The formulation and stability of dispersible or chewable tablets containing anti retroviral drugs

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B. Pharm

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Preface

This dissertation is part of the fulfilment of the requirements for the degree Magister Scientiae. Research sources from many different authors were quoted to verify the methods used during the study. This dissertation is written in Harvard format. A complete bibliography of all the publications is included. Furthermore a table with abbreviations used in the text is included; many of the abbreviations are unique to the publications quoted. Metric units were used during the study. Terms used during the study can either be found in the BP, USP or ICH guidelines used during the study (see bibliography). The most current publications were used where applicable or available.

Abstract

The formulation and stability of novel dosage forms of anti retroviral drugs

Lamivudine (3TC), Nevirapine (NVP) and Zidovudine (AZT) is indicated as part of antiretroviral combination therapy for the treatment of Human Immunodeficiency Virus (HIV) infected adults and children who present clinical or immunological evidence of progression of the disease. Current dosage forms of these drugs in single or dual-therapy include tablets, coated tablets and oral solutions.

Three problem areas are currently experienced with the above mentioned dosage forms. Firstly tablets and coated tablets, although they provide good stability and therefore shelf life, cannot be easily administered to children and geriatrics due to the fact that they cannot swallow tablets. Oral solutions on the other hand can be easily administered to children and geriatrics but provides poor stability and shelf life especially in harsh African conditions. Thirdly, according to UNICEF, extemporaneous preparation of pediatric suspensions from solid adult dosage forms can reduce the stability of the drugs as well as the bioavailability.

The aim of this study was to formulate a novel dosage form that will provide a solution for the above-mentioned problems.

Combinations of the three drugs were formulated in a dispersible or chewable tablet. Preformulation studies were carried out to determine the compatibility of the drugs and the excipients used in the formulae. Four batches of tablets were manufactured, each containing a different amount of the three drugs mentioned above, in accordance with WHO requirements. Accelerated stability tests were carried out at different temperatures and degrees of humidity to determine the stability of the drugs in the dosage form. Physical tests that were carried out on the tablets include assay, hardness, friability, loss on drying, disintegration time, dissolution and uniformity of mass, diameter and thickness.

Initial studies revealed that 3TC were the least stable of the three drugs in watery solutions. 3TC was least stable in a solution at a high pH (NaOH in water), it was

more stable at a lower pH (HCl in water) and most stable at neutral conditions (Water). NVP was also less stable at lower pH conditions in solution. The dosage form complied with requirements for rapid disintegration and palatability in solution. The dosage form can be classified as a dispersible as well as a chewable tablet.

Uittreksel

Formulering en stabileitstoetsing van 'n nuwe anti retrovirale doseervorm

Lamivudien (3TC), Nevirapien (NVP) en Zidovudien (AZT) is aangedui as deel van die behandeling van Menslike Imuniteitsgebrek Virus (MIV) in volwassenes sowel as kinders waarin progressie van die siektetoestand plaasvind. Huidige doseervorme bevat bogenoemde middels as enkelmiddel of dualistiese kombinasies in tablette, bedekte tablette en orale suspensies.

Drie probleme word huidiglik ondervind met die genoemde doseervorme. Eerstens kan tablette en bedekte tablette; alhoewel hul goeie stabiliteitseienskappe en 'n lang raklewe verseker; kan nie maklik deur kinders en bejaardes gebruik word nie as gevolg van probleme om harde tablette in te sluk. Orale suspensies kan maklik toegedien word aan kinders sowel as bejaardes. Ongelukkig is orale suspensies egter chemies en fisies minder stabiel en besit dus 'n korter raklewe as vloeistofvrye doseervorme, bv. tablette. Geneesmiddels en formuleringshulpstowwe ondergaan makliker chemiese en mikrobiologiese afbraak in vloeistofvorm as in hul droë vorm veral in Afrika klimaatskondisies. Derdens; volgens UNICEF kan doseervorme, wat uit vaste doseervorme berei word vir pasiënte deur gesondheidsorgpersoneel, nie die verlangde biobeskikbaarheid of raklewe verseker nie.

Die doel van die studie is om 'n doseervorm te ontwikkel wat bogenoemde probleme sal oplos.

Preformuleringstudies is uitgevoer om vas te stel of die geneesmiddels verenigbaar is met mekaar en moontlike tablethulpstowwe wat in die formulerings gebruik gaan word. Verkillende hoeveelhede van elke geneesmiddel is geformuleer in 'n dispergeerbare of koubare tablet. Vier lotte tablette is vervaardig met elke lot wat 'n verkillende hoeveelheid van elke geneesmiddel bevat in ooreenstemming met die vereistes van die Wêreld Gesondheids-Organisasie (WGO). Fisiese toetse soos gespesifiseer in die onderskeie farmakopiëe is op elke lot uitgevoer en sluit in geneesmiddelinhoud, hardheid, afsplitsing, vogverlies tydens droging, disintegrasie-tyd, dissolusie en massa-, deursnee- en diktebepalings. Die tablette is by

verskillende versnelde stabilitieitskondisies (verhoogde temperatuur en vogtigheid) geplaas vir 'n maksimum periode van 3 maande om te bepaal of enige chemiese of fisiese veranderinge in die doseervorme plaasgevind het.

Aanvanklike studies het getoon dat 3TC die minste stabiel van die drie geneesmiddels is in waterige oplossings. Die geneesmiddel was die minste stabiel by 'n hoë pH (NaOH in water), meer stabiel by 'n lae pH (HCl in water) en die meeste stabiel in neutrale kondisies (Water). NVP was minder stabiel by lae pH toestande in oplossing.

Al die tablet-lotte voldoen aan vereistes soos gestel deur die onderskeie farmakopeë. Die tablette kan geklassifiseer word as dispergeerbare- sowel as koubare-tablette.

Aims and Objectives

Acquired Immune Deficiency Syndrome (AIDS) have been known to healthcare professionals for more than twenty years. Although no known cure for the virus (Human Immunodeficiency Virus) (HIV) causing the disease is known, many effective treatments to reduce the mortality rate are available. Treatments include a healthy lifestyle as well as drug treatment.

Azidothymidine or shortly Zidovudine was one of the very first anti retroviral drugs used in the treatment of AIDS. The drug was initially administered as mono-therapy to patients presenting with HIV progression. Unfortunately resistance to the drug quickly developed due to the rapid mutation rate of the virus. Many drugs are currently available for the treatment of AIDS and new drugs are constantly under development.

Mono-therapy of HIV infection is something of the past and multiple drug treatment replaced the AZT mono-therapy of the 1980's. AZT is currently administered to patients in combination with other first- (NRTI's), second- (NNRTI's) and third generation (PI's) anti retroviral drugs. The use of multiple drug treatment resulted in a decrease in drug resistance. Furthermore the treatment of patients is varied with other multiple drug combinations after a period of treatment to reduce the possibility of resistance to one or more of the drugs.

Unfortunately the amount of drug combinations available is dependant on the possibility of drug-drug interactions in regimens, biological contra-indications *in vivo* and possible cross-resistance with similar drugs. Most of the commercially available treatment regimes contain a single or dual combination of drugs. Patients are prescribed more than one regimen at multiple intervals and with different amounts of each regimen on a daily basis. This kind of treatment confuses the patient and may ultimately lead to the non-adherence to the treatment.

Azidothymidine, Lamivudine and Nevirapine have been used successfully in multiple drug treatment of AIDS patients. Successful clinical trials with the combination have been performed in India. The drugs are fairly inexpensive and readily available worldwide.

The main objectives of this study therefore are to:

- determine the chemical and physical compatibility of the above mentioned anti retrovirals with each other and excipients used to manufacture the dosage form.
- develop different regimen strengths to treat both children and adults.
- develop dispersible tablet formulations containing AZT, NVP and 3TC in combination.
- develop and validate a HPLC method for the simultaneous analysis of the drugs in the above mentioned formulations.
- evaluation of the physical characteristics in accordance to pharmacopoeial specifications.
- stability testing and evaluation of the physical- and chemical characteristics after 3 months of accelerated conditions.
- evaluation of results and drawing of conclusions.

Chapter 1

HIV, AIDS and anti retrovirals used in the treatment of AIDS

1.1 Introduction: HIV, AIDS

The acquired immunodeficiency syndrome (AIDS) epidemic is caused by the infection with the human immunodeficiency virus (HIV). HIV is a retrovirus of which the mature virions contain two single stranded RNA molecules surrounded by a nucleocapsid and an outer lipid envelope. Type HIV-1 is the main cause for infections worldwide although HIV-2 is the cause for infections in West Africa. Infection with HIV is characterised by viral replication and CD4 lymphocyte depletion which results in a profound immunodeficiency (Raffanti *et al.*, 2001:1349). The end result of the immune deficiency is secondary infections primarily cancer-like Karposi's sarcoma, tuberculosis, cryptosporidium (gastro-enteritis), herpes zoster, oral and skin lesions. Death occurs as a result of the opportunistic infections (Webb, 1997:4).

Unidentified cases of HIV were reported as early as 1959 in sub-Saharan Africa and in the late 1970's. The first official notification of the epidemic was in June 1981 by the Centre of Disease Control (CDC) in the United States of America (Perrow *et al.*, 1990:15).
The proposition that HIV was inoculated to humans by early experimental oral polio vaccines (OPV CHAT) in the 1950's, derived from macaque kidney cells seems highly unlikely. The exact origin of the HIV virus is unknown although it contains similar characteristics as Simian Immunodeficiency Virus (SIV) found in certain species of primates in central Africa (Berry *et al.*, 2004:1-9).

In 2003 more than 5 million people were infected with HIV, 700 000 of which were children. Ninety five percent of child infections were vertical mother-to-child and 90% of these children live in sub-Saharan Africa. According to reports it is estimated that 500 000 children are currently in need of antiretroviral therapy worldwide. In 2003 some 490 000 child deaths under the age of 14 were due to AIDS, an estimated 17% of al AIDS deaths were among children (UNICEF, 2004:1).

The HIV reverse transcriptase enzyme is very prone to errors resulting in rapid evolution in genetic diversity causing rapid development of resistance to antiretroviral agents. The understanding of the pathogenesis of the virus led to the development of many new effective drugs. Three classes of anti retrovirals are currently available: Nucleoside HIV reverse transcriptase inhibitors, Non-nucleoside HIV reverse transcriptase inhibitors and HIV protease inhibitors (Raffanti *et al.*, 2001:1349).

1.2 Drugs indicated for the treatment of HIV infections

The first effective agent against HIV discovered in 1987 was Zidovudine a nucleoside reverse transcriptase inhibitor (NRTI). Unfortunately the NRTI's could delay HIV infection only temporarily. The search for more effective agents led to the discovery of the nonnucleoside reverse transcriptase inhibitors (NNRTI) of which Nevirapine was the first. In 1990 Saquinavir, the first protease inhibitor was developed (Raffanti *et al.*, 2001:1352).

Reverse transcriptase is an enzyme that converts viral RNA into proviral DNA before incorporating it into the host cell chromosome. NRTI's prevent infection of uninfected host cells but has little effect on already infected cells. The NRTI's are substrates for reverse transcriptase. NRTI's have to be phosphorylated by the host cell enzymes in the cytoplasm to become active. The nucleoside reverse transcriptase inhibitors lack a 3'-hydroxyl group, incorporation into the DNA terminates chain elongation (Raffanti *et al.*, 2001:1353).

1.2.1 Nucleoside reverse transcriptase inhibitors

NRTI's were the first generation of anti retrovirals. Examples include: Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir and Emtricitabine (Wikipedia, 2006: NRTI's). Zidovudine (AZT) was initially approved as monotherapy. Subsequently it was approved in combination therapy with zalcitabine and lamivudine (3TC). Studies with AZT demonstrated clinical and survival benefits in patients with AIDS and those with symptomatic and asymptomatic HIV infection who had a CD4+ T-lymphocyte count of 500 cells per cubic millimetre or less. Limited clinical benefits were due to incomplete suppression of HIV replication and emergence of resistant strains. AZT is currently approved for the treatment of HIV infection in combination regimens with PI, NRTI and NNRTI's. Combination regiments can achieve long term viral suppression with partial reconstitution of the immune system (Fischl, 2003:23).

1.2.2 Protease Inhibitors

Protease Inhibitors were the second generation of anti retrovirals developed for the treatment of AIDS and Hepatitis. PI's prevent viral replication by inhibiting the activity of the enzyme (protease) used to cleave viral proteins before assembly of a new virion. Examples of PI's include: Saquinavir, Ritonavir, Indinavir, Nelfinavir, Amprenavir, Lopinavir, Atazanavir, Fosamprenavir and Tipranavir (Wikipedia, 2006: PI's).

1.2.3 Nonnucleoside reverse transcriptase inhibitors

NNRTI's were the third generation of anti retrovirals. Examples include: Nevirapine, Delavirdine and Efavirenz (Wikipedia, 2006: NRTI's).

Zidovudine, Lamivudine and Nevirapine are approved for use in the United States (Table 1.1), by the WHO (Table 1.4) and South African health authorities (Table 1.3).

Table 1.1: Antiretroviral agents approved for use in the US (Raffanti *et al.*, 2001:1354)

GENERIC NAME	DEVELOPMENTAL OR OTHER NAME	RELATIVE ANTIVIRAL EFFECT
Nucleoside reverse transcriptase inhibitors		
Zidovudine	AZT; azidothymidine	++
Didanosine	ddI; dideoxyinosine	++
Stavudine	D4T	++
Zalcitabine	ddC; dideoxycytidine	+
Lamivudine	3TC	++
Abacavir	1592U89	+++
Nonnucleoside reverse transcriptase inhibitors		
Nevirapine	BI-RG-587	+++
Efavirenz	DMP266	+++
Delavirdine		+++
Protease inhibitors		
Saquinavir	L-735,524	++
Indinavir	ABT-538	+++
Ritonavir	VX-478; 141W94	+++
Nelfinavir	ABT-378	+++
Amprenavir		+++
Lopinavir		+++

1.3 Treatment guidelines in South Africa

According to the South African department of health, initiation of therapy is started in a patient if the CD4 count is ≤ 200 or the patient is symptomatic with WHO Stage IV (Table 1.2) condition irrespective of the CD4 count (Naidoo et al., 2005:2- 3). The WHO states that initiation of therapy should be started if the CD4 count is ≤ 200 with WHO stage I or II HIV disease; CD4 count 200-350 with WHO Stage III disease or WHO Stage IV HIV disease irrespective of the CD4 count (Naidoo *et al.*, 2005:2- 3).

Table 1.2: WHO staging system for HIV infection in adults and adolescents (Naidoo, 2004:40)

Stage I : 1. Asymptomatic 2. Persistent generalized lymphadenopathy Performance scale 1: asymptomatic, normal activity
Stage II : 1. Weight loss < 10 % of body weight 2. Minor mucocutaneaous manifestations (e.g. seborrhoea, prurigo, oral ulcers, fungal nail infections, angular cheilitis) 3. Herpes zoster within the last 5 years 4. Recurrent upper respiratory tract infection (URTI), e.g. bacterial sinusitis And/or performance scale 2: symptomatic, normal activity
Stage III : 1. Weight loss > 10 % of body weight 2. Chronic diarrhoea > 1 month 3. Prolonged fever > 1 month 4. Oral candidiasis 5. Oral hairy leucoplakia 6. Pulmonary TB 7. Severe bacterial infections (pneumonia, pyomyositis) And/or performance scale 3: bedridden < 50 % of the day during the last month
Stage IV : 1. HIV wasting syndrome: weight loss of > 10 % of body weight, plus either unexplained chronic diarrhoea (>1 month) or chronic weakness and unexplained prolonged fever (>1 month) 2. Pneumocystis carinii pneumonia(PCP) 3. CNS toxoplasmosis 4. Cryptosporidiosis and diarrhoea > 1 month 5. Extra pulmonary Cryptococcus 6. Cytomegalovirus infection other than liver, spleen or lymph node 7. Herpes simplex infection; visceral or mucocutaneous > 1 month 8. Progressive multifocal leuco-encephalopathy (PML) 9. Any disseminated endemic mycosis 10. Oesophageal, tracheal or pulmonary candidiasis 11. Atypical mycobacteriosis disseminated 12. Non-typhoid salmonella septicaemia 13. Extra pulmonary TB 14. Lymphoma 15. Kaposi's sarcoma 16. HIV encephalopathy And/or performance scale 4: bedridden > 50 % of the day during the last month

Initiation of therapy in ART-naïve patients is two NRTI's one from each category 1 and 2 (Table 1.3) plus 1 NNRTI from category 4. Patients with a viral load of less than 50 000 copies per ml use 3 NRTI's one from each category 1 to 3. Protease inhibitors are reserved for second line defence with two new NRTI's from group 1 to 3 (Gibbon, 2001:310).

Table 1.3: Categories of antiretroviral drugs in South Africa (Gibbon, 2001:310)

Category 1 (NRTI)	**Category 2 (NRTI)**	**Category 3 (NRTI)**	**Category 4 (NNRTI)**	**Category 5 (PI)**
Stavudine Zidovudine	Didanosine Lamivudine Zalcitabine	Abacavir	Nevirapine Efavirenz	Nelfinavir Indinavir ± Ritonavir Saquinavir ± Ritonavir Lopinavir ± Ritonavir (Combination product) Ritonavir

1.4 Commercially available dosage forms or combinations of drugs

Anti retroviral drugs are offered in a variety of dosage forms and dosage strengths to suite most of the patient needs (Table 1.4). Although paediatric dosage forms contain an amount of drug suitable for treatment of children, the administered dosage must still be calculated according to every patient's weight (UNICEF, 2004:2).

Many single drug regimens are currently available (Table 1.5). The single drugs can be combined with each other during Highly Active Anti Retroviral Therapy (HAART). The biggest problem with these combinations is patient compliance. Most patients find it very difficult to accept a combination of more than two dosage forms to be taken simultaneously. The WHO recommends the use of a combination of at least 3 anti retroviral drugs (Table 1.6) as predetermined by the country where applicable. Combination dosage forms are preferred if adequate safety and efficacy data are available for the combination dosage form (WHO, 2002:7).

Long term safety and clinical trials have been performed in India on fixed dose combinations (FDCs) of anti retrovirals amongst anti retroviral naïve patients. Formulation combinations of AZT/3TC/NVP and d4T/3TC/NVP were assessed in two studies at two private tertiary referral centres.

An improvement in mean CD4 counts was observed. The largest improvement in CD4 count occurred during the first 3-6 months of initiation of HAART and was sustained for up to 2 years. The most commonly encountered side effects were rash (6.9%) and hepatitis (3.2%) occurring within 1-12 weeks of initiating therapy (Pujari *et al.*, 2003:1).

During the development of a FDC drug it is important to ensure compatibility of the active substances in the formulation. Good bioavailability should also be considered when developing a FDC.

No incompatibilities were observed in the formulation of AZT/3TC/NVP and therefore the substances can be formulated in a FDC (Pujari *et al.*, 2003:4)

Table 1.4: WHO essential drug list for ART and dosages for each drug (WHO, 2002:7-8)

Nucleoside reverse transcriptase inhibitors	
Abacavir (ABC)	Tablet, 300mg (as sulphate) Oral solution, 100mg (as sulphate)/5ml
Didanosine (ddI)	Buffered chewable, dispersible tablet, 25mg, 50mg, 100mg, 150mg, 200mg Buffered powder for oral solution, 100mg, 167mg, 250mg packets Unbuffered enteric coated capsule, 125mg, 200mg, 250mg, 400mg
Lamivudine (3TC)	Tablet, 150mg Oral solution 50 mg/5ml
Stavudine (d4T)	Capsule 15mg, 20mg, 30mg, 40mg Powder for oral solution, 5mg/5ml
Zidovudine (ZDV or AZT)	Tablet, 300mg Capsule 100 mg, 250 mg Oral solution or syrup, 50mg/5ml Solution for IV infusion injection, 10 mg/ml in 20-ml vial
Non-nucleoside reverse transcriptase inhibitors	
Efavirenz (EFV or EFZ)	Capsule, 50mg, 100mg, 200mg Oral solution, 150mg/5ml
Nevirapine (NVP)	Tablet 200 mg Oral suspension 50 mg/5-ml
Protease inhibitors	
Indinavir (IDV)	Capsule, 200mg, 333mg, 400mg (as sulphate)
Ritonavir	Capsule, 100mg Oral solution 400mg/5ml
Lopinavir + Ritonavir (LPV/r)	Capsule, 133.3mg + 33.3mg Oral solution, 400mg + 100mg/5ml
Nelfinavir (NFV)	Tablet, 250mg (as mesilate) Oral powder 50mg/g
Saquinavir (SQV)	Capsule, 200mg

Table 1.5: List of commercial single drug regimens available (Arora, 2003:2)

Anti retroviral drug and dosages	**Registered trademark and manufacturing company**
1. Lamivudine Tablets 150/300 mg	Epivir® Tablets, GSK, USA
2. Zidovudine Tablets 300 mg	Retrovir® Tablets GSK, USA
3. Nevirapine Tablets 200 mg	Viramune® Tablets, Boehringer, USA
4. Stavudine Caps 20/30/40 mg	Zerit® Capsules, BMS, USA
5. Indinavir Capsules 400 mg	Crixivan® Capsules, Merck, USA
6. Efavirenz Capsules 200 mg	Sustiva® Capsules, BMS, USA
7. Efavirenz Tablets 600 mg	Sustiva® Tablets, BMS, USA
8. Abacavir Tablets 300 mg	Ziagen® Tablets, GSK, USA
9. Nelfinavir Tablets 250/625mg	Viracept® Tablets, Agouron, USA
10. Didanosine DR capsules 125/200/250/400mg	Videx® EC Tablets, BMS, USA
11. Didanosine Chewable Tablets 25/50/100/200mg	Videx® Chewable, BMS, USA
12. Nevirapine Suspension 50mg/5ml	Viramune®, Susp, Boehringer, USA
13. Zidovudine Liquid 50mg/5ml	Retrovir® Liquid, GSK, USA
14. Lamivudine Liquid 50mg/5ml	Epivir® Liquid, GSK, USA

Table 1.6: List of commercial combination drug regimens available (Arora, 2003:3)

Anti retroviral drugs and dosages	**Registered trademark and manufacturing company**
Lamivudine + Zidovudine Tablets 150+300 mg	Combivir®, GSK, USA
Abacavir + Lamivudine + Zidovudine Tablets 300+150+300 mg	Trizivir®, GSK, USA
Lamivudine + Stavudine Tablets 150+30/40mg	Ranbaxy, India
Lamivudine + Nevirapine + Stavudine Tablets 150+200+30/40mg	Ranbaxy, India
Lamivudine + Nevirapine + Zidovudine Tablets 150+200+300mg	Ranbaxy, India

1.5 Reasons for new dosage form development

An enormous variety and different combinations of anti retroviral drugs are commercially available as can be seen in tables 1.5 and 1.6. Due to the large variety of dosage forms in different strengths and drug combinations it is difficult for the primary health care professional to decide which medication should be supplied to the wide variety of patients e.g. infants to adults. Infants and children have different drug requirements compared to adults. Dosage regimens are usually calculated according to lean body weight or body surface area for children. The World Health Organization requires dosage regimens that can be easily administered to a variety of age groups. Lamivudine, Zidovudine and Nevirapine are all included in the World Health Organization (WHO) list for essential anti retrovirals as seen in table 1.4. Due to the proven effectiveness of these three drugs it was considered for inclusion in the new formulation.

After careful consideration of the three ARV dose ranges (UNICEF, 2004: Annex 1, Table 1a), the following dosage strengths for development of the dispersible tablet formulations were compiled (Table 1.7).

Table 1.7: Combination and strengths of anti retrovirals used in dispersible tablet formulations during the study.

Formulation 1: 10 mg Lamivudine and 20 mg Nevirapine per tablet	Infants 3-6 kg two tablets twice daily dispersed in liquid. (WHO: 20 mg 3TC and 40 mg NVP BD)
Formulation 2: 15 mg Lamivudine, 37.5 mg Nevirapine and 25 mg Zidovudine per tablet	Infants 6-10 kg two tablets twice daily dispersed in liquid. (WHO: 25 mg 3TC, 75 mg NVP and 50 mg ZDV BD)
Formulation 3: 25 mg Lamivudine, 50 mg Nevirapine and 50 mg Zidovudine per tablet	Children 10-15 kg two tablets twice daily dispersed in liquid. (WHO: 50 mg 3TC, 100 mg NVP and 70 mg ZDV BD)
Formulation 4: 75 mg Lamivudine, 100 mg Nevirapine and 150 mg Zidovudine per tablet	Children 15-20 kg one tablet daily and 20-29 kg two tablets twice daily dispersed in liquid. (WHO: 15-20 kg – 75 mg 3TC, 150 mg NVP and 100 mg ZDV BD) (WHO: 20 -29 kg and adults – 100-150 mg 3TC, 200 mg NVP and 150 mg ZDV BD)

Due to the difficulty to adhere to a variety of age groups, estimated formulations were developed. In instances where the correct strength of a dosage is not available the dosage given can be halved, doubled or combined with one of the other formulations depending on the situation. Due to unavailability of equipment scored tablets could not be manufactured. Note that for infants' 3-6 kg bodyweight Zidovudine was excluded due to the inability of small infants to metabolize the drug resulting in the development of serious side effects. The above formulations developed can therefore cater for a wide variety of age groups and adheres to the guidelines set by the WHO. Current formulations cater for either children or adults and in most cases a combination of dosage forms have to be taken or prepared by the health care worker (UNICEF, 2004:1).

1.6 Summary

A current challenge is the treatment of the paediatric population infected with HIV. Special considerations have to be taken when treating infants and children. As children grow, physiological changes produce differences in absorption, distribution,

metabolism and excretion of drugs and the need arise for different dosing and treatment options. Regimens need to be palatable, should be stable once mixed even with foodstuffs and should not have complex food requirements. The dispensing method need to be simple as the parents may be sick, elderly or illiterate. Current regimens require children to take frequent doses of unpalatable syrups or suspensions, many of which require cold storage, have a limited shelf life and are very costly. Once the child reaches 10 kg or more the child requires large quantities of the regimen leading to the extemporaneous preparation of mixtures from adult dosage forms. This can lead to dangerous under- or over dosing if providers and caregivers are not guided appropriately. The paediatric dosage forms are usually much more costly than the adult regimens (UNICEF, 2004:4).

The preferred dosage forms for paediatrics are solid dosage forms e.g. granules, chewable or crushable tablets. The patient weight at which the dosage forms should be offered will vary from 10 – 12 kg depending on the ease of administration, acceptance and cost (UNICEF, 2004:7).

Syrups, suspensions or dissolvable formulations of the following remain the best options: Zidovudine, Abacavir and Lamivudine or Nevirapine or Lopinavir/Ritonavir (UNICEF, 2004:7).

Recommendations for solid formulations are:

- Used by child as soon as possible when a child can swallow (child weight > 10 kg).
- Suitable dosage forms to provide dose ranges by 2 - 3 kg weight band for small children and 10 kg weight band for bigger children.
- Granulate, crushable or dispersible tablet.
- Stable with a long shelf life even at high temperatures and humidity.
- Scored tablets.
- Suitable masking of bad taste (UNICEF, 2004:7).

A disadvantage of the FDCs, particularly the formulations containing nevirapine is that physicians may initiate treatment with these regimes without a lead-in dose and therefore increasing the risk of development of adverse effects like hepatitis or rash (Pujari *et al.*, 2003:5).

CHAPTER 2

Physicochemical properties of drugs and excipients used in the formulations

2.1 Lamivudine

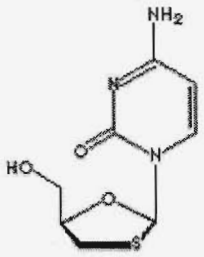

Figure 2.1: The *cis* enantiomer of 2'-deoxy-3'-thiacytidine (Budavari *et al.*, 2001:5364)

Molecular Formulae:	$C_8H_{11}N_3O_3S$
Molecular Weight:	229.259
Variants:	(2R, 5R)-form, **(2R, 5S)-form** and (2S, 5R)-form
Melting points:	153-156; **160-162**; 145-147 °C respectively
Pharmacological active isomer:	(2R, 5R)-form
Physical description:	Crystal, white to of-white (Et_2O/MeOH) (Pharma Source)
Solubility:	Approximately 70mg/ml in water at 20 °C. (Budavari *et al.*, 2001:5364)

2.1.1 Structure

Lamivudine (Figure 2.1) is a pyrimidine nucleoside analogue that contains a sulphur atom in place of the 3' carbon of the ribose ring. The compound was originally synthesized in a racemic mixture (BCH-189) and was subsequently separated into positive and negative enantiomers. 3TC is the negative enantiomer; the ribose ring is in a position opposite to the ribose ring position found in physiologic nucleosides and most nucleoside analogues (Eron, 2003:84).

2.1.2 Pharmacology

3TC must be metabolised to the triphosphorylated form to be an active antiviral compound. 3TC-triphosphate competes with deoxycytidine-triphospate (dCTP), an endogenous nucleoside for binding in the HIV reverse transcriptase binding site. The incorporation of 3TC-triphosphate into the elongating DNA molecule results in chain termination. 3TC lacks the 3'-hydroxyl group required for the 5' to 3' linkage required for DNA synthesis. Both positive- and negative enantiomers have activity against the HIV virus. The positive enantiomer is slightly more cytotoxic than the negative enantiomer. 3TC is active against HIV-1, HIV-2 and hepatitis B.

3TC has been shown to be synergistic with other antiretroviral agents i.e. nucleoside analogues (zidovudine, stavudine, didanosine), protease inhibitors and nucleoside reverse transcriptase inhibitors in inhibiting the HIV-1 virus. Three drug combinations of 3TC/ZDV/saquinavir, 3TC/ZDV/d4T, 3TC/ZDV/nevirapine and 3TC/ZDV/delavirdine have been shown to be synergistic or additive *in vitro*. 3TC interfere with the phosphorylation of zalcitabine (ddC), likely because they are both cytosine analogues. 3TC and ddC are thus antagonists and a combination of these two is not recommended during HAART therapy (Eron, 2003:84).

2.1.3 Pharmacokinetics

3TC has favourable oral bioavailability of 82% in male HIV patients. Foodstuffs have little effect on the bioavailability of the drug. Similar oral bioavailability has been shown in tablet and oral solutions although intra-subject variability has been shown with the tablet formulations. The bioavailability of the drug is somewhat lower in infants with a bioavailability of 66% in one study. 3TC enters cells by passive diffusion. 3TC is phosphorylated more actively in resting lymphocytes than active ones.

The half-life of 3TC is 3-4 hours in serum; clearance is dependent on weight and renal function and is not influenced by gender, disease stage, CD4+ T-lymphocyte count or race.

Studies have shown no change in pharmacokinetic parameters after 24 weeks of continuous dosing. The drug has low protein binding in plasma and seems to cross most physiological membranes with ease. 3TC clearance is prolonged in neonates compared to older children. 70% of the drug is excreted unchanged by the kidneys and requires dose adjustment for patients with renal impairment. 3TC can be cleared by haemodialysis and requires no change in dose for such patients due to the large volume of distribution. Pharmacokinetic changes have not been shown for patients with impaired hepatic function (Eron, 2003:85).

2.1.4 Toxicology

Toxicity occurs as a result of the affinity for human DNA polymerases. Most of the nucleoside analogues have a greater affinity for HIV-1 reverse transcriptase than for human polymerases. Neutropenia was observed in higher than normal doses as well as a decrease in neutrophil counts. Higher haemoglobin counts were reported in the treatment with 3TC alone. During combination therapy with 3TC and AZT neutropenia and nausea was the most common adverse effects (Eron, 2003:86).

