COMBINATION ANTIRETROVIRAL THERAPY: FORMULATION OF A POWDER FOR ORAL SUSPENSION CONTAINING LAMIVUDINE, ZIDOVUDINE AND NEVIRAPINE

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"May every young scientist remember and not fail to keep his eyes open for the possibility that an irritating failure off his apparatus to give consistent results may once or twice in a lifetime conceal an important discovery."

-Patrick Blackett-

To my dear parents and supporting family.

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ABSTRACT

The human race has had to endure a variety of disabling and life threatening diseases which include leprosy, plaque, Black Death and numerous other infamous viruses, none of which awaken terror like the Human Immunodeficiency Virus (HIV). HIV infection has produced one of the most dreaded epidemics of the twentieth century, or rather, of all time, leaving no country or race untouched. Until this time, no cure for this disease has been discovered, and sufferers have to tolerate the frustrating adverse effects of the numerous Antiretroviral (ARV) drugs that have to be taken in order to live an average life. Whilst combination antiretroviral therapy has proven to be the most effective approach in treating HIV positive patients, Fixed Dose Combination (FDC) therapy seem to assure better patient adherence.

An oral liquid dosage formulation is desired for paediatric HIV patients, and for those who cannot swallow other oral dosage forms such as capsules and tablets. The focus of this study was to combine lamivudine, zidovudine and nevirapine into a powder for oral suspension, and to identify incompatibilities and other factors that could possible affect the physical and chemical stability of the formulation. Formulations were designed according to the UNICEF/WHO (2004) dosage suggestions.

The first step in the product development was an investigative literature study into the stability and possible interactions between the three active pharmaceutical ingredients (APIs) and excipients. Compatibility was confirmed by means of DSC and High Performance Liquid Chromatography (HPLC) -studies. The optimal mixing time for the powders for suspension was determined before formulation of the bulk formulae began.

Six suspension formulations according to patient mass were formulated containing the dosage régime for one month as suggested by WHO. Sodium propyl hydroxybenzoate and sodium methyl hydroxybenzoate were added as preservatives.

The physical and chemical stability of pharmaceutical substances do play a vital role in the success of a specific product. Stability tests, physical and chemical, and preservative efficacy studies were therefore performed on all six formulations over a period of twelve weeks, at three temperature conditions, namely 25°C+60%RH, 30°+65%RH and 40°C+75%RH. In that period, in-use stability testing, to ensure the stability during patient use, was also conducted.

Results obtained for pH, relative density, viscosity and moisture content indicated that no significant changes took place during the accelerated stability testing, as well as the in-use test period. There was a change in the physical appearance, especially the colour, with time at the 30°C+65%RH and 40°C+75%RH storage conditions, as well as during in-use testing after 12 weeks.

Dissolution results showed that lamivudine, zidovudine and nevirapine were immediately dissolved, with 100% dissolution after 10 minutes. The assay results for lamivudine, zidovudine and nevirapine remained within the specification of 90-110%, but a downward trend in lamivudine assay was observed in the 300 ml and 900 ml formulation, where a result of 89.3% was measured after 12 weeks at 40°C+75%RH.

Results for sodium methyl hydroxybenzoate were all within the 90-110% limit. Results higher than the upper limit of 110% were calculated for sodium propyl hydroxybenzoate in some samples. This could be due to integration inconsistencies during assay due to the very small peak. Preservative Efficacy Test (PET) results were all within the USP 28 specifications, even during in-use studies, and confirmed the effectiveness of the preservative system.

In conclusion it can be said that lamivudine, zidovudine and nevirapine were successfully combined in a FDC for use in paediatric, adult and geriatric AIDS patients.

The powder for suspension formulations have definite marketing possibilities and could become an essential part in the fight against AIDS.

UITTREKSEL

Die mensdom het deur die eeue heen met 'n verskeidenheid ondraaglike en lewensgevaarlike siektetoestande te doen gehad waaronder melaatsheid, pes, die Swart Dood en ander dodelike virusse, waarvan Menslike Immuniteitsgebrek-virus (MIV) van die laaste groep die grootste vrees aanwakker. MIV-infeksie is die oorsaak van een van die mees gevreesde epidemies van die twintigste eeu, of eerder, van alle tye, en laat geen land of ras onaangeraak nie. Tot en met die hede is daar nog geen kuur vir Verworwe Immuniteitsgebrek -sindroom (VIGS) ontdek nie. VIGS-pasiënte moet die frustrerende newe-effekte van die vele antiretrovirale (ARV) geneesmiddels verdra om 'n gemiddelde lewe te lei. Hoewel kombinasie antiretrovirale terapie 'n koste-effektiewe wyse is om MIV-positiewe pasiënte te behandel, is vastedosis terapie dalk die antwoord op beter pasiënt meewerkendheid.

'n Orale vloeistof doseervorm word benodig vir pediatriese MIV pasiënte en vir diegene wat dit moeilik vind om vaste orale doseervorme in te neem, bv. tablette en kapsules. Die fokus van hierdie studie was om lamivudien, zidovudien en nevirapien in 'n poeier vir orale suspensie te kombineer, en sekere overenigbaarhede en ander faktore wat die fisiese en chemiese stabiliteit van die formulerings kan beïnvloed, te identifiseer. Formules is ontwerp volgens UNICEF/WGO (2004) doserings riglyne.

Die eerste stap in die nuwe produkontwikkeling was 'n uitgebreide literatuurstudie om die stabiliteit en moontlike interaksies tussen die drie aktiewe bestanddele en hulpstowwe te ondersoek. Verenigbaarheid was bevestig met behulp van DSC en HPLC-studies. Die optimale mengtyd vir die poeiers vir suspensie is bepaal voordat formulering van laboratoriumlotte begin het.

Ses suspensieformules, gegrond op die pasiënt se liggaamsmassa, is geformuleer en bevat die behandelingsdosis vir een maand soos voorgestel deur die Wêreld Gesondheids Organisasie (WGO). Natriummetielhidroksiebensoaat en natriumpropielhidroksiebensoaat is ingesluit in die formules as preserveermiddels.

Die fisiese- en chemiese stabiliteit van farmaseutiese produkte speel 'n kernrol in die sukses van die betrokke produk. Stabiliteitstoetse, fisies en chemies, en preserveermiddeleffektiwiteitstoetse is uitgevoer op al ses formules oor 'n periode van twaalf weke by drie temperatuurtoestande, naamlik 25°C+60%RH, 30°C+65%RH en 40°C+75%RH. In hierdie tydperk is in-gebruik studies, om die stabiliteit gedurende pasient gebruik te verseker, ook gedoen.

Resultate verkry vir pH, relatiewe digtheid, viskositeit en voginhoud dui daarop dat geen noemenswaardige veranderinge tydens versnelde stabiliteitstoetsing of die in-gebruik toetsing plaasgevind het nie. Daar was 'n verandering in die fisiese voorkoms, veral t.o.v. kleur, waargeneem by monsters gestoor by 30°C+65%RH en 40°C+75%RH, asook by monsters tydens in-gebruik studies, na 12 weke.

Dissolusieresultate wys dat lamivudien, zidovudien en nevirapien dadelik oplos, met 100% dissolusie na 10 minute. Die konsentrasie van lamivudien, zidovudien en nevirapien het binne die spesifikasies van 90-110% gebly, maar 'n afwaartse neiging in lamivudien konsentrasie is waargeneem in die 300 ml en 900 ml formulerings, waar 'n waarde van 89.3% na 12 weke in laasgenoemde geval gemeet is.

Resultate vir natriummetielhidroksiebensoaat konsentrasie was binne die 90-110% limiet. Resultate hoër as die boonste limiet is bereken vir natrium propiel hidroksiebensoaat in sekere monsters, wat toegeskryf kan word aan moontlike foutiewe integrasie parameters tydens HPLC-analise. Preserveermiddeleffektiwiteitstoetse (PET) se positiewe uitslag, tydens versnelde stabiliteit en in-gebruik toetsing, het die effektiwiteit van die preserveermiddels bevestig.

Samevattend kan gesê word dat lamivudien, zidovudien en nevirapien suksesvol gekombineer is in 'n vaste dosis kombinasie vir die gebruik in pediatriese, volwasse en bejaarde VIGS-pasiënte.

Die poeier vir suspensie formulerings het definitiewe bemarkingspotensiaal en kan 'n essensiële rol speel in die bekamping van VIGS.

AIM AND OBJECTIVES

Lamivudine (3 TC), zidovudine (AZT), and nevirapine (NVP) are antiretroviral drugs used in the Highly Active Antiretroviral Treatment (HAART) regime for paediatric, adult and geriatric AIDS sufferers across the world (Gibbon, 2001:310). There is a desperate need for a Fixed Dose Combination (FDC) product which combines the abovementioned drugs in a single product which can be easily administered to paediatric, adult and geriatric patients, preferably in a liquid dosage form.

The aim of this study was to adhere to the need for FDCs for paediatric, adult and geriatric use. The stability of lamivudine, zidovudine and nevirapine in combination has never been studied. Literature studies suggested that the three actives might be more stable in pH ranges 4-7. The insoluble nature of nevirapine also posed a problem, forcing the formulation of a suspension, instead of a solution.

The main objectives of this study were:

- Development and validation of a stability indicating assay method for the simultaneous determination of the APIs and preservatives present in the formulation.
- To formulate a FDC in the form of a powder for oral suspension, with acceptable organoleptic properties.
- The determination of the physical and chemical stability of the formulated powder for suspension during accelerated stability conditions.
- To determine the chemical and physical stability of the reconstituted suspension during in-use testing to simulate patient use.
- To establish the effectiveness of the preservatives chosen.
- To explore the marketing possibilities of these newly formulated products.

GLOSSARY

API - Active Pharmaceutical Ingredient

ART - Antiretroviral Therapy

ARV - Antiretroviral

BD - Twice a day

CDC - Center for disease control

DSC - Differential Scanning Calorimetry

FDC - Fixed Dose Combination

HAART - Highly Active Antiretroviral Therapy

HPLC - High Performance Liquid Chromatography

IPEC - International Pharmaceutical Excipient Council

LAV - Lymphadenopathy Associated Virus

QID -Four times a day

SIV - Simian Immunodeficiency

TDS - Three times a day

UNAIDS – Joint United Nations Programme on HIV/AIDS

UNICEF – United Nations Childrens Fund

WHO - World Health Organisation

CHAPTER 1

HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

"One may not reach the dawn save by the path of the night."

-Kahlil Gibran-

1.1 INTRODUCTION

HIV infection has produced one of the most feared epidemics of the twentieth century. It has spread world wide, leaving no race or country unaffected (Cossarizza & Kaplan, 2002: ix). "The acquired immunodeficiency syndrome (AIDS) epidemic is one of the greatest challenges facing the medical community today" (Raffanti & Haas, 2002:1349).

Since the summer of 1981, when the first cases of AIDS were starting to emerge, an estimated 38 million adults and children were living with the human immunodeficiency virus (HIV). The AIDS death toll up to 2003 was an estimated 2.9 million. At the rate at which this merciless disease is being spread an additional 14,000 new HIV infections can be added to the list, daily (UNAIDS, 2004).

1.2 DISCOVERY OF HIV

Although it is clear today that HIV is the cause of AIDS, its precise origin remains unknown. According to Parsyn (2005:433) recent findings suggest that HIV-1 and -2 might have been transmitted from primates to humans 15 or 20 years ago, but earlier unrecognised infections might have occurred.

In 1959 the first recorded specimen of HIV was discovered in a blood specimen obtained at Leopoldville (now Kinshasa) in the Belgian Congo. This was the first known death caused by AIDS (Natural treatments for AIDS, 1998).

According to the theory of Bette Korber, the disease can be traced to a single viral ancestor that could have emerged between 1910 to 1950. Korber contends, through an analysis done at the Los Alamos National Laboratory in New Mexico, that the pandemic may have come from one or more infected humans around 1930 (Natural treatment for AIDS, 1998).

American philosopher, Louis Pascal, brought forward another highly controversial, but plausible theory, first spelt out in 1987. According to him the early cases of AIDS originated in the Central African states of Congo, Rwanda or Burundi. It was this area that was subjected to trials of a live polio vaccine on as many as 300,000 men, women and children (Natural treatment for AIDS, 1998).

Pascal's argument was that the abovementioned vaccine, which was grown from cultures obtained from chimpanzee kidneys, might have carried the virus. Dr Albert Sabin (polio researcher) had reported that such a batch had been contaminated with an unknown virus (Natural treatment for AIDS, 1998).

Reports of Kaposi's sarcoma and *Pneumocystis carinii* pneumonia in young men began to appear in the early 1980's, and it was subsequently realised they were both homosexual and immuno compromised (Adler, 1993:1). Though the condition became known early on as AIDS, its cause or modes of transmission were not immediately clear.

It was in 1983 at the Institut Pasteur, Paris, that Barrè-Sinoussi, Montagnier and colleagues discovered HIV and named it Lymphadenopathy Associated Virus (LAV). In 1984 Popovic, Gallio and co-workers described the development of cell lines infected with the virus, and designated the virus LAV, HTLV-III (Mortimer, 1993:5). Viruses isolated from patients with AIDS in America, Central Africa and Europe were all the same virus, and it was named HIV-1 (Mortimer, 1993:5).

During 1985 another retrovirus, different from HIV-1, was isolated in patients living in West Africa or having West African connections. Institut Pasteur, Paris, named it LAV-2, better known today as HIV-2 (Moretimer, 1993:5).

This particular HIV-2 virus is closely related to the Simian Immunodeficiency Virus (SIV) carried by African green monkeys. SIV causes AIDS-like disease in captive rhesus monkeys, thus supporting the theory that at some stage HIV-1 and HIV-2 might have been transmitted from primates to humans (Mortimer, 1993:5).

1.3 TRANSMISSION OF HI-VIRUS

The most common mode of transmission of the virus throughout the world is unprotected sexual intercourse with multiple partners, whether it is vaginal- or anal intercourse is irrelevant (Natural treatment for AIDS, 1998).

Other methods of transmission are:

- Through the receipt of infected blood or blood products and donated organs. Donating blood does not run the risk of disease contraction since the needles used for such purposes are always sterile. Dried blood is not infectious since the HI-virus is unable to survive outside the human body beyond a short period of time (Natural treatment for AIDS, 1998).
- From mother to child, which occurs in utero and at birth.
- Through therapeutic procedures e.g. surgical operations.
- By re-use or sharing of contaminated needles used by drug abusers.
- Through the breast milk of an infected mother (Adler, 1993:2).

HIV has also been isolated from samples of lymphocytes, cervical secretions, cerebrospinal fluid, tears, urine and cell free plasma.The concentrations of the virus in the different fluids vary considerably and therefore cannot without a doubt be considered as sure means of HIV transmission (Adler, 1993:2).

AIDS is not contracted through:

- Touching, hugging, kissing.
- Drinking or eating from utensils used by an infected person.
- Sharing toilet seats.
- By any other mode of casual contact, including working, socialising and living with infected people (Natural treatments for AIDS, 1998).

No evidence of transmission through saliva has been documented, but it is sure that the virus is not spread through social or casual contact, nor is it air-borne, water-borne or transmitted through mosquitoes (Natural treatment for AIDS, 1998).

1.4 LIFE CYCLE OF THE HI-VIRUS

HIV can be called "immunosuppressive" because it infects the cells of the immune system and ultimately leads to their demolition (Beverley & Sattentau, 1993:9). HIV is an RNA (ribonuclease) retrovirus that infects $CD4^+$ lymphocytes, dendritic cells and macrophages (Raffanti & Haas, 2001:1350). The reproduction of HI-virus in the human body (scheme 1.1) can be divided into six steps (Raffanti & Haas, 2001:1350-1351).

Step 1

↓

Binding: HIV has proteins on its surface with a high affinity for the $CD4^+$ receptor on the T4 cell. The viral capsid is released into the host cell.

Step 2

↓

Reverse transcription: Viral enzyme (reverse transcriptase) makes a pro-viral DNA copy of the RNA.

Step 3

↓

Integration: Integrase (viral enzyme) hides the pro-viral DNA in the cell's DNA, which produces new HI viruses in the nucleus.

Step 4

↓

Transcription: Strands of viral DNA in the nucleus separate, enzymes create messenger RNA (mRNA).

Step 5

↓

Translation: Each section of mRNA is translated into a specific protein building block to form a part of a new HI-virus.

Step 6

↓

Viral Assembly: Protease (viral enzyme) cuts long proteins into smaller proteins which become elements of new HI-viruses or enzymes.

Scheme 1.1: Reproduction of the HI virus in the human body.

Once the new viral units are assembled they leave the host cell and create new viruses that are able to infect new cells, and undergo reproduction (Raffanti & Haas, 2002:1350-1351).

1.5 DEFINITION OF AIDS

Although we use the term 'disease' when referring to AIDS, AIDS is strictly speaking not a specific illness. It is a collection of many conditions that manifest in specific parts of the body, as a result of a severely weakened immune system. The HI-virus weakens the immune system over a period of time which differs from patient to patient, and gradually prevents the body's immune system from fighting pathogens that invade the body (UNAIDS, 2004).

Originally, AIDS was defined by the Centre for Disease Control (CDC) in North-America as occurring in a person:

(a) With a reliably diagnosed disease that is moderately indicative of an underlying cellular immune deficiency.

(b) Who has no known underlying cause of cellular immune deficiency or any other cause of immune suppression associated with the disease (Adler, 1993:1).

A change in definition took place in September 1987 and the definition now includes encephalopathy, HIV wasting syndrome and a wider range of diseases indicative of AIDS such as oesophageal candidosis, extrapulmonary cryptococcosis and progressive multifocal leucoencephalopathy (Adler, 1993:1).

Adult patients are divided into three groups:

a) Those without laboratory evidence of an HIV infection, but with positive diagnosis of an indicator disease.

b) Those with laboratory evidence of the presence of an HIV infection, regardless of the presence of other causes of immunodeficiency, or any of the specified indicator diseases whether diagnosed presumptively or definitively.

c) Those with laboratory evidence against an HIV infection. AIDS is diagnosed in this group only when all the other major causes of immunodeficiency have been excluded - for example, a high dose of any long term systemic corticosteroid or other immunosuppressive cytotoxic disease - and the patient has had unequivocal *Pneumocystis carinii* pneumonia or any

other disease indicative of AIDS and a T helper ($CD4^+$) lymphocyte count of less than $0.4x10^9/\ell$ (Adler, 1993:1).

Children with HIV are in two respects defined differently from adults:

a) In children, recurrent or multiple serious bacterial infections and lymphoid interstitial pneumonitis or pulmonary lymphoid hyperplasia are accepted as indicative of AIDS.

b) More strict criteria are used for children who are less than five months old and whose mothers are thought to have been infected with HIV during the child's perinatal period (Adler, 1993:1).

The CDC published an expanded definition of AIDS in December 1992, which includes persons with confirmed HIV infection who have a $CD4^+$ T lymphocyte count of less than $0.2x10^9/\ell$, irrespective of clinical manifestations. Along with the abovementioned criteria, three new AIDS indicative diseases were added to the existing list, namely pulmonary tuberculosis, bacterial pneumonia and invasive cervical cancer (Adler, 1993:2).

1.6 PATHOLOGY OF HIV

A clinical problem is triggered by HIV and snowballs into the development of the disease, from the time of seroconversion (primary HIV) and ending with AIDS and death (Mindel & Tenant-Flowers 2001:17).

Classification systems for the HIV disease are based on the presence of clinical symptoms and signs, $CD4^+$ count, investigative signs and the availability of HIV screening (Mindel & Tenant-Flowers 2001:17).

The World Health Organization (WHO) staging system for HIV infection in adults and adolescents divides patients into four stages of which some of the symptoms are mentioned below:

- Stage 1: Asymptomatic
- Stage 2: Weight loss <10% body weight

  Minor mucotaneous manifestations

  Recurrent upper respiratory tract infections

- Stage 3: Weight loss >10% body weight

  Chronic diarrhoea > 1 month

  Oral candidacies

  Prolonged fever > 1 month

  Pulmonary TB

  Bacterial infections

- Stage 4: HIV wasting syndrome: Weight loss >10% body weight

  Chronic diarrhoea or weakness and prolonged fever

  Pneumocystis carinii pneumonia

  Kaposi's sarcoma.

1.7 TREATMENT FOR HIV

The swift discovery of effective antiretroviral therapy (ART) was made possible by already developed drug treatment programmes for existing diseases including hypertension, cancer and other viral infections (Raffanti & Haas, 2002:1352).

1.7.1 GOALS FOR ANTIRETROVIRAL THERAPY

The Primary goals of ART are:

- To reduce the viral load to undetectable levels of less than 400 copies/ml.
- To restore and/or preserve the immunological function in the body.
- To improve the quality of life.
- Reduction of HIV related morbidity and mortality (Naidoo, 2005:1).

ART is generally recommended if:

- The patient manifests significant symptoms related to HIV.
- Extra pulmonary TB is diagnosed (Naidoo, 2005:40).

- A viral load greater than 5000-10000 copies/ml, is present.
- The $CD4^+$ cell count is below $500/mm^3$ (AIDS Information Center, 1999).

Inappropriate drug prescription may cause development of drug resistance, as HIV mutates rapidly and there is a high viral turnover (Gibbon, 2001:308).

Standardised treatment regimes for HIV are based on Highly Active Antiretroviral Treatment (HAART), a **triple therapy** regimen designed to minimise drug resistance and poor antiretroviral efficiency (Naidoo, 2005:1). Advantages of HAART include:

- HIV replication suppression, which in turn causes partial immune recovery.
- Development of resistance is prevented with the suppression of viral replication.
- Patient adherence can be improved by simplifying dosage regimes (Gibbon, 2001:308) (Refer to tables 1.2, 1.3, and 1.4).

Guidelines for the use of HIV treatments advocate the use of **two Nucleoside Reverse Transcriptase Inhibitor's (NRTIs) + one Non Nucleoside Reverse Transcriptase Inhibitor (NNRTI)** or **one Protease Inhibitor (PIs)** (Naidoo, 2005:1).

Mono therapy, because of its high level of resistance and poor antiretroviral efficiency, is reserved for the prevention of mother to child transmission, ideally when the mother has a viral load of less than 1000 copies/ml and is asymptomatic (Naidoo, 2005:1).

Dual drug therapy also has a high level of resistance and poor antiretroviral efficacy, but the treatment may be continued if the patient is already on it and the viral load and $CD4^+$ count goals are maintained (Naidoo, 2005:1-2).

Table 1.1: Examples of ARV drugs currently on the market.

Drug Class	Drug Name	Examples of Trade Names	Abbreviations
NRTI	• Zidovudine • Stavudine • Abacavir • Didanosine • Emtricitabine • Lamivudine • Zalcitabine	Retrovir® Zerit® Ziagen® Videx® Emtriva® Epivir® Hivid®	AZT/ ZDV d4T ABC ddI FTC 3TC ddC
NNRTI	• Nevirapine • Efavirenz • Delavirdine	Viramune® Stocrin® Rescriptor®	NVP EFV DLV
PI	• Indinavir • Ritonavir • Nelfinavir • Amprenavir • Saquinavir	Crixivan® Norvir® Viracept® Agenerase® Invrase®	IDV RTV NFV APV SQV
FI	• Enfluvirtide	Fuzeon®	T20

1.7.2 ARVs: CLASSIFICATION AND MECHANISM OF ACTION

All ARVs are designed to inhibit specific steps in the viral reproduction cycle. Each class of ARV inhibits an HIV enzyme directly (NNRTIs) or indirectly (NRTIs) (Refer to paragraph 1.4).

Refer to scheme 1.1 for the reproduction cycle of the HI-virus in the human body.

- **Nucleoside Reverse Transcriptase Inhibitors (NRTIs):** Inhibit the enzyme "reverse transcriptase" by mimicking the building blocks of HIV DNA.
- **Non Nucleoside Reverse Transcriptase Inhibitors (NNRTIs):** Inhibit the enzyme reverse transcriptase directly.
- **Protease Inhibitors (PIs):** Inhibit the protease enzyme needed for late stage HIV production.
- **Fusion Inhibitors (FIs):** Inhibit GP-41 mediated fusion, works synergistically with all other ARV classes (Naidoo, 2005:37).

Table 1.1 lists examples of ARV drugs in each category currently on the market.

1.7.3 ARVs: ADVERSE EFFECTS

All ARVs have extreme adverse effect that cause one of the foremost problems in treating HIV patients, namely poor patient adherence. This not only leads to therapeutic failure, but also causes the HI-virus to develop a certain kind of resistance towards specific ARVs.

All patients will not experience the same adverse effects when treated with certain ARVs, but the following will normally occur when treated with:

- **NRTIs:** Pancreatitis
  Peripheral neuropathy
  Hypersensitivity reactions (Abacavir)
  Headache
- **NNRTIs:** Maculopapular rashes (severe or life threatening)
  CNS effects (abnormal dreams, headache…)
  Depression
  Diarrhoea
  Hepatitis
- **PIs:** Lipodystrophy
  Hypercholesterolaemia
  Hypertriglyceridaemia
  Insulin resistance (Gibbon, 2001:313-319).
- **FIs** Hypersensitive reactions
  Immune-mediated reactions

Respiratory distress

Nausea and vomiting (United States Department of Health and Human services: 2005:19).

(Refer to tables 1.5, 1.6 and 1.7 for further discussion of adverse effects and possible patient adherence problems).

1.7.4 AVAILABLE TREATMENT REGIMES AND DOSAGE FORMS

When treating a patient for HIV, there are numerous treatment regimes to consider.

All ARVs present with a high incidence of adverse effects, ranging from a mere headache to life-threatening Stevens-Johnson syndrome. To ensure patient adherence, a treatment regime with minimum adverse effects and maximum therapeutic satisfaction should be identified.

In the following tables the advantages and disadvantages of three categories of ARVs will be discussed.

Table 1.2: Advantages and disadvantages of Nucleoside Reverse Transcriptase Inhibitor (NRTI) treatment (United States Department of Health and Human services: 2005:40).

Advantages and disadvantages of NRTI-treatment	
Advantages	**Disadvantages**
• Minimal drug-drug interactions • Protease inhibitor and NNRTI-sparing • Only limited cross resistance among NRTIs • Better adherence than PIs • Combination therapy is co-formulated as a single pill; low pill burden	• Life threatening cases of lactic acidosis and hepatic steatosis are rare but serious • Use of ZDV/3TC/ABC (Trizivir) co-formulation has potential for ABC hypersensitive reaction

Table 1.3: Advantages and disadvantages of Non-nucleoside reverse transcriptase inhibitor (NNRTI) treatment (United States Department of Health and Human Services: 2005:42).

Advantages and disadvantages of NNRTI treatment	
Advantages	**Disadvantages**
• Protease inhibitor- sparing • Less fat maldistribution and less dyslipidaemia than protease inhibitors • Lower pill burden than PIs (solid dosage forms) • Easier to adhere to than PI- based regimes	• Serious and potentially life threatening hepatic toxicity and skin rashes (Stevens-Johnson Syndrome) • Potential for various drug interactions due to metabolism via hepatic enzymes • Cross-resistance may occur among NNRTIs

Table 1.4: Advantages and disadvantages of Protease inhibitor (PI) treatment (United States Department of Health and Human Services: 2005:43).

Advantages and disadvantages of PI treatment	
Advantages	**Disadvantages**
• NNRTI- sparing • Targets viral reverse transcriptase and protease enzymes (two steps in the viral replication cycle) • Multiple mutations are required for resistance to develop against PIs	• Potential for multiple drug interactions due to metabolism via hepatic enzymes • Poor palatability of liquid preparations • Metabolic complications including fat maldistribution and insulin resistance

Table 1.5: **The different dosage forms available for nucleoside reverse transcriptase inhibitors** (United States Department of Health and Human services: 2005:1-7).

Drug Name	**Dosage Forms**	**Combination Therapy**	**Dose per day (single drug)**	**Possible Adherence problems**
Abacavir (ABC)	Paediatric oral solution: 20 mg/ml. Tablets: 300 mg.	- Tablets: 300 mg ZDV +150mg 3TC+ 300 mg ABC. - Tablets: 300 mg 3TC+ 600 mg ABC	Neonatal: Not used Paediatric: (age: >3 months) 8 mg/kg/twice a day. Adolescents: (age: 13-24 years) Not used Adults: 300 mg (2 tablets) or 600 mg (1 tablet).	
Didanosine (ddI)	Paediatric powder for oral solution: 10 mg/ml. Chewable tablets: 25 mg, 50 mg, 100 mg, 150 mg, 200 mg. Delayed release capsules: 125 mg, 200 mg, 250 mg, 400 mg.	- No fixed dose combinations available.	Neonatal: (age: <3 months) 100 mg/m^2 of body surface area every 12 hours. Paediatric: (age: >3 months) 120 mg/ m^2 of body surface area every 12 hours. Adolescents/adults: ≥60 kg: 400 mg daily, <60 kg: 250 mg daily.	In resource-poor settings, it is complicated to determine dose according to m^2 of body surface area, due to lack of equipment and staff.

Table 1.5: (Continue)

Drug Name	Dosage Forms	Combination Therapy	Dose per Day (single drug)	Possible Adherence problems
Emtricitabine (FTC)	Oral solution: 10 mg/ml (investigational). Capsules: 200 mg.	- Tablets: 200 mg FTC+ 300 mg TDF.	Neonatal: Not used Paediatric: (age: three months-17 years) 6mg/kg twice a day (>33 kg: 200mg capsule daily). Adolescents/adults: 200 mg capsule daily OR 24 ml (240 mg) oral solution daily.	Fixed dose combination therapy should not be used in patients with creatinine clearance of <30 m/minute. In resource-poor settings creatinine clearance may be difficult to determine due to lack of staff, training and insufficient equipment.
Lamivudine (3TC)	Solution: 5mg /ml and 10 mg/ml. Tablets: 100 mg, 150 mg and 300 mg.	- Tables: 300 mg ZDV+150 mg 3TC. - 300 mg ZDV+150 mg 3TC+300mg ABC. - 300 mg 3TC+600 mg ABC.	Neonatal: (<30 days) 2 mg/kg twice a day. Paediatric: 4 mg/kg twice a day. Adolescent: (age: ≥ 16 years) Body weight: ≥ 50 kg: 300 mg daily <50 kg: 4 mg/kg (maximum dose: 150mg) twice a day.	Assuming that a neonatal baby weighs 2 kg, the dose would then be 0.8 ml twice a day. In resource-poor settings, it could be difficult to measure the correct dose due to insufficient staff, equipment or training.

Table 1.5: (Continue)

Drug Name	**Dosage Forms**	**Combination Therapy**	**Dose per Day (single drug)**	**Possible Adherence problems**
Stavudine (d4T)	Solution: 1 mg/ml Capsules: 15 mg, 20 mg, 30 mg and 40 mg.	- No fixed dose combinations available.	Neonatal: (age: birth-13 days) 0.5 mg/kg every twelve hours. Paediatric: (age: 14 days-up to a weight of 30 kg) 1 mg/kg every twelve hours. Adolescents: (weight ≥ 30 kg) 40 mg twice a day. Adults: (weight ≥ 60 kg) 40 mg twice daily, (< 60 kg) 30 mg twice daily.	Because there is no fixed dose combination therapy available, patients will be forced to take many tablets or capsules daily in order to receive combination ARV therapy.
Zalcitabine (ddC)	Tablets: 0.375 mg and 0.75 mg	- No fixed dose combinations available.	Neonatal: Not used Paediatric: Not suitable for use in children under the age of 13 years. Adolescents: (aged ≥ 13 years) 0.75 mg three times a day Adults: 0.75 mg three times a day.	Because there is no fixed dose combination therapy available, patients will be forced to take many tablets or capsules daily in order to receive combination ARV therapy.

Table 1.5: (Continue)

Drug Name	Dosage Form	Combination Therapy	Dose per Day (single drug)	Possible adherence problems
Zidovudine (ZDV, AZT)	Syrup: 10 mg/ml. Concentrate for IV injection: 10 mg/ml. Capsules: 100 mg. Tablets: 300 mg.	- Tablets: 300 mg ZDV+150 mg 3TC. - 300 mg ZDV+150mg 3TC+300 mg ABC.	Neonatal: (age: <6 weeks) Oral, 2 mg/kg every six hours. IV 1.5 mg/kg every six hours. Paediatric: (age 6 weeks to 12 years) Oral, 160 mg/m^2 of body surface area every eight hours. IV (Intermittent infusion) 120 mg/ m^2 of body surface area every six hours. (Continuous infusion) 20 mg/ m^2 of body surface area per hour. Adolescent: (age: ≥12 years) 200 mg three times a day or 300 mg twice a day. Adults: 200 mg three times a day or 300 mg twice a day.	In resource-poor settings, it is complicated to determine dose according to m^2 of body surface area, due to lack of equipment and staff.

