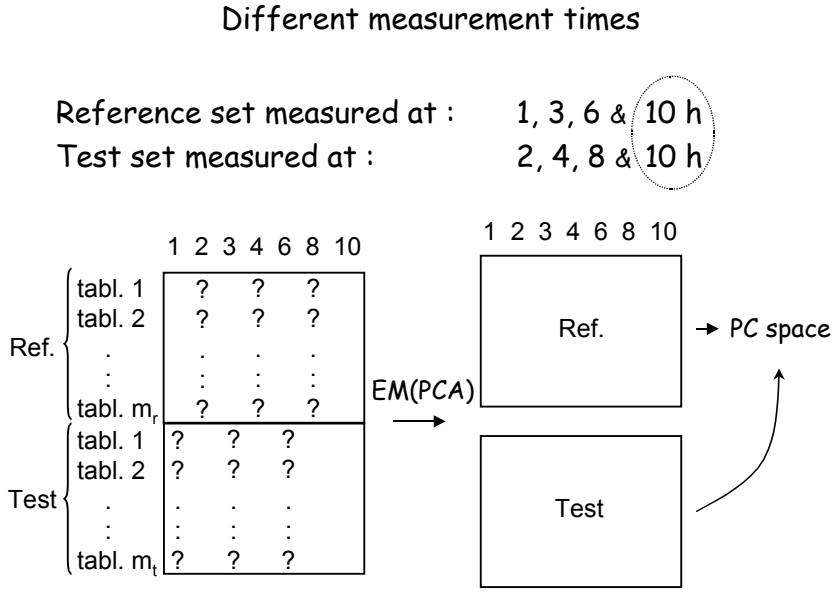
The EM procedure can be summarized as follows:

1. Replace the missing elements in data matrix X with their initial estimates, for instance, the mean values for the corresponding variable.
2. Perform PCA of the completed data set.
3. Predict the missing value(s) using a limited number of significant principal components (PC's). This results in a matrix of the predicted values $\hat{\mathbf{X}}$.
4. Replace the missing elements in the original matrix X with their predicted values from $\hat{\mathbf{X}}$ (the observed values of X remain unchanged).
5. Repeat steps 2 to 4 till convergence.

The result is the original data matrix X in which the missing elements are replaced by the predicted values from the EM(PCA) algorithm. It is important to emphasize that the values for the missing data are optimized in order to be analyzed further by PCA.

As regards to dissolution testing, a special case of missing data is encountered when the data sets to be compared are measured at different time points. The missing values (time points) for each data set are replaced by the predicted values obtained by the EM(PCA) algorithm after which PCA is performed on the recomposed, originally incomplete data set. The procedure is schematically illustrated in Fig. 4.11. EM can only be successfully applied if the sets of data have at least one common sample time point.

*Figure 4.11: Schematic illustration of EM/PCA procedure on two sets of dissolution data measured at different sample time points*




In the case of missing values in one of the dissolution sets, two approaches are possible for bootstrapping:

*EM/BOOT/PCA:* the missing values in matrix $\mathbf{X}_{\mathbf{miss}}$ ($m \times p$) are first replaced by their values estimated by EM(PCA) to yield a matrix $\mathbf{X}$ ($m \times p$). Next, $n$ bootstrap matrices ($\mathbf{X}_1$, $\mathbf{X}_2$,…, $\mathbf{X}_n$) are formed, all with size ($m \times p$). After computation of the $n$ column mean vectors with size ($1 \times p$), an ($n \times p$) matrix is formed followed by PCA.

*BOOT/EM/PCA*: starting from matrix $\mathbf{X}_{\mathbf{miss}}$ ($m \times p$) with missing values, first $n$ bootstrap matrices, also with missing values, are formed. Each of the $n$ bootstrap matrices is then completed using EM (PCA). After construction of the ($n \times p$) matrix of column means, PCA is performed. The results obtained after *BOOT/EM/PCA* are similar to those from *EM/BOOT/PCA*, but the former method is much slower to perform.

## 4.3 Materials and methods

### *4.3.1 Laboratories*

The dissolution tests of the same batches of ciprofloxacin and metronidazole tablets were performed at the quality control laboratory of the Tanzania Pharmaceutical Industries (TPI) (Arusha, Tanzania) and at the Laboratory of Pharmaceutical Technology, Ghent University (Ghent, Belgium) by the same analyst.

### *4.3.2 Drug samples*

Eight ciprofloxacin and seven metronidazole generic tablet formulations available on the Tanzanian market were purchased from pharmacies in Tanzania. The innovator brand for metronidazole (Rhône Poulenc Rhorer) was obtained from a pharmacy in Belgium. The samples were allocated a code shown in Table 4.1.

*Table 4.1: Manufacturers of and the code allocated to the samples*

| Metronidazole tablets | | Ciprofloxacin tablets | |
|---|---|---|---|
| **Manufacturer** | **Code** | **Manufacturer** | **Code** |
| ACE Laboratories | A | Aurobindo | 1 |
| Flamingo Pharmaceuticals | F | Cadila Pharmaceuticals | 2 |
| Intas Pharmaceuticals | I | Egyptian Pharmaceuticals | 3 |
| Medopharm | M | Flamingo Pharmaceuticals | 4 |
| Shelys Pharmaceuticals | S | Freudun Pharmaceuticals | 5 |
| S Kant Health Care | SK | Gracure | 6 |
| TPI | T | Intas Pharmaceuticals | 7 |
| Rhône Poulenc Rhorer | R | S Kant Health Care | 8 |

### *4.3.3 Equipment*

In Belgium the dissolution apparatus was a VK 7010 system linked to a VK 8000 automatic sampler (Vankel Technology, Cary, USA). The UV analysis of the samples was performed using a Lambda 12 UV/VIS spectrophotometer (Perkin Elmer, Norwalk, USA).

In Tanzania the dissolution apparatus was an Erweka DT06 (Erweka Darmstadt, Germany). The analysis of the samples was performed using a 551 UV/VIS spectrophotometer (Perkin Elmer, Norwalk, USA).

### *4.3.4 Materials*

Belgium

Metronidazole was supplied by Alpha Pharma (Zwevegem, Belgium), ciprofloxacin hydrochloride by Bayer (Leverkusen, Germany) and hydrochloric acid was obtained from Vel (Leuven, Belgium).

Tanzania

Metronidazole was supplied by Sigma (Steinheim, Germany), while ciprofloxacin hydrochloride was obtained from Pentex Miles (Kankakee, USA). Hydrochloric acid was obtained from BDH Chemicals (Poole, England).

### *4.3.5 Dissolution method*

For the metronidazole formulations the USP Method 1 at 100 rpm was used. The dissolution medium in each vessel was 900 ml of 0.1N HCl maintained at 37 ± 0.5°C. Aliquots (5 ml) of the dissolution media were withdrawn after 10, 20, 30, 40, 50 and 60 min. The samples were withdrawn automatically using sampling probes and manually using a 5 ml syringe for the tests conducted in Belgium and Tanzania, respectively. In both laboratories the dissolution samples were analysed by UV at 278 nm.

For the ciprofloxacin formulations the USP Method 2 at 50 rpm was used. The dissolution medium in each vessel was 900 ml distilled water maintained at 37 ± 0.5°C. Aliquots (5 ml) of the dissolution media were withdrawn manually (using a 5 ml syringe) after 5, 15, 30 and 45 min for the dissolution tests performed in Tanzania. In Belgium, the samples were automatically withdrawn after 5, 10, 15, 20, 25 and 30 min using sampling probes. The dissolution samples were analysed by UV at 276 nm.

Software

All programs used for PCA analysis were written in MATLAB (Version 4.0, the MathWorks, Natick, MA, USA).

## 4.4 Results and discussion

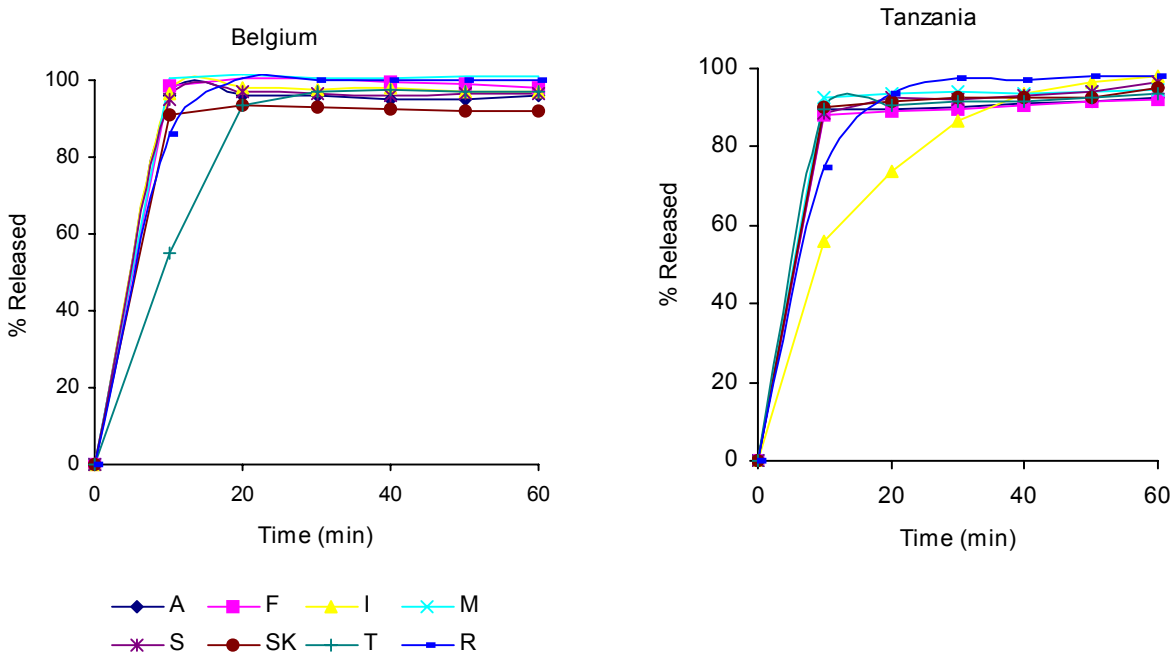
### *4.4.1 Metronidazole formulations*

#### *4.4.1.1 Dissolution profiles*

Fig. 4.12 shows the dissolution profiles of metronidazole. All formulations satisfy the USP 24 tolerance limits for dissolution as they released more than 85% of the labelled drug within 1 hr.

The dissolution profiles show that most of the formulations had a fast drug release as within 10 minutes more than 80% of the drug had been released (Fig. 4.12). However, for both sets of data there is one formulation having an initial slower release rate: the Intas formulation (code I) for the data obtained in Tanzania, while for data obtained in Belgium it was the TPI formulation (code T).

*Figure 4.12: Dissolution profiles of metronidazole formulations analysed in Belgium and Tanzania*



#### 4.4.1.2 Comparison by similarity factor $f_2$

The similarity factor was calculated for each formulation by arbitrarily taking the data from Belgium as a reference. Similarity factor analysis (Table 4.2) showed that only two formulations had a value less than 50: the Intas and TPI formulations having $f_2$ factors of 35 and 41, respectively. As described previously these formulations had initially a slower dissolution rate: the Intas batch for samples analysed in Tanzania and the TPI batch for those analysed in Belgium (Fig. 4.12).

*Table 4.2: The $f_2$ values for the metronidazole dissolution profiles*

| Formulation | $f_2$ value |
|---|---|
| ACE | 64 |
| Flamingo | 51 |
| Intas | 34 |
| Medopharm | 57 |
| Shelys | 69 |
| S Kant | 68 |
| TPI | 41 |
| Rhône Poulenc Rhorer | 63 |

#### 4.4.1.3 Comparison by BOOT/PCA

The data from Intas and TPI formulations were analysed by the BOOT/PCA technique. The results show that there is no overlap between the data measured in the two laboratories (Fig. 4.13 and 4.14). There is a difference on the scores mainly along PC1 (size), indicating that there are significant inter-laboratory differences on percent drug release of both formulations. As an example, the BOOT/ PCA of the dissolution data for the Shelys formulation (which had an $f_2$ value greater than 50) is shown, the overlap of the scores indicating that there was similarity between the data obtained in the two laboratories (Fig. 4.15).

*Figure 4.13: PCA/BOOT normalized scores plot of the dissolution data obtained in Belgium and Tanzania for the Intas formulation (o Belgium, ★ Tanzania)*

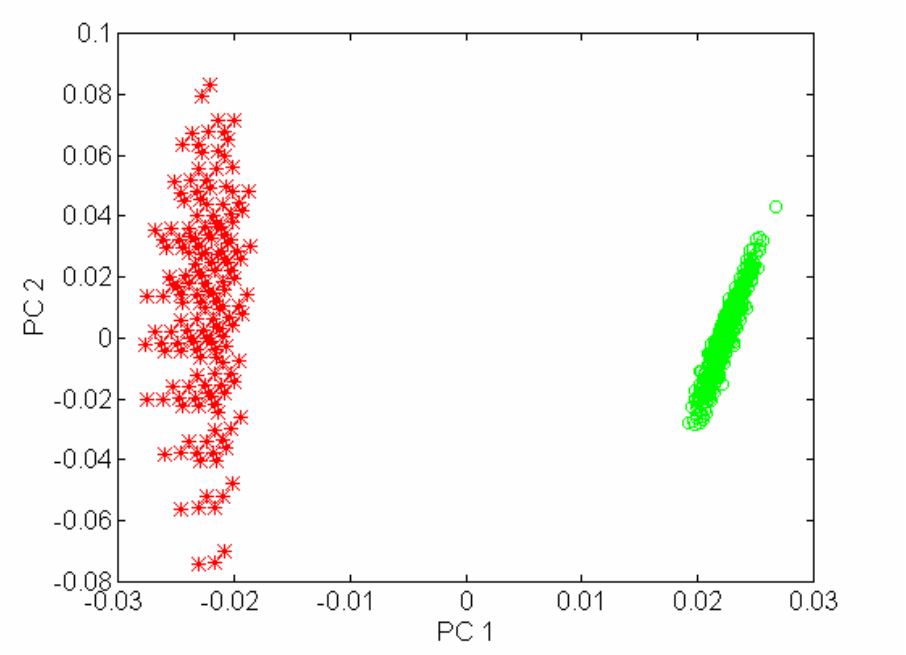



*Figure 4.14: PCA/BOOT normalized scores plot of the dissolution data obtained in Belgium and Tanzania for the TPI formulation. (o Belgium, ★ Tanzania)*

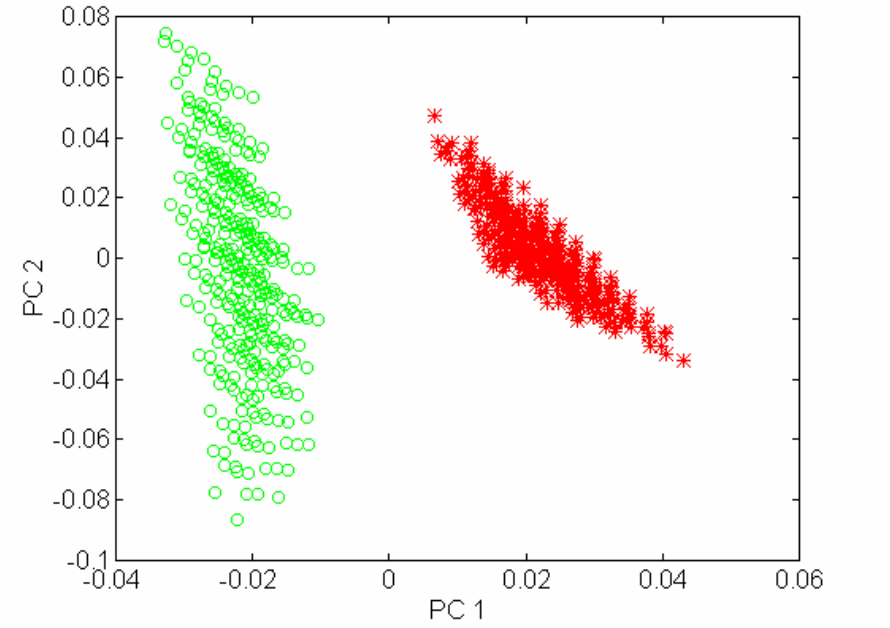
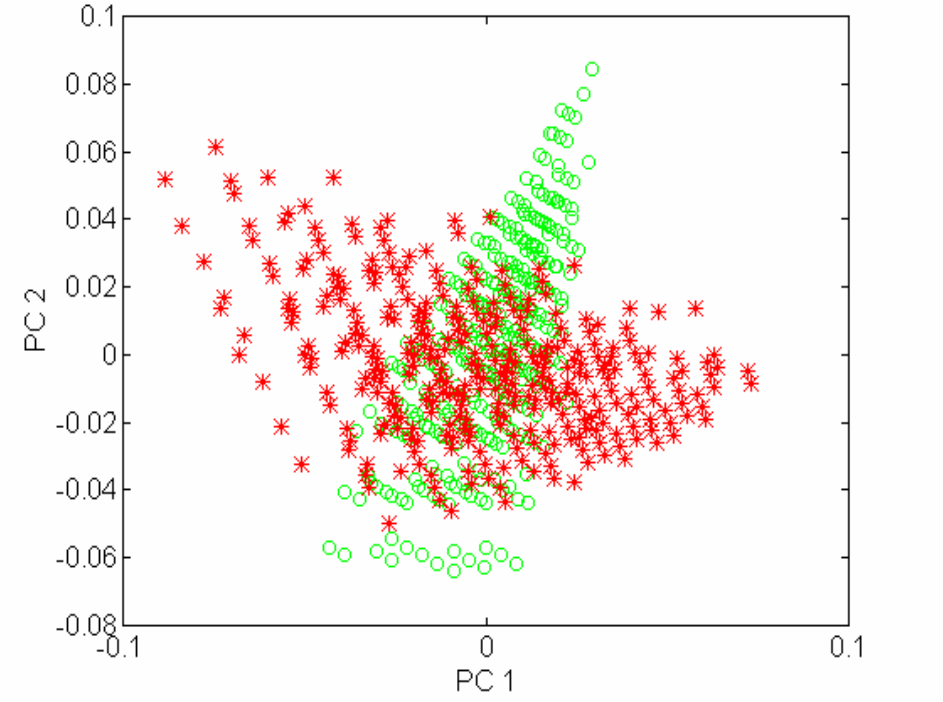


*Figure 4.15: PCA/BOOT normalized scores plot of the dissolution data obtained in Belgium and Tanzania for the Shelys formulation. (o Belgium, ★ Tanzania)*




The BOOT/PCA technique was also used to compare the data of the eight formulations obtained in the two laboratories. This was done by projecting in a single BOOT/PCA scores plot the data of all 8 formulations from one laboratory. With the exception of the Intas formulation, the scores of the other formulations from the data obtained in Tanzania are closely together along PC1 (Fig. 4.17). However, the scores for the data obtained in Belgium are relatively spread (Fig. 4.16). This indicates that there are differences on the PC1 and PC2 scores (percent dissolved and shape of the dissolution profile) for these formulations. However the differences illustrated by this method are probably an overinterpretation and not pharmaceutically significant.

*Figure 4.16: PCA normalized scores plot of the dissolution data obtained in Belgium after BOOT/PCA of the data from the metronidazole formulations*

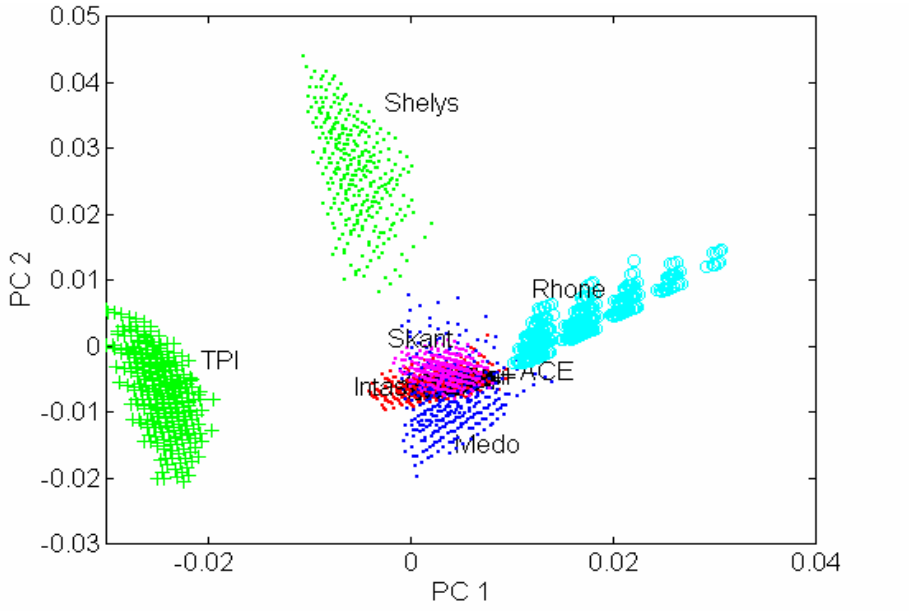
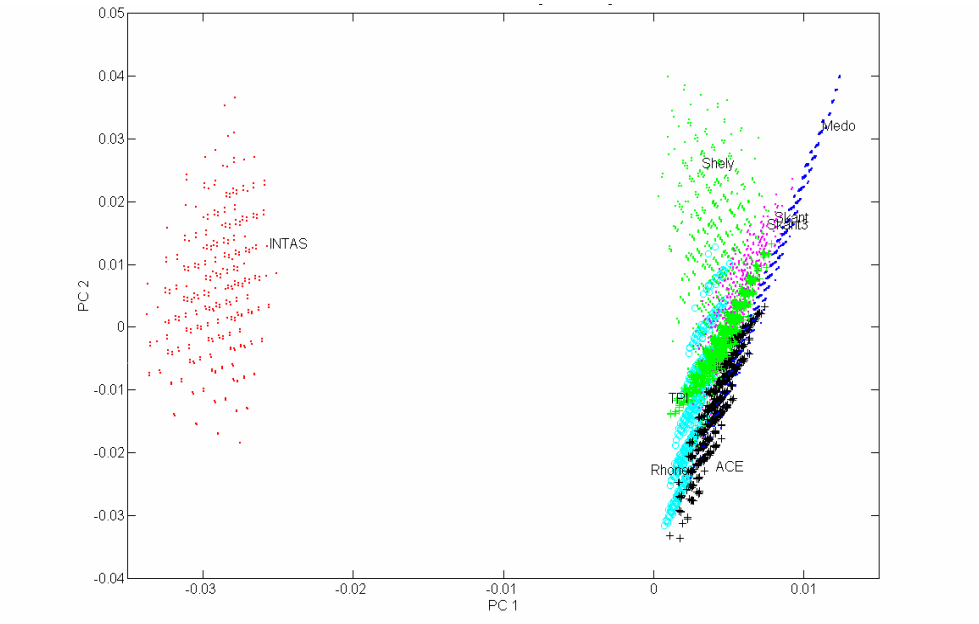



*Figure 4.17: PCA normalized scores plot of the dissolution data obtained in Tanzania after BOOT/PCA of the data from the metronidazole formulations*

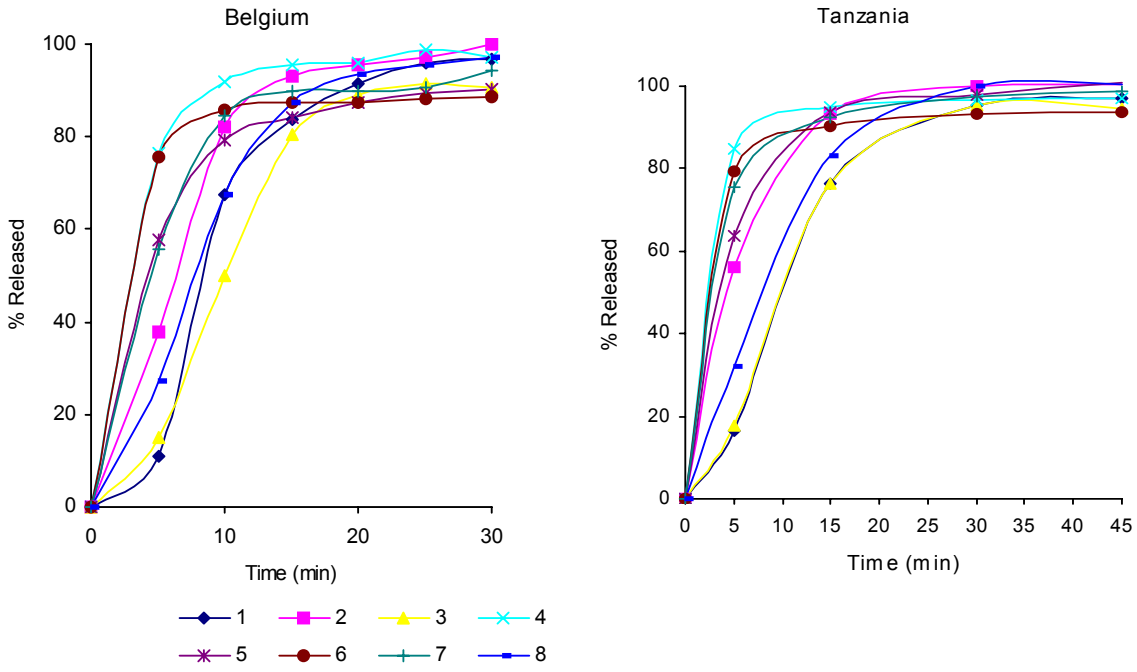


### 4.4.2 Ciprofloxacin formulations

#### 4.4.2.1 Dissolution profiles

The dissolution profiles show variations between formulations on the percent drug release from 0 to 15 min for both the inter- and intra-laboratory dissolution data (Fig. 4.18).