2.1.5 3TC in combination with other RTI's

According to Eron (2003:90) Nevirapine with 3TC and AZT have also been shown to be highly active therapy, even in individuals with HIV RNA levels of more than 100,000 copies per millilitre in a comparative study with NVP/3TC/ZDV.

2.1.6 Drug interactions with 3TC

A decrease in renal clearance has been observed during the co-administration with trimethoprim - sulphamethoxazole (Eron, 2003:96). The adjustment of 3TC, trimethoprim or sulphamethoxazole is not required unless the patient is renal impaired (Raffanti *et al.*, 2001:1359) (Gibbon, 2001:313).

2.1.7 Recommended dosages

150 mg orally four times daily for adolescents and adults with a body weight of more than 50 kg and 2 mg/kg every 12 hours for adults weighing less than 50 kg. In children the dose is 4 mg/kg every 12 hours up to 300 mg daily. Dose adjustments are required for persons with renal impairment. 3TC can be administered with or without food (Eron, 2003:96).

2.2 Nevirapine

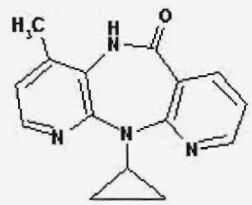

Figure 2.2: 11-cyclopropyl-5,11-dihydro-4-methyl-6H-Dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (Budavari *et al.*, 2001:6515)

Molecular Formulae: $C_{15}H_{14}N_4O$

Molecular Weight: 266.302

Melting points: 247-249; 253-254; °C

Physical description: Crystal, white to of-white (EtOAc) (Pharma Source)

Solubility: Approximately 0.1mg/ml in water (20 °C) at neutral pH, highly soluble pH < 3. (Budavari *et al.*, 2001:6515)

2.2.1 Structure

Nevirapine is a dipyridodiazepine derivative nonnucleoside reverse transcriptase inhibitor. The chemical structure of the drug differs from other commercially available NNRTI's and antivirals (McEvoy, 2002:692).

2.2.2 Pharmacology

Nevirapine (NVP) is a NNRTI of HIV-1(Sweetman, 2002:637). It inhibits virus replication by binding directly to reverse transcriptase (RT) in a pocket adjacent to

the catalytic site of the enzyme. The drug binds to RT and causes conformational change that inactivates the enzyme preventing polymerization of viral RNA to DNA. NVP is very specific for HIV-1 RT and does not interfere with human DNA polymerization enzymes. NVP can enter most cells affected by the virus unlike NRTI's which is active in only certain cell groups. The characteristic that makes NVP so unique is that it do not require intracellular phosphorylation to become active. NVP can also bind to viral RT in the plasma thus decreasing the viral load in the cells as well as in the plasma.

Cross resistance with other NNRTI's (efavirenz and delavirdine) can occur but there is no record of cross resistance with PI's (Montaner *et al.*, 2003:134).

2.2.3 Pharmacokinetics

Oral absorption of NVP is very high for both tablet and oral solutions (>90%). Within 4 hours peak concentrations of 2.0±0.4 mg/ml were reached following a 200 mg oral dose. NVP peak concentrations increase linearly following multiple doses. Absorption is not severely affected by the simultaneous intake of food or antacids (Montaner *et al.*, 2003:134). NVP binds 60% to plasma proteins. It crosses the blood brain barrier (45% of plasma concentrations), placenta and is distributed in the breast milk. Half life of the drug can vary from 25 to 45 hours and metabolised mainly in the liver (Sweetman, 2002:637).

2.2.4 Toxicology

The most prevalent side effects during the treatment with NVP include: Skin rash or Stevens-Johnson syndrome, liver enzyme induction and hepatotoxicity (Montaner *et al.*, 2003:134) (Sweetman, 2002:637).

2.2.5 Drug interactions with NVP

The simultaneous administration of NVP and Ketoconazole may lead to increased levels of NVP or reduce the levels of Ketoconazole. CYP450 Isoenzyme inducers may reduce the plasma levels of NVP. NVP may reduce the levels of oral contraceptives (ethinyl estradiol or norethindone). The co-administration of NVP with certain Protease Inhibitors is not recommended due to overlapping metabolic pathways that may lead to the reduction in PI plasma levels (Montaner *et al.*, 2003:141-142) (Sweetman, 2002:637).

2.2.6 Recommended dosages

Initial dosages of 200 mg daily for 14 days then 200 mg twice daily for adults. 4 mg per kg body-weight daily for 14 days then 7 mg twice daily for children 2 months to 8 years. 4 mg per kg daily for 14 days then 4 mg twice daily for children 8 to 16 years (Sweetman, 2002:637).

2.3 Zidovudine

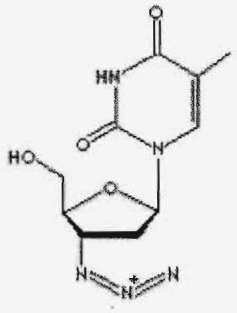

Figure 2.3: 3'-Azidothymidine. 3'-Azido-3'-deoxythymidine (Budavari *et al.*, 2001:10180)

Molecular Formulae: $C_{10}H_{13}N_5O_4$

Molecular Weight: 267.244

Melting points: 106-112; 119-121 (after drying) °C

Physical description: Crystal, white to of-white needles (Et_2O) (Pharma Source)

Solubility: Approximately 25 mg/ml in water at 25°C. (Budavari *et al.*, 2001:10180)

2.3.1 Structure

Zidovudine is a dideoxynucleoside reverse transcriptase inhibitor. AZT differs structurally from thymidine in that it contains a 3'-azido group rather than a 3'-hydoxyl

group. The replacement of the 3'-hydoxyl group in the nucleoside results in the inability of AZT to form phosphodiester linkages in this position and preventing DNA chain elongation (McEvoy, 2002:739).

2.3.2 Pharmacology

AZT is a synthetic thymidine analogue that is phosphorylated to a diphospate and then to the active zidovudine 5'-triphosphate which interfere with viral transcriptase and elongation of viral DNA chain (Fischl, 2003:23) (Sweetman, 2002:647).

2.3.3 Toxicology

AZT is haemotoxic. AZT should not be administered to patients with anaemia, bone-marrow suppression, reduced liver and kidney function, neonates with hiper-bilirubinemia and the elderly (Sweetman, 2002:646).

2.3.4 Pharmacokinetics

AZT is readily absorbed from the GI-tract with peak concentrations within 0.5 to 1.5 hours. Simultaneous intake of food decreases plasma concentrations up to 50%. The half-life of AZT is 0.78 to 1.93 hours; the triphosphorylated form has a half-life of 3 to 4 hours. AZT is eliminated through conjugation and urinary excretion. AZT is not significantly metabolised by the CYTP450 enzymes. AZT crosses the blood-brain barrier, placenta and is excreted in the breast milk (Fischl, 2003:23-25) (Sweetman, 2002:647).

2.3.5 Drug interactions with AZT

AZT and Stavudine, Ribavirin in combination have an antagonistic activity (Fischl, 2003:23-25). AZT undergoes glucoronidation. Drugs that undergoes glucoronidation delay the metabolism of AZT. Simultaneous use of AZT with myelosuppresive or nephrotoxic drugs are not recommended (Sweetman, 2002:646).

2.3.6 Recommended dosages

Oral doses of 500 – 600 mg daily in divided doses may be given for adults (Fischl, 2003:34-35). For children over 3 months of age 360 to 480mg per m^2 body-surface daily in 3 or 4 divided doses. Doses should not exceed 200mg every 6 hours (Sweetman, 2002:648).

2.4 Aspartame

Chemical name:	*N*-α-$_L$-Aspartyl-$_L$-phenylalanine 1-methyl ester [22839-47-0]
Empirical formula:	$C_{14}H_{18}N_2O_5$
Molecular weight:	294.31
Function:	Sweetening agent, 180-200 times the sweetness of sucrose, 1g = 17 kJ (4 kcal)
Description:	Off white odourless crystalline powder with an intense sweet taste
Melting point:	246-247°C
Solubility:	Sparingly soluble in water
Incompatibilities:	DSC studies indicate possible interactions with dibasic calcium phosphate and magnesium stearate (Kibbe, 2000:27)

Reason for inclusion in formulation

Aspartame is approved for use in beverages, desserts and pharmaceutical products. It discolours in the presence of ascorbic acid or tartaric acid. It is a possible carcinogenic substance and should not be overused (Mendes *et al.*, 1989:388).

Aspartame has an extended sweetness compared to other sweeteners. It is exceptionally stable at room temperature and 50% humidity. Quantities of 3 to 8 mg are normally used per tablet (Peck *et al.*, 1989:120).

2.5 Carboxymethylcellulose Sodium

Chemical name:	Cellulose, carboxymethyl ether, sodium salt [9004-32-4]
Molecular weight:	90,000-700,000
Function:	Coating agent, disintegrant, tablet binder, stabilizing agent, viscosity-increasing agent, water absorbing agent
Description:	Off white odourless granular powder
Melting point:	Browns at 227, chars at 252 °C
Solubility:	Insoluble in water, easily dispersible
Incompatibilities:	Interactions with strongly acidic solutions, soluble salts of iron and other metals, precipitates with ethanol, form complexes with gelatine, pectin, collagen and precipitate certain positively charged proteins (Kibbe, 2000:87-90)

Reason for inclusion in formulation

Cellulose materials ensure that tablets are tough but moderately hard. The molecular weight of the cellulose affects its viscosity and swelling properties (Peck *et al.*, 1989:107).

The cross linked form of carboxymethylcellulose (Ac-di-sol™) is accepted as a tablet disintegrant. It is insoluble in water but have a high affinity for it. Ac-di-sol™ is classified as a 'super-disintegrant' (Peck *et al.*, 1989:109).

2.6 Cellulose, Microcrystalline

Chemical name:	Cellulose [9004-34-6]
Empirical formula:	$(C_6H_{10}O_5)_{220}$
Molecular weight:	36,000
Function:	Adsorbent, suspending agent, tablet and capsule diluent, tablet disintegrant
Description:	Off white odourless crystalline powder composed of porous particles, commercially available in different particle sizes and moisture grades
Melting point:	Chars at 260-270 °C
Solubility:	Insoluble in water, moisture content < 5% w/w, hygroscopic
Incompatibilities:	Strong oxidizing agents (Kibbe, 2000:102-105)

Reason for inclusion in formulation

Microcrystalline cellulose (Avicel™) is a good disintegrant, even at concentrations as low as 10%. It acts as a disintegrant by allowing water into the tablet structure by means of capillary pores breaking the hydrogen bonds between adjacent bundles of cellulose microcrystals (Peck *et al.*, 1989:109-110).

Microcrystalline cellulose is available in two grades. Avicel PH 101™ is the original product widely used in direct compression formulations. Avicel PH 102™ is more agglomerated and with a larger particle size than the original. Avicel PH 102™ posses better flow properties with no decrease in compressibility than the original product (Shangraw, 1989:210).

2.7 Colloidal silicon dioxide

Chemical name: Silicon dioxide [7631-86-9]

Empirical formula: SiO_2

Molecular weight: 60.08

Function: Adsorbent, anti caking agent, glidant, suspending agent, tablet disintegrant, viscosity-increasing agent

Description: Light, loose, bluish-white coloured, odourless, tasteless, non-gritty amorphous powder

Melting point: - °C

Solubility: Insoluble in water, soluble in hydrofluoric acid

Incompatibilities: - (Kibbe, 2000:143-144)

Reason for inclusion in formulation

Silicon dioxide (Cab-O-Sil™, Aerosil™) is an absorbent that are capable of retaining large quantities of liquids without becoming wet and are still able to retain its good flow properties.

Silicon dioxide can be included in a tablet formula to act as a glidant and absorbent (Peck *et al.*, 1989:121).

2.8 Croscarmellose sodium

Chemical name: Cellulose, carboxymethyl ether, sodium salt, and cross linked [74811-65-7]

Empirical formula: Cross linked polymer of carboxymethylcellulose sodium

Molecular weight:	See carboxymethylcellulose sodium
Function:	Tablet and capsule disintegrant
Description:	Off white odourless granular powder
Melting point:	- °C
Solubility:	Insoluble in water, swells 4-8 times its original volume on contact with water
Incompatibilities:	Strong acids and soluble salts of iron and other metals (Kibbe, 2000:160-161)

Reason for inclusion in formulation

Carboxymethylcellulose acts as a disintegrant depending on its ability to swell when in contact with water. More effective disintegrants are available and it is only useful as a disintegrant when used in conjunction with other disintegrants for example microcrystalline cellulose (Bandelin, 1989:177).

2.9 Magnesium stearate

Chemical name:	Octadecanoic acid magnesium salt [557-04-0]
Empirical formula:	$C_{36}H_{70}MgO_4$
Molecular weight:	591.34
Function:	Tablet and capsule lubricant
Description:	Fine white, precipitated or milled, impalpable powder of low bulk density, faint odour of stearic acid and characteristic taste

Melting point: 117-150 or 126-130 (high purity) °C

Solubility: Insoluble in water

Incompatibilities: Strong acids, alkalis, iron salts, strong oxidizing materials, aspirin, some vitamins and alkaloid salts (Kibbe, 2000:305-308)

Reason for inclusion in formulation

Magnesium Stearate is widely used as a non-toxic excipient in the pharmaceutical, cosmetic and food industries. It is used as a lubricant in the manufacturing process of tablets and capsules in concentrations between 0.25-5% (Kibbe, 2000:305-306).

2.10 Saccharin sodium

Chemical name: 1,2-Benzisothiazol-3(2H)-one 1,1-dioxide, sodium salt [6155-57-3]

Empirical formula: $C_7H_4NNaO_3S$

Molecular weight: 205.16

Function: Sweetening agent, 300 times the sweetness of sucrose

Description: White odourless or aromatic crystalline powder with an intense sweet taste and metal aftertaste

Melting point: Decomposes

Solubility: Soluble in water

Incompatibilities: - (Kibbe, 2000:457-459)

Reason for inclusion in formulation

Saccharin is approved as a sweetener in chewable tablets. A concern with saccharin is a bitter after taste that can be reduced with the addition of 1% sodium chloride in the formulation (Peck, 1989:120).

2.11 Sodium starch glycolate

Chemical name:	Sodium carboxymethyl starch [9063-38-1]
Empirical formula:	-
Molecular weight:	500,000-1,000,000
Function:	Tablet and capsule disintegrant
Description:	Off white odourless crystalline, tasteless, free flowing powder with oval or spherical granules 30-100 µm diameter and less spherical particles 10-35 µm
Melting point:	Chars at 200 °C
Solubility:	Insoluble in water
Incompatibilities:	Ascorbic acid (Kibbe, 2000:501-504)

Reason for inclusion in formulation

Sodium starch glycolate (Explotab™, Primogel™) is a modified starch that acts as a super disintegrant. This modified starch can increase in volume by 200 to 300% in water while natural dried starches increase in volume from 10 to 25%.

A concern with the use of these modified starches in compressed tablets is that increased temperature and humidity conditions increase disintegration time. Increased disintegration time leads to a reduction in dissolution rate of the active substances (Peck *et al.*, 1989:109).

Chapter 3

Preformulation

3.1 Introduction

Although preformulation may be defined as the science of physicochemical characterization of candidate drugs, studies to determine the formulation conditions for drugs may also be defined as preformulation. These studies influence the product design and should be performed as early as possible during the development of the product. These studies should be performed on a need-to-know basis to streamline the development process (Steele, 2004:175).

The role of excipients in drug formulations are; to produce a product that is stable, uniform in content and quality and may even influence the delivery of a drug to desired location at the desired rate (Lund, 1994:192).

Variation in physical properties of product ingredients may result in problems during the production and use of a product. During modern development and production processes quality assurance is essential and is usually built into the production process from preparation of the raw ingredients to manufacturing of the product (Sneader, 1986:20).

The goals of preformulation are the following (Carstensen, 1998:239):

1. Establish the necessary physicochemical parameters of a drug
2. Determine its kinetic rate profile
3. Establish its physical characteristics
4. Establish its compatibility with common excipients

Lamivudine, Nevirapine and Zidovudine are not new drug substances. These drugs are marketed extensively in different patented formulations (Sweetman, 2002:636; 637; 645). Physicochemical parameters, kinetic rate profiles and physical characteristics are easily obtainable from different publications (Budavari *et al.*, 2001:5364; 1163; 1809) (Pharma source, 1998).

3.2 Analysis of drugs

3.2.1 Particle size

Particle size of drugs and excipients can influence the flow characteristics of the direct compression formulae. Smaller drug particles have higher surface area to volume ratios than larger particles. A large surface area ensures a rapid dissolution rate given sufficient liquid adsorption on the surface of the particle can occur. The usual problem with small particles is aggregation, leading to poor flow characteristics and insufficient mixing of the tablet powder. Large particles usually have sufficient flow characteristics but an insufficient dissolution rate. Ideally particles have to produce sufficient flow characteristics and small enough to ensure a rapid dissolution rate (Lund, 1994:181).

3.2.2 Solubility

Drugs are usually less stable when in a solution. Factors that may affect solubility of a drug include pKa, temperature, common ion effect, solubilisation and crystal purity (Lund, 1994:185-189). The solubility of lamivudine and zidovudine is 70 mg/ml and 25 mg/ml at 20 to 25°C respectively. Nevirapine has a solubility of 0.1 mg/ml at a neutral pH but is highly soluble at a pH beneath 3 (Budavari *et al.,* 2001:5364; 6514; 10180).

3.2.3 Crystal properties

Most of the drug substances are crystalline materials. Crystalline structures are substance molecules packed in an orderly and reproducible manner. The excipients used in the manufacture of tablets vary from crystalline materials to amorphous polymers. During the formulation process the crystallinity of substances will greatly affect the physical properties of the compounds and also the properties of the product.

Polymorphism is when the molecules of a solid exist in more than one packing arrangement. Monotropic polymorphism is when only one polymorph of a substance is stable and the metastable form changes to the stable form within time. Enantiotrophic polymorphism is when more than one stable polymorph exists under different experimental conditions, a change in pressure or temperature may alter the stable form. Monotropic polymorphs have different melting points, the polymorph with

the highest melting point being the most stable. The metastable form will have a faster dissolution rate than the stable form (Lund, 1994:179).

3.2.4 Thermal analysis (DSC)

Differential Scanning Calorimetry (DSC) is based on the principle that a substance undergoes physical and chemical change when heated. During these reactions heat is either absorbed (endothermic) or evolved (exothermic). The substance to be investigated is heated simultaneous with a thermally inert material or empty pan (reference) in separate sample holders. Temperature differences between the samples upon heating are measured by differential thermocouples. As soon as the sample becomes hotter or colder than the reference sample a current is produced in the thermocouple. Depending on the properties of the current endothermic or exothermic data can be obtained. Thermal data are usually presented graphically on a plot of sample temperature to reference standard temperature. Endothermic reactions are shown as downward peaks and exothermic as upward peaks (Webb, 1958:1-6) (Blažek, 1973:152-157).

Thermal analysis (TA) has the following applications during preformulation testing:

1. Characterization of materials e.g. polymorphic form of drug
2. Determining of possible drug and excipient interactions
3. Determining of material purity

During most studies differential scanning calorimetry (DSC) is usually the method of choice (Lund, 1994:193). DSC is useful for purity determination in samples, utilising the colligative property that impurities depress the melting point of a substance. Different polymorphs of a substance usually have different melting points (Lund, 1994:194).

An important use of DSC during preformulation is to screen for possible drug and excipient interactions. The use of thin layer chromatography (TLC) and high performance chromatography (HPLC) during preformulation is too time-consuming for determining of drug and excipient interactions. TLC and HPLC methods involve storage of mixtures at elevated temperatures and humidities and assaying it at different time intervals, this method may take several weeks (Lund, 1994:195).

DSC is less time-consuming and may take several hours to complete. During the DSC screening process 1:1 mixtures of a drug and excipient are mixed, weighed on a micro-balance, filled and crimped into an aluminium pan. The pan is then heated in a DSC at a rate of 5 to 10°C/minute. An interaction is suspected if a change in response is detected for the mixture compared to the responses of the two substances analyzed separately. The absence of a peak, additional peak, changes in the peak shape, changes in peak onset temperature or maxima and changes in peak heights may be the result of interactions (Lund, 1994:195).

DSC is a guide for possible interactions but other analytical techniques that are more sensitive can be utilized to verify any interactions (Lund, 1994:195).
According to Steele (2004:228) most drugs are prone to interactions with Mg-stearate and lactose. Although Mg-stearate is present in minute quantities (1%) in direct compression formulations and although other excipients may result in larger interactions than expected it is worthwhile to examine its interaction.

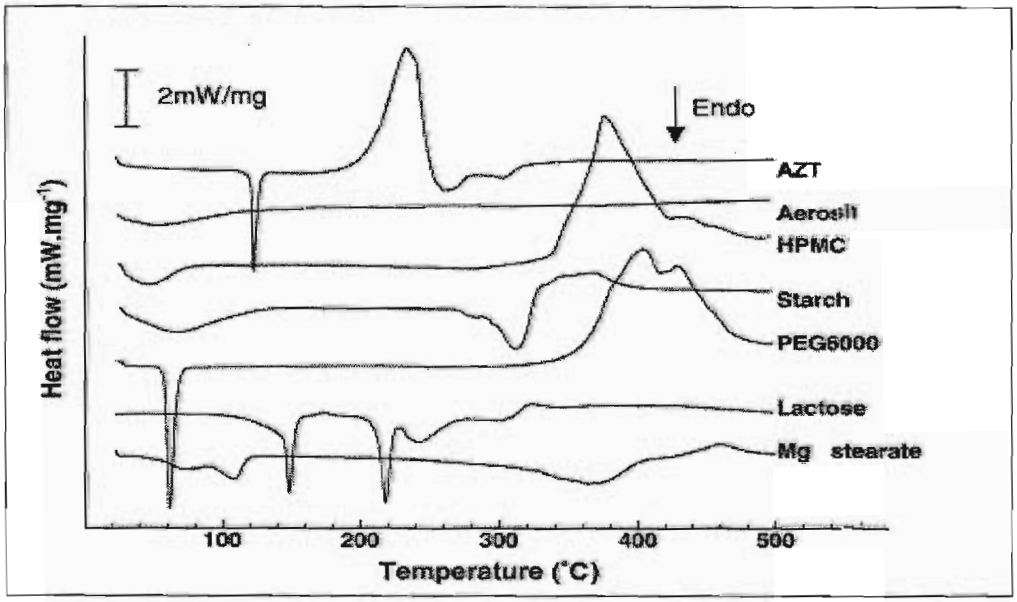

Figure 3.4: Example of a compatibility DSC-thermogram overlay of commonly used direct compression excipients and Zidovudine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1} (Araújo, 2003:311).

3.2.5 Method of DSC analysis

1. A list of all the drugs and excipients and each substance's melting point were compiled from literature:

Lamivudine	153-156; **160-162**; 145-147°C
Nevirapine	247-249; 253-254°C
Zidovudine	106-112; 119-121°C
Magnesium stearate	**117-150**; 126-130°C
Avicel PH102™	Chars at 260-270°C
Explotab™	Chars at 200°C
Aerocil™	Unknown
Sodium saccharin	Chars
Xylitab™	Chars
Passion fruit flavouring	Unknown
Kollidon™	Chars

2. An upper limit for the analysis of the substances was determined. A limit of 400°C was used in all DSC determinations.
3. Apparatus: A Mettler-Toledo Micro Balance, and Mettler-Toledo DSC 822^e, Germany with auto sampler was used for all determinations. $Star_e$ Software v. 9.00 Gmbh was used for sample analysis and $Star_e$ Software v. 9.00_a Gmbh was used for data analysis. Both the microbalance and DSC were valid and calibrated.
4. For initial determinations each drug and excipient was weighed separately (2-4 mg) and crimped in aluminium pans.
5. For compatibility studies a 1:1 mixture of each drug together with the other drugs and excipients were weighed and crimped in aluminium pans.
6. A method of analysis, sequence and the weight of each sample analysed were entered into the software.
7. The samples were placed into the auto sampler and the sequence started.

3.2.6 DSC analytical results

The melting points of the drugs were determined and the following results were obtained:

Lamivudine (Fig. 3.5)	176.99°C
Nevirapine (Fig. 3.6)	245.08°C

Zidovudine (Fig. 3.7)	123.66°C
Magnesium stearate (Fig. 3.8)	117.60°C

Although single test runs were performed of al the excipients, melting points could not be determined conclusively.

Compatibility between combinations of the drugs was determined. Mixtures of Lamivudine/Nevirapine, Lamivudine/Zidovudine and Nevirapine/Zidovudine were analysed.

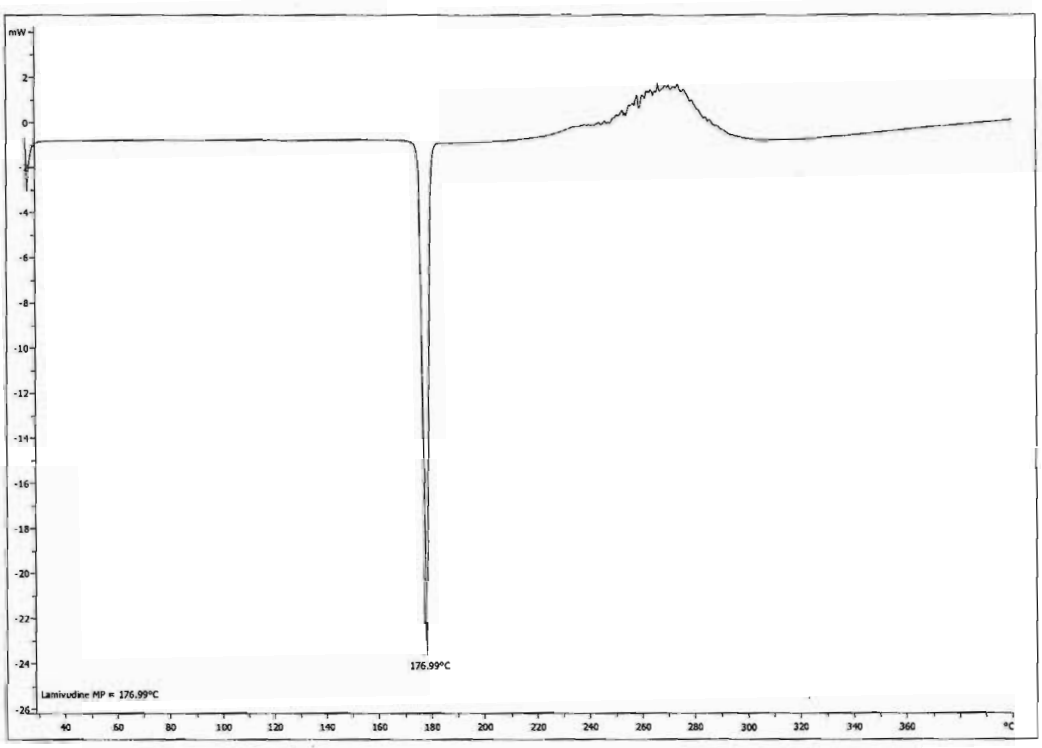

Figure 3.5: DSC-thermogram of Lamivudine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

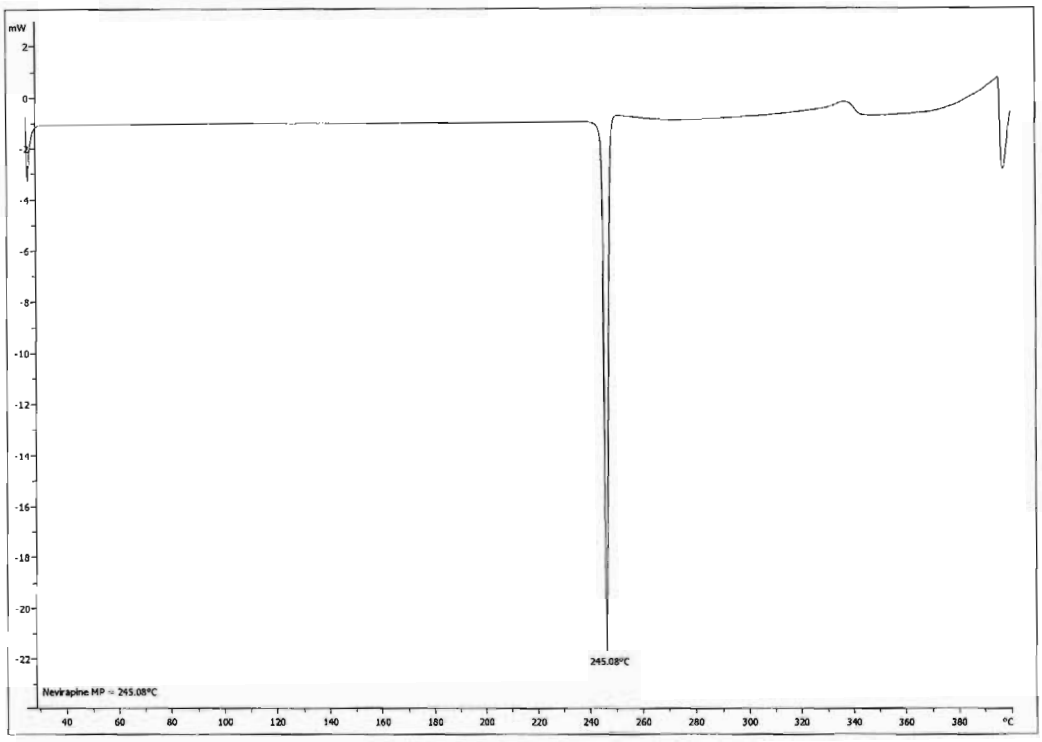

Figure 3.6: DSC-thermogram of Nevirapine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

Although melting points determined during DSC studies differ slightly from theoretical melting points in literature, the melting points of each substance was determined for baseline melting points during compatibility studies.

Endotherms or energy absorption are indicated as a downward peak according to convention in all the thermograms. The opposite applies to exotherms, although not used during this study.

Possible incompatibilities between two substances can be seen when a depression or shift of melting points (endotherms) occur when the two substances are combined and analyzed in the same way as the single substances.

Where incompatibilities are detected it is only an indication of possible interactions between substances at that temperature.