Table 1.6: The different dosage forms available for non nucleoside reverse transcriptase inhibitors (United States Department of Health and Human Services: 2005:8-9).

Drug Name	Dosage Form	Combination Therapy	Dose per Day (single drug)	Possible Adherence Problems
Delavirdine (DLV)	Tablets: 100 mg and 200 mg.	No fixed dose combinations available.	Neonatal: Not used Paediatric: Not used Adolescent: (age ≥ 16 years) 400 mg three times a day. Adult: 400 mg three times a day.	Due to the fact that there is no fixed dose combination therapy available, patients will be forced to take numerous tablets or capsules a day in order to receive combination ARV therapy.
Nevirapine (NVP)	Suspension: 10 mg/ml Tablets: 200 mg	No fixed dose combinations available	Neonatal: 5 mg/kg OR 120 mg/m^2 of body surface area daily for 14 days, followed by 120 mg/m^2 of body surface area twice a day for 14 days, followed by 200 mg/m^2 of body surface area twice a day. Paediatric: 120-220 mg/m^2 of body surface area twice daily OR (if aged ≥ 8 years) 4 mg/kg twice daily (If aged < 8 years) 7 mg/kg twice a day. Adolescents/adults: 200 mg twice a day.	In resource-poor settings, it is complicated to determine dose according to m^2 of body surface area, due to lack of equipment and staff. Because there is no fixed dose combination therapy available, patients will be forced to take many tablets or capsules a day, in order to receive combination ARV therapy.

Table 1.7: **The different dosage forms available for protease inhibitors** (United States Department of Health and Human Services: 2005:12-18).

Drug Name	**Dosage Form**	**Combination Therapy**	**Dose per Day (single drug)**	**Possible Adherence Problems**
Amprenavir (APV)	Paediatric Oral solution: 15 mg/ml. Capsules: 50 mg and 150 mg.	- No fixed dose combinations available.	Neonatal: Not used Paediatric: (age 4 to 12 years) or (13 to16 years weighing < 50 kg) Oral solution: 17 mg/kg three times a day. Capsules: 20 mg/kg twice a day. Children aged 13 to 16 years weighing > 50 kg: Oral solution: 1400 mg twice a day. Adolescents: (weighing ≥ 50 kg or age > 16 years): Capsules: 1200 mg twice a day. Adults: Capsules: 1200 mg twice a day.	Because there is no fixed dose combination therapy available, patients will be forced to take many tablets or capsules daily in order to receive combination ARV therapy. The oral solution contains 550 mg propylene glycol/ml and 46 IU vitamin E/ml. Patients should therefore be switched to capsules as soon as they are able to take a capsule. The pill burden will be as high as 16 tablets a day, and could cause poor patient adherence.

Table 1.7: (Continue)

Drug Name	Dosage Form	Combination Therapy	Dose per Day (single drug)	Possible Adherence Problems
Indinavir (IDV)	Capsules: 100 mg, 200 mg, 333 mg and 400 mg.	- No fixed dose combinations are available.	Neonatal: Not used Paediatric: Not approved for use in children. Adolescent: 800 mg every eight hours. Adults: 800 mg every eight hours.	A liquid formulation for IDV is not available. Patients who have trouble swallowing the capsules might not adhere to the treatment regime.
Nelfinavir (NFV)	Powder for oral suspension: 50 mg one level gram scoop, 200 mg per level teaspoon. Tablets: 250 mg and 625 mg.	- No fixed dose combinations are available.	Neonatal: Not used Paediatric: (aged 2-13 years) 45-55 mg/kg twice a day. Adolescent: 1250 mg twice a day OR 750 mg three times a day. Adults: 1250 mg twice a day OR 750 mg three times a day.	

Table 1.7: (Continued)

Drug Name	Dosage Form	Combination Therapy	Dose per Day (single drug)	Possible Adherence Problems
Ritonavir (RTV)	Oral solution: 80 mg/ml. Capsules: 100 mg.	- Soft capsules: 133.3 mg Lopinavir (LPV) +33.3 mg RTV. - Oral solutions: 80 mg LPV+ 20mg/ml RTV.	Neonatal: Not approved for use in patients under the age of one month. Norvir® (ritonavir oral solution) contains 43%/v alcohol. Paediatric: (age > 1 month) 350-400 mg per m^2 of body surface area twice a day. Adolescents: 600 mg twice a day. Adults: 600 mg twice a day.	Severe nausea and vomiting occur during the first few weeks of treatment. In resource-poor settings, it is complicated to determine dose according to m^2 of body surface area, due to lack of equipment and staff.
Saquinavir (SQV)	Soft gel capsules: 200 mg Hard gel capsules: 200 mg Film-coated tablets: 500 mg.	- Capsules: 400 mg SQV + 400 mg RTV.	Neonatal: Not used Paediatric: Not used Adolescent: (age >16 years) 1200 mg (soft gel capsule) three times a day. Adults: 1200 mg (soft gel capsules) three times a day.	High pill burden (18 capsules a daily) could cause poor patient adherence. Sun exposure may cause photosensitivity reactions; sunscreen or protective clothing must be worn, which could be too hot in summer.

Table 1.8: **The different dosage forms available for Fusion Inhibitors** (United States department of health and human services: 2005:19).

Drug Name	**Dosage Form**	**Combination Therapy**	**Dose per Day (single drug)**	**Possible Adherence Problems**
Enfuvirtide (T-20)	Injection: 108 mg (reconstruction 1.1ml sterile water will deliver 90 mg/ml)	- No fixed dose combinations are available.	Neonatal: Not used Paediatric: (aged < 6 years) 2 mg/kg twice a day injected subcutaneously into the upper arm, abdomen or anterior thigh. Adolescent: (age > 16 years) 90 mg twice a day injected subcutaneously into the upper arm, abdomen or anterior thigh. Adult: 90 mg twice a day injected subcutaneously into the upper arm, abdomen or anterior thigh.	98% of patients experience local injection site reactions including: pain, erythema, nodules, pruritis and ecchymosis. Duration of the local injection site reaction is 3-7 days. If every injection causes a local injection site reaction, and each reaction takes 3-7 days to heal, the patient will be in constant discomfort and pain for the duration of the treatment.

1.7.5. PRINCIPLES FOR PAEDIATRIC TREATMENT

A set of principles for treating paediatric patients with currently available ARV formulations in resource-poor settings has been set by UNICEF and the WHO (UNICEF/WHO, 2004).

For small infants (<10 kg) syrups, solutions and dissolvable formulations containing:

- Zidovudine, abacavir and lamivudine,
- Nevirapine
- Lopinavir or Ritonavir

are listed as the best option (UNICEF/WHO, 2004).

The following ARVs are not recommended due to dispensing-, acceptability problems or need for refrigeration:

- stavudine liquid
- didanosine sachets
- nelfinavir powders.

UNICEF/WHO recommends that infants and children above 10 kg should switch to available solid dosage forms as soon as they can be tolerated. If difficulty in swallowing is experienced, dosage forms i.e. tablets, may be halved (UNICEF/WHO, 2004).

As the number of paediatric patients grows, frequent dosage changes are required, which can be a complicated matter when solid dosage forms are used. Liquid or suspension formulations can be adjusted according to weight more accurately.

UNICEF and the WHO pointed out three fixed dose combinations (FDC) already available for adults, for which a need for paediatric formulations exists:

- d4T+3TC+NVP
- ZDV+3TC+NVP
- ZDV+3TC+ABC (UNICEF/WHO: 2004).

1.8 CONCLUSION

ARV treatment should be started with one goal in mind, i.e. to ensure a better and longer quality life, for all users.

Careful consideration has been given to each of the treatments dosage forms in this chapter. Different dosage forms do play an enormous role in patient adherence. A treatment regime such as the PIs that requires a patient to swallow up to 18 tablets or capsules each day is sure to fail over a long period of time. Furthermore FIs, having only the injection as the administering route, will also be a less tolerated treatment due to the pain caused during administration.

Treatment regimes should be easily understood by the patient self, as well as by the patient's caregiver. Dosages requiring the measurement of m^2 of body surface area can easily be administered wrongly in resource-poor settings due to the lack of training or equipment (UNICEF/WHO, 2004).

HAART suggests that two NRTIs and one NNRTI are to be used in ARV therapy, as mentioned in paragraph 1.7.1. Lamivudine (NRTI), zidovudine (NRTI) and nevirapine (NNRTI) are three ARVs that are suitable for neonatal, paediatric, adult and geriatric use.

If a paediatric patient aged 4 months (weight: 6.5 kg) received HAART, the patient would be expected to take the following medication:

- Zidovudine (50 mg/5ml): 6 ml 12-hourly.
- Nevirapine (50 mg/5ml): 2.6 ml per day for 14 days, then 4.55 ml twice a day.
- Lamivudine (10 mg/ml): 2.6 ml twice a day.

The abovementioned HAART treatment is very complex and could easily be misunderstood in resource-poor settlings. Furthermore, it would add up to 26.3 ml of medication per day.

Geriatric patients receiving HAART consisting of the same drug regime will be expected to take 4 tablets daily consisting of:

- Zidovudine (300 mg) in combination with Lamivudine (150 mg): 1 tablet twice a day
- Nevirapine (200 mg): 1 tablet a day for 14 days, then 1 tablet twice a day.

There is no doubt that these patients will adhere to the treatment regime at first, but it is speculated that they will, as their health declines, have difficulty swallowing the solid dosage forms, influencing their compliance.

Fixed dose combination therapy is the answer to the question being asked all over the world, namely what is to be done to improve patient adherence when it comes to ARV treatment regimes?

Unfortunately, very few fixed dose combinations exist in the form of a solution or suspension.

An FDC containing lamivudine, zidovudine and nevirapine in the form of a powder for oral suspension would address the abovementioned problems of patient adherence to a treatment regime as it would reduce the number of daily doses significantly. It was therefore decided combine the ARVs lamivudine, zidovudine and nevirapine in a powder for oral suspension.

The chemical, physical and pharmacological characteristics of these three ARVs, will be discussed in detail in the next chapter.

CHAPTER 2

ACTIVE PHARMACEUTICAL INGREDIENTS (APIs): LAMIVUDINE, ZIDOVUDINE AND NEVIRAPINE

"It is through science that we prove, but through intuition that we discover."

- Henri Poincare-

2.1 LAMIVUDINE

Lamivudine (3TC) is a nucleoside reverse transcriptase inhibitor (NRTI), cytosine nucleoside analog that shows activity against HIV-1 and HIV-2, as well as (*in vitro*) hepatitis B virus (Yuen *et al.*, 1995:1174; Raffanti & Haas, 2001:1358).

Lamivudine has two critical centres (Yuen *et al.*, 1995:1174) which was found to be the negative enantiomer of 2'-deoxy-3'thiacytidine, which is less toxic and has greater antiviral activity than the positive enantiomer (Raffanti & Haas, 2001:1358). Through intracellular kinases, it undergoes anabolic phosphorylation to form the active metabolite, lamivudine 5'-triphosphate, which prevents HIV-1 replication (Johnson *et al.*, 1999:41).

2.1.1 CHEMICAL PROPERTIES

Lamivudine is a white to off-white crystalline solid (GlaxoSmithKline, 2003:1).

- **Molecular formula:** $C_8 H_{11} N_3 O_3 S$
- **CAS Registry Number:** 134678-17-4
- **CAS Name:** (2R-*cis)*-4-Amino-1-[-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] 2(1*H)*-primidinone
- **Additional Name(s):** (-)-2'-deoxy-3'-thiacytidine;

  3'-thia-2', 3'-dideoxycytidine;

- **CX Number:** X1010490-8
- **Molecular Weight:** 229.25
- **Percent Composition:** C 41.91%, H 4.84%, N 18.33%, O 20.94%, S 13.99%
- **Melting Range:** 172-178°C
- **Solubility:** 70 mg/ml, in water at 20°C (Merck, 1999).

2.1.2 STABILITY

Nguyen *et al.* (1995:1671-1682) conducted a study of the preservative efficacy and chemical stability of lamivudine in oral liquid formulations. The chemical stability was mainly influenced by the pH, and it was found that lamivudine stability improved when the pH increased from 4.5 to 7.5 (Nguyen *et al.*, 1995:1671-1682).

2.1.3 ANALYTICAL METHODS FOR DETECTION

Methods of identifying lamivudine in oral solution and human plasma usually include high performance liquid chromatography (HPLC) with or without ultraviolet detection (Hoetelmans *et al.*, 1998:387-394).

2.1.4 ABSORPTION, DISTRIBUTION AND ELIMINATION

- Lamivudine reaches peak plasma levels in approximately one hour and shows a high oral bioavailability of 80% in or without the presence of food (Raffanti & Haas, 2001:1359; Gibbon, 2001:313).
- Plasma protein binding of lamivudine is minimal (Gibbon, 2001:313).
- About 70% of lamivudine is primarily excreted unchanged in the urine (Raffanti & Haas, 2001:1359; Gibbon, 2001:313).

2.1.5 ADVERSE EFFECTS

In general, lamivudine tends to cause far fewer adverse effects than most other NRTIs and is therefore frequently used in combination with other ARVs when treating paediatric and geriatric patients (Gibbon, 2001:313).

The following adverse effects may occur:

- Peripheral neuropathy and pancreatitis.
- Vomiting, nausea, upper abdominal pain.
- Headache, fatigue, fever.
- Rash, pruritis and sweating.

There have been reports of elevated liver enzymes (Gibbon, 2001:313).

2.1.6 PAEDIATRIC AND ADULT DOSE

Dosing adjustments are required when treating patients with renal impairment due to HIV, chronic hepatitis and old age. An increased ability to clear the drug is shown by paediatric patients, and therefore they require a higher mg/kg dose than adults (Johnson *et al.*, 1999:41-46).

Neonates less than 30 days old are given 2 mg/kg twice daily. Paediatric patients between 3 months and 12 years of age receive 4 mg/kg twice daily with a maximum of 150 mg per day (Gibbon, 2001:313; Taketomo *et al.*, 2001:553).

The adult dose is 150 mg every 12 hours and for patients weighing less than 50 kg, 2 mg/kg 12 hourly (Gibbon, 2001:313; Taketomo *et al.*, 2001:553).

2.2 ZIDOVUDINE

Zidovudine (AZT) is a nucleoside reverse transcriptase inhibitor (NRTI), a synthetic thymidine analogue and has antiretroviral activity against HIV-1 and HIV-2, as well as human T-cell lymphotrophic virus (HTLV) (Goodman *et al.*, 2001:1353). The drug's selectivity of action against HIV can be ascribed to its high affinity as a substrate for the enzyme viral reverse transcriptase, compared to mammalian cellular DNA polymerases (Collins & Unadkat, 1989:1).

2.2.1 CHEMICAL PROPERTIES

Zidovudine is a crystalline, odourless and white to off-white solid (Sethi, 1991:732).

- **Molecular formula:** $C_{10}H_{13}N_5O_4$
- **CAS registry number:** [30516-87-1]
- **CAS names :** 3'-Azido-3'-deoxythymidine
- **Additional name(s):** Azidothymidine;

AZT

- **CX number:** X1001940-4
- **Molecular weight:** 267.24
- **Percent composition:** C 44.94%, H 4.90%, N 26.21%, O 23.95%
- **Melting range:** 121-125°C
- **Solubility:** Soluble in water, 25 mg/ml (Merck, 1999).

2.2.2 STABILITY

Zidovudine was placed in solution (isotonic phosphate buffer) with a pH of 7.4 and found to be stable. No degradation was detected within 30 hours at 37°C (Kim & Chien, 1995:1062). Kawaguchi *et al.* (1989:1944) found zidovudine to be stable in aqueous solution over the pH range 1.0-10.8.

Thermal analysis of zidovudine was conducted by Araújo *et al.* (2003:313). Zidovudine showed decomposition in three steps, the first intermediate product being thymine. Thereafter followed decomposition of thymine and elimination of a carbon residue (Araújo *et al.*, 2003:313).

2.2.3 ABSORPTION, DISTRIBUTION AND ELIMINATION

- Zidovudine is completely absorbed through the gastrointestinal tract and has a bioavailability of 65% due to first-pass

metabolism by conversion to 5-glucuronyl zidovudine (Sethi, 1991:750; Gibbon, 2001: 315; Goodman *et al.*, 2001: 1354).

- Zidovudine shows good penetration of the central nervous system and has a plasma protein binding of approximately 30% (Sethi, 1991:752; Gibbon, 2001:315).
- Zidovudine is metabolised in the liver by the enzyme glucuronidase, to the major inactive metabolite 3`-azido-3`deoxy-5-0-β-D-glucopyranosyl thymidine, which is excreted in the urine as metabolite (65-75%) and as unchanged drug (15-17%) (Sethi, 1991:753; Gibbon, 2001:315).

2.2.4 ADVERSE EFFECTS

The most common adverse effects reported with the use of zidovudine are anaemia and leucopenia, mainly neutropenia, manifesting a few weeks after starting treatment (Gibbon, 2001:315).

Other adverse effects include:

- Headache, insomnia, nausea, myalgia and rarely lactic acidosis, seizures, confusion and hepatotoxicity (Gibbon, 2001:315; Taketomo *et al.*, 2000:990).
- Zidovudine may cause muscle damage possibly by inhibiting mitochondrial DNA polymerase-γ, associated with reduced amounts of mitochondrial DNA (Raffanti & Haas, 2001: 1354).

Zidovudine should be used with caution in patients with bone marrow suppression or anaemia (Gibbon, 2001:315; Taketomo *et al.*, 2000:990).

2.2.5 PAEDIATRIC AND ADULT DOSE

When treating patients who suffer from age-related renal impairment, dosage adjustments should be considered (Gibbon, 2001:315).

The standard neonatal dose may be excessive when treating premature infants, and it is advised that the dose be changed to 1.5 mg/kg every 12 hours

from birth to 2 weeks, thereafter, 2 mg/kg every 8 hours (Taketomo *et al.*, 2000:991). Paediatric dose for children between 3 months and 12 years is 180 mg/m^2 of body surface area every 12 hours, with a maximum of 800 mg per day (Gibbon, 2001:315).

The oral adult dose is 300 mg every 12 hours, which can be reduced to 250 mg 12-hourly if needed. Mother-to-child transmission prevention requires an oral dose of 300 mg 12-hourly for the last 4 weeks of pregnancy, to be increased to 300 mg every 3 hours from the onset of labour to delivery (Gibbon, 2001:315).

2.3 NEVIRAPINE

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that also has potent activity against HIV-1, but has no mentionable activity against HIV-2 or other retroviruses (AIDS Information Center, 1999). Nevirapine binds directly to the allosteric site on reverse transcriptase and inhibits the activities of both RNA and DNA dependent DNA polymerase (Kaul *et al.*, 2004:843). The drug works by diffusing into the cell and binding to the enzyme, reverse transcriptase, causing inactivation of the enzyme due to conformational changes (AIDS Information Center, 1999).

2.3.1 CHEMICAL PROPERTIES

Nevirapine is derived from the series of dipyridodiazepones and is presented as the free base form. There is one polymorphic form and one pseudopolymorphic form in which the active substance can exist; anhydrous and hemihydrate. Both forms are white to off-white crystalline powders (Viramune: Scientific Discussion, 2000).

- **Molecular formula:** $C_{15}H_{14}N_4O$
- **CAS registry number :** [129618-40-2]
- **CAS names :** 11-Cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2`,3`-*e*][1,4]diazepin-6-one
- **Additional names:** BI-RG-587

- **Molecular weight :** 266.30
- **Percent composition:** C 67.65%, H 5.30%, N 21.04%, O 6.01%.
- **Melting range :** 247°-249°C
- **Solubility:** Almost insoluble in water (0.1 mg/ml), sparingly soluble in dichloromethane R and methanol R (Merck, 1999).

2.3.2 STABILITY

The anhydrous form of nevirapine was found to be non-hygroscopic and even when exposed to 92% relative humidity for 24 months at 30°C, conversion to the hemihydrate did not occur (Viramune: Scientific Discussion, 2000).

Stability tests were done on several pilot and production size batches of nevirapine, and results show that nevirapine was extremely stable during the study of 24 months under stressed conditions. No degradants were detected (Viramune: Scientific Discussion, 2000).

Chan Li *et al.* (2000:249) subjected nevirapine samples to stress by adding base, acid, UV light, hydrogen peroxide, as well as heat and humidity. An assay was performed on the stressed samples to determine the percentage of remaining nevirapine. Table 2.1 summarizes the stress conditions together with the results obtained.

Table 2.1: The influence of various stress conditions on the stability of nevirapine samples (Chan Li *et al.*, 2000:249).

Stress conditions	Duration	Peak integrity	% Nevirapine remaining
1N HCl	31 days	pure	29.64
1N NaOH	31 days	pure	81.50
3% H_2O_2	31 days	pure	71.75
Light (600 foot candles)	31 days	pure	99.99
Temperature and humidity 40°C/75%RH	31 days	pure	99.81

Table 2.1 illustrates that nevirapine is more stable at basic pH conditions and is not influenced by excessive light or humidity.

2.3.3 ABSORPTION, DISTRIBUTION AND ELIMINATION

- Nevirapine is rapidly and readily absorbed after oral administration and has a bioavailability of less than 90%.
- It is widely distributed with a V_d of 1.21 ℓ/kg, crosses the placenta and is excreted in the breast milk.
- 45% of the plasma concentration is present in the cerebrospinal fluid, protein binding is 60%.
- Nevirapine is metabolised by the cytochrome P-450 isozymes, metabolism occurs more rapidly in paediatric patients than in adults.

- $T_{½}$ = 45 (single dose), auto induction of the metabolism occurs after 2-3 weeks and causes $T_{½}$ to decrease to 25-30 hours after multiple dosing.
- 81.3% is excreted in the urine as metabolites, 10.1% in the faeces and more than 3% is excreted in the urine as parent drug (Taketomo *et al.*, 2000:684; Gibbon, 2001:316).

2.3.4 ADVERSE EFFECTS

Of all of the adverse effects of nevirapine, the most infamous of all is the rash that occurs in the first six weeks of treatment. It usually manifests as maculopapular erythematous cutaneous eruptions with or without pruritis, and can be seen on the face, trunk and extremities (Taketomo *et al.*, 2000:684).

Other frequent adverse effects include:

- Fever, headache, sedation, nausea, diarrhoea, elevated liver enzymes and myalgia (Gibbon, 2001:316; Taketomo *et al.*, 2000:684).

2.3.5 PAEDIATRIC AND ADULT DOSE

Nevirapine must be administered with caution in patients suffering from renal of hepatic failure or dysfunction (Taketomo *et al.*, 2000:684). It is also advised that frequent liver function monitoring should take place during treatment. When patients present with a severe rash or rash accompanied by abnormal liver function tests, treatment must be discontinued immediately (Gibbon, 2001:316).

When nevirapine is administered orally, neonates to three months receive 5 mg/kg once daily for 14 days, followed by 120 mg/kg twice a day for 14 days, then 200 mg/m^2 every 12 hours (Taketomo *et al.*, 2000:684).

An initial dose of 4 mg/kg once a day for 14 days is prescribed for children aged between two months and eight years. If no rash or adverse effects occur, the dose can be increased to 4 mg/kg twice daily. The maintenance dose

for this age group is 7 mg/kg every 12 hours, the maximum dose being 200 mg every 12 hours (Gibbon, 2001:316; Taketomo *et al.*, 2000:684).

Adults and adolescents are treated with an initial dose of 200 mg once a day for 14 days. In the absence of a rash and adverse effects, the dose can be increased to a full dose, with a maximum of 200 mg every 12 hours (Gibbon, 2001:316; Taketomo *et al.*, 2000:684).

2.4 CONCLUSION

The chemical stability and dosage regime for each of the three chosen ARVs was discussed. In theory, the chemical characteristics of these three APIs lead us to believe that they seem to be compatible, and seem to be able to remain stable when combined in an ARV powder for oral suspension.

In the next chapter, characteristics and advantages of the oral suspension will be discussed along with the excipients needed to formulate a pharmaceutically elegant suspension.

CHAPTER 3

SUSPENSION FORMULATION

**"Your results should be reproducible-
all experiments should fail in the same manner."**

- Anon-

3.1 THE PHARMACEUTICAL SUSPENSION

Suspensions can be defined as "preparations containing finely divided drug particles distributed somewhat uniformly throughout a vehicle in which the drug exhibits a minimum degree of solubility" (Ansel, 1976:142).

There are two basic forms of suspensions commercially available:

- Ready-to-use: the active substance is already suspended or dispersed in a solution, with or without excipients or stabilisers.
- Dry oral powders: containing the active ingredient and the excipients used to ensure proper suspending or dispersing of the active substance, which immediately, when in contact with the proper dilution agent e.g. distilled water, results in a proper suspension suitable for immediate oral administration (Ansel, 1976:142).

3.1.1 ADVANTAGES OF ORAL SUSPENSIONS

The advantages of the use of liquid oral suspensions are endless and depend somewhat on the characteristics of the active ingredient used in the particular suspension.

One of the main reasons for preparing an oral suspension is the instability of certain actives in solution and their stability in suspension. In this instance the oral suspension provides chemical stability whilst a liquid dosage form is still provided (Ansel, 1976:143).

Patients who suffer from HIV present with minor to major oral and throat infections, which cause severe pain when swallowing items such as food, tablets or capsules. An oral suspension is more suitable for these patients.

Further advantages include:

- Liquid dosage forms provide greater convenience in administration when very large doses are required.
- Medicine can be administered to infants and children with ease (Ansel, 1976:143).

3.1.2 DESIRED CHARACTERISTICS OF A PHARMACEUTICAL SUSPENSION

When developing a pharmaceutically elegant delivery system, various factors should be taken into consideration. Amongst other things, the system should be chemically stable, acceptable to the user, and therapeutically efficient, to name but a few (Ansel, 1976:143).

Features that apply to pharmaceutical suspensions are:

- A slow settling rate.
- The suspension should be readily suspended when shaken gently.
- The particle size of the suspended powder should remain more or less constant throughout long periods of storage.
- When the suspension is poured, it must flow evenly from the container (Ansel, 1976:143).

Pharmaceutical excipients make it possible for the formulator to create an elegant suspension that exhibits all of these characteristics.

3.2 PHARMACEUTICAL EXCIPIENTS

An excipient can be defined as "an inert substance, which is added to a drug to provide bulk, e.g. in tablet formulae" (Pharmaceutical Technology: 2003).

According to the International Pharmaceutical Excipient Council (IPEC) pharmaceutical excipients are: "Any substance other than the active drug or pro-drug which has been appropriately evaluated for safety and is included in a drug delivery system to either:

- Aid processing of the system during manufacture, or
- Protect, support or enhance stability, bioavailability or patient acceptability, or
- Assist in product identification, or
- Enhance any other attribute of the overall safety and effectiveness of the drug product during storage or use" (Garg *et al.*, 2001:15).

Various excipients were used to ensure that the formulated ARV suspensions comply with these standards for a pharmaceutical suspension.

The excipients were:

- Sodium saccharin
- Sorbitol
- Sodium citrate
- Citric acid
- Methylcellulose
- Sodium methyl hydroxybenzoate
- Sodium propyl hydroxybenzoate
- Flavourant (passion fruit)

Each of the excipients used will be discussed shortly in the following paragraphs.

3.2.1 SODIUM SACCHARIN

Sodium saccharin is an intense sweetener regularly used in food products, drinks, oral hygiene products and a range of other generally used products. It has a sweetening power more than 500 times that of sucrose.

Its main function in pharmaceutical formulations, is masking unpleasant taste characteristics or to enhance flavour systems (Russel & Thurgood, 2003:532). This makes it the ideal sweetener for a formulation containing the extremely bitter nevirapine API.

3.2.1.1 DESCRIPTION

Sodium saccharin is a white odourless crystalline powder with an intense sweet taste, and a metallic aftertaste (Russel & Thurgood, 2003:532).

3.2.1.2 STORAGE AND STABILITY

Under normal conditions, sodium saccharin tends to be quite stable. No decomposition is found when it is stored in bulk. The decomposition product, ammonium-*o*-sulpho benzoic acid, is formed when exposed to high temperatures (125°C) and low pH (pH 2) for an hour or longer (Russel & Thurgood, 2003:533).

Sodium saccharin should be stored in a dry, cool place, in a tightly sealed container (Kibbe, 2000:262).

3.2.1.3 SAFETY AND ADVERSE EFFECTS

Considerable controversy has arisen surrounding the question of safety of sodium saccharin since the late 1970s. This has led extensive research into this topic. The World Health Organization (WHO) has published a temporary maximum daily intake for sodium saccharin, namely 2.5 mg/kg body weight (Russel & Thurgood, 2003:533).

The Committee on Toxicity of Chemicals in food, Consumer Products and Environment (COT) has set the maximum daily intake for sodium saccharin at up to 5 mg/kg body weight.

Adverse effects of sodium saccharin include:

- Urticaria with pruritis, and
- Photosensitisation reactions (Russel & Thurgood, 2003:532).

3.2.2 SORBITOL

Sorbitol is commonly used in pharmaceutical and cosmetic formulations as a sweetening agent, plastieizer and humectant. It is used in this particular formulation for it`s sweetening characteristics, as well as its stabilising effect on drug suspensions (Nash. 2003:596).

3.2.2.1 STORAGE AND STABILITY

Sorbitol is hygroscopic and should be stored in an airtight container, in a cool dry surrounding. Because sorbitol is chemically inert, it can be used in formulations with nearly every other excipient (Nash. 2003:597).

3.2.2.2 SAFETY AND ADVERSE EFFECTS

Sorbitol occurs naturally in different fruits and berries, and is absorbed from the gastrointestinal tract more slowly than sucrose. It is also better tolerated by diabetics than sucrose (Nash, 2003:598).

It is advised that sorbitol be used in quantities no greater than 20 g/day in adults. Sorbitol is metabolised to fructose by the liver and can be dangerous when administered to fructose-intolerant patients (Winek, 1999:68).

3.2.3 SODIUM CITRATE

Sodium citrate is mainly used in the food and medicinal industries as an alkalising agent, emulsifying agent and sequestering agent. It was included in these ARV formulations because of its capacity to act as a buffering agent (Amidon, 2003:560).

Many of the excipients included in the suspensions function best within a specific pH range (Amidon, 2003:560) and it was found that the chemical stability of lamivudine was mainly influenced by the pH. Stability improved when the pH increased from 4.5 to 7.5 (Nguyen *et al.*, 1995:1671-1682).

3.2.3.1 STORAGE AND STABILITY

Sodium citrate is a stable material which should be stored in airtight containers in a cool dry place (Amidon, 2003:561).

3.2.3.2 SAFETY AND ADVERSE EFFECTS

Although sodium citrate can be regarded as a safe, non-toxic and non-irritant excipient, extreme consumption may cause general gastrointestinal discomfort and diarrhoea (Amidon, 2003:561).

Adult doses are as much as 15 g of sodium citrate per day, in divided doses (Amidon, 2003:561).

3.2.4 CITRIC ACID

Citric acid is widely used in the pharmaceutical industry as an acidifying agent or buffering agent. Other functional categories include antioxidants, plasticiser and flavour enhancer (Amidon, 2003:158). It was included into this formulation because of its buffering and flavour enhancing capacity.

3.2.4.1 STORAGE AND STABILITY

Bulk citric acid should be stored in a cool dry place in an airtight container. Citric acid tends to loose water of crystallisation in dry air, or if temperatures rise to 40°C and higher (Amidon, 2003:159).

3.2.4.2 SAFETY AND ADVERSE EFFECTS

Citric acid is generally regarded as a nontoxic excipient when consumed orally. It occurs naturally in the human body, especially in the skeleton. Frequent consumption may lead to teeth erosion (Amidon, 2003:159).

Patients who suffer from renal failure and receive aluminum compounds to control phosphate absorption are advised not to consume citric acid or products containing citric acid as an excipient (Amidon, 2003:159).

3.2.5 METHYLCELLULOSE

Methylcellulose is commonly used in oral, as well as topical pharmaceutical formulations. Different grades of viscosity of methylcellulose are used in various pharmaceutical formulations. High viscosity grades are used in tablet formulations as disintegrators, low to medium grades function as binding agents and low viscosity grades as suspending or thickening agents in oral suspensions (Allen & Luner, 2003:386).

Of the three actives present in the formulated ARV suspensions, nevirapine has the poorest solubility characteristics, and thus it was of cardinal importance than a suspending agent was added to ensure that the correct dose of nevirapine was administered to the patient.