*Figure 4.18: Dissolution profiles of ciprofloxacin formulations analysed in Belgium and Tanzania*




#### 4.4.2.2 Comparison by similarity $f_2$ factor

For the ciprofloxacin formulations the percentage drug dissolved were measured at different sample time points. There are two possibilities to compare the data.

The first possibility is to compare the values between laboratories using the data measured in Belgium taken as a reference. However, EM/BOOT/PCA must be performed on each set of data to generate the missing data points as shown in the example in Table 4.3.

*Table 4.3: An example of missing data generated after EM/PCA using 5 PC's on the Aurobindo dissolution data. The replaced values are shown in bold*

| Laboratory | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 | 45 |
| **Belgium** | | | | | | | |
| tablet 1 | 36.0 | 77.3 | 89.0 | 94.0 | 98.9 | 100.0 | **99.4** |
| tablet 2 | 15.5 | 72.7 | 86.7 | 92.0 | 98.8 | 98.5 | **99.4** |
| tablet 3 | 2.5 | 72.7 | 84.6 | 95.2 | 96.2 | 95.4 | **94.2** |
| tablet 4 | 1.28 | 40.4 | 76.5 | 84.9 | 93.7 | 96.5 | **96.9** |
| tablet 5 | 8.0 | 68.2 | 83.7 | 92.2 | 96.7 | 95.9 | **96.5** |
| tablet 6 | 3.4 | 68.1 | 82.9 | 90.2 | 92.5 | 94.7 | **95.2** |
| **Tanzania** | | | | | | | |
| tablet 1 | 2.6 | **28.6** | 68.8 | **74.1** | **81.2** | 89.9 | 95.2 |
| tablet 2 | 2.2 | **33.7** | 77.0 | **81.9** | **90.9** | 100.1 | 98.7 |
| tablet 3 | 1.6 | **40.7** | 74.1 | **81.3** | **88.9** | 92.8 | 95.7 |
| tablet 4 | 30.0 | **49.7** | 76.5 | **79.9** | **87.5** | 92.6 | 98.1 |
| tablet 5 | 11.9 | **40.0** | 74.8 | **80.5** | **87.3** | 94.1 | 96.6 |
| tablet 6 | 22.4 | **48.9** | 77.8 | **85.2** | **88.8** | 94.8 | 95.4 |

After performing EM(PCA) to generate the missing data, the $f_2$ factor for each of the eight sets of measurements is calculated taking the measurements made in Belgium as a reference. The $f_2$ values obtained are shown in Table 4.4.

*Table 4.4: The $f_2$ values of ciprofloxacin formulations computed after EM/PCA of the ciprofloxacin data*

| Formulation | $f_2$ value |
|---|---|
| Aurobindo | 46 |
| Cadila | 53 |
| Egyptian | 68 |
| Flamingo | 71 |
| Freudun | 54 |
| Gracure | 70 |
| Intas | 55 |
| S Kant | 65 |

The $f_2$ analysis shows that the dissolution profiles of seven of the eight formulations measured in Belgium and Tanzania are pharmaceutically similar ($f_2 \geq 50$).

The second possibility is to compare the eight formulations with each other using data obtained in the same laboratory. Since the data of all eight formulations in each laboratory are measured at the same sampling times EM is not necessary.

There are 28 possible combinations to compare the eight brands in pairs: 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 2-3, 2-4,.... Using the data obtained in Tanzania only 8 of the 28 $f_2$ factors are above the limit of 50. Using the data obtained in Belgium 9 of the 28 $f_2$ factors are above 50. Of the combinations with an $f_2$ factor above 50, only five pairs (1-3, 5-6, 5-7, 6-7 and 4-6) are common between both laboratories although their $f_2$ values were not identical (Table 4.5).

*Table 4.5: Pair-wise comparison of the ciprofloxacin formulations using $f_2$ factors computed from the original dissolution data and from data after EM/PCA: combinations with $f_2$ factors $\geq 50$*

| Belgium | | Tanzania | |
|---|---|---|---|
| **Pair** | **$f_2$ value** | **Pair** | **$f_2$ value** |
| 1-3 | 55 | 1-3 | 73 |
| 1-8 | 58 | 2-5 | 60 |
| 2-7 | 53 | 3-8 | 53 |
| 2-8 | 55 | 4-6 | 68 |
| 3-8 | 50 | 4-7 | 64 |
| 4-6 | 55 | 5-6 | 52 |
| 5-6 | 55 | 5-7 | 61 |
| 5-7 | 70 | 6-7 | 68 |
| 6-7 | 53 | **4-5** | **50*** |
| **4-7** | **51*** | | |

***** $f_2$–factor was above 50 only after EM/PCA

The pairwise comparison is also done by calculating $f_2$ after EM(PCA) to test the influence of EM(PCA) algorithim. The results (Table 4.5) show that 9 and 10 of the 28 combinations have $f_2$ values $\geq$ 50 for the data measured in Tanzania and

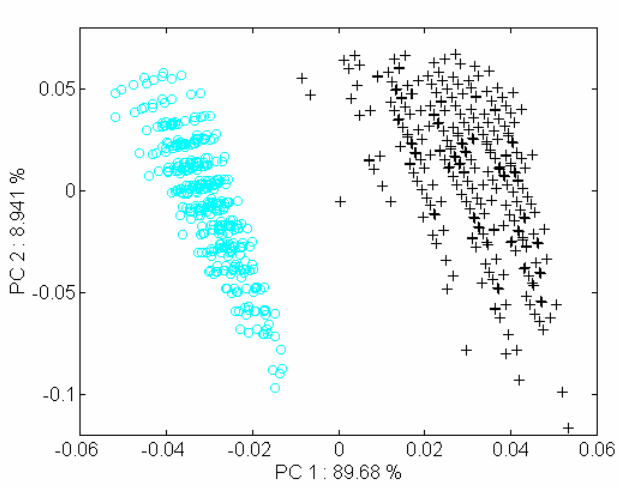
Belgium, respectively: the same formulations as without EM(PCA) plus one additional combination. This demonstrates that the influence of EM(PCA) on the results is minimal as the number of combinations with $f_2$ values ≥ 50 increased marginally.

Pairwise comparison of the formulations shows that the dissolution profiles vary widely even for the data obtained from the same laboratory. This is not surprising as the dissolution profiles (Fig. 4.16) show differences in drug release during the initial 15 minutes. However, the pharmaceutical relevance of these differences will probably be negligible.

#### 4.4.2.3 Comparison by BOOT/PCA

From the $f_2$ analysis of the inter-laboratory dissolution data, the profiles of the Aurobindo formulation (code 1) were not equivalent. The inter-laboratory data from this formulation were also compared using the BOOT/PCA technique. Since the data obtained in Tanzania and in Belgium were measured at different sample time points, the EM(PCA) algorithm was applied to generate the missing data before BOOT/PCA was done. The results of EM/BOOT/PCA for the Aurobindo formulation are shown in Fig. 4.19.

*Figure 4.19: The PCA /BOOT normalised scores plot for the Aurobindo formulation after EM/BOOT/PCA of dissolution data obtained in (+) Belgium and (o) Tanzania*



There is no overlap of the scores obtained from the two laboratories. The differences on PC1 scores indicate that there is a significant difference between the percent drug dissolved from this formulation for the data obtained in the two laboratories.

The EM/BOOT/PCA technique was also used to compare the data of the eight formulations by projecting in a single PC1/PC2 scores plot the dissolution data of the formulations measured in one laboratory. To check the influence of the EM(PCA) algorithm on the observed results, the BOOT/PCA was also used to compare the eight brands using the original dissolution data measured in Tanzania and in Belgium, respectively (Fig. 4.20 and 4.21).

*Figure 4.20: The normalized scores plot after EM/BOOT/PCA of the dissolution data obtained in Belgium and Tanzania for the ciprofloxacin formulations*

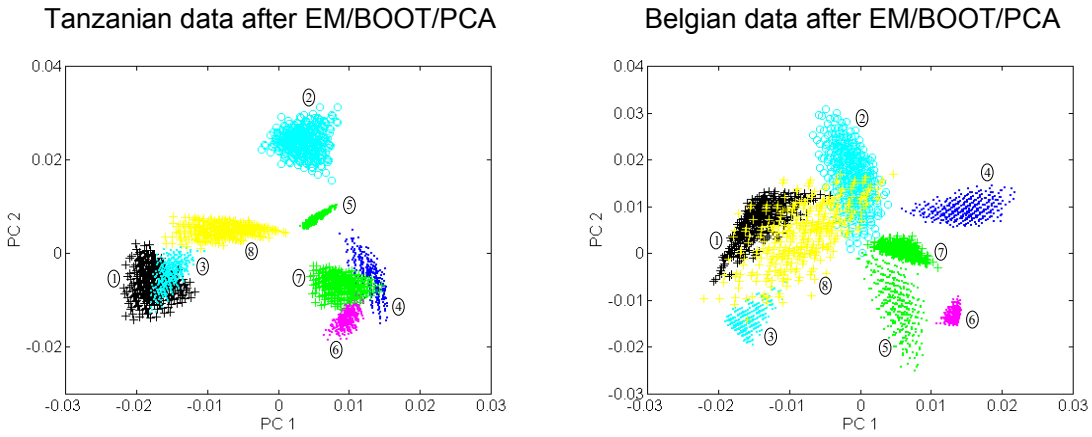
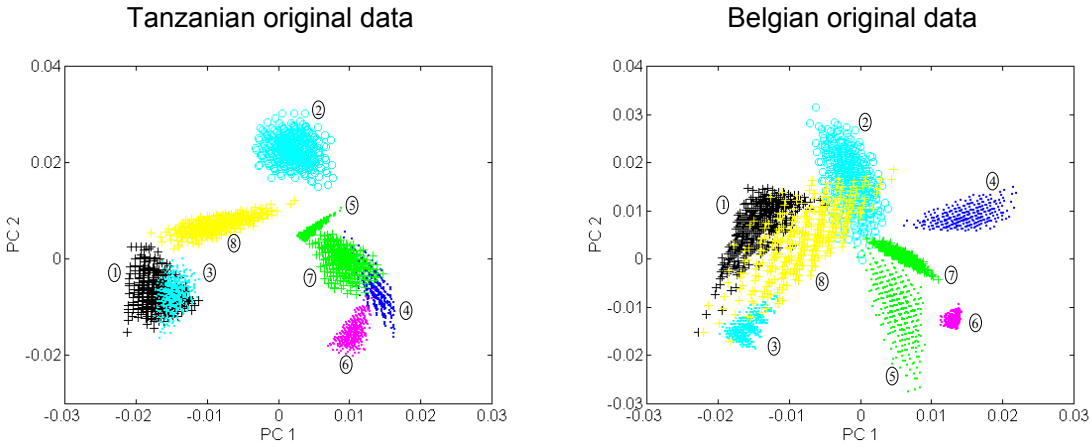



*Figure 4.21: BOOT/PCA normalized scores plot of the dissolution data obtained in Belgium and Tanzania for the ciprofloxacin formulations*




The EM/BOOT/PCA scores plot obtained shows differences in the dissolution data of the batches measured in both laboratories: the dissolution profiles differ in the percent drug release (PC1) as well as in the shape (PC2).

Comparing the data from the two laboratories, it is evident that the EM/BOOT/PCA method is more discriminative compared with the $f_2$ analysis. Using $f_2$ analysis seven out of the eight formulations are considered pharmaceutically similar ($f_2$ > 50); but with EM/BOOT/PCA more variations are observed.

For the Tanzanian data, the paired combination with $f_2$ factor above 50 (Table 4.5) are projected close to each other in the EM/BOOT/PCA plot (pairs 1-3, 4-6, 4-7, 5-7 and 6-7 had the highest $f_2$ values). The other combinations (2-5, 3-8 and 5-6) are somewhat further apart in the EM/BOOT/PCA, plot but have similar scores on PC1.

The EM/BOOT/PCA plot for the tablets analysed in Belgium showed similar results: the pairs 1-8, 2-7, 2-8, 3-8 and 5-7 are situated close to each other and all had a similarity factor greater than 50. The other combinations considered pharmaceutically equivalent (1-3, 4-6 and 6-7) were also closer together and have similar scores on PC1.

Comparing the EM/BOOT/PCA and BOOT/PCA plots (Fig 4.20 and 4.21) for the data set from each country showed that EM had little influence on the scores obtained for the formulations. The formulations are projected in almost the same areas of the PC1/PC2 plot. Similar results were also observed when computing $f_2$ factors from data obtained after performing the EM(PCA) algorithm.

The evaluation of the dissolution data using different methods has yielded different results. Using the USP 24 guidelines for dissolution tests all sets of data obtained from the two laboratories meet the tolerance limits for dissolution. On this basis, the dissolution data measurements performed in Belgium and Tanzania are considered equivalent.

Evaluation of the dissolution data using the $f_2$ factor (that takes into consideration all sampling points) showed that it is more discriminating than the USP 24 method. On the basis of $f_2$ analysis there are some differences between the data measured in the two countries: 6 out of the 8 metronidazole and 7 out of 8 ciprofloxacin formulations were considered pharmaceutically equivalent. This may be considered as a minor variation.

The BOOT/PCA comparison demonstrated that it was a good method that could visualize the statistical differences between sets of dissolution data. The BOOT/PCA method is more discriminative than the $f_2$ factor as more variations

between dissolution data of the different formulations are observed. There are more differences between the measurements performed in the two laboratories for the two drugs. Although there were statistical differences between the inter-laboratory dissolution data it is most likely that these differences are not pharmaceutically important.

## 4.5 Conclusion

The evaluation by $f_2$ factor and PCA/BOOT of the dissolution data obtained at both labolatories has revealed inter-laboratory differences between dissolution profiles of the same batch of a formulation. The extent of the differences between inter-laboratory data was however small. Although these differences were statistically significant, it could not be concluded if they were pharmaceutically significant. The in vivo significance of the differences between the dissolution profiles based on the $f_2$ analysis needs to be investigated.

## 4.6 References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# 5. INFLUENCE OF DISSOLUTION RATE ON THE BIOAVAILABILITY OF TWO ACETYLSALICYLIC ACID TABLET FORMULATIONS

## 5.1 Introduction

Acetylsalicylic acid (ASA) is widely used for the analgesic, anti-inflammatory and antipyretic actions of the drug itself and of its metabolite salicylic acid. In addition, as it inhibits platelet aggregation (action solely attributed to ASA), low doses of ASA are recommended as a prophylaxis therapy in reducing the risk of ischemic heart attacks, myocardial infarction and thrombolytic arterial diseases. The drug is included in the national essential drug list of Tanzania for its analgesic, anti-inflammatory and antipyretic effects.

The in vitro dissolution of immediate release ASA formulations (three generic and one innovator brand) on market in Tanzania has been investigated in chapter 3. The in vitro drug release from the formulations was determined immediately after purchase and after 3 and 6 months of storage under simulated tropical conditions (40°C, 75% RH) by performing dissolution tests as recommended in the USP 24. All formulations obtained from the Tanzanian market failed the dissolution test.

The aim of the study is to determine whether the different in vitro dissolution characteristics of the acetylsalicylic acid formulations significantly influence their bioavailability.

Since the strength of most ASA-formulations on market in Tanzania is 300 mg and the innovator brand from Bayer (previously analysed in Chapter 3) is of 500 mg strength, an immediate release formulation containing 300 mg ASA and having market authorisation in Belgium (Dispril® 300) was chosen as a reference formulation for the in vivo study.

## 5.2 In vitro evaluation

Acetylsalicylic acid tablets from Reckit & Coleman (Belgium) (Dispril® 300, batch 01D10) and from Shelys Pharmaceutical Industries (Tanzania) (batch 068) were

evaluated. The Shelys formulation had the lowest drug dissolution amongst the formulations sampled from the Tanzanian market. The dissolution properties of the samples were evaluated before the bioavailability study.

Dissolution test

The in vitro drug release of the tablet formulations was determined by performing a dissolution test as described in the USP 24 and detailed in chapter 3.

Equipment

A dissolution tester VK 7010 linked to an automatic sampler VK 8000 (VanKel Technology, Cary, USA) was used for the dissolution tests. A Lambda 12 UV/VIS spectrophotometer (Perkin Elmer, Norwalk, USA) was used to determine the drug concentrations of the samples.

## 5.3 In vivo evaluation

Clinical protocol

Ten female volunteers participated in the study after giving informed consent. The age of the volunteers ranged from 20 to 34 years (mean: 25 yrs), their weight from 45 to 74 kg (mean: 61 kg) and their body mass index from 18.1 to 26.2 kg/m$^2$ (mean: 21.6 kg/m$^2$). The volunteers were healthy as proven by medical history, physical examination, electrocardiogram and lab tests (haematological and urine). Volunteers with suspected history of alcoholism, barbiturate abuse, allergic bronchospasms, bleeding disorders and allergy to ASA or non-steroidal analgesic agents were excluded from the study. Pregnant or lactating females or females with childbearing potential without adequate contraception were also excluded from the study. The Ethics Committee of Ghent University Hospital approved the clinical protocol.

Prior and concomitant therapy

During the entire trial, subjects were required not to use any medication other than the trial medication. The exceptions to this rule were paracetamol and oral contraceptives. Subjects were allowed to use paracetamol up to 3 days before drug administration in each period. The use of paracetamol could be allowed by

the clinical investigator (no more than 3 x 500 mg per day and no more than 3 g per week) for the treatment of headache or other pain. Oral contraceptives (OAC) were allowed. In case paracetamol or OAC were used, the dose and dosage regimen were recorded on the Concomitant Therapy Form that formed a part of the Case Report Form (CRF).

Procedure

The subjects fasted for at least 10 hrs before drug administration. Drinking of water was allowed up to 2 hrs before drug administration. The subjects were required not to consume grapefruit, grapefruit juice or beverages containing alcohol or quinine 24 hrs before and 24 hrs after drug dosing per period. The subjects were required to take the whole tablet together with 200 ml of water. From 2 hrs after dosing, intake of water was allowed. A standard breakfast and lunch were given 3 and 6 hrs post dosing, respectively. The standard breakfast consisted of four slices of bread, one slice of ham, one slice of cheese, butter, jelly and two cups of decaffeinated coffee or tea with, if desired, milk and/or sugar. The subjects remained in the testing facility for 15 hrs after receiving the dose.

Randomisation

The study was an open randomized 2-period cross-over design. The washout time between periods was 3 days. In each period subjects were given a single dose of 300 mg acetylsalicylic acid as an immediate release tablet. Subjects entering the study were allocated a number from 1 to 10. The randomisation scheme shown in Table 5.1 was used to assign the subjects to either of the two treatments:

- ✓ A1: (Dispril® from Reckitt & Coleman)
- ✓ A2: (acetylsalicylic acid tablets from Shelys Pharmaceutical Industries).

*Table 5.1: Randomisation scheme*

| Subject | Period 1 | Period 2 |
|---|---|---|
| 1 | A1 | A2 |
| 2 | A2 | A1 |
| 3 | A1 | A2 |
| 4 | A2 | A1 |
| 5 | A1 | A2 |
| 6 | A2 | A1 |
| 7 | A1 | A2 |
| 8 | A2 | A1 |
| 9 | A1 | A2 |
| 10 | A2 | A1 |

Blood Sampling

Venous blood samples of 5 ml (to obtain about 2 ml of plasma) were taken from an antecubital vein within one hour before and 0.17 (10 min), 0.33 (20 min), 0.5 (30 min), 0.67 (40 min), 0.83 (50 min), 1, 1.33 (1 hr 20 min), 1.67 (1 hr 40 min), 2, 2.5, 3, 4, 6, 8, 11, 15 and 24 hrs after drug administration. Blood samples were collected in ice-chilled tubes (heparinized) and centrifuged for 10 minutes at 1500g and 4°C within 20 min of collection. While awaiting centrifugation the samples were kept in an icebox. Plasma was immediately aspirated into plastic tubes previously labelled with the investigator's name, trial number, CRF ID, subject initials, date and sampling time, sealed by means of polyethylene stoppers and stored at –20 °C for a maximum of 24 hr and then at -80°C until analysis.

### *5.3.1 Plasma drug analysis*

A validated bioanalytical method for the simultaneous determination of acetylsalicylic acid (ASA) and salicylic acid (SA) in a limited volume of human plasma using ESI(-)-LC-MS/MS was developed by the Laboratory of Bioanalysis (Faculty of Pharmaceutical Sciences, Ghent University) (Bouche et al., 2003).

#### 5.3.1.1 Materials

Reagents and solutions

ASA, SA and the internal standard (IS) 3-methyl-SA were obtained from Sigma-Aldrich (Bornem, Belgium). All solvents(water, acetonitrile, methanol) were of HPLC-grade and purchased from Merck (Darmstadt, Germany). The other used products were of analytical grade and include ammonia (UCB, Leuven, Belgium), formic acid (FA, Fluka Chemie, Buchs Switzerland), acetic acid (AA, Sigma-Aldrich, Bornem, Belgium), sulphuric acid, sodium sulphate (both Merck, Darmstadt, Germany) and sodium tungstate (UCB, Leuven, Belgium).

Stock and calibration solutions of ASA were prepared in acetonitrile, complemented with FA (2%) and dried on sodium sulphate for at least 48 h. As such, ASA solutions remained stable for at least one month, with respect to decomposition of ASA to SA. Stock and calibration solutions of ASA and the IS were prepared in acetonitrile. All solutions were stored at –20°C in brown glass recipients and used no longer than one month. The stock concentration of ASA and SA was 1.25 mg/ml, while that of the IS-solution was 1.0 mg/ml.

#### 5.3.1.2 Methods

Apparatus

The analytical set-up consisted of a 2695 Alliance LC chromatographic system (Waters, Manchester, UK) linked to a triple quadrupole MS instrument (Quattro Ultima, Waters-Micromass MS Technologies, Manchester, UK). The MS interface was used in the electrospray negative ion mode (ESI (-)).