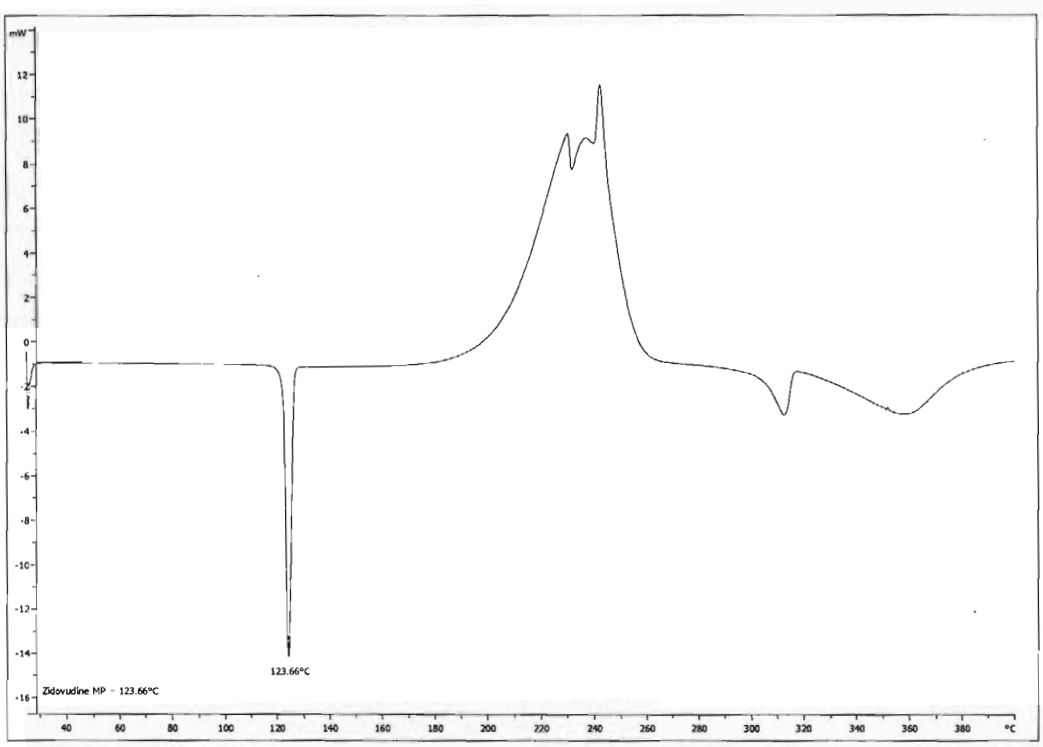

Figure 3.7: DSC-thermogram of Zidovudine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

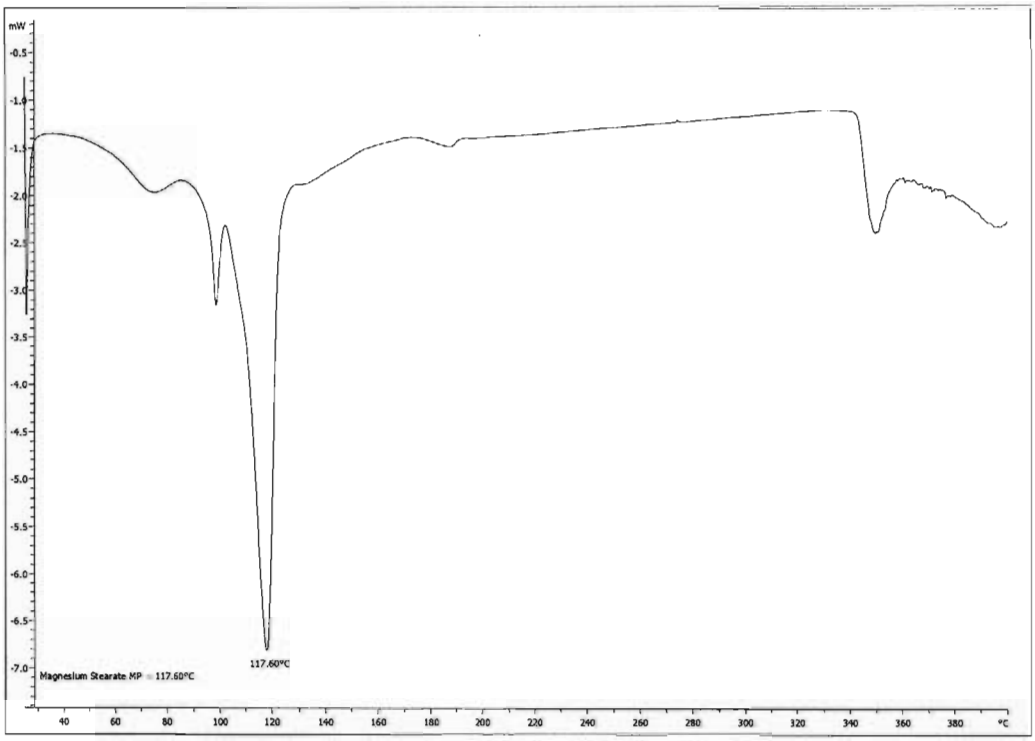

Figure 3.8: DSC-thermogram of Magnesium Stearate in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

Compatibility between drugs and Magnesium stearate were considered more important than the other excipients:

- Interactions between drugs and magnesium stearate were mentioned in literature.
- Magnesium stearate has a definite melting point compared to most other tablet excipients that usually chars when heated to high temperatures.
- Magnesium stearate is an important tablet excipient although used in minute quantities in most tablets.

Therefore magnesium stearate was considered separate from the other tablet excipients. Compatibility with this excipient was determined although it was used in commercial formulations containing the drugs (Steele, 2004:228).

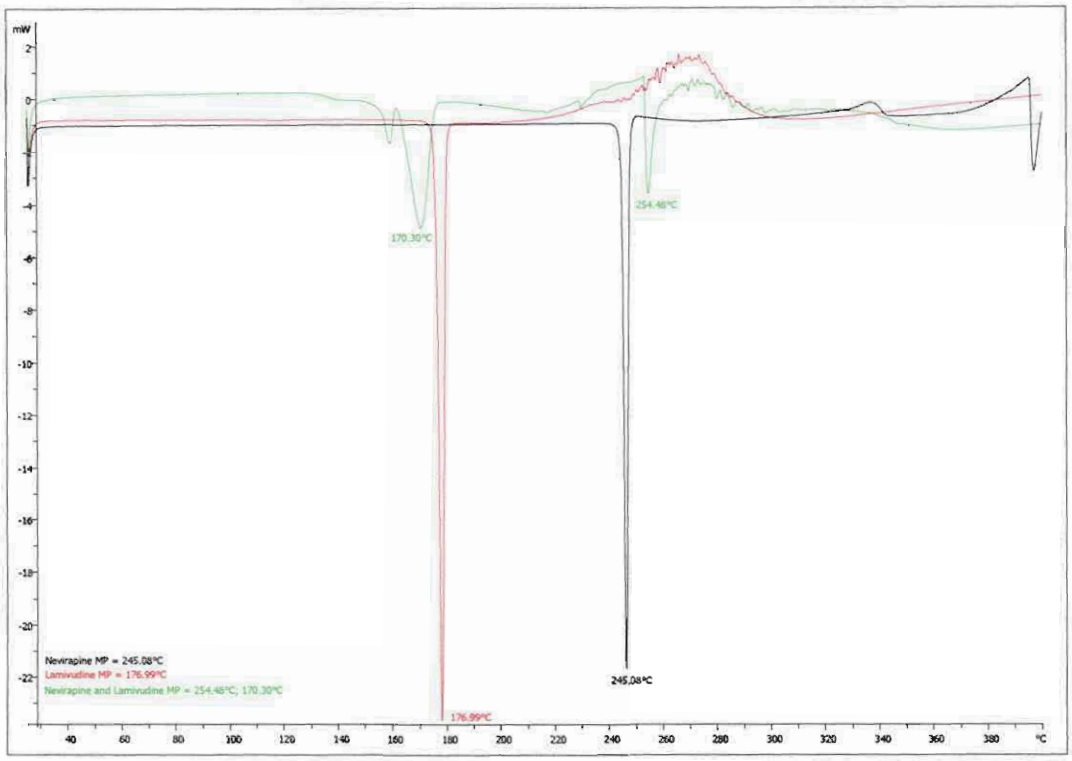

Figure 3.9: Compatibility DSC-thermogram overlay of Lamivudine and Nevirapine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

An interaction between Lamivudine and Nevirapine was detected (Fig. 3.9).

The exotherm of both the drugs was suppressed compared to the thermograms of the separate drugs. Lamivudine's endotherm was suppressed from ± -23 mW to ± -5 mW and Nevirapine's endotherm was suppressed from ± -22 mW to ± -4 mW.

A shift in endotherms occurred. The endotherm of Lamivudine was at a lower temperature than endotherm of Lamivudine alone. Nevirapine's endotherm was at a higher temperature in the mixture compared to the endotherm of the single drug.
An endotherm shift from 176.99°C to 170.30°C in Lamivudine was detected and 245.08°C to 254.48°C in Nevirapine.

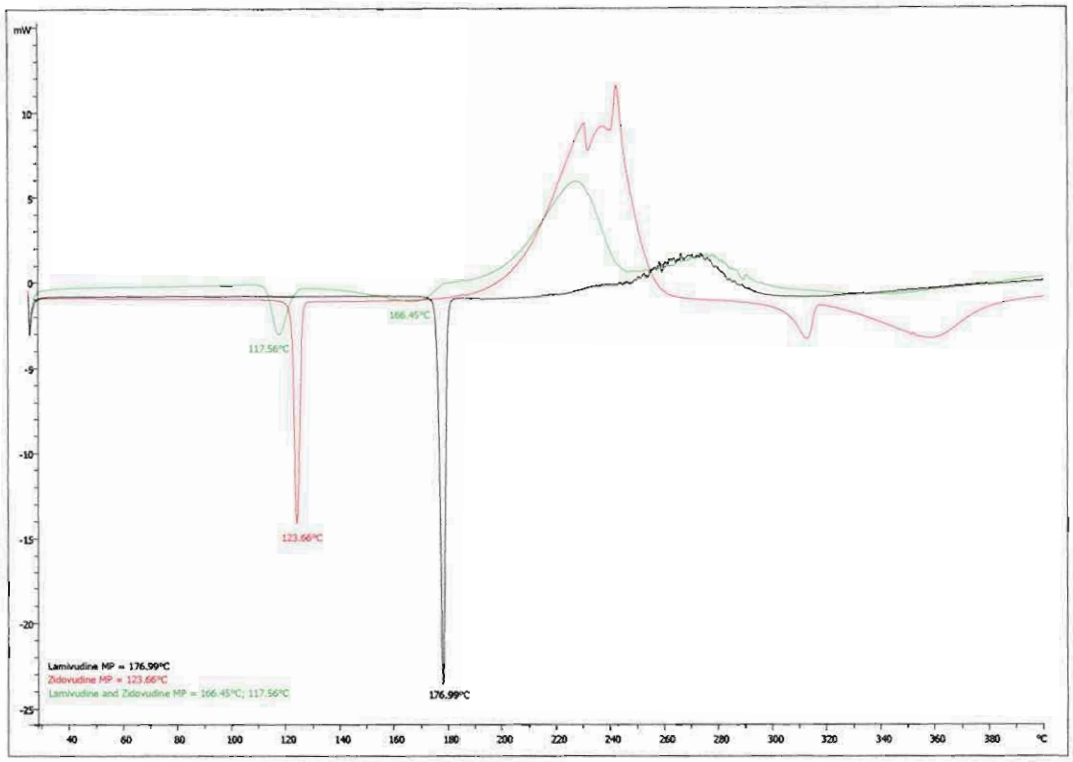

Figure 3.10: Compatibility DSC-thermogram overlay of Lamivudine and Zidovudine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

An interaction between Lamivudine and Zidovudine was detected (Fig. 3.10).

The endotherm of both the drugs was suppressed compared to the thermograms of the separate drugs. Lamivudine's endotherm was suppressed from ± -23 mW to ± -1 mW and Zidovudine's endotherm was suppressed from ± -14 mW to ± -3 mW.

A shift in endotherms occurred. The endotherm of Lamivudine was at a lower temperature than endotherm of Lamivudine alone. Zidovudine's endotherm was at a lower temperature in the mixture compared to the endotherm of the single drug.
An endotherm shift from 176.99°C to 166.45°C in Lamivudine was detected and 123.66°C to 117.56°C in Zidovudine.

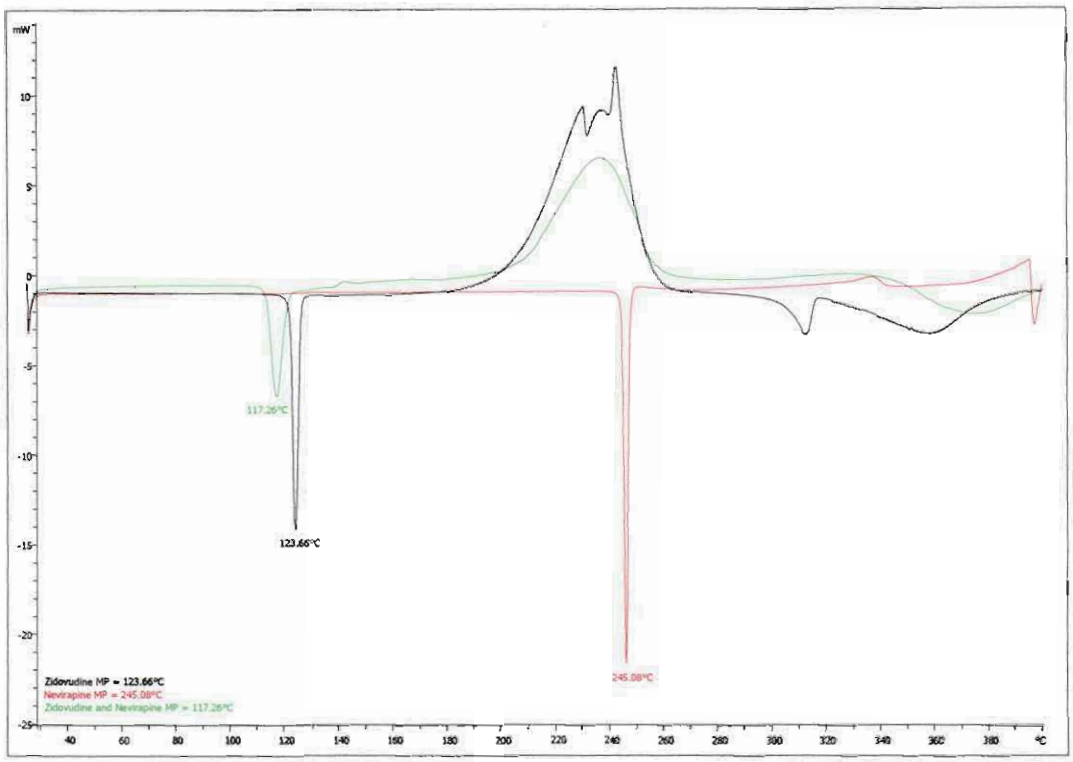

Figure 3.11: Compatibility DSC-thermogram overlay of Nevirapine and Zidovudine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

An interaction between Nevirapine and Zidovudine was detected (Fig. 3.11).

The endotherm of both the drugs was suppressed compared to the thermograms of the separate drugs. Nevirapine's endotherm was suppressed or cancelled completely and Zidovudine's endotherm was suppressed from ± -14 mW to ± -7 mW.

A shift in endotherms occurred. Zidovudine's endotherm was at a lower temperature in the mixture compared to the endotherm of the single drug.
An endotherm shift from 123.66°C to 117.26°C in Zidovudine was detected.

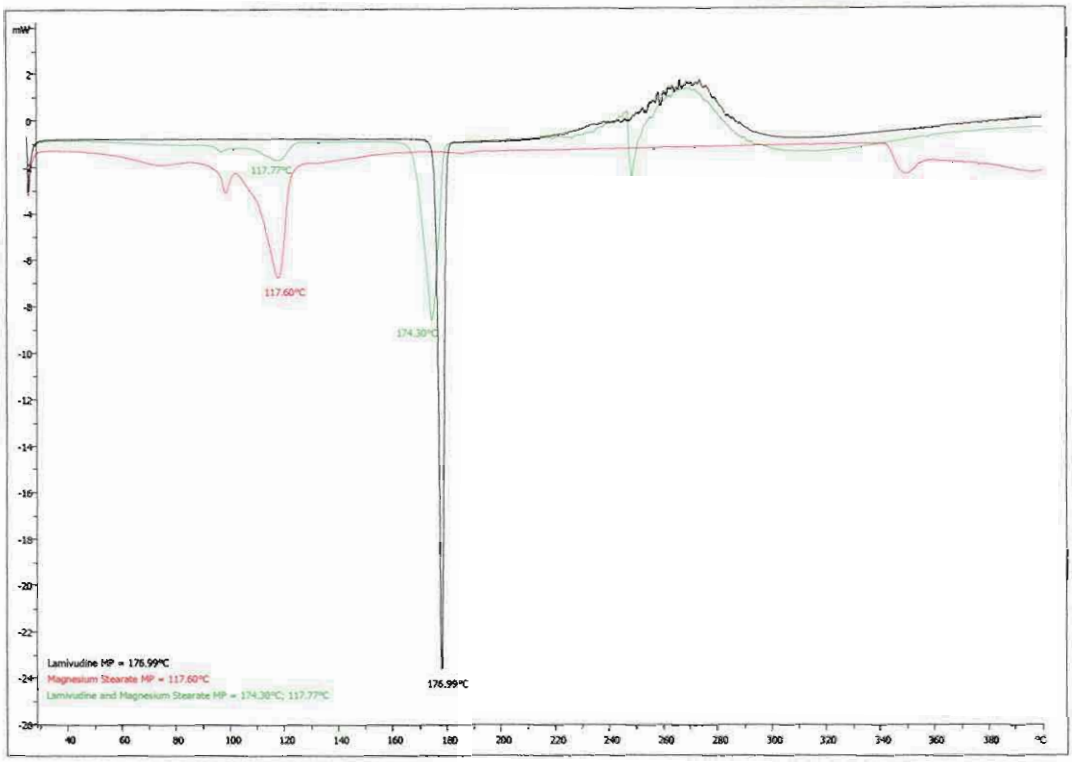

Figure 3.12: Compatibility DSC-thermogram overlay of Lamivudine and Magnesium Stearate in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

An interaction between Lamivudine and Magnesium stearate was detected (Fig. 3.12).

The endotherm of both the substances was suppressed compared to the thermograms of the separate substances. Lamivudine's endotherm was suppressed from ± -24 mW to ± -9 mW and Magnesium stearate's endotherm was suppressed from ± -7 mW to ± -2 mW.

A shift in endotherms occurred. Lamivudine's endotherm was at a lower temperature in the mixture compared to the endotherm of the single drug. Magnesium stearate's endotherm was at a slightly higher temperature than the single substance.
An endotherm shift from 176.99°C to 174.30°C in Lamivudine and a shift from 117.60°C to 117.77°C in Magnesium stearate were detected.

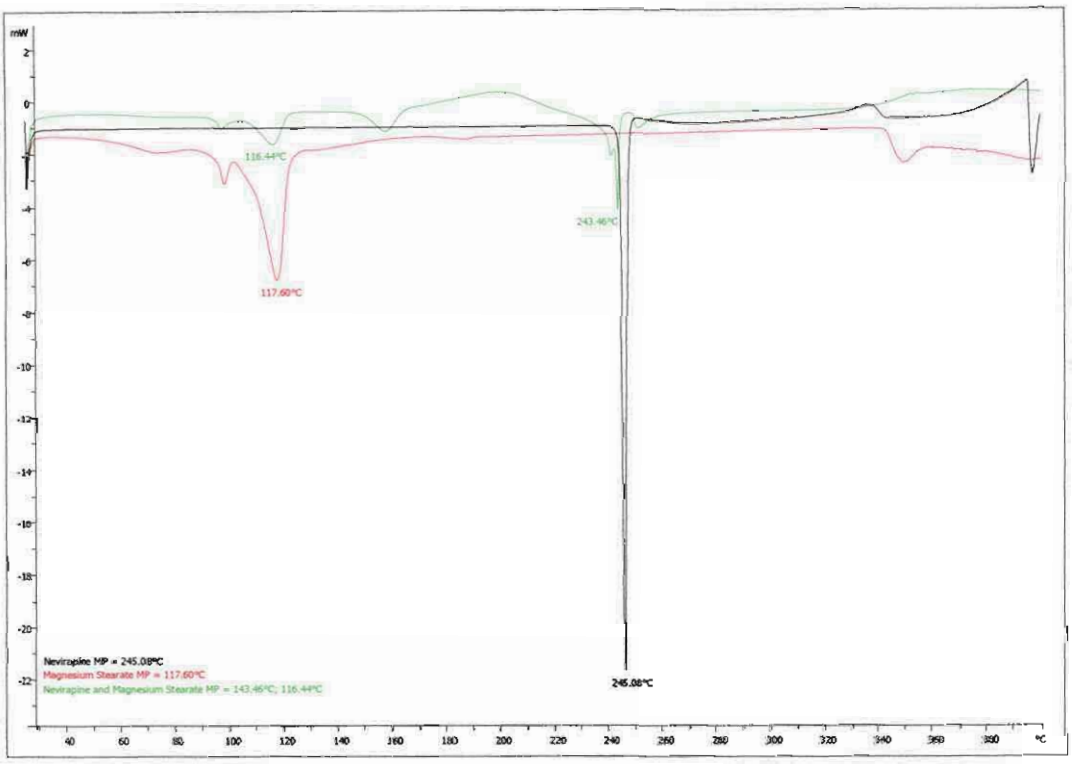

Figure 3.13: Compatibility DSC-thermogram overlay of Nevirapine and Magnesium Stearate in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

An interaction between Nevirapine and Magnesium stearate was detected (Fig. 3.13).

The endotherm of both the substances was suppressed compared to the thermograms of the separate substances. Nevirapine's endotherm was suppressed from ± -22 mW to ± -4 mW and Magnesium stearate's endotherm was suppressed from ± -7 mW to ± -2 mW.

A shift in endotherms occurred. Both Nevirapine's and Magnesium stearate's endotherms was at a lower temperature in the mixture compared to the endotherms of the single substances. An endotherm shift from 245.08°C to 243.46°C in Nevirapine and a shift from 117.60°C to 116.44°C in Magnesium stearate were detected.

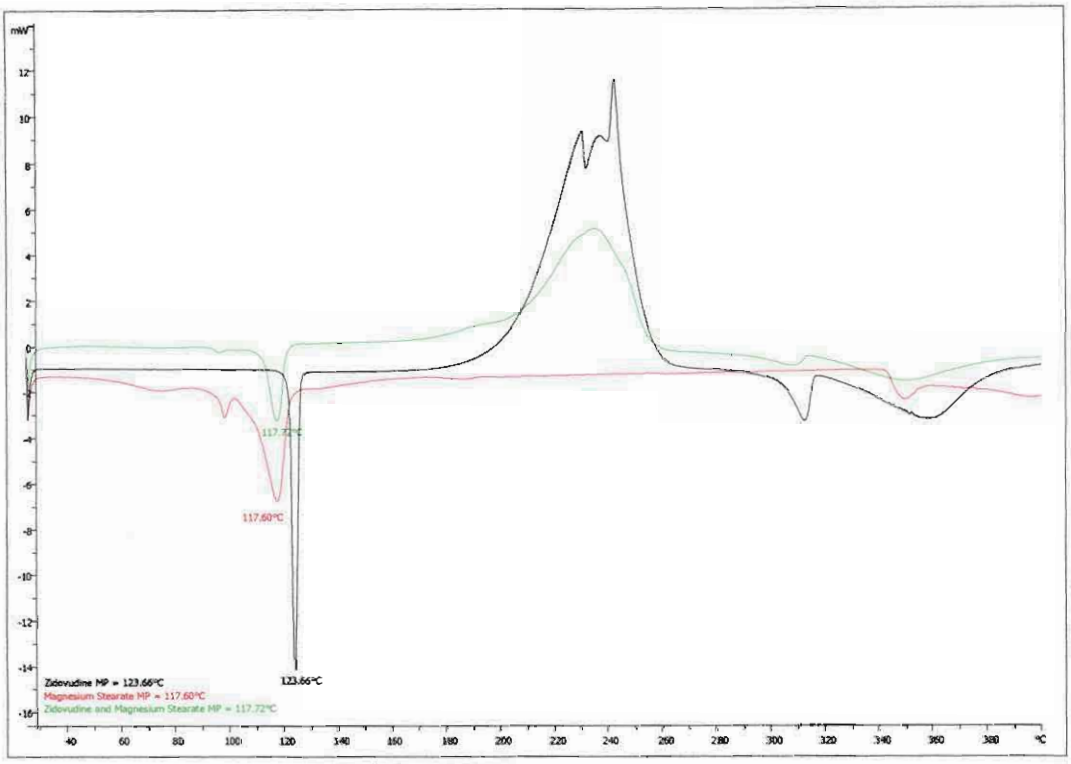

Figure 3.14: Compatibility DSC-thermogram overlay of Zidovudine and Magnesium Stearate in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

An interaction between Zidovudine and Magnesium stearate was detected (Fig. 3.14).

The endotherm of both the substances was suppressed compared to the thermograms of the separate substances. In the thermogram of the mixture of the two substances only one endotherm could be detected. The melting points of both substances are within ± 5°C of each other leading to a possible overlap of melting points. Zidovudine's endotherm was at ± -15 mW, Magnesium stearate's endotherm was at ± -7 mW and the endotherm of the mixture was suppressed to ± -4 mW.

A shift in the endotherm occurred. Zidovudine's endotherm was at a lower temperature and Magnesium stearate's endotherm was at a higher temperature in the mixture compared to the endotherms of the single substances. An endotherm shift from 123.66°C to 117.72°C in Zidovudine and a shift from 117.60°C to 117.72°C in Magnesium stearate were detected.

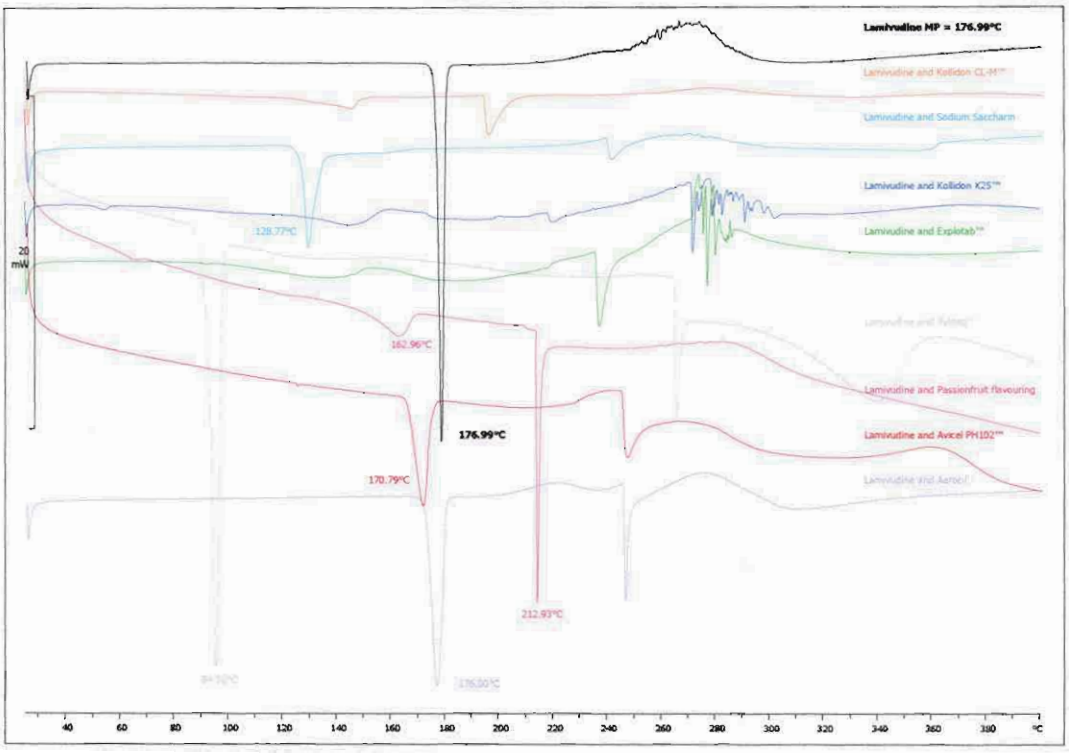

Figure 3.15: Compatibility DSC-thermogram overlays of Lamivudine and tablet excipients in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

According to compatibility DSC overlay thermograms of Lamivudine in a 1:1 combination with tablet excipients (Fig. 3.15), interactions occurred with all the tablet excipients.

Depressions and shifts in endotherms were detected in most of the tablet excipients when combined with Lamivudine.

Most of the tablet excipients are synthetic or semi-synthetic organic compounds and therefore determination of a definite melting point is very difficult.

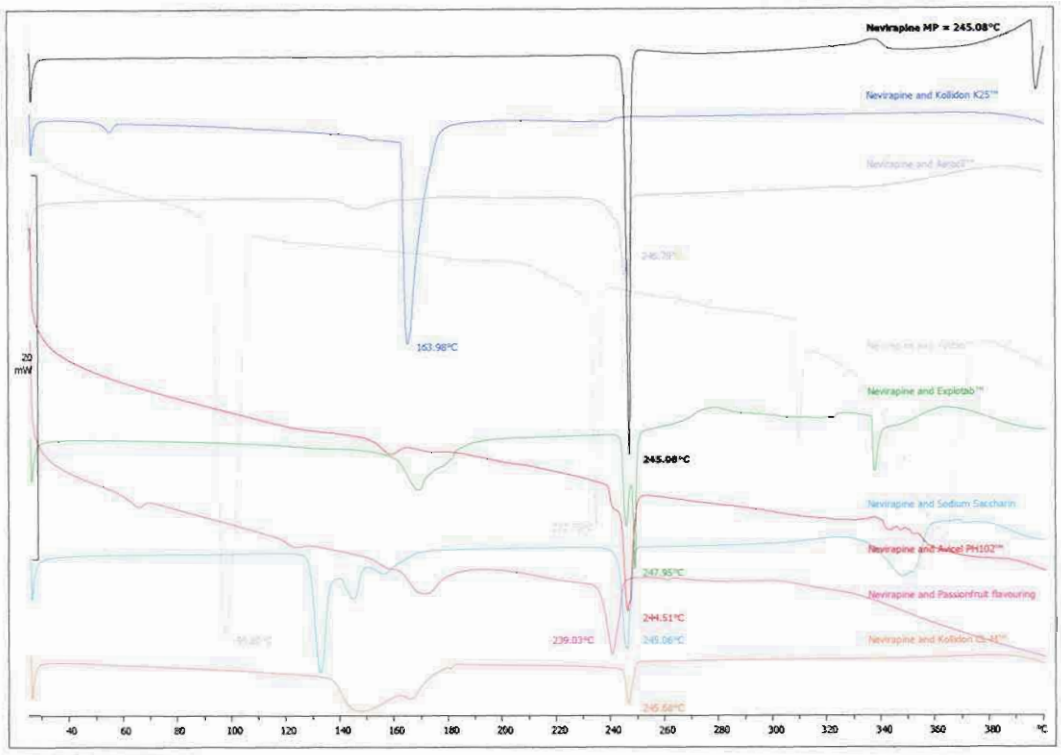

Figure 3.16: Compatibility DSC-thermogram overlays of Nevirapine and tablet excipients in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

According to compatibility DSC overlay thermograms of Nevirapine in a 1:1 combination with tablet excipients (Fig. 3.16), interactions occurred with all the tablet excipients.

Depressions and shifts in endotherms were detected in most of the tablet excipients when combined with Nevirapine.