3.2.5.1 STORAGE AND STABILITY

Methylcellulose powder shows slight hygroscopic characteristics and should be stored in an airtight container. In solution, it seems to be very stable between the pH values of 3 to 11 at 25°C. When exposed to conditions of extreme acidity, the viscosity of methylcellulose is reduced (Allen & Luner, 2003:387).

Methylcellulose solutions tend to be affected by microbial spoilage, and it is advised that an antimicrobial preservative should be present in such formulations (Allen & Luner, 2003:387).

3.2.5.2 SAFETY AND ADVERSE EFFECTS

Methylcellulose is widely used in food, as well as cosmetic formulations, and is regarded as a non-irritant, generally safe substance (Allen & Luner, 2003:388). Most of the adverse effects listed below relate to the excessive use of methylcellulose as a bulk laxative and are not applicable when used as an excipient in pharmaceutical suspension formulations (Allen & Luner, 2003:388).

- Flatulence
- Oesophageal obstruction
- Gastrointestinal distension.

3.2.6 SODIUM METHYL HYDROXY BENZOATE AND SODIUM PROPYL HYDROXYBENZOATE

Due to the use of excipients that are sensitive to microbial organisms it is important to include an antimicrobial preservative to improve pharmaceutical stability of the suspension. Sodium methyl hydroxybenzoate is such an agent, which can be used alone or in combination with other hydroxybenzoates such as propyl hydroxybenzoate (Rieger, 2003:392).

Sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate sodium salts are more soluble than the other hydroxybenzoate salts (Rieger, 2003:392), and were therefore used in these particular ARV suspensions.

3.2.6.1 STORAGE AND STABILITY

Aqueous solutions at pH values ranging from 3 to 6 were shown to be stable for up to four years at room temperature. The hydroxybenzoates are most active against microbials at pH levels ranging from 4 to 8, and are active against yeasts and moulds, but less active against bacteria. Gram-positive bacteria are more affected by the preservatives than gram-negative bacteria (Rieger, 2003:391).

3.2.6.2 SAFETY AND ADVERSE EFFECTS

Sodium methyl- and sodium propyl hydroxybenzoate are widely used as antimicrobials in food, medicinal and cosmetic formulations. To date no systemic reactions to hydroxybenzoates have been reported. The WHO has set acceptable daily intake for sodium methyl- and sodium propyl hydroxybenzoate at no more than 10 mg/kg body weight (Rieger, 2003:392).

3.3 CONCLUSION

The excipients discussed, have characteristics that make their inclusion in the formulation for an ARV powder for oral suspension extremely valuable.

In theory, the combination of lamivudine, Zidovudine, nevirapine and these excipients looks promising. However, it remains important to conduct a compatibility study to prove what was found in theory.

Compatibility studies were conducted with the aid of differential scanning calorimetry (DSC) and high performance liquid chromatography (HPLC), and will be discussed in chapter 4.

CHAPTER 4

PREFORMULATION

"Why think? Why not try the experiment?"

- John Hunter-

Synthetic and organic programs initiated by several companies between the 1950's and 1960's, paved the way for preformulation. Pharmacists were faced with the task of rapid formulation and needed a fast and dependable way to formulate intelligently (Carstensen & Rhodes, 2000:238).

When attempting to recognise an incompatibility in a 10-component product, many hours in the laboratory are lost. It would therefore be sensible and cost effective to prematurely investigate the drugs' incompatibilities beforehand (Carstensen & Rhodes, 2000:238).

4.1 GOALS FOR PREFORMULATION

Preformulation studies aim to:

- Determine the compatibility of the APIs with commonly used excipients.
- Establish the kinetic rate profile of the actives.
- Identify the physical characteristics of the actives.
- Ascertain the essential physicochemical parameters of a new drug substance (Carstensen & Rhodes, 2000: 239).

4.2 COMPATABILITY TESTING

Before attempting the initial formulation the majority of product development groups perform compatibility tests. The standard is to make up a mixture of 1:1 drug and

excipient to determine which excipients may be used with the drug (Carstensen & Rhodes, 2000:252).

Commonly used methods include chemical assay, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), differential scanning calorimetry (DSC) and microcalorimetric methods (Carstensen & Rhodes, 2000:252).

The methods chosen for this preformulation study were DSC and HPLC.

4.2.1 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

During the formulation of new drug products, it is useful to have readily accessible knowledge of any drug – drug, or excipient – drug interactions (Botha *et al.*, 1987:346).

Differential scanning calorimetry (DSC) is an accurate and straightforward method of optimising formulations (Ultrasensitive Calorimetry for the Life Sciences, 2005). Potential compatibilities and incompatibilities of several commonly used pharmaceutical excipients can be evaluated by using DSC (Mura *et al.*, 1998:747).

Interpretation of thermal data is not always simple. Nevertheless, DSC screening for compatibility provides further knowledge concerning possible interactions (Botha *et al.*, 1987:347).

A study to determine the compatibility of picotamide with common excipients namely palmitic acid, stearic acid, stearyl alcohol, PEG 20 000 and sorbitol, was carried out using thermal analysis by Mura *et al.* (1998:747). Results showed that interactions were mainly due to dissolution in the melted excipient.

Botha *et al.* (1987:345) conducted a DSC study to identify possible drug – drug interactions in polypharmaceuticals intended for the alleviation of the symptoms of colds and flu. Results indicated that several drugs used in the formulation were incompatible.

4.2.1.1 MATERIALS

The compatibility of Lamivudine, zidovudine and nevirapine with the following excipients was examined: sodium saccharin, sorbitol, sodium citrate, citric acid, methylcellulose, sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate.

4.2.1.2 METHOD

Lamivudine, zidovudine, nevirapine and excipients were combined in physical mixtures of ratios 1:1 active and excipient. The samples were considered uniform.

Weighed samples (5 – 10 mg, Mettler M3 Microbalance, Schwerzenbach, Switzerland) of the individual components and physical mixtures were scanned in aluminium pans with a perforated lid in the 25 - 300°C temperature range under static air. The Shimadzu DSC – 50 (Shimadzu, Tokyo, Japan) was used, nitrogen purge flow was 35 ml/min and the heating rate was 10°C/min. Indium was used for calibration purposes. At least two replicates were made for each DSC thermogram.

4.2.1.3 RESULTS

From the results summarised in table 4.1, it is evident that the only incompatibility detected using DSC studies was between zidovudine and citric acid.

In figure 4.1 the DSC thermogram of the interaction between zidovudine and citric acid is given.

4.2.1.4 DISCUSSION

When two substances are mixed, the purity of each of the involved substances is reduced, which generally results in lower melting points (Botha *et al.*, 1987:347). Incompatibilities can be identified when the peaks

- Disappear
- Shift
- Grow larger
- Another peak appears.

In figure 4.1.1 the exothermic reaction (c) is the thermogram of citric acid. It is clear that in thermogram (b), the physical mixture of zidovudine and citric acid, the exothermic reaction is not as clear as in thrmogram (c). This may indicate an incompatibility.

Table 4.1: DSC results of the compatibility study between lamivudine, zidovudine, nevirapine and various excipients.

APIs and Excipients	Active Pharmaceutical Ingredients (APIs)		
	Lamivudine	Zidovudine	Nevirapine
Lamivudine	_	√	√
Zidovudine	√	_	√
Nevirapine	√	√	_
Sorbitol	√	√	√
Sodium saccharin	√	√	√
Sodium citrate	√	√	√
Citric acid	√	X	√
Sodium methyl hydroxybenzoate	√	√	√
Sodium propyl hydroxybenzoate	√	√	√
Methylcellulose	√	√	√
Flavourant	√	√	√

X - Incompatible

√ - no interaction, therefore compatible

Thermogram (a) represents zidovudine. It is clear that in thermogram (b) the melting endotherm of zidovudine is minimized and shifted to a lower temperature range. This may indicate an incompatibility.

The extreme conditions that the samples were subjected to during DSC testing were extraordinary. During formulation and patient administration the formulation would never be under similar extreme conditions. These extremities may be the cause of the interactions.

It was decided to perform further studies with the aid of HPLC.

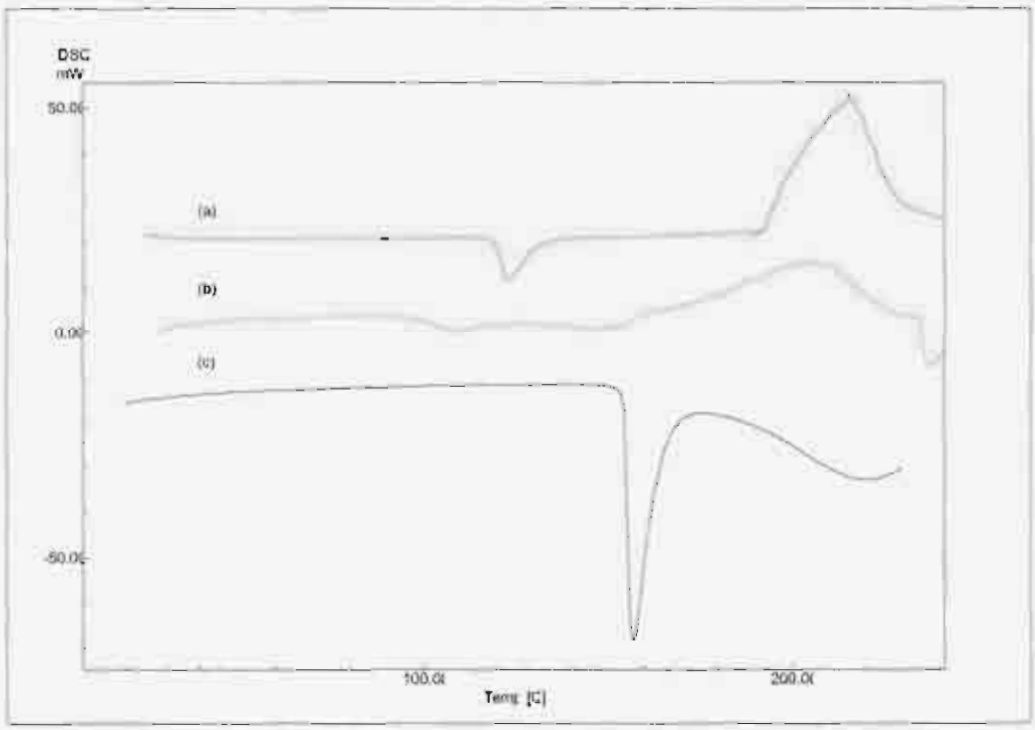

Figure 4.1: DSC thermograms of zidovudine (a), citric acid (c) and 1:1 physical mixture of zidovudine and citric acid (b).

4.2.2 HIGH PEREFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High performance liquid chromatography has many applications, including identification, separation, purification and quantification of various compounds

(Applications for HPLC:2005). Each compound should have a characteristic peak under certain chromatographic conditions (Applications for HLPC, 2005).

4.2.2.1 METHOD

Samples containing 1:1 ratios of active and excipient were prepared and diluted with methanol to 100 ml as stipulated in annexure G, paragraph 2.

The Hewlett Packard Agilent 1100 series HPLC was used together with the G1322A degasser, G1312A binary pump and the G1329A ALS autosampler. Chromatographic conditions are described in Annexure G.

4.2.2.2 RESULTS

No incompatibility between lamivudine, zidovudine, nevirapine and the various excipients could be detected by HPLC studies (table 4.2). In figures 4.2 and 4.3 the chromatograms of zidovudine and the physical mixture of zidovudine and citric acid can be seen.

Table 4.2: HPLC results of the compatibility study between lamivudine, zidovudine, nevirapine and various excipients.

APIs and Excipients	Active Pharmaceutical Ingredients (APIs)		
	Lamivudine	Zidovudine	Nevirapine
Lamivudine	_	√	√
Zidovudine	√	_	√
Nevirapine	√	√	_
Sorbitol	√	√	√
Sodium saccharin	√	√	√
Sodium citrate	√	√	√
Citric acid	√	√	√
Sodium methyl hydroxybenzoate	√	√	√
Sodium propyl hydroxybenzoate	√	√	√
Methylcellulose	√	√	√
Flavourant	√	√	√

√ - no interaction, therefore compatible

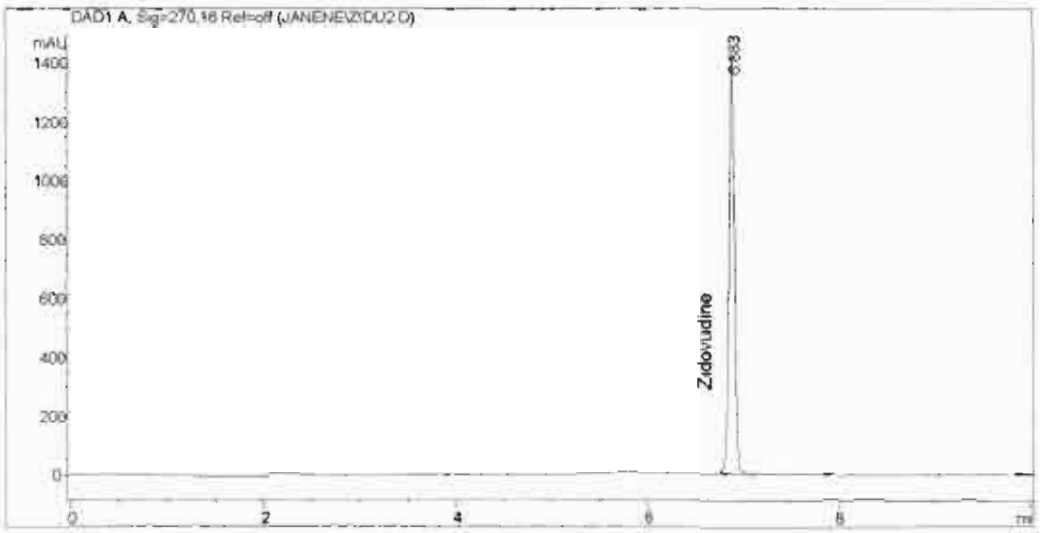

Figure 4.2: HPLC chromatogram of zidovudine during the HPLC compatibility study.

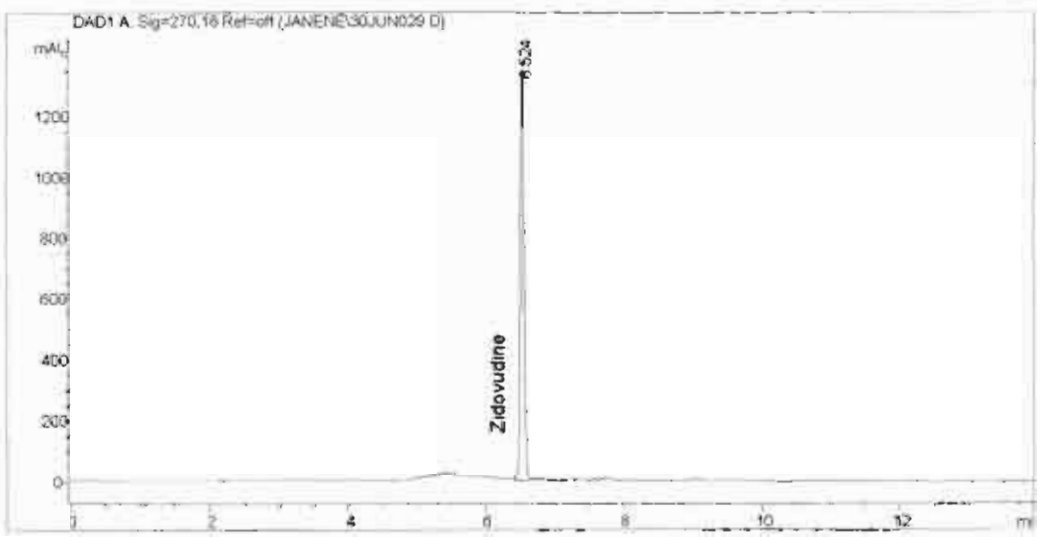

Figure 4.3: HPLC chromatogram of a 1:1 mixture of zidovudine and citric acid during the HPLC compatibility study.

4.2.2.3 DISCUSSION

The HPLC compatibility tests confirmed the DSC results obtained for lamivudine and nevirapine, but showed compatibility between zidovudine and citric acid. Peak purity was performed to determine the purity of the zidovudine peak, and it was found to be satisfactory.

After careful consideration of both the DSC and HPLC results, it was decided to include citric acid the formulations as discussed in chapter 5.

4.3 CONCLUSION

The aim of this chapter was to determine the compatibility or incompatibility of lamivudine, zidovudine, nevirapine and excipients. Two analytical procedures were preformed, namely DSC and HPLC.

Results obtained from DSC testing provided no evidence of incompatibility between lamivudine, nevirapine and the excipients. Zidovudine was compatible with all excipients other than citric acid. A further compatibility study was preformed by using HPLC to verify the results obtained through DSC studies.

The HPLC compatibility tests provided results that confirmed those obtained through DSC for lamivudine, nevirapine and excipients. Results obtained for zidovudine compatibility with citric acid through HPLC testing was different from results obtained from DSC screening. HPLC provided evidence of a compatibility between zidovudine and citric acid.

The extreme conditions to which samples were subjected to during DSC testing were extraordinary. During formulation and patient administration the formulation would never be under similar extreme conditions. These extremities may be the cause of the interactions.

It was therefore decided to include citric acid in the formulations for ARV powder for suspension for paediatric, adult and geriatric patients.

Determination of an ideal mixing time for the bulk formulated powders is also discussed. Discussions of the specific formulas for ARV powders for suspension, together with the dosage regimes and containers follow in chapter 5.

CHAPTER 5

ANTIRETROVIRAL SUSPENSION FORMULATION

"Anything can be made to work if you fiddle with it long enough"

-Wyszowski's Law-

Powders for oral suspension were formulated, as shown in Table 5.5. In order to ensure that an optimum amount of each of the APIs, as well as each preservative is present in every container, an ideal mixing time had to be determined. The mixing time experiment will be discussed in paragraph 5.5 and onward.

5.1 MATERIALS USED IN THE FORMULATIONS

5.1.1 ACTIVE PHARMACEUTICAL (APIs) INGREDIENTS

Details of the APIs that were used in all the formulations in this study are given in table 5.1.

Table 5.1: **APIs used in the formulations.**

APIs	Supplier	Batch Number	Grade
Lamivudine	Xiamen Mchem Laboratories Ltd, Yiemen, China.	040801	99.9%
Zidovudine	Xiamen Mchem Laboratories Ltd, Yiemen, China.	040903	100.4%
Nevirapine (Anhydrous)	Fischer Chemicals AG, Zürich, Switzerland.	100003	99.2%

5.1.2 PRESERVATIVES

Table 5.2 lists the preservatives used in all ARV formulations.

Table 5.2: Preservatives used in the formulations.

Preservative	Supplier	Grade
Sodium methyl hydroxybenzoate	Merck, Damstadt, Germany.	USP
Sodium propyl hydroxybenzoate	Merck, Damstadt, Germany.	USP

5.1.3 EXCIPIENTS

Table 5.3 lists all the excipients used in the ARV formulations.

Table 5.3: Excipients used in the formulations.

Material	Supplier	Function	Grade
Sorbitol	Cerestar, Vilvoorde, Belgium.	Sweetener	98%
Sodium saccharin	Merck, Damstadt, Germany.	Sweetener	USP
Citric acid	Roche, Switzerland.	Flavour enhancer	USP
Sodium citrate	Merck, Damstadt, Germany.	Buffering agent	USP
Methylcellulose	Shin-Etsu Chemical Co, Ltd, Niigata, Japan.	Suspending agent	USP
Flavourant (Passion fruit)	International Flavours and Fragrances, South Africa.	Flavourant	USP

5.2 PAEDIATRIC ARV DOSE RANGES

The six different ARV powder formulations for suspension were designed, based on recommendations made by the WHO during a technical consultation in Geneva (UNICEF/WHO: 2004). Five of the formulations covered the different weight stages during the first years of the paediatric patient's life. The sixth formulation addressed the need of adult and geriatric patients experiencing difficulty when swallowing solid dosage forms. Table 5.4 contains the dosage regime for each weight category as specified by the WHO.

Table 5.4: ARV dosage range as specified by UNICEF/WHO (2004).

Weight (kg)	Dose	Lamivudine	Zidovudine	Nevirapine
3-6	2.5 ml BD*	15 mg/2.5 ml	-------	40 mg/2.5 ml
6-10	5 ml BD	25 mg/5 ml	50 mg/5 ml	75 mg/5 ml
10-15	7 ml BD	50 mg/7 ml	100 mg/7 ml	100 mg/7 ml
15-20	10 ml BD	75 mg/10 ml	100 mg/10 ml	150 mg/10 ml
20-29	15 ml BD	100 mg/15 ml	200 mg/15 ml	100 mg/15 ml
Adults	16.6 ml BD	150 mg/16.6 ml	275 mg/16.6 ml	200 mg/16.6 ml

*BD- Twice a day.

5.3 CONTAINERS

Clear PVC was chosen for the containers for the powders for suspension due to its durability. The target group of the patients using the ARV treatment live in resource poor settings and, in most cases, are only able to visit the clinic or hospital once a month.

Glass containers have a higher risk of shattering, and in such cases, the ARV suspension would be unsuitable for use, leaving the patient without medicinal care for a period of up to one month.

A transparent container was chosen to enable the patient or caregiver to identify and rectify sedimentation. This will ensure that the patient receives a more uniform dose. Other advantages are that the patient or caregiver may quickly identify changes such as separation of the suspension and physical changes such as colour changes.

Six different sized containers were needed for the six different volumes of suspension. Unfortunately, the volumes of the formulated suspensions were non-standard and PVC containers matching the volume of every suspension could not be found.

Containers with volumes of 150 ml, 300 ml, 500 ml and 1000 ml were used.

5.4 METHOD OF FORMULATION

The objective for the formulation of the powders for suspension was to provide patients with ARV treatment in suspension form for a period of one month. Therefore, based on the information given in table 5.4, the volume needed for patients (3-6 kg) would be the daily dose (5 ml) multiplied by 30 days, thus 150 ml (1 month's supply). The volumes for the other dosage ranges were determined in the same manner. The final formulae for the powders for suspension are given in table 5.5. The specific volumes of water necessary for reconstitution of the suspension are given in table 5.6.

Bulk quantities of these formulations were prepared, and underwent thorough mixing. The mixing process and the determination thereof are discussed in paragraph 5.5. The correct amount of mixed powder was placed into different sizes of clear PVC bottles (as discussed in paragraph 5.3) and sealed with white PVC screw tops.

Table 5.5: ARV powder for suspension formulations.

	Amount per volume of reconstituted suspension					
APIs and Excipients	**150 ml**	**300 ml**	**420 ml**	**600 ml**	**900 ml**	**1000 ml**
Nevirapine	2.40 g	4.50 g	6.00 g	9.00 g	6.00 g	12.00 g
Zidovudine	-------	3.00 g	6.00 g	6.00 g	12.00 g	16.5 g
Lamivudine	0.90 g	1.50 g	3.00 g	4.50 g	6.00 g	9.00 g
Sorbitol	60.0 g	120 g	168 g	240 g	360 g	400 g
Sodium saccharin	0.225 g	0.45 g	0.70 g	0.90 g	1.35 g	1.50 g
Sodium citrate	1.50 g	3.00 g	4.20 g	6.00 g	6.00 g	6.00 g
Citric acid	1.05 g	2.10 g	2.94 g	4.20 g	6.30 g	7.00 g
Sodium methyl hydroxybenzoate	0.225 g	0.45 g	0.63 g	0.90 g	1.35 g	1.50 g
Sodium propyl hydroxybenzoate	0.03 g	0.06 g	0.084 g	0.12 g	0.18 g	0.20 g
Methylcellulose	0.75 g	1.50 g	2.10 g	3.00 g	4.50 g	5.00 g
Flavourant	0.26 g	0.52 g	0.728 g	1.04 g	1.56 g	1.73 g

Table 5.6: Reconstitution of the ARV suspension.

ARV suspension formulation (ml)	Amount of purified water needed for reconstitution of the suspension (ml)
• 150	100
• 300	200
• 420	281
• 600	400
• 900	598
• 1000	667

5.5 DETERMINATION OF MIXING TIME

In pharmacy, the mixing objectives during every formulation process are, 1) to obtain dosage units that will each hold the identical quantity of active substance and, 2) to produce thousands of these uniform batches (Lantz & Schwartz, 1990:1).

Unfortunately, the mixing process will never yield a perfect mix. Perfect meaning "that state in which any sample removed from the mixture will have exactly the same composition as all the other samples taken from the mix" (Lantz & Schwartz, 1990:1).

There are alternatives for creating the ideal mix. These are randomisation and ordered mixing (Lantz & Schwartz, 1990:13).

Ordered mixing comprises various mechanisms for creating a nearly perfect mix, namely mechanical mixing, coating, and adhesion (Lantz & Schwartz, 1990:13). Mechanical mixing was used to mix these particular ARV formulations. This can be described as follows:

- the desired mixture is obtained by dividing and recombining the powder substances until the desired subdivision unit is obtained. However, if particle adhesion is not present, segregation of the mix could take place when handled (Lantz & Schwartz, 1990:15).

5.5.1 EXPERIMENTAL PROCEDURE

Due to the extremely high cost of the active substances present in the ARV formulations, it was decided that the mixing experiment would be conducted using the formulation for the 300 ml ARV suspension, and not for each formulation separately. The data retrieved would represent the mixing time used for all the formulations.

The formulation for the 300 ml ARV suspension was prepared eightfold and placed in eight separate, clearly marked glass jars. Each jar was emptied into the mixer separately, and the mixer was turned on for the specified duration. The eight jars represented eight different mixing time trials, namely 2, 4, 6, 8, 10, 12, 14 and 16 minutes.

Samples were taken after each time trial from the left, right, and middle parts of the mixer.

The Turbula rx 20003 mixer (Switzerland) was used in the experiment. Rotation speed was set at 40 rpm and samples were taken from different parts of the mixer container at each specified time interval. The samples were assayed for API content according to the HPLC method described in Annexure G, and the ideal mixing time was determined.

5.5.2 RESULTS

The results obtained by using HPLC analysis to determine the amount of active present in every sample are given in table 5.7.

Table 5.7: Theoretical values and results of the determination of mixing time for powder for suspension.

Actives	Theoretical Value	Mixing Time (minutes) and Experimental Values (mg /5ml).							
		2 min	4min	6min	8min	10min	12min	14min	16min
Lamivudine	25mg/5ml	20.4	24.8	25.3	26.5	28.9	31.1	25.7	23.5
Zidovudine	50mg/5ml	28.2	37.9	49.2	49.8	56.0	59.5	46.7	50.9
Nevirapine	75mg/5ml	68.9	71.3	75.9	76.6	87.9	82.7	72.8	69.1
Sodium methyl hydroxybenzoate	0.15%m/v	0.07	0.09	0.10	0.14	0.16	0.15	0.14	0.19
Sodium propyl hydroxybenzoate	0.02%m/v	0.08	0.03	0.03	0.02	0.03	0.03	0.02	0.03

5.5.2.1.1 APIs

The results of the mixing time for lamivudine, zidovudine and nevirapine are presented graphically in figure 5.1.

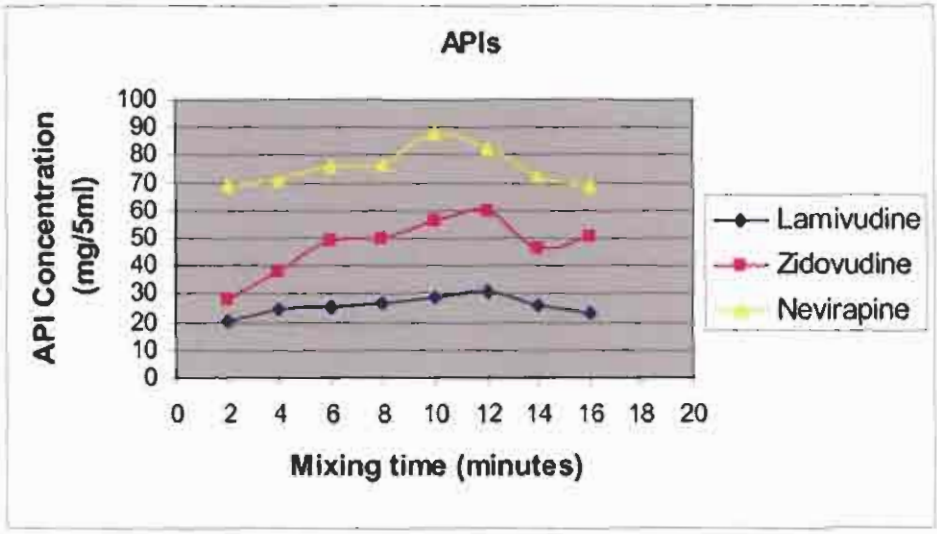

Figure 5.1: Graph of the mixing time results obtained for lamivudine, zidovudine and nevirapine.

5.5.2.1.1 DISCUSSION

The optimum theoretical concentrations per 5 ml for lamivudine, zidovudine, and nevirapine were 25 mg, 50 mg, and 75 mg respectively. The graph shows that, after six minutes, the optimum concentration for all three APIs has been reached. When considering the results for the three actives, it can be concluded that the ideal mixing time for the ARV powder for suspension is six minutes

5.5.2.2 PRESERVATIVES

The results of the mixing time for sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate are presented graphically in figures 5.2 and 5.3.

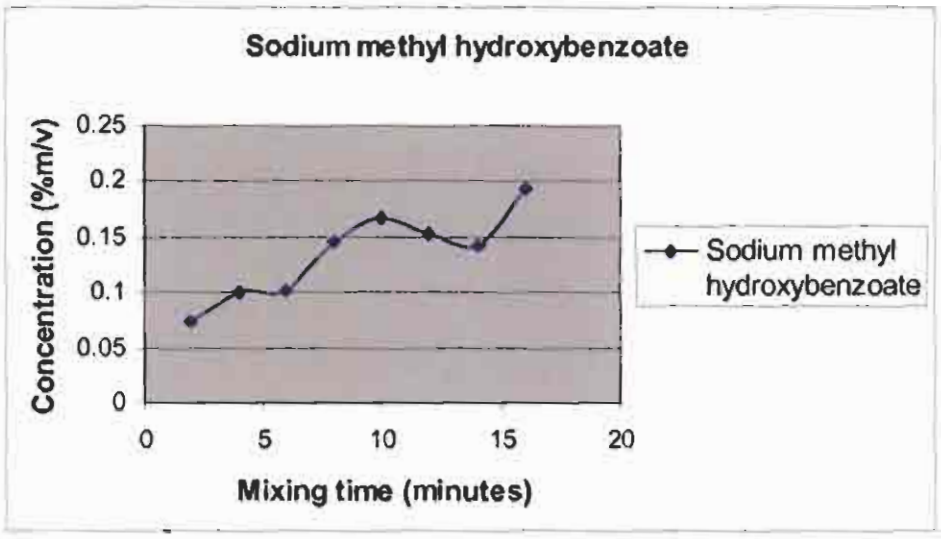

Figure 5.2: Graph of the mixing time results for sodium methyl hydroxybenzoate.

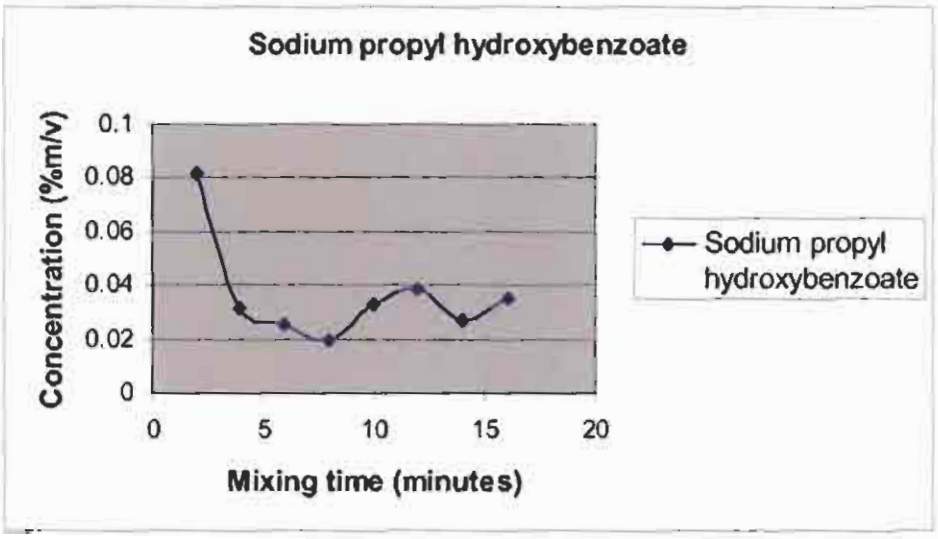

Figure 5.3: Graph of the mixing time results for sodium propyl hydroxybenzoate.