Sample pre-treatment

Samples were allowed to thaw at room temperature. As soon as the liquefied state was reached (i.e. no longer than 20 min), 250 µl of crude plasma was sampled and immediately acidified with 10µl of sulphuric acid (residual pH 1). After addition of the IS-solution (20 µl, concentration 0.1 mg/ml) and 40 µl of acetonitrile (similar to the calibration standards) ultra-fast sample clean-up was achieved by protein precipitation with 50 µl of a 10% sodium tungstate solution. Samples were then shaken vigorously for 15 seconds and centrifuged at 6000 rpm for 10 min

(Biofuge, Heraeus Instruments, Germany). Subsequently, the obtained clear supernatant was transferred into autosampler vials.

Chromatographic conditions

Chromatographic separation between ASA and SA was achieved by injecting 15 µl of the clear supernatant on a Waters XTerra MS $C_8$ column (3.5 µm particle size, 150x2.1 mm). In order to protect the analytical column, a guard column was coupled to the analytical column (Hypersil BDS, 3.5 µm particle size, 20x2.1 mm, Alltech, Deerfield, IL). The guard column was replaced after approximately 150 injections.

Total run time was 15 min. The mobile phase consisted of a gradient of water: acetonitrile in a ratio 9:1 (A) and acetonitrile : water in ratio 9:1 (B). Both eluents were complemented with 0.02% acetic acid, which resulted in a pH of 3.1. Detailed LC-gradient conditions are given in Table 5.2.

*Table 5.2: Overview of the LC-gradient conditions*

| Time (min) | A% | B% | Flow rate (ml/min) |
|---|---|---|---|
| 0.0 | 80 | 20 | 0.3 |
| 1.9 | 80 | 20 | 0.3 |
| 2.0 | 80 | 20 | 0.2 |
| 3.0 | 80 | 20 | 0.2 |
| 3.5 | 30 | 70 | 0.2 |
| 4.5 | 30 | 70 | 0.2 |
| 5.5 | 80 | 20 | 0.2 |
| 15.0 | 80 | 20 | 0.2 |

Due to the chemical instability of ASA (basic hydrolysis of the ester-function), it is of prime importance that sample preparation and chromatographic separation are achieved under acidic conditions. Nevertheless, as ASA and SA are both acidic compounds, they need to be analysed in the ESI negative ion mode. Therefore post-column decomposition of ASA to SA was applied inside the LC-tubing leading the column eluent to the mass spectrometer. To that end, a 0.5 M ammonia

solution was added to the column eluent, via a T-piece immediately behind the analytical column and using an auxiliary pump (LC3-XP pump, Pye Unicam, Cambridge, UK; pumping rate 50 µl/min). As a result, 250 µl/min of eluent with a pH of 10 entered the ESI interface.

During the first minutes of the analytical run (0-2 min), the eluent mixture was directed to a waste bin by means of a divert valve, and this due to the highly acidic nature of the injected supernatant, containing sulphuric acid. Afterwards, the valve was automatically switched, thus guiding the eluent mixture to the mass spectrometer for ionisation and detection.

The high column flow at the beginning of the analytical run (0.3 ml/min) favoured the wash-out of residual sulphuric acid and salts from the analytical column, thereby decreasing MS interface deterioration as well as ionisation suppression of the target components.

LC/MS-conditions

Both compounds were analysed on a triple quadrupole mass spectrometer (Quattro Ultima, Waters Micromass MS-technologies, Manchester, UK) in the ESI negative ion mode, by application of multiple reaction monitoring (MRM). An overview of the optimised and applied ESI(-) MS/MS conditions for the investigated compounds is given in Table 5.3.

*Table 5.3: Overview of the applied ESI(-) MS/MS conditions.*

| Compound | Precussor ion | | Product ion (m/z) | Cone voltage (V) | Collision energy (eV) |
|---|---|---|---|---|---|
| | ion | m/z | | | |
| ASA | $[M-H]^-$ | 137.0 | 93.1 | 30 | 18 |
| | | 179.0 | 137.0 | | |
| SA | $[M-H]^-$ | 137.0 | 93.1 | 53* | 18 |
| | | 137.0 | 65.1 | | |
| IS (3-MeSA) | $[M-H]^-$ | 151.0 | 107.0 | 30 | 16 |
| | | 151.0 | 95.0 | | |

* Detuned to extend the dynamic range of the MS-detector

Quantification was performed based on peak area ratios (ASA/IS and SA/IS), using reconstructed mass fragmentograms. For quantification purposes the following transitions were monitored: *m/z* 137.0 to 93.1 for ASA and SA and 151.0 to 107.0 for the internal standard. The other product ions, mentioned in Table 5.3, were monitored as qualifier ions.

As explained above, ASA enters the mass spectrometric detector after basic decomposition to SA. Logically, the same ion transition, as in the case of SA, is monitored. However, the genuine precursor ion of ASA (*m/z* 179) was also followed as some sort of identity confirmation for ASA: a small residual peak was always present at the retention time of ASA. This peak represents a very small amount of ASA not being decomposed to SA. Validation results however, clearly shows that this residual amount of ASA does not compromise accurate and reproducible quantification of ASA, thereby indicating that the decomposition of ASA to SA is reproducible. By application of the described LC-MS/MS conditions, the retention times were 4.7 min, 6.4 min and 10.6 min for ASA, SA and the IS, respectively.

#### *5.3.1.3 Pharmacokinetic and statistical analysis*

The peak plasma concentration ($C_{max}$) and the time to reach the peak plasma concentration ($t_{max}$) were obtained from the individual plasma concentration vs. time profiles. The area under the plasma concentration/time curve to 24 hrs post dosing ($AUC_{0\text{-}24hrs}$) was calculated by linear trapezoidal summation. The terminal elimination rate constant ($\lambda_z$) was determined by log-linear regression of the terminal points of the plasma concentration/time curve and the half-life time ($t_{0.5}$) was defined as $0.693/\lambda_z$.

A computer program MW/Pharm software package (v.3.01, Mediware 1987-1991, Utrecht, The Netherlands) was used for the pharmacokinetic analysis.

Statistical analysis of the pharmacokinetic data was performed using a two-way ANOVA. The data were tested for normal distribution with the Kolmogorov-Smirnov test. The homogeneity of variances was tested with the Levene's test. To further compare the effects of the different treatments a multiple comparison among pairs of means was performed using the Scheffe test with $p < 0.05$ as

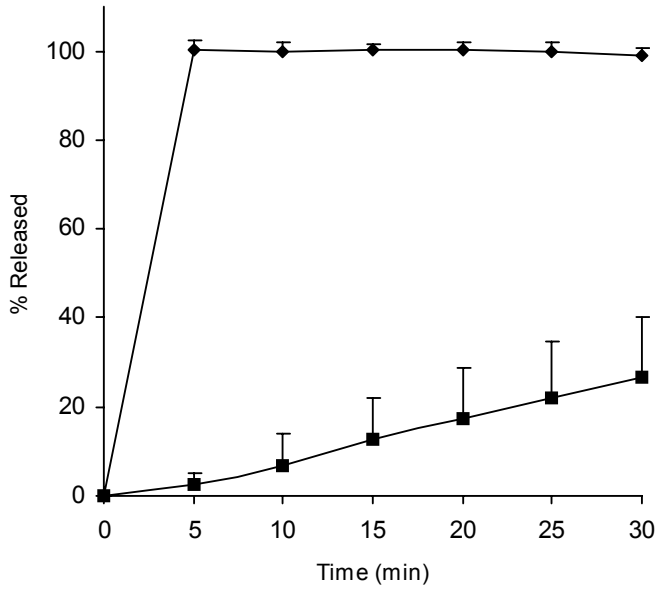
significance level. For all statistical analysis the program SPSS version 10.0 was used.

## 5.4 Results

### *5.4.1 In vitro drug release*

The percent drug released within 30 min from the Dispril®-tablets and the Shelys formulation is 100% and 27%, respectively (Fig. 5.1). Dispril® showed a very fast dissolution rate, having released its entire drug content within the first 5 min. Within this interval the release from the Shelys formulation was only 2.4%.

*Figure 5.1: Dissolution profiles of Dispril® tablets (♦) and acetylsalicylic acid tablets of Shelys (■). USP 24 requirement: more than 80% released within 30 min*



### *5.4.2 Bioavailability*

As ASA is extensively metabolised in the gut and in plasma into salicylic acid (SA) the bio-analytical method used was optimised to measure the plasma concentration of both molecules. The individual plasma concentration/time profiles of the 10 subjects for both drugs (ASA and SA) are shown in Figures 5.2 and 5.3, while the individual pharmacokinetic parameters obtained from the plasma concentration/time profiles are shown in Tables 5.4 and 5.5.

For both formulations, plasma concentrations above the limit of quantitation (LOQ) were already observed in all subjects after 10 min. In case of Dispril® the individual plasma concentrations in the first 10 min ranged from 1.1 to 4.3 mg/l (median 2.1 mg/l) and 2.2 to 9.2 mg/l (median 3.7 mg/l) for ASA and SA, respectively. The corresponding values for the Shelys formulation were 0.1 to 1.8 mg/l (median 0.6 mg/l) and 0.1 to 4.9 mg/l (median 1.8 mg/l). In all subjects and for both formulations, the plasma concentration of acetylsalicylic acid was below the limit of detection (LOD) after 4 hrs, while the plasma concentration of salicylic acid remained above LOQ up to 15 hrs.

*Figure 5.2: Individual salicylic acid plasma concentration/time profiles (n=10) following administration of a single dose of 300 mg acetylsalicylic acid: Dispril® (A1); Shelys formulation (A2)*

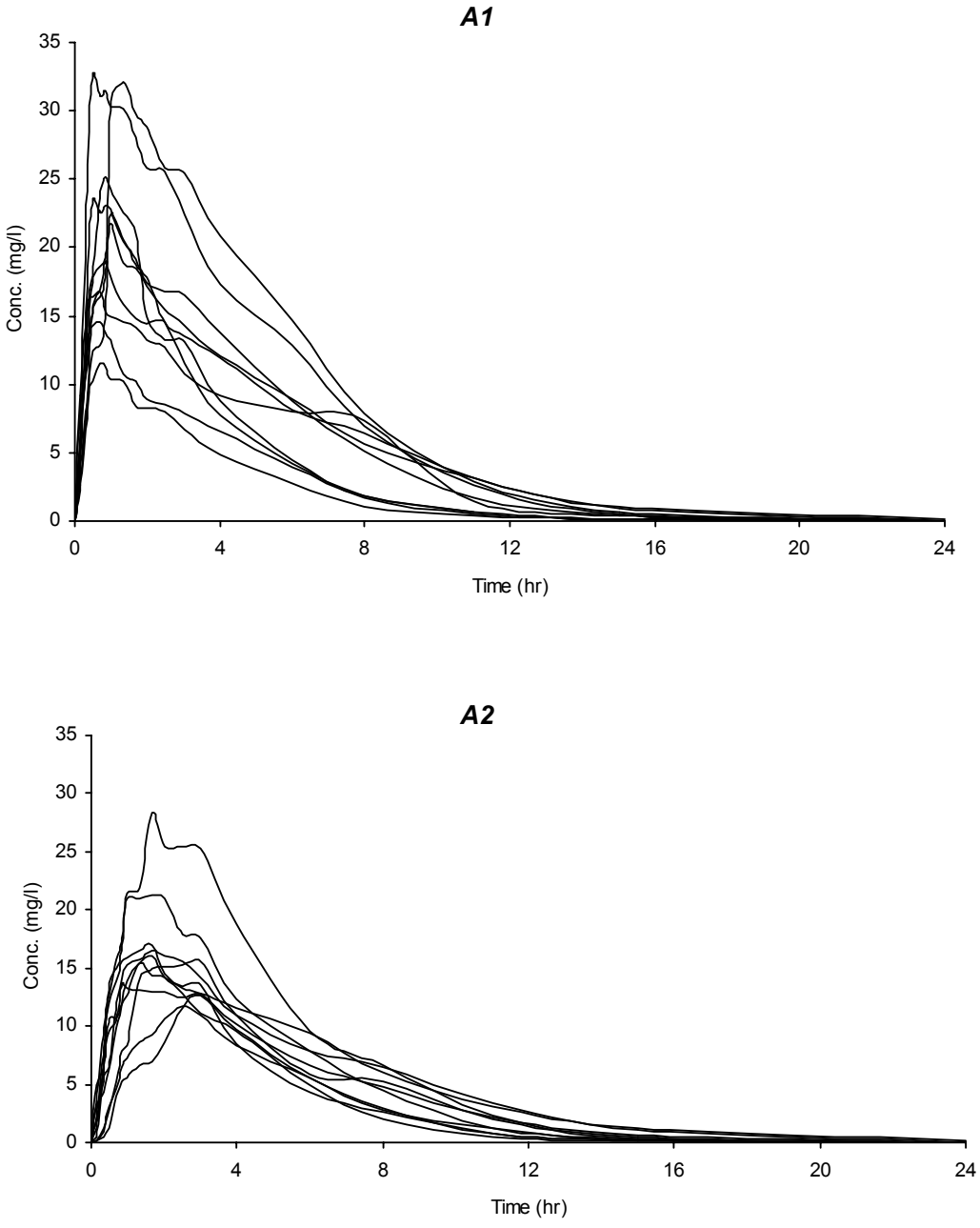


*Figure 5.3: Individual acetylsalicylic acid plasma concentration/time profiles (n=10) following administration of a single dose of 300 mg acetylsalicylic acid: Dispril® (A1); Shelys formulation (A2)*

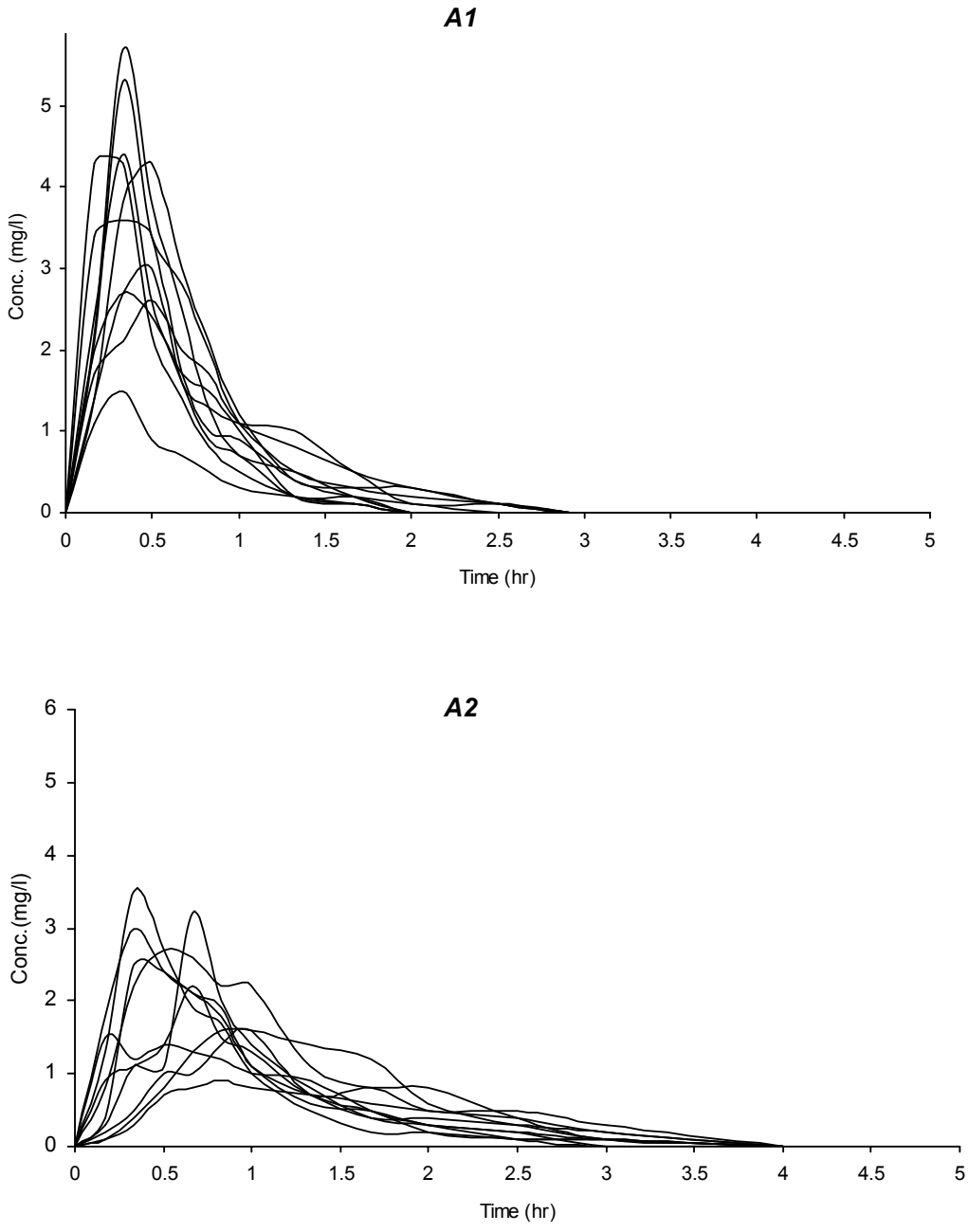


*Table 5.4: Individual $AUC_{0\text{-}24hr}$ and $C_{max}$ for acetylsalicylic acid and salicylic acid following administration of a single dose of 300 mg acetylsalicylic acid*

| Subject | Formulation and drug | | | |
|---|---|---|---|---|
| | Dispril® | | Shelys | |
| | ASA | SA | ASA | SA |
| | $AUC_{0\text{-}24hr}$ (µg.hr/ml) | | | |
| 1 | 2.48 - | 119.53 | 2.69 | 109.40 |
| 2 | 2.98 | 167.80 | 3.53 | 145.30 |
| 3 | 3.27 | 81.54 | 2.11 | 71.83 |
| 4 | 0.96 | 45.72 | 2.07 | 73.20 |
| 5 | 2.64 | 121.71 | 3.08 | 108.00 |
| 6 | 2.61 | 172.76 | 2.42 | 90.66 |
| 7 | 3.12 | 73.18 | 2.27 | 62.45 |
| 8 | 2.29 | 58.51 | 1.66 | 63.25 |
| 9 | 2.37 | 100.89 | 2.53 | 69.33 |
| 10 | 2.03 | 121.15 | 2.24 | 112.58 |
| **Mean** | **2.48** | **106.28** | **2.46** | **93.30** |
| **SD** | **0.66** | **42.85** | **0.54** | **26.43** |
| | $C_{max}$ (µg/ml) | | | |
| 1 | 2.6 | 23.4 | 3.0 | 21.3 |
| 2 | 5.7 | 32.6 | 2.7 | 28.3 |
| 3 | 5.3 | 25.2 | 1.5 | 15.4 |
| 4 | 1.5 | 11.6 | 2.2 | 16.0 |
| 5 | 3.6 | 22.4 | 2.5 | 13.7 |
| 6 | 3.0 | 32.1 | 1.6 | 15.5 |
| 7 | 4.3 | 19.0 | 1.6 | 12.7 |
| 8 | 4.4 | 14.6 | 0.9 | 11.7 |
| 9 | 4.3 | 16.8 | 3.5 | 17.0 |
| 10 | 2.7 | 21.8 | 3.2 | 16.5 |
| **Mean** | **3.7** | **21.9** | **2.3** | **16.8** |
| **SD** | **1.3** | **6.9** | **0.8** | **4.8** |

*Table 5.5: Individual $t_{max}$ values (hr) for acetylsalicylic acid and salicylic acid following administration of a single 300 mg dose of acetylsalicylic acid*

| Subject | $t_{max}$ (hr) | | | |
|---|---|---|---|---|
| | ASA | | SA | |
| | Dispril® | Shelys | Dispril® | Shelys |
| 1 | 0.5 | 0.3 | 0.5 | 1.7 |
| 2 | 0.3 | 0.5 | 0.5 | 1.7 |
| 3 | 0.3 | 0.1 | 0.8 | 1.3 |
| 4 | 0.3 | 0.6 | 0.6 | 1.7 |
| 5 | 0.3 | 0.3 | 1.0 | 0.8 |
| 6 | 0.5 | 0.9 | 1.3 | 3.0 |
| 7 | 0.5 | 1.0 | 0.8 | 3.0 |
| 8 | 0.3 | 0.8 | 0.7 | 2.5 |
| 9 | 0.2 | 0.3 | 0.7 | 1.6 |
| 10 | 0.3 | 0.6 | 1.0 | 1.6 |
| **Mean** | **0.4** | **0.6** | **0.8** | **1.9** |
| **Range** | **0.2 – 0.5** | **0.2 – 1.0** | **0.5 – 1.3** | **0.8 – 3.0** |

Figure 5.4 shows the mean plasma concentration/time profiles for ASA and SA, and Table 5.6 the mean (arithmetic) pharmacokinetic parameters and the statistical evaluation.

The mean $C_{max}$ obtained for the Shelys formulation was lower for both ASA and SA; their respective values being 39 and 23% lower than those obtained from Dispril®. Similarly, this formulation yielded a higher $t_{max}$ for both drugs. In case of salicylic acid, the mean $t_{max}$ of the Shelys formulation is about twice as high.