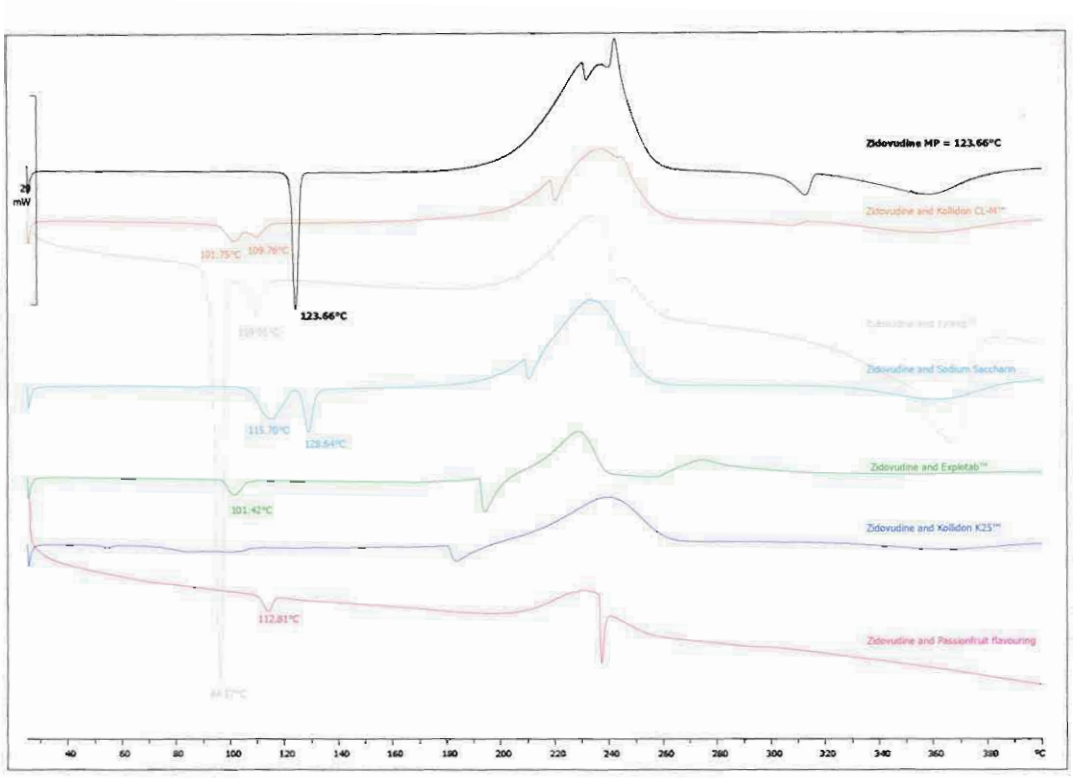

Figure 3.17: Compatibility DSC-thermogram overlays of Zidovudine and tablet excipients in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

According to compatibility DSC overlay thermograms of Zidovudine in a 1:1 combination with tablet excipients (Fig. 3.17), interactions occurred with all the tablet excipients.

Depressions and shifts in endotherms were detected in most of the tablet excipients when combined with Zidovudine.

3.2.7 Conclusions of DSC study

According to DSC compatibility studies all the tablet excipients have incompatibilities with the three drugs at high temperatures.

According to patented and commercial formulations these tablet excipients may be used in combination with the drugs although possible interactions may occur at high temperatures.

Therefore preformulation studies to determine the compatibility between drugs and excipients by means of DSC are inconclusive.

Short term studies to determine the compatibility of drugs and excipients in combination dissolved in a liquid (water) by means of HPLC may be necessary.

Furthermore long term accelerated stability studies on the formulation (tablet) may give a true indication of the stability and compatibility of the drugs in combination with all the excipients.

Table 3.1: Patented formulation excipients versus excipients used in the formulations during the study.

Excipient	**Patented formulation** (Zakarian *et al.*, 2003:2, pat. No. 6,605,301, 12 August 2003)	**Kess™** (Filaxis Kess™, 2005:1)	**Kess Complex™** (Filaxis Kess Complex™, 2005:1)	**Formulations during the study**
Lamivudine	×	✓	×	✓
Zidovudine	×	×	✓	✓
Nevirapine	×	×	✓	✓
Microcrystalline cellulose	✓	✓	✓	✓
Sodium starch glycolate	✓	✓	✓	✓
Colloidal silica dioxide	✓	✓	✓	✓
Magnesium stearate	✓	✓	✓	✓
Polysorbate (Tween 80™)	✓	×	✓	✓
Kollidon CL-M™ (PVP)	✓	×	×	✓
Explotab™ (Sodium starch glycolate)	✓	×	×	✓
Xylitab™ (Xylitol)	✓	×	×	✓
Sodium-Saccharin	✓	×	×	✓

Due to the inconclusiveness of the DSC studies, the fact that some of the excipients are used in commercial formulations (Table 3.1) and the absence of major interactions during further stability indicating studies the excipients and amounts used during the study can be justified.

3.4 Summary

Although many products containing different combinations and amounts of the drugs are available in the market, the compatibility data of these drugs with different excipients and drug stability data are not easily obtainable in literature.

Particle sizes do not seem to pose a problem; the drugs and excipients have good flow properties for direct compression techniques.

Interactions were detected during DSC compatibility studies. Further investigation with more sensitive methods e.g. HPLC during the stability studies will give a true indication of possible interactions.

3TC and AZT are readily soluble in neutral water under normal conditions. NVP is practically insoluble in water under normal conditions. Solubility does not seem to pose a problem as the formulation will be a crude dispersion during use. Solubility problems during assay can be overcome by using the co-solvent methanol (Lund, 1994:188) and dissolutions can be performed at a pH < 3 (Budavari *et al.,* 2001:6515).

A general assumption is that more effort must be put into specifying and controlling the ingredients and method of manufacture for complex regimens. This is especially important for fixed dose combinations rather than products containing a single active substance (Walters, 2003:1-2).

Chapter 4

Formulation of dispersible tablets

4.1 Introduction

Tablets are manufactured from powders. Tablet powders consist of solid particles surrounded by spaces (air). Powders are not true solids; powders can flow, resist deformation under pressure and can be compressed. Factors that may influence these properties of the powder include solid or fluid interactions and cohesion between particles (Davies, 2004:381).

During the formulation of tablets; equilibrium between the bioavailability, chemical-physical stability and feasibility of the product should be considered.

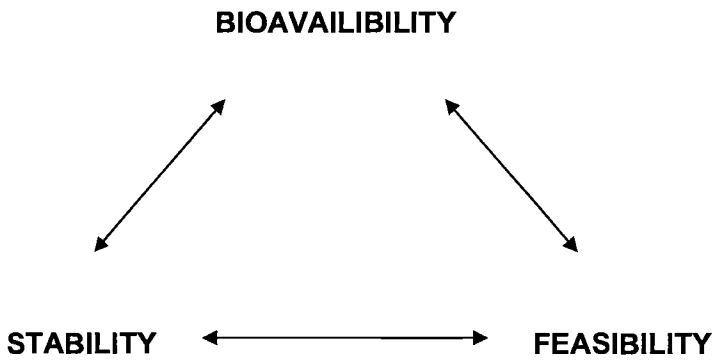

Figure 4.1: Relationship between bioavailability, stability and feasibility of a dosage form (Davies 2004:403).

Changes to the formulation of the dosage-form to optimize one of the above mentioned properties may negatively affect the remaining properties of the dosage-form (Figure 4.1). Equilibrium in the formulation of immediate release dosage forms is especially important e.g. an increase in particle size to enhance the flow ability of the powder may negatively affect the bioavailability of the formulation during dissolution (Davies, 2004:403).

According to Davies (2004:406) tablet formulations should possess the following properties:

1. Tablet powder must exhibit good flow properties, especially in direct compression manufacturing.
2. The tablet powder must exhibit good compacting properties.
3. Sufficient and compatible lubrication of the powder.
4. Sufficient disintegration and dissolution tablet properties.
5. An elegant tablet appearance.

Five different manufacturing techniques are available for tablet manufacture (Davies, 2004:432).

1. Direct compression: Tablet powder requires no pre-treatment before commencing with the tabletting procedure.
2. Wet granulation (Aqueous): Addition of a liquid (usually water) and binder to the tablet powder; formation of granules, drying, milling and sieving before commencing with the tabletting procedure.
3. Wet granulation (non-Aqueous): The same procedure as for aqueous wet granulation except for the use of a volatile non-aqueous solvent and the use of vacuum pumps to remove the solvent.
4. Dry granulation (slugging): Formation of crude tablets from the tablet powder in a tablet press, milling and sieving of the particles to a specified size before commencing with the tabletting procedure.
5. Dry granulation (roller compaction): Same as slugging except for the use of rollers to form granules (Davies, 2004:432).

Tablets are manufactured by a tablet press. Two kinds of presses are available, single punch (eccentric) and rotary. Outputs vary from 3000 to 1 million tablets per minute depending on the type of apparatus. The principle of tablet presses remains the same. Tablet powder is filled to a specific depth in a die and compressed between two punches, the upper punch is then removed and the lower punch moves upward to eject the tablet. Due to the slow manufacturing speed of the single presses, these presses are rarely used during the full scale manufacturing process. Single tablet presses are used during formulation studies and in development

laboratories because they require relatively small amounts of material to produce a batch of tablets (Davies, 2004:403-405).

4.2 Formulation process

Before a formulation could be compiled many different patented formulations available were examined. Both anti retroviral and other drug formulations were studied. The availability and necessity of excipients were examined and considered. The study of patented formulations was only considered as a guide to examine the excipient properties at different concentrations in different combinations. Furthermore, different manufacturing techniques were examined and experiments with different manufacturing techniques e.g. granulation and direct compression performed to optimize the manufacturing of the formulations during the study.

The following claims were made for the formulation and manufacture of a United States patented dispersible formulation (Zakarian *et al.*, 2003:2, pat. No. 6,605,301, 12 August 2003) containing one or more of a macrolide antibiotic/s.

4.2.1 Guidelines regarding excipients used in the patented formulation (Zakarian *et al.*, 2003:2, pat. No. 6,605,301, 12 August 2003):

1. Disintegrants a mixture of (Polyvinylpyrrolidone) PVP and Croscarmellose sodium in proportions of between 1 and 25% of the total weight of the tablet. PVP: Croscarmellose sodium ratios are between 1:1 and 4:1.

2. Sweeteners are chosen from aspartame, saccharin sodium, acesulfame potassium, ammonium glycerinate and mixtures thereof. When using two sweetners use in ratios of between 1:1 to 2:1 and 1 to 20% of the tablet weight.

3. One of the following excipients: diluent, surfactant, lubricant, glidant and one or more flavourants.

4. At least one diluent from the group consisting of microcrystalline cellulose, lactose, hydroxypropyl methyl cellulose or pregelatinized starch.

5. Microcrystalline cellulose in proportions of 5 to 50% of the tablet weight.

6. One surfactant consisting of polysorbate or sodium lauryl sulfate in proportions of between 0.1 to 3% of tablet mass.

7. Magnesium stearate as lubricant between 0.5 to 3% and colloidal silica as glidant between 0.5 to 3 % of tablet weight.

8. Mint, chocolate, caramel, vanilla, strawberry or liquorice flavourants and mixtures thereof between 0.5 to 15% of tablet mass.

Mint flavour:	1 to 7% of tablet mass
Vanilla/Caramel:	1 to 10% of tablet mass

4.2.2 Amount of substances used in the patented formulation:

(Zakarian *et al.*, 2003:2, pat. No. 6,605,301, 12 August 2003)

Drug	37.50%
Crospovidone	2.25%
Croscarmellose sodium	4.25%
Polysorbate	0.38%
Microcrystalline cellulose	38.12%
Aspartame	8.00%
Saccharin sodium	4.00%
Mint flavour	4.00%
Colloidal silica	0.50%
Magnesium stearate	1.00%

4.2.3 Manufacturing method:

A. Mix active ingredients with 30 to 60% of the quantity of disintegrant(s) intended to be present in the tablets.

B. Granulate the mixture with water and at least one surfactant.

C. Dry the granules.

D. Add the remaining 40 to 70% of the disintegrant(s), sweetener(s), diluent, lubricant, glidant and flavourant.

E. Compress the resulting mixture.

4.2.4 Tablet properties of the patented formulation:

Tablets must be able to disintegrate completely within 3 minutes when placed in liquid such as water, leading to a suspension that can easily be made homogeneous by stirring it (Zakarian *et al.*, 2003:2-9).

The patented formulation was examined to determine the concentrations of excipients used in a dispersible formulation. The concentrations of excipients used in the formulations are completely unique to the study and the patented formulation was merely used as a guide.

Formulations containing anti retrovirals were examined to determine the relationship between the type and amounts of excipients included in the formulations and the anti retrovirals. None of the anti retroviral formulations examined were dispersible formulations. A correlation between the amounts of drug and excipients could therefore not be evaluated.

The following formulations from Filaxis were examined:

Kess™ (Filaxis Kess™, 2005:1)

Each tablet contains the following:

Lamivudine	150.00 mg
Microcrystalline cellulose	130.00 mg
Sodium starch glycolate	13.07 mg
Colloidal silica dioxide	1.00 mg
Magnesium stearate	6.15 mg

Kess Complex™ (Filaxis Kess Complex™, 2005:1)

Each tablet contains the following:

Lamivudine	150.00 mg
Zidovudine	300.00 mg
Microcrystalline cellulose	270.00 mg
Sodium starch glycolate	22.50 mg
Colloidal silica dioxide	2.25 mg
Magnesium stearate	5.60 mg

Coating:

Eudragit RD 100™	7.70 mg
Polysorbate 80™	4.40 mg
Polyethylene glycol 6000	1.70 mg
Talc powder	8.60 mg
Titanium dioxide	4.30 mg

4.3 Manufacturing formula

After examination, consideration and experimentation with the patented and commercial tablet formulations, the following formulations were developed (Table 4.1 to 4.4).

4.3.1 Amounts of drugs and excipients used in the tablet formulations during the study

Table 4.1: Drugs and excipients in formula 1 (1000 mg tablet)

Substance	Amount per tablet (%)	Amount per tablet (mg)
Lamivudine	1.00	10.00
Nevirapine	2.00	20.00
Avicel PH-102™	77.12	771.20
Kollidon CL-M™ (PVP)	1.00	10.00
Explotab™ (Sodium starch glycolate)	4.00	40.00
Xylitab™ (Xylitol)	5.00	50.00
Sodium-Saccharin	4.00	40.00
Flavourant (Passion fruit)	4.00	40.00
Aerosil™ (Colloidal silica)	0.50	5.00
Tween-80™ (Polysorbate)	0.38	3.80
Magnesium-Stearate	1.00	10.00

Table 4.2: Drugs and excipients in formula 2 (1000 mg tablet)

Substance	Amount per tablet (%)	Amount per tablet (mg)
Lamivudine	1.50	15.00
Nevirapine	3.75	37.50
Zidovudine	2.50	25.00
Avicel PH-102™	72.37	723.70
Kollidon CL-M™ (PVP)	1.00	10.00
Explotab™ (Sodium starch glycolate)	4.00	40.00
Xylitab™ (Xylitol)	5.00	50.00
Sodium-Saccharin	4.00	40.00
Flavourant (Passion fruit)	4.00	40.00
Aerosil™ (Colloidal silica)	0.50	5.00
Tween-80™ (Polysorbate)	0.38	3.80
Magnesium-Stearate	1.00	10.00

Table 4.3: Drugs and excipients in formula 3 (1000 mg tablet)

Substance	Amount per tablet (%)	Amount per tablet (mg)
Lamivudine	2.50	25.00
Nevirapine	5.00	50.00
Zidovudine	5.00	50.00
Avicel PH-102™	67.62	676.20
Kollidon CL-M™ (PVP)	1.00	10.00
Explotab™ (Sodium starch glycolate)	4.00	40.00
Xylitab™ (Xylitol)	5.00	50.00
Sodium-Saccharin	4.00	40.00
Flavourant (Passion fruit)	4.00	40.00
Aerosil™ (Colloidal silica)	0.50	5.00
Tween-80™ (Polysorbate)	0.38	3.80
Magnesium-Stearate	1.00	10.00

Table 4.4: Drugs and excipients in formula 4 (1000 mg tablet)

Substance	Amount per tablet (%)	Amount per tablet (mg)
Lamivudine	7.50	75.00
Nevirapine	10.00	100.00
Zidovudine	15.00	150.00
Avicel PH-102™	47.62	476.20
Kollidon CL-M™ (PVP)	1.00	10.00
Explotab™ (Sodium starch glycolate)	4.00	40.00
Xylitab™ (Xylitol)	5.00	50.00
Sodium-Saccharin	4.00	40.00
Flavourant (Passion fruit)	4.00	40.00
Aerosil™ (Colloidal silica)	0.50	5.00
Tween-80™ (Polysorbate)	0.38	3.80
Magnesium-Stearate	1.00	10.00

4.3.2 Manufacturing method of tablets during study:

1. Active substances and excipients were obtained from the suppliers. The certificates of analysis of all the active substances were checked.

2. A suitable manufacturing environment and equipment were prepared.

3. Excipients and drug substances were weighed and mixed according to the order in table 4.1 to 4.4 except for the magnesium stearate. It was mixed in a V-mixer for 15 minutes. After initial mixing the magnesium stearate was added and the formula were mixed for a further 10 minutes.

4. The tablet powder was compressed by a single tablet press with a punch diameter of 16 mm. Press parameters were adjusted until a product of consistent quality was achieved. 1500 tablets of 1 g each per batch were manufactured.

5. Initial tablet tests were performed.

6. Remaining tablets were stored in PVC bottles at 25 °C; 60% RH, 30°C; 60% RH and 40 °C; 80% RH consecutively.

7. After 4, 8 and 12 weeks stability tests were performed on each batch.

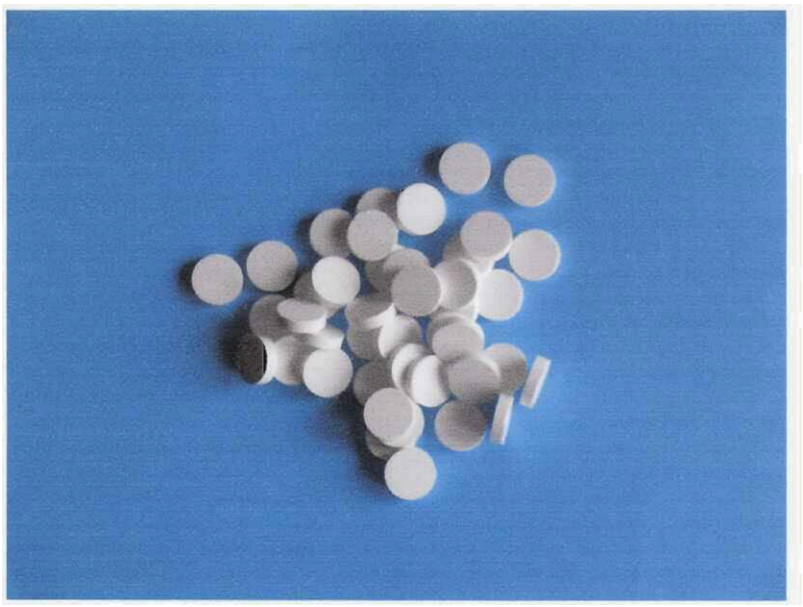

Figure 4.1: Tablets manufactured during above mentioned procedure. Diameter ± 16mm and Thickness ± 4mm.

4.4 Conclusion

The main objective during the formulation procedure was to develop formulations that exhibit ideal properties for its intended application.

Due to the large drug amounts in the formulations, large amounts of disintegrants were included to ensure a decreased disintegration time. Microcrystalline cellulose was included for its filling as well as disintegration properties. Large amounts of Avicel™ were included (47.62 – 77.12%) to ensure tablets that comply with physical requirements and Explotab™ to reduce the disintegration time.

Initially a wet granulation method was considered. Due to the good flow and compression properties of Avicel PH102™ direct compression was examined and used as the method of choice.

The tablet powder revealed good flow and lubrication properties during tabletting. Tablet mass varied within 5% of the theoretical tablet mass and therefore uniformity of dosage form have been achieved. Initial disintegration experiments revealed that all the formulations disintegrated within 1 minute after introduction into water (50 ml at ambient temperature).

Chapter 5

Method development and Validation for simultaneous determination of Lamivudine, Nevirapine and Zidovudine

This chapter describes the method that was developed and validated as part of the study to determine the quantity of each drug in the formulations in combination with the other drugs.

5.1 Chromatographic conditions

Liquid chromatography was used to quantify the active substances during the study. The analytical procedure was performed under the following conditions.

Analytical instrument:	HP1100 series HPLC equipped with a gradient pump, auto sampler, UV detector and Chemstation Rev. A.08.03 data acquisition and analysis software or equivalent.
Column:	Luna C18-2 column, 150 x 4.6 mm, 5µm, 100 Å pores, 17.8% carbon load, endcapped, Phenomenex, Torrance, CA was used.
Mobile phase:	Acetonitrile/water with 0.2% triethylamine, pH adjusted to 7.0 with phosphoric acid or ammonium hydroxide
Gradient:	8% acetonitrile at 1.5 minutes, then to 65% between 8 to 10 minutes and 8% after 10.10 minutes.
Flow rate:	1.0 ml/min.
Injection volume:	10 µl.
Detection:	UV at 270 nm, 16 nm bandwidth.
Retention time:	Approximately ±3.7, ±6.5 and ±7.7 minutes for lamivudine, zidovudine and nevirapine respectively.
Stop time:	15 minutes
Solvents:	Assay - Methanol (1.25% during standard preparation) and deionised water (Milli Q).

Dissolution - Methanol (1.25% during standard preparation) and 0.1M HCl 900 ml for dissolution buffer.

5.2 Sample preparation

1. Weigh approximately 2 g of powdered tablet accurately into a tared 100 ml volumetric flask.
2. Dissolve in 50ml methanol, sonicate for 10 minutes.
3. Make up to volume with deionised water (Assay) or 0.1M HCl (Dissolution).
4. Dilute 5 ml of this solution to 50 ml with deionised water.
5. Centrifuge the solution for 10 minutes at 2500 rpm.
6. Transfer the clear supernatant into separate vials.
7. Inject into the chromatograph (6 injections).

5.3 Standard preparation

1. Weigh approximately 150 mg lamivudine, 200 mg nevirapine and 300 mg zidovudine accurately and dissolve in 50 ml methanol in a 100 ml volumetric flask with sonication for 10 minutes.
2. Allow to cool to room temperature and make up to volume with deionised water.
3. Dilute 5 ml of this solution to 50 ml with deionised water.
4. Transfer into separate vials.
5. Inject into the chromatograph (3 injections).

5.4 Calculations

The tablet (Formula 4) contains 7.5 mg/50 ml lamivudine, 10 mg/50 ml nevirapine and 15 mg/50ml zidovudine. The prepared sample will contain 150 µg/ml lamivudine, 200 µg/ml nevirapine and 300 µg/ml zidovudine.

$$\text{mg drug/5 ml} = \frac{\text{SAR X (mass of std. mg) X (std. Potency) X wt/ml}}{\text{STR X sample mass (g) X 100}}$$

SAR = sample peak area

STR = standard peak area

Wt/ml = weight per ml of the solution in g

100 are to compensate for standard potency

5.5 System suitability parameters

- Make six injections of a standard solution.
- Calculate the relative standard deviation of the peak areas obtained.
- Calculate the number of theoretical plates and the resolution between the lamivudine, nevirapine and the zidovudine peaks.
- Use the tangent method to calculate the parameters.
- The system is suitable to perform the analysis if the following criteria are met:

1. RSD of 2 injections not more than 2 %.
2. The column must have more than 10,000 theoretical plates for lamivudine, 30,000 plates for zidovudine and 45,000 for nevirapine.
3. The resolution between the lamivudine and zidovudine peaks must be more than 7.6 (ICH Q2(R1), 1994:10).

5.6 Validation test procedure and acceptance criteria

5.6.1 Specificity

Specificity tests include identification, assay and impurity tests. Suitable identification tests should be able to discriminate between compounds with closely related structures. The procedure may be confirmed by obtaining positive results when a sample containing the analyte is compared to a standard containing the reference analyte and negative results are obtained with a placebo sample.

For assay, discrimination of the analyte in the presence of impurities should be demonstrated. This can be done either by spiking a standard containing the known analyte with impurities or degradation products or if impurities are not available by comparing a standard to a sample stored under relevant stress conditions as specified in the pharmacopoeia (ICH Q2(R1), 1994:6-7).

5.6.2 Linearity

The ability of an analytical procedure to produce test results that are directly proportional to the concentration of active substances in a sample within a given range (Hong *et al.* 2000:366-367).

A linear relationship should be evaluated across a concentration range of the analytical procedure. It may be demonstrated through analysis of dilutions of a stock solution containing the drug substance (ICH Q2(R1), 1994:8).

Method:

1. A standard solution is prepared as described under standard preparation
2. Variable volumes are injected into the chromatograph to obtain standards from 60-120% of the expected sample concentration.

Acceptance criteria:
Linear regression analysis should yield a regression coefficient (R^2) of ≥ 0.99

5.6.3 Range

Range is normally derived from linearity studies. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure (ICH Q2(R1), 1994:8).

5.6.4 Accuracy

Indication of the closeness of the experimental value to the true value. Measurement of the percentage active substance recovered by assay compared to a standard preparation .It should be established across the specified range of the analytical procedure (Hong *et al.* 2000:361) (ICH Q2(R1), 1994:9).

Method:

1. Weigh approximately 150 mg lamivudine, 200 mg nevirapine and 300 mg zidovudine accurately and dissolve in 50 ml methanol in a 100 ml volumetric flask with sonication for 10 minutes.
2. Weight approximately 1350 mg of the tablet excipient mixture and dissolve in 50 ml methanol in a 100 ml volumetric flask with sonication for 10 minutes.
3. Allow to cool to room temperature and make up to volume with deionised water.

Standard:

4. Dilute 4 ml of solution (1.) to 50 ml with deionised water – 80%
5. Dilute 5 ml of solution (1.) to 50 ml with deionised water – 100%
6. Dilute 6 ml of solution (1.) to 50 ml with deionised water – 120%
7. Dilutions were made in triplicate.
8. Inject into the chromatograph in duplicate.

Samples:

9. Transfer 4 ml of solution (1.) and (2.) respectively into a 50 ml flask and dilute to volume with deionised water – 80%
10. Transfer 5 ml of solution (1.) and (2) respectively into a 50 ml flask and dilute to volume with deionised water – 100%
11. Transfer 6 ml of solution (1.) and (2.) respectively into a 50 ml flask and dilute to volume with deionised water – 120%
12. Filter 10 ml of each mixture through a syringe driven filter, discarding the first 5 ml of filtrate.
13. Transfer 2 ml of the filtrate to separate vials.
14. Inject into the chromatograph in duplicate.

Placebo:

15. Filter 10 ml of the excipient mixture (2.) through a syringe driven filter, discarding the first 5 ml of filtrate.
16. Transfer 2 ml of the filtrate to a vial.
17. Inject into the chromatograph.

Acceptance criteria:

Recovery must be between 98% and 102%

5.6.5 Precision

The closeness of data values to each other for a number of measurements under the same analytical conditions. It refers to the distribution of test results around their average. It is expressed as the percent relative standard deviation for a statistical significant number of samples (Hong *et al.* 2000:361-362).

Precision is part of the validation procedure for assay and quantitive determination of impurities (ICH Q2(R1), 1994:10).

5.6.5.1 Intra-day precision (repeatability)

Results of the HPLC method operating over a short time interval under the same conditions; also termed intra-assay precision. Injector performance and analysis of samples are evaluated during this procedure (Hong *et al.* 2000:362).

Method:

1. Weigh approximately 1.6 (a.), 2 (b.) and 2.4 (c.) g tablet powder accurately and dissolve in 50 ml methanol in three 100 ml volumetric flasks with sonication for 10 minutes. Prepare each sample in triplicate.
2. Allow to cool to room temperature and make up to volume with deionised water.
3. Dilute 5 ml of solution (a.) to 50 ml with deionised water – 80%
4. Dilute 5 ml of solution (b.) to 50 ml with deionised water – 100%
5. Dilute 5 ml of solution (c.) to 50 ml with deionised water – 120%
6. Filter 10 ml of each mixture through a syringe driven filter, discarding the first 5 ml of filtrate.
7. Transfer 2 ml of the filtrate to separate vials.
8. Inject into the chromatograph in duplicate.

Acceptance criteria: Intra-day precision < 2%

5.6.5.2 Intermediate precision

Variations within the laboratory environment are assessed. The reliability of the method in an environment different from the developmental method is examined (Hong *et al.* 2000:362) (ICH Q2(R1), 1994:10).

5.6.5.3 Reproducibility

Collaborative studies between two different laboratories. Not required if intermediate precision is achieved (Hong *et al.* 2000:362).

Reproducibility should be considered when the analytical procedure is standardised for inclusion in a pharmacopoeia (ICH Q2(R1), 1994:10).

5.6.6 Ruggedness

5.6.6.1 Sample solution stability

Method:

1. Prepare a sample as described under sample preparation.
2. Inject the sample in the chromatograph.
3. Leave the sample and re-analyse over a period of 24 hours.

Acceptance criteria:

Samples should be analyzed within the period before degradation of 2% can occur. If degradation does occur, precaution should be taken to compensate for the loss (ICH Q2(R1), 1994:10).

5.6.6.2 System repeatability

Method:

A standard should be injected six times consecutively to determine the repeatability of peak areas and retention times.

Acceptance criteria:

Peak areas and retention times should have a RSD ≤ 2% (ICH Q2(R1), 1994:10).

5.6.6.3 Robustness

Measurement of a methods capacity to remain unaffected by small, deliberate variations in some parameters and provide an assurance of its reliability during normal use (Hong *et al.* 2000:368).

Method:

Deliberate changes are made to the chromatographic conditions to determine the method's tolerance towards changes. Tolerance to changes to the flow, wavelength, injection volume, gradient and the use of similar column by different manufacturers should be determined.

Acceptance criteria:

A variance in the chromatographic conditions of 5% should be allowed for the method.

5.6.7 System suitability

Method:

- Chromatographic performance should be calculated e.g. retention time, capacity factor, USP peak tailing factor, resolution between peaks and repeatability.
- Set realistic performance limits that should be met before an analysis could be performed.

Acceptance criteria:

USP tailing factor of less than 1.5. Theoretical plates per column should be above 10,000; 30,000 and 45,000 respectively for each analyte and the RSD between 2 injections should not be more than 2% (ICH Q2(R1), 1994:10).

5.7 Validation results

During the validation of lamivudine, nevirapine and zidovudine simultaneously the following results were obtained as summarized (Table 5.1):

Table 5.1: Summary of test results

Test type	Results
Specificity	Complies (ICH Q2(R1), 1994:6-7)
Linearity	Lamivudine: R^2 = 0.9991 Nevirapine: R^2 = 0.9999 Zidovudine: R^2 = 0.9992 (ICH Q2(R1), 1994:8)
Range	Lamivudine = 0.9 – 348.2 µg/ml Nevirapine = 0.9 – 350.6 µg/ml Zidovudine = 0.9 – 349.8 µg/ml
Accuracy	Lamivudine = 98.10% Nevirapine = 98.60% Zidovudine = 100.1% (Hong *et al.* 2000:361) (ICH Q2(R1), 1994:9)
Precision	Lamivudine: RSD ≤ 0.94% Nevirapine: RSD ≤ 1.45% Zidovudine: RSD ≤ 0.84% (ICH Q2(R1), 1994:10)
Ruggedness	Complies (ICH Q2(R1), 1994:10)
Robustness	Complies (ICH Q2(R1), 1994:10)

5.7.1 Specificity

A chromatograph of a placebo sample solution containing all the tablet excipients is illustrated in fig 5.1. Peaks with retention times of lamivudine, zidovudine and

nevirapine in a standard solution is illustrated in figure 5.2 to 5.5 and in a sample solution illustrated in figure 5.6 (ICH Q2(R1), 1994:6-7).