5.5.2.2.1 DISCUSSION

The optimum theoretical concentrations per 5 ml for sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate are 0.15 mg and 0.02 mg respectively. When considering the results shown in figures 5.2 and 5.3, it is clear that the ideal mixing time for the two preservatives is eight minutes.

The three APIs have an ideal mixing time of six minutes, but also show satisfactory results after a mixing time of eight minutes. The two preservatives have the desired concentrations after eight minutes.

After reviewing the results of the mixing time experiment, it was decided that a mixing time of eight minutes would be suitable for the ARV suspension.

5.6 CONCLUSION

The aim of this study was to create an ARV powder for suspension for paediatric, adult, and geriatric patients. Five different ARV powder formulations for suspension were specifically created for the different weight categories during the paediatric patient's life, namely 3-6, 6-10, 10-15, 15-20, and 20-29 kg. A sixth formulation was created for adult and geriatric use.

Specific volumes of each powder for suspension were created to supply the patient with one month's ARV treatment. Each volume was determined by using the daily dosage as recommended by UNICEF/WGO (2004).

Mixing time experiments were preformed on laboratory batches to determine the ideal mixing time. Results showed that a mixing time of eight minutes would be most suitable for this formulation.

Various laboratory batches of the six ARV powder formulations for suspension were created, and underwent thorough mixing for eight minutes. Thereafter the powders for suspension were placed in clear PVC containers and were sealed with a screw cap.

These laboratory samples underwent stability testing for a period of three months. Reasons for stability testing together with the stability testing programme and the stability tests will be discussed in the subsequent chapter.

CHAPTER 6

STABILITY TESTING: A VITAL QUALITY ATTRIBUTE FOR DRUG PRODUCTS

"There never was anything by the wit of man so well devised or so sure established which hath not in the continuance of time become corrupted..."

-Thomas Cranmer-

All things formed by the human hand, from the exquisite Parthenon to the frivolous milkshake, are subject to discomposure. Pharmaceuticals in this case are no different (Carstensen & Rhodes, 2000:2).

The rate of degradation of an assortment of drug products varies dramatically. If appropriately packed and stored, some drug products maintain integrity for more than ten years. Other products such as radiopharmaceuticals are, in the best of environments, only usable for two to three days (Carstensen & Rhodes, 2000:2).

6.1. REASONS FOR STABILITY TESTING

Stability is an indispensable property of drug products; this causes stability evaluation to be one of the most essential procedures in drug development and manufacturing. Reasons for stability testing are:

- Patient safety and wellbeing.
- To keep a reputable name as a manufacturer.
- Stability testing may be compulsory because of legal requirements.
- In the future, a database can be used for formulation of different products (Carstensen & Rhodes, 2000:11).

6.2 MECHANISMS OF DEGRADATION

6.2.1 CHEMICAL

Oxidation, solvolysis and reduction are all methods of chemical degradation. Kinetics and the knowledge thereof are of immense assistance in dealing with chemical decomposition (Carstensen & Rhodes, 2000:12).

6.2.2 PHYSICAL

Vibration, abrasion and temperature fluctuations are all causes of physical decomposition, which can be tested for with methods such as suspension resuspendability and sedimentation rate, tablet friability, ect. (Carstensen & Rhodes, 2000:12).

6.2.3 BIOLOGICAL AND MICROBIOLOGICAL

All over the world, microbiological and non-microbiological organisms play a role in product degradation and cause stability problems (Carstensen & Rhodes, 2000:10).

6.3 ADVERSE EFFECTS DUE TO INSTABILITY

A variety of mechanisms exist by which pharmaceutical products may decompose as discussed in paragraph 6.2. This results in an immense array of adverse effects.

6.3.1 DECOMPOSITION OF APIs

Loss of the API is of great significance when conducting stability studies on pharmaceutical products. Generally any product that contains less than 90% of the API stated on the label is regarded as being of unacceptable quality (Carstensen & Rhodes, 2000:3).

6.3.2 ESCALATION IN API CONCENTRATION

For certain pharmaceutical products, loss of vehicle may result in an elevated concentration of the active drug. One example of such behaviour by a drug product is

lidocaine gels. Sometimes perfusion bags allow solvent to evaporate and cause the product in the bag to present an increase in API concentration (Carstensen & Rhodes, 2000:5).

6.3.3 LOSS OF CONTENT UNIFORMITY

The suspension is the drug delivery system that is most likely to lose its uniformity of content with time. Determination of sedimentation volume and redispersion is therefore incorporated into the stability protocol (Carstensen & Rhodes, 2000:5).

6.3.4 DECLINE OF MICROBIOLOGICAL STATUS

Though it is not expected that all pharmaceutical products be sterile (totally devoid of all forms of both vegetative and spore life), the microbiological status of all drug delivery systems is of great concern (Carstensen & Rhodes, 2000:5).

There are two probable ways in which the microbiological status of a product can change considerably over time:

- Micro-organisms present in the product during manufacture may reproduce and increase the quantity of organisms in the product.
- Package integrity is compromised during storage or distribution (Carstensen & Rhodes, 2000:5).

Other adverse effects include: Loss of package reliability, decrease in label quality, decline in pharmaceutical elegance and patient acceptability, and the formation of toxic degradation products (Carstensen & Rhodes, 2000:7).

6.4 STABILITY PROGRAMME

Six ARV formulations were prepared in this study, namely 150 ml, 300 ml, 420 ml, 600 ml, 900 ml and 1000 ml powder for suspension (Refer to chapter 5).

The laboratory batches of these formulations were put on a stability programme for three months according to the International Conference on Harmonisation (ICH) on technical requirements for the registration of pharmaceuticals for human use (ICH 1996 and 2003).

6.4.1 STORAGE TEMPERATURES

The physical and chemical stability of preparations should be determined over a wide range of temperatures. Laboratory batches were stored at three temperatures according to ICH conditions. Controlled storage facilities were used during the stability period.

- 25°C + 60 % RH
- 30°C + 65 % RH
- 40°C + 75 % RH

6.4.2 STABILITY TESTS CONDUCTED

All tests were done using calibrated and / or validated test apparatus, where appropriate.

Stability tests conducted on the ARV suspension laboratory batches included:

- Visual assessment
- pH
- Relative density
- Viscosity
- Moisture content
- Resuspendability and sedimentation rate
- API release (dissolution)
- API and preservative concentration assay
- Preservative efficacy

Table 6.1 lists the stability tests preformed, as well as the intervals after which the tests were preformed on the laboratory batches of the ARV powders for suspension.

Table 6.1: Stability tests conducted on laboratory batches of all six ARV powders for suspension.

Tests	Formulation form		Test intervals				
	Powder	Reconstituted Suspension	Initial	Initial in-use	6 weeks	12 weeks	12 weeks in use
Visual assessment	√	√	√	√	√	√	√
pH	—	√	√	√	√	√	√
Relative density	—	√	√	√	√	√	√
Viscosity	—	√	√	√	√	√	√
Moisture content	√	—	√	—	√	√	—
Resuspendability	—	√	√	√	√	√	√
Sedimentation rate	—	√	√	√	√	√	√
API release (dissolution)	—	—	—	—	—	√	—
API and preservative assay	—	√	√	√	√	√	√
Preservative efficacy (PET)	—	√	√	—	—	√	√

controlled storage temperature in degrees Celsius – 25, 30 or 40 degrees. The number in

6.4.2.1 IN-USE STABILITY TESTS

Stability testing of the suspension after reconstitution, should be conducted to provide information for the labeling on the on the suspension, the storage conditions and the in-use period of the reconstituted suspension (ICH stability guideline under storage conditions).

In-use stability testing was performed on the reconstituted suspension at the initial time interval and after 12 weeks storage at 25°C+60%RH, 30°C+65%RH and 40°C+75%RH. To simulate patient usage, the reconstituted suspension was kept at uncontrolled room temperature out of direct sunlight for a period of 30 days. Each morning and late afternoon, the correct dose for each suspension was removed from the container. Full testing was performed after 15 and 30 days.

6.4.2.2 BATCH NUMBERS

The powder for ARV suspension, as previously mentioned, underwent an extensive range of stability indicative tests. The different time and temperature parameters will be indicated in the following manner in the tables of results.

The duration (in weeks) of each stability test is represented by the first number present, i.e. 12 W, represents a period of twelve weeks. The in-use stability testing on day 15 or 30, is represented by the number 15 or 30, i.e. 30. The following figure represents the controlled storage temperature in degrees Celsius – 25, 30 or 40 degrees. The number in bracket represents the percentage relative humidity at which the powder for suspension was stored.

Example: 12 W.30/ 30 (65) = Twelve weeks, 30 days, 30°C+65% RH.

6.5 TEST METHODS

All tests were carried out under Good Laboratory Practice (GLP) conditions, to ensure the accuracy of the test results. All tests were performed on the reconstituted suspensions except for the determination of moisture content, where dry powder samples were used (Refer to table 6.1).

Reconstitution of the ARV suspensions: Each of the ARV powders for suspension requires a specific amount of purified water for reconstitution. Refer to chapter 5, table 5.6 for the specified amounts of purified water to be added to the different formulations.

6.5.1 VISUAL ASSESSMENT

A visual assessment of each stored laboratory batch was carried out. Colour, odour and physical appearance were examined.

6.5.2 pH

The pH was measured using the Mettler Toledo MP 220 pH meter. The pH meter was calibrated with buffer solutions of pH 4 and 7 before use.

6.5.3. RELATIVE DENSITY

An Anton Paar DMA 38 Density/Specific Gravity/Concentration meter was used to determine the relative density of the reconstituted suspension. The instrument was calibrated before use.

6.5.4 VISCOSITY

The viscosity of the test samples was measured using a Brookfield Programmable Model DV-II + Viscometer, together with the Helipath stand and the Labcon low temperature water bath. The temperature was set at 25°C and the SC4-18 spindle was used to determine the viscosity. The spindle rotated for 10 minutes at speeds increasing at 1-minute intervals from 0.3, 0.6, 1.5, 3, 6, 12, 30, 60 and back to 30 rpm. Ten readings were taken of each sample.

6.5.5 MOISTURE CONTENT

The moisture content of each of the powders for suspension was determined using the Metrohm Karl Fisher 701KF Titrino, along with the 703Ti stand. A mass of 0.25 g of each sample was weighed. Samples were tested in duplicate.

6.5.6 RESUSPENDABILITY AND SEDIMENTATION RATE

Resuspendability and sedimentation rate tests were done on each of the laboratory batches. During testing 100 ml of the reconstituted suspension was poured into a 100 ml volumetric cylinder and left for 24 hours. After 24 hours the volume of sedimentation formed may not be more than 5 cm. The sediment must also be resuspended within 1 minute of vigorous shaking.

6.5.7 DISSOLUTION

6.5.7.1 APPARATUS

- VanKel 7000
- USP apparatus 2 – paddle

6.5.7.2 METHOD

- Stirring speed: 50 rpm
- Dissolution medium: 900 ml 0.1 M HCl at pH 1.25
- Dissolution sample: 5 ml reconstituted suspension
- Withdrawal times: 10, 15, 20, 30 and 45 minutes
- Volume withdrawn: 5 ml

The amount of active released after each time interval was determined by means of HPLC (Refer to chapter 7-12 for results and discussions).

6.5.8 ASSAY: LAMIVUDINE, ZIDOVUDINE, NEVIRAPINE AND PRESERVATIVES

The Hewlett Packard Agilent 1100 series HPLC instrument was used, together with the G1322A degasser, G1312A binary pump, and the G1329A ALS autosampler. Methanol was used as a solvent to enhance the solubility of nevirapine, as it has low solubility in water.

Standard preparation: Refer to Annexure, G paragraph 4.

Sample preparation: Refer to Annexure, G paragraph 3.

6.5.9 PRESERVATIVE EFFCTIVENESS TESTING (PET)

Sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate were used as preservatives in the formulations in this study. This test determined the ability of these preservatives to prevent contamination of the formulated product by microbial organisms. This test was done on the initial formulation and after three months at 25°C+60% RH, 30°C+65% RH and 40°C+75% RH.

The PET was subcontracted to Cosi Pharmaceuticals. All test samples were compared to standards set for category 3 products in USP 28 (USP 28:2242), namely > 2.0 log reduction at 14 days and no increase at 28 days for

- *E. coli*,
- *P. aeruginosa*
- *S. aureus*

And no increase from initial inoculum for

- *A. niger*
- *C. albicans*

6.6 CONCLUSION

Stability testing is a vital part of pharmaceutical product development. In this chapter, all test procedures performed on the laboratory batches of the ARV powders for suspension were discussed. All tests were performed under GLP conditions and comply with the set standards as mentioned in USP 28.

The results of the test procedures described in this chapter are discussed in chapters 7-12, and are graphically represented in Annexures A to F. The data gathered during these test procedures will be discussed separately in these chapters and relevant conclusions will be drawn.

CHAPTER 7

RESULTS AND DISCUSSION: POWDER FOR SUSPENSION FOR PAEDIATRIC PATIENTS: 3-6 KG

"Unnamed Law: If it happens, it must be possible."

-Anon-

The powder for suspension that, if reconstituted, has a volume of 150 ml and provides ARV treatment for paediatric patients with a body mass of 3-6 kg for 1 month, was subjected to accelerated stability testing for a period of twelve weeks (refer to chapter 6 for the discussion of the stability program).

The results of each test will be discussed and graphic presentations of these results can be seen in annexure A-F.

7.1 VISUAL ASSESSMENT

7.1.1 RESULTS

Visual assessment was performed on reconstituted suspensions. Initial laboratory samples were stored at uncontrolled room temperature. Results for the visual assessment are given in Table 7.1.

Table 7.1: Visual assessment results of the 150 ml reconstituted ARV suspension, during stability and in-use testing.

Visual assessment			
Initial	**Storage temperatures**	**6 Weeks**	**12 Weeks**
Snow white, homogeneous thick white suspension.	25°C+60%RH	Snow white, uniform suspension.	White, milky suspension, larger particles than initially visible are present.
	30°C+65%RH	White uniform suspension.	Off white suspension, larger particles than initially visible are present.
	40°C+75%RH	White suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
In-use stability			
	Storage temperatures	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	Snow white, homogeneous thick suspension.	Snow white, homogeneous thick suspension.
12 Weeks	25°C+60%RH	Milky white suspension, larger particles than initially visible are present.	Milky white suspension, larger particles than initially visible are present.
	30°C+65%RH	Off white suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
	40°C+75%RH	Off white, larger particles than initially visible are present.	Creamy white larger particles than initially visible are present.

7.1.2 DISCUSSION

A change in colour in the reconstituted powder for suspension from white to off-white was observed after storage at 30°C+65%RH and 40°C+75%RH for 12 weeks. The physical appearance showed a slight decomposition. Much larger dispersed particles, than were initially noticed were present throughout the suspension. The passion fruit flavour of the suspension remained stable throughout the stability and in-use test periods.

7.2 pH

7.2.1 RESULTS

The pH results after accelerated stability and in-use testing for the reconstituted suspension are given in table 7.2.

Table 7.2: pH results of the reconstituted ARV suspension after accelerated stability and in-use stability testing.

pH			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
4.54	25°C+60%RH	4.63	4.55
	30°C+65%RH	4.69	4.53
	40°C+75%RH	4.66	4.57
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	4.50	4.47
12 Weeks	25°C+60%RH	4.54	4.51
	30°C+65%RH	4.59	4.53
	40°C+75%RH	4.53	4.52

7.2.2 DISCUSSION

There was no significant change in the pH of the reconstituted suspension after accelerated stability testing for a period of 12 weeks at three different temperatures. pH values during the in-use stability, representing patient usage, also remained unchanged.

7.3 RELATIVE DENSITY

7.3.1 RESULTS

The relative density results after accelerated stability and in-use stability testing are given in table 7.3.

Table 7.3: Relative density results (g/ml) of the reconstituted ARV suspension after accelerated stability and in-use studies.

Relative density (g/ml)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.156	25°C+60%RH	1.153	1.153
	30°C+65%RH	1.162	1.153
	40°C+75%RH	1.168	1.151
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature.	1.156	1.155
12 Weeks	25°C+60%RH	1.153	1.151
	30°C+65%RH	1.149	1.147
	40°C+75%RH	1.148	1.145

7.3.2 DISCUSSION

The relative density of the reconstituted ARV powder for suspension remained relatively stable during accelerated stability testing over a period of 12 weeks, as well as during in-use testing for 15 and 30 days.

7.4 VISCOSITY

7.4.1 RESULTS

The viscosity results for the reconstituted suspension are given in table 7.4.

Table 7.4: **Viscosity results (cp) of the reconstituted ARV suspension during accelerated stability and in-use testing.**

Viscosity (cp)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
48.5	25°C+60%RH	48.5	49.6
	30°C+65%RH	48.5	48.4
	40°C+75%RH	48.4	48.5
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	48.5	48.5
12 Weeks	25°C+60%RH	49.7	49.7
	30°C+65%RH	48.5	48.5
	40°C+75%RH	48.5	48.5

7.4.2 DISCUSSION

During the 12 week stability test period, the viscosity values of the reconstituted ARV suspension remained stable. During the in-use stability test period, no change was observed in the viscosity values.

7.5 MOISTURE CONTENT

7.5.1 RESULTS

The moisture content results for the ARV powder for suspension are listed in table 7.5.

Table 7.5: **Moisture content of the ARV powder for suspension during the accelerated stability test period.**

Moisture content (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.068	25°C+60%RH	1.075	1.076
	30°C+65%RH	1.091	1.079
	40°C+75%RH	1.087	1.089

7.5.2 DISCUSSION

The moisture content of the ARV powder for suspension showed no significant change over a period of 12 weeks during the accelerated stability study.

7.6 RESUSPENDABILITY AND SEDIMENTATION RATE

7.6.1 RESULTS

All the samples tested complied with the set standards, namely not more than 5 cm of sediment should be present after 24 hours, and the sediment formed should be easily resuspended.

7.7 API RELEASE (DISSOLUTION)

7.7.1 RESULTS

The dissolution results for the reconstituted ARV powder for suspension are presented in figures 7.1-7.2.

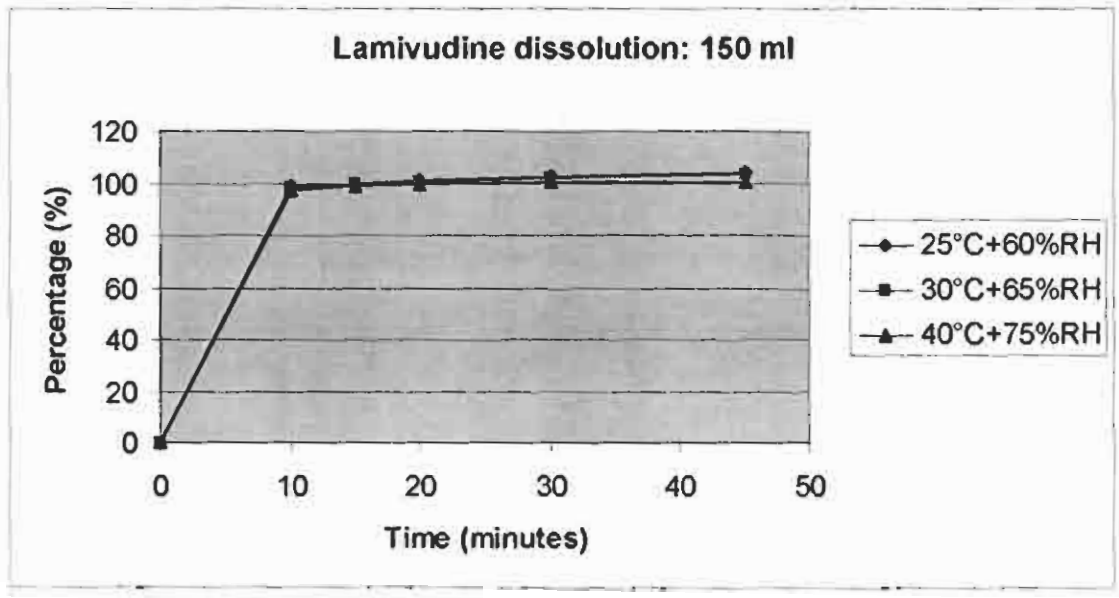

Figure 7.1: Dissolution results of lamivudine in 150 ml formulation obtained after 12 weeks accelerated stability testing.

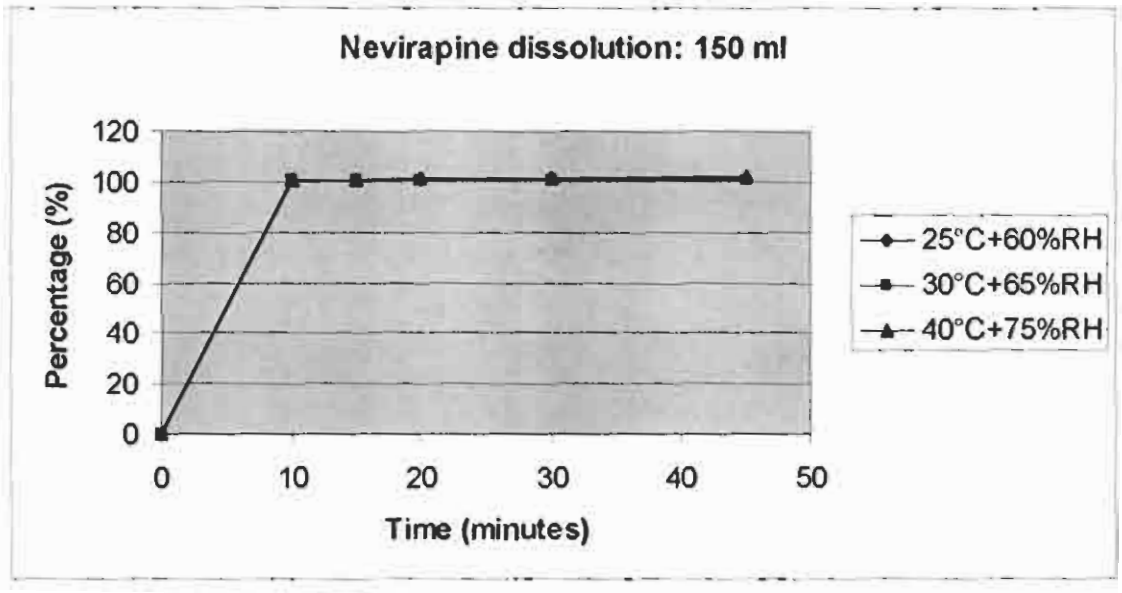

Figure 7.2: Dissolution results of nevirapine in 150 ml formulation obtained after 12 weeks accelerated stability testing.

7.7.2 DISCUSSION

The dissolution results obtained after 12 weeks accelerated stability testing showed that lamivudine and nevirapine were immediately dissolved with a dissolution of 101% and 102% respectively after 10 minutes.

7.8 API AND PRESERVATIVE CONCENTRATION ASSAY

7.8.1 LAMIVUDINE

The assay results for lamivudine during the accelerated stability and in-use stability study testing are given in table 7.6.

Table 7.6: **Lamivudine assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.**

Lamivudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
103.4	25°C+60%RH	100.5	104.2
	30°C+65%RH	101.9	100.8
	40°C+75%RH	100.4	97.8
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature.	102.6	99.8
12 Weeks	25°C+60%RH	97.8	97.2
	30°C+65%RH	96.0	95.9
	40°C+75%RH	96.6	96.1

7.8.2 NEVIRAPINE

The assay results for nevirapine during the accelerated stability and in-use stability study testing are given in table 7.7.

Table 7.7: Nevirapine assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Nevirapine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
95.5	25°C+60%RH	90.6	96.3
	30°C+65%RH	91.1	94.0
	40°C+75%RH	92.6	93.3
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature.	85.7	94.8
12 Weeks	25°C+60%RH	95.6	92.4
	30°C+65%RH	94.2	93.2
	40°C+75%RH	93.1	92.9

7.8.3 DISCUSSION

A downward trend in the assay for lamivudine was observed, especially after storage for 12 weeks at 40°C+75%RH. However, all results were within the specified limits of 90 -110% for stability testing.

The assay results for nevirapine showed variation between test intervals. All results were within the 90 -110% limit, except for the assay value obtained after storage for 15 days at uncontrolled room temperature during the in-use testing at the initial stage. This result could be attributed to laboratory error.

7.8.4 RESULTS: SODIUM METHYL HYDROXYBENZOATE

The assay results for sodium methyl hydroxybenzoate are given in table 7.8.

Table 7.8: **Sodium methyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.**

Sodium methyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.157	25°C+60%RH	0.161	0.154
	30°C+65%RH	0.163	0.158
	40°C+75%RH	0.142	0.157
In-use stability testing			
	Storage conditions	**15 Days**	**30 Day**
Initial	Uncontrolled room temperature.	0.158	0.149
12 Weeks	25°C+60%RH	0.152	0.152
	30°C+65%RH	0.159	0.158
	40°C+75%RH	0.152	0.149

7.8.5 RESULTS: SODIUM PROPYL HYDROXYBENZOATE

The assay results for sodium propyl hydroxybenzoate are given in table 7.9.

Table 7.9: Sodium propyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium propyl hydroxybenzoate (%m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.020	25°C+60%RH	0.022	0.020
	30°C+65%RH	0.023	0.021
	40°C+75%RH	0.023	0.020
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.020	0.021
12 Weeks	25°C+60%RH	0.021	0.020
	30°C+65%RH	0.020	0.020
	40°C+75%RH	0.020	0.021

7.8.6 DISCUSSION

Theoretical concentrations for sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate are 0.15% m/v and 0.02% m/v respectively. Sodium propyl hydroxybenzoate concentrations accelerated at the 6 weeks time period at 30°C and 40°C. Accept for the above mentioned acceleration, results obtained during the accelerated stability trial and in-use stability remained well within the limit of 90 – 110% (0.135 – 0.165% m/v for sodium methyl hydroxybenzoate and 0.018 – 0.022% m/v for sodium propyl hydroxybenzoate).

7.9 PRESERVATIVE EFFICACY TESTING (PET)

7.9.1 RESULTS

The PET results for the initial, 6 weeks, 12 weeks and 12 weeks 30 days laboratory batches can be seen in tables 7.10-7.19.

Table 7.10: Preservative Efficacy Test results of the initial ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.11: Preservative Efficacy Test results of the 6 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.12: Preservative Efficacy Test results of the 6 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.13: Preservative Efficacy Test results of the 6 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.14: Preservative Efficacy Test results of the 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.15: Preservative Efficacy Test results of the 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.16: Preservative Efficacy Test results of the 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.17: Preservative Efficacy Test results of the 12 weeks 25°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.18: Preservative Efficacy Test results of the 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 7.19: Preservative Efficacy Test results of the 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

7.9.2 DISCUSSION

All laboratory samples complied with the requirements for category 3 products as described in USP 28. Therefore it can be concluded that the preservatives chosen for the

formulation were effective in protecting the suspension from microbial contamination, even after simulating patient usage for a period of 30 days.

7.10 CONCLUSION

In this chapter, various test samples of the 150 ml ARV powder for suspension, after reconstitution with distilled water, underwent extensive testing during a period of twelve weeks and in-use testing for thirty days. The test samples represented lamivudine and nevirapine at concentrations of 15 mg/2.5 ml and 40 mg/2.5 ml.

The ARV powder for suspension was stored at three storage conditions, namely 25°C+60%RH, 30°C+65%RH and 40°C+75%RH. The in-use stability tests were performed on reconstituted suspensions kept at uncontrolled room temperature.

Visual assessment tests included assessment of the colour, flavour and physical appearance of the test samples. Changes in colour drastically occurred in the 12 W.30/ 30 (65) and the 12 W.30/ 40 (75), were it changed from snow white to a watery see-through colour. The physical appearance underwent change, solid particles were dispersed throughout the suspension, which appeared during the accelerated stability period. This may cause an incorrect dose of ARVs to be administered to the patient

Relative density showed negligible decline. The viscosity and the pH remained stable. The moisture content of the powder for suspension was unchanged throughout the stability testing period. All samples tested complied with the specifications for sedimentation rate and resuspendability.

Dissolution results indicated that both lamivudine and nevirapine were immediately dissolved after 10 minutes.

By using the stability indicating HPLC method developed and described in Annexure G, the concentration of lamivudine and nevirapine was determined over the stability test period. A downward trend in the lamivudine assay values was observed but all test results remained within the 90-110% specification. Nevirapine assay results showed inconsistency, but remained within the specifications of 90-110%, except for the 15 day in-use sample after 12 weeks at 25°C+60%RH. Laboratory error could be responsible for the result of 85.7% which was obtained.

Preservative concentration of sodium methyl hydroxybenzoate was within specification throughout the accelerated and in-use testing period, sodium propyl hydroxybenzoate concentrations at storage temperatures of 30°C and 40°C were accelerated during the 6 weeks stability testing, 12 week stability and in-use testing revealed concentrations in the specified ranges. These results were confirmed by the positive outcome of the PET testing.

From these findings it can be concluded that the 150 ml ARV powder for suspension has potential as a paediatric ARV treatment for patients with a body weight of 3-6 kg. Further stability testing including a longer stability period will be required to more accurately reflect the stability potential of this formulation.

CHAPTER 8

RESULTS AND DISCUSSION: POWDER FOR SUSPENSION FOR PAEDIATRIC PATIENTS: 6-10 KG

"Research is to see what everybody else has seen, and think what nobody else has thought."

-Albert Szent-Györgi-

The powder for suspension that, if reconstituted, has a volume of 300 ml and provides ARV treatment for paediatric patients with a body mass of 6-10 kg for 1 month, was subjected to accelerated stability testing for a period of twelve weeks (refer to chapter 6 for the discussion of the stability program).

The results of each test will be discussed and graphic presentations of these results can be seen in annexure A-F.

8.1 VISUAL ASSESSMENT.

8.1.1 RESULTS

Visual assessment was performed on reconstituted suspensions. Initial laboratory samples were stored at uncontrolled room temperature. Results for the visual assessment are given in Table 8.1.

Table 8.1: Visual assessment results of the 300 ml reconstituted ARV suspension, during stability and in-use testing.

Visual assessment			
Initial	**Storage temperatures**	**6 Weeks**	**12 Weeks**
Snow white, homogeneous thick white suspension.	25°C+60%RH	Snow white, uniform suspension.	White suspension, larger particles than initially visible are present.
	30°C+65%RH	White uniform suspension.	Off white, uniform suspension.
	40°C+75%RH	White suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
In-use stability			
	Storage temperatures	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	Snow white, homogeneous thick suspension.	Snow white, homogeneous thick suspension.
12 Weeks	25°C+60%RH	Off white suspension, larger particles than initially visible are present.	Milky white suspension, larger particles than initially visible are present.
	30°C+65%RH	Off white suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
	40°C+75%RH	Off white suspension, larger particles than initially visible are present.	Creamy white suspension, larger particles than initially visible are present.

8.1.2 DISCUSSION

Some changes in the physical appearance of the suspension were apparent. The flavour stayed identical throughout the whole test period, but the colour changed from white to off-white after 12 weeks storage at 30°C+65%RH and 40°C+75%RH.

After 12 weeks larger particles than those initially observed, were present at all storage temperatures, including the in-use samples at 15 and 30 days

8.2 pH

8.2.1 RESULTS

The pH results after accelerated and in-use stability testing for the reconstituted suspension are given in table 8.2.

Table 8.2: pH results of the reconstituted ARV suspension after accelerated stability and in-use stability study.

pH			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
4.68	25°C+60%RH	4.65	4.46
	30°C+65%RH	4.77	4.52
	40°C+75%RH	4.66	4.53
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	4.60	4.58
12 Weeks	25°C+60%RH	4.42	4.40
	30°C+65%RH	4.52	4.51
	40°C+75%RH	4.53	4.50

8.2.2 DISCUSSION

The pH of the reconstituted suspension stayed relatively unchanged during the accelerated stability and in-use studies.

8.3 RELATIVE DENSITY

8.3.1 Results

The relative density results after accelerated and in-use stability testing for the reconstituted suspension are given in table 8.3.

Table 8.3: Relative density results (g/ml) of the reconstituted ARV suspension after accelerated stability and in-use studies.

Relative density (g/ml)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.159	25°C+60%RH	1.158	1.149
	30°C+65%RH	1.157	1.154
	40°C+75%RH	1.157	1.152
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	1.159	1.159
12 Weeks	25°C+60%RH	1.146	1.145
	30°C+65%RH	1.151	1.150
	40°C+75%RH	1.149	1.147

8.3.2 DISCUSSION

The relative density of the reconstituted suspension remained stable over the stability and in-use test periods.