*Figure 5.4: Mean (n=10) acetylsalicylic acid (A) and salicylic acid (B) plasma concentration/time profiles following administration of a single dose of 300 mg acetylsalicylic acid: Dispril® (◆); Shelys formulation (■).*

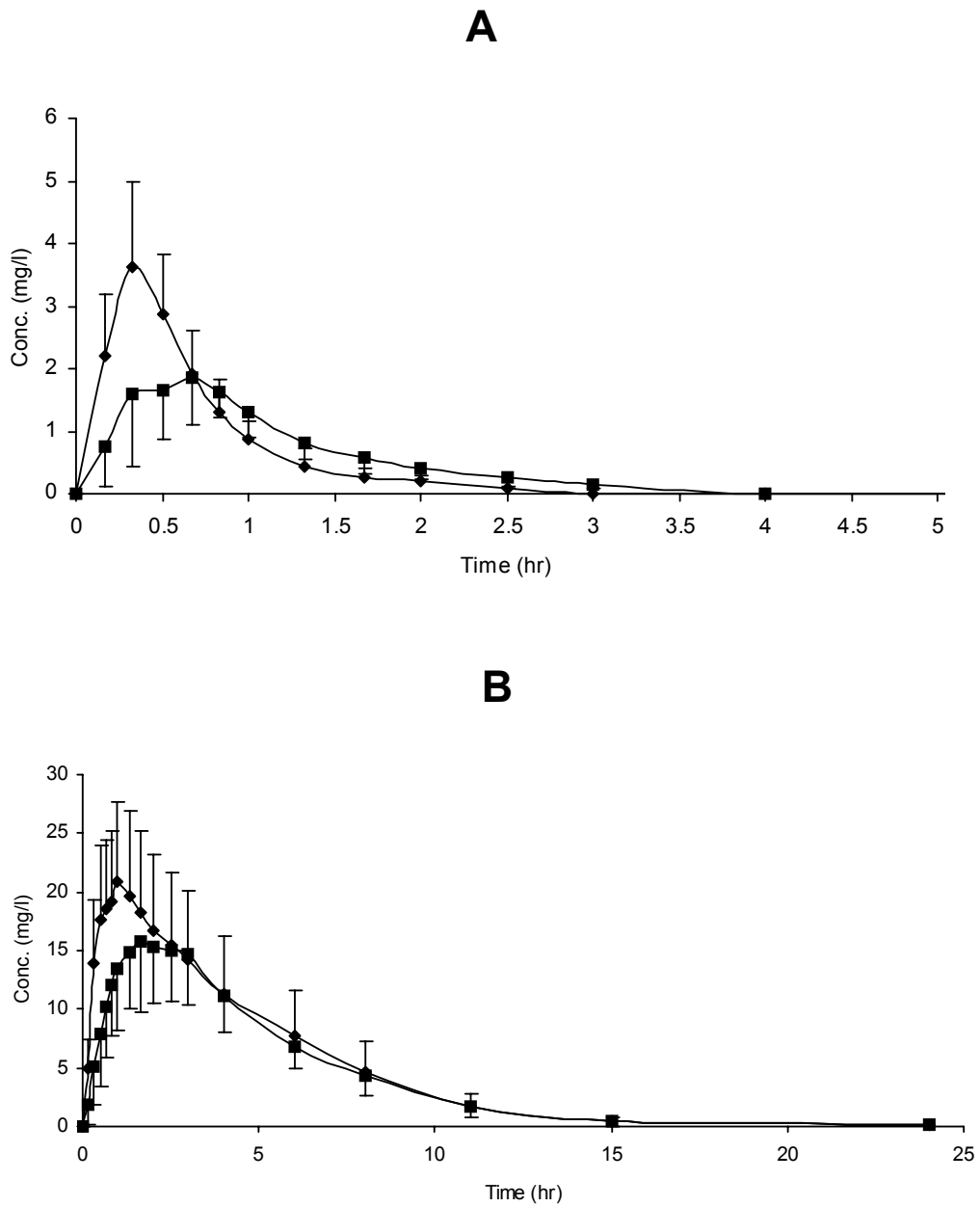


*Table 5.6: Mean (n=10)(arithmetic) pharmacokinetic parameters (± SD) for ASA and SA following oral administration of a single dose of 300 mg acetylsalicylic acid.*

| Parameter | Formulation | |
|---|---|---|
| | Dispril® | Shelys |
| **Acetylsalicylic acid** | | |
| $AUC_{0-24hr}$ (µg.hr/ml) | 2.48 ± 0.66 | 2.46 ± 0.54 |
| $C_{max}$ (µg/ml) | 3.7 ± 1.3** | 2.3 ± 0.8* |
| $C_{max}/AUC_{0-24hr}$ (hr) | 1.51 ± 0.31** | 0.92 ± 0.31* |
| $t_{max}$ (hr) | 0.4 ± 0.1** | 0.6 ± 0.3* |
| $t_{0.5}$ (hr) | 0.3 ± 0.1 | 0.5 ± 0.1 |
| **Salicylic acid** | | |
| $AUC_{0-24h}$ (µg.hr/ml) | 106.28 ± 42.85 | 93.30 ± 26.43 |
| $C_{max}$ (µg/ml ) | 21.9 ± 6.9 | 16.8± 4.8 |
| $C_{max}/AUC_{0-24hr}$ ($hr^{-1}$) | 0.22 ± 0.05 | 0.18 ± 0.03 |
| $T_{max}$ (hr) | 0.8 ±0.3** | 1.9 ± 0.7* |
| $t_{0.5}$ (hr) | 2.3 ± 0.6 | 2.0± 0.4 |

* Significantly different from **, the corresponding value of the other formulation ($p < 0.05$, Scheffe test).

Statistical analysis of the pharmacokinetic data shows that the two formulations yielded similar $AUC_{0-24h}$ values for both ASA and SA, indicating that the extent of absorption of the two drugs from both formulations was similar. The absorption rate parameters ($C_{max}$, $t_{max}$, and $C_{max}/AUC_{0-24h}$) obtained from Dispril® (for both ASA and SA) were higher compared with those of the Shelys formulation. In the case of ASA, the difference between the corresponding parameters of the two formulations was significant ($p < 0.05$), indicating that ASA from the Dispril® is absorbed faster compared with the Shelys formulation.

However, while there was no significant difference between $C_{max}$ of SA measured for both formulations, Dispril® achieved its $C_{max}$ faster as the $t_{max}$ of this formulation was significantly shorter than that of Shelys.

## 5.5 Discussion

Most pharmacokinetic data reported in literature are from studies where the administered dose is at least 500 mg of acetylsalicylic acid. Since the elimination kinetics of this drug are dose-dependent it is not possible to compare the literature values in a linear model. AUC and $C_{max}$ values of 7.1 mg.h/l and 5.5 mg/l, and of 179.7 mg.h/l and 33.4 mg/l for ASA and SA, respectively, have been reported after administration of a single 500 mg dose acetylsalicylic acid (Siegmund et al., 1998). The values obtained in this study are in a similar range.

This study aimed at investigating whether the very low in vitro drug release obtained from the Shelys formulation would result into a poor bioavailability (hence efficacy) of this formulation. The extent of absorption for both formulations was similar (for both SA and ASA) indicating that with respect to this primary pharmacokinetic parameter, the two formulations have a similar in vivo behaviour. Since the inhibition of the platelet aggregation (which is solely attributed to acetylsalicylic acid) is irreversible and dose dependent (Burch et al., 1978; Patrignani et al., 1982), there will probably be no difference in this activity between both formulations as their AUC's of ASA were similar.

The rate of absorption from the Shelys formulation (as evidenced by $t_{max}$ and $C_{max}$/AUC of both ASA and SA) was significantly lower compared with that of Dispril®, an observation consistent with the in vitro drug release from both ASA formulations. Nevertheless, the two formulations yielded a maximum salicylic acid plasma concentration ($C_{max}$) in excess of 90.0 mg/l, which is well above the minimal level (20 mg/l) required for effective analgesic action of salicylic acid (Dollery, 1999). Thus the efficacy of both formulations to relieve pain will probably be similar although Dispril®, having a shorter $t_{max}$, could have a faster onset of action.

The results show that although there was a large difference in the in vitro drug release between both formulations, the observed difference in $C_{max}$ between the formulations (for both ASA and SA) was not of the same magnitude. Similar observations have been reported in the literature for nitrofurantoin tablets (Gouda et al., 1987); where tablet formulations having very low in vitro drug release exhibited only a moderate reduction in $C_{max,}$ while the AUC was unaffected. These

observations highlight the limited predictive power of the in vitro dissolution tests about the in vivo drug availability and calls for a cautious approach when predicting in vivo efficacy from in vitro data.

## 5.6 Conclusion

The pharmacokinetic data showed that despite the large difference in in vitro drug release, the two formulations had similar bioavailability profiles with regard to the extent of drug absorption. However, the rate of absorption from the Shelys formulation was significantly lower compared to that of Dispril®. Although the Shelys formulation had a moderately lower $C_{max}$, its influence on the clinical efficacy will probably be insignificant as $C_{max}$ is in excess of the minimum plasma concentration required for analgesia.

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# 6. INFLUENCE OF TROPICAL CLIMATIC CONDITIONS ON BIOAVAILABILITY OF ENTERIC COATED DICLOFENAC SODIUM TABLET FORMULATIONS

## 6.1 Introduction

The stability of the in vitro dissolution of diclofenac sodium from four enteric-coated tablet formulations marketed in Tanzania has been previously described in Chapter 3. The in vitro drug release and drug content from the formulations was determined before and after storage for 6 months at simulated tropical conditions (75% RH, 40°C). Immediately after purchase, the in vitro drug release of all formulations was above the USP 24 tolerance limits for drug release. Upon storage at simulated tropical conditions the drug release of the innovator brand (Voltaren® 50 mg) from Novartis and of the formulation from Remedica remained stable for the entire test duration. However, the drug release of the Intas and the Camden formulations decreased dramatically: the formulation from Camden released only 10% of the drug after 45 min of dissolution testing in phosphate buffer (pH 6.8). The diclofenac content in all formulations remained stable during the 6 months period.

The objective of this work is to investigate whether the observed trends of the in vitro drug release after exposure to simulated tropical conditions are reflected in vivo. The formulations with the best and worst stability of the in vitro drug release, Voltaren® 50 (Novartis) and Diclo® 50 (Camden), respectively, were investigated.

## 6.2 In vitro evaluation

### Study plan

Samples from Novartis (Voltaren® 50, batch 00D03BT) and Camden (Diclo® 50, batch 000523) were purchased in Belgium and Tanzania, respectively. Part of the tablets was stored at ambient conditions for 3 months, whereas the remaining part was stored for 3 months at the stability test conditions (40°C and 75% RH). After 3

months the samples were evaluated for their in vitro drug release and in vivo drug availability.

Dissolution tests

**USP 2 method**

The in vitro drug release of the tablet formulations was determined by performing a dissolution test in an acid medium (0.1N HCl) for 2.0 hr, thereafter the tablets were transferred into a phosphate buffer (pH 6.8) and tested for 50 min. The volume of the dissolution media was 900 ml and was maintained at 37 ± 0.5°C. The rotational speed of the paddles was 50 rpm. A 5 ml sample was taken after 2 hr testing in the acid medium. During the buffer stage, samples (5 ml) were taken at 10, 20, 30, 40, 45 and 50 min.

**USP 3 method**

The diclofenac tablets from the Camden formulation stored at simulated tropical conditions were tested for their dissolution characteristics using reciprocating cylinders (USP 3 apparatus). The test was first performed in acid medium (0.1N HCl) for 2.0 hr, thereafter the tablets were transferred into a phosphate buffer (pH 6.8) and tested for 50 min. The volume of the dissolution media in both cases was 250 ml and was maintained at 37 ± 0.5°C. The apparatus was operated at 21 dips per min. A 2 ml sample was taken after 2 hr testing in acid medium. During the buffer stage, samples (2 ml) were taken at 10, 20, 30, 40, 45 and 50 min.

Disintegration test.

The tablets from Camden stored at simulated tropical conditions were tested for their disintegration properties as described in the European Pharmacopoeia for enteric coated tablets: a 2 hr test was conducted in 1000 ml 0.1N HCl, thereafter the acid solution was replaced with a phosphate buffer pH 6.8 and the test was continued for a further 1 hr. Both solutions were maintained at 37 ± 0.2°C and the apparatus was operated at 30 cycles per min.

Equipment

A dissolution tester VK 7010 linked to an automatic sampler VK 8000 (VanKel Technology, Cary, USA) was used for the USP method 2 test. A VK Bio Dis

reciprocating cylinder tester linked to an automatic sampler VK 8000 (VanKel Technology, Cary, USA) was used for the USP 3 method test.

The Pharma Test PTZ E apparatus (Pharma Test, Hainburg, Germany) was used for the disintegration test.

## 6.3 In vivo evaluation

Clinical protocol

Twelve volunteers (8 males) participated in the study after giving an informed consent. The age of the volunteers ranged from 19 to 38 years (mean 24 yrs). Their weight ranged from 59 to 88 kg (mean 65 kg) and their body mass index from 19.8 to 27.1 kg/$m^2$ (mean 22.4 kg/$m^2$). The volunteers were healthy as proven by medical history, physical examination, electrocardiogram, and lab tests (haematological and urine). Volunteers with suspected history of alcoholism, barbiturate abuse and allergy to diclofenac, aspirin and/or nonsteroidal analgesic agents were excluded from the study. Pregnant, lactating females or females with childbearing potential without adequate contraception were also excluded from the study. The Ethics Committee of Ghent University Hospital approved the clinical protocol.

Prior and concomitant therapy

During the entire trial, subjects were required not to use any medication other than the trial medication. The exceptions to this rule were paracetamol and oral contraceptives. Subjects were allowed to use paracetamol up to 3 days before drug administration in each period. The use of paracetamol could be allowed by the clinical investigator (no more than 3 x 500 mg per day and no more than 3 g per week) for the treatment of headache or other pain. Oral contraceptives (OAC) were allowed. In case paracetamol or OAC were used, the dose and dosage regimen were recorded on the Concomitant Therapy Form which formed a part of the Case Report Form (CRF).

Procedure

The subjects fasted for at least 10 hr before drug administration. Drinking of water was allowed up to 2 hr before drug administration. The subjects were required not

to consume grapefruit, grapefruit juice or beverages containing alcohol or quinine between 24 hr before and 16 hr after drug dosing per period. The subjects were required to take the whole tablet together with 200 ml of water. From two hr after dosing, intake of water was allowed. A standard breakfast was given 3 hr post dosing and lunch 6 hr post dosing. The standard breakfast consisted of four slices of bread, one slice of ham, one slice of cheese, butter jelly and two cups of decaffeinated coffee or tea with, if desired, milk and/or sugar. The subjects remained in the testing facility for 16 hr after receiving the dose.

Randomisation

The study was an open randomized 4-period cross-over design. The washout time between periods was 3 days. In each period subjects were given a single dose of 50 mg diclofenac sodium as an enteric coated tablet.

Subjects entering the study were allocated a number from 1 to 12. The randomisation scheme shown in Table 6.1 was used to assign the subjects to either of the four treatments:

- ✓ D1 (Diclo® 50 stored for 3 months at ambient conditions)
- ✓ D2 (Diclo® 50 stored for 3 months at simulated tropical conditions)
- ✓ D3 (Voltaren® 50 stored for 3 months at ambient conditions)
- ✓ D4 (Voltaren ® 50 stored for 3 months at simulated tropical conditions)

*Table 6.1: Randomisation scheme*

| Subject | Period 1 | Period 2 | Period 3 | Period 4 |
|---|---|---|---|---|
| 1 | D1 | D2 | D3 | D4 |
| 2 | D2 | D3 | D4 | D1 |
| 3 | D3 | D4 | D1 | D2 |
| 4 | D4 | D1 | D2 | D3 |
| 5 | D2 | D3 | D4 | D1 |
| 6 | D3 | D4 | D1 | D2 |
| 7 | D4 | D1 | D2 | D3 |
| 8 | D1 | D2 | D3 | D4 |
| 9 | D3 | D4 | D1 | D2 |
| 10 | D4 | D1 | D2 | D3 |
| 11 | D1 | D2 | D3 | D4 |
| 12 | D2 | D3 | D4 | D1 |

Blood Sampling

Venous blood samples of 5 ml (to obtain 2 ml of plasma) were taken from an antecubital vein within one hr before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 16 hr after the drug administration. Blood samples were collected in heparinized tubes and centrifuged for 10 min at 1500g within 2 hr after collection. Separated plasma was aspirated with a disposable pipette and transferred in plastic tubes. The tubes were sealed by means of polyethylene stoppers, and labelled with the investigator's name, trial number, CRF identity, subject initials, date and time of sampling. Samples were stored at -20°C until assayed.

### *6.3.1 Plasma drug analysis*

Plasma diclofenac concentrations were measured by a reversed-phase HPLC with UV detection according to the method described by Giagoudakis and Markantonis (1998).

#### *6.3.1.1 Materials*

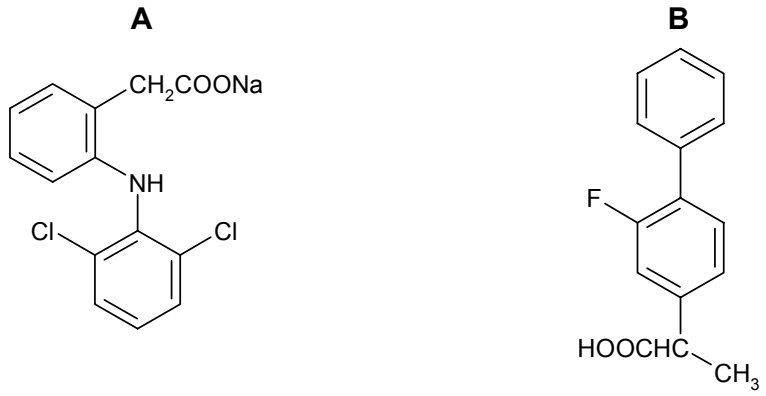
All solvents were of HPLC grade and all reagents were of analytical grade. Diclofenac sodium was supplied by Alpha Pharma (Zwevegem, Belgium) flurbiprofen was obtained from Sigma Aldrich (Steinheim, Germany). Sodium acetate anhydrous, acetic acid, orthophosphoric acid and n-hexane were obtained from Vel (Leuven, Belgium). Acetonitrile, methanol and isopropyl alcohol were obtained from Biosolve (Valkenswaard, The Netherlands).

#### *6.3.1.2 Methods*

Standard solutions

Stock solutions containing 160 mg/l diclofenac sodium and 50 mg/l of flurbiprofen (internal standard) (Fig 6.1) were prepared in methanol. These were diluted in methanol (1:100) to make solutions of 1.6 µg/ml and 0.5 µg/ml diclofenac sodium and flurbiprofen, respectively. From the diclofenac sodium solution, working solutions of 16, 40, 80, 400, 800, 1200, and 1600 ng/ml were made by appropriate dilutions in methanol. The working solution for the internal standard was 500 ng/ml.

*Figure 6.1: Structural formula of diclofenac sodium (A) and flurbiprofen (B)*




Extraction procedure

To 500 µl of a plasma sample with unknown drug concentration or to 500 µl blank plasma, 250 µl of the internal standard solution (flurbiprofen) was added and the mixture was vortexed for 30 s. Subsequently, 1 ml of 2.5M orthophosphoric acid was added followed by 1 min vortexing. Extraction was achieved by adding 3 ml of an n-hexane/isopropyl alcohol mixture (80:20 v/v), followed by 2 min vortex mixing. The mixture was centrifuged for 10 min at 3000 rpm. Using a Pasteur pipette, the upper organic layer was transferred to a 10 ml disposable tube and evaporated to dryness at 37°C under a gentle stream of dry nitrogen. The residue was reconstituted in 200 µl mobile phase and a 25 µl aliquot was injected into the loop.

Chromatographic conditions

The mobile phase consisted of a mixture of 0.1M sodium acetate (pH adjusted to 6.5 with 10% acetic acid) and acetonitrile (70/30, v/v) and was pumped at a flow rate of 1 ml/min through the column. Plasma concentrations were monitored by UV absorbance at 280 nm.

The HPLC equipment (Lachorm, Merck, Darmstadt, Germany) comprised of a L 7100 isocratic pump, an integrator L 7000 and a UV-detector (Spectra system UV 2000, Spectra Physics, Darmstadt, Germany). Chromatographic separation was achieved using a column LiChrospher RP-18® (5 µm) and guard column LiChrospher 100 RP-18® (5 µm).

#### 6.3.1.3 *Validation of the analytical method*

The method used to determine the diclofenac sodium plasma concentration was validated as recommended by Shah et al. (1992) and revised in the FDA Guidance for industry (2001).

#### 6.3.1.4 *Pharmacokinetic and statistical analysis*

The peak plasma concentration ($C_{max}$), the time to reach the peak plasma concentration ($t_{max}$) and the time to the onset of absorption (lag time) were obtained from the individual plasma concentration vs. time profiles. The area under the plasma concentration-time curve to 16 hr post dosing ($AUC_{0\text{-}16hr}$) was calculated by linear trapezoidal summation. Terminal elimination rate constant ($\lambda_z$) was determined by log-linear regression of the terminal points of the plasma concentration-time curve and the half-life time ($t_{0.5}$) was defined as $0.693/\lambda_z$.

The MW/Pharm software package (v.3.01, Mediware 1987-1991, Utrecht, The Netherlands) was used for the pharmacokinetic analysis.

Statistical analysis of the pharmacokinetic data was performed using a two-way ANOVA. The data were tested for normal distribution with the Kolmogorov-Smirnov test. The homogeneity of variances was tested with the Levene's test. To further compare the effects of the different treatments a multiple comparison among pairs of means was performed using the Scheffe test with $p < 0.05$ as significance level. For all statistical analysis the program SPSS version 10.0 was used.

## 6.4 Results

### *6.4.1 Validation of the bioanalytical method*

#### 6.4.1.1. *Linearity*

The calibration range (20-2000 ng/ml) was chosen on the basis of expected plasma concentrations after single dose administration of a 50 mg enteric diclofenac sodium tablet (Giagoudas and Markantonis, 1998; Shimamoto et al., 2000; Terhaag et al., 2000). Seven calibration standards (20, 50,100, 500, 1000, 1500 and 2000 ng/ml) were made by spiking 500 µl blank plasma with 250 µl of the appropriate diclofenac sodium working solution and 250 µl of internal standard

working solution. A blank sample was included in the calibration curve to ensure that no interfering components were co-eluted with the drug and the internal standard or introduced during preparation of the standards. The mean calibration curve (n = 9) of the diclofenac concentration versus the ratio of peak areas of diclofenac sodium and the internal standard was calculated.

The calibration curve obtained showed that the analytical method is linear over the entire concentration range: Y = 0.0040x (± 0.0002) + 0.0062 (± 0. 0025) with a coefficint of determination ($R^2$) of 0.9990 (± 0.00008).

#### *6.4.1.2 Limits of detection and quantitation*

The limit of detection is defined as the minimum detectable level of the analyte under the assay conditions. It has been defined as the concentration of the analyte that produces a response equivalent to the blank signal plus three times the standard deviation of the blank. The blank signal is defined as the mean of the Y intercept of the calibration curves. From regression analysis of the calibration curves, the limit of detection (LOD) of this method was determined at 2 ng/ml.

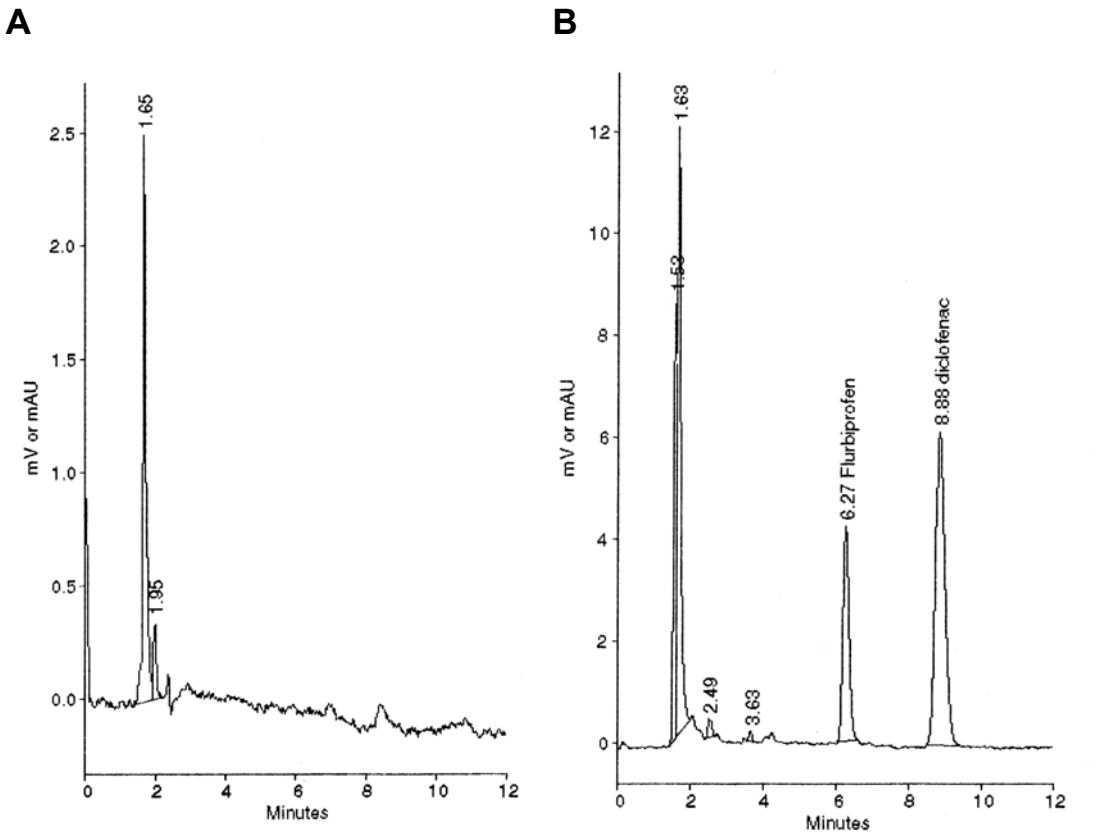
The limit of quantitation (LOQ) is defined as the minimum concentration of the analyte in the matrix that can be determined with an acceptable precision, accuracy and variability. The precision, accuracy and variability were determined using a minimum of five samples to calculate the coefficient of variation and accuracy. The lowest concentration in the calibration curve is usually taken as the limit of quantitation (LOQ) provided it can be reliably quantitated with an acceptable accuracy (FDA guidance: RSD less than 20% and accuracy between 80 and 120%). For this analytical method the lowest concentration that was determined with an acceptable precision and accuracy is 20 ng/ml. The RSD obtained for this concentration was 11.5% with an accuracy of 91.3%.