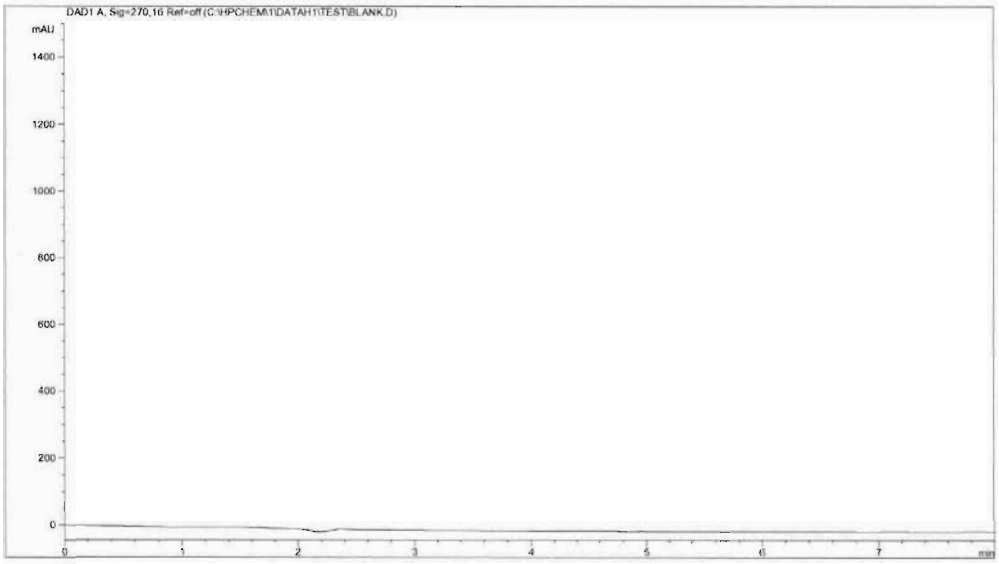

Figure 5.1: Graphic representation of placebo in a solution.

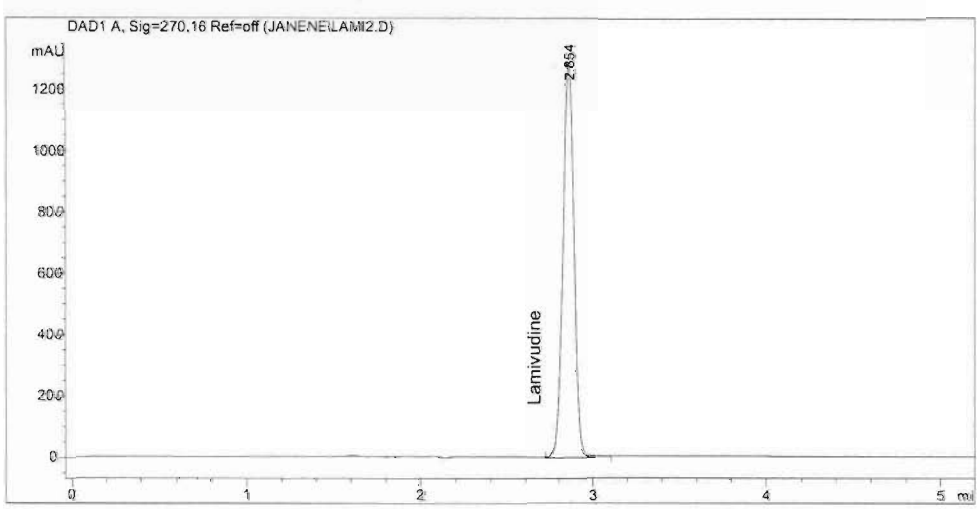

Figure 5.2: Graphic representation of the retention time of lamivudine in solution.

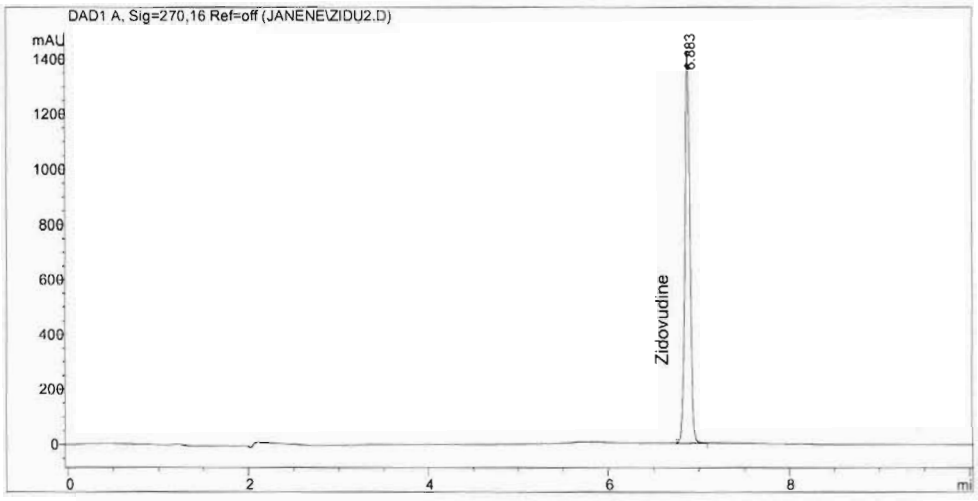

Figure 5.3: Graphic representation of the retention time of zidovudine in solution.

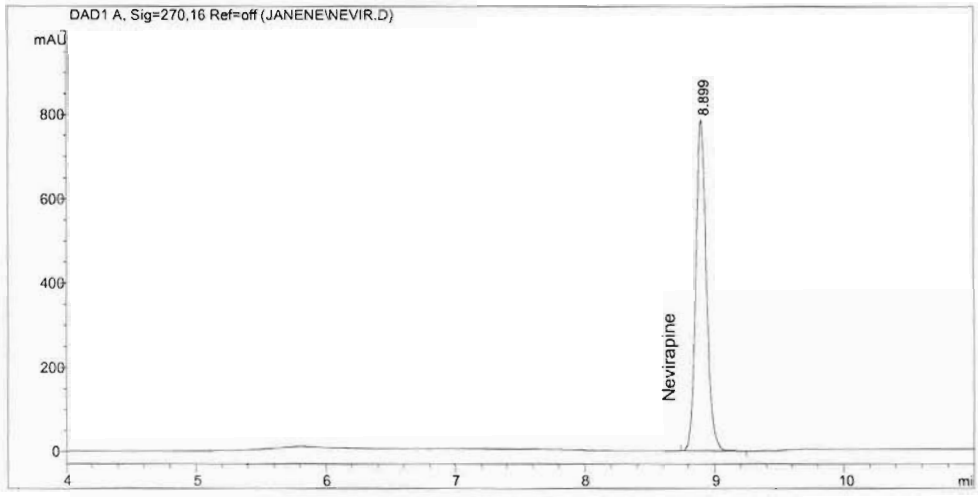

Figure 5.4: Graphic representation of the retention time of nevirapine in solution.

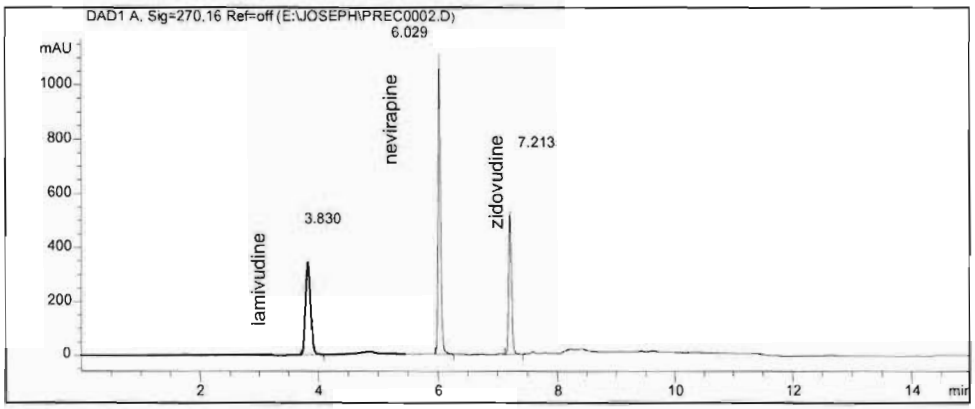

Figure 5.5: Graphic representation of the retention times of lamivudine, zidovudine and nevirapine in a standard solution.

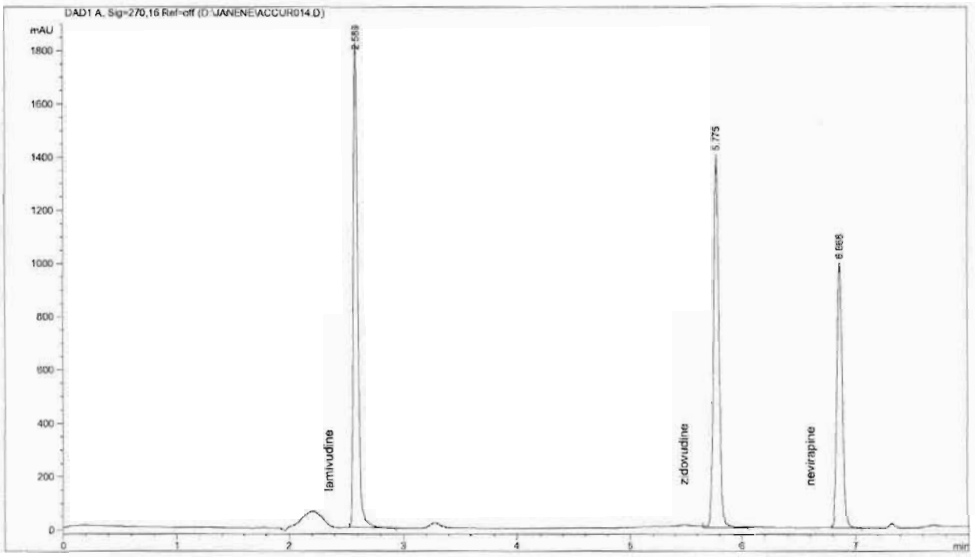

Figure 5.6: Graphic representation of the retention times of lamivudine, zidovudine and nevirapine in a sample solution.

The retention times observed in Fig. 5.5 and 5.6 of the active substances correlate with the times of the individual substances observed in Fig. 5.2 to 5.4. The chromatogram of the placebo excipient solution Fig. 5.1 indicates that no overlapping interferences between the active substances and the tablet excipients were detected. The method under validation is specific for the active substances (ICH Q2(R1), 1994:6-7).

5.7.2 Linearity

Table 5.2: Peak area and concentration for lamivudine

µg/ml	Area		Mean
0.9	22.87	23.61	23.24
1.7	47.20	46.99	47.10
3.5	93.39	93.96	93.68
5.2	137.46	140.08	138.77
7.0	186.79	186.83	186.81
8.7	233.70	233.51	233.61
3.5	79.35	77.56	78.46
7.0	154.69	154.79	154.74
13.9	310.47	310.45	310.46
20.9	465.9	466.1	466.00
27.9	622.18	62.182	342.18
34.8	778.9	778.78	778.84
34.8	787.47	777.21	782.34
69.6	1554.99	1552.15	1553.57
139.3	3110.68	3098.81	3104.75
208.9	4669.86	4655.18	4662.52
278.6	6182.14	6153.42	6167.78
348.2	7557.43	7785.78	7671.61

Table 5.3: Regression parameters of lamivudine

		Lower 95%	Upper 95%
R^2	0.999114		
Intercept	-4.37997	-47.2294	38.46948
Slope	22.13184	21.78249	22.48119

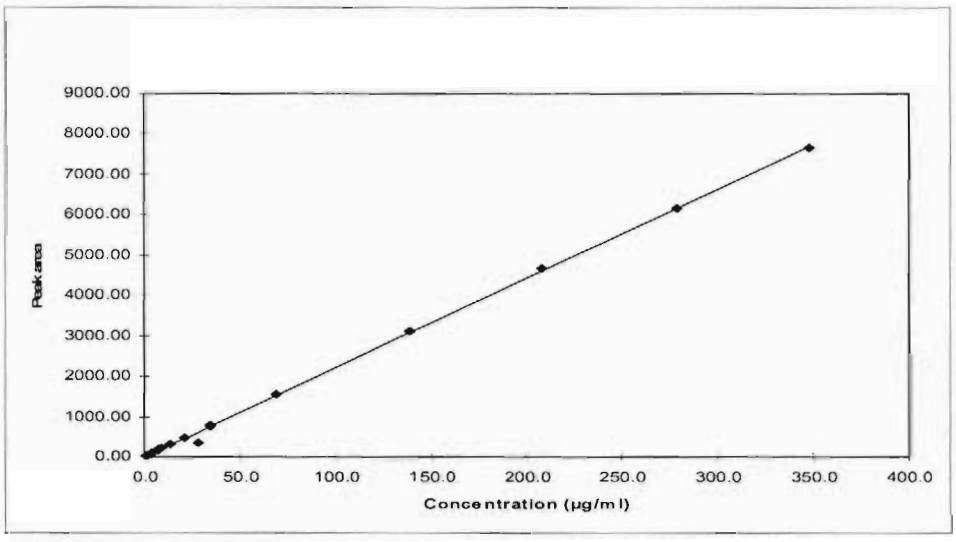

Figure 5.4: Linear regression curve for lamivudine

Table 5.4: Peak area and concentration for nevirapine

µg/ml	Area		Mean
0.9	15.38	15.88	15.63
1.8	30.88	30.09	30.49
3.5	60.81	61.06	60.94
5.3	90.91	91.66	91.29
7.0	121.76	121.55	121.66
8.8	152.97	151.92	152.45
3.5	51.88	51.30	51.59
7.0	101.44	102.02	101.73
14.0	204.97	204.85	204.91
21.0	306.75	306.95	306.85
28.0	412.31	411.83	412.07
35.1	515.88	516.41	516.15
35.1	521.78	525.64	523.71
70.1	1034.67	1042.02	1038.35
140.3	2072.23	2071.95	2072.09
210.4	3104.07	3101.49	3102.78
280.5	4112.85	4098.81	4105.83
350.6	5058.78	5162.09	5110.44

Table 5.5: Regression parameters of nevirapine

R^2	0.999953	Lower 95%	Upper 95%
Intercept	8.955198	2.424111	15.48629
Slope	14.60493	14.55205	14.65781

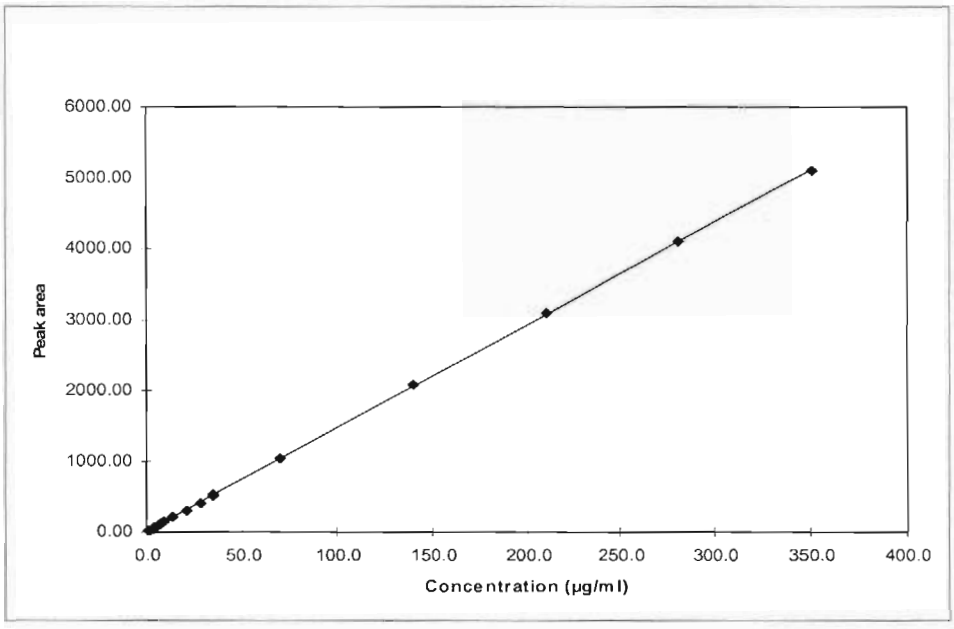

Figure 5.5: Linear regression curve for nevirapine

Table 5.6: Peak area and concentration for zidovudine

µg/ml	Area		Mean
0.9	19.41	20.68	20.05
1.7	39.89	39.37	39.63
3.5	80.62	82.02	81.32
5.3	119.52	121.03	120.28
7.0	162.01	155.94	158.98
8.5	198.51	198.65	198.58
3.5	65.62	64.98	65.30
7.0	130.45	131.05	130.75
14.0	263.50	262.35	262.93
21.0	395.3	393.27	394.29
28.0	522.79	536.42	529.61
35.0	667.13	672.41	669.77
35.0	675.62	678.84	677.23
70.0	1351.79	1358.88	1355.34
139.9	2684.45	2687.7	2686.08
209.9	3987.15	3985.36	3986.26
279.8	5210.8	5206.54	5208.67
349.8	6234.87	6366.41	6300.64

Table 5.7: Regression parameters of zidovudine

R^2	0.999241	Lower 95%	Upper 95%
Intercept	28.50334	-4.51478	61.52145
Slope	18.34325	18.07529	18.61122

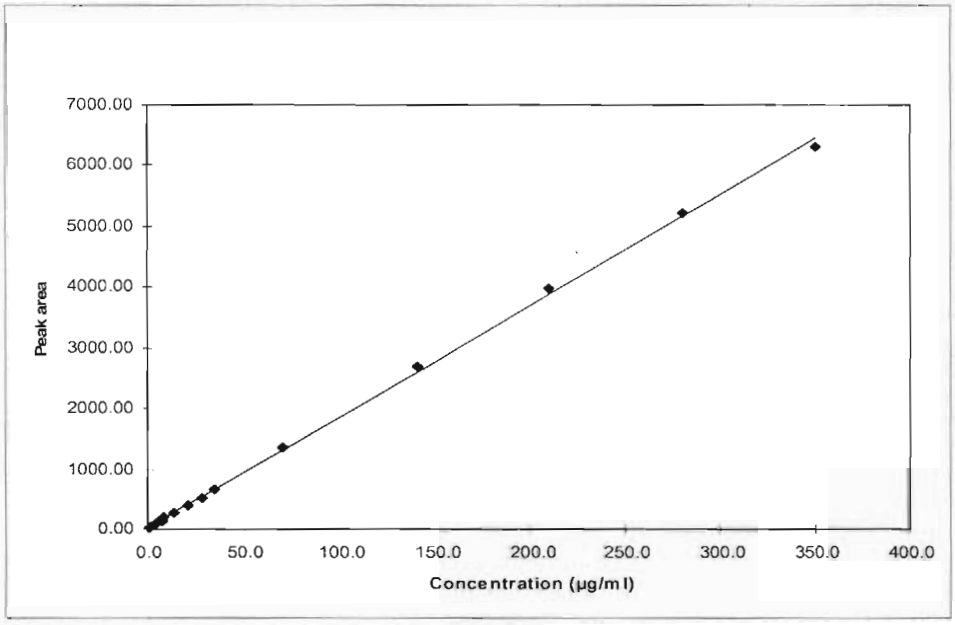

Figure 5.6: Linear regression curve for zidovudine

A R^2 with a decimal factor of at least three 9's were achieved during linearity studies of the individual substances. It indicates that the concentration variations in each substance result in a linear variation in the peak area, within limits obtained during chromatography (ICH Q2(R1), 1994:8).

5.7.3 Accuracy

Table 5.8: Percentage lamivudine recovered

Conc. spiked µg/ml	Area		Mean area	Recovery µg/ml	Recovery %
120.5	2663.2	2708.3	2686	118.4	98.3
120.5	2690.2	2678	2684	118.4	98.2
120.5	2669.8	2679.3	2675	117.9	97.9
150.6	3379.9	3366.1	3373	148.7	98.8
150.6	3325.8	3330.3	3328	146.8	97.4
150.6	3379.8	3368.7	3374	148.8	98.8
180.7	4009.6	3982.6	3996	176.2	97.5
180.7	3979.7	4005.9	3993	176.1	97.4
180.7	4062.7	4032.2	4047	178.5	98.8

Table 5.9: Confidence intervals for lamivudine

Statistical analysis	
Mean	98.1
SD	0.5
% SD	0.6
95% confidence intervals	
Lower limit	97.5
Upper limit	98.7
Estimated median	98.2
Confidence Level (95.0%)	0.6

Over the concentration range of 120.5 – 180.7 µg/ml (Table 5.8) for lamivudine the method yielded an accuracy of 98.1% (Table 5.9).

Table 5.10: Percentage nevirapine recovered

Conc. spiked µg/ml	Area		Mean area	Recovery µg/ml	Recovery %
160.2	2420.99	2422.1	2422	158.5	98.9
160.2	2406.63	2410.83	2409	157.6	98.4
160.2	2411.91	2405.69	2409	157.6	98.4
200.2	3036.86	3035.64	3036	198.7	99.2
200.2	3007.79	3003.94	3006	196.7	98.2
200.2	3030.02	3043.98	3037	198.7	99.3
240.2	3610.74	3601.79	3606	236.0	98.2
240.2	3602.85	3600.9	3602	235.7	98.1
240.2	3633.88	3622.52	3628	237.4	98.8

Table 5.11: Confidence intervals for nevirapine

Statistical analysis	
Mean	98.6
SD	0.4
% SD	0.4
95% confidence intervals	
Lower limit	98.1
Upper limit	99.2
Estimated median	98.4
Confidence Level (95.0%)	0.6

Over the concentration range of 160.2 – 240.2 µg/ml (Table 5.10) for nevirapine the method yielded an accuracy of 98.6% (Table 5.11).

Table 5.12: Percentage zidovudine recovered

Conc. spiked µg/ml	Area		Mean area	Recovery µg/ml	Recovery %
240.1	5017.54	5020.22	5019	241.7	100.7
240.1	4984.41	4992.95	4989	240.2	100.1
240.1	5001.79	5005.29	5004	240.9	100.3
300.1	6277.67	6285.28	6281	302.5	100.8
300.1	6232.53	6230.71	6232	300.1	100.0
300.1	6280.54	6270.57	6276	302.2	100.7
360.12	7447.19	7440.05	7444	358.4	99.5
360.12	7454.76	7377.68	7416	357.1	99.2
360.12	7478.17	7474.97	7477	360.0	100.0

Table 5.13: Confidence intervals for zidovudine

Statistical analysis	
Mean	100.1
SD	0.5
% SD	0.5
95% confidence intervals	
Lower limit	99.7
Upper limit	100.6
Estimated median	100.1
Confidence Level (95.0%)	0.4

Over the concentration range of 240.1 – 360.12 µg/ml (Table 5.12) for zidovudine the method yielded an accuracy of 100.1% (Table 5.13).

Concentration ranges of a standard compared to the concentration ranges of a sample indicated that the method yielded sufficient accuracy for the individual substances. The accuracy achieved for the individual substances is within limits 98 – 102% (Hong *et al.* 2000:361) (ICH Q2(R1), 1994:9).

5.7.4 Precision

Table 5.14: Intra-day precision of lamivudine

Mass (µg/ml)	Area			Conc.µg/ml	%Recovery
86.530	1963.34	1958.37	1961	86.5	99.9
86.580	1988.89	1970.83	1980	87.3	100.8
86.520	1974.88	1978.94	1977	87.2	100.8
108.130	2437.02	2425.43	2431	107.2	99.1
108.170	2440.89	2447.96	2444	107.8	99.6
108.140	2437.02	2435.73	2436	107.4	99.3
129.800	2927.97	2941.35	2935	129.4	99.7
129.880	2954.13	2943.33	2949	130.0	100.1
129.760	3015.23	3009.69	3012	132.8	102.4

Mean	100.20
SD	0.94
%RSD	0.94

Table 5.15: Intra-day precision of nevirapine

Mass (µg/ml)	Area			Conc.µg/ml	%Recovery
90.060	1417.29	1401.87	1410	91.9	102.1
90.450	1415.53	1370.21	1393	90.8	100.4
90.000	1403.54	1375.09	1389	90.6	100.7
112.560	1725.89	1731.68	1729	112.7	100.2
112.690	1711.48	1714.45	1713	111.7	99.1
112.690	1777.55	1740.15	1759	114.7	101.8
135.390	2026.89	2046.23	2037	132.8	98.1
135.320	2025.99	2056.18	2041	133.1	98.4
135.130	2037.51	2024.58	2031	132.5	98.0

Mean	99.87
SD	1.45
%RSD	1.45

Table 5.16: Intra-day precision of zidovudine

Mass (µg/ml)	Area			Conc.µg/ml	%Recovery
175.940	3532.7	3534.8	3534	176.2	100.1
176.050	3580	3586.7	3583	178.7	101.5
175.920	3581.78	3583.88	3583	178.6	101.5
219.860	4430.48	4434.83	4433	221.0	100.5
219.940	4414.87	4407.04	4411	219.9	100.0
219.880	4402.74	4408.27	4406	219.7	99.9
263.940	5245.7	5260.26	5253	261.9	99.2
264.080	5312.09	5281.02	5297	264.1	100.0
263.840	5393.49	5384.39	5389	268.7	101.8

Mean	100.52
SD	0.85
%RSD	0.84

Table 5.17: Inter-day precision of lamivudine

	Day 1 %Recovery	Day 2 %Recovery	Day 3 %Recovery	Between days
	98.6	97.2	96.0	-
	97.8	96.4	96.6	-
	97.7	97.1	96.3	-
Mean	98.03	96.90	96.30	97.08
SD	0.40	0.36	0.24	1.12
RSD%	0.41	0.37	0.26	1.16

Table 5.19: Inter-day precision of nevirapine

	Day 1 %Recovery	Day 2 %Recovery	Day 3 %Recovery	Between days
	101.2	99.1	98.4	-
	100.5	100.9	98.6	-
	101.9	99.6	99.7	-
Mean	101.20	99.87	98.90	99.99
SD	0.57	0.76	0.57	0.94
RSD%	0.56	0.76	0.58	0.94

Table 5.21: Inter-day precision of zidovudine

	Day 1 %Recovery	Day 2 %Recovery	Day 3 %Recovery	Between days
	103.1	104.5	103.2	-
	104.2	103.8	102.6	-
	102.7	105.1	103.4	-
Mean	103.34	104.48	103.07	103.63
SD	0.63	0.55	0.34	0.61
RSD%	0.61	0.52	0.33	0.59

Intra-day precision was within limits of 4% for lamivudine, nevirapine and zidovudine.

Inter-day precision was within the limits of 4% SD for all three drugs.

Both intra- and inter-day precision were achieved. Therefore the assumption can be made that the distribution of the test results will remain constant within limits near the average within and between days under analytical conditions (ICH Q2(R1), 1994:10).

5.7.5 Ruggedness

Table 5.23: Stability results of lamivudine, zidovudine and nevirapine

	Lamivudine		Zidovudine		Nevirapine	
Time (hours)	Peak Area	% Recovery	Peak Area	% Recovery	Peak Area	% Recovery
0	3152.00	100.0	5197.50	100.0	4076.65	100.0
1	3180.05	100.9	5293.63	101.8	4075.92	100.0
2	3178.48	100.8	5246.63	100.9	4079.34	100.1
3	3186.04	101.1	5250.38	101.0	4094.10	100.4
4	3198.57	101.5	5227.63	100.6	4099.85	100.6
5	3151.36	100.0	5261.08	101.2	4078.24	100.0
6	3153.18	100.0	5337.70	102.7	4080.59	100.1
7	3151.36	100.0	5333.08	102.6	4078.24	100.0
8	3154.09	100.1	5337.69	102.7	4081.76	100.1

9	3161.36	100.3	5350.00	102.9	4091.18	100.4
10	3141.36	99.7	5316.15	102.3	4065.29	99.7
11	3145.91	99.8	5323.85	102.4	4071.18	99.9
12	3138.64	99.6	5311.54	102.2	4061.76	99.6
Mean	3161	100.3	5291	101.8	4080	100.1
SD	17.97	0.57	47.25	0.91	10.30	0.25
RSD %		0.57		0.89		0.25

Variations were less than 5% for all analytes.

Standard deviations of 0.57%, 0.89% and 0.25% for Lamivudine, Zidovudine and Nevirapine peak areas respectively were recovered. All peak areas were within the specified limit.

The recovery of all the analytes were within limits during the period of analysis and the samples are stable during the period. Therefore the method complies with ruggedness specifications (ICH Q2(R1), 1994:10).

5.7.6 Repeatability

Table 5.24: System repeatability for lamivudine, zidovudine and nevirapine

	Lamivudine		Zidovudine		Nevirapine	
	Area	Retention time (minutes)	Area	Retention time (minutes)	Area	Retention time (minutes)
1	3169.4	3.249	5197.5	6.390	4086.1	7.623
2	3206.0	3.317	5293.6	6.400	4086.8	7.621
3	3171.7	3.220	5246.6	6.380	4089.7	7.617
4	3145.3	3.240	5250.4	6.393	4082.4	7.624
5	3190.4	3.240	5227.6	6.390	4082.0	7.620
6	3155.6	3.290	5261.1	6.400	4094.8	7.624
Mean	3173.1	3.259	5246.1	6.392	4086.9	7.622
SD	20.34	0.033	29.45	0.007	4.38	0.003
RSD %	0.64	1.023	0.56	0.107	0.11	0.033

Standard deviations were less than 2% for both the peak areas as well as retention times for all analytes. Deviations of 0.64%, 0.56% and 0.11% were detected for Lamivudine, Zidovudine and Nevirapine peak areas in six readings. Deviations of 1.023%, 0.107% and 0.033% were detected for above mentioned analytes' retention times in six readings.

Repeatibility was achieved during the validation (ICH Q2(R1), 1994:10).

5.7.7 System suitability

Table 5.25: System suitability parameters obtained during an analysis of the three analytes

	Lamivudine	Zidovudine	Nevirapine
Retention Time (Minutes)	2.589	5.775	6.868
Capacity factor (k')	0.212	1.703	2.215
USP Peak tailing factor	1.090	0.998	1.040
Efficiency: Plates per column	19131	65079	89860
Resolution between previous peak (Tangent method)	-	38.522	11.990
Repeatability (%)	0.64	0.56	0.11

In Table 5.25 the system suitability parameters of the three analytes are given for an analysis performed.

All parameters fall within limits mentioned previously (5.6.7 System suitability page 82). System suitability was also achieved during the analytical procedures and therefore the system and parameters used during the analysis of the substances is suitable (ICH Q2(R1), 1994:10).

Chapter 6

Stability testing

Stability testing is performed on drug products to determine if the products retain its full efficacy up to the end of its expiry date. Organoleptic, physico-chemical, chemical and microbial test results must be within the predefined tolerance ranges up to the end of the expiry date (Grimm *et al.* 1993:17).

The following aspects must be considered in order for the quality of the product to be assured by the stability information:

- Shelf-life – Expiration date must refer to the area in which the product is to be distributed
- Storage conditions – For certain products shelf-life can only be guaranteed if specific storage instructions are complied with.
- Stability in opened container – For certain products it is necessary to specify the time within which a product must be used after opening of the container.
- Overage – Certain products require an overage in order to achieve an acceptable shelf-life and may only be added if the formulation is already optimized (Grimm *et al.* 1993:17-18).

Factors that may influence the stability of a product include internal-, manufacturing- and external factors (Grimm *et al.* 1993:18).

Internal factors include:

- Reactivity of the active ingredients, excipients and packaging materials.
- Interactions between active ingredients, excipients and packaging materials or a combination thereof.

Factors relating to manufacture:

- Batch size

- Equipment used
- Sequence in which the constituents are added
- Difference in quality of materials used

External factors:

- Temperature
- Humidity
- Light
- Oxygen
- pH (Grimm *et al.* 1993:18).

6.1 Principles

The stages of stability testing during the development of a new drug product can be divided in six steps (Grimm *et al.* 1993:20-21):

1. Tests with the active substances.
2. Preformulation and formulation findings for the clinical- and final dosage form.
3. Stress and accelerated tests with selected formulations and packaging materials.
4. Long term testing.
5. Follow-up tests.
6. Tests in the event of changes during the production process e.g. formulation, apparatus changes.