8.4 VISCOSITY

8.4.1 RESULTS

The viscosity results after accelerated and in-use stability testing for the reconstituted suspension are given in table 8.4.

Table 8.4: Viscosity (cp) results of the reconstituted ARV suspension during accelerated stability and in-use testing.

Viscosity (cp)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
22.4	25°C+60%RH	22.4	20.3
	30°C+65%RH	20.3	20.2
	40°C+75%RH	20.3	20.2
In use-stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	20.3	20.2
12 Weeks	25°C+60%RH	20.1	20.1
	30°C+65%RH	20.2	20.2
	40°C+75%RH	20.2	20.2

8.4.2 DISCUSSION

No significant change from the initial value for viscosity was observed after 12 weeks accelerated stability testing, as well as during in-use testing.

8.5 MOISTURE CONTENT

8.5.1 RESULTS

The moisture content results after accelerated stability testing for the powder suspension are given in table 8.5.

Table 8.5: Moisture content of the ARV powder for suspension during the accelerated stability test period.

Moisture content (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.126	25°C+60%RH	1.131	1.137
	30°C+65%RH	1.129	1.132
	40°C+75%RH	1.126	1.131

8.5.2 DISCUSSION

The moisture content of the ARV powder for suspension remained stable over the stability test period of 12 weeks.

8.6 RESUSPENDABILITY AND SEDIMENTATION RATE

8.6.1 RESULTS

All samples tested complied with the set standards, namely not more than 5 cm sediment should be present after 24 hours, and the sediment formed should be easily resuspended.

8.7 API RELEASE (DISSOLUTION)

8.7.1 RESULTS

The dissolution results for the reconstituted ARV powder for suspension are presented in figures 8.1-8.3.

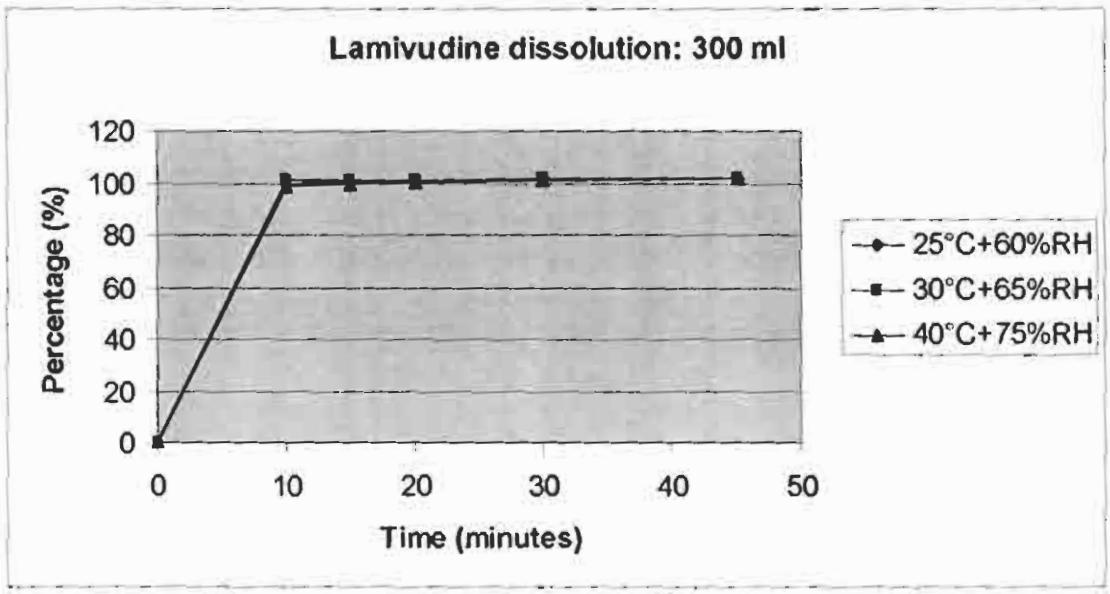

Figure 8.1: Dissolution results of lamivudine in 300 ml formulation obtained after 12 weeks accelerated stability testing.

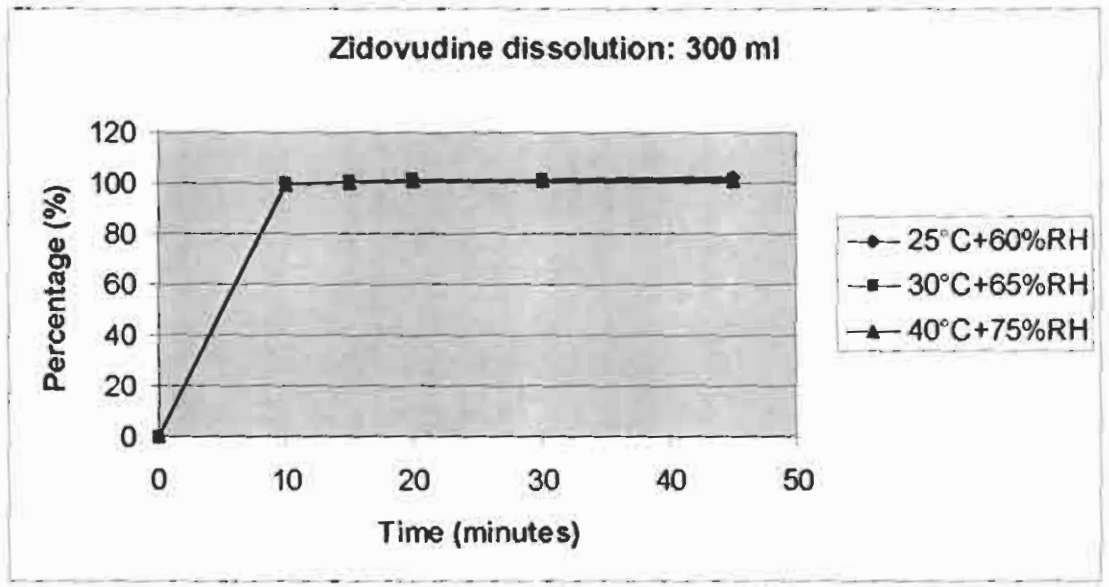

Figure 8.2: Dissolution results of zidovudine in 300 ml formulation obtained after 12 weeks accelerated stability testing.

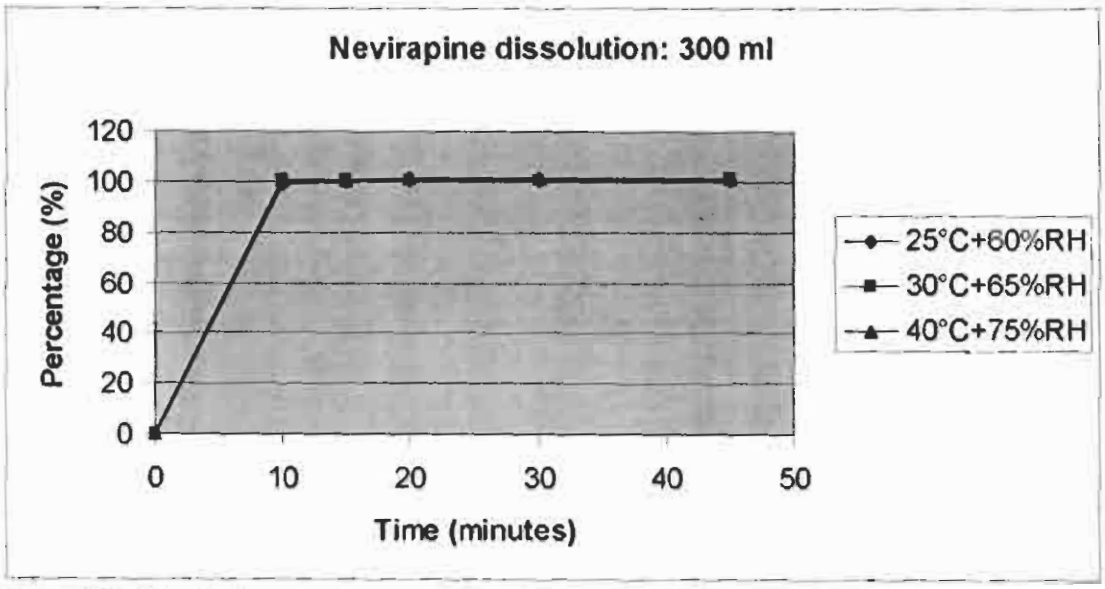

Figure 8.3: Dissolution results of nevirapine in 300 ml formulation obtained after 12 weeks accelerated stability testing.

8.7.2 DISCUSSION

The dissolution results obtained after 12 weeks accelerated stability testing showed that lamivudine, zidovudine and nevirapine were immediately dissolved with a dissolution of 103%, 102% and 100% respectively after 10 minutes.

8.8 API AND PRESERVATIVE CONCENTRATION ASSAY

8.8.1 RESULTS: LAMIVUDINE

The assay results for lamivudine during the accelerated stability and in-use stability study testing are given in table 8.6.

Table 8.6: Lamivudine assay results after 12 weeks accelerated stability testing and 30 days in-use stability study.

Lamivudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
100.0	25°C+60%RH	101.8	97.4
	30°C+65%RH	101.7	100.6
	40°C+75%RH	103.6	104.6
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	101.9	95.9
12 Weeks	25°C+60%RH	97.3	96.9
	30°C+65%RH	90.2	90.1
	40°C+75%RH	91.9	89.3

8.8.2 RESULTS: ZIDOVUDINE

The assay results for zidovudine during the accelerated stability and in-use stability study testing are given in table 8.7.

Table 8.7: Zidovudine assay results after 12 weeks accelerated stability testing and 30 days in-use stability study.

Zidovudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
99.4	25°C+60%RH	107.0	103.7
	30°C+65%RH	103.4	93.6
	40°C+75%RH	105.3	97.0
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	102.0	97.1
12 Weeks	25°C+60%RH	102.5	100.6
	30°C+65%RH	93.3	92.8
	40°C+75%RH	95.2	95.3

8.8.3 RESULTS: NEVIRAPINE

The assay results for nevirapine during the accelerated stability and in-use stability study testing are given in table 8.8.

Table 8.8: Nevirapine assay results after 12 weeks accelerated stability testing and 30 days in-use stability study.

Nevirapine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
98.5	25°C+60%RH	96.5	92.6
	30°C+65%RH	91.8	95.1
	40°C+75%RH	96.3	94.9
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	87.3	94.2
12 Weeks	25°C+60%RH	96.6	91.3
	30°C+65%RH	91.8	95.7
	40°C+75%RH	96.4	92.9

8.8.4 DISCUSSION

The assay results obtained for lamivudine, zidovudine and nevirapine were all within the specifications of 90-110%.

There was a definite downward trend in the assay results obtained for lamivudine during the in-use study with the 30 day result after 12 weeks at 40°C+75%RH below the limit of 90%. In-use assay values for zidovudine and nevirapine showed inconsistency with the result for 15 days for the initial sample, namely 87.3%, below 90%. This could be due to experimental error.

8.8.5 RESULTS: SODIUM METHYL HYDROXYBENZOATE

The assay results for sodium methyl hydroxybenzoate are given in table 8.9.

Table 8.9: Sodium methyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium methyl hydroxybenzoate (%m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.147	25°C+60%RH	0.150	0.149
	30°C+65%RH	0.149	0.152
	40°C+75%RH	0.151	0.151
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.147	0.147
12 Weeks	25°C+60%RH	0.152	0.148
	30°C+65%RH	0.149	0.151
	40°C+75%RH	0.151	0.154

8.8.6 RESULTS: SODIUM PROPYL HYDROXYBENZOATE

The assay results for sodium propyl hydroxybenzoate are given in table 8.10.

Table 8.10: Sodium propyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium propyl hydroxybenzoate (%m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.021	25°C+60%RH	0.025	0.025
	30°C+65%RH	0.026	0.023
	40°C+75%RH	0.023	0.020
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.022	0.018
12 Weeks	25°C+60%RH	0.023	0.024
	30°C+65%RH	0.024	0.022
	40°C+75%RH	0.021	0.021

8.8.7 DISCUSSION

Theoretical concentrations for sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate are 0.15% m/v and 0.02% m/v respectively. All results obtained for sodium methyl hydroxybenzoate during the accelerated stability trial and in-use stability remained well within the limit of 90 – 110% (0.135 – 0.165% m/v).

Some of the results obtained for sodium propyl hydroxybenzoate were above the limit of 110%. Due to the very small peaks representing sodium propyl hydroxybenzoate in the HPLC chromatogram, the above specification results could be due to faulty integration parameters.

8.9 PRESERVATIVE EFFICACY

8.9.1 RESULTS

The PET results for the initial, 6 weeks, 12 weeks and 12 weeks 30 days laboratory batches can be seen in tables 8.11-8.20.

Table 8.11: Preservative Efficacy Test results of initial ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.12: Preservative Efficacy Test results of 6 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.13: Preservative Efficacy Test results of 6 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.14: Preservative Efficacy Test results of 6 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.15: Preservative Efficacy Test results of 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.16: Preservative Efficacy Test results of 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.17: Preservative Efficacy Test results of 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.18: Preservative Efficacy Test results of 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.19: Preservative Efficacy Test results of 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 8.20: Preservative Efficacy Test results of 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

8.9.2 DISCUSSION

All laboratory samples complied with the requirements for category 3 products as described in USP 28. Therefore it can be concluded that the preservatives chosen for the

formulation were effective in protecting the suspension from microbial contamination, even after simulating patient usage for a period of 30 days.

8.10 CONCLUSION

This chapter summarised results for various test samples of the 300 ml ARV powder for suspension, which underwent extensive testing during a period of twelve weeks and in-use testing for thirty days. The test samples represented lamivudine, zidovudine and nevirapine at concentrations of 25 mg/ 5 ml, 75 mg/ 5 ml and 50 mg/ 5 ml, respectively.

The ARV powder for suspension was stored at three storage conditions, namely 25°C+60%RH, 30°C+65%RH and 40°C+75%RH. The in-use stability tests were performed at uncontrolled room temperature.

Visual assessment tests included assessment of the colour, flavour and physical appearance of the test samples. No changes in flavour occurred during the stability period. Colour changed from snow white to off-white and watery, especially the test samples stored at 40°C+75%RH, which suggests temperatures as high as 40°C to be too high for the physical stability of the suspension.

The results obtained for pH, relative density, viscosity and moisture content did not change significantly throughout the stability testing period. All samples complied with the set specifications for sedimentation rate and resuspendability.

Dissolution results indicated that lamivudine, zidovudine and nevirapine were immediately dissolved after 10 minutes.

Assay testing by means of HPLC showed a downward trend in the results obtained for lamivudine during in-use testing. Results obtained for zidovudine and nevirapine showed some inconsistency, but remained within the specification limit of 90-110%, except for the 15 days in-use sample at the initial stage where a below specification result was obtained for nevirapine. This result could be due to experimental error.

Results obtained for sodium methyl hydroxybenzoate remained stable and within the limit of 90-110%. Above specification results were obtained for sodium propyl hydroxybenzoate in some samples. PET testing confirmed efficacy of the preservative system used.

The deterioration in the physical appearance of the suspension might be grounds for poor patient compliance. Further stability testing including a longer stability period will be required to more accurately reflect the stability potential of this formulation.

From these findings it can be concluded that the 300 ml ARV powder for suspension has potential as a paediatric ARV treatment for patients with a body weight of 6-10 kg.

CHAPTER 9

RESULTS AND DISCUSSION: POWDER FOR SUSPENSION FOR PAEDIATRIC PATIENTS: 10-15 KG

"It's a good morning exercise for a researcher to discard a pet hypothesis every day before breakfast."

-Konrad Lorenz-

The powder for suspension that, if reconstituted, has a volume of 420 ml and provides ARV treatment for paediatric patients with a body mass of 10-15 kg for 1 month, was subjected to accelerated stability testing for a period of twelve weeks (refer to chapter 6 for the discussion of the stability program).

The results of each test will be discussed and graphic presentations of these results can be seen in annexure A-F.

9.1 VISUAL ASSESSMENT.

9.1.1 RESULTS

Visual assessment was performed on reconstituted suspensions. Initial laboratory samples were stored at uncontrolled room temperature. Results for the visual assessment are given in Table 9.1.

Table 9.1: Visual assessment results of the 420 ml reconstituted ARV suspension, during stability and in-use testing.

Visual assessment			
Initial	**Storage temperatures**	**6 Weeks**	**12 Weeks**
Snow white, homogeneous thick white suspension.	25°C+60%RH	Snow white, uniform suspension.	White, milky suspensions, larger particles than initially visible are present.
	30°C+65%RH	White uniform suspension.	Off white suspensions, larger particles than initially visible are present.
	40°C+75%RH	White suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
In-use stability			
	Storage temperatures	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	Snow white, homogeneous thick suspension.	Snow white, homogeneous thick suspension.
12 Weeks	25°C+60%RH	Milky white suspension, larger particles than initially visible are present.	Milky white suspension, larger particles than initially visible are present.
	30°C+65%RH	Off white suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
	40°C+75%RH	Off white suspension, larger particles than initially visible are present.	Creamy white suspension, larger particles than initially visible are present.

9.1.2 DISCUSSION

Some changes in the physical appearance of the suspension were apparent. The flavour stayed identical throughout the whole test period, but the colour changed from white to off-white after 12 weeks storage at 30°C+65%RH and 40°C+75%RH.

After 12 weeks larger particles than those initially observed, were present at all storage temperatures, including the in-use samples at 15 and 30 days

9.2 pH

9.2.1 RESULTS

The pH results after accelerated and in-use stability testing for the reconstituted suspension are given in table 9.2.

Table 9.2: pH results of the reconstituted ARV suspension after accelerated stability and in-use stability study.

pH			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
4.75	25°C+60%RH	4.70	4.65
	30°C+65%RH	4.77	4.65
	40°C+75%RH	4.72	4.64
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	4.75	4.65
12 Weeks	25°C+60%RH	4.65	4.63
	30°C+65%RH	4.65	4.61
	40°C+75%RH	4.64	4.60

9.2.2 DISCUSSION

The reconstituted suspension showed little change in pH over the accelerated stability and in-use test periods.

9.3 RELATIVE DENSITY

9.3.1 RESULTS

The relative density results after accelerated and in-use stability testing for the reconstituted suspension are given in table 9.3.

Table 9.3: Relative density results (g/ml) of the reconstituted ARV suspension after accelerated stability and in-use studies.

Relative density (g/ml)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.160	25°C+60%RH	1.161	1.159
	30°C+65%RH	1.160	1.157
	40°C+75%RH	1.163	1.160
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	1.161	1.160
12 Weeks	25°C+60%RH	1.156	1.155
	30°C+65%RH	1.154	1.152
	40°C+75%RH	1.158	1.156

9.3.2 DISCUSSION

The relative density of the reconstituted ARV suspension remained satisfactory during the accelerated stability and in-use stability test periods. No significant change in density was observed.

9.4 VISCOSITY

9.4.1 RESULTS

The viscosity results after accelerated and in-use stability testing for the reconstituted suspension are given in table 9.4.

Table 9.4: Viscosity (cp) results of the reconstituted ARV suspension during accelerated stability and in-use testing.

Viscosity (cp)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
50.7	25°C+60%RH	50.7	47.5
	30°C+65%RH	52.8	48.4
	40°C+75%RH	52.8	48.7
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	52.8	52.8
12 Weeks	25°C+60%RH	46.9	46.3
	30°C+65%RH	47.2	46.8
	40°C+75%RH	47.9	47.4

9.4.2 DISCUSSION

No significant change in the viscosity results obtained was observed after accelerated and in-use stability testing.

9.5 MOISTURE CONTENT

9.5.1 RESULTS

The moisture content results after accelerated stability testing for the powder for suspension are given in table 9.5.

Table 9.5: Moisture content of the ARV powder for suspension during the accelerated stability test period.

Moisture content (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.209	25°C+60%RH	1.221	1.223
	30°C+65%RH	1.219	1.220
	40°C+75%RH	1.216	1.224

9.5.2 DISCUSSION

The moisture content of the ARV powder for suspension remained stable over the accelerated stability test period of 12 weeks.

9.6 RESUSPENDABILITY AND SEDIMENTATION RATE

9.6.1 RESULTS

All samples tested complied with the set standards, namely not more than 5 cm sediment should be present after 24 hours, and the sediment formed should be easily resuspended.

9.7 API RELEASE (DISSOLUTION)

9.7.1 RESULTS

The dissolution results for the reconstituted ARV powder for suspension are presented in figures 9.1-9.3.

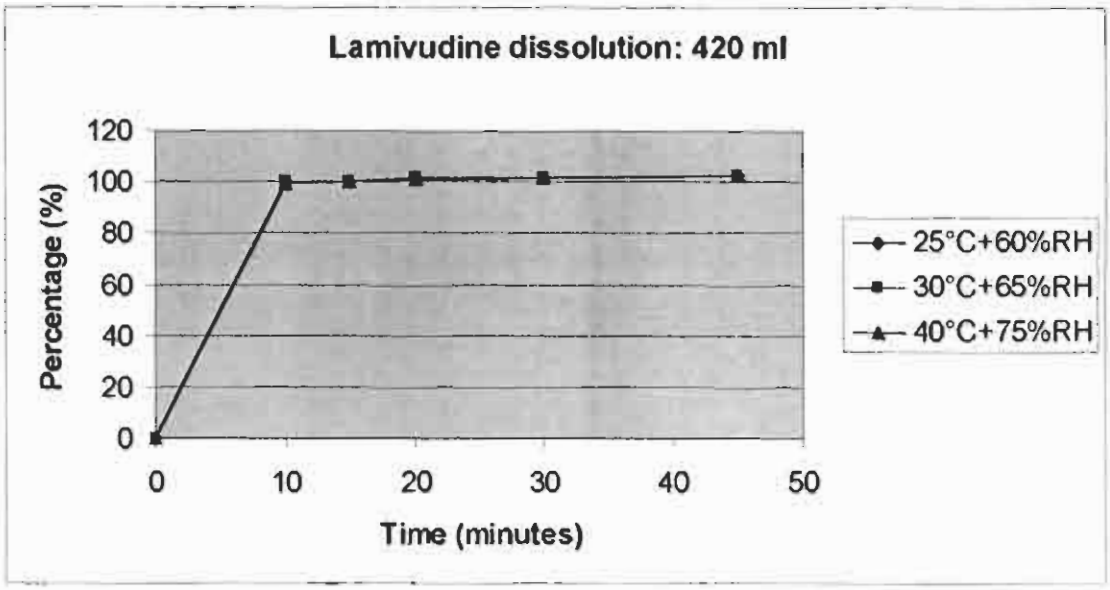

Figure 9.1: Dissolution results of lamivudine in 420 ml formulation obtained after 12 weeks accelerated stability testing.

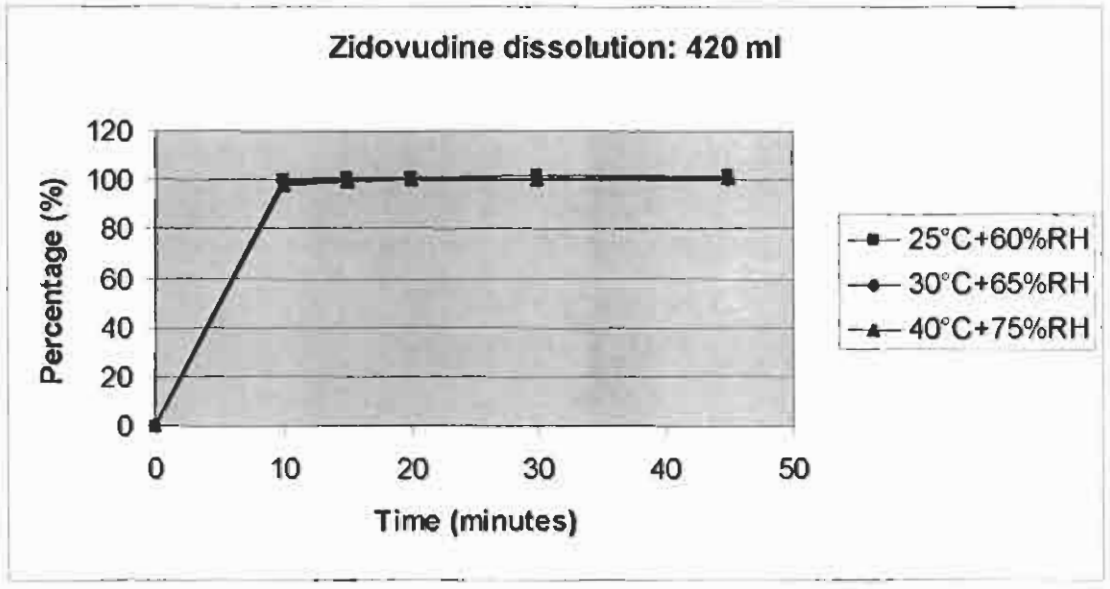

Figure 9.2: Dissolution results of zidovudine in 420 ml formulation obtained after 12 weeks accelerated stability testing.

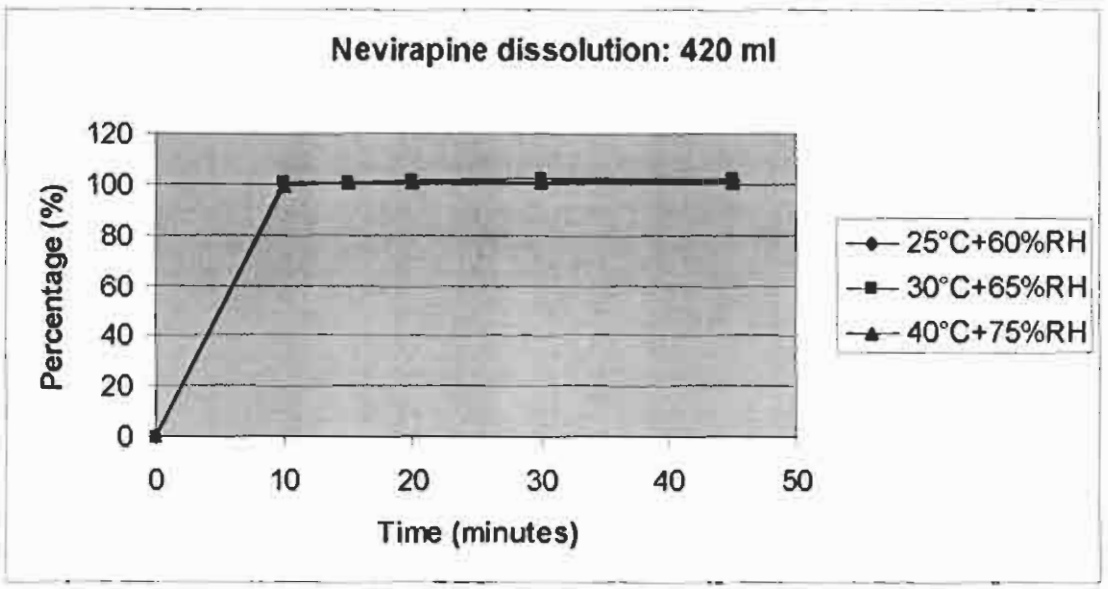

Figure 9.3: **Dissolution results of nevirapine in 420 ml formulation obtained after 12 weeks accelerated stability testing.**

9.7.2 DISCUSSION

The dissolution results obtained after 12 weeks accelerated stability testing showed that lamivudine, zidovudine and nevirapine were immediately dissolved with a final dissolution concentration of 103%, 101% and 101% respectively after 10 minutes.

9.8 API AND PRESERVATIVE CONCENTRATION ASSAY

9.8.1 RESULTS: LAMIVUDINE

The assay results for lamivudine during the accelerated stability and in-use stability study testing are given in table 9.6.

Table 9.6: Lamivudine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Lamivudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
100.5	25°C+60%RH	105.9	92.5
	30°C+65%RH	101.7	101.5
	40°C+75%RH	101.5	92.1
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	103.1	102.1
12 Weeks	25°C+60%RH	98.7	97.9
	30°C+65%RH	92.7	91.7
	40°C+75%RH	96.2	95.5

9.8.2 RESULTS: ZIDOVUDINE

The assay results for zidovudine during the accelerated stability and in-use stability study testing are given in table 9.7.

Table 9.7: Zidovudine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Zidovudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
100.9	25°C+60%RH	105.1	100.8
	30°C+65%RH	98.1	92.7
	40°C+75%RH	108.8	95.2
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	101.5	102.2
12 Weeks	25°C+60%RH	98.7	97.9
	30°C+65%RH	92.3	91.7
	40°C+75%RH	96.1	95.5

9.8.3 RESULTS: NEVIRAPINE

The assay results for nevirapine during the accelerated stability and in-use stability study testing are given in table 9.8.

Table 9.8: Nevirapine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Nevirapine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
111.4	25°C+60%RH	91.5	94.9
	30°C+65%RH	95.0	97.6
	40°C+75%RH	93.4	100.5
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	103.4	101.5
12 Weeks	25°C+60%RH	95.0	93.2
	30°C+65%RH	96.2	96.3
	40°C+75%RH	95.6	94.3

9.8.2 DICUSSION

Assay results obtained for lamivudine, zidovudine and nevirapine during accelerated and in-use stability were all within the specification of 90-110%, with one exception, nevirapine concentration was extremely high during initial testing. Laboratory error could be responsible for the result of 111.4% obtained.

9.8.4 RESULTS: SODIUM METHYL HYDROXYBENZOATE

The assay results for sodium methyl hydroxybenzoate are given in table 9.9.

Table 9.9: **Sodium methyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.**

Sodium methyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.153	25°C+60%RH	0.139	0.147
	30°C+65%RH	0.141	0.155
	40°C+75%RH	0.144	0.151
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.149	0.151
12 Weeks	25°C+60%RH	0.153	0.154
	30°C+65%RH	0.160	0.159
	40°C+75%RH	0.152	0.152

9.8.5 RESULTS: SODIUM PROPYL HYDROXYBENZOATE

The assay results for sodium propyl hydroxybenzoate are given in table 9.10.

Table 9.10: Sodium propyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium propyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.021	25°C+60%RH	0.026	0.024
	30°C+65%RH	0.023	0.026
	40°C+75%RH	0.021	0.019
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.023	0.021
	Storage conditions	**15 Days**	**30 Days**
12 Weeks	25°C+60%RH	0.029	0.022
	30°C+65%RH	0.019	0.020
	40°C+75%RH	0.023	0.019

9.8.6 DISCUSSION

Theoretical concentrations for sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate are 0.15% m/v and 0.02% m/v respectively. All results obtained for sodium methyl hydroxybenzoate during the accelerated stability trial and in-use stability remained well within the limit of 90 – 110% (0.135 – 0.165% m/v).

Some of the results obtained for sodium propyl hydroxybenzoate were above the limit of 110%. Due to the very small peaks representing sodium propyl hydroxybenzoate in the HPLC chromatogram, the above specification results could be due to faulty integration parameters.

9.9 PRESERVATIVE EFFICACY

9.9.1 RESULTS

The PET results for the initial, 6 weeks, 12 weeks and 12 weeks 30 days laboratory batches can be seen in tables 9.11-9.20.

Table 9.11: **Preservative Efficacy Test results of initial ARV reconstituted suspension.**

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.12: **Preservative Efficacy Test results of 6 weeks 25°C+60%RH ARV reconstituted suspension.**

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.13: Preservative Efficacy Test results of 6 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.14: Preservative Efficacy Test results of 6 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.15: Preservative Efficacy Test results of 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.16: Preservative Efficacy Test results of 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.17: Preservative Efficacy Test results of 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.18: Preservative Efficacy Test results of 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.19: Preservative Efficacy Test results of 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 9.20: Preservative Efficacy Test results of 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

9.9.2 DISCUSSION

All laboratory samples complied with the requirements for category 3 products as described in USP 28. Therefore it can be concluded that the preservatives chosen for the

formulation were effective in protecting the suspension from microbial contamination, even after simulating patient usage for a period of 30 days.

9.10 CONCLUSION

This chapter summarised results for various test samples of the 420 ml ARV powder for suspension, which underwent extensive testing during a period of twelve weeks and in-use testing for thirty days. The test samples represented lamivudine, zidovudine and nevirapine at concentrations of 50 mg/ 7 ml, 100 mg/ 7 ml and 100 mg/ 7 ml, respectively.

The ARV powder for suspension was stored at three storage conditions, namely 25°C+60%RH, 30°C+65%RH and 40°C+75%RH. The in-use stability tests were performed at uncontrolled room temperature.