#### *6.4.1.3 Selectivity*

The extraction and chromatographic procedures were capable of separating the plasma constituents from the drug and the internal standard. At a flow rate of 1 ml/min retention times of about 6.5 min for flurbiprofen and about 9.0 min for diclofenac were recorded. There were no interfering peaks at these retention times as evidenced by the representative chromatograms of blank plasma and of plasma

(spiked with the internal standard) obtained from a volunteer 2.5 hr after administration of a 50 mg diclofenac sodium enteric coated tablet (Fig. 6.2).

*Figure 6.2: The chromatograms of blank plasma (A) and of plasma (spiked with IS) obtained from a volunteer 2.5 hr after administration of a 50 mg diclofenac sodium tablet (B)*




### *6.4.1.4 Precision*

Precision refers to the variation or the scatter of the measurements around the mean value. It is also the measure of reproducibility of the whole analytical method including the sample preparation, extraction, reconstitution and sample analysis.

The within day (repeatability) and between day (reproducibility) precision was calculated as the relative standard deviation (RSD) of the mean peak area obtained after repeated injection.

The repeatability of the analytical method was determined for each concentrations of the calibration standards by calculating the variations in the measured peak area for the same sample injected at different times during the same day. For reproducibility, the samples were injected at different days. The results for precision of the method are shown in Table 6.2.

A relative standard deviation (RSD) between 5.4% and 11.2% and between 3.1% and 11.8% was obtained for repeatability and reproducibility, respectively. These values were not greater than the acceptance criteria of 15%.

*Table 6.2: Intra- (repeatability) and inter-day (reproducibility) precision for the determination of diclofenac sodium in plasma (n = 8)*

| Concentration (ng/ml) | Precision (RSD %) | |
|---|---|---|
| | Intra-day | Inter-day |
| 20 | 8.3 | 11.5 |
| 50 | 8.0 | 8.6 |
| 100 | 9.8 | 4.1 |
| 500 | 11.0 | 3.1 |
| 1000 | 11.2 | 10.5 |
| 1500 | 5.4 | 11.8 |
| 2000 | 7.0 | 7.2 |

### *6.4.1.5 Accuracy*

Accuracy describes the closeness of the mean test result (obtained by applying the method) to the true concentration of the analyte. Accuracy was determined by comparing the measured diclofenac sodium concentrations and the spiked concentrations in plasma. The results of the measured diclofenac sodium concentrationsfrom the spiked plasma samples and the RSD-value at each concentration are summarized in Table 6.3. At all concentrations tested the mean value did not deviate by more than 10% from the nominal concentration. The RSD

values fall within the acceptance level (FDA guidance: RSD less than 15%, except for the LOQ where it should not exceed 20%).

*Table 6.3: Accuracy (n = 8) for the determination of diclofenac sodium in plasma*

| | Concentration (ng/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **20** | **50** | **100** | **500** | **1000** | **1500** | **2000** |
| Mean | 18.3 | 49.1 | 93.4 | 486.4 | 1029.7 | 1506.3 | 2028.2 |
| RSD (%) | 11.5 | 7.4 | 8.2 | 2.5 | 5.9 | 6.1 | 4.5 |
| % of nominal conc. | 91.5 | 98.2 | 93.4 | 97.3 | 103.0 | 100.4 | 101.4 |

### *6.4.1.6 Recovery*

Recovery is defined as the concentration of diclofenac sodium found in the spiked samples after the extraction procedure. Appropriate concentrations of diclofenac sodium methanolic solutions were added to blank plasma to make concentrations of 20, 50, 100, 500, 1000, 1500 and 2000 ng/ml. After extraction and reconstitution, their peak areas were compared with those obtained by injecting the same concentration of diclofenac sodium in mobile phase. Recovery was calculated as the ratio of the peak area of the extracted sample versus the unextracted sample multiplied by 100. The coefficient of variation of the recovery at different concentrations was also determined.

Table 6.4 shows that values obtained for recovery were above 75% in the 20 to 2000 ng/ml concentration range and were reproducible (RSD between 3.1 and 9.7%). These values are within the minimum value required for recovery (75%) with a maximum RSD of 15%.

*Table 6.4: Recovery of diclofenac sodium and flurbiprofen for the determination of diclofenac sodium in plasma (n = 8)*

| | Conc. (ng/ml) | Recovery (%) | RSD (%) |
|---|---|---|---|
| Diclofenac sodium | 20 | 85.2 | 9.2 |
| | 50 | 95.3 | 9.1 |
| | 100 | 95.3 | 9.7 |
| | 500 | 94.8 | 6.1 |
| | 1000 | 90.2 | 3.1 |
| | 1500 | 93.0 | 4.4 |
| | 2000 | 98.3 | 4.9 |
| Flurbiprofen | 625 | 97.2 | 5.6 |

### *6.4.1.7 Stability of diclofenac sodium in plasma*

The stability of the drug in plasma was determined at concentrations of 50, 500 and 1500 ng/ml in plasma stored at -20°C for 2 months. Analyses were performed after 30 and 60 days. The determined concentrations and standard deviation, shown in Table 6.5, indicated that the drug was stable in plasma for at least 60 days.

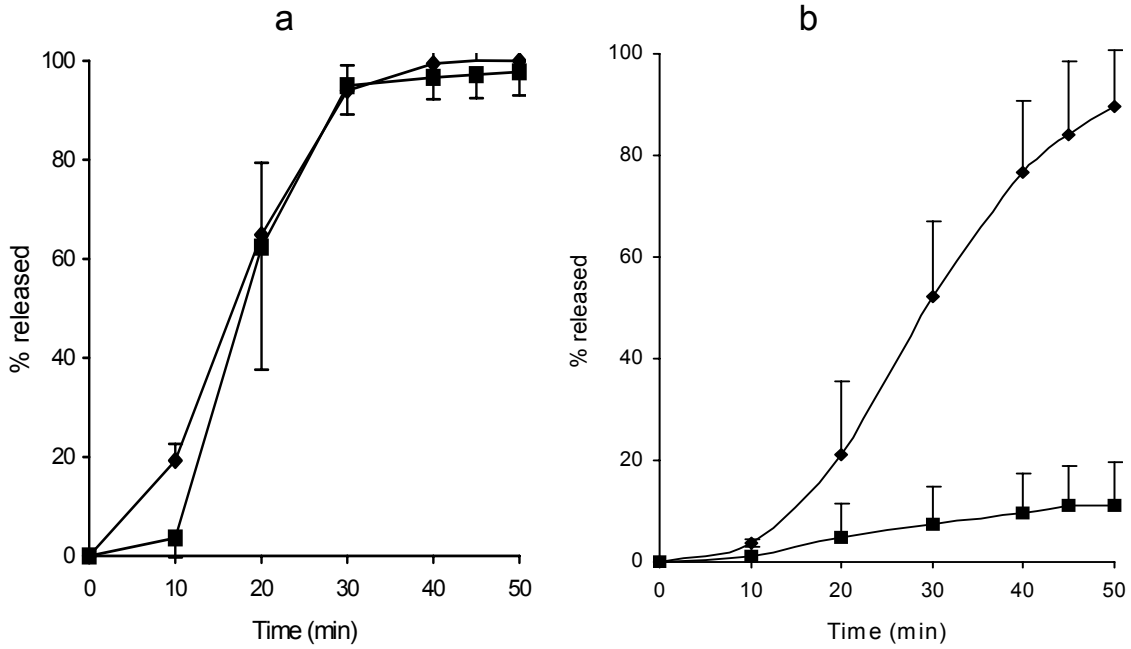
*Table 6.5: Stability of diclofenac sodium in plasma after storage at -20°C (n = 8)*

| Theoretical conc. (ng/ml) | Actual concentration (ng/ml)(± SD) after | |
|---|---|---|
| | 30 days | 60 days |
| 50 | 49.7 ± 3.1 | 49.2 ± 3.4 |
| 500 | 494.0 ± 20.7 | 511.0 ± 22.9 |
| 1000 | 1063.0 ± 56.7 | 1002.0 ± 53.6 |

### 6.4.2 *In vitro drug release*

The profiles obtained after dissolution testing (Fig 6.3) show that there was a remarkable difference between the stability of the two formulations after 3 months storage under simulated tropical conditions. The percent drug release from the Novartis formulation remained at 100% after 3 months of storage under simulated tropical conditions, while the release of the Camden formulation after 45 min decreased from 84% to 11%. The tablets from this formulation failed to disintegrate during the entire dissolution test, retaining their original shape and forming a plastic mass.

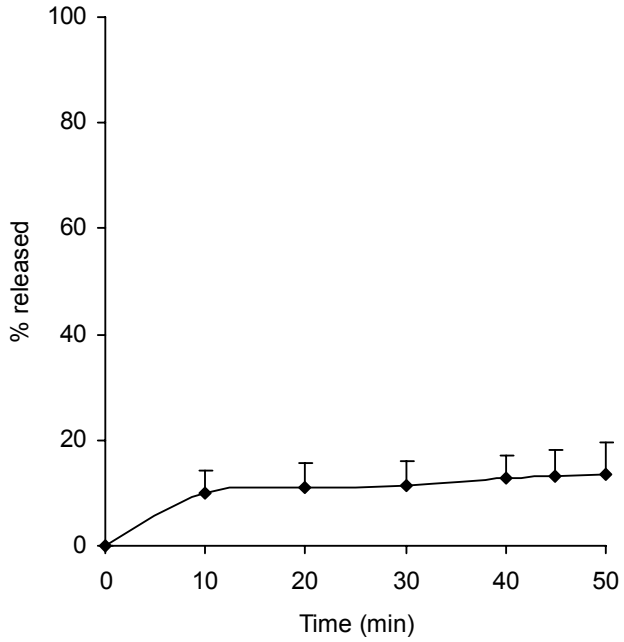
*Figure 6.3: Dissolution profiles of Voltaren® 50 (a) and Diclo® 50 (b) tablets stored for 0 (◆) and 3 (■) months at simulated tropical conditions (75% RH, 40°C), following dissolution testing in a phosphate buffer medium (pH 6.8) using the USP 2 apparatus*




The dissolution profile of the Camden formulation stored at simulated tropical conditions and obtained using the reciprocating cylinder method (USP method 3) was similar to the one obtained using the USP 2 method (Fig. 6.4). The percent drug release after 45 min in phosphate buffer pH 6.8 was 13%. The tablets failed to disintegrate for the entire duration of the test as they retained their original

shape and formed a plastic mass. When performing a disintegration test as described in the European Pharmacopoeia, all 6 tablets of the Camden formulation failed to disintegrate after 2 hr in a 0.1N HCl and 1 hr in a phosphate buffer (pH 6.8).

*Figure 6.4: Dissolution profiles of Diclo® 50 tablets stored for 3 months at simulated tropical conditions (75% RH, 40°C) following dissolution testing in phosphate buffer (pH 6.8) using the USP 3 apparatus*




### *6.4.3 In vivo drug availability*

As one subject did not follow the study protocol (the tablet was broken in two and not taken as a whole as it was required by the protocol) the plasma concentration profiles and pharmacokinetic data of this volunteer (number 8) were not included in the data analysis The individual diclofenac plasma concentrations of 11 subjects after single administration of a 50 mg enteric-coated diclofenac sodium tablet exposed to different storage conditions are shown in Fig. 6.5 and 6.6, and the individual pharmacokinetic parameters in Table 6.6 and 6.7. Since in most subjects the plasma drug concentration was below the limit of detection at 10 hr, the graphs have been truncated at 10 hr.

*Figure 6.5: Individual diclofenac plasma concentration/time profiles (n=11) after administration of a single dose of 50 mg diclofenac sodium as an enteric coated tablet: Diclo ®50 stored at ambient conditions (D1); Diclo® 50 stored at simulated tropical conditions (D2)*

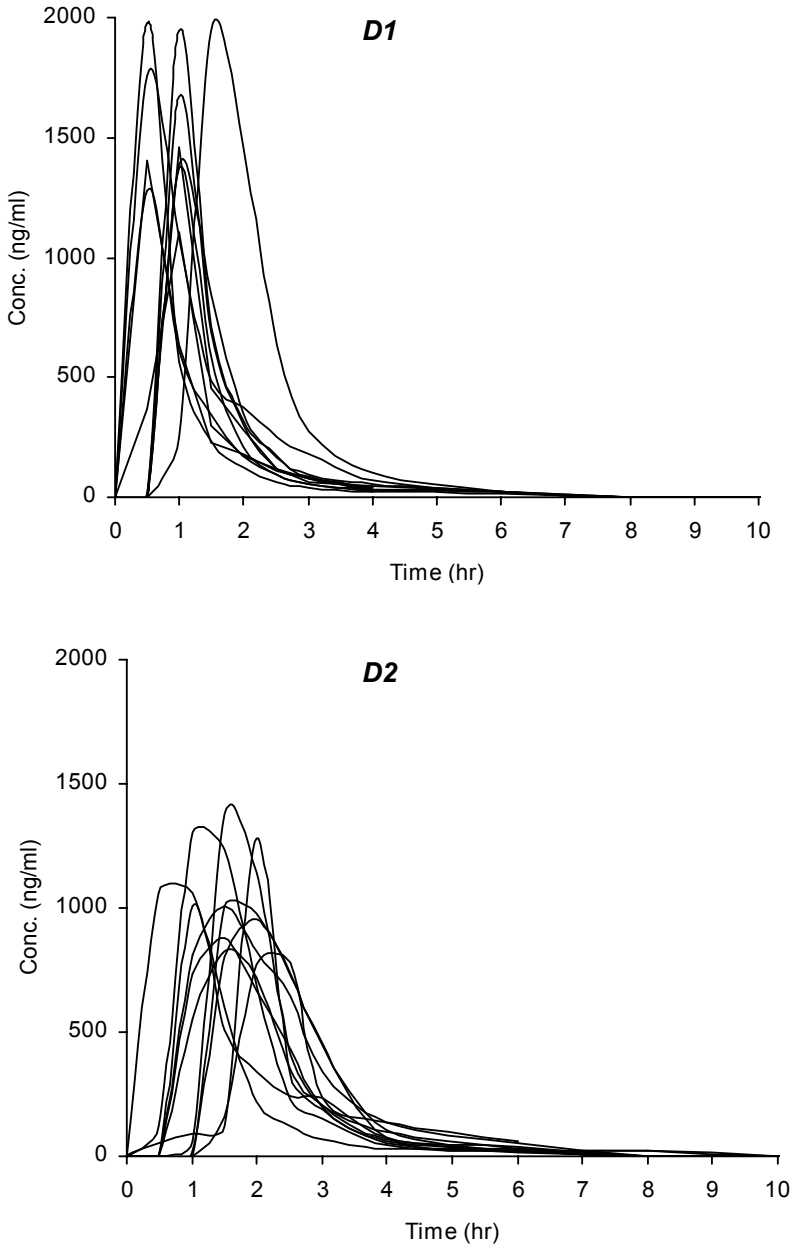


*Figure 6.6: Individual diclofenac plasma concentration/time profiles (n=11) after administration of a single dose of 50 mg diclofenac sodium as an enteric coated tablet: Voltaren® 50 stored at ambient conditions (D3); Voltaren® 50 stored at simulated tropical conditions (D4)*

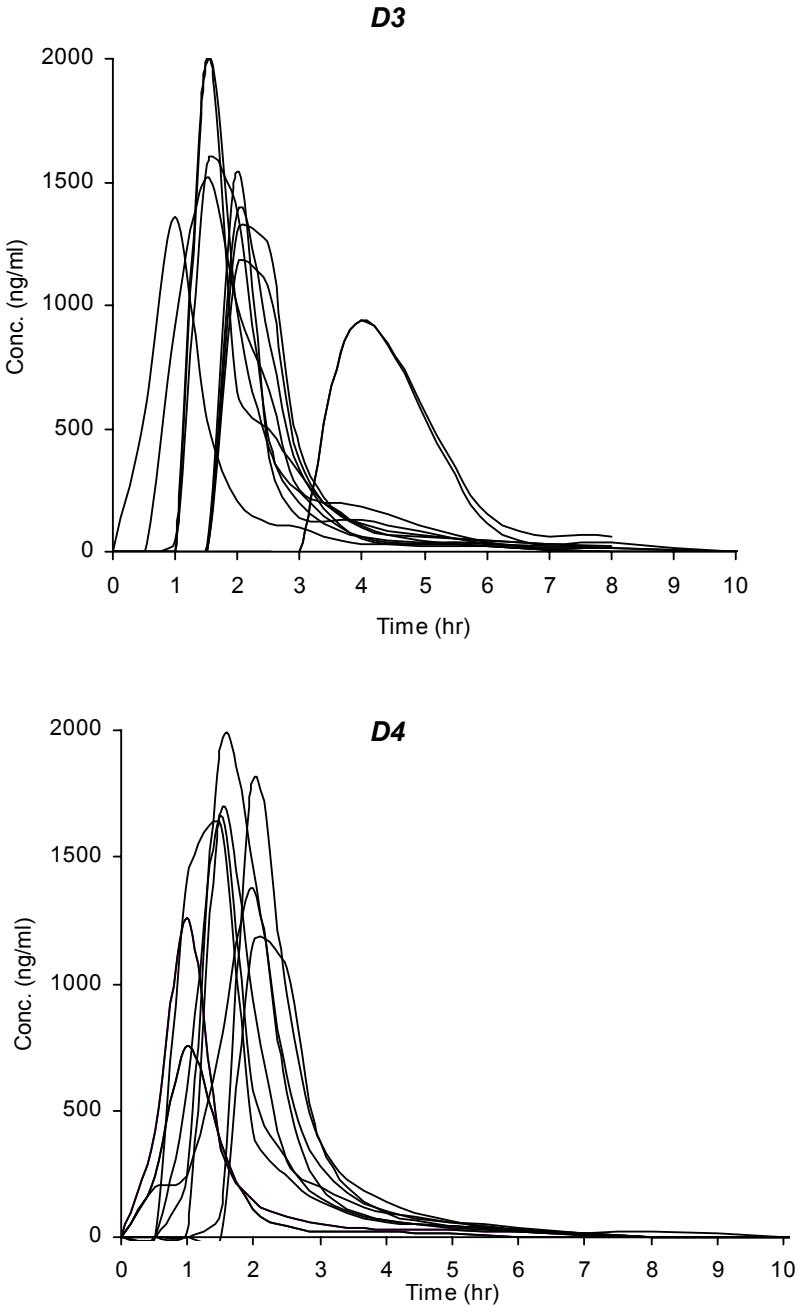


*Table 6.6: Individual $AUC_{0\text{-}16hr}$ and $C_{max}$ values of 11 subjects following administration of a single dose of 50 mg diclofenac sodium as an enteric coated tablet*

| Subject | Formulation[a] | | | |
|---|---|---|---|---|
| | D1[a] | D2[a] | D3[a] | D4[a] |
| | **$AUC_{0\text{-}16hr}$ (ng.hr/ml)** | | | |
| 1 | 1045 | 1357 | 2119 | 2008 |
| 2 | 1578 | 1963 | 1497 | 1961 |
| 3 | 1109 | 1260 | 1418 | 1497 |
| 4 | 1040 | 1021 | 1186 | 766 |
| 5 | 1529 | 1888 | 2192 | 1455 |
| 6 | 1867 | 1749 | 1505 | 1166 |
| 7 | 1299 | 1587 | 1510 | 1817 |
| 9 | 2570 | 2155 | 1623 | 2091 |
| 10 | 1438 | 1559 | 1783 | 2770 |
| 11 | 2653 | 1919 | 2218 | 1793 |
| 12 | 1454 | 1768 | 2583 | 1942 |
| **Mean** | **1598** | **1656** | **1785** | **1751** |
| **SD** | **559** | **339** | **431** | **526** |
| | **$C_{max}$ (ng/ml)** | | | |
| 1 | 1103 | 777 | 1576 | 1905 |
| 2 | 1406 | 1006 | 1159 | 1674 |
| 3 | 1457 | 1010 | 938 | 1159 |
| 4 | 1371 | 1282 | 1543 | 754 |
| 5 | 1282 | 1376 | 1996 | 1728 |
| 6 | 2000 | 1064 | 1358 | 1261 |
| 7 | 1671 | 877 | 1374 | 1375 |
| 9 | 2000 | 1004 | 938 | 1625 |
| 10 | 1949 | 826 | 1297 | 1944 |
| 11 | 1944 | 1299 | 1998 | 1803 |
| 12 | 1383 | 954 | 1523 | 1665 |
| **Mean** | **1597** | **1043** | **1427** | **1536** |
| **SD** | **327** | **198** | **356** | **362** |

[a](D1: Diclo® 50 stored for 3 months at ambient conditions; D2: Diclo® 50 stored for 3 months at simulated tropical conditions; D3: Voltaren® 50 stored for 3 months at ambient conditions; D4: Voltaren® 50 stored for 3 months at simulated tropical conditions).

*Table 6.7: Individual $t_{max}$ values (hr) of 11 subjects following administration of a single dose of 50 mg diclofenac sodium as an enteric coated tablet*

| Subject | $t_{max}$ (hr) | | | |
|---|---|---|---|---|
| | D1[a] | D2[a] | D3[a] | D4[a] |
| 1 | 1.0 | 2.5 | 1.5 | 1.0 |
| 2 | 0.5 | 1.5 | 2.0 | 1.5 |
| 3 | 1.0 | 1.0 | 4.0 | 2.0 |
| 4 | 1.0 | 2.0 | 2.0 | 1.0 |
| 5 | 0.5 | 1.5 | 1.5 | 1.0 |
| 6 | 0.5 | 1.0 | 1.0 | 1.0 |
| 7 | 1.0 | 1.5 | 2.0 | 2.0 |
| 9 | 0.5 | 1.5 | 4.0 | 1.5 |
| 10 | 1.0 | 1.5 | 2.0 | 1.5 |
| 11 | 1.5 | 1.0 | 1.5 | 2.0 |
| 12 | 1.0 | 2.0 | 1.5 | 1.5 |
| **Mean** | **0.9** | **1.5** | **2.0** | **1.5** |
| **Range** | **0.5 - 1.5** | **1.0 - 2.5** | **1.5 - 4.0** | **1.0 - 2.0** |

[a](D1: Diclo® 50 stored for 3 months at ambient conditions; D2: Diclo® 50 stored for 3 months at simulated tropical conditions; D3: Voltaren® 50 stored for 3 months at ambient conditions; D4: Voltaren® 50 stored for 3 months at simulated tropical conditions).

The arithmetic mean diclofenac plasma concentration/time profiles of both formulations are shown in Fig. 6.7, and the mean (arithmetic) pharmacokinetic parameters in Table 6.8.