Suitable test methods are required for each test parameter. The test or analytical methods must be validated and the apparatus used qualified. For assay determination it is therefore possible to determine whether the active ingredient content has changed in relation to its initial value during the course of storage. For assay testing the following should be considered in the validation (Grimm *et al.* 1993:22-23):

- Specificity
- Linearity of active ingredient and decomposition products

- Accuracy
- Precision. Relative standard deviation (RSD) should be < 2%
- Limit of quantitation for the decomposition products which should be at least 0.5% with reference to the active ingredient
- Ruggedness
- Availability of documentation

Specifications and tolerances must take manufacturing, analysis and tolerable changes during storage into account during stability testing. Tolerances must be established for all test parameters. To ascertain the discriminatory power of the individual test procedures a relative standard deviation is used for assay as well as hardness, disintegration and dissolution rate testing. When these test data have been collected, significant statistical changes can be determined and it is possible to establish tolerances for tolerable changes (Grimm *et al.* 1993:23).

6.2 Storage conditions

During stability testing the changes occurring in a dosage form during storage is assessed and stability information compiled from the results. The extent of the changes that occur during stability testing is influenced by the storage conditions. Storage parameters such as temperature and relative humidity are the most important stability determining factors (Grimm *et al.* 1993:23-24).
Accelerated stability tests are used to:

- Identify the weaknesses of an active substance or formulation
- Make stability predictions
- Obtain information on extreme loading during transport

To make sufficient stability predictions a relationship between long-term storage conditions and acceleration tests should exist. Stability predictions can only be made if an increase in reaction temperatures can be extrapolated. Extrapolation is only possible if the laws of reaction kinetics apply, the activation energy of the substance is not temperature dependant and the reaction mechanism does not change in the temperature range for the extrapolated temperatures.

Factors that change reaction kinetics include: Loss of moisture to different degrees at individual temperatures. Increase in concentration of active substance or ion strength to different degrees in semi-solid and liquid dosage forms. This can be prevented by using water impermeable packaging materials.
Reaction kinetics can not predict organoleptic and physico-chemical changes (Grimm *et al.* 1993:23-26).

A distinction is drawn between testing for chemical changes (40 to 80 °C) of an active in a dosage form and testing for organoleptic and physico-chemical changes (below 40 °C). The rate of decomposition can be accelerated to such a rate that results are available within 3 months on the basis of the laws of reaction kinetics. For reliable stability predictions at least three temperatures are required.
The following conditions are required for solid dosage forms: 25°C ± 2°C/60% ± 5% Relative Humidity (RH), 30°C ± 2°C/65% ± 5% RH and 40°C ± 2°C/75% ± 5% RH (ICH Q1A(R2), 2003:3) (Grimm *et al.* 1993:26-27).

6.3 Testing procedure

The following conditions were available: 25°C/60% RH, 30°C/65% RH and 40°C/75% RH.

Four batches of tablets were manufactured. Baseline testing were performed one each batch at 0 weeks. Each batch of tablets was packed in 100's in white 500 ml PVC screw cap containers. The containers were labelled and placed at the above mentioned temperatures.

At 4, 8 and 12 weeks one container of each batch was removed from the stability environment. Each container was left for 24 hours to equilibrate the container temperature to room temperature before proceeding with the tablet tests.

6.4 Tablet tests

The pharmacopoeia specifies that all tests should be performed in a regulated laboratory in accordance with good laboratory practice. Protective equipment and work practices should be followed at all times. The person/s performing the tests should be familiar with the safety data of the materials used during the procedure (USP 2005:6) (SOP ADM 23).
Knowledge of laboratory procedures, apparatus used during the procedure and good laboratory practice is essential to perform any analytical procedure (SOP EQ 02). Safety equipment e.g. laboratory coat, latex gloves, safety glasses and respirator were worn where applicable in accordance with the material safety data sheet (MSDS) and standard operating procedures (SOP ADM 23).

Metric measurements were used during all the analytical procedures. All the analytical terms and abbreviations are in accordance with the pharmacopoeia (USP 2005:6-11). All equipment was valid, calibrated and validated analytical procedures were used.

Waste products were discarded in accordance with operating procedures (SOP ADM 10).

6.4.1 Description

The ICH guideline specifies that a qualitative description should be provided for all new drug products e.g. size, shape and colour. Any changes thereof during manufacture or storage should be investigated and appropriate action taken (ICH Q6A, 1999:7).

6.4.2 Identification

The identity of the active substance should be established in the new product and the method should be able to discriminate between compounds of closely related structures. Identification by a single chromatographic retention time is not regarded as being specific. The use of two chromatographic procedures where the separation is based on different principles or combination of tests in a single procedure e.g. HPLC/UV DAD, HPLC/MS or GC/MS is acceptable (ICH Q6A, 1999:7).

6.4.3 Assay

Assay of each batch of tablets were performed in accordance to USP standards (USP 2005:2050). The USP specifies that zidovudine tablets should contain not less than 90.0% and not more than 110.0% of the labelled amount of zidovudine (USP 2005:2050).

In the ICH guideline stability indicating assay to determine the active substance content is considered compulsory during new drug product development (ICH Q6A, 1999:7).

Method:

- Ten tablets were selected randomly from the applicable batch and weighed on an analytical balance (SOP BAL 01).
- A standard solution containing the same concentration of drugs present in the tablet was prepared (see 5.3 Standard preparation, page 77).
- The 10 tablets were powdered in a pestle and mortar.
- Weigh approximately 2 g of powdered tablet accurately into a tared 100 ml volumetric flask.
- Dissolve in 50ml methanol, sonicate for 10 minutes.
- Make up to volume with deionised water.
- Dilute 5 ml of this solution to 50 ml with deionised water.
- Centrifuge the solution for 10 minutes at 2500 rpm.
- Transfer the clear supernatant into separate vials.
- Inject into the chromatograph in duplicate.
- Both samples and standards were prepared in duplicate.
- Samples and standards were analyzed quantitively for the substances by means of LC (USP 2005:2050).
- Assay testing was performed at 0, 4, 8 and 12 weeks during stability testing.

6.4.4 Hardness

- The test apparatus was programmed to measure tablet thickness, diameter (mm) and hardness (N).
- The ten tablets were analyzed.
- A summary of the results were printed with mean, minimum and maximum forces measured (BP 2005: A377).

6.4.5 Friability

The measurement of tablet friability is regarded as a supplement to other physical strength measurements such as tablet breaking force (BP 2005:A377 and Ph. Eur. Method 2.9.7).

Pharma-Test friabilator was used during the procedure (SOP FRB 01). The apparatus consists of a drum, diameter 283-291 mm, dept 36-40 mm of transparent polished polymer. The one side of the drum is removable and an inside curved projection with radius 75.5-85.5 extends from the middle of the drum to the outer wall. The drum is attached to the horizontal axis of a device that rotates at 25 ± 1 rpm (BP 2005:A377).

Method:

- Ten tablets from each batch were brush-cleaned and weighed (BP 2005:A377).
- The tablets were then exposed to the tumbling conditions of the friabilator for 4 minutes (25 rpm).
- Afterwards they were removed and again brush-cleaned before commencing with the second weighing procedure.
- The mean weight difference of the tablets before and after the procedure was recorded.
- After each procedure the tablets were powdered for the assay testing.
- Care was taken not to cross contaminate the tablets with the previous lot of tablets that were subjected to friability. This was done through cleaning the apparatus properly before commencing with the test (SOP FRB 01).

- The test apparatus was programmed to measure tablet thickness, diameter (mm) and hardness (N).
- The ten tablets were analyzed.
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- The mean weight difference of the tablets before and after the procedure was recorded.
- After each procedure the tablets were powdered for the assay testing.
- Care was taken not to cross contaminate the tablets with the previous lot of tablets that were subjected to friability. This was done through cleaning the apparatus properly before commencing with the test (SOP FRB 01).

6.4.6 Uniformity of mass

The test is performed on tablets to ensure the consistency of dosage units in a batch. Each unit should contain an amount of active substance within the narrow range around the label claim. Uniformity of mass is performed on tablets that contain more than 25 mg or 25% of active substance per tablet (BP, 2005:A294) (USP, 2005:2505).

- Ten tablets are selected randomly from each batch.
- Each tablet was weighed on a calibrated microbalance and the mass recorded (SOP BAL 01).
- The difference between the recorded masses is expressed as a percentage of the theoretical tablet mass.

6.4.7 Loss on drying

The amount of water or any volatile substance present in a batch of tablets can be detected during the procedure. For substances that contain water as the only volatile constituent the procedure Water determination is appropriate (USP 2005:2429).

Method:

- Not less than four tablets per batch were selected randomly and powdered.
- The amount of tablet powder equivalent to one tablet (1g) was accurately weighed in a wide, shallow screw cap glass bottle with a heat resistant screw cap. Both bottle and caps were numbered to allow ease of identification.
- After the weighing procedure the caps were removed, the bottles were gently tilted to distribute the powder evenly and both the bottles and caps placed in the oven.
- The samples were placed in a pre-heated oven at a given temperature (105°C) for 5 hours.
- The samples were then removed from the oven, caps replaced and placed in a desiccator to allow cooling to room temperature.
- The samples were weighed and the weight difference between the initial mass and final mass recorded and expressed as a percentage of the initial powder mass (USP 2005:2429).

6.4.8 Dissolution

Dissolution is an indication of the bioavailability of a tablet. It is also an indication of the disintegration and solubilisation tempo (Abrahamsson, 2004:241). The dissolution test is used to determine the dissolution rate of the active ingredients of solid dosage forms (BP 2005:A272).

Disintegration testing is not obligatory compendially and has been replaced by the dissolution test. Disintegration testing is still being used during stability programs and sometimes a correlation between disintegration and dissolution do exist (Carstensen *et al.* 2000:299).
In vitro dissolution studies during research and development are performed for the following reasons:

- Validation of the manufacturing process.
- Investigation of effects of different storage conditions on the dosage form.
- For batch quality control purposes.
- As a surrogate for bioequivalence studies (Abrahamsson, 2004:241).

In vitro dissolution methods for batch quality control may not be sufficient for all the different aims of dissolution. The dissolution method and conditions may be adapted to serve its purpose (Abrahamsson, 2004:241).

Dissolution testing should be able to predict both the drug release and dissolution processes. Dissolution tempo of the active substances will be affected by its physicochemical properties (solubility, diffusivity), particle properties of the drug (surface area and polymorphism of the active substances) and formulation properties (wetting, solubilisation) (Abrahamsson, 2004:241).

It is useful if *in vitro* dissolution testing provides some correlation with *in vivo* data or physiological conditions in the gastro intestinal tract. Careful considerations should be taken when selecting a dissolution medium or dissolution apparatus (Abrahamsson, 2004:241).

Changes in disintegration due to storage usually happen rapidly, usually within 12 weeks at room temperature. No meaningful correlations exist of disintegration and dissolution profiles at high temperatures to profiles at low temperatures. Changes in

disintegration profiles can rationally be determined at 4, 8 and 12 weeks at room temperature (Carstensen *et al.* 2000:309).

The type of apparatus used depends on the physico-chemical characteristics of the dosage form. All the parts of the apparatus must be inert and preferably allow observation of the preparation being examined (BP 2005:A272).

For the dissolution of zidovudine tablets both the BP and USP specifies the use of the following parameters:

Medium:	Water; 900 ml
Apparatus 2 (Paddle):	50 rpm
Time:	30 minutes
Tolerance:	Not less than 80% of the labelled amount of zidovudine dissolved within 30 minutes (USP 2005:2050).

No pharmacopoeial specifications were available for the dissolution of the triple-drug combination present in the formulations. The dissolution method for zidovudine was altered to accommodate the dissolution of the other drugs, especially nevirapine (USP 2005:2050).

Choice of dissolution medium

The choice of the dissolution medium is dependant on the type of study performed, the following aspects should be considered when selecting a dissolution medium.

- Pharmacopoeial conditions and recommendations.
- Sensitivity of the formulation to different medium factors.
- Active substance solubility and stability at different pH values.
- Resemblance to *in vivo* data or gastro intestinal conditions (Abrahamsson, 2004:247).

Choice of apparatus and stirring rate

The choice of these parameters depends on pharmacopoeial recommendations, variability in dissolution test results and correlation with *in vivo* data. Stirring rate is

important as a too slow rate may not sufficiently disperse the particles and in the process delay the dissolution process. Unfortunately a too fast stirring rate is also not advisable as differences in dissolution rate between different batches or dosage forms may not be detectable (Abrahamsson, 2004:247).

Sampling intervals

For bioequivalence studies multi-point sampling intervals is recommended for immediate release dosage forms (Abrahamsson, 2004:251).

Parameters:

Medium:	0.1 M HCl pH adjusted to 2.0 ± 0.5; 900 ml (SOP PH 05)
Apparatus 2 (Paddle):	50 rpm
Time:	5 ml filtered with a Millipore Millex–HV Hydrophobic PVDF 0.45 µm™ filter at 5; 10; 15; 20 and 30 minutes

Method:

- A calibrated VanKel 7000™ dissolution apparatus, water bath regulated vesels (900 ml) regulating the vessel temperature at 37°C ± 0.5 without an autosampler was used during dissolution testing (SOP DIS 01). The apparatus consists of six glass beakers with paddles stirring the medium present in the beakers. The temperature of the medium and speed of the paddles can be controlled during the procedure.
- 0.1 M HCl dissolution medium was prepared and divided in 900 ml aliquots into the beakers and allowed to reach operating temperature (37.0 °C ± 0.5) whilst being stirred by the paddles.
- Six tablets were randomly selected from each batch; weighed and each weight recorded.
- The six tablets were then introduced into the beakers at time 0 minutes.
- After the elapse of different time intervals, aliquots were withdrawn from each beaker, discarding the first 3ml from the filter and transferred to HPLC vials.

- Standards were prepared in duplicate (see 5.2 Standard preparation, page 77) and the standards and samples were analysed quantitively by HPLC. Note that dissolution medium was used for the dilutions of the standard solutions during dissolution standard preparation.

Chapter 7

Test results

7.1 Manufacturing observations

All tablet formulations were manufactured on a single tablet press. An un-bevelled punch with a diameter of 16 mm was used. The mixed tablet powders were introduced to the hopper in aliquots to ensure sufficient powder flow. A small hopper with a volume of ± 500 ml was used. The whole tablet press, punches and hopper were cleaned after and before the manufacture of each formulation. The tablet press operated at a speed of ± 100 tablets per minute. Before a batch of tablets was manufactured, the press parameters were adjusted to produce tablets that comply with specifications. No problems with powder-flow and powder-lubrication were encountered during the manufacturing process. Problems with the adjustment of the tablet press parameters were encountered during the manufacture of formula 4.

Tablet description

Round tablets with a diameter of 16 mm and thickness of 4 mm were manufactured. The tablets have a white to off-white colour with no markings, flat smooth surface and sharp edges.

7.2 Assay

The aim of assay testing is to determine the amount of active substances in a pharmaceutical regimen and compare the amounts to the theoretical or labelled amount. Results are expressed as a percentage of the labelled amount. Limits for deviations are given in the pharmacopoeia and may differ between different pharmacopoeias. Limits are stricter for more potent drugs present in lower concentrations. Limits are usually between 90-110% but may differ for different active substances and pharmacopoeias.

Initial results at 0 weeks are performed to determine if the manufacturing process went according to plan and no formulation problems were encountered.

Although the deviation of all formulas falls within limits of the pharmacopoeia, little stability data can be derived if a large degree of standard deviation is present. A very small standard deviation is the ideal for stability testing. To achieve such a small deviation the formulation and manufacturing process have to adhere to the strictest procedures. It is especially hard to adhere to these strict standards when manufacturing tablets as a perfect mix is not possible but rather a random mix (Davies, 2004:388).

Formulation problems were encountered with formulation 1 as seen in the initial assay results (Table 7.1). Possible problems with mixing or 'demixing' were encountered.

Improper mixing or 'demixing' during manufacture seems to pose a problem. Uniformity of mass and hardness testing results may reveal the true identity of the problem.

Table 7.1: Amounts of each drug present in formula 1 during assay testing.

0 Weeks Initial	Lamivudine % Tablet	Zidovudine % Tablet	Nevirapine % Tablet
	95.59; 2.93% SD	-	97.01; 0.25% SD

4 Weeks	Temperature & humidity	Lamivudine % Tablet	Zidovudine % Tablet	Nevirapine % Tablet
	25°C; 60% RH	96.62; 1.84% SD	-	95.96; 1.44% SD
	30°C; 60% RH	96.52; 1.06% SD	-	95.08; 0.35% SD
	40°C; 75% RH	94.66; 1.01% SD	-	94.25; 0.18% SD
8 Weeks	25°C; 60% RH	99.47; 1.12% SD	-	94.35; 0.13% SD
	30°C; 60% RH	97.36; 0.93% SD	-	93.46; 1.11% SD
	40°C; 75% RH	96.65; 1.24% SD	-	92.32; 0.39% SD
12 Weeks	25°C; 60% RH	97.63; 0.10% SD	-	94.12; 0.02% SD
	30°C; 60% RH	96.10; 2.40% SD	-	95.51; 0.36% SD
	40°C; 75% RH	96.55; 0.18% SD	-	96.88; 0.60% SD

No signs of chemical decomposition could be observed in any of the active substances during the stability studies of formulation 1 (Table 7.1).

Note that formulation 1 does not contain any Zidovudine.

Table 7.2: Amounts of each drug present in formula 2 during assay testing.

0 Weeks Initial	Lamivudine % Tablet	Zidovudine % Tablet	Nevirapine % Tablet
	98.01; 0.61% SD	100.30; 1.94% SD	100.55; 0.95% SD

4 Weeks	Temperature & humidity	Lamivudine % Tablet	Zidovudine % Tablet	Nevirapine % Tablet
	25°C; 60% RH	98.47; 0.12% SD	100.40; 0.51% SD	97.97; 0.53% SD
	30°C; 60% RH	96.81; 1.30% SD	97.92; 1.12% SD	97.51; 1.28% SD
	40°C; 75% RH	100.27; 1.08% SD	95.41; 0.37% SD	95.49; 0.13% SD
8 Weeks	25°C; 60% RH	99.97; 0.38% SD	101.40; 1.39% SD	95.58; 0.51% SD
	30°C; 60% RH	98.29; 0.40% SD	100.23; 0.11% SD	94.78; 0.89% SD
	40°C; 75% RH	99.08; 0.61% SD	100.50; 0.36% SD	94.22; 0.65% SD
12 Weeks	25°C; 60% RH	96.44; 0.77% SD	100.08; 0.87% SD	98.82; 0.47% SD
	30°C; 60% RH	98.81; 0.65% SD	98.29; 0.01% SD	98.28; 0.14% SD
	40°C; 75% RH	97.54; 0.43% SD	99.68; 1.02% SD	98.89; 0.18% SD

For formulation 2 (Table 7.2) no signs of degradation in any of the active substances could be observed and therefore verifying the results obtained during formula 1. In this formulation the standard deviation was smaller than observed in formulation 1.

Table 7.3: Amounts of each drug present in formula 3 during assay testing.

0 Weeks Initial	Lamivudine % Tablet	Zidovudine % Tablet	Nevirapine % Tablet
	103.87; 0.51% SD	99.52; 3.64% SD	97.82; 1.16% SD

4 Weeks	Temperature & humidity	Lamivudine % Tablet	Zidovudine % Tablet	Nevirapine % Tablet
	25°C; 60% RH	98.69; 2.33% SD	96.67; 0.60% SD	98.11; 1.93% SD
	30°C; 60% RH	96.78; 0.45% SD	94.72; 0.32% SD	96.81; 2.68% SD
	40°C; 75% RH	99.27; 0.03% SD	93.68; 1.65% SD	98.26; 2.35% SD
8 Weeks	25°C; 60% RH	101.31; 0.33% SD	97.01; 0.36% SD	92.82; 1.01% SD
	30°C; 60% RH	101.42; 0.62% SD	98.61; 0.10% SD	94.23; 0.45% SD
	40°C; 75% RH	98.83; 0.21% SD	96.98; 1.16% SD	91.53; 0.63% SD
12 Weeks	25°C; 60% RH	100.89; 0.91% SD	96.03; 0.86% SD	97.61; 0.02% SD
	30°C; 60% RH	99.65; 0.98% SD	97.56; 0.36% SD	101.47; 0.71% SD
	40°C; 75% RH	102.49; 3.86% SD	99.91; 1.79% SD	98.71; 0.34% SD

In formula 3 again no degradation can be observed (Table 7.3).

Table 7.4: Amounts of each drug present in formula 4 during assay testing.

0 Weeks	Lamivudine % Tablet	Zidovudine % Tablet	Nevirapine % Tablet
	103.34; 2.52% SD	96.55; 0.05% SD	100.94; 1.82% SD

4 Weeks	Temperature & humidity	Lamivudine % Tablet	Zidovudine % Tablet	Nevirapine % Tablet
	25°C; 60% RH	99.16; 0.06% SD	99.01; 0.37% SD	95.19; 0.58% SD
	30°C; 60% RH	99.74; 0.88% SD	95.14; 0.03% SD	96.23; 0.66% SD
	40°C; 75% RH	97.86; 0.33% SD	93.39; 0.39% SD	109.19; 0.24% SD
8 Weeks	25°C; 60% RH	100.89; 0.67% SD	104.25; 0.24% SD	94.47; 3.05% SD
	30°C; 60% RH	100.29; 1.00% SD	103.19; 0.78% SD	97.31; 0.07% SD
	40°C; 75% RH	99.73; 1.74% SD	100.06; 0.01% SD	96.12; 3.11% SD
12 Weeks	25°C; 60% RH	102.37; 0.14% SD	98.93; 0.68% SD	100.44; 0.45% SD
	30°C; 60% RH	102.28; 0.61% SD	98.61; 0.35% SD	100.45; 1.91% SD
	40°C; 75% RH	102.08; 0.64% SD	98.67; 0.60% SD	99.30; 1.77% SD

None of the formulations seem to pose stability problems at any stage of stability testing (Table 7.1-7.4). No unidentified peaks could be observed during assay testing in any of the formulations; it correlates with the chromatogram of the tablet excipients in a placebo solution. The retention times of the active substances in the samples correlate with the retention times of the active substances in the standards.

A slight discolouration of the formulations at 40°C was observed. Discolouration seems to pose the biggest problem, the formulas at the higher temperatures discoloured from an off-white to a slight yellow. The discolouration does not seem to be the result of instability in the active substances but rather an excipient instability problem.

For mean substance determinations formulas 2 to 4 were used. Formula 1 does not contain zidovudine. Any possible interactions due to the presence of zidovudine might be excluded if this formula was used to calculate the mean concentrations.

Formulations with smaller standard deviations may in future be required to determine if any chemical degradation of active substances occurred during the 12 week stability testing procedure.

Assay results revealed that no chemical degradation of any of the three drugs occurred during stability testing in the tablets.

No drug excipient interactions could be seen in any of the formulations during HPLC analysis, contradicting the DSC results during preformulation.

No degradation products were detected during HPLC analysis, confirming that no chemical degradation occurred.

The discolouration of the tablets may be attributed to one or more of the organic excipients used in the formulations. This physico-chemical instability does not seem to pose a problem to stability of the drugs.

7.3 Hardness

Hardness, diameter and thickness tests were performed on all batches and conditions to determine if the formulations underwent any physical changes during stress conditions.

Table 7.5: Average tablet hardness for formula 1 (N).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	112.82; 7.63% SD	87.75; 9.95% SD	91.18; 13.69% SD	86.96; 4.90% SD
30°C; 60%	112.82; 7.63% SD	82.39; 4.94% SD	87.54; 9.43% SD	78.67; 8.00% SD
40°C;75%	112.82; 7.63% SD	75.23; 10.64% SD	81.10; 10.70% SD	75.89; 7.42% SD

Table 7.6: Average tablet hardness for formula 2 (N).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	92.39; 8.35% SD	76.91; 13.62% SD	72.73; 11.36% SD	79.23; 9.90% SD
30°C; 60%	92.39; 8.35% SD	82.51; 9.15% SD	74.37; 7.59% SD	75.89; 8.41% SD
40°C; 75%	92.39; 8.35% SD	76.72; 5.43% SD	77.34; 4.13% SD	71.48; 7.40% SD

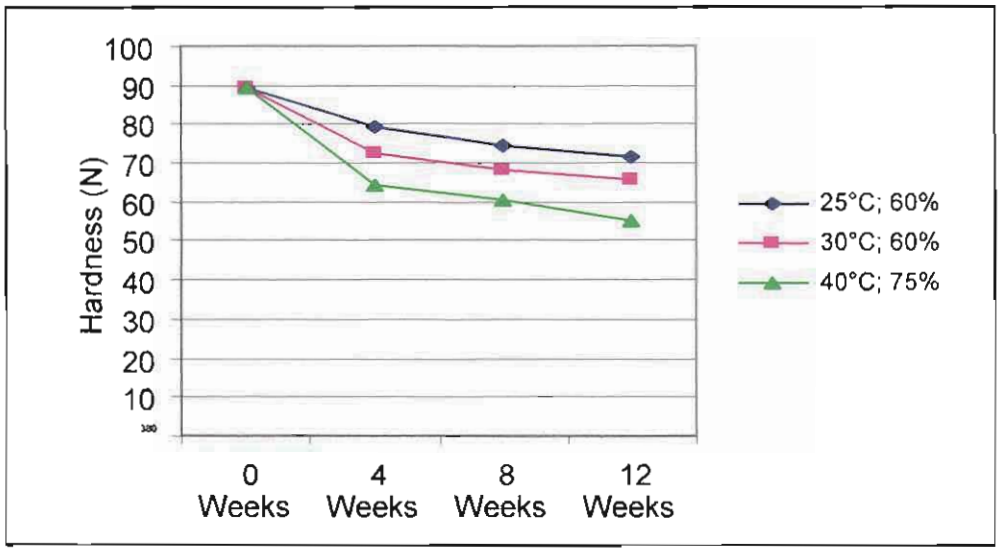
Table 7.7: Average tablet hardness for formula 3 (N).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	89.2; 20.85 % SD	79.32; 13.68% SD	74.45; 12.91% SD	71.35; 10.08% SD
30°C; 60%	89.2; 20.85 % SD	72.5; 14.59% SD	68.03; 15.44% SD	65.93; 10.83% SD
40°C; 75%	89.2; 20.85 % SD	64.25; 10.28% SD	60.45; 7.13% SD	54.89; 14.42% SD

Table 7.8: Average tablet hardness for formula 4 (N).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	82.59; 23.44% SD	68.61; 32.85% SD	84.53; 29.89% SD	69.68; 25.76% SD
30°C; 60%	82.59; 23.44% SD	61.85; 8.86% SD	75.71; 23.28% SD	61.61; 14.43% SD
40°C; 75%	82.59; 23.44% SD	72.08; 17.08% SD	67.93; 16.73% SD	72.39; 25.91% SD

No significant changes could be observed during the hardness testing of formulations 1, 2 and 4. A decrease in tablet hardness can be observed for formula 3 (Table 7.5 – 7.8).



Graph 7.1: Decrease in tablet hardness of formula 3 at different stages of stability testing.

In formula 3 a seemingly linear decrease in hardness can be seen (Graph 7.1).

A possible explanation for the decrease in hardness may be due to relaxation of bonds between adjacent particles of the tablet binders or partial hydration of the disintegrants due to the increased temperature and humidity conditions (Carstensen *et al.* 2000:296).

Hardness of all the batches of tablets was determined to indicate whether it was able to withstand any physical stress conditions. The pharmacopoeia specify that tablets

have to be hard enough to withstand physical stress but still be able to disintegrate rapid enough to comply with disintegration specifications.

7.4 Diameter

Table 7.9: Average tablet diameter formula 1 (mm).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	16.19; 0.05% SD	16.25; 0.09% SD	16.24; 0.07% SD	16.23; 0.06% SD
30°C; 60%	16.19; 0.05% SD	16.26; 0.06% SD	16.27; 0.06% SD	16.26; 0.06% SD
40°C; 75%	16.19; 0.05% SD	16.29; 0.08% SD	16.31; 0.07% SD	16.31; 0.07% SD

Table 7.10: Average tablet diameter formula 2 (mm).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	16.21; 0.08% SD	16.25; 0.07% SD	16.26; 0.07% SD	16.23; 0.06% SD
30°C; 60%	16.21; 0.08% SD	16.25; 0.07% SD	16.28; 0.06% SD	16.26; 0.05% SD
40°C; 75%	16.21; 0.08% SD	16.27; 0.05% SD	16.31; 0.04% SD	16.32; 0.08% SD

Table 7.11: Average tablet diameter formula 3 (mm).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	16.19; 0.12% SD	16.23; 0.06% SD	16.25; 0.08% SD	16.22; 0.07% SD
30°C; 60%	16.19; 0.12% SD	16.26; 0.08% SD	16.26; 0.07% SD	16.26; 0.07% SD
40°C; 75%	16.19; 0.12% SD	16.27; 0.18% SD	16.34; 0.08% SD	16.35; 0.08% SD

Table 7.12: Average tablet diameter formula 4 (mm).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	16.18; 0.09% SD	16.23; 0.08% SD	16.23; 0.09% SD	16.23; 0.12% SD
30°C; 60%	16.18; 0.09% SD	16.24; 0.13% SD	16.26; 0.06% SD	16.26; 0.07% SD
40°C; 75%	16.18; 0.09% SD	16.26; 0.07% SD	16.32; 0.07% SD	16.33; 0.11% SD

A mean increase in diameter from 0 to 12 weeks can be seen in all formulas.

An increase in diameter with increased stress conditions can also be observed.

The largest increase in mean diameter can be seen for al formulas at 40°C; 75% than any other temperature or humidity (Table 7.9 – 7.12). This increase in diameter is due to expansion of the microcrystalline cellulose (disintegrant) in the structure of the tablet when exposed to high levels of humidity.

7.5 Thickness

Table 7.13: Average tablet thickness formula 1(mm).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	4.11; 0.44% SD	4.22; 0.17% SD	4.22; 0.30% SD	4.21; 0.28% SD
30°C; 60%	4.11; 0.44% SD	4.23; 0.14% SD	4.24; 0.26% SD	4.25; 0.32% SD
40°C; 75%	4.11; 0.44% SD	4.27; 0.36% SD	4.28; 0.35% SD	4.30; 0.36% SD

Table 7.14: Average tablet thickness formula 2(mm).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	4.08; 0.66% SD	4.18; 0.37% SD	4.18; 0.44% SD	4.17; 0.45% SD
30°C; 60%	4.08; 0.66% SD	4.17; 0.26% SD	4.21; 0.32% SD	4.21; 0.30% SD
40°C; 75%	4.08; 0.66% SD	4.22; 0.50% SD	4.26; 0.43% SD	4.27; 0.32% SD

Table 7.15: Average tablet thickness formula 3(mm).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	4.05; 0.51% SD	4.11; 0.36% SD	4.13; 0.19% SD	4.13; 0.29% SD
30°C; 60%	4.05; 0.51% SD	4.15; 0.49% SD	4.15; 0.49% SD	4.16; 0.34% SD
40°C; 75%	4.05; 0.51% SD	4.19; 0.61% SD	4.25; 0.44% SD	4.27; 0.34% SD

Table 7.16: Average tablet thickness formula 4(mm).

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	4.07; 0.52% SD	4.12; 1.38% SD	4.02; 2.54% SD	4.10; 3.10% SD
30°C; 60%	4.07; 0.52% SD	4.14; 0.62% SD	4.18; 0.66% SD	4.18; 0.43% SD
40°C; 75%	4.07; 0.52% SD	4.19; 0.26% SD	4.25; 0.63% SD	4.28; 0.70% SD

A mean increase in thickness from 0 to 12 weeks can be seen in all formulas.