Visual assessment tests included assessment of the colour, flavour and physical appearance of the test samples. No changes in flavour occurred during the stability period. Colour changed from snow white to watery yellow, especially the test samples stored at 40°C+75%RH, which suggests temperatures as high as 40°C to be too high for the physical stability of the suspension.

The results obtained for pH, relative density, viscosity and moisture content did not change significantly throughout the stability testing period. All samples complied with the set specifications for sedimentation rate and resuspendability.

Dissolution results indicated that lamivudine, zidovudine and nevirapine were immediately dissolved after 10 minutes.

HPLC determination of assay concentrations for lamivudine, zidovudine and nevirapine showed that all results obtained were within the limit of 90-110%, except for the initial value obtained for nevirapine. The result of 111.4% could be due to experimental error.

Results obtained for sodium methyl hydroxybenzoate remained stable and within the limit of 90-110%. Above specification results were obtained for sodium propyl hydroxybenzoate in some samples. PET testing confirmed efficacy of the preservative system used.

Further stability testing including a longer stability period will be required to more accurately reflect the stability potential of this formulation.

From these findings it can be concluded that the 420 ml ARV powder for suspension has potential as a paediatric ARV treatment for patients with a body weight of 10-15 kg.

CHAPTER 10

RESULTS AND DISCUSSION: POWDER FOR SUSPENSION FOR PAEDIATRIC PATIENTS: 15-20 KG

"Medicinal scientists are nice people, but you should not let them treat you."

-August Bier-

The powder for suspension that, if reconstituted, has a volume of 600 ml and provides ARV treatment for paediatric patients with a body mass of 15-20 kg for 1 month, was subjected to accelerated stability testing for a period of twelve weeks (refer to chapter 6 for the discussion of the stability program).

The results of each test will be discussed and graphic presentations of these results can be seen in annexure A-F.

10.1 VISUAL ASSESSMENT

10.1.1 RESULTS

Visual assessment was performed on reconstituted suspensions. Initial laboratory samples were stored at uncontrolled room temperature. Results for the visual assessment are given in Table 10.1.

Table 10.1: Visual assessment results of the 600 ml reconstituted ARV suspension, during stability and in-use testing.

Visual assessment			
Initial	**Storage temperatures**	**6 Weeks**	**12 Weeks**
Snow white, homogeneous thick white suspension.	25°C+60%RH	Snow white, uniform suspension.	White suspension, larger particles than initially visible are present.
	30°C+65%RH	White uniform suspension.	White suspension, larger particles than initially visible are present.
	40°C+75%RH	White suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
In-use stability			
	Storage temperatures	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	Snow white, homogeneous thick suspension.	Snow white, homogeneous thick suspension.
12 Weeks	25°C+60%RH	White suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
	30°C+65%RH	Off white suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
	40°C+75%RH	Watery yellow suspension, larger particles than initially visible are present.	Watery yellow suspension, larger particles than initially visible are present.

10.1.2 DISCUSSION

During the accelerated stability and in-use stability program, the laboratory samples showed significant change in colour and physical appearance. The visual test results revealed change in the colour from snowy white to watery yellow as the stability test period progressed. The flavour of the suspension remained stable throughout the accelerated stability and in-use stability test period.

Larger dispersed particles than those initially observed were present at 40°C+75%RH after 6 weeks and 12 weeks accelerated stability, as well as after 12 weeks at 30°C+65%RH and in-use testing after 12 weeks.

10.2 pH

10.2.1 RESULTS

The pH results after accelerated and in-use stability testing for the reconstituted suspension are given in table 10.2.

Table 10.2: pH results of the reconstituted ARV suspension after accelerated stability and in-use stability study.

pH			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
4.76	25°C+60%RH	4.71	4.64
	30°C+65%RH	4.60	4.57
	40°C+75%RH	4.73	4.71
In use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	4.66	4.59
12 Weeks	25°C+60%RH	4.62	4.60
	30°C+65%RH	4.53	4.51
	40°C+75%RH	4.68	4.65

10.2.2 DISCUSSION

The reconstituted suspension showed little change in pH over the accelerated stability and in-use test periods.

10.3 RELATIVE DENSITY

10.3.1 RESULTS

The relative density results after accelerated and in-use stability testing for the reconstituted suspension are given in table 10.3.

Table 10.3: Relative density results (g/ml) of the reconstituted ARV suspension after accelerated stability and in-use studies.

Relative density (g/ml)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.156	25°C+60%RH	1.156	1.154
	30°C+65%RH	1.158	1.156
	40°C+75%RH	1.157	1.156
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	1.159	1.159
12 Weeks	25°C+60%RH	1.150	1.149
	30°C+65%RH	1.152	1.150
	40°C+75%RH	1.154	1.153

10.3.2 DISCUSSION

The relative density of the reconstituted suspension remained stable over the accelerated stability and in-use test periods.

10.4 VISCOSITY

10.4.1 RESULTS

The viscosity results after accelerated and in-use stability testing for the reconstituted suspension are given in table 10.4.

Table 10.4: Viscosity (cp) results of the reconstituted ARV suspension during accelerated stability and in-use testing.

Viscosity (cp)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
49.6	25°C+60%RH	49.6	47.5
	30°C+65%RH	52.3	51.2
	40°C+75%RH	52.3	50.1
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	52.3	52.4
12 Weeks	25°C+60%RH	47.2	46.2
	30°C+65%RH	49.7	47.1
	40°C+75%RH	49.4	48.5

10.4.2 DISCUSSION

During the accelerated stability and the in-use stability test periods the viscosity of the reconstituted ARV suspension remained relatively stable.

10.5 MOISTURE CONTENT

10.5.1 RESULTS

The moisture content results after accelerated stability testing for the powder for suspension are given in table 10.5.

Table 10.5: Moisture content of the ARV powder for suspension during the accelerated stability test period.

Moisture content (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.025	25°C+60%RH	1.029	1.030
	30°C+65%RH	1.030	1.033
	40°C+75%RH	1.028	1.031

10.5.2 DISCUSSION

The moisture content of the ARV powder for suspension remained stable over the accelerated stability period. No significant change in results was observed.

10.6 RESUSPENDABILITY AND SEDIMENTATION RATE

10.6.1 RESULTS

All samples tested complied with the set standards, namely not more than 5 cm sediment should be present after 24 hours, and the sediment formed should be easily resuspended.

10.7 API RELEASE (DISSOLUTION)

10.7.1 RESULTS

The dissolution results for the reconstituted ARV powder for suspension are presented in figures 10.1-10.3.

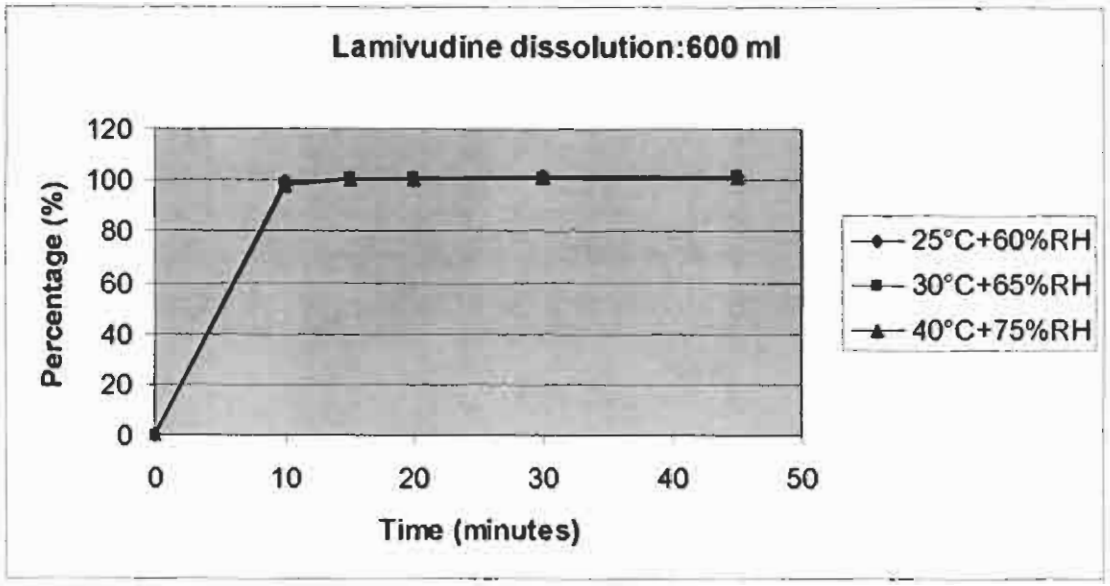

Figure 10.1: Dissolution results of lamivudine in 600 ml formulation obtained after 12 weeks accelerated stability testing.

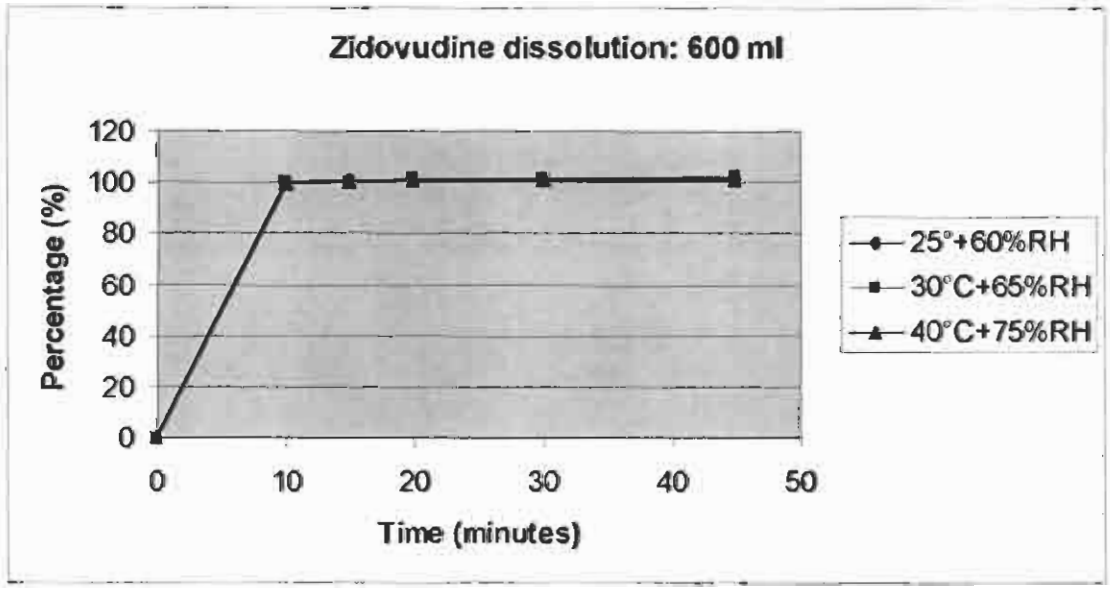

Figure 10.2: Dissolution results of zidovudine in 600 ml formulation obtained after 12 weeks accelerated stability testing.

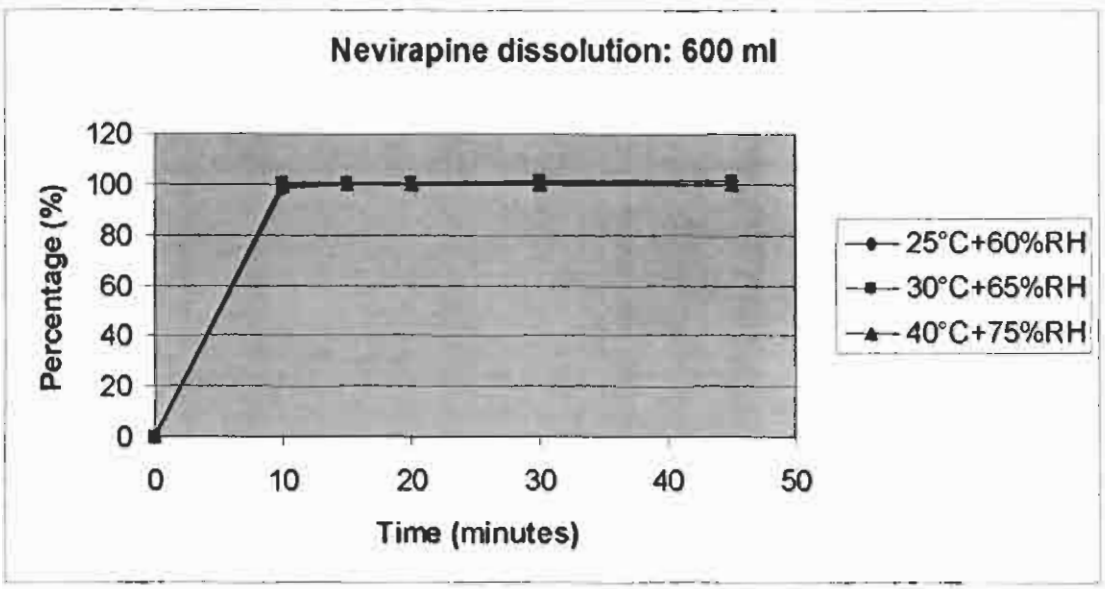

Figure 10.3: Dissolution results of nevirapine in 600 ml formulation obtained after 12 weeks accelerated stability testing.

10.7.2 DISCUSSION

The dissolution results obtained after 12 weeks accelerated stability testing showed that lamivudine, zidovudine and nevirapine were immediately dissolved with a dissolution of 102%, 102% and 101% respectively after 10 minutes.

10.8 API AND PRESERVATIVE CONCENTRATION ASSAY

10.8.1 RESULTS: LAMIVUDINE

The assay results for lamivudine during the accelerated stability and in-use stability study testing are given in table 10.6.

Table 10.6: Lamivudine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Lamivudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
100.3	25°C+60%RH	99.1	97.8
	30°C+65%RH	99.1	98.4
	40°C+75%RH	98.5	98.3
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	104.4	97.3
12 Weeks	25°C+60%RH	97.4	96.2
	30°C+65%RH	97.7	97.0
	40°C+75%RH	98.0	97.7

10.8.2 RESULTS: ZIDOVUDINE

The assay results for zidovudine during the accelerated stability and in-use stability study testing are given in table 10.7.

Table 10.7: Zidovudine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Zidovudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
101.4	25°C+60%RH	103.7	97.4
	30°C+65%RH	99.1	100.8
	40°C+75%RH	99.5	98.6
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	107.9	96.7
12 Weeks	25°C+60%RH	96.7	96.4
	30°C+65%RH	98.2	97.8
	40°C+75%RH	98.4	98.0

10.8.3 RESULTS: NEVIRAPINE

The assay results for nevirapine during the accelerated stability and in-use stability study testing are given in table 10.8.

Table 10.8: Nevirapine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Nevirapine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
101.8	25°C+60%RH	94.5	90.9
	30°C+65%RH	95.1	93.7
	40°C+75%RH	95.2	96.2
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	99.2	93.9
12 Weeks	25°C+60%RH	91.6	90.9
	30°C+65%RH	92.9	91.4
	40°C+75%RH	94.8	95.0

10.8.4 DISCUSSION

Lamivudine, zidovudine and nevirapine assay results obtained during accelerated stability testing and in-use testing were all within the specification of 90-110%

10.8.5 RESULTS: SODIUM METHYL HYDROXYBENZOATE

The assay results for sodium methyl hydroxybenzoate are given in table 10.9.

Table 10.9: Sodium methyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium methyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.152	25°C+60%RH	0.162	0.159
	30°C+65%RH	0.157	0.151
	40°C+75%RH	0.154	0.158
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.155	0.159
12 Weeks	25°C+60%RH	0.161	0.159
	30°C+65%RH	0.154	0.155
	40°C+75%RH	0.155	0.151

10.8.6 RESULTS: SODIUM PROPYL HYDROXYBENZOATE

The assay results for sodium propyl hydroxybenzoate are given in table 10.10.

Table 10.10: Sodium propyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium propyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.024	25°C+60%RH	0.027	0.019
	30°C+65%RH	0.027	0.020
	40°C+75%RH	0.026	0.023
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.024	0.021
12 Weeks	25°C+60%RH	0.024	0.021
	30°C+65%RH	0.018	0.019
	40°C+75%RH	0.023	0.024

10.8.7 DISCUSSION

Theoretical concentrations for sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate are 0.15% m/v and 0.02% m/v respectively. All results obtained for sodium methyl hydroxybenzoate during the accelerated stability trial and in-use stability remained well within the limit of 90 – 110% (0.135 – 0.165% m/v).

Some of the results obtained for sodium propyl hydroxybenzoate were above the limit of 110%. Due to the very small peaks representing sodium propyl hydroxybenzoate in the HPLC chromatogram, the above specification results could be due to faulty integration parameters.

10.9 PRESERVATIVE EFFICACY

10.9.1 RESULTS

The PET results for the initial, 6 weeks, 12 weeks and 12 weeks 30 days laboratory batches can be seen in tables 10.11-10.20.

Table 10.11: Preservative Efficacy Test results of initial ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.12: Preservative Efficacy Test results of 6 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.13: Preservative Efficacy Test results of 6 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.14: Preservative Efficacy Test results of 6 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.15: Preservative Efficacy Test results of 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.16: Preservative Efficacy Test results of 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.17: Preservative Efficacy Test results of 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.18: Preservative Efficacy Test results of 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.19: Preservative Efficacy Test results of 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 10.20: Preservative Efficacy Test results of 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

10.9.2 DISCUSSION

All laboratory samples complied with the requirements for category 3 products as described in USP 28. Therefore it can be concluded that the preservatives chosen for the formulation were effective in protecting the suspension from microbial contamination, even after simulating patient usage for a period of 30 days.

10.10 CONCLUSION

This chapter summarised results for various test samples of the 600 ml ARV powder for suspension, which underwent extensive physical and chemical testing during a period of twelve weeks and in-use testing for thirty days. The test samples represented lamivudine, zidovudine and nevirapine at concentrations of 75 mg/ 10 ml, 150 mg/ 10 ml and 100 mg/ 10 ml, respectively.

The ARV powder for suspension was stored at three storage conditions, namely 25°C+60%RH, 30°C+65%RH and 40°C+75%RH. The in-use tests were performed at uncontrolled room temperature.

Visual assessment tests included assessment of the colour, flavour and physical appearance of the test samples. No changes in flavour occurred during the stability period. Colour changed from snow white to off-white and watery, especially the test samples stored at 40°C+75%RH, which suggests temperatures as high as 40°C to be too high for the physical stability of the suspension.

The results obtained for pH, relative density, viscosity and moisture content did not change significantly throughout the stability testing period. All samples complied with the set specifications for sedimentation rate and resuspendability.

Dissolution results indicated that lamivudine, zidovudine and nevirapine were immediately dissolved after 10 minutes.

HPLC determination of assay concentrations for lamivudine, zidovudine and nevirapine showed that all results obtained were within the limit of 90-110%.

Results obtained for sodium methyl hydroxybenzoate remained stable and within the limit of 90-110%. Above specification results were obtained for sodium propyl hydroxybenzoate in some samples. PET testing confirmed efficacy of the preservative system used.

Further stability testing including a longer stability period will be required to more accurately reflect the stability potential of this formulation.

From these findings it can be concluded that the 600 ml ARV powder for suspension has potential as a paediatric ARV treatment for patients with a body weight of 15-20 kg.

CHAPTER 11

RESULTS AND DISCUSSION: POWDER FOR SUSPENSION FOR PAEDIATRIC PATIENTS: 20-29 KG

"There are no such things as applied sciences,
only applications of science."

-Louis Pasteur-

The powder for suspension that, if reconstituted, has a volume of 900 ml and provides ARV treatment for paediatric patients with a body mass of 20-29 kg for 1 month, was subjected to accelerated stability testing for a period of twelve weeks (refer to chapter 6 for the discussion of the stability program).

The results of each test will be discussed and graphic presentations of these results can be seen in annexure A-F.

11.1 VISUAL ASSESSMENT

11.1.1 RESULTS

Visual assessment was performed on reconstituted suspensions. Initial laboratory samples were stored at uncontrolled room temperature. Results for the visual assessment are given in Table 11.1.

Table 11.1: Visual assessment results of the 900 ml reconstituted ARV suspension, during stability and in-use testing.

Visual assessment			
Initial	**Storage temperatures**	**6 Weeks**	**12 Weeks**
Snow white, homogeneous thick white suspension.	25°C+60%RH	Snow white, uniform suspension.	Snow white suspension, larger particles than initially visible are present.
	30°C+65%RH	White uniform suspension.	Off white suspension, larger particles than initially visible are present.
	40°C+75%RH	White suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
In-use stability			
	Storage temperatures	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	Snow white, homogeneous thick suspension.	Snow white, homogeneous thick suspension.
12 Weeks	25°C+60%RH	Milky white suspension, larger particles than initially visible are present.	Milky white suspension, larger particles than initially visible are present.
	30°C+65%RH	Off white suspension, larger particles than initially visible are present.	Creamy white suspension, larger particles than initially visible are present.
	40°C+75%RH	Creamy white suspension, larger particles than initially visible are present.	Creamy white suspension, larger particles than initially visible are present.

11.1.2 DISCUSSION

A change in colour in the reconstituted powder for suspension from white to off-white was observed after storage at 30°C+65%RH and 40°C+75%RH for 12 weeks. The physical appearance showed a slight decomposition. Much larger dispersed particles, than were initially noticed were present throughout the suspension. The passion fruit flavour of the suspension remained stable throughout the stability and in-use test periods.

11.2 pH

11.2.1 RESULTS

The pH results after accelerated and in-use stability testing for the reconstituted suspension are given in table 11.2.

Table 11.2: pH results of the reconstituted ARV suspension after accelerated stability and in-use stability study.

pH			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
4.55	25°C+60%RH	4.51	4.49
	30°C+65%RH	4.54	4.52
	40°C+75%RH	4.46	4.39
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	4.49	4.45
12 Weeks	25°C+60%RH	4.45	4.43
	30°C+65%RH	4.48	4.45
	40°C+75%RH	4.36	4.34

11.2.2 DISCUSSION

During the accelerated stability and in-use stability test period, the pH of the reconstitutes suspension remained relatively stable.

11.3 RELATIVE DENSITY

11.3.1 RESULTS

The relative density results after accelerated and in-use stability testing for the reconstituted suspension are given in table 11.3.

Table 11.3: Relative density results (g/ml) of the reconstituted ARV suspension after accelerated stability and in-use studies.

Relative density (g/ml)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.157	25°C+60%RH	1.156	1.155
	30°C+65%RH	1.155	1.155
	40°C+75%RH	1.155	1.155
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	1.158	1.157
12 Weeks	25°C+60%RH	1.153	1.151
	30°C+65%RH	1.154	1.152
	40°C+75%RH	1.152	1.152

11.3.2 DISCUSSION

No significant change in the relative density results was observed after accelerated stability for 12 weeks and in-use testing for 30 days.

11.4 VISCOSITY

11.4.1 RESULTS

The viscosity results after accelerated and in-use stability testing for the reconstituted suspension are given in table 11.4.

Table 11.4: Viscosity (cp) results of the reconstituted ARV suspension during accelerated stability and in-use testing.

Viscosity (cp)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
48.5	25°C+60%RH	48.5	49.6
	30°C+65%RH	49.6	51.2
	40°C+75%RH	49.6	50.3
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	49.6	49.5
12 Weeks	25°C+60%RH	49.2	48.6
	30°C+65%RH	50.4	49.8
	40°C+75%RH	49.9	49.1

11.4.2 DISCUSSION

The viscosity of the reconstituted suspension showed no significant change during the accelerated stability and in-use test periods.

11.5 MOISTURE CONTENT

11.5.1 RESULTS

The moisture content results after accelerated stability testing for the powder suspension are given in table 11.5.

Table 11.5: Moisture content of the ARV powder for suspension during the accelerated stability test period.

Moisture content (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.261	25°C+60%RH	1.306	1.319
	30°C+65%RH	1.324	1.329
	40°C+75%RH	1.317	1.324

11.5.2 DISCUSSION

The moisture content of the ARV powder for suspension showed no significant change during the accelerated stability test period.

11.6 RESUSPENDABILITY AND SEDIMENTATION RATE

11.6.1 RESULTS

All the samples tested complied with the set standards, namely not more than 5 cm of sediment should be present after 24 hours, and the sediment formed should be easily resuspended.

11.7 API RELEASE (DISSOLUTION)

11.7.1 RESULTS

The dissolution results for the reconstituted ARV powder for suspension are presented in figures 11.1-11.3.

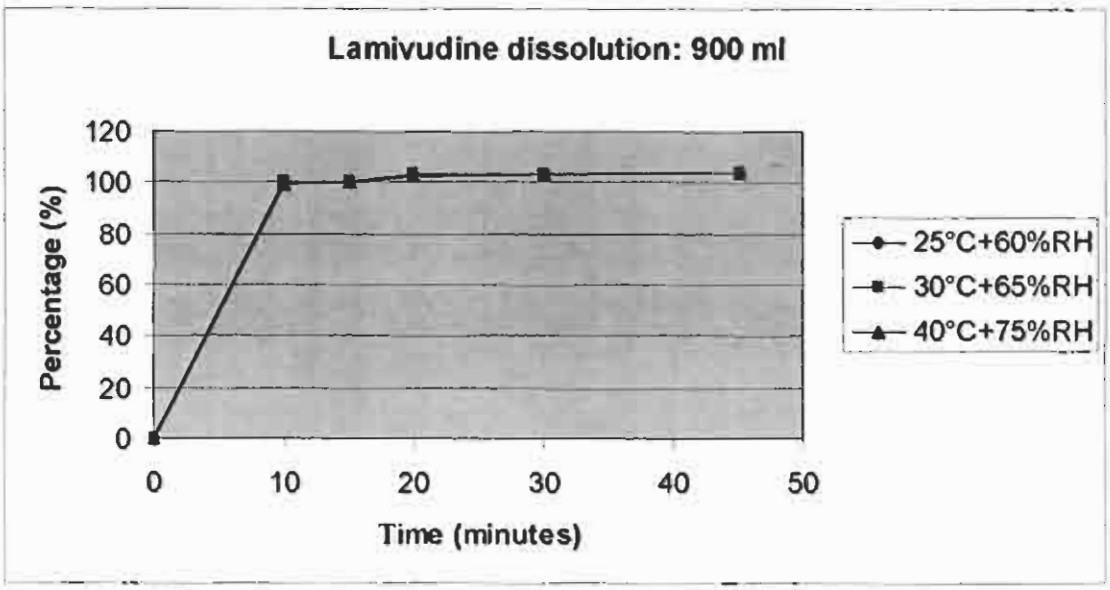

Figure 11.1: Dissolution results of lamivudine in 900 ml formulation obtained after 12 weeks accelerated stability testing.

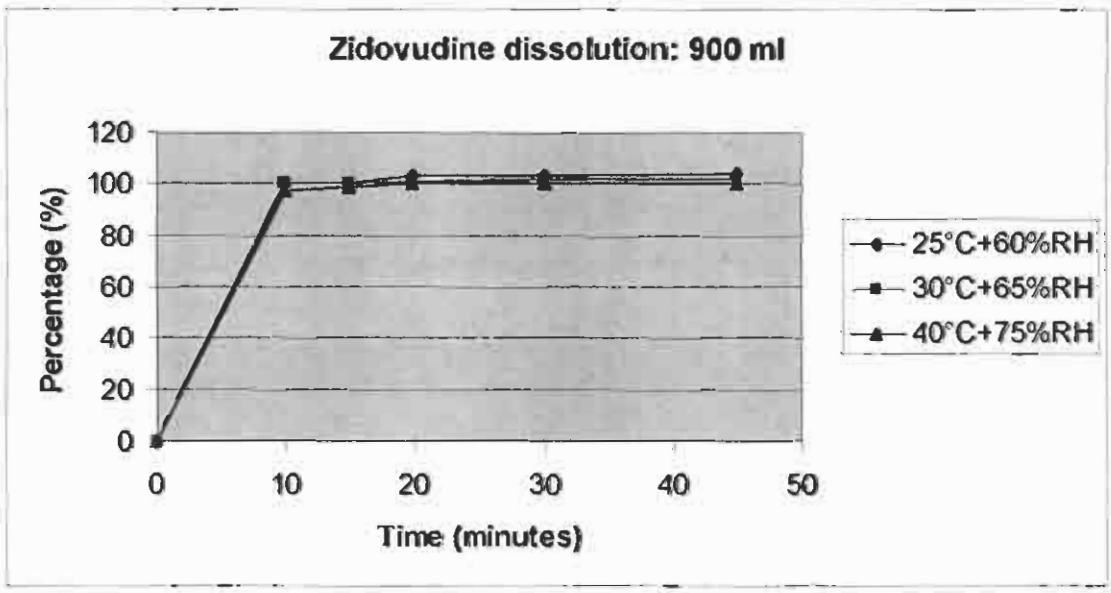

Figure 11.2: Dissolution results of zidovudine in 900 ml formulation obtained after 12 weeks accelerated stability testing.

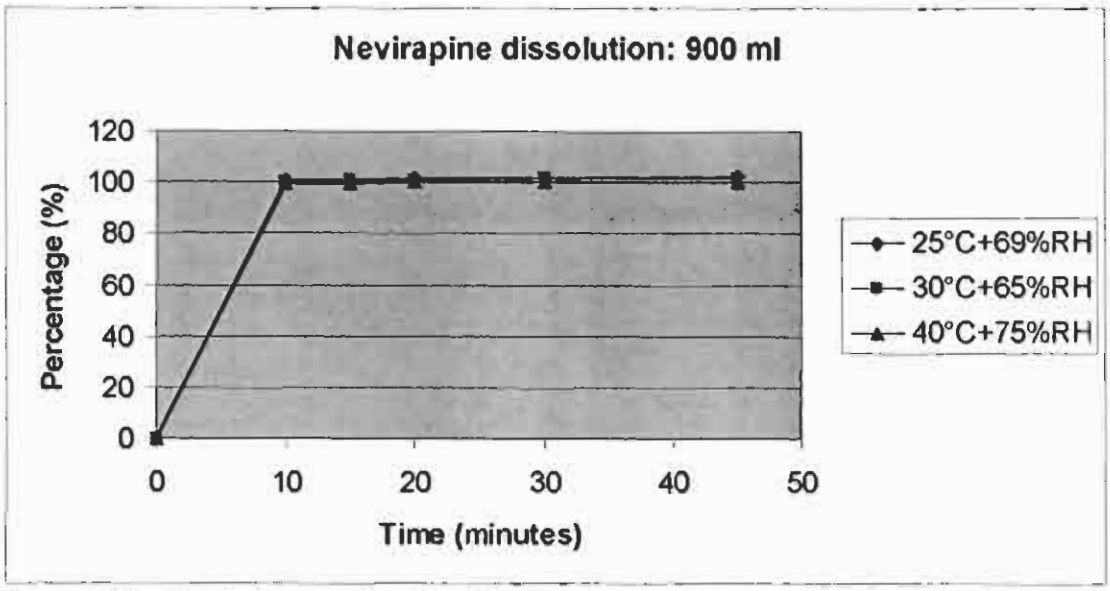

Figure 11.3: Dissolution results of nevirapine in 900 ml formulation obtained after 12 weeks accelerated stability testing.

11.7.2 DISCUSSION

The dissolution results obtained after 12 weeks accelerated stability testing showed that lamivudine, zidovudine and nevirapine were immediately dissolved with a final dissolution concentration of 104%, 103% and 101% respectively after 10 minutes.

11.8 API AND PRESERVATIVE CONCENTRATION ASSAY

11.8.1 RESULTS: LAMIVUDINE

The assay results for lamivudine during the accelerated stability and in-use stability study testing are given in table 11.6

Table 11.6: **Lamivudine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.**

Lamivudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
100.6	25°C+60%RH	101.8	102.1
	30°C+65%RH	99.0	106.1
	40°C+75%RH	95.3	96.7
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	101.2	98.5
12 Weeks	25°C+60%RH	98.8	98.2
	30°C+65%RH	95.1	94.5
	40°C+75%RH	93.7	93.1

11.8.2 RESULTS: ZIDOVUDINE

The assay results for zidovudine during the accelerated stability and in-use stability study testing are given in table 11.7.

Table 11.7: Zidovudine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Zidovudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
103.3	25°C+60%RH	102.4	103.9
	30°C+65%RH	102.6	100.5
	40°C+75%RH	100.9	104.6
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	102.6	94.8
12 Weeks	25°C+60%RH	103.9	102.9
	30°C+65%RH	100.4	99.5
	40°C+75%RH	103.7	101.5

11.8.3 RESULTS: NEVIRAPINE

The assay results for nevirapine during the accelerated stability and in-use stability study testing are given in table 11.8.