*Figure 6.7: Mean (n=11) diclofenac plasma concentration / time profiles following administration of a single dose of 50 mg diclofenac sodium: Diclo® 50 (A) and Voltaren® 50 (B) stored for 3 months at ambient conditions (♦) and at simulated tropical conditions (■)*

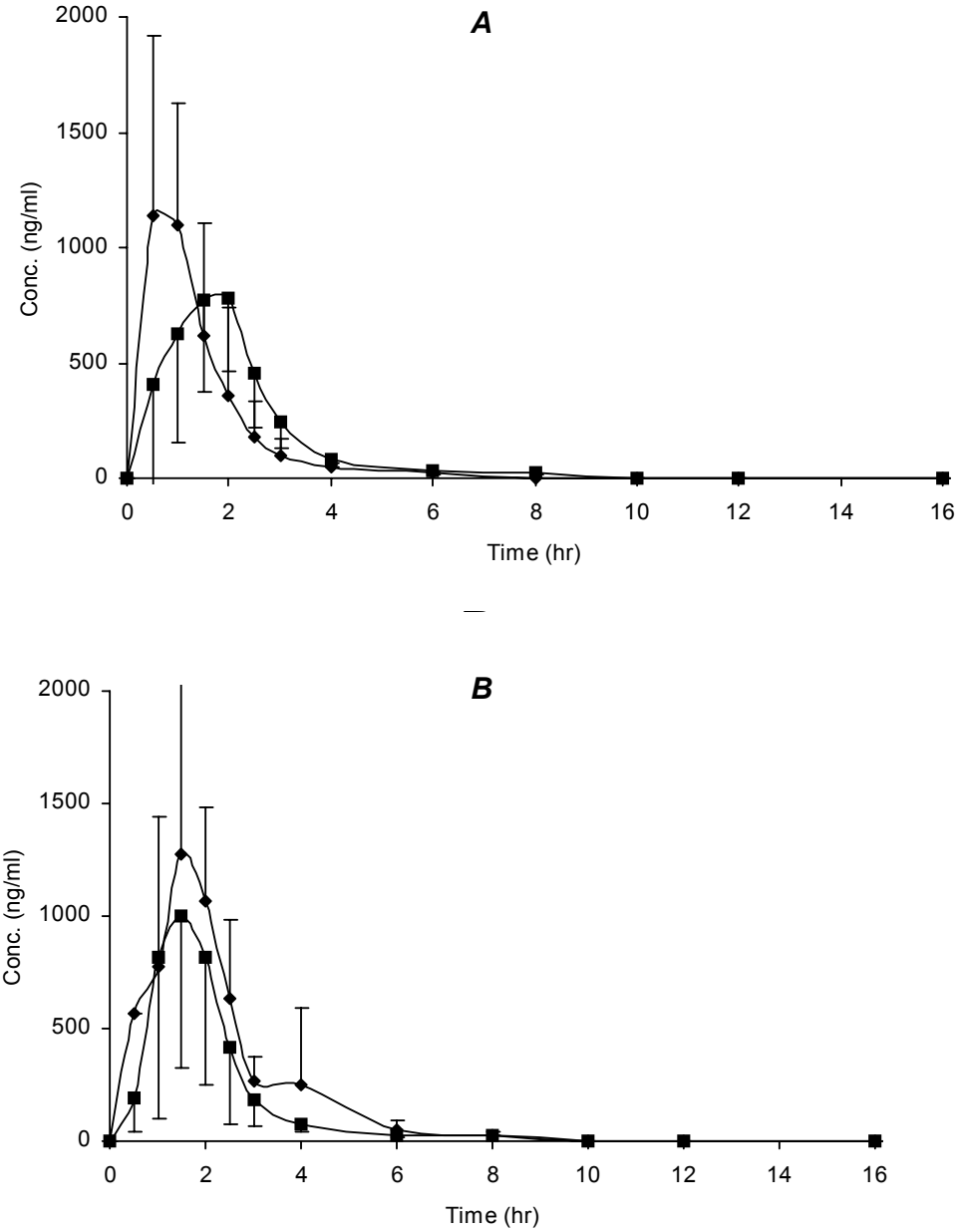


*Table 6.8: Mean (n=11)(arithmetic) pharmacokinetic parameters (± SD) following administration of a single dose of 50mg diclofenac sodium as enteric coated tablets*

| Parameter | Formulation[a] | | | |
|---|---|---|---|---|
| | D1 | D2 | D3 | D4 |
| AUC $_{0\text{-}16hr}$ (ng.hr/ml) | 1598 ± 559 | 1656 ± 339 | 1785 ± 431 | 1751 ± 526 |
| $C_{max}$ (ng/ml) | 1597 ± 327** | 1043 ± 197* | 1427 ± 357 | 1535 ± 361 |
| $C_{max}$/AUC ($hr^{-1}$) | 1.1 ± 0.2** | 0.7 ± 0.2* | 0.8 ± 0.2 | 0.9 ± 0.2 |
| $t_{max}$ (hr) | 0.9 ± 0.3* | 1.5 ± 0.5 | 2.0 ± 1.0** | 1.5 ± 0.5 |
| lag time (hr) | 0.7 ± 0.3* | 1.0 ± 0.3 | 1.8 ± 1.2** | 0.9 ± 0.5 |
| $t_{0.5}$ (hr) | 1.4 ± 0.8 | 1.5 ± 0.5 | 1.5 ± 0.4 | 1.5 ± 0.6 |

*significantly different from ** ($p < 0.05$)

[a](D1: Diclo® 50 stored for 3 months at ambient conditions; D2: Diclo® 50 stored for 3 months at simulated tropical conditions; D3: Voltaren® 50 stored for 3 months at ambient conditions; D4: Voltaren® 50 stored for 3 months at simulated tropical conditions).

Comparison of the mean AUC values of the two formulations stored at the different test conditions showed that they were not significantly different, indicating that there was no difference in the extent of drug absorption from the formulations stored at the ambient conditions and those stored under simulated tropical conditions. The extent of absorption from the Novartis and the Camden formulations stored at ambient conditions were also not significantly different.

The mean $C_{max}$ obtained from the Novartis tablets exposed to the different storage conditions was not significantly different. The storage conditions did not influence the rate of absorption from the Novartis tablets. As observed during the dissolution tests, the in vitro drug release from this formulation was also not affected by storage at high temperature and high relative humidity.

The $C_{max}$ values obtained for the Camden formulation showed that the samples exposed to simulated tropical conditions yielded a significantly lower $C_{max}$ compared to those exposed to ambient conditions. The $C_{max}$ for the Novartis and Camden samples that were exposed to ambient conditions were not significantly different.

The parameter $C_{max}$/AUC was proposed by Endrenyi et al. (1991) as a better measure for the absorption rate. Dividing $C_{max}$ by AUC compensates for the

influence of intra-individual variability in clearance on $C_{max.}$ Comparison of the $C_{max}$ /AUC values showed that there were no significant differences between the absorption rate from the Novartis formulations exposed to the different storage conditions. However, there was a significant difference between the ratios obtained from the Camden formulation samples exposed to the different storage conditions.

The time to the onset of absorption (lag time) showed a wide inter-subject variation (ranging from 0.5 to 4 hr). Statistical analysis of the log transformed data showed no significant differences of this parameter between the Novartis formulation exposed to the different storage conditions. Similar observations were made for the Camden samples. Comparing the two formulations stored at ambient temperatures, the lag time of the Camden formulations was shorter than the one observed for the Novartis formulation and the difference was statistically significant.

The $t_{max}$ values varied between the subjects, but the variances were normally distributed. This parameter of the Novartis samples stored at ambient and simulated tropical conditions was not significantly different. However, the $t_{max}$ value of the Camden formulation exposed to ambient conditions was significantly shorter than that of the Novartis formulation stored under similar conditions.

## 6.5 Discussion

The HPLC method used to determine diclofenac in plasma was sensitive, accurate and reproducible. The limits of detection and linearity range achieved were similar to the ones reported by Giagoudakis and Markantonis (1998). The pharmacokinetic parameters obtained in this experiment were similar to ones reported in literature. AUC values of 1262 ng.h/ml after the administration of a single dose of 50 mg diclofenac sodium as enteric coated tablet have been reported by Terhaag et al. (2000). $C_{max}$ values of 1285 ng/ml and 1400 ng/ml were also obtained by Walter and von Niecieck (2001) and Hanses et al. (1995), respectively. The terminal half-life (1.5 hr) was similar for both formulations and was in agreement with the values (2 hr) reported in literature (Davies and Anderson, 1997).

The in vitro dissolution and bioavailability of the Novartis formulation were not influenced by the storage conditions. The evaluation of the main bioavailability parameters (AUC and $C_{max}$) obtained for this formulation showed that the bioavailability remained unchanged when stored at high humidity and high temperature. Thus there is a great degree of assurance that the efficacy of this formulation will remain unchanged during distribution and storage in countries with Class IV tropical climatic conditions. On the contrary, the in vitro drug release from the Camden formulation was reduced dramatically after 3 months of storage under simulated tropical conditions. Although the extent of absorption (AUC) of samples exposed to the different storage conditions was similar, $C_{max}$ after exposure to simulated tropical conditions was significantly lower than that from the samples stored at ambient conditions. These observations suggest that the formulation has not been optimized to withstand the storage and distribution in countries with a tropical climate (class IV).

The dissolution tests carried out using the USP 2 and USP 3 methods for the Camden samples stored under simulated tropical conditions gave similar results: the drug release was very low and the tablets failed to disintegrate. However, the pharmacokinetic parameters obtained for this formulation show that the extent of absorption (AUC) was not significantly altered, but the rate of drug absorption ($C_{max}$) was only moderately lower. The poor relationship between the in vitro dissolution and bioavailability of oral solid dosage forms stored at high temperature and high relative humidity has also been reported for nitrofurantoin tablets (Gouda et al., 1984) and etodolac capsules (Dey et al., 1993). When operated at low agitation rates (5 dips/min), the USP apparatus 3 creates hydrodynamic conditions equivalent to those obtained by USP Method 2 at 50 rpm and also similar dissolution profiles in case of drugs with high to moderate solubility (Yu et al., 2002). In this study the USP Method 3 was operated at higher agitation rates yet the dissolution profile was not different from that obtained with the USP Method 2 at 50 rpm. This indicates that other factors rather than the insufficient hydrodynamic stress created by the dissolution apparatus are responsible for the failure of the dissolution process to predict the in vivo observations. The in vivo drug release is not only affected by agitation intensity,

but also by presence of peristaltic destruction forces (Kamba et al., 2002), digestive enzymes and bile salts in the gastrointestinal fluids.

## 6.6 Conclusion

Storage of enteric coated diclofenac sodium tablets under simulated tropical conditons has shown that the combination of temperature and high humidity (as is found in tropical countries) may cause changes in the formulation, leading to a decrease of the in vitro drug release even when the drug content remained stable. The observed changes on the in vitro drug release were not reflected to the same magnitude in the bioavailability of the formulation. The USP 2 and 3 dissolution tests were too conservative and not predictive of the in vivo performance of the enteric coated diclofenac tablets stored under simulated tropical conditions. The results of in vitro drug release tests have to be interpreted with caution when considering changes in dissolution characteristics due to storage conditions (high temperature / high humidity). The use of simulated gastric and intestinal fluids as a medium and dissolution test parameters simulating the peristaltic destruction forces during the dissolution testing of oral solid dosage forms exposed to conditions of high temperature and high humidity should be investigated.

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# 7. INFLUENCE OF TROPICAL CLIMATIC CONDITIONS ON BIOAVAILABILITY OF CIPROFLOXACIN TABLET FORMULATIONS

## 7.1 Introduction

Ciprofloxacin, a quinoline carboxylic acid, is a broad spectrum anti-microbial agent that is active against both Gram-negative and Gram-positive pathogenic bacteria, many of which are resistant to other commonly used antibiotics such as penicillins and aminoglycosides. Consequently, in countries where infectious diseases are widespread it is considered as an essential life saving drug. In the Tanzanian Standard Treatment Guideline (Ministry of Health Tanzania, 1997) it is recommended as the drug of choice for the treatment of infections of the respiratory tract, middle ear, paranasal sinuses, abdomen, skin, soft tissue, bones and joints. It is also indicated for septicaemia and prophylaxis in patients undergoing abdominal surgery.

In the biopharmaceutical drug classification scheme, ciprofloxacin is considered a class II drug having a low solubility and high permeability. As observed for other drugs in this class, different formulations may present a variable absorption and hence a variable bioavailability if their dissolution characteristics are not similar (Amidon et al., 1995).

Presently there are 35 ciprofloxacin formulations registered for the Tanzanian market (Pharmacy Board Tanzania, personal communication, 2002). With such a large number of formulations on the market, there is a possibility of variations in the bioavailability between brands as a result of differences in raw material, formulation and/or process technology between the manufacturers. There is no information available about the bioavailability of most formulations on market in Tanzania. Furthermore, the influence of tropical storage conditions (as is found in most parts of the country) on the bioavailability has not been investigated.

The in vitro dissolution of 9 ciprofloxacin tablet formulations marketed in Tanzania has been previously described in Chapter 3. The in vitro drug release and drug content from the formulations was determined before and after a stability test for 6

months at simulated tropical conditions (75% RH, 40°C). When analysed immediately after purchase the in vitro drug release of all formulations was above the USP 24 tolerance limits for dissolution. Upon 6 months of storage at simulated tropical conditions the drug release of all formulations had not changed, their dissolution profiles obtained before and after storage being similar. The in vitro dissolution of a solid oral dosage form is not always predictive of its in vivo behaviour. Furthermore, there is an extremely large price difference between the innovator brand and the other formulations, hence one may expect that the lower priced generics may have been produced using inferior excipients and/or process technology possibly influencing bioavailability.

In this chapter, the influence of tropical storage on the quality of ciprofloxacin tablet formulations is investigated by conducting a human bioavailability study on tablet samples of two ciprofloxacin formulations stored for 3 months at ambient and at simulated tropical conditions. The two formulations were chosen on the basis of having the best in vitro quality parameters (drug content and drug release) amongst the 9 formulations previously evaluated (section 3).

## 7.2 In vitro evaluation

### Study plan

Samples from Bayer (Ciproxin® 500, batch 01D10) and Cadila (Ciprodac® 500, batch E9003) were purchased in Belgium and Tanzania, respectively. A part of the tablets was stored at ambient conditions for 3 months, whereas the remaining part was stored for 3 months at simulated tropical conditions (40°C and 75% RH). After 3 months the samples were evaluated for their in vitro drug release and in vivo drug availability.

### Dissolution tests

The in vitro drug release of the tablet formulations was determined by performing a dissolution test as described by the USP 24 and detailed in Chapter 3.

### Equipment

A dissolution tester VK 7010 linked to an automatic sampler VK 8000 (VanKel Technology, Cary, USA) was used for the dissolution tests. A Lambda 12 UV/VIS

spectrophotometer (Perkin Elmer, Norwalk, USA) was used for determining the drug concentrations of the samples.

## 7.3 In vivo evaluation

Clinical protocol

Twelve volunteers (7 females) participated in the study after giving informed consent. The age of the volunteers ranged from 19 to 41 years (mean: 26 yrs), their weight from 55 to 94 kg (mean: 72 kg) and their body mass index from 20.6 to 27.2 kg/$m^2$ (mean: 24.0 kg/$m^2$). The volunteers were healthy as proven by medical history, physical examination, electrocardiogram, and lab tests (haematological and urine). Volunteers with suspected history of alcoholism, barbiturate abuse and allergy to ciprofloxacin or fluoroquinolones were excluded from the study. Pregnant or lactating females or females with childbearing potential without adequate contraception were also excluded from the study. The Ethics Committee of Ghent University Hospital approved the clinical protocol.

Prior and concomitant therapy

During the entire trial, subjects were required not to use any medication other than the trial medication. The exceptions to this rule were paracetamol and oral contraceptives. Subjects were allowed to use paracetamol up to 3 days before drug administration in each period. The use of paracetamol could be allowed by the clinical investigator (no more than 3 x 500 mg per day and no more than 3 g per week) for the treatment of headache or other pain. Oral contraceptives (OAC) were allowed. In case paracetamol or OAC are used, the dose and dosage regimen was recorded on the Concomitant Therapy Form that formed a part of the Case Report Form (CRF).

Procedure

The subjects fasted for at least 10 hr before drug administration. Drinking of water was allowed up to 2 hr before drug administration. The subjects were required not to consume grapefruit, grapefruit juice or beverages containing alcohol or quinine between 24 hr before and 32 hr after drug dosing per period. The subjects were required to take the whole tablet together with 200 ml of water. From 2 hr after dosing, intake of water was allowed. A standard breakfast and lunch were given 3 and 6 hr post dosing, respectively. The standard breakfast consisted of four slices of bread, one slice of ham, one slice of cheese, butter, jelly and two cups of decaffeinated coffee or tea with, if desired, milk and/or sugar. The subjects remained in the testing facility for 15 hr after receiving the dose.

Randomisation

The study was an open randomized 4-period cross-over design. The washout time between periods was 5 days. In each period subjects were given a single tablet containing 500 mg ciprofloxacin. Subjects entering the study were allocated a number from 1 to 12. The randomisation scheme shown in Table 6.1 was used to assign the subjects to either of the four treatments:

- ✓ C1 (Ciprodac® 500 mg stored for 3 months at ambient conditions)
- ✓ C2 (Ciprodac® 500 stored for 3 months at simulated tropical conditions)
- ✓ C3 (Ciproxin® 500 stored for 3 months at ambient conditions)
- ✓ C4 (Ciproxin ® 500 stored for 3 months at simulated tropical conditions).

*Table 6.1: Randomisation scheme*

| Subject | Period 1 | Period 2 | Period 3 | Period 4 |
|---|---|---|---|---|
| 1 | C1 | C2 | C3 | C4 |
| 2 | C2 | C3 | C4 | C1 |
| 3 | C3 | C4 | C1 | C2 |
| 4 | C4 | C1 | C2 | C3 |
| 5 | C2 | C3 | C4 | C1 |
| 6 | C3 | C4 | C1 | C2 |
| 7 | C4 | C1 | C2 | C3 |
| 8 | C1 | C2 | C3 | C4 |
| 9 | C3 | C4 | C1 | C2 |
| 10 | C4 | C1 | C2 | C3 |
| 11 | C1 | C2 | C3 | C4 |
| 12 | C2 | C3 | C4 | C1 |

Blood Sampling

Venous blood samples of 5 ml were taken from an antecubital vein 45 min before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 15, 24 and 32 hr after the drug administration. Blood samples were collected in heparinized tubes and centrifuged for 10 min at 1500g within 2 hr after collection. Separated plasma (about 2 ml) was aspirated with a disposable pipette and transferred into plastic tubes. The tubes were sealed by means of polyethylene stoppers, and labelled with the investigator's name, trial number, CRF identity, subject initials, date and time of sampling. Samples were stored at -20°C until assayed.

### *7.3.1 Plasma drug analysis*

Plasma ciprofloxacin concentrations were measured by a reversed-phase HPLC-method with fluorescence detection (Limberg and Buggé, 1994).

#### *7.3.1.1 Materials*

All solvents were of HPLC grade and all reagents were of analytical grade. Ciprofloxacin was purchased from Roig Pharma (Barcelona, Spain), pipemidic acid from Sigma Aldrich (Steinheim, Germany) and tetrabutyl ammonium hydroxide

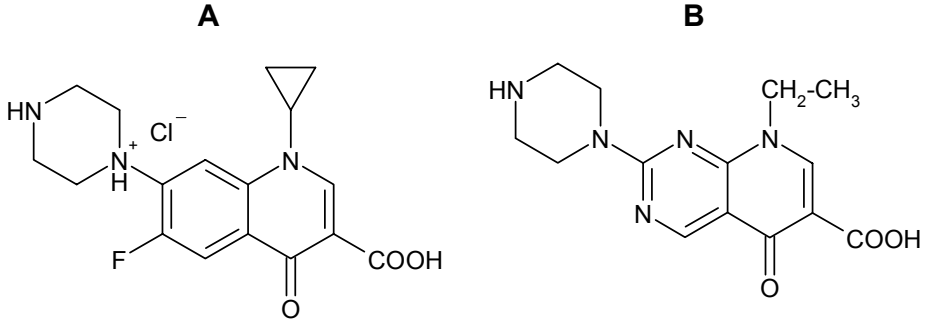
from Fluka Chemie (Buchs, Switzerland). Orthophosphoric acid and hydrochloric acid were obtained from Vel (Leuven, Belgium), while acetonitrile was obtained from Biosolve (Valkenswaard, The Netherlands).

### *7.3.1.2 Methods*

Standard solutions

Stock solutions containing 90 µg/ml ciprofloxacin hydrochloride and 72 µg/ml pipemidic acid as an internal standard (Fig 7.1) were prepared in diluent (water/phosphoric acid/acetonitrile; 990:1.7:64 v/v). The pH of the diluent was adjusted to 3.0 with 0.2N sodium hydroxide. Working solutions of 18, 9, 5.4, 2.7, 0.9 and 0.45 µg ciprofloxacin hydrochloride per ml were made by appropriate dilution in the solvent mixture. The working solution of the internal standard (7.2 µg/ml) was similarly made. The stock solutions were protected from light, kept at 4°C and used within 5 days.

*Figure 7.1: The structural formula of ciprofloxacin hydrochloride (A) and the internal standard pipemidic acid (B).*




Sample preparation

To 200 µl of a plasma sample with unknown drug concentration 50 µl of the internal standard solution (pipemidic acid) was added and the mixture was vortexed for 30 s. Protein precipitation was achieved by the addition of 200 µl of glycine buffer (pH 2.8) followed by 1 min vortexing. The mixture was centrifuged for 15 min at 4000 rpm. The upper layer was transferred into a test tube and a 10 µl aliquot was injected into the HPLC system. A calibration curve was prepared by spiking 150 µl

blank plasma with 50 µl of a ciprofloxacin standard solution and 50 µl of the internal standard solution and the same deproteination procedure was followed for this mixture.

Chromatographic conditions

The HPLC system consisted of a LaChrom L 7100 isocratic pump (Merck, Darmstadt, Germany). Separations were performed on a Purosphere RP-C18® (3 mm internal diameter) column packed with 5 µm particles. A guard column Purosphere 100 RP-18® (5 µm) was used to protect the analytical column. The column temperature was not standardized, but was maintained at room temperature.

The mobile phase consisted of a mixture of water, orthophosphoric acid (85%), acetonitrile and the ion pairing reagent tetrabutyl ammonium hydroxide (0.001M) in a ratio of 990: 1.7: 64: 60 (v/v). The pH of the mobile phase was 2.9 and the flow rate was set at 0.56 ml/min. Drug concentration was monitored using a LaChrom L 7480 fluorescence detector at an excitation wavelength ($\lambda_{ex}$) of 278 nm and an emission wavelength ($\lambda_{em}$) of 450 nm. Peak integrations were carried out using a LaChrom L7000 integrator.

#### *7.3.1.3 Validation of the analytical method*

The method used to determine the ciprofloxacin plasma concentration was validated as recommended by Shah et al. (1992) and revised in the FDA Guidance for industry (2001).

#### *7.3.1.4 Pharmacokinetic and statistical analysis*

The peak plasma concentration ($C_{max}$) and the time to reach the peak plasma concentration ($t_{max}$) were obtained from the plasma concentration / time profiles. The area under the plasma concentration-time curve to 32 hr post dosing ($AUC_{0-32hr}$) was calculated by linear trapezoidal summation. The terminal elimination rate constant ($\lambda_z$) was determined by log-linear regression of the terminal points of the plasma concentration-time curve and the half-life time ($t_{0.5}$) was defined as $0.693/\lambda_z$. The MW/Pharm software package (v.3.01, Mediware 1987-1991, Utrecht, The Netherlands) was used for the pharmacokinetic analysis.