An increase in thickness with increased stress conditions can also be observed.

The largest increase in mean thickness can be seen for al formulas at 40°C; 75% RH than any other temperature or humidity (Table 7.13 – 7.16). This increase in thickness is due to expansion of the microcrystalline cellulose (disintegrant) in the structure of the tablet when exposed to high levels of humidity.

This increase in the overall size of the tablet is caused by the hydration of the microcrystalline cellulose and other dissintegrants resulting in partial swelling of the tablet (Carstensen *et al.* 2000:296).

The increase in size is minimal and can not be detected with the unaided eye. A slight increase in size does not pose a problem to stability or patient compliance as the tablet is not swallowed whole by the patient.

7.6 Friability

Friability testing was performed on all the batches of tablets and at all stress conditions. The objective during friability testing is to determine the physical stability of the tablets when exposed to mechanical stress procedures.

Table 7.17: Amount of tablet mass (%) lost due to friability for formula 1.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	0.78	0.97	1.12	1.25
30°C; 60%	0.78	1.09	1.85	1.46
40°C; 75%	0.78	1.29	2.05	1.67

Table 7.18: Amount of tablet mass (%) lost due to friability for formula 2.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	1.22	1.05	1.48	1.47
30°C; 60%	1.22	1.34	1.87	1.52
40°C; 75%	1.22	1.39	2.3	1.81

Table 7.19: Amount of tablet mass (%) lost due to friability for formula 3.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	1.53	1.28	1.69	1.63
30°C; 60%	1.53	1.3	1.97	1.8
40°C; 75%	1.53	2.05	3	2.66

Table 7.20: Amount of tablet mass (%) lost due to friability for formula 4.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	1.8	1.54	2.09	1.61
30°C; 60%	1.8	2.1	2.91	2.02
40°C; 75%	1.8	1.66	3.19	2.68

A mean increase in loss from 0 to 12 weeks can be seen in all formulas.

An increase in loss with increased stress conditions can also be observed.

The largest increase in mean loss can be seen for al formulas at 40°C; 75% RH than any other temperature or humidity (Table 7.17 – 7.20).

A possible explanation for an increase in diameter, thickness and friability may possibly be explained again by hydration or expansion of binder/disintegrant particles (microcrystalline cellulose) in the tablet matrix when exposed to increased levels of humidity (Carstensen *et al.* 2000:296).

The pharmacopoeia specifies a maximum loss of 1% during friability testing (BP, 2005) (USP, 2005).

During friability testing a loss of more than 3% were recorded for formula 4 at 12 weeks at the highest temperature. This loss may again be attributed to hydration of certain tablet excipients. A second factor that might have influenced the friability occurred during the manufacture of the tablets. The tablets were manufactured in a single tablet press with a punch diameter of 16mm. The punch surface was flat and the corners were not edged to produce a tablet with rounded corners. These sharp corners of the tablets were easily damaged and caused an increase in the loss during friability.
A possible solution for the friability problem may be to use edged or bevelled punches or coating the tablet after manufacture. The easiest approach would be to pack the tablets in such a way to minimize mechanical stress during transport.

7.7 Uniformity of mass

Uniformity of mass is performed on a batch of tablets to determine whether the tabletting procedure produced a batch of tablets that contained an identical amount of tablet powder in each tablet.

Table 7.21: Average tablet mass (g) formula 1.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	1.0411; 1.09% SD	1.0413; 1.61% SD	1.0464; 1.72% SD	1.0510; 0.85% SD
30°C; 60%	1.0411; 1.09% SD	1.0381; 0.95% SD	1.0509; 1.25% SD	1.0450; 1.14% SD
40°C; 75%	1.0411; 1.09% SD	1.0349; 1.18% SD	1.0453; 1.10% SD	1.0448; 1.27% SD

Table 7.22: Average tablet mass (g) formula 2.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	1.0127; 1.36% SD	1.0186; 1.49% SD	1.0219; 1.71% SD	1.0325; 1.53% SD
30°C; 60%	1.0127; 1.36% SD	1.0295; 1.25% SD	1.0264; 1.01% SD	1.0343; 1.37% SD
40°C; 75%	1.0127; 1.36% SD	1.0358; 1.03% SD	1.0415; 0.46% SD	1.0455; 1.36% SD

Table 7.23: Average tablet mass (g) formula 3.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	1.0054; 2.51% SD	1.0198; 1.45% SD	1.0139; 1.66% SD	1.0177; 1.49% SD
30°C; 60%	1.0054; 2.51% SD	1.0119; 1.93% SD	1.0097; 1.72% SD	1.0109; 1.28% SD
40°C; 75%	1.0054; 2.51% SD	1.0122; 1.08% SD	1.0111; 1.07% SD	1.0066; 1.82% SD

Table 7.24: Average tablet mass (g) formula 4.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	1.1128; 26.42% SD	1.0031; 2.16% SD	1.0051; 2.46% SD	1.0095; 4.17% SD
30°C; 60%	1.1128; 26.42% SD	1.0058; 0.81% SD	1.0257; 2.52% SD	1.0127; 1.60% SD
40°C; 75%	1.1128; 26.42% SD	1.0182; 1.72% SD	1.0174; 2.01% SD	1.0276; 3.03% SD

The average masses of the all the batches remained relatively constant at increased stress conditions and at different intervals (Table 7.21 – 7.24).

No large losses or gain in mass could be detected during uniformity of mass.

A slight mechanical problem with the parameters of the tablet press did occur during the manufacture of formula 4. Tablet formulas 1 to 3 required the same adjustments to the tablet press. Formula 4 was different and the formulator had to make repeated adjustments to achieve a reproducible product that complied with standards.

7.8 Loss on drying

Loss of moisture content during drying is performed to examine the amount of moisture (water) that was absorbed by a tablet during stability testing under increased temperature and humidity conditions.

The amount of moisture present in a tablet is important to ensure a stable product. Too little moisture in a tablet may cause it to be brittle and unable to withstand mechanical stress. Too much moisture may result in chemical decomposition of drugs or enable microbiological growth.

Drying in an oven was used for the tablets as the tablets contained organic excipients that may possibly react with the reactants if submitted to Karl Fisher titration.

An amount of ± 1g of tablet powder was submitted to drying at 105°C for 5 hours to ensure sufficient drying without decomposition of any of the tablet excipients.

Table 7.25: Amount of tablet moisture (%) lost due to drying formula 1.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	5.00	5.08	4.94	4.61
30°C; 60%	5.00	5.08	5.18	4.88
40°C; 75%	5.00	5.26	5.15	4.96

Table 7.26: Amount of tablet moisture (%) lost due to drying formula 2.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	4.76	5.08	4.83	4.52
30°C; 60%	4.76	4.95	5.02	4.71
40°C; 75%	4.76	4.74	4.96	4.88

Table 7.27: Amount of tablet moisture (%) lost due to drying formula 3.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	4.53	4.72	4.63	4.27
30°C; 60%	4.53	4.80	4.64	4.48
40°C; 75%	4.53	4.70	4.82	4.47

Table 7.28: Amount of tablet moisture (%) lost due to drying formula 4.

Temperature & % Relative Humidity	0 Weeks	4 Weeks	8 Weeks	12 Weeks
25°C; 60%	3.67	3.78	3.84	3.52
30°C; 60%	3.67	3.78	3.93	3.68
40°C; 75%	3.67	3.57	3.86	3.67

No significant absorption of moisture could be detected in any one formulation during exposure to stress conditions over the 12 week period at any of the stress conditions.

An increase in the amount of moisture for formula 4 down to 1 can be seen, with formula 1 containing the most moisture of al the formulas (Table 7.25 – 7.28).

The formulations containing the most amounts of excipients gained the most amount of moisture. This gain in moisture was the result of the microcristaline cellulose, this excipient that was most abundant in formula one, absorbed moisture from the atmosphere resulting in a mass gain of the tablets.

A mass gain was also recorded with increased humidity during the stability testing. The formulas that were exposed to the higher humidities gained more moisture than those exposed to the lower humidities.

7.9 Dissolution

Disintegration is when a tablet breaks into its main components when introduced to a liquid (BP, 2005:A268).

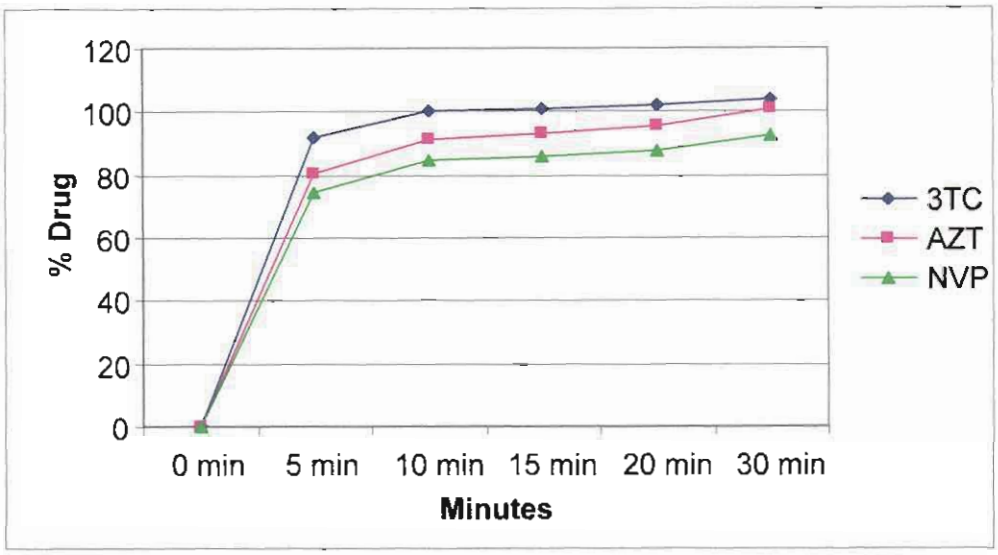
Dissolution is when particles of drug inside a solution dissolve to form a drug solution (BP, 2005:A272).

The dissolution procedure can be used as an indication of the disintegration as well as the dissolution tempo of a tablet. Dispersible tablets have to disintegrate very rapidly to ensure the patient is able to consume the solution easily.

Dissolution tests were performed only on formula 4. Formula 4 contained the most amounts of drugs and least amount of disintegrants. Due to the difficulty of nevirapine to dissolve in watery mediums at high concentrations only formula 4 was used for comparative dissolution testing.

Dissolution parameters:

- Dissolution medium: 900ml 0.1M HCl in purified water per vessel and pH adjusted to 2
- Dissolution apparatus: VanKel 7000^{TM}; Paddles; 50 rpm; 37°C ± 0.5
- Extraction times: 5; 10; 15; 20 and 30 minutes



Graph 7.2: Initial (0 Weeks) dissolution results for tablet formula 4.

All the tablets disintegrated within 1 minute after introduction into the vessels during initial dissolution testing.

At 5 minutes more than 70% of the NVP was dissolved and more than 80% of the 3TC and AZT were dissolved (Graph 7.2).

More than 90% of all the drugs were dissolved after 30 minutes (Graph 7.2).

Table 7.30: Dissolution results at 4 weeks of stability testing (Formula 4).

3TC	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	78.51	89.66	99.4	102.84	105.15
30°C;60% RH	95.94	106.26	109.58	110.67	112.48
40°C;75% RH	88.23	98.3	102.7	104.78	105.86

AZT	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	74.34	86.25	96.57	100.8	102.58
30°C;60% RH	44.48	98.6	103.49	107.66	110.21
40°C;75% RH	80.06	92.95	97.19	99.86	102.32

NVP	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	69.6	79.74	88.89	92.57	93.25
30°C;60% RH	81.09	90.21	94.4	96.81	99.47
40°C;75% RH	75.36	86.84	90.92	93.61	97.02

At 4 weeks all the tablets disintegrated within 1 minute after introduction into the vessels.

At 5 minutes more than a half of all the drugs was dissolved and at 30 minutes more than 90% of the drugs was dissolved (Table 7.30).

Table 7.31: Dissolution results at 8 weeks of stability testing (Formula 4).

3TC	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	71.72	88.94	95.28	101.64	100.53
30°C;60% RH	85.27	95.15	97.67	98.81	99.65
40°C;75% RH	80.8	97.8	100.15	100.85	101.33

AZT	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	69.28	88.45	97.32	106.04	106.98
30°C;60% RH	79.66	90.47	95.88	98.05	100.05
40°C;75% RH	76.29	87.44	96.67	98.84	99.95

NVP	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	62.32	79.12	85.04	89.6	91.21
30°C;60% RH	73.66	83.01	86.93	88.94	90.96
40°C;75% RH	69.21	85.04	88.88	90.68	92.42

At 5 minutes more than a 60% of all the drugs was dissolved and at 30 minutes more than 90% of the drugs was dissolved (Table 7.31).

Table 7.32: Dissolution results at 12 weeks of stability testing (Formula 4).

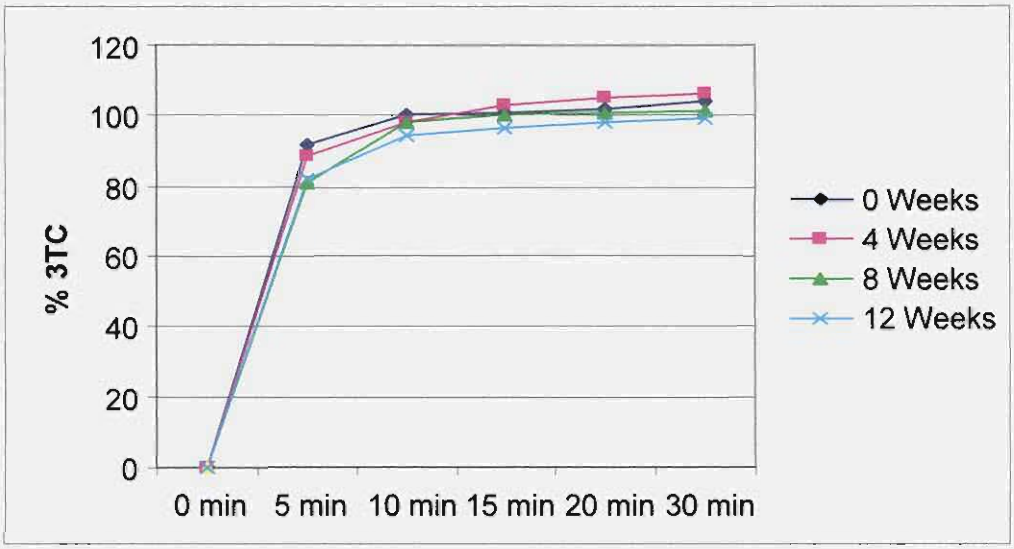
3TC	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	87.69	93.81	96.45	97.7	99.09
30°C;60% RH	92.12	98.41	99.57	100.17	100.91
40°C;75% RH	81.97	94.04	96.35	97.93	99.02

AZT	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	80.07	89.42	92.7	94.84	97.49
30°C;60% RH	81.2	88.9	91.73	93.44	95.8
40°C;75% RH	75.46	88.18	92.22	94.92	97.28

NVP	5 min	10 min	15 min	20 min	30 min
25°C;60% RH	74.81	82.69	85.81	87.78	90.86
30°C;60% RH	77.77	85.78	88.14	89.41	91.58
40°C;75% RH	72.33	83.09	86.7	89.34	91.19

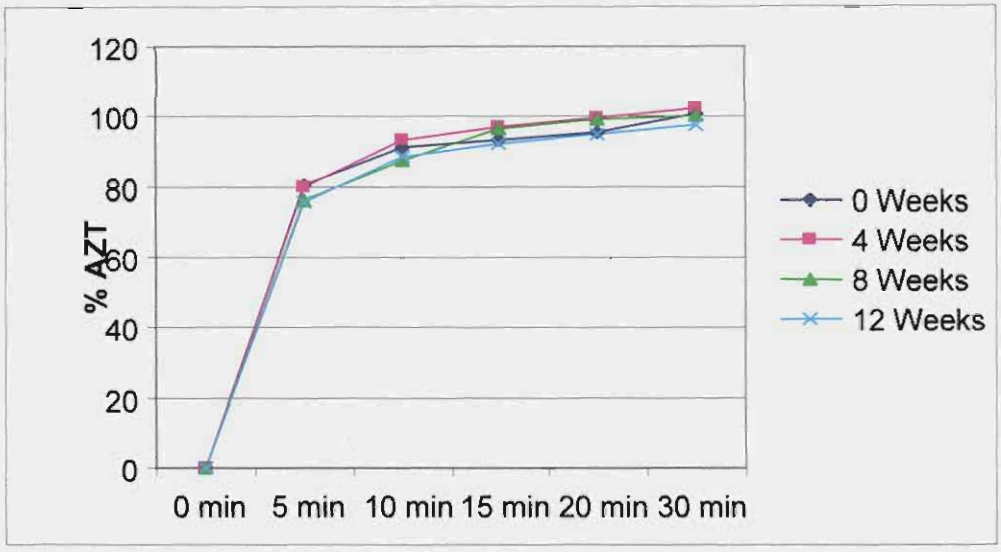
At 12 weeks all the tablets disintegrated within 1 minute after introduction into the vessels and no decrease in disintegration time could be seen.

At 5 minutes more than a 70% of all the drugs was dissolved and at 30 minutes more than 90% of the drugs was dissolved (Table 7.32).



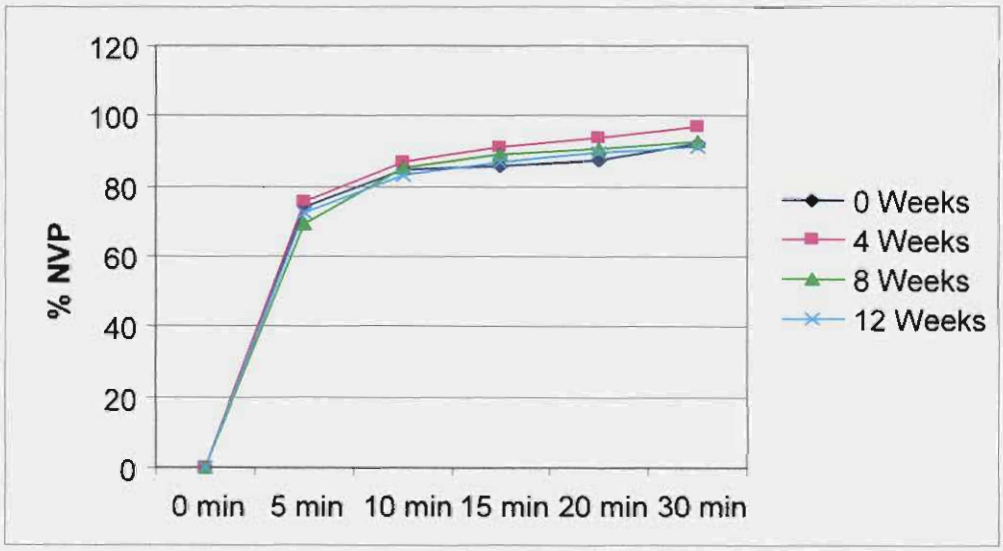
Graph 7.3: Drug release of lamivudine at 40°C; 75% RH for 0 to 12 weeks stability testing (Formula 4).

The dissolution rate of lamivudine during the stability testing seems to remain the same. A slight decrease in the dissolution rate can be seen at 12 weeks (Graph 7.3). The decrease in dissolution rate may be explained by a possible decrease in disintegration rate.



Graph 7.4: Drug release of zidovudine at 40°C; 75% RH for 0 to 12 weeks stability testing (Formula 4).

The dissolution rate of zidovudine during the stability testing remains the same and no increase or decrease in dissolution rate could be seen (Graph 7.4).



Graph 7.5: Drug release of nevirapine at 40°C; 75% RH for 0 to 12 weeks stability testing (Formula 4).

The dissolution rate of nevirapine during the stability testing remains the same and no increase or decrease in dissolution rate could be seen (Graph 7.5).

Not one of the formulations revealed any drug degradation or increase/decrease in drug release rate during stability testing. All the formulations disintegrated within 1 minute and dissolved to more than 90% within 30 minutes.

All formulations comply with standards determined by the pharmacopoeia and proved to be stable during stability testing.

7.10 Summary

Assay

All four of the tablet formulations were stable during stability testing. Assay results were within limits set by the pharmacopoeia (BP, 2005) (USP, 2005).

Hardness

All the batches were hard enough to comply with these standards. In some of the formulas there seem to be a decrease in hardness during stability testing. This decrease in hardness may be the result of hydration of the microcrystalline cellulose (Carstensen *et al.* 2000:296).

Diameter and thickness

A slight increase in diameter and thickness were observed during stability testing. This increase was the most at the higher temperatures and humidities.

Friability

Friability seems to pose a problem during stability testing of the tablets. Some of the formulations did not comply with pharmacopoeial standards.

Uniformity of mass

All the tablet formulas complied with the standards set by the pharmacopoeia (BP, 2005) (USP, 2005).

Loss on drying

Loss during drying testing did not reveal a gain or loss of moisture due to stability testing. Though an initial increase in moisture was recorded no mean increase can be seen.

Dissolution

More than 90% of all the drugs dissolved within 30 minutes of dissolution testing. All the formulations comply with specifications determined by the pharmacopoeia (BP, 2005) (USP, 2005).

Chapter 8

Conclusions

8.1 Reason for development of formulation

Many formulations of anti retroviral medication are available or being developed (Arora, 2003:2). Few of these formulations are suitable for developing countries. Most of the developing countries are situated in rural areas and subjected to extreme climatic conditions e.g. Africa, South-America and Southern Asia (UNICEF, 2004:7).

Most anti retroviral medications are unaffordable to patients in these countries. Patients using these medications may be from disadvantaged communities with little formal education. Patients that are fortunate enough to receive medication in many cases struggle with the side effects and adherence to a large amount of regimens to be taken daily. Children are even more severely affected by the pandemic. If adherence to treatment is difficult for adult patients then one may ask in what situation are the children in these countries (UNICEF, 2004:4)?

Therefore the formulation of more affordable generic anti retrovirals that are suitable for conditions in these countries is essential. A triple-drug, affordable, robust formulation, easy to administer, with long dosage intervals and with proven effectiveness are required (UNICEF, 2004:4) (Pujari *et al.*, 2003:5).

Formulations adhering to these properties are being developed in India. The triple-drug combination 3TC/AZT/NVP has already undergone large clinical trials in India and has been found effective during HAART. Generic companies in India are developing these and similar triple-drug regimens (Pujari *et al.*, 2003:5).

The development of more effective, user friendly and most important quality regimens can not be overemphasized.

Dispersible or chewable dosage forms are ideal in this situation. These formulations are stable in extreme climates, easy to administer, affordable, light and compact to transport to developing countries (UNICEF, 2004:7).

8.2 Drugs and excipients used in the formulations

Most pharmaceutical formulations contain inactive excipients in combination with the active substances. The reason for the inclusion of excipients is obvious: to improve the formulation characteristics (Lund, 1994:192).

Firstly the inclusion of more than one active substance may pose a problem. Active substances may undergo chemical reactions when combined in a formulation. These substances may antagonize each others effects, not necessarily pharmacological but in other biological ways (Carstensen, 1998:239).

Secondly the inclusion of excipients in combination with each other and active substances may pose a problem. Some excipients may react chemically with each other or the active substances. Some excipients may have a biological effect or reduce the biological effects of the active substances (Carstensen, 1998:239).

All the drugs and excipients used in the formulation must improve the regimen to produce a product with the required characteristics (Carstensen, 1998:239).

Excipients can not be included only for esthetical value, this is especially important for formulations destined for developing countries. The addition of unnecessary or costly excipients is expensive and may produce a product that is not suitable for the developing countries.

Therefore the selection of excipients used in this formulation is materials used the world over. All the excipients are easily obtainable at an affordable cost from different manufacturers. Some of the materials may not be ideal for the formulation e.g. artificial sweeteners. Inclusion of these materials may be justified by the unique situation in which the regimens will be distributed.

The specific excipient selection was included in the formulation to produce a tablet that can be used either as a dispersible or a chewable tablet. Most of the excipients included are ideal; producing a product with the necessary pharmacopoeial characteristics, quality and stability in extreme climatic conditions.

8.3 Preformulation

Preformulation studies usually refer to the studies performed on a new chemical entity. In this context some of the procedures used during the study of a new chemical entity do overlap with procedures performed to produce a new dosage form of an existing chemical entity (Steele, 2004:175).

In this context preformulation refers to the study of the physico-chemical compatibility of active substances and excipients in the new formulation. During the preformulation study test procedures used during quality and stability testing are validated (Steele, 2004:175).

Incompatibilities between all drugs and excipients were observed during the preformulation DSC studies. A HPLC method for the quantification of the three drugs in the dosage forms during assay and dissolution testing was developed and validated.

8.4 Formulation

The formulation process is the shortest but most critical during the production of the dosage form. Any problems during the production of the dosage form will severely affect test results performed on it thereafter (Davies, 2004:432).

The production process of direct compressed tablets consists of three steps. Firstly the drugs and excipients sizes must be reduced (milling). This step is only necessary if the particle sizes of the materials used are too big. Secondly the drugs and excipients must be mixed in the right amounts and added in the right order. Lastly the tablet powder must be compressed by a suitable tablet press (Davies, 2004:432).

Four formulations were formulated during this study. The reason for formulating four formulations are to produce a range of regimens suitable for a whole population e.g. infants, children, adolescents and adults. Another reason is that formulation problems with one or more of the formulations may easily be detected when comparing it with the rest of the formulations.

Formulations 1 to 3 did not pose any severe problems. The tablet masses varied within 5% of the theoretical tablet mass. Content uniformity and physical characteristics were also within specifications and ideal.

Formulation 4 did pose a problem. The tablet masses varied within 10% of the theoretical mass. The content uniformity did not pose a problem but physical characteristics did vary. A possible reason for this variation in tablet mass may be attributed to incorrect adjustment of the tablet press. Unfortunately, the only tablet press available at the time of this study was an ancient machine with worn out setting adjustors. This had a negative impact on the study that was beyond the control of the student.
Problems were encountered with the adjustment of the apparatus during the production of the fourth formula.

Although the fourth formula posed some problems the rest of the formulations complied with standards and good results were obtained from these formulations.

8.5 Test results

Formulation 1 to 3 complied with pharmacopoeial standards for dispersible and chewable tablets during initial tablet testing. Initial test results were used as a baseline for any changes occurring during stability procedures. The remaining tablets were packed and labelled in PVC containers and placed in stability chambers at different conditions for up to 12 weeks. Samples were removed at 4, 8 and 12 weeks and tested. Stability testing was performed to determine the physical stability characteristics of the different formulations rather than the degradation or shelf life characteristics of the formulations and drugs.

The following tests were performed on all the formulations at 0, 4, 8 and 12 weeks of stability testing:

- Description of tablet characteristics
- Assay testing of active substances
- Mass variation of formulations
- Tablet dimensions and hardness

- Friability
- Loss of moisture content during drying
- Drug release rate – Dissolution testing

8.5.1 Tablet characteristics

No severe visual changes were detected in any of the formulations. A slight discolouration from off-white to yellow occurred in all the formulations from 0 to 12 weeks of stability testing. This discolouration was barely visible with the unaided eye. No other visual changes e.g. tablet surface, size or volume of tablets occurred during the stability procedure.

Deterioration in the odour of the flavourings did occur. The formulations were initially very aromatic; to such extent that the formulation laboratory smelled like a confectionary factory. Unfortunately the aroma slowly deteriorated during stability testing and after 12 weeks in stability the tablets tasted like flavoured paper.

Fortunately the artificial sweeteners did not fade during stability testing. The stability of xylitol to microbial decomposition may pose a future problem as it is a natural sweetener that may act as a medium for microbial growth.

Microcrystalline cellulose is not nearly an ideal excipient for chewable tablets as it has the same consistency as cardboard and leaves a dry feeling in the mouth. It is for this reason that xylitol was included; with little success. Microbial degradation of microcrystalline cellulose may also pose a future problem as no preservatives were added in any of the formulations.

Another concern is the bitter taste of the nevirapine. Masking of the bitter taste is very difficult in this situation. Addition of flavourings cannot completely mask the taste and an alternative approach may be necessary. An alternative approach may be the use of an anion exchange resin. Incorporation of nevirapine in the resin and the resin-complex in the formulation may produce a formulation without a bitter taste (Khan, 1976:1).

Although the formulation comply with visual standards a lot of changes in the palatability and microbial stability have to be done before any of the formulations are suitable to ensure patient compliance.

8.5.2 Assay testing

All the formulations initial assay tests complied with standards in the pharmacopoeia. Compliance with these standards does not necessarily ensure that the formulations have the preferred bioavailability *in vivo*. Furthermore the specifications of the pharmacopoeia are only guidelines to the preferred limits of a regimen.

The limits achieved during this study were not nearly stringent enough to determine if any decomposition of the active substances occurred during stability testing. A relative standard deviation of < 1% in content uniformity was not achieved.

The reason for assay testing during the procedure was for short term degradation or shelf-life determination of the product. Effective degradation studies may require long term stability testing.

This deviation of contents may be due to two unfortunate events during the formulation procedure:

- Insufficient mixing procedures of the tablet powder
- Demixing during tabletting

Assay was performed on all the formulations in order to determine if formulation problems occurred and to determine if any physico-chemical interactions were present within the formulation.

No interactions between any of the drugs or excipients were detected during stability assay determination.

8.5.3 Mass variation

According to pharmacopoeial specifications mass variation is determined for tablet formulations containing more than a specified amount of active substances.

During this study mass variation testing were performed to determine if any changes in physical characteristics during stability testing occurred. Possible absorption or loss of moisture may be detected during this procedure. This procedure can be utilized in conjunction with loss of volatile substances during drying and possible verification of data may be achieved.

Fortunately no significant increase or decrease in mean tablet mass in any of the formulations could be detected. A possible reason for this may be that the moisture absorption was so small that it was undetectable against the deviation in tablet masses. According to loss during drying an increase of moisture loss did occur (<1%).

8.5.4 Dimensions

Changes in the dimensions of the formulations (thickness and diameter) may be due to moisture loss or absorption. Measurements were taken automatically during determination of tablet hardness.

Measurements were < 1 mm and possibility of inaccurate measurements was a reality. Small tablet particles on the surface of the tablet could possibly interfere with the measurements. Care was taken to ensure that no excessive particles were present on the tablet surface before measurements were taken.

Small increases in the mean diameter of some of the formulations were detected during stability testing (< 0.2 mm). Increases were not severe to be detected by the unaided eye.

A small mean increase in thickness of the formulations was also detected during stability testing (< 0.3 mm). The increase in thickness is larger than the increase in diameter when the results are expressed as a fraction of the initial tablet dimensions.

Changes in the structure of the tablets did occur during the stability testing procedure. Increases in the tablet dimensions may possibly be attributed to moisture absorption.

Fortunately the changes in tablet dimensions are barely detectable and seem to be insignificant to this study.

8.5.5 Hardness

Pharmacopoeial specifications for tablet hardness indicate that tablets have to be hard enough to withstand the maximum amount of mechanical stress and still be able to comply with other specifications. Pharmacopoeial guidelines do not specify numerical values for tablet hardness.

During the manufacturing process of tablets a few factors may determine the hardness of the product for example:

1. Compliance to pharmacopoeial specifications, especially dissolution e.g. tablets that are too hard may fail to disintegrate.
2. Tablet press wear e.g. the harder a tablet is pressed the more wearing will occur on the apparatus reducing its lifespan.
3. Type of tablet e.g. chewable tablets will be formulated to be softer to ensure the patient is able to chew it easily.