Table 11.8: Nevirapine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Nevirapine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
117.2	25°C+60%RH	91.3	98.1
	30°C+65%RH	92.7	93.2
	40°C+75%RH	97.6	97.0
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	120.4	104.0
12 Weeks	25°C+60%RH	93.1	92.8
	30°C+65%RH	93.0	92.6
	40°C+75%RH	92.9	91.6

11.8.4 DISCUSSION

Assay results obtained for lamivudine, zidovudine and nevirapine during accelerated and in-use testing were all within the specification of 90-110%, with the exception of nevirapine results at the initial and 15 days in-use at the initial stage. Both these results were above specification. Experimental error could be responsible for the above specification values obtained.

11.8.5 RESULTS: SODIUM METHYL HYDROXYBENZOATE

The assay results for sodium methyl hydroxybenzoate are given in table 11.9.

Table 11.9: Sodium methyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium methyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.151	25°C+60%RH	0.148	0.146
	30°C+65%RH	0.157	0.158
	40°C+75%RH	0.142	0.152
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.146	0.148
12 Weeks	25°C+60%RH	0.146	0.146
	30°C+65%RH	0.158	0.157
	40°C+75%RH	0.152	0.152

11.8.6 RESULTS: SODIUM PROPYLHYDROXYBENZOATE

The assay results for sodium propyl hydroxybenzoate are given in table 11.10

Table 11.10: Sodium propyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium propyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.024	25°C+60%RH	0.029	0.019
	30°C+65%RH	0.031	0.026
	40°C+75%RH	0.024	0.026
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.021	0.021
12 Weeks	25°C+60%RH	0.024	0.025
	30°C+65%RH	0.026	0.022
	40°C+75%RH	0.021	0.024

11.8.7 DISCUSSION

Theoretical concentrations for sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate are 0.15% m/v and 0.02% m/v respectively. All results obtained for sodium methyl hydroxybenzoate during the accelerated stability trial and in-use stability remained well within the limit of 90 – 110% (0.135 – 0.165% m/v).

Some of the results obtained for sodium propyl hydroxybenzoate were above the limit of 110%. Due to the very small peaks representing sodium propyl hydroxybenzoate in the HPLC chromatogram, the above specification results could be due to faulty integration parameters.

11.9 PRESERVATIVE EFFICACY

11.9.1 RESULTS

The PET results for the initial, 6 weeks, 12 weeks and 12 weeks 30 days laboratory batches can be seen in tables 11.11-11.20.

Table 11.11: Preservative Efficacy Test results of initial ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.12: Preservative Efficacy Test results of 6 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.13: Preservative Efficacy Test results of 6 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.14: Preservative Efficacy Test results of 6 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.15: Preservative Efficacy Test results of 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.16: Preservative Efficacy Test results of 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.17: Preservative Efficacy Test results of 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.18: Preservative Efficacy Test results of 12 weeks 30 days 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.19: Preservative Efficacy Test results of 12 weeks 30 days 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 11.20: Preservative Efficacy Test results of 12 weeks 30 days 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

11.9.2 DISCUSSION

All laboratory samples complied with the requirements for category 3 products as described in USP 28. Therefore it can be concluded that the preservatives chosen for the formulation were effective in protecting the suspension from microbial contamination, even after simulating patient usage for a period of 30 days.

11.10 CONCLUSION

This chapter summarized results for various test samples of the 900 ml ARV powder for suspension, which underwent extensive testing during a period of twelve weeks and in-use testing for thirty days. The test samples represented lamivudine, zidovudine and nevirapine at theoretical concentrations of 100 mg/ 15 ml, 100 mg/ 15 ml and 200 mg/ 15 ml, respectively.

The ARV powder for suspension was stored at three storage conditions, namely 25°C+60%RH, 30°C+65%RH and 40°C+75%RH. The in-use stability tests were preformed at uncontrolled room temperature.

Visual assessment tests included assessment of the colour, flavour and physical appearance of the test samples. No changes in flavour occurred during the stability period. Colour changed from snow white to a creamy colour. Test samples stored at 40°C+75%RH displayed a change in colour six weeks earlier than the samples stored at 25°C+60%RH and 30°C+65%RH. This may suggest the suspension to be more physically stable at 25°C+60%RH and 30°C+65%RH.

The results obtained for pH, relative density, viscosity and moisture content did not change significantly throughout the stability testing period. All samples complied with the set specifications for sedimentation rate and resuspendability.

Dissolution results indicated that lamivudine, zidovudine and nevirapine were immediately dissolved after 10 minutes.

HPLC determination of assay concentrations for lamivudine, zidovudine and nevirapine showed that all results obtained were within the limit of 90-110%, except for the initial and 15 days in-use value at the initial stage for nevirapine. Laboratory error could be responsible for the above specification results in these two cases.

Results obtained for sodium methyl hydroxybenzoate remained stable and within the limit of 90-110%. Above specification results were obtained for sodium propyl hydroxybenzoate in some samples. PET testing confirmed efficacy of the preservative system used.

Although most assay values of this formulation do comply with the set standards, the deterioration of the physical appearance of the suspension might be grounds for poor patient compliance. Further stability testing including a longer stability period will be required to more accurately reflect the stability potential of this formulation.

From these findings it can be concluded that the 900 ml ARV powder for suspension has potential as a paediatric ARV treatment for patients with a body weight of 20-29 kg.

CHAPTER 12

RESULTS AND DISCUSSION: POWDER FOR SUSPENSION FOR ADULT AND GERIATRIC PATIENTS

"In theory, there is no difference between theory and practice,
But in practice there is a great deal of difference."

-Anon-

The powder for suspension that, if reconstituted, has a volume of 1000 ml and provides ARV treatment for adult and geriatric patients for 1 month, was subjected to accelerated stability testing for a period of twelve weeks (refer to chapter 6 for the discussion of the stability program).

The results of each test will be discussed and graphic presentations of these results can be seen in annexure A-F.

12.1 VISUAL ASSESSMENT

12.1.1 RESULTS

Visual assessment was performed on reconstituted suspensions. Initial laboratory samples were stored at uncontrolled room temperature. Results for the visual assessment are given in Table 12.1.

Table 12.1: **Visual assessment results of the 1000 ml reconstituted ARV suspension, during stability and in-use testing.**

Visual assessment			
Initial	**Storage temperatures**	**6 Weeks**	**12 Weeks**
Snow white, homogeneous thick white suspension.	25°C+60%RH	Snow white, uniform suspension.	White, milky suspension, larger particles than initially visible are present.
	30°C+65%RH	White uniform suspension.	Off white suspension, larger particles than initially visible are present.
	40°C+75%RH	White suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
In-use stability			
	Storage temperatures	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	Snow white, homogeneous thick suspension.	Snow white, homogeneous thick suspension.
12 Weeks	25°C+60%RH	Milky white suspension, larger particles than initially visible are present.	Milky white suspension, larger particles than initially visible are present.
	30°C+65%RH	Off white suspension, larger particles than initially visible are present.	Off white suspension, larger particles than initially visible are present.
	40°C+75%RH	Creamy white suspension, larger particles than initially visible are present.	Watery yellow suspension, larger particles than initially visible are present.

12.1.2 DISCUSSION

During the visual assessment, a change in the physical appearance of the reconstituted suspension was clearly seen. Larger than originally marked particles were dispersed throughout the suspension after 6 weeks storage at 40°C+75%RH and 12 weeks at 30°C+65%RH and 40°C+75%RH, as well as in all the in-use samples after 12 weeks. Further changes in colour were obvious, especially during the 12 week 15 days in-use testing and onward. The passion fruit flavour of the suspension remained stable throughout the accelerated stability and in-use stability test periods.

12.2 pH

12.2.1 RESULTS

The pH results after accelerated and in-use stability testing for the reconstituted suspension are given in table 12.2.

Table 12.2: pH results of the reconstituted ARV suspension after accelerated stability and in-use stability study.

pH			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
4.50	25°C+60%RH	4.54	4.51
	30°C+65%RH	4.56	4.47
	40°C+75%RH	4.59	4.54
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	4.52	4.52
12 Weeks	25°C+60%RH	4.49	4.47
	30°C+65%RH	4.45	4.44
	40°C+75%RH	4.50	4.49

12.2.2 DISCUSSION

During the accelerated stability and in-use stability study the pH of the reconstituted suspension showed no significant change.

12.3 RELATIVE DENSITY

12.3.1 Results

The relative density results after accelerated and in-use stability testing for the reconstituted suspension are given in table 12.3.

Table 12.3: Relative density results (g/ml) of the reconstituted ARV suspension after accelerated stability and in-use studies.

Relative density (g/ml)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.156	25°C+60%RH	1.158	1.156
	30°C+65%RH	1.157	1.155
	40°C+75%RH	1.156	1.153
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	1.158	1.157
12 Weeks	25°C+60%RH	1.154	1.152
	30°C+65%RH	1.153	1.152
	40°C+75%RH	1.150	1.149

12.3.2 DISCUSSION

The relative density of the 1000 ml reconstituted suspension remained relatively stable during the accelerated stability and in-use testing period.

12.4 VISCOSITY

12.4.1 RESULTS

The viscosity results after accelerated and in-use stability testing for the reconstituted suspension are given in table 12.4.

Table 12.4: Viscosity (cp) results of the reconstituted ARV suspension during accelerated stability and in-use testing.

Viscosity (cp)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
52.3	25°C+60%RH	52.3	51.6
	30°C+65%RH	51.7	50.3
	40°C+75%RH	51.6	50.8
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	51.7	51.7
12 Weeks	25°C+60%RH	50.0	49.6
	30°C+65%RH	49.7	49.2
	40°C+75%RH	50.1	49.5

12.4.2 DISCUSSION

During the accelerated stability test and in-use stability testing period, the viscosity of the reconstituted suspension showed no significant changes.

12.5 MOISTURE CONTENT

12.5.1 RESULTS

The moisture content results after accelerated stability testing for the powder for suspension are given in table 12.5.

Table 12.5: Moisture content of the ARV powder for suspension during the accelerated stability test period.

Moisture content (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
1.438	25°C+60%RH	1.468	1.470
	30°C+65%RH	1.471	1.476
	40°C+75%RH	1.478	1.483

12.5.2 DISCUSSION

No significant change in the moisture content of the powder for suspension was observed after accelerated stability testing.

12.6 RESUSPENDABILITY AND SEDIMENTATION RATE

12.6.1 RESULTS

All the samples tested complied with the set standards, namely not more than 5 cm of sediment should be present after 24 hours, and the sediment formed should be easily resuspended.

12.7 API RELEASE (DISSOLUTION)

12.7.1 RESULTS

The dissolution results for the reconstituted ARV powder for suspension are presented in figures 12.1-12.3.

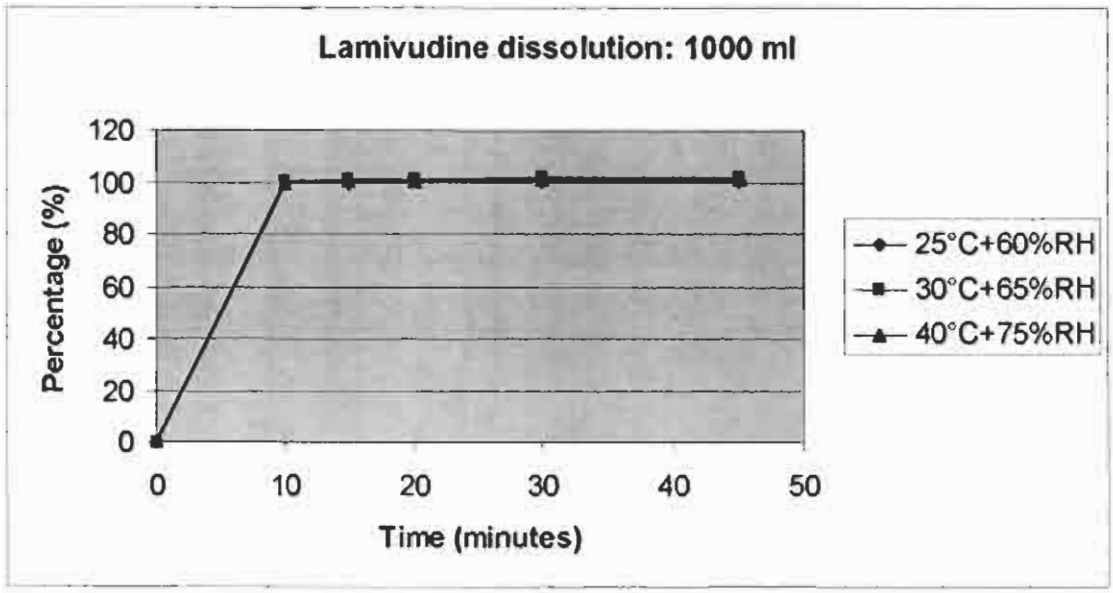

Figure 12.1: Dissolution results of lamivudine in 1000 ml formulation obtained after 12 weeks accelerated stability testing.

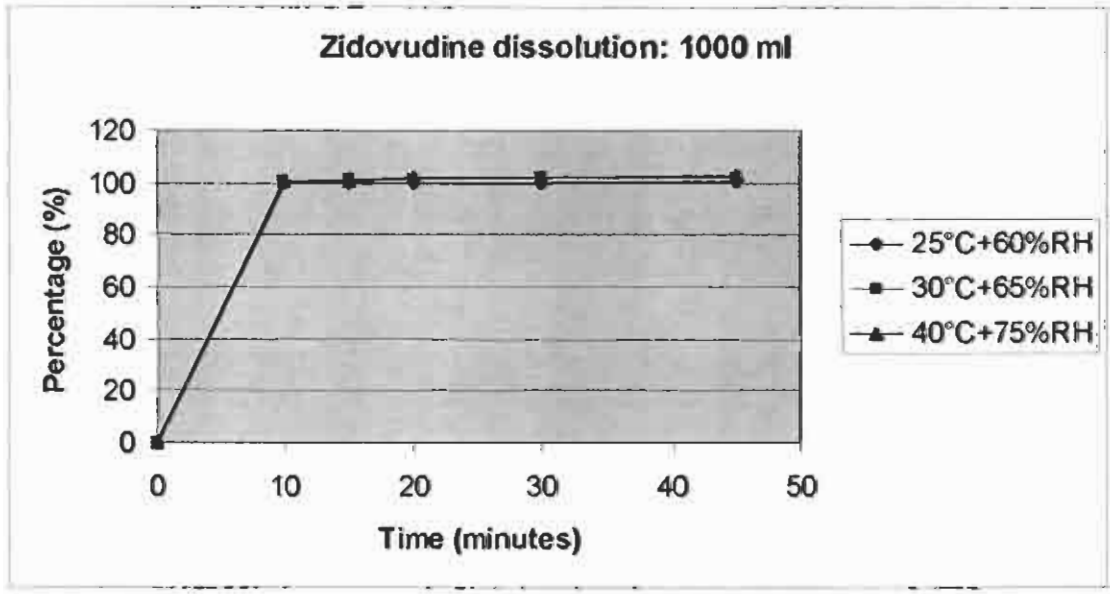

Figure 12.2: Dissolution results of zidovudine in 1000 ml formulation obtained after12 weeks accelerated stability testing.

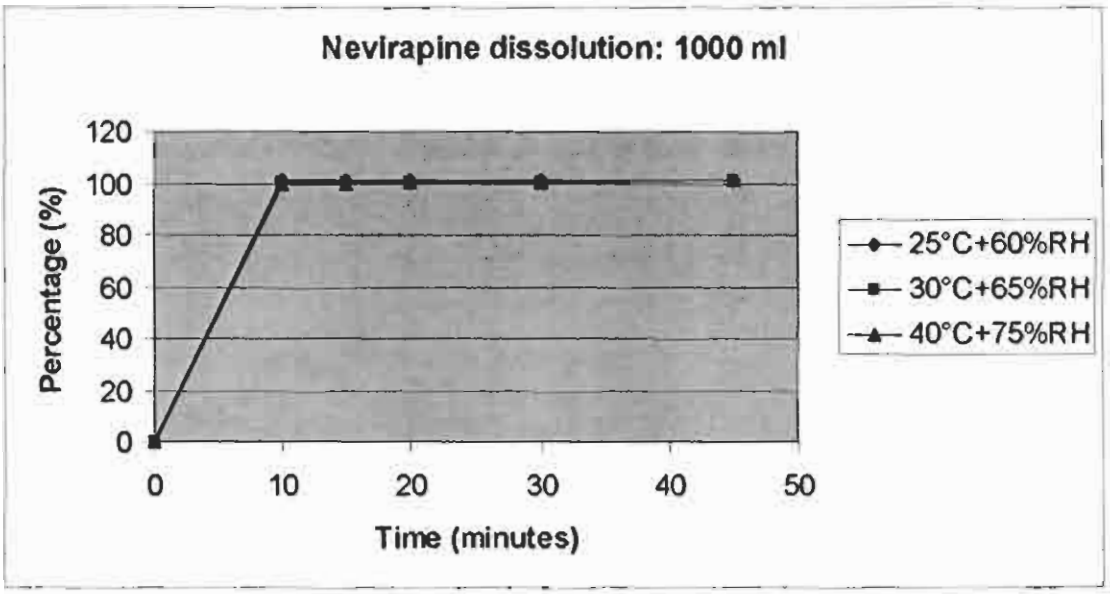

Figure 12.3: Dissolution results of nevirapine in 1000 ml formulation obtained after 12 weeks accelerated stability testing.

12.7.2 DISCUSSION

The dissolution results obtained after 12 weeks accelerated stability testing showed that lamivudine, zidovudine and nevirapine were immediately dissolved with a dissolution of 101%, 103% and 102% respectively after 10 minutes.

12.8 API AND PRESERVATIVE CONCENTRATION ASSAY

12.8.1 RESULTS: LAMIVUDINE

The assay results for lamivudine during the accelerated stability and in-use stability study testing are given in table 12.6.

Table 12.6: **Lamivudine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.**

Lamivudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
100.6	25°C+60%RH	99.3	97.0
	30°C+65%RH	103.0	94.7
	40°C+75%RH	99.3	93.8
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	98.4	99.4
12 Weeks	25°C+60%RH	97.0	96.6
	30°C+65%RH	94.7	94.3
	40°C+75%RH	94.7	93.9

12.8.2 RESULTS: ZIDOVUDINE

The assay results for zidovudine during the accelerated stability and in-use stability study testing are given in table 12.7.

Table 12.7: Zidovudine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Zidovudine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
99.7	25°C+60%RH	97.9	94.2
	30°C+65%RH	93.0	94.0
	40°C+75%RH	97.0	93.2
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	98.2	95.5
12 Weeks	25°C+60%RH	94.4	93.2
	30°C+65%RH	94.4	95.0
	40°C+75%RH	93.2	92.6

12.8.3 RESULTS: NEVIRAPINE

The assay results for nevirapine during the accelerated stability and in-use stability study testing are given in table 12.8.

Table 12.8: Nevirapine assay results after 12 weeks accelerated stability study and 30 days in-use stability study.

Nevirapine (%)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
99.8	25°C+60%RH	94.8	93.0
	30°C+65%RH	91.9	95.2
	40°C+75%RH	94.3	94.0
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	90.2	96.1
12 Weeks	25°C+60%RH	90.5	91.6
	30°C+65%RH	91.2	90.8
	40°C+75%RH	92.2	91.9

12.8.4 DISCUSSION

A downward trend in the assay results obtained for lamivudine, zidovudine and nevirapine was observed after accelerated and in-use stability testing. However, all results remained within the 90-110% specification.

12.8.5 RESULTS: SODIUM METHYL HYDROXYBENZOATE

The assay results for sodium methyl hydroxybenzoate are given in table 12.9.

Table 12.9: Sodium methyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium methyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.162	25°C+60%RH	0.158	0.166
	30°C+65%RH	0.151	0.154
	40°C+75%RH	0.153	0.159
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.162	0.160
12 Weeks	25°C+60%RH	0.169	0.161
	30°C+65%RH	0.159	0.152
	40°C+75%RH	0.158	0.160

12.8.6 RESULTS: SODIUM PROPYL HYDROXYBENZOATE

The assay results for sodium propyl hydroxybenzoate are given in table 12.10.

Table 12.10: Sodium propyl hydroxybenzoate assay results after 12 weeks accelerated stability testing and 30 days in-use stability testing.

Sodium propyl hydroxybenzoate (% m/v)			
Initial	**Storage conditions**	**6 Weeks**	**12 Weeks**
0.021	25°C+60%RH	0.022	0.025
	30°C+65%RH	0.028	0.026
	40°C+75%RH	0.021	0.024
In-use stability testing			
	Storage conditions	**15 Days**	**30 Days**
Initial	Uncontrolled room temperature	0.022	0.019
12 Weeks	25°C+60%RH	0.025	0.027
	30°C+65%RH	0.026	0.025
	40°C+75%RH	0.020	0.018

12.8.7 DISCUSSION

Theoretical concentrations for sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate are 0.15% m/v and 0.02% m/v respectively. Results obtained for sodium methyl hydroxybenzoate during the accelerated stability trial and in-use stability showed a slight incline in concentration of the laboratory sample stored at 25°C+60%RH, all of the other laboratory samples remained well within the limit of 90 – 110% (0.135 – 0.165% m/v) during the in-use and stability testing.

Some of the results obtained for sodium propyl hydroxybenzoate were above the limit of 110%. Due to the very small peaks representing sodium propyl hydroxybenzoate in the HPLC chromatogram, the above specification results could be due to faulty integration parameters.

12.9 PRESERVATIVE EFFICACY

12.9.1 RESULTS

The PET results for the initial, 6 weeks, 12 weeks and 12 weeks 30 days laboratory batches can be seen in tables12.11-12.20.

Table 12.11: **Preservative Efficacy Test results of initial ARV reconstituted suspension.**

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.12: **Preservative Efficacy Test results of 6 weeks 25°C+60%RH ARV reconstituted suspension.**

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.13: Preservative Efficacy Test results of 6 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.14: Preservative Efficacy Test results of 6 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.15: Preservative Efficacy Test results of 12 weeks 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.16: Preservative Efficacy Test results of 12 weeks 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.17: Preservative Efficacy Test results of 12 weeks 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.18: Preservative Efficacy Test results of 12 weeks 30 days 25°C+60%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.19: Preservative Efficacy Test results of 12 weeks 30 days 30°C+65%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

Table 12.20: Preservative Efficacy Test results of 12 weeks 30 days 40°C+75%RH ARV reconstituted suspension.

Test Organism	Initial Inoculum	Log Unit Reduction			Specified limits for category 3 products
		Day 7	Day 14	Day 28	
E. coli	2.0×10^3	> 3.0	> 3.0	> 3.0	>2.0 log reduction at 14 days and no increase at 28 days.
P. aeruginosa	2.4×10^3	> 3.0	> 3.0	> 3.0	
S. aureus	2.1×10^3	> 3.0	> 3.0	> 3.0	
A. niger	1.6×10^3	> 3.0	> 3.0	> 3.0	No increase from initial inoculum
C. albicans	2.0×10^3	> 3.0	> 3.0	> 3.0	

12.9.2 DISCUSSION

All laboratory samples complied with the requirements for category 3 products as described in USP 28. Therefore it can be concluded that the preservatives chosen for the formulation were effective in protecting the suspension from microbial contamination, even after simulating patient usage for a period of 30 days.

12.10 CONCLUSION

This chapter summarised results for various test samples of the 1000 ml ARV powder for suspension, which underwent extensive testing during a period of twelve weeks and in-use testing for thirty days. The test samples represented lamivudine, nevirapine and zidovudine at theoretical concentrations of 150 mg/ 16.6 ml, 200 mg/ 16.6 ml and 275 mg/ 16.6 ml, respectively.

The ARV powder for suspension was stored at three storage conditions, namely 25°C+60%RH, 30°C+65%RH and 40°C+75%RH. The in-use stability tests were preformed at uncontrolled room temperature.

Visual assessment tests included assessment of the colour, flavour and physical appearance of the test samples. No changes in flavour occurred during the stability period. Colour dramatically changed from snow white to a watery yellow. A change in physical appearance was apparent in test samples stored for 6 weeks at 40 C+75%RH, and 12 weeks 25°C+60%RH and higher, as well as the in-use samples after 12 weeks storage.

The results obtained for pH, relative density, viscosity and moisture content did not change significantly throughout the stability testing period. All samples complied with the set specifications for sedimentation rate and resuspendability.

Dissolution results indicated that lamivudine, zidovudine and nevirapine were immediately dissolved after 10 minutes.

HPLC determination of assay concentrations for lamivudine, zidovudine and nevirapine showed that all results obtained were within the limit of 90-110%.

Results obtained for sodium methyl hydroxybenzoate indicated a slight increase in concentration of the laboratory samples stored at 25°C+60%RH for 12 weeks during in-use and stability studies, the rest of the laboratory samples remained stable and within the limit of 90-110%. Above specification results were obtained for sodium propyl hydroxybenzoate in some samples. PET testing confirmed efficacy of the preservative system used.

Although the assay values of this formulation do comply with the set standards the deterioration of the physical appearance of the suspension might be grounds for poor patient compliance.

Further stability testing including a longer stability period will be required to more accurately reflect the stability potential of this formulation.

From these findings it can be concluded that the 1000 ml ARV powder for suspension has potential as an adult and geriatric ARV treatment for patients who experience difficulty when swallowing solid dosage forms.

CHAPTER 13

SUMMARY AND CONCLUSION

"The great tragedy of science – the slaying of a beautiful hypothesis by an ugly fact."

- T.H. Huxley-

Fixed Dose Combination (FDC) of Antiretroviral (ARV) drugs could in future become somewhat of a comfort for those who suffer from HIV or AIDS. Because of the user friendliness of FDCs, patient adherence will improve remarkably. Workloads upon the shoulders of medical staff and caregivers will drastically decrease and wrongly administered dosages due to complicated dosage regimes will be something of the past.

Before the completion of this study, no FDC containing lamivudine, zidovudine and nevirapine has been available.

13.1 SUMMARY

The focus of this study was to combine lamivudine, zidovudine and nevirapine into an oral powder for suspension. Six powder for suspension formulas were prepared, of which five contained the dosage regimes for five growth stages during the paediatric HIV / AIDS patient's life. The sixth formulation contained the dosage regime for adult and geriatric HIV/AIDS patients as suggested by UNICEF/WHO (2004).

All six formulations were subjected to accelerated stability studies over a period of twelve weeks. Initial and twelve week batches underwent in-use stability testing, where the stability of the reconstituted suspensions during patient usage was studied.

Assay determination of APIs and preservative content was done by means of HPLC. Physical stability of the formulations were investigated by conducting pH, relative density, viscosity, moisture content, sedimentation rate and resuspendability experiments..

Nevirapine on two occasions did present an extremely low assay concentration during in use stability testing. This may be due to faulty laboratory equipment used at the specific time.

The pH, relative density, viscosity and moisture content of the formulations remained stable throughout the given stability periods. Sedimentation rate and resuspendability results also complied with the set standards. Visual assessment of the reconstituted suspensions did indicate possible instability. Large dispersed particles, which were absent during initial testing, were present after 12 weeks storage at 30°C+65%RH and 40°C+75%RH, as well as during in-use testing after 12 weeks. The colour of the reconstituted suspensions also changed over time, gradually turning from snow white to a watery yellow colour. The sweet passion fruit flavour remained stable during accelerated stability and in-use periods.

Dissolution results showed that lamivudine, zidovudine and nevirapine were immediately dissolved, with 100% dissolution after 10 minutes. The assay results for lamivudine, zidovudine and nevirapine remained within the specification of 90-110%, but a downward trend in lamivudine assay was observed in the 300 ml and 900 ml formulation, where a result of 89.3% was measured after 12 weeks at 40°C+75%RH.

Results for sodium methyl hydroxybenzoate indicated a slight increase in concentration in the 150ml and 1000ml container, the rest of the laboratory batches remained within the 90-110% limit throughout accelerated stability and in-use testing. Above specification results were also obtained for sodium propyl hydroxybenzoate in some samples which could be ascribed to faulty integration parameters due to the very small peak representing sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate in the HPLC chromatogram.

Preservative efficacy testing confirmed the sufficiency of the preservative system used in the formulations.

13.2 CONCLUSION

The effective treatment of HIV and AIDS in this day and age is dependent on three major factors, namely effective medicinal therapy, uncomplicated dosage regimes and patient compliance. The formulated FDC containing lamivudine, zidovudine and nevirapine in a powder for oral suspension has the possibilities to become an essential part in the fight against HIV and AIDS.

There are additional requirements that need further investigation which include:

- Conducting accelerated stability studies over a longer period of time.
- Possible reformulation of the products to eliminate visual instability.
- Container volumes that match the exact volume of the reconstituted suspension.
- *In vivo* testing of these six ARV powders for oral suspension.

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CONFERENCE CONTRIBUTIONS

Conference contributions

1. De Villiers, J.O., Lötter, A.P. & Swanepoel, E. 2005. The combination of lamivudine, zidovudine and nevirapine in a powder for oral suspension. Poster presented at the 20th annual conference of the Academy of Pharmaceutical Sciences, September 2005, Port Elizabeth, South Africa.

The Combination of Lamivudine, Zidovudine and Nevirapine In a Powder for Oral Suspension.

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Purpose:

Combination antiretroviral therapy has proven to be the most effective approach in treating HIV positive patients. An oral liquid dosage formulation is desired for paediatric HIV patients, and for those who cannot swallow other oral dosage forms such as capsules and tablets. The focus of this study was to identify incompatibilities, and other factors that could possibly affect the chemical stability of Nevirapine, Zidovudine and Lamivudine in combination in a powder for oral suspension. Six suspension formulations according to patient mass were made containing the dosage régime for one month as suggested by the WHO. Sodium propylhydroxybenzoate and sodium methyl-hydroxybenzoate were added as preservatives.

Methods:

A preformulation study was preformed to eliminate any incompatibility between the excipients and the actives. The formulated products were submitted to accelerated stability studies for a three month period at 25°/60%RH, 30°C/65%RH and 40°C/75%RH. HPLC was used to determine the concentration of the active ingredients. Preservative efficacy tests were done to determine the effectiveness of the preservative system. Other tests included appearance, pH, viscosity, density, resuspendability and rate of sedimentation. Also, an in use stability study was done to simulate patient use.

Results:

No incompatibilities were detected between the excipients and the actives. There was no change in odor or physical appearance of the formulations, the suspensions had a satisfactory sedimentation rate, and the sediment was easily resuspended. The pH, viscosity and densities of the formulations were relatively constant. The assay values of all actives and preservatives remained within specification. Preservative efficacy results proved the effectiveness of the preservative system.

Conclusion:

The results obtained indicate that Lamivudine, Zidovudine, Nevirapine and excipients are compatible and can successfully be combined in a powder for oral suspension.

The Combination of Lamivudine, Zidovudine and Nevirapine In a Powder for Oral Suspension.

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Figure 1 3D Structure of the HI-virus.

Background

Combination antiretroviral therapy has proven to be the most effective approach in treating HIV positive patients. An oral liquid dosage formulation is desired for paediatric HIV patients, and for those who cannot swallow other oral dosage forms such as capsules and tablets.

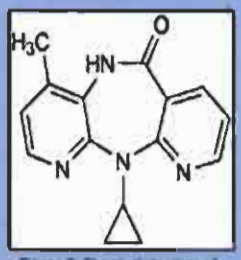

Figure 2 Chemical structure of Nevirapine

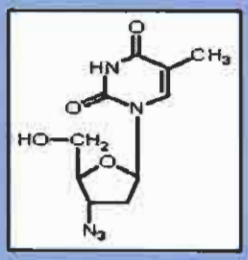

Figure 3 Chemical structure of Zidovudine.

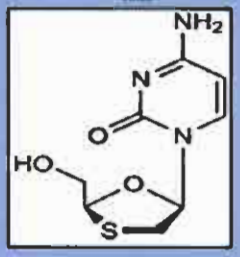

Figure 4 Chemical structure of Lamivudine

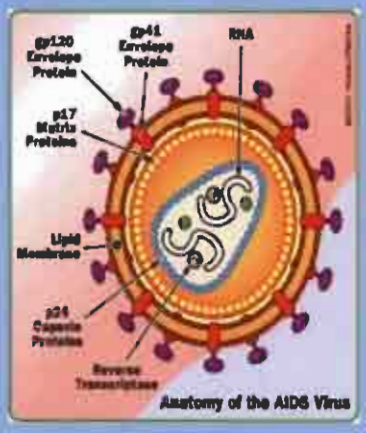

Figure 5 Anatomy of the HI-Virus.

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTs) which binds to the enzyme reverse transcriptase adjacent to the catalytic site and causes conformational changes that inactivate the enzyme (Hardman et al., 2001:1360). Zidovudine and Lamivudine are nucleoside reverse transcriptase inhibitors (NRTs) both competing with thymidine triphosphate for incorporation into the viral DNA and causes termination of the viral DNA chain (Hardman et al., 2001:1358).