Statistical analysis of the pharmacokinetic data was performed using a two-way ANOVA. The data were tested for normal distribution with the Kolmogorov-Smirnov test. The homogeneity of variances was tested with the Levene's test. To further compare the effects of the different treatments a multiple comparison among pairs of means was performed using the Scheffe test with $p < 0.05$ as significance level. For all statistical analysis the program SPSS version 10.0 was used.

## 7.4 Results

### *7.4.1 Validation of the bioanalytical method*

#### *7.4.1.1. Linearity*

The lower limit of the calibration range (0.05 µg/ml) was chosen on the basis of the reported plasma concentrations after a single oral dose of 500 mg ciprofloxacin (Plaisance et al., 1987; Maya et al., 2001). Six calibration standards (0.05, 0.1, 0.3, 0.6, 1.0, and 2.0 µg/ml) were made by spiking blank plasma with the appropriate amounts of ciprofloxacin hydrochloride solution. The concentration of the internal standard (pipemidic acid) was 0.8 µg/ml. A blank sample was included in the calibration curve to ensure that no interfering components were co-eluted with the drug and the internal standard or introduced during preparation of the standards.

The mean calibration curve (n = 8) of the ciprofloxacin concentration versus the ratio of peak areas of ciprofloxacin and the internal standard was calculated.

The calibration curve showed that the analytical method is linear over the entire concentration range (0.05 - 2.0 µg/ml): $Y = 5.11618x$ (± 0.0863) + 0.0193 (± 0. 03548) with a coefficient of determination ($R^2$) of 0.9996 (± 0.0005).

#### *7.4.1.2 Limits of detection and quantitation.*

The limit of detection (LOD) was defined as the concentration of the analyte that produced a response equivalent to the blank signal plus three times the standard deviation of the blank signal (mean of the Y-intercept of the calibration curves). From regression analysis of the calibration curve, the limit of detection of this method was determined at 0.02 µg/ml.

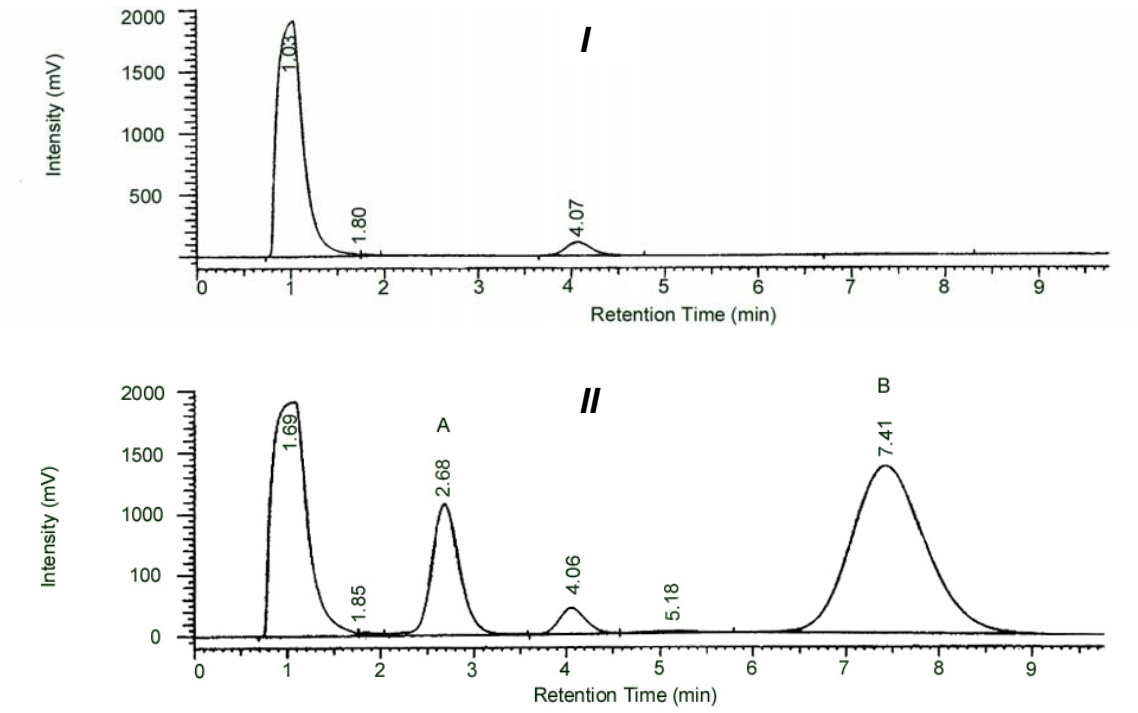
The limit of quantitation (LOQ) was defined as the lowest concentration in the calibration curve. For this analytical method the limit of quantitation (LOQ) was

determined at 0.05 µg/ml, at this concentration the accuracy being 96.0% and the relative standard deviation (RSD) 7.1% (FDA guidance: RSD of the LOQ must be less than 20% and the accuracy between 80 and 120%).

### *7.4.1.3 Selectivity*

The chromatographic procedures were capable of separating the plasma constituents from the drug and the internal standard. The retention times were about 2.5 and 7.5 min for pipemidic acid and ciprofloxacin, respectively. There were no interfering peaks at these retention times as evidenced by the representative chromatograms of blank plasma and of plasma (spiked with the internal standard) obtained from a volunteer 3.0 hr after administration of a 500 mg ciprofloxacin tablet (Fig. 7.2).

*Figure 7.2: The chromatograms of blank plasma (I) and of plasma (spiked with IS) obtained from a volunteer 3.0 hr after administration of a 500 mg ciprofloxacin tablet (II) with A and B being the peaks for pipemidic acid and ciprofloxacin, respectively*



### 7.4.1.4 Precision

The precision of the analytical method was determined at concentrations of 0.05, 0.6 and 2.0 µg ciprofloxacin per ml plasma by calculating the variations of the peak areas for the same sample injected at different times during the same day (repeatability). For reproducibility, the samples were injected at different days. The results for precision of the method are shown in Table 7.2. The relative standard deviations (RSD) obtained for the within and between day analysis were both less than the acceptance criteria of 15%.

*Table 7.2: Intra- (repeatability) and inter-day (reproducibility) precision for the determination of ciprofloxacin in plasma (n = 8)*

| Conc. (µg /ml) | Precision (RSD %) | |
|---|---|---|
| | Intra-day | Inter-day |
| 0.05 | 6.6 | 7.9 |
| 0.60 | 3.6 | 5.3 |
| 2.00 | 2.6 | 3.4 |

### 7.4.1.5 Accuracy

The accuracy was determined by comparing the measured ciprofloxacin concentrations and the spiked concentrations in plasma. The mean results and the RSD at each concentration level are summarized in Table 7.3. The mean value did not deviate by more than 10% from the true value and the RSD values are within the acceptance level (FDA guidance: RSD less than 15%, except for the LOQ where it should not exceed 20%).

*Table 7.3: Accuracy (n = 8) for the determination of ciprofloxacin in plasma*

| Concentration (µg/ml) | Accuracy(%) | RSD |
|---|---|---|
| 0.05 | 96.0 | 7.1 |
| 0.60 | 103.3 | 3.7 |
| 2.00 | 100.5 | 2.0 |

### 7.4.1.6 Recovery

50 µl of the appropriate ciprofloxacin standard solution was added to blank plasma to make concentrations of 0.05, 0.6 and 2.0 *µ*g/ml. After protein precipitation, their peak areas were compared with those obtained by injecting the same concentration of ciprofloxacin in mobile phase. Recovery was calculated as the ratio of the peak area of the plasma samples divided by the peak areas of the mobile phase samples, multiplied by 100. The relative standard deviation of the recovery at different concentrations was also determined. The recovery of pipemidic acid at 0.8 *µ*g/ml was similarly determined. Table 7.4 shows that the values obtained for recovery were above 90% and reproducible (RSD between 3.1 and 7.1%). These values are above the minimum value required for recovery (i.e. 75%) with a maximum RSD of 15%.

*Table 7.4: Recovery of ciprofloxacin and pipemidic acid for the determination of ciprofloxacin in plasma (n = 8)*

| | Conc. (µg/ml) | Recovery (%) | RSD (%) |
|---|---|---|---|
| Ciprofloxacin | 0.05 | 94.5 | 7.0 |
| | 0.60 | 95.3 | 3.1 |
| | 2.00 | 98.3 | 2.0 |
| Pipemidic acid | 0.80 | 95.8 | 7.1 |

### 7.4.1.7 Stability of ciprofloxacin in plasma

The stability of the drug in plasma was determined at concentrations of 0.05, 0.60 and 2.00 µg/ml in plasma stored at -20°C. Analyses were performed after 15 and 30 days. The determined concentrations and standard deviation, shown in Table 7.5, indicated that the drug was stable in plasma for at least 30 days.

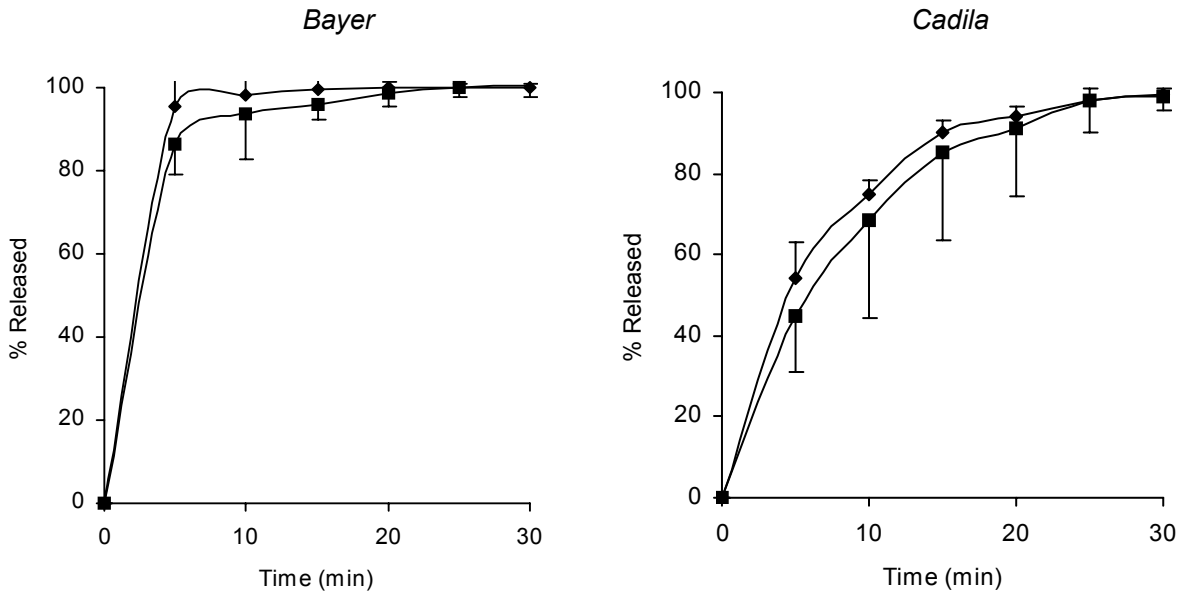
*Table 7.5: Stability of ciprofloxacin in plasma stored at -20°C for 30 days (n=8)*

| Theoretical conc. (µg/ml) | % of the theoretical concentration found (± SD) after | |
|---|---|---|
| | 15 days | 30 days |
| 0.05 | 94.0 ± 8.0 | 94.0 ± 8.0 |
| 0.60 | 96.7 ± 5.0 | 102.0 ± 5.0 |
| 2.00 | 101.1 ± 4.2 | 99.2 ± 3.8 |

### *7.4.2 In vitro drug release*

The in vitro drug release of the two formulations is shown in Fig. 7.3. For the Bayer formulation stored at ambient conditions, the drug release after 30 min of dissolution testing was 100%, releasing more than 90% of drug within the first 5 min. The drug release was not influenced by storage at simulated tropical conditions. The drug release from the Cadila formulation after 30 min was 100%. However, the initial drug release was slower (55% in the first 5 min) than that of the Bayer formulation. Similar to the Bayer formulation, storage for 3 months at simulated tropical conditions did not influence the drug release. These results are consistent with the ones previously obtained after performing dissolution tests on different batches of the same formulations from the same manufacturers (Chapter 3).

*Figure 7.3: Dissolution profiles of the Bayer and Cadila formulations obtained after 30 min dissolution testing on tablets stored for 3 months at ambient conditions (◆) and at simulated tropical conditions (■)*




### 7.4.3 *In vivo drug availability*

The four 'treatments' were well tolerated and no drug related adverse effects were reported by the volunteers during the study. The individual ciprofloxacin plasma concentrations after single administration of a 500 mg ciprofloxacin tablet exposed to different storage conditions are shown in Fig. 7.4 and 7.5, and the individual pharmacokinetic parameters in Table 7.6 and 7.7.

*Figure 7.4*: Individual ciprofloxacin plasma concentration/time profiles (n=12) after administration of a single dose of 500 mg ciprofloxacin as a Ciprodac® 500 tablet stored at ambient conditions (C1) and at simulated tropical conditions (C2)

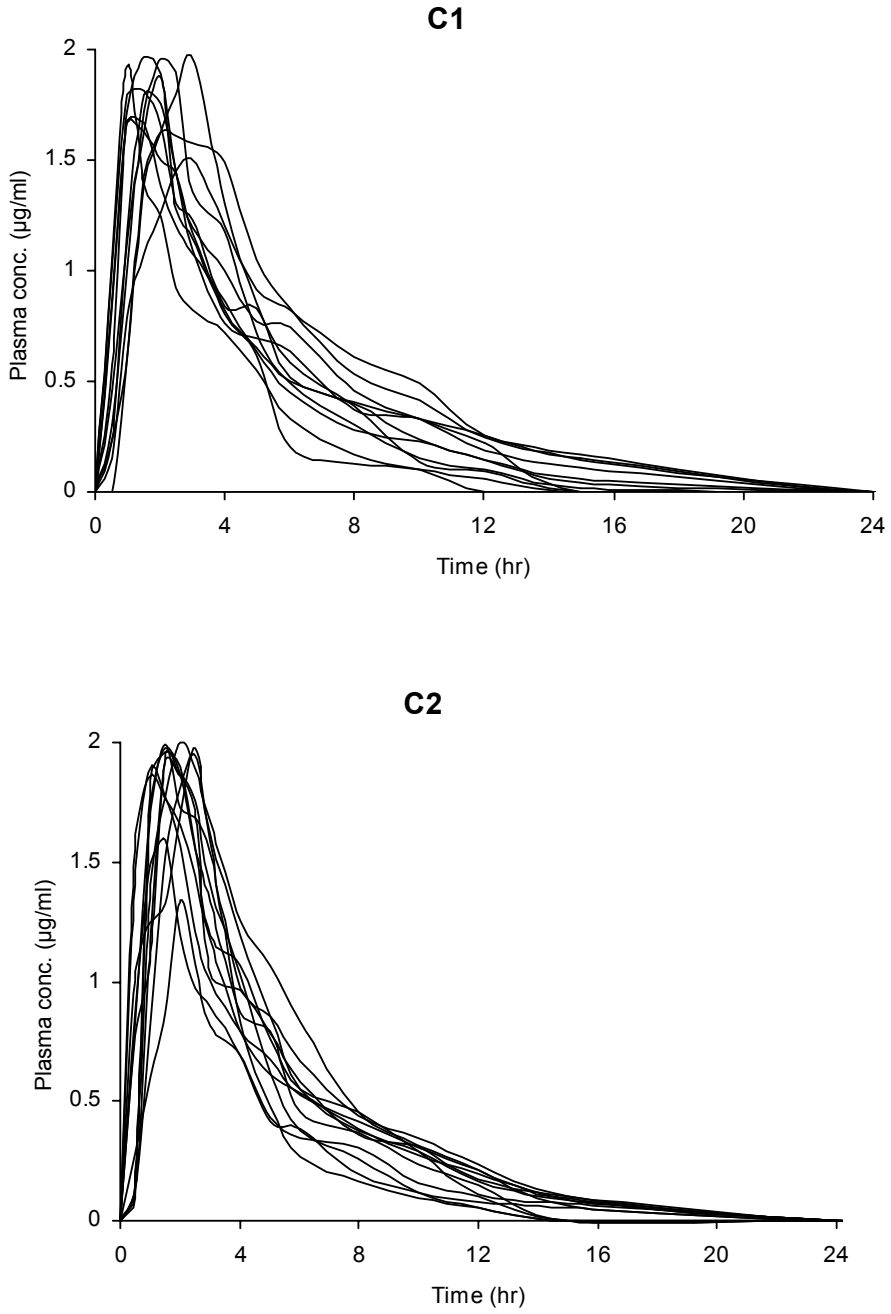


*Figure 7.5: Individual ciprofloxacin plasma concentration/time profiles (n=12) after administration of single dose of 500 mg ciprofloxacin as a Ciproxin® 500 tablet stored at ambient conditions (C3) and at simulated tropical conditions (C4)*

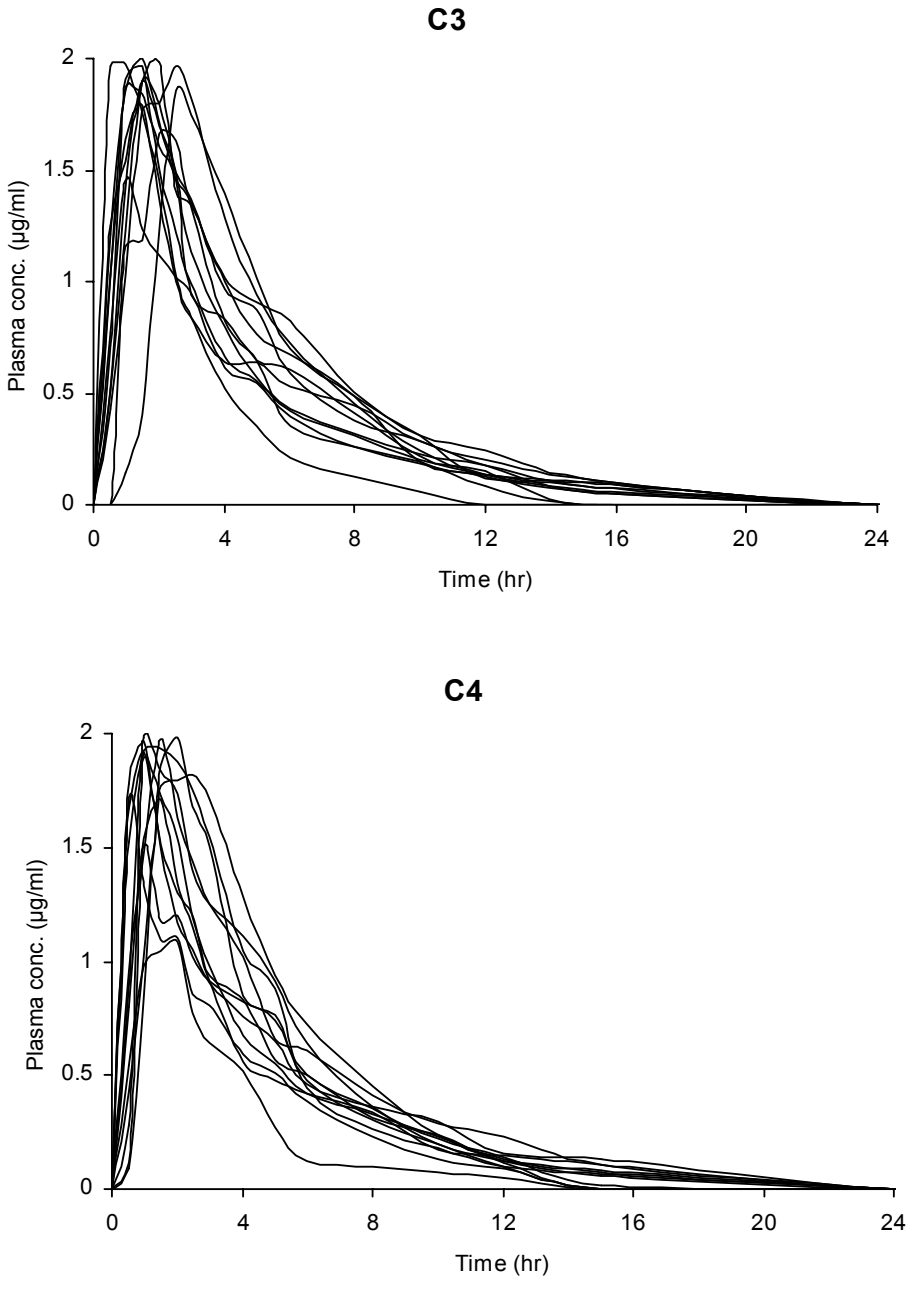


*Table 7.6: Individual $AUC_{0-32hr}$ and $C_{max}$ values of 12 subjects following administration of a single dose of 500 mg ciprofloxacin*

| Subject | Formulation[a] | | | |
|---|---|---|---|---|
| | C1 | C2 | C3 | C4 |
| | **$AUC_{0-32hr}$ (µg.hr/ml.)** | | | |
| 1 | 10.12 | 9.18 | 9.32 | 9.00 |
| 2 | 8.14 | 5.54 | 10.74 | 6.27 |
| 3 | 6.45 | 5.89 | 8.32 | 8.27 |
| 4 | 6.37 | 7.01 | 9.16 | 8.05 |
| 5 | 8.70 | 8.31 | 6.28 | 4.15 |
| 6 | 10.34 | 10.98 | 10.31 | 9.34 |
| 7 | 8.60 | 9.46 | 6.93 | 9.94 |
| 8 | 10.70 | 9.31 | 8.76 | 9.02 |
| 9 | 11.03 | 10.70 | 10.05 | 7.76 |
| 10 | 8.97 | 8.55 | 6.83 | 7.94 |
| 11 | 10.69 | 7.86 | 9.07 | 7.31 |
| 12 | 8.51 | 9.62 | 8.16 | 9.84 |
| **Mean** | **9.05** | **8.53** | **8.66** | **8.07** |
| **SD** | **1.58** | **1.72** | **1.42** | **1.63** |
| | **$C_{max}$ (µg/ml)** | | | |
| 1 | 1.82 | 1.93 | 1.90 | 1.94 |
| 2 | 1.68 | 1.34 | 1.98 | 1.70 |
| 3 | 1.87 | 1.59 | 1.96 | 1.85 |
| 4 | 1.92 | 1.84 | 1.87 | 1.92 |
| 5 | 1.95 | 1.98 | 1.98 | 1.09 |
| 6 | 1.92 | 1.96 | 1.97 | 1.95 |
| 7 | 1.96 | 1.95 | 1.66 | 1.82 |
| 8 | 1.50 | 1.89 | 1.76 | 1.97 |
| 9 | 1.63 | 1.99 | 1.96 | 1.51 |
| 10 | 1.80 | 1.98 | 1.45 | 1.98 |
| 11 | 1.97 | 1.95 | 1.86 | 1.71 |
| 12 | 1.67 | 1.86 | 1.85 | 1.93 |
| **Mean** | **1.81** | **1.85** | **1.85** | **1.78** |
| **SD** | **0.16** | **0.19** | **0.16** | **0.26** |

[a](C1: Ciprodac® 500 stored for 3 months at ambient conditions; C2: Ciprodac® 500 stored for 3 months at simulated tropical conditions, C3: Ciproxin® 500 stored for 3 months at ambient conditions C4: Ciproxin® 500 stored for 3 months at simulated tropical conditions).