Taking all the factors into account it may sometimes be difficult to formulate a tablet that will comply with all these conditions.

All the tablets that were formulated were hard enough to withstand a moderate amount of stress without breakage or deformation. Results were verified by friability testing where none of the tablets 'capped' and the maximum loss was < 3% per tablet. Though the tablets were hard enough, all of the formulations disintegrated completely within 1 minute after introduction into water.

A mean decrease in tablet hardness was observed in all formulations during stability testing. A nearly linear decrease was observed in formula 3 at all conditions over the 12 week period. The decrease in hardness was not too severe to negatively influence the formulation characteristics.

A correlation between hardness and friability can be observed. The harder formulations (1 and 2) is less prone to friability than the softer formulations (3 and 4).

8.5.6 Friability

Determination of the loss of tablet mass by means of mechanical stress (friability) is part of the hardness testing procedure. Loss of not more than 1% of most tablets during friability is accepted as ideal by pharmacopoeial specifications.

A loss of less than 1% was not achieved during the tests.

Dispersible or chewable formulations are formulated 'softer' than regular oral tablets. These formulations are also larger than tablets that have to be swallowed whole. This two factors result in tablets that are more prone to friability than normal oral tablets.

A third problem that attribute to an increased friability was the shape of the punch used during the manufacturing process.

An increase in loss due to friability was detected in all formulations during increased stability conditions.

An increase in loss due to friability was detected within formulations. A maximum loss of 3% was determined for formula 4 with a decrease down to formula 1.

Increased stress conditions had a negative effect on the hardness of all the formulations. Formulations containing a higher fraction of drugs in the formula revealed a larger loss due to friability.

8.5.7 Loss of moisture

Moisture content of the formulations was determined by drying. The formulations did not contain any volatile substances other than water. Water content of the formulations was determined by drying known quantities of tablet powder at a specified temperature for a certain time interval. The mass difference of the dried powder was expressed as a fraction of the initial powder mass.

Chemical titration for determining the water content was not performed. The reasons for determining the water content by loss during drying is the following:

- None of the formulations contained any volatile solvents e.g. ethanol.
- A natural sweetener and flavourings were present in all the formulations and possible interference of the titration agents may pose a problem.
- All the formulations were stable at the drying temperature ensuring that only water loss was determined.
- All the samples were analysed at the same time interval, climatic conditions e.g. humidity conditions was the same for all the samples.
- Ease of operation and the ability to analyze more than one sample simultaneously.

All the formulations had a moisture loss of < 5%. An increased loss in moisture content was detected.

A linear increase in loss of moisture content during stability testing was detected in certain formulations with increased stability conditions.

No conclusive linear increases in moisture loss were detected in any of the formulations during stability testing at a given stability condition.

Increasing moisture loss at higher stability conditions indicates that moisture was absorbed by the formulations during the process and that the rate of absorption was dependant on the stability conditions.

8.5.8 Dissolution

Dissolution testing was performed on formulation 4 during stability testing.

Multi-point dissolution testing was performed to determine the release rate of the drugs during the stability procedure.

The difference between the dissolution testing performed during quality control and stability testing is the amount of sample extractions.

During quality control very few sample extractions are usually made for the time interval of the dissolution test. Pharmacopoeial specifications usually specify that not less than 80% (Zidovudine, USP 2005:2050) of the active substance dissolve within 30 minutes.

During stability testing more than one sample extractions are necessary. The objective of dissolution during stability testing is to determine the release rate of the drugs. To determine the release rate it is necessary to gather an amount of data points that is statistically significant.

Five data points were determined during the dissolution procedure. A time interval of 30 minutes was used to correlate with the time interval for dissolution of Zidovudine tablets (USP, 2005:2050). A time interval of more than 30 minutes would be unnecessary due to the rapid disintegration rate of the tablets.

All the formulations disintegrated within 1 minute of introduction into the dissolution medium.

Samples were withdrawn after 5, 10, 15, 20 and 30 minutes. Drug concentrations were more than 90% for all drugs, at all conditions after 30 minutes.

No conclusive changes in drug release rate were observed for any drugs at any of the stability conditions.

8.6 Conclusion

The dosage form developed during the study adheres to guidelines set by the World Health Organization concerning the type and amount of drugs. In most instances this single regimen can replace a variety of dosage forms currently taken on a daily basis by a variety of patients. The tablets can be dispersed in a small amount of water e.g. 20 ml for infants and children. Due to the stability and reduced mass the dosage forms will possibly be more suited to socio-economic and climatic conditions in the developing countries. The tablets comply with most of the standards of the different pharmacopoeias.

ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AZT	Azidothymidine, Zidovudine, ZDV
CDC	Centre for disease control
CNS	Central nerve system
CYP450	Cytochrome P450
dCTP	Deoxycytidine-triphospate
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
FDC	Fixed dose combination
GI	Gastro intestinal
HAART	Highly active anti retroviral treatment
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
LC	See HPLC
MSDS	Material safety data sheet
NaOH	Sodium hydroxide
NVP	Nevirapine
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Nonnucleoside reverse transcriptase inhibitor
OPV	Oral polio vaccine
PCP	Pneumocystis carinii pneumonia
PI	Protease inhibitor
PML	Multifocal leucoencephalopathy
RH	Relative humidity
RNA	Ribonucleic acid
RT	Reverse transcriptase
RTI	Reverse trancriptase inhibitor
SIV	Simian immunodeficiency virus
SOP	Standard operating procedure
TB	Tuberculosis
UNICEF	United Nations children fund
URTI	Upper respiratory tract infection
WHO	World health organization

ZDV	See AZT
3TC	Lamivudine

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Annexure A: Poster presented at 26th Annual Conference of the Academy of Pharmaceutical Sciences at Nelson Mandela Metropolitan University, Port Elizabeth, 29 September to 1 October 2005.

The formulation and stability of novel dosage forms of anti retroviral drugs

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Purpose

Formulation and stability testing of a triple-drug combination, anti-retroviral, dispersible tablet. Dosages of each drug has to comply to different weights or age groups[4] (Table 1).

Background

Lamivudine(3TC)[1] and Zidovudine (AZT)[2] are both nucleoside reverse transcriptase inhibitors (NRTI's), Nevirapine (NVP)[3] is a nonnucleoside reverse transcriptase inhibitor (NNRTI). All of these drugs are indicated for the treatment of AIDS in combination with other NNRTI, NRTI, or PI's. Many dual- and triple-drug formulations are currently available. Very few of these formulations are versatile and cost effective enough to comply to African conditions. Little stability data is available to support the effectiveness of the current formulations in these conditions. Formulations are not versatile enough to administer to a wide variety of age groups and patients with disabilities[4].

Methods

Possible formulations containing common pharmaceutical excipients were developed. 3TC, AZT, NVP and excipients were obtained from the suppliers. Compatibility studies were done on the drugs and excipients by means of DSC (Shimadzu DSC-50). Four different formulations were prepared by means of direct compression techniques. Initial USP and BP compliance studies were performed. Samples were packed in 250 ml white PVC jars at 25°C = 65%, 30 °C = 65% and 40 30 °C = 75% RH. At 0, 4, 8 and 12 weeks the samples were tested for mass uniformity, hardness, diameter, thickness (Pharma Test, typePTB 103, Switzerland), friability, loss on drying, assay and dissolution (HPLC, Hewlet Packard 1050). Dissolutions were performed at pH 1.2, due to the poor solubility of NVP at higher pH's. Both assays and dissolutions were analyzed quantitively by means of HPLC. None of the retention times interfered with each other (Figure 1).

Results

The tablets comply to the USP and BP specifications for dispersible tablets at 0, 4 and 8 weeks. Mass uniformity was between the limits ± 5 % for the theoretical mass (1000mg). Hardness was enough to ensure a minimum loss during friability (max 3 % loss on 4th formulae) but soft enough for the tablet to be used as either a chewable tablet and to allow the tablet to disperse within 1 minute in water. To reduce chipping of the sides, friability can be reduced by using beveled stamps in the tablet press (note sharp edges in Figure 2). Diameter and thickness increased (less than 1 %) at higher temperatures and humidity. Loss on drying were constant at all temperatures but increased inter formulae with the highest amount of loss in the formulae containing the most excipients and decreasing with the increase of active substances. Assay for all formulae were between 98-102 % of the labeled amount up to 8 weeks at all temperatures and humidity's. Dissolution were performed, not less than 80 % of all the drugs were dissolved within 30 minutes.

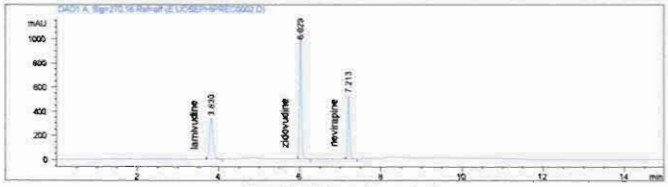

Figure 1: HPLC diagram of the retention times

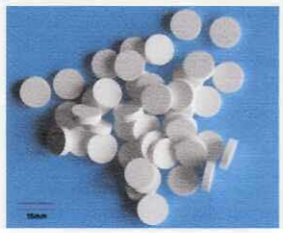

Figure 2: Image of the actual tablets. (Note: Diameter 16 mm, Thickness 4 mm)

Table 1: Amount of each drug per formulae (Note: 1 Tablet = 1000mg)

Formulae	mg 3TC/Tablet	mg NVP/Tablet	mg AZT/Tablet	Dose per weight or age [4]
1	10	20	0	3 - 6 Kg : 2 Tabs, 2 x day
2	15	37.5	25	6 -10 Kg : 2 Tabs, 2 x day
3	25	50	50	10 -15 Kg : 2 Tabs, 2 x day
4	75	100	150	15 - 29 Kg : 1Tab, 2 x day and Adults : 2 Tabs, 2 x day

Conclusion

Although the study have not been completed, all results indicate that the triple drug combination tablets are stable enough to ensure sufficient shelf life and compliance to specifications of the pharmacopoeia. The tablets comply to both chewable and dispersible specifications. The patient can therefore either chew or disperse the tablet according to his/her preference. The tablets cannot be prepared as a suspension for later use due to the lack of preservatives, buffers and suspending agents.

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Annexure B: Paper submitted for publication in Drug Development and Industrial Pharmacy.

The formulation and stability of dispersible or chewable tablets containing anti retroviral drugs

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Abstract

At present there are no single dosage form containing lamivudine, zidovudine and nevirapine. Such a combination is needed to fulfil the proposed dosage schedule of the WHO. Four dispersible/ chewable tablets with varying amounts of the actives were formulated. Preliminary accelerated stability tests indicate that the formulations should remain stable for at least 2 years. The tablets are easy to administer and ideal for compliance with the WHO dosage schedule.

1. Introduction

The acquired immunodeficiency syndrome (AIDS) epidemic is caused by the infection with the human immunodeficiency virus (HIV). HIV is a retrovirus of which the mature virions contain two single stranded RNA molecules surrounded by a nucleocapsid and an outer lipid envelope. Type HIV-1 is the main cause for infections worldwide although HIV-2 is the cause for infections in West Africa. Infection with HIV is characterised by viral replication and CD4 lymphocyte depletion which results in a profound immunodeficiency (Raffanti *et al.*, 2001:1349).

The end result of the immune deficiency is secondary infections primarily cancer-like Kaposi's sarcoma, tuberculosis, cryptosporidium (gastro-enteritis), herpes zoster, oral and skin lesions. Death occurs as a result of the opportunistic infections (Webb, 1997:4).

1.1 Nucleoside reverse transcriptase inhibitors

Nucleoside Reverse Transcriptase Inhibitors (NRTI's) were the first generation of anti retrovirals. Examples include: Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir and Emtricitabine (Wikipedia, 2006: NRTI's). Zidovudine (AZT) was initially approved as monotherapy. Subsequently it was approved in combination therapy with zalcitabine and lamivudine (3TC). Studies with AZT demonstrated clinical and survival benefits in patients with AIDS and those with symptomatic and asymptomatic HIV infection who had a CD4+ T-lymphocyte count of 500 cells per cubic millimetre or less. Limited clinical benefits were due to incomplete suppression of HIV replication and emergence of resistant strains. AZT is

currently approved for the treatment of HIV infection in combination regimens with PI, NRTI and NNRTI's. Combination regiments can achieve long term viral suppression with partial reconstitution of the immune system (Fischl, 2003:23).

1.2 Protease Inhibitors

Protease Inhibitors (PI's) were the second generation of anti retrovirals developed for the treatment of AIDS and Hepatitis. PI's prevent viral replication by inhibiting the activity of the enzyme (protease) used to cleave viral proteins before assembly of a new virion. Examples of PI's include: Saquinavir, Ritonavir, Indinavir, Nelfinavir, Amprenavir, Lopinavir, Atazanavir, Fosamprenavir and Tipranavir (Wikipedia, 2006: PI's).

1.3 Nonnucleoside reverse transcriptase inhibitors

Nonnucleoside Reverse Transcriptase Inhibitors (NNRTI's) were the third generation of anti retrovirals. Examples include: Nevirapine, Delavirdine and Efavirenz (Wikipedia, 2006: NRTI's).

An enormous variety and different combinations of anti retroviral drugs are commercially available. Due to the large variety of dosage forms in different strengths and drug combinations it is difficult for the primary health care professional to supply medication to the wide variety of patients e.g. infants to adults. Infants and children have different drug requirements compared to adults. Dosage regimens are usually calculated according to lean body weight or body surface area for children. The World Health Organization requires dosage regimens that can be easily administered to a variety of age groups. Lamivudine, zidovudine and nevirapine are all included in the WHO list for essential anti retrovirals. Anti retroviral drugs are offered in a variety of dosage forms and dosage strengths to suit most of the patient needs. Although paediatric dosage forms contain an amount of drug suitable for treatment of children, the administered dosage must still be calculated according to every patient's weight (UNICEF, 2004:2).

The single drugs can be combined with each other during Highly Active Anti Retroviral Therapy (HAART). The biggest problem with these combinations is patient compliance. Most patients find it very difficult to accept a combination of more than two dosage forms to be taken simultaneously. The WHO recommends the use of a combination of at least 3 anti retroviral drugs as predetermined by the country where applicable. Combination dosage forms are preferred if adequate safety and efficacy data are available for the combination dosage form (WHO, 2002:7).

Clinical trials have been performed successfully on patients with the triple combination of the three anti retrovirals, lamivudine, zidovudine and nevirapine in India (Pujari *et al.*, 2003).

Due to the proven effectiveness of lamivudine (3TC), nevirapine (NVP) and zidovudine (AZT) in combination therapy it was considered for inclusion in a new formulation. The objective of this study was to prove that the development of a dosage form containing all three of the above mentioned drugs was possible. Although drug regimens already exist containing the drugs in single and dual

combinations, none exist that contain all three. Stability data on the three drugs in a dosage form are not available and therefore special attention was given to the stability aspects of the drugs in the dosage form under development. Furthermore the new dosage form would have new characteristics making it more suitable for a wider group of patients and possibly solve some problems currently mentioned by UNICEF and WHO.

2. Experimental

2.1 Analytical apparatus

All procedures were performed in a controlled laboratory environment on verified analytical equipment supplied and serviced by the Research Institute for Industrial Pharmacy. Initial compatibility studies were performed by means of Differential Scanning Calorimetry (DSC) on a Mettler-Toledo DSC 822^e, Germany with auto sampler. Star$_e$ Software v. 9.00 Gmbh was used for sample analysis and Star$_e$ Software v. 9.00$_a$ Gmbh was used for data analysis. An Agilent 1100 series HPLC equipped with a gradient pump, auto sampler, diode array UV detector and Chemstation Rev. A.08.03 data acquisition and analysis software was used for all assay determinations. All analytical apparatus used for pharmacopoeial testing complied with BP, 2005 and USP, 2005 standards.

2.2 Materials

Analytical reagents Acetonitrile, Hydrochloric Acid, Methanol and Triethylamine were obtained from Merck and Sigma-Aldrich (South Africa) whilst lamivudine batch no.040801, zidovudine batch no.040903 was obtained from Xiamen Mchem Laboratories, China and Nevirapine batch no. 1001003 from Cipla, India. Tablet excipients were obtained from different manufacturers through the Research Institute of Industrial Pharmacy, North West University and subjected to their quality control procedures in compliance with pharmacopoeial standards.

2.3 DSC conditions

DSC was performed on single drugs, excipients and dual mixtures. Samples were weighed on a micro-balance (± 2 mg) into aluminium crucibles, capped and crimped. Each sample was analysed in a nitrogen atmosphere 50ml.min^{-1} and heating at 10°C.min^{-1}.

2.4 Chromatographic conditions

All HPLC experiments were performed on a Luna C18-2 column, 150 x 4.6 mm, 5µm (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile/ water with 0.2% triethylamine, pH adjusted to 7.0 with phosphoric acid or ammonium hydroxide. The mobile phase was filtered through a 0.45µm nylon membrane filter. Chromatography was performed at room temperature using a flow rate of 1 ml/min. A gradient of 8% acetonitrile from 0 to 1.5 minutes, then to 65% between 8 to 10 minutes and 8% after 10.10 minutes was used. Run time was set at 15 minutes. The volume of each injection was 10 µl. Eluents were monitored on photo-diode array detector at wavelength of 270 nm.

2.5 Standard preparation

Approximately 150 mg lamivudine, 200 mg nevirapine and 300 mg zidovudine were accurately weighed and dissolve in 50 ml methanol in a 100 ml volumetric flask with sonication for 10 minutes. The

solution was allowed to cool to room temperature and made up to volume with deionised water. 5 ml of this solution was diluted to 50 ml with deionised water and transferred into separate vials.

2.6 Sample preparation

Approximately 2 g of powdered tablet were accurately weighed into a 100 ml volumetric flask. The sample was dissolved in 50ml methanol by sonication for 10 minutes and made up to volume with deionised water (Assay) or 0.1M HCl (Dissolution). 5 ml of this solution was diluted to 50 ml with deionised water/ 0.1 M HCl. This solution was centrifuged for 10 minutes at 2500 rpm and the clear supernatant transferred into separate vials.

2.7 Method validation

The method was developed and validated in-house. Validation was performed according to ICH guidelines (ICH, 2005).

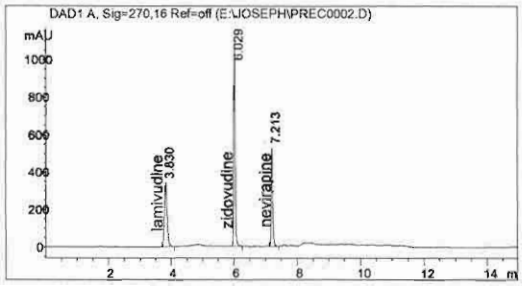

Figure 1: Chromatogram of a standard solution of lamivudine, zidovudine and nevirapine.

2.8 Tablet manufacture

Four different formulations were manufactured during the study. Each formulation's drug content correlates roughly to guidelines set by the WHO (Table 1). The tablets were manufactured with easily obtainable excipients and by means of direct compression techniques.

Table 1: Combination and strengths of anti retrovirals used in dispersible tablet formulations.

Formulation 1: 10 mg Lamivudine and 20 mg Nevirapine per tablet	Infants 3-6 kg two tablets twice daily dispersed in liquid. (WHO: 20 mg 3TC and 40 mg NVP BD)
Formulation 2: 15 mg Lamivudine, 37.5 mg Nevirapine and 25 mg Zidovudine per tablet	Infants 6-10 kg two tablets twice daily dispersed in liquid. (WHO: 25 mg 3TC, 75 mg NVP and 50 mg ZDV BD)
Formulation 3: 25 mg Lamivudine, 50 mg Nevirapine and 50 mg Zidovudine per tablet	Children 10-15 kg two tablets twice daily dispersed in liquid. (WHO: 50 mg 3TC, 100 mg NVP and 70 mg ZDV BD)
Formulation 4: 75 mg Lamivudine, 100 mg Nevirapine and 150 mg Zidovudine per tablet	Children 15-20 kg one tablet daily and 20-29 kg two tablets twice daily dispersed in liquid. (WHO: 15-20 kg – 75 mg 3TC, 150 mg NVP and 100 mg ZDV BD) (WHO: 20 -29 kg and adults – 100-150 mg 3TC, 200 mg NVP and 150 mg ZDV BD)

All the formulations contained the following excipients (Table 2):

Table 2: Drugs and excipients in formula 1-4 (1000 mg tablet)

Substance	Amount per tablet (%)	Function
Lamivudine	-	Drug
Nevirapine	-	Drug
Zidovudine	-	Drug
Avicel PH-102™	-	Filler, disintegrant
Kollidon CL-M™ (PVP)	1.00	Disintegrant
Explotab™ (Sodium starch glycolate)	4.00	Disintegrant
Xylitab™ (Xylitol)	5.00	Sweetener
Sodium-Saccharin	4.00	Sweetener
Flavouring (Passion fruit)	4.00	Flavouring
Aerosil™ (Colloidal silica)	0.50	Glidant
Tween-80™ (Polysorbate)	0.38	Solubilizer
Magnesium-Stearate	1.00	Lubricant

Manufacturing method:

1. Active substances and excipients were obtained from the suppliers. The certificates of analysis of all the active substances were checked.
2. A suitable manufacturing environment and equipment were prepared.
3. Excipients and drug substances were weighed and mixed according to the order in table 2 except for the magnesium stearate. It was mixed in a V-mixer for 15 minutes. After initial mixing the magnesium stearate was added and the formula was mixed for a further 10 minutes.
4. The tablet powder was compressed by a single tablet press with a punch diameter of 16 mm. Press parameters were adjusted until a product of consistent quality was achieved. 1500 tablets of 1 g each per batch were manufactured.
5. Initial tablet tests were performed.
6. Remaining tablets were packaged in PVC bottles at 25 °C; 60% RH, 30°C; 60% RH and 40 °C; 75% RH consecutively.
7. After 4, 8 and 12 weeks stability tests were performed on each batch.

3. Results

3.1 DSC Analysis

Thermal compatibility between the three drugs and excipients were determined. Compatibility thermograms between the three drugs and excipients can be seen in Figures 2 to 7. Possible interactions were observed with all combinations during DSC studies. Depressions and shifts in the melting points were observed during the simultaneous analysis of all the drugs with excipients.

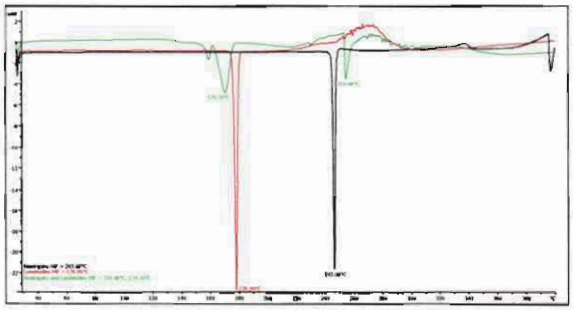

Figure 2: Compatibility DSC-thermogram overlay of Lamivudine and Nevirapine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

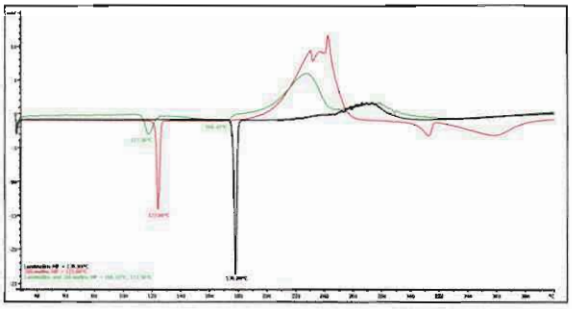

Figure 3: Compatibility DSC-thermogram overlay of Lamivudine and Zidovudine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

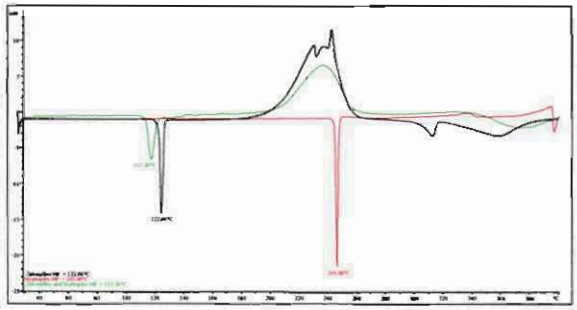

Figure 4: Compatibility DSC-thermogram overlay of Nevirapine and Zidovudine in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

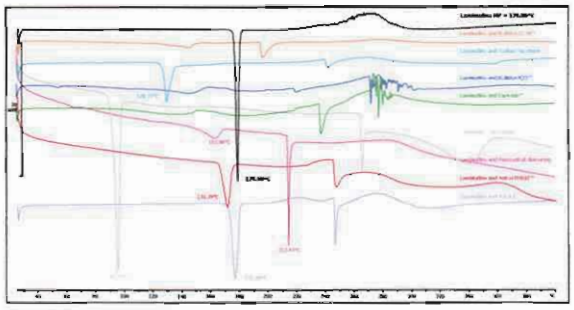

Figure 5: Compatibility DSC-thermogram overlays of Lamivudine and tablet excipients in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

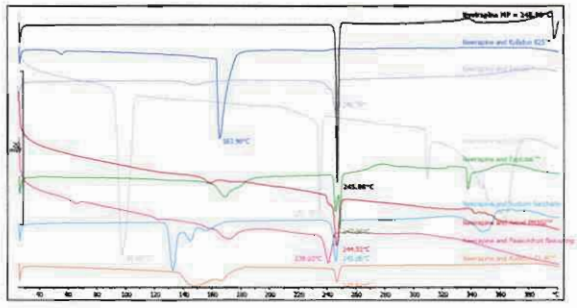

Figure 6: Compatibility DSC-thermogram overlays of Nevirapine and tablet excipients in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

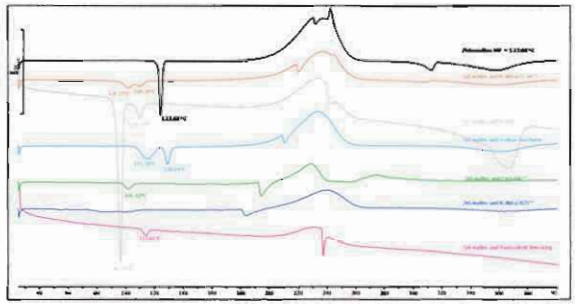

Figure 7: Compatibility DSC-thermogram overlays of Zidovudine and tablet excipients in a nitrogen atmosphere 50ml.min^{-1} and heating 10°C.min^{-1}

3.2 *HPLC Validation*

Specificity for lamivudine, zidovudine and nevirapine complies with ICH standards for simultaneous analysis of all three drugs in both standard and sample solutions. Linearity complies and R^2-values for lamivudine = 0.9991, nevirapine = 0.9999 and zidovudine = 0.9992. The following concentration ranges were tested: Lamivudine = 0.9 – 348.2 µg/ml, nevirapine = 0.9 – 350.6 µg/ml and zidovudine = 0.9 – 349.8 µg/ml. Accuracy values of 98.10%, 98.60% and 100.1% were achieved. Precision with a RSD of less than ≤ 0.94%, ≤ 1.45% and ≤ 0.84% were achieved. Both ruggedness and robustness complied with ICH standards (ICH, 2005).

3.3 *Pharmacopoeial test results*

Pharmacopoeial tests were performed on all the formulations manufactured at initial, 4, 8 and 12 weeks (Table 3). The following tests were performed: Assay, loss on drying, mass uniformity, diameter, thickness, hardness, friability and dissolution testing.

Table 3: Results obtained during pharmacopoeial testing of all the formulations during stability testing

Test type	Pharmacopoeial limits (BP, 2005)	Results obtained
Assay	90-110% of label claim	Complies
Hardness	-	54.89-112.82 N
Diameter	-	< 1mm change
Thickness	-	< 1mm change
Friability	< 1% of tablet mass	≤ 3.19 %
Uniformity of mass	< 5% of tablet mass	Complies
Loss on drying	-	≤ 5% per tablet and < 2% loss/gain of moisture
Dissolution	90% of drug within 30 min	> 70% of all drugs within 5 min and 100 ± 5% within 30 min

Dissolution testing was performed only on formula 4. Dissolution testing were performed using paddles, 50 rpm and 900 ml 0.1 M HCl pH adjusted to 2.0 ± 0.5 with extraction time intervals at 5, 10, 15, 20 and 30 minutes.

No severe changes in drug release rate were observed in any one of the three drugs at any of the stability conditions. The drug release rate remains fairly constant at 40°C and 75% humidity during the 12 week stability period as seen in figure 8, 9 and 10 with all drugs.

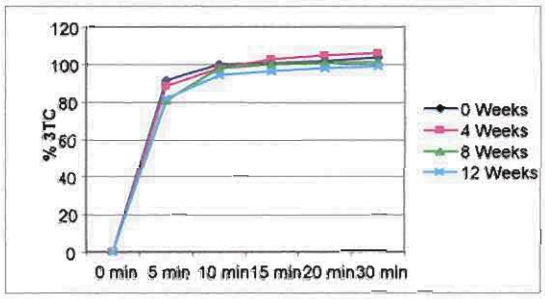

Figure 8: Drug release of lamivudine at 40°C; 75% RH for 0 to 12 weeks stability testing (Formula 4).

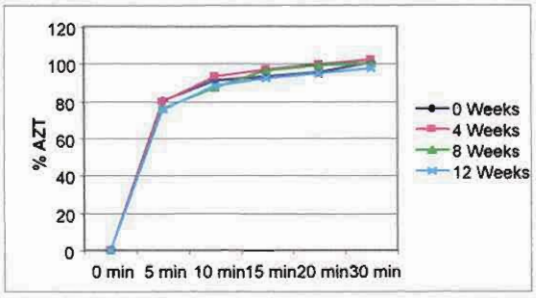

Figure 9: Drug release of zidovudine at 40°C; 75% RH for 0 to 12 weeks stability testing (Formula 4).

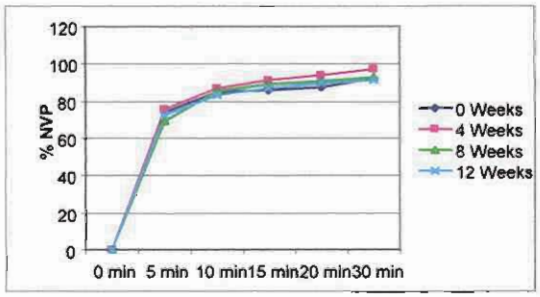

Figure 10: Drug release of nevirapine at 40°C; 75% RH for 0 to 12 weeks stability testing (Formula 4).

4. Conclusions

Problems with possible chemical interactions were detected during compatibility DSC studies between drugs and excipients. Due to the inconclusiveness of the results and the fact that formulations containing the drugs in combination with each other and the excipients were commercially available worldwide, further studies with the formulations were performed. Unfortunately problems were encountered during the manufacture of the formulations. The tablet press used was an antique single press dating from the 1950's in a bad condition, also the punches used edges were not shaped so that the tablets manufactured had sharp edges resulting in an increase in friability. During initial studies at 0 weeks all the formulations complied with pharmacopoeial specifications (BP, 2005) (USP, 2005) except for friability that was too high. During the stability test period of 12 weeks none of the four tablet formulations revealed any severe changes in physical or chemical composition. Assay and dissolution testing were used as an indication of the stability of the drugs in the formulations during stability testing. No severe changes were detected during stability assay and dissolution testing. The conclusion can therefore be made that all the formulations formulated during this study are both physically and chemically stable when subjected to accelerated stability conditions.

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