Materials and methods

A preformulation study was performed to eliminate any incompatibility between the excipients and the actives. The formulated products were submitted to accelerated stability studies for a three month period at 25°C/60%RH, 30°C/65%RH and 40°C/75%RH. HPLC was used to determine the concentration of the active ingredients. Preservative efficacy tests were done to determine the effectiveness of the preservative system. Other tests included appearance, pH, viscosity, density, resuspendability and rate of sedimentation. Also, an in-use stability study was done to simulate patient use.

Results

No incompatibilities were detected between the excipients and the actives. There was no change in odor or physical appearance of the formulations, the suspensions had a satisfactory sedimentation rate, and the sediment was easily resuspended. The pH, viscosity and densities of the formulations showed no significant change. The assay values of all actives and preservatives remained within specification. Preservative efficacy results proved the effectiveness of the preservative system. The results below show the assay values of Zidovudine, Nevirapine and Lamivudine in the 60ml suspension in concentrations specified by the WHO for children with a body weight of between 15-20kg.

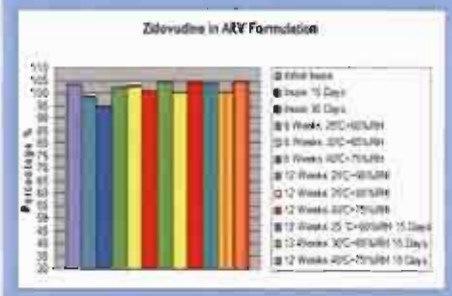

Figure 6 The concentration (%) Zidovudine in the ARV formulation.

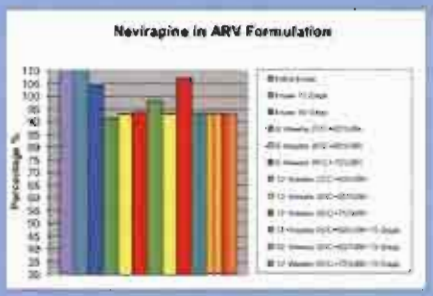

Figure 7 The concentration (%) Nevirapine in the ARV formulation.

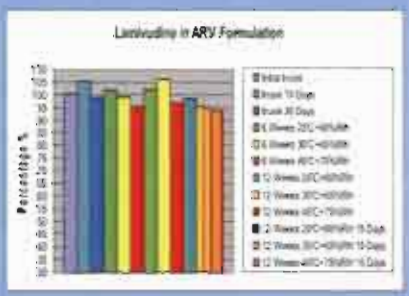

Figure 8 The concentration (%) Lamivudine in the ARV formulation.

Conclusion

The results obtained indicate that Lamivudine, Zidovudine, Nevirapine and excipients are compatible and can successfully be combined in a powder for oral suspension.

Hardman et al., 2001:1360; Hardman et al., 2001:1358

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ANNEXURES

- **ANNEXURE A** GRAPHIC REPRESENTATION: pH
- **ANNEXURE B** GRAPHIC REPRESENTATION: RELATIVE DENSITY
- **ANNEXURE C** GRAPHIC REPRESENTATION: VISCOSITY
- **ANNEXURE D** GRAPHIC REPRESENTATION: MOISTURE CONTENT
- **ANNEXURE E** GRAPHIC REPRESENTATION: API ASSAYS
- **ANNEXURE F** GRAPHIC REPRESENTATION: PRESERVATIVE ASSAYS
- **ANNEXURE G** HPLC METHOD VALIDATION

ANNEXURE A

pH: Initial in-use stability study

- **150 ml** **A2**
- **300 ml** **A2**
- **420 ml** **A3**
- **600 ml** **A3**
- **900 ml** **A4**
- **1000 ml** **A4**

pH: Accelerated stability study

- **150 ml** **A5**
- **300 ml** **A5**
- **420 ml** **A6**
- **600 ml** **A6**
- **900 ml** **A7**
- **1000 ml** **A7**

pH: 12 Week in-use stability study

- **150 ml** **A8**
- **300 ml** **A8**
- **420 ml** **A9**
- **600 ml** **A9**
- **900 ml** **A10**
- **1000 ml** **A10**

A.1: pH: INITIAL IN USE STUDY

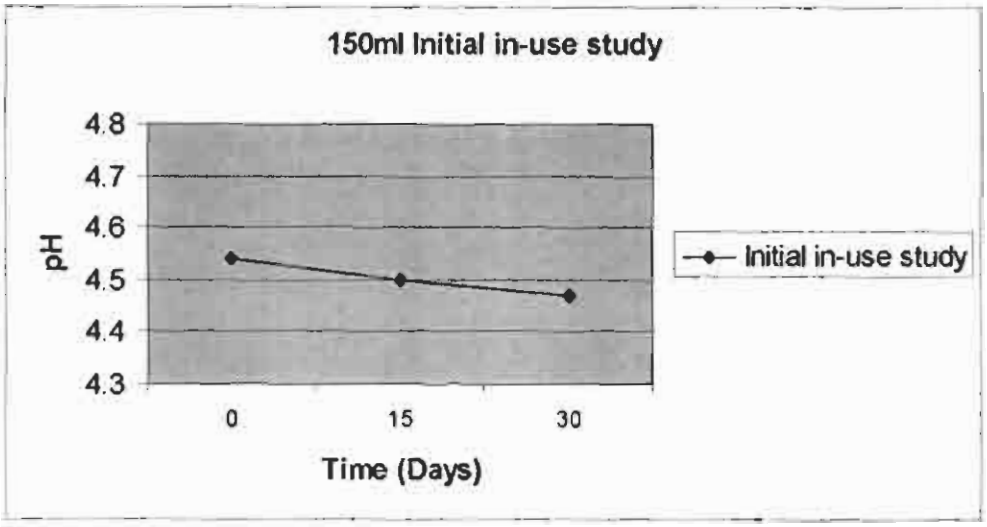


Figure A.1.1: Graphic representation of the pH results obtained for the 150 ml ARV suspension during in-use stability testing.

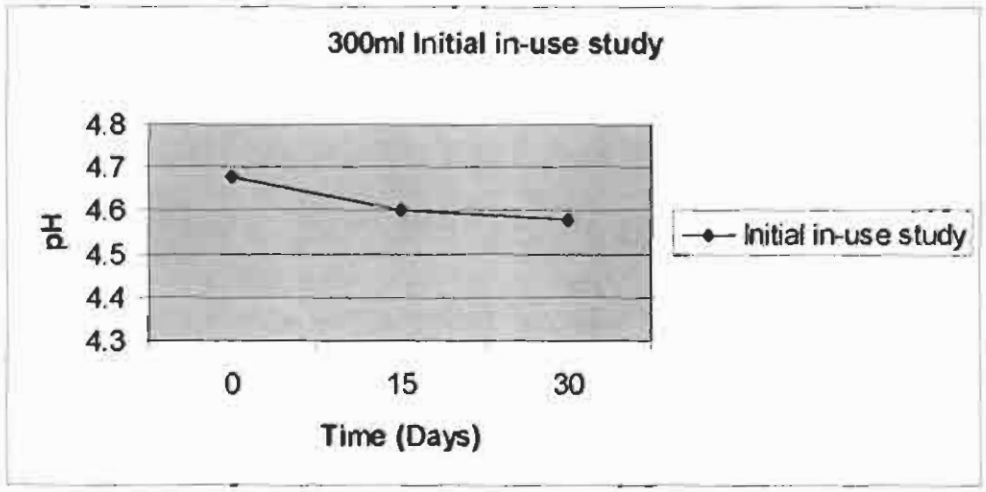


Figure A.1.2: Graphic representation of the pH results obtained for the 300 ml ARV suspension during in-use stability testing.

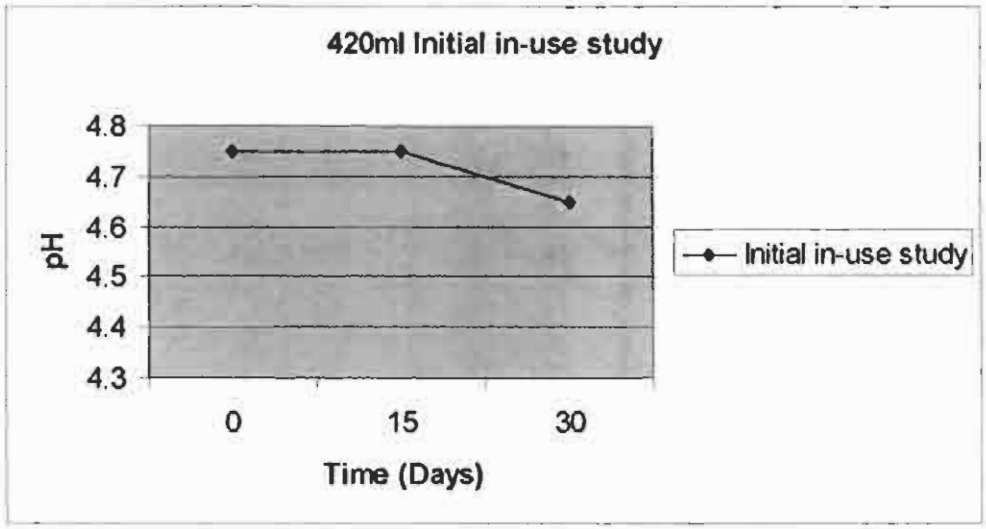


Figure A.1.3: Graphic representation of the pH results obtained for the 420 ml ARV suspension during in-use stability testing.

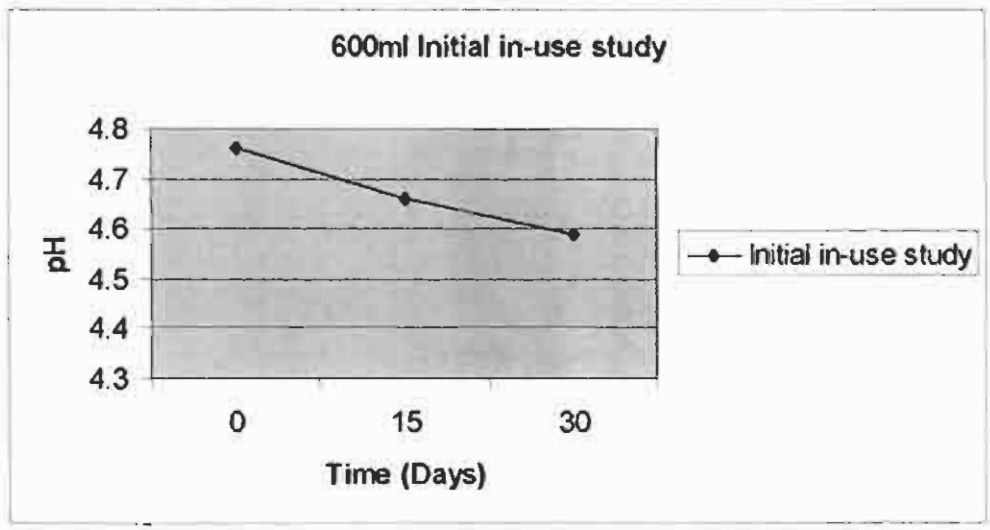


Figure A.1.4: Graphic representation of the pH results obtained for the 600 ml ARV suspension during in-use stability testing

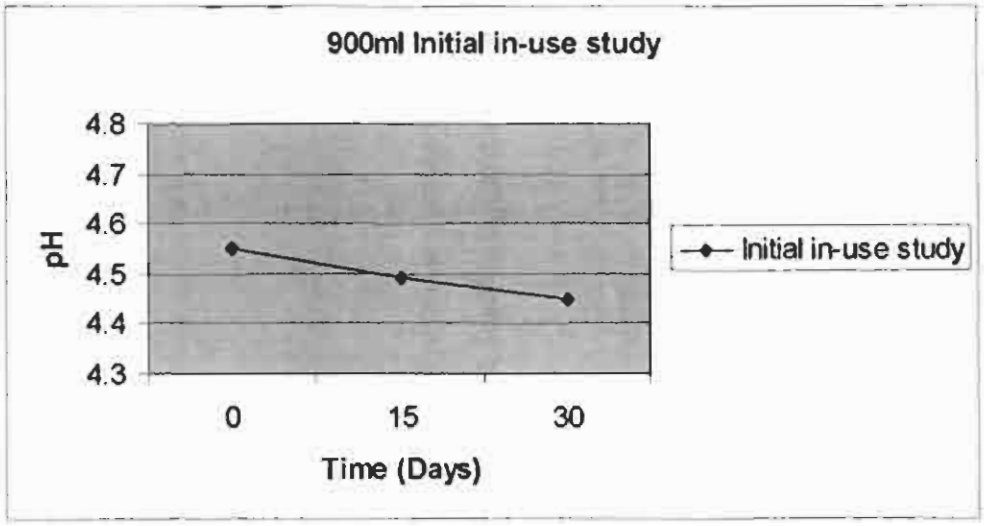


Figure A.1.5: Graphic representation of the pH results obtained for the 900 ml ARV suspension during in-use stability testing.

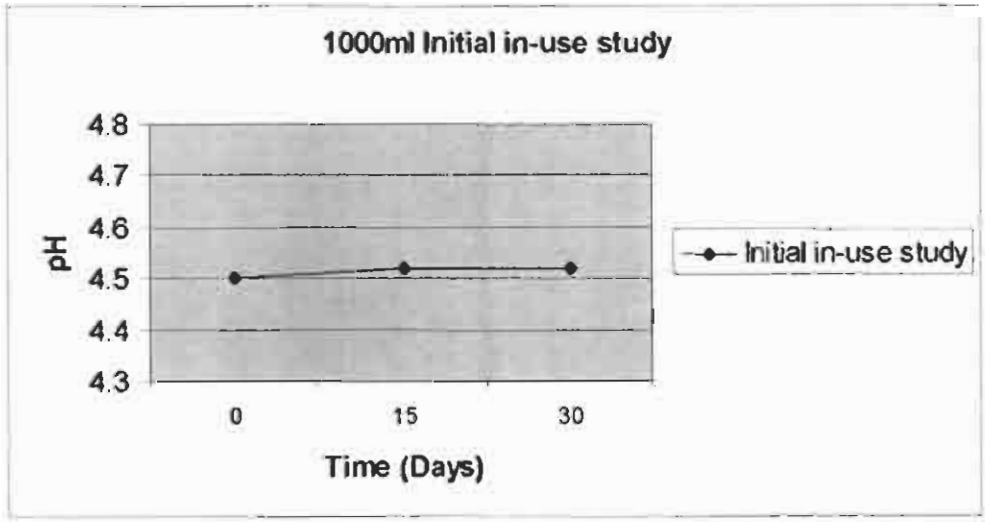


Figure A.1.6: Graphic representation of the pH results obtained for the 1000 ml ARV suspension during in-use stability testing.

A.2: pH STABILITY STUDY

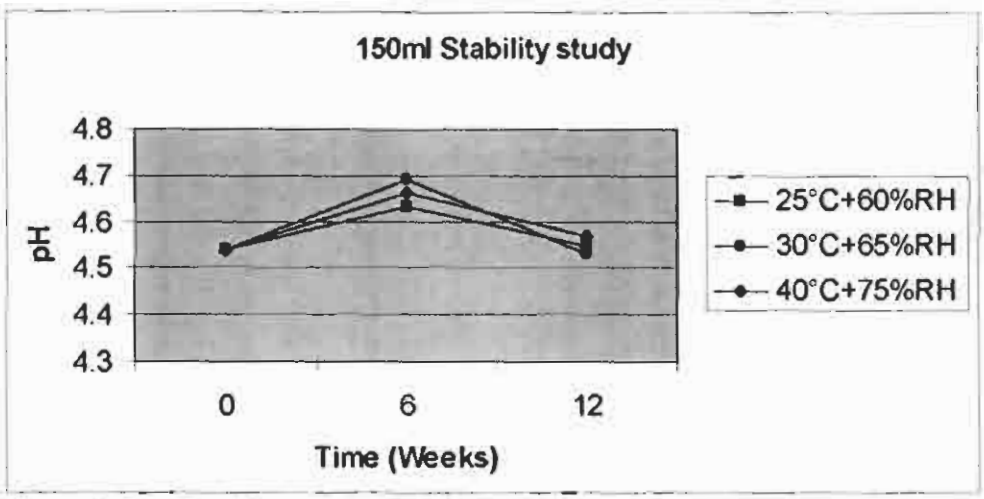


Figure A.2.1: Graphic representation of the pH results obtained for the 150 ml ARV suspension during accelerated stability testing.

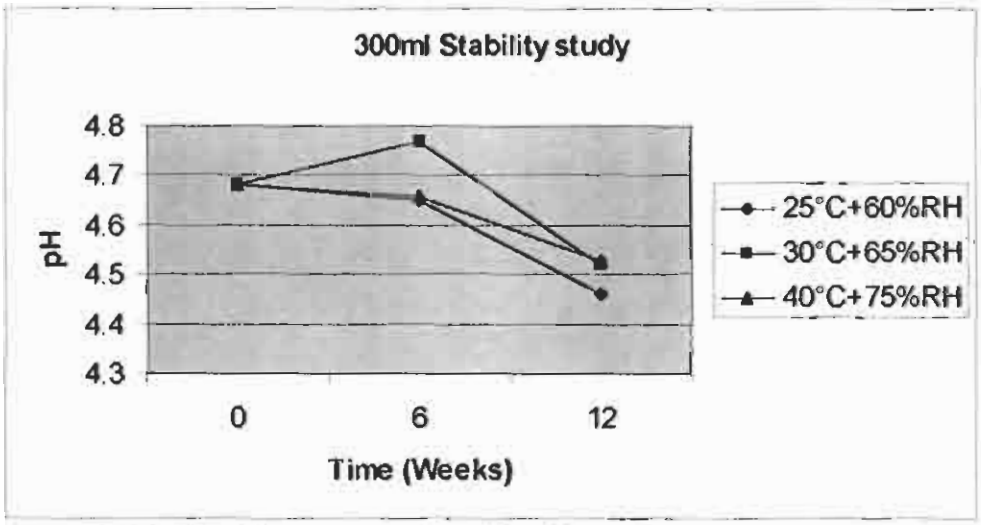


Figure A.2.2: Graphic representation of the pH results obtained for the 300 ml ARV suspension during accelerated stability testing.

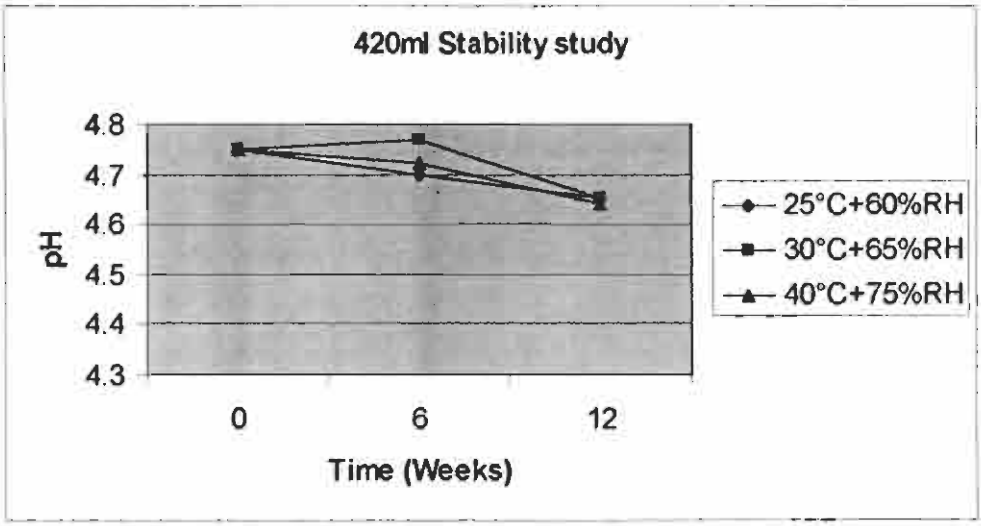


Figure A.2.3: Graphic representation of the pH results obtained for the 420 ml ARV suspension during accelerated stability testing.

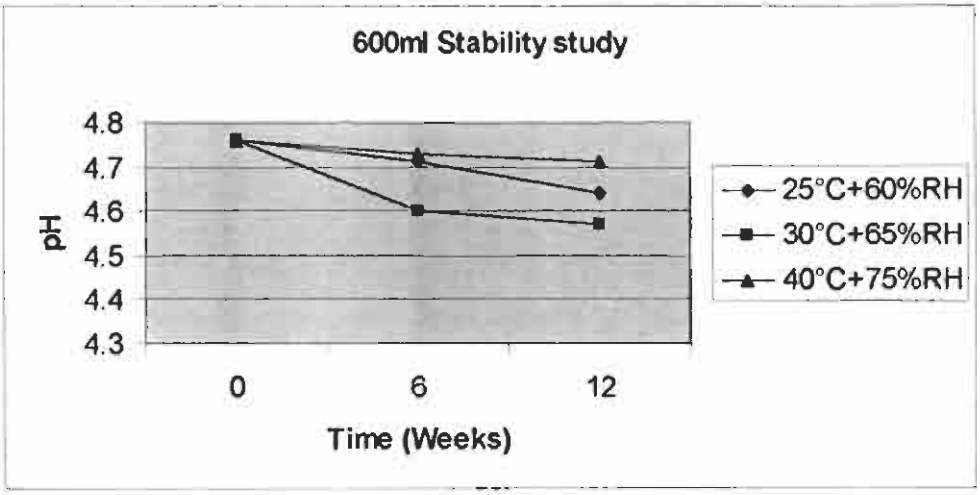


Figure A.2.4: Graphic representation of the pH results obtained for the 600 ml ARV suspension during accelerated stability testing.

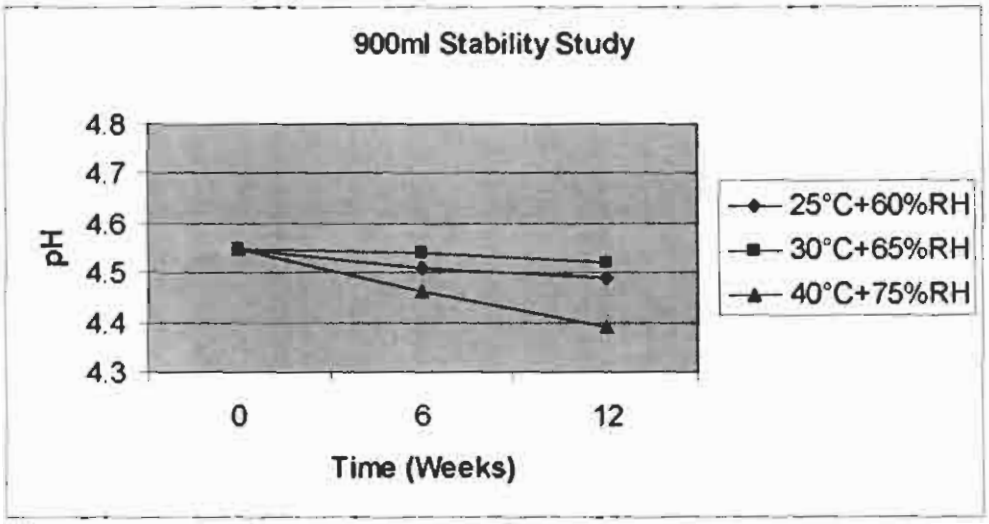


Figure A.2.5: Graphic representation of the pH results obtained for the 900 ml ARV suspension during accelerated stability testing.

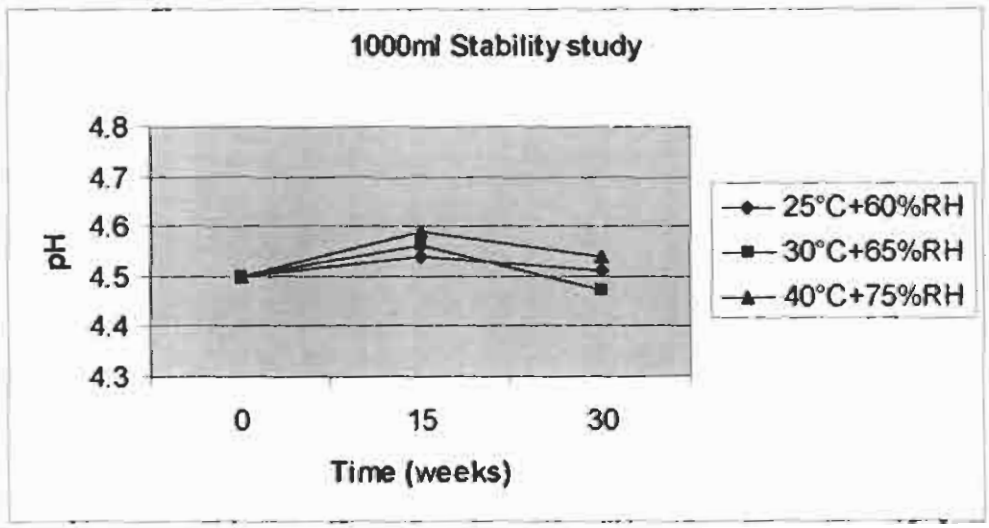


Figure A.2.6: Graphic representation of the pH results obtained for the 1000 ml ARV suspension during accelerated stability testing.

A.3: pH STABILITY STUDY

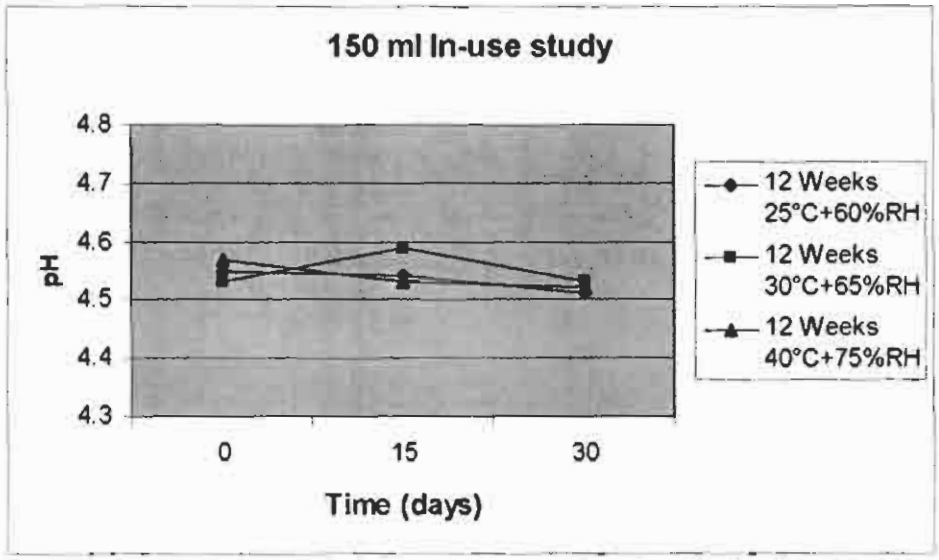


Figure A.3.1: Graphic representation of the pH results obtained for the 150 ml ARV suspension during 12 week in-use stability testing.

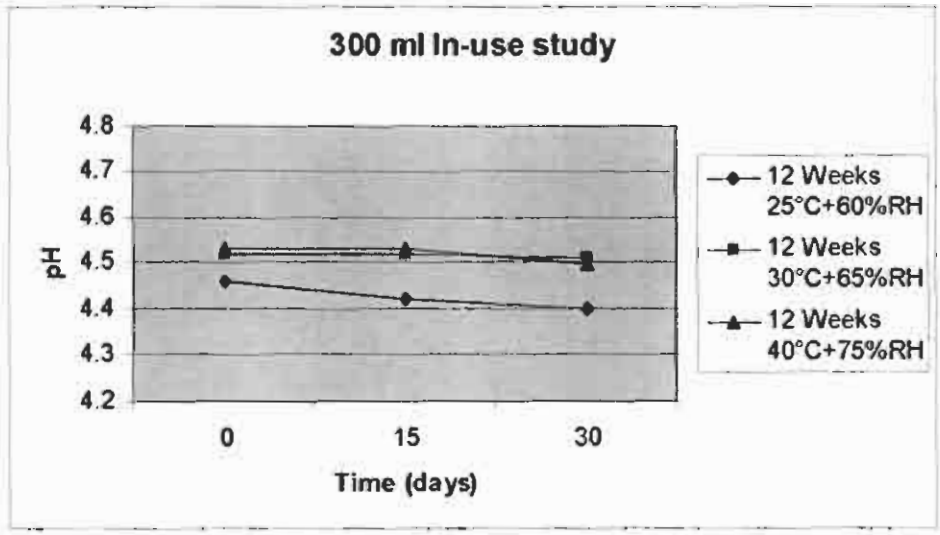


Figure A.3.2: Graphic representation of the pH results obtained for the 300 ml ARV suspension during 12 week in-use stability testing.

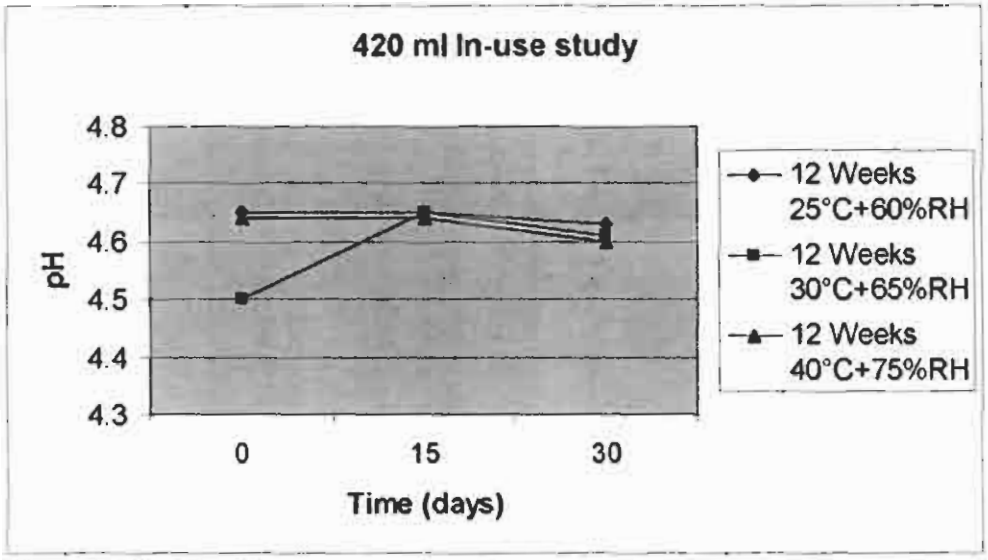


Figure A.3.3: Graphic representation of the pH results obtained for the 420 ml ARV suspension during 12 week in-use stability testing.

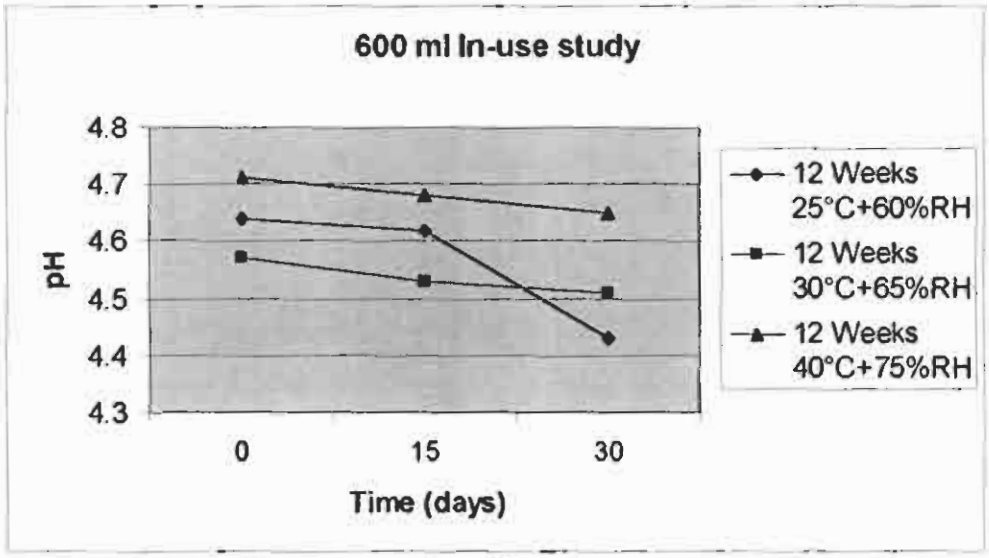


Figure A.3.4: Graphic representation of the pH results obtained for the 600 ml ARV suspension during 12 week in-use stability testing