*Table 7.7: Individual $t_{max}$ values (hr) of 12 subjects following administration of a single dose of 500 mg ciprofloxacin tablet*

| Subject | $t_{max}$ | | | |
|---|---|---|---|---|
| | C1[a] | C2[a] | C3[a] | C4[a] |
| 1 | 1.0 | 1.5 | 1.5 | 1.5 |
| 2 | 1.0 | 2.0 | 1.5 | 0.5 |
| 3 | 2.0 | 1.5 | 1.5 | 1.0 |
| 4 | 1.0 | 1.5 | 1.0 | 1.0 |
| 5 | 1.0 | 2.5 | 0.5 | 2.0 |
| 6 | 2.0 | 2.0 | 2.5 | 1.5 |
| 7 | 2.0 | 2.0 | 2.0 | 2.0 |
| 8 | 3.0 | 1.0 | 1.5 | 1.0 |
| 9 | 2.0 | 1.0 | 1.5 | 1.0 |
| 10 | 1.5 | 1.5 | 1.0 | 2.0 |
| 11 | 1.5 | 2.0 | 2.5 | 1.5 |
| 12 | 1.0 | 1.0 | 1.5 | 1.5 |
| **Mean** | **1.75** | **1.63** | **1.54** | **1.42** |
| **Range** | **1.0 - 3.0** | **1.0 – 2.5** | **0.5 – 2.5** | **0.5 – 2.5** |

[a](C1: Ciprodac® 500 stored for 3 months at ambient conditions; C2: Ciprodac® 500 stored for 3 months at simulated tropical conditions, C3: Ciproxin® 500 stored for 3 months at ambient conditions C4: Ciproxin® 500 stored for 3 months at simulated tropical conditions)

The arithmetic mean ciprofloxacin plasma concentration/time profiles of both formulations are shown in Fig. 7.6, and the mean (arithmetic) pharmacokinetic parameters in Table 7.8.

*Figure 7.6: Mean (n=12) ciprofloxacin plasma concentration/time profiles following administration of a single dose of 500 mg ciprofloxacin as Ciprodac® 500 (A) and Ciproxin® 500 (B) tablets stored for 3 months at ambient conditions (◆) and at simulated tropical conditions (■)*

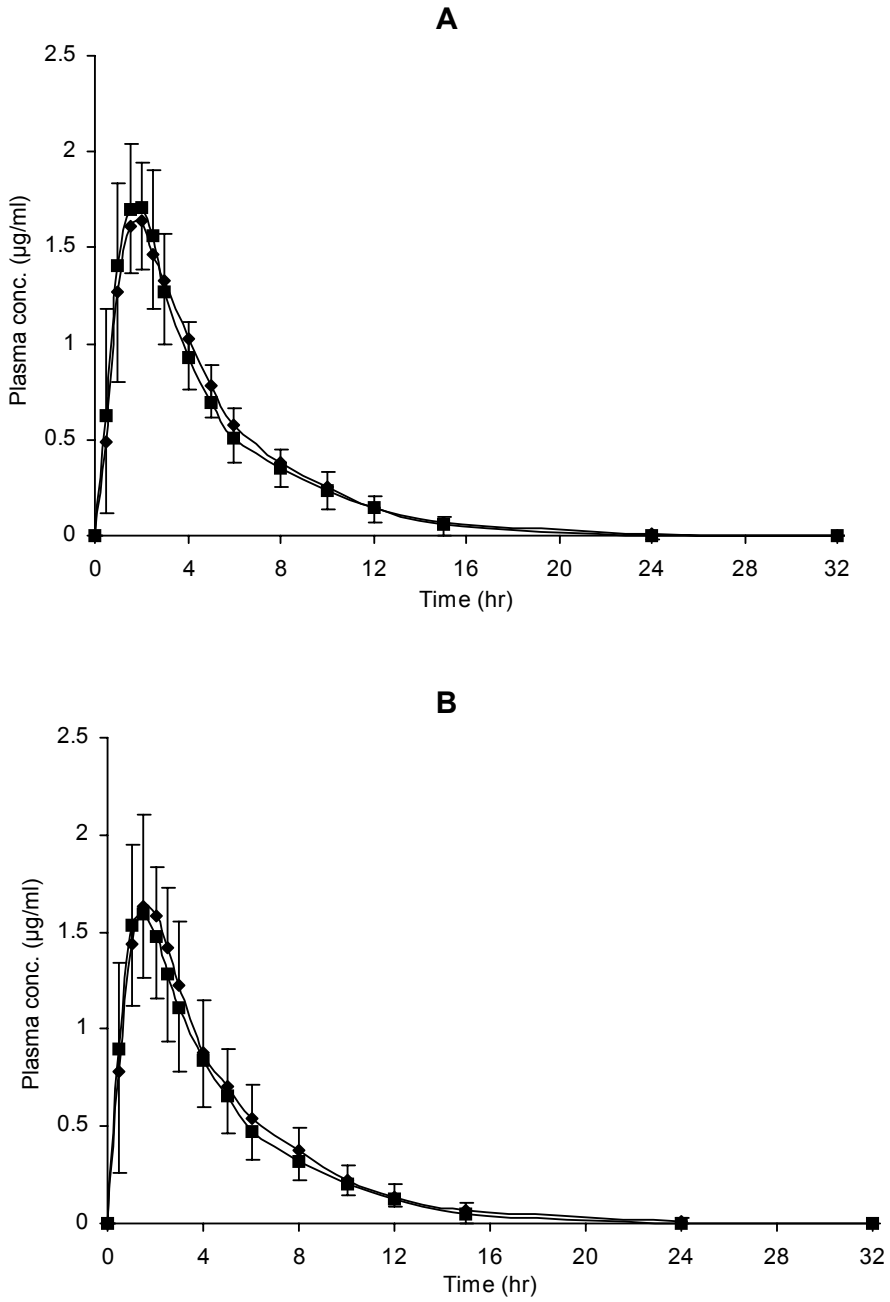


*Table 7.8: Mean (n=12)(arithmetic) pharmacokinetic parameters (± SD) following administration of a single dose of 500 mg ciprofloxacin tablet*

| Parameter | Formulation[a] | | | |
|---|---|---|---|---|
| | C1 | C2 | C3 | C4 |
| AUC $_{0\text{-}32hr}$ (µghr/ml) | 9.05 ± 1.58 | 8.53 ± 1.72 | 8.66 ± 1.42 | 8.07 ± 1.63 |
| $C_{max}$ (µg/ml) | 1.81 ± 0.16 | 1.85 ± 0.19 | 1.85 ± 0.16 | 1.78 ± 0.26 |
| $C_{max}$/AUC ($hr^{-1}$) | 0.21 ± 0.05 | 0.22 ± 0.03 | 0.22 ± 0.04 | 0.22 ± 0.03 |
| $t_{max}$ (hr) | 1.75 ± 0.72 | 1.63 ± 0.48 | 1.54 ± 0.58 | 1.42 ± 0.51 |
| $t_{0.5}$ (hr) | 4.00 ± 1.15 | 3.97 ± 1.09 | 3.95 ± 1.02 | 3.93 ± 0.99 |

[a](C1: Ciprodac® 500 stored for 3 months at ambient conditions; C2: Ciprodac ® 500 stored for 3 months at simulated tropical conditions, C3: Ciproxin® 500 stored for 3 months at ambient conditions C4: Ciproxin® 500 stored for 3 months at simulated tropical conditions).

The Kolmogorov-Smirnov analysis showed that the individual $AUC_{0\text{-}32hr}$, $C_{max}$ and $t_{max}$ values were normally distributed. Moreover, the Levene's test showed a homogeneous distribution of the variances for these parameters with no significant subject influence.

The mean $AUC_{0\text{-}32hr}$, $C_{max}$ and $t_{max}$ values obtained from the two formulations were similar and independent of storage conditions. The values did not change significantly after storage at simulated tropical conditions for 3 months. This shows that the extent of drug absorption, the maximum plasma concentration and the time to achieve this concentration in plasma from the two formulations were not significantly different ($p > 0.05$) and not influenced by the high humidity / high temperature storage conditions.

The parameter $C_{max}$/AUC has been recommended to compare the absorption rate between formulations (Endrenyi et al., 1991; Tothfalus and Endrenyi, 1995) as dividing $C_{max}$ by AUC compensates for the influence of intra-individual variability in clearance on $C_{max.}$ As observed for $C_{max}$ and $t_{max}$, (parameters that indicates the absorption rate) there was no significant difference on the $C_{max}/AUC_{0\text{-}32hr}$ values obtained for the two formulations, even after storage at simulated tropical conditions. This shows that the formulations had a similar absorption rate that was not influenced by storage at high temperature and high humidity. Since

ciprofloxacin is a drug with a high permeability, this observation indicates that the dissolution characteristics of the two formulations in the gastrointestinal tract were similar and in vitro dissolution is a good indicator for the bioavailability for such drugs.

## 7.5 Discussion

The pharmacokinetic parameters obtained in this study were similar to the ones reported in literature. In a review on the pharmacokinetics of ciprofloxacin, Vancebryan et al. (1990) has reported AUC-values ranging from 6.78 to 13.5 µg.hr/ml and $C_{max}$-values from 1.51 to 3.23 µg/ml after oral administration of a single 500 mg tablet. The terminal half-life ($t_{0.5}$) and $t_{max}$ of both formulations were similar and in agreement with the values (3.3-5.04 hr and 1.0-1.45 hr, respectively) reported by these authors.

The in vivo drug availability from the two formulations mirrored the in vitro drug dissolution characteristics as was expected: ciprofloxacin has a high permeability, therefore dissolution is the rate limiting step in its absorption. Since the 9 formulations from the Tanzanian market had similar dissolution characteristics it can be assumed that they would have similar bioavailability profiles.

This observation illustrates an example where the Essential Drugs Concept (EDC) can be of immense benefit to a poor nation if it is judiciously implemented. The market price of the innovator brand was at least 20 times higher compared with that of the other formulations (Table 3.7), yet it had a similar bioavailability profile compared with the generic brand. When the necessary mechanisms for monitoring the quality of drugs on market have been put in place by the regulatory authorities, encouraging the use of generics could save a lot of resources. On the contrary when the quality of drugs on market cannot be ensured, the presence of cheap generics may lead to loss of confidence by both prescribers and users who may prefer the expensive brands on the assumption that the higher the price the better the quality.

## 7.6 Conclusion

The pharmacokinetic parameters obtained from the two formulations show that all drugs had satisfactory and comparable in vivo release profiles, even after exposure to tropical storage conditions. Clinically the formulations are expected to be similarly effective. The encouragement to use cheaper generic brand as stipulated in the Essential Drugs Concept should be complemented by the establishment of a functioning quality assurance mechanism that continuously monitors the quality and efficacy of drugs on market.

## 7.7 References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# 8. GENERAL CONCLUSIONS AND RECOMMENDATIONS

Increasing access to effective and affordable essential drugs is one of the objectives of any rational national drug policy. The WHO recommends the essential drugs concept as the best strategy for such an endeavour, especially to developing countries where meagre resources are available. Reports about the presence of substandard drugs on the market even when anecdotal, risks promoting irrational drug use, hence denting the benefits achievable by adoption of such a strategy. This study aimed at providing information on the quality of drugs on market in Tanzania.

The in vitro tests revealed that although all formulations contained the labelled drug in sufficient quantities, some formulations had not been optimised for stability in tropical climatic conditions. Essential drug formulations with a fixed dose combination showed a poor dissolution of one component (the lesser soluble component of the combination). This raises a concern, as the effectiveness of such drugs depends on the synergistic action exerted by both components. It is recommended that similar studies be done on more of such drug formulations as they are increasingly being recommended for infectious diseases since their use could delay the emergence of resistant microorganisms.

The bioavailability determination of acetylsalicylic acid and enteric coated diclofenac sodium tablets showed a poor in vitro / in vivo correlation. Since in vivo tests cannot be routinely used as quality control tests, efforts should be made to optimize the current in vitro dissolution test parameters to make the dissolution test predictive of bioavailability.

In general this study has shown that the prevalence of substandard drugs in Tanzanian is not as widely spread as was expected from literature reviews. Institution of a functioning quality assurance system is the most reliable deterrent against substandard drugs. In addition, such a system would provide to the general public information on the quality of drugs on market thus promote public confidence on the drug supply system and hence stimulate rational drug use.

# 9. SUMMARY

The quality and affordability of drugs is one of the major public health concerns in many developing countries. To address the problem of affordability in these countries, the WHO initiated the Essential Drugs Concept (EDC) and encouraged its member states to adopt this concept as a strategic policy to ensure that effective drugs to treat the majority of diseases in a community are constantly made available at affordable prices. Tanzania adopted the concept into its national drug policy, but it has since then lacked facilities to control and monitor the quality of drugs on market (an essential component of the EDC). As a result, there has been little information on the quality of drugs marketed in the country. In addition to safeguarding the general public against the hazards of substandard drugs, such information is necessary to build up public confidence in the health system and promoting rational drug use.

This thesis is aimed at evaluating the quality of the most commonly used essential drugs and at investigating the influence of tropical storage conditions on the in vitro and in vivo drug availability from different formulations of these drugs marketed in Tanzania. In Chapter 1 a literature review is presented about the health policy and the current situation of pharmaceutical market in Tanzania and also about the global concerns on the prevalence of substandard/counterfeit drugs in developing countries. The objectives of the study are outlined in Chapter 2.

57 formulations of 9 essential drugs were sampled from major importers and distributors of pharmaceuticals for the Tanzanian market. During collection of the samples it was noted that there existed a large price difference between innovator and generic brands. In chapter 3, the in vitro quality parameters (drug content, dissolution and the influence of tropical climatic conditions (Class IV: 75% relative humidity / 40°C) on the in vitro dissolution) were determined. The results showed that there were no fake drugs and that drug content in all formulations was within pharmacopoeia specifications. Only few formulations had an in vitro drug release that was below the USP 24 dissolution tolerance limits. Furthermore, the dissolution rate of some formulations that previously met the USP 24 requirements, failed after 6 months storage at simulated tropical conditions.

Inter-laboratory differences between sets of dissolution data of the same batch of a formulation are explored in chapter 4. Dissolution tests were performed in Tanzania and Belgium on cipofloxacin and metronidazole formulations. For each formulation the sets of data are compared using the FDA's similarity factor $f_2$ and Principal Component Analysis (a technique that enables visualisation of similarities or differences between sets of multivariate data). The evaluation showed only minor differences between the data obtained at both laboratories. The similarity of the between laboratory dissolution data could be explained by the fact that validated equipment was used and that the tests were performed by the same analyst, hence highlighting the importance of training the personnel working in a quality control laboratory.

The bioavailability of two acetylsalicylic acid (ASA) formulations(Dispril® and a formulation manufactured by Shelys is presented in Chapter 5. The latter formulation had a 100% in vitro drug release within 5 min, while the former released only 27% of the labelled drug after 30 min of dissolution test. Evaluation of the primary pharmacokinetic parameters obtained in the study showed that the extent of drug absorsption (for both ASA and its main metabolite salicylic acid) from the Shelys formulation was similar to that of Dispril®. However the Shelys formulation yielded a significantly lower $C_{max}$ and had a longer $t_{max}$ indicating that the rate of absorption from this formulation was lower. The effect of a lower absorption rate from this formulation on the efficacy (other than delayed onset of pain relief) will probably be insignificant as the $C_{max}$ obtained for SA was above the minimal concentration required for analgesia.

In Chapter 6 the influence of tropical storage conditions on the bioavailability of diclofenac sodium tablets was investigated by performing a 4-period cross-over study involving tablets of the innovator brand (Novartis) and a generic brand (Camden). The bioavailability profiles of the Novartis and Camden tablets stored at ambient conditions were similar, as were their dissolution profiles. The bioavailability of the Novartis tablets was not influenced by storage at high temperature and high relative humidity. Although the Camden formulation had its in vitro dissolution significantly reduced after exposure to Class IV conditions, its bioavailability showed only a moderate reduction in the rate of drug absorption, while the extent of drug absorption was not significantly altered. These

observations indicated that some formulations available on the Tanzanian market were not optimized for stability in tropical climates and highlighted the possibility of a poor in vitro / in vivo correlation.

In Chapter 7 the influence of tropical climatic conditions on bioavailability of two ciprofloxacin formulations was investigated by performing a 4-period crossover study on 12 subjects. In vitro, all ciprofloxacin formulations had similar potencies, in vitro dissolution and stability profiles. The pharmacokinetic parameters obtained showed that the two formulations had similar bioavailability profiles that were not influenced by storage at tropical climatic conditions. Since ciprofloxacin is a low solubility and high permeability drug, it can be presumed that all 9 formulations would have similar bioavailability profiles as their dissolution and stability profiles were similar (despite a very large price difference between the innovator and generic brands). The results show the importance of encouraging the use of generic versions (especially in developing countries) when the necessary quality control measures have been instituted.

# 10. SAMENVATTING

In veel ontwikkelingslanden is de kwaliteit en de kostprijs van geneesmiddelen één van de voornaamste zorgen binnen de gezondheidssector. Om het probleem van de kostprijs in deze landen aan te pakken, heeft de WHO het Essential Drugs Concept (EDC) opgesteld en zijn leden aangemoedigd om dit concept op te nemen in hun nationaal gezondheidsbeleid. Deze strategie verzekert dat er continu geneesmiddelen beschikbaar zijn die doeltreffend zijn tegen het merendeel van de ziektes welke de bevolking treffen en dit tegen een aanvaardbare kostprijs. Tanzania heeft dit concept opgenomen in zijn nationaal geneesmiddelenbeleid, maar er is een tekort aan faciliteiten om de kwaliteit van de geneesmiddelen beschikbaar op de markt daadwerkelijk te controleren (een essentieel onderdeel van het EDC). Hierdoor is er zeer weinig informatie beschikbaar is over de kwaliteit van de geneesmiddelen beschikbaar op de Tanzaniaanse markt. Deze informatie is niet alleen noodzakelijk om de bevolking te vrijwaren van de gevaren van verbonden aan geneesmiddelen van mindere kwaliteit, maar ook om het vertrouwen in het gezondsheidssysteem op te bouwen bij de bevolking en om een rationeel geneesmiddelen gebruik aan te moedigen.

Deze thesis heeft als doel de kwaliteit te evalueren van een aantal frequent gebruikte essentiële geneesmiddelen en te onderzoeken wat de invloed is van tropische bewaaromstandigheden op de in vitro en in vivo beschikbaarheid van deze geneesmiddelen uit de verschillende formulaties die op de Tanzaniaanse markt verkocht worden. In Hoofdstuk 1 werd een literatuuroverzicht gegeven van het gezondheidsbeleid en de huidige situatie op de farmaceutische markt in Tanzania en ook van de mondiale bezorgheid die er is omwille van de aanwezigheid van vervalse geneesmiddelen in ontwikkelingslanden. De doelstellingen van dit onderzoek zijn geschetst in Hoofstuk 2.

Bij belangrijkste invoeders en groothandelaars van farmaceutische producten voor de Tanzaniaanse markt werden stalen genomen van 57 formulaties van 9 essentiële geneesmiddelen. Tijdens het verzamelen van de stalen werd opgemerkt dat er grote prijsverschillen bestaan tussen het originele gepatenteerde product en het generisch equivalent. In Hoofdstuk 3 werden de volgende in vitro kwaliteitsparameters bepaald: geneesmiddelengehalte, geneesmiddelvrijstelling en

de invloed van een tropisch klimaat (75% relatieve vochtigheid/ 40°C) op de in vitro dissolutie. De resultaten toonden aan dat er tussen de geanalyseerde producten geen vervalste geneesmiddelen waren en dat het geneesmiddelengehalte in alle formulaties binnen de specificaties van de United States Farmacoppee (USP 24) viel. Enkele fomulaties hadden een in vitro geneesmiddelenvrijstelling lager dan de limieten gesteld in de USP 24. Bovendien voldeed de geneesmiddelvrijstelling van sommige formulaties niet meer aan de gestelde USP 24 eisen na 6 maanden bewaring onder gesimuleerde tropische condities.

In Hoofstuk 4 werden de dissolutiedata geëvalueerd van hetzelfde lot van een formulatie geanalyseerd in verschillende laboratoria. Dissolutietesten werden uitgevoerd in Tanzania en België op ciprofloxacine- en metronidazoletabletten. Voor elke formulatie werden de data vergeleken door middel van de FDA's similarity factor $f_2$ and Principal Component Analysis (een techniek die toelaat om de verschillen tussen sets van multivariabele data weer te geven). Uit de evaluatie bleken er slechts kleine verschillen tussen de data bekomen in beide laboratoria te bestaan. De gelijkaardige resultaten kunnen verklaard worden door het feit dat gevalideerde toestellen werden gebruikt en dat de testen werden uitgevoerd door dezelfde operator. Dit benadrukt het belang van training van het personeel dat tewerkgesteld wordt in een laboratorium voor kwaliteitscontrole.

De biologische beschikbaarheid van twee acetylsalicylzuur (ASA) formulaties (Dispril® en een formulatie geproduceerd door de firma Shelys) is voorgesteld in Hoofdstuk 5. De eerstgenoemde formulatie stelde in vitro zijn volledige geneesmiddelendosis binnen de 5 minuten, terwijl de laatste formulatie slechts 2% van het geneesmiddel vrijstelde binnen hetzelfde tijdsinterval en slechts 27% na 30 min. De farmacokinetische parameters toonden dat de hoeveelheid geabsorbeerd geneesmiddel (AUC)(voor zowel ASA als zijn voornaaste metaboliet salicylzuur) na toediening van de Shelys formulatie analoog is aan de AUC-waarden bekomen na inname van een Dispril®-tablet. De Shelys formulatie had echter een significant lagere $C_{max}$ en een hogere $t_{max}$. Dit wijst op een lagere absorptiesnelheid uit deze formulatie, waarvan het effect op de efficiëntie van het preparaat waarschijnlijk niet significant zal zijn, aangezien de $C_{max}$-waarden bekomen voor salicylzuur hoger zijn dan de minimale concentratie vereist voor het analgetisch effect.

In Hoofdstuk 6 werd de invloed van tropische bewaaromstandigheden op de biologische beschikbaarheid van natriumdiclofenac onderzocht, dit zowel voor het originele gepatenteerde product van Novartis als voor een generische vorm van de firma Camden. De biologische beschikbaarheid van Novartis- en Camden-tabletten bewaard bij kameromstandigheden waren gelijkaardig, net zoals hun dissolutieprofielen. De biologische beschikbaarheid van de Novartis-formulatie werd niet beïnvloed door bewaring bij hoge temperatuur en hoge relatieve vochtigheid. Dit in tegenstelling met de Camden formulatie blootgesteld aan gesimuleerde tropische condities waar de in vitro dissolutie significant daalde. In vivo vertoonde deze formulatie echter slechts een geringe daling van de geneesmiddelen absorptiesnelheid, terwijl de mate waarin het geneesmiddel geabsorbeerd werd (AUC) niet significant wijzigde. Deze observaties wijzen op het feit dat sommige formulaties welke beschikbaar op de Tanzaniaanse markt, niet geoptimaliseerd zijn naar stabiliteit toe in een tropische klimaat en benadrukken het belang van een goede in vitro/ in vivo correlatie.

In Hoofdstuk 7 werd de invloed van tropische bewaaromstandigheden op de biologische beschikbaarheid van twee ciprofloxacine formulaties onderzocht. In vitro hadden alle ciprofloxacine formulaties gelijkaardige in vitro dissolutie– en stabiliteitsprofielen. De farmacokinetische parameters toonden dat de twee formulaties dezelfde biologische beschikbaarheidsprofielen hadden en dat deze niet beïnvloed werden door bewaring bij gesimuleerde tropische condities. Aangezien ciproflaxacine een slecht oplosbaar geneesmiddel is met een hoge permeabiliteit en hun dissolutie- en stabiliteitsprofielen hetzelfde waren, kan verondersteld worden dat alle formulaties een analoge biologische beschikbaarheid zullen hebben (ondanks de grote prijsverschillen tussen het originele product en de generische equivalenten). Deze resultaten wijzen op het belang van het aanmoedigen van het gebruik van generische producten (voornamelijk in ontwikkelingslanden) wanneer de noodzakelijke kwaliteitscontroles zijn ingesteld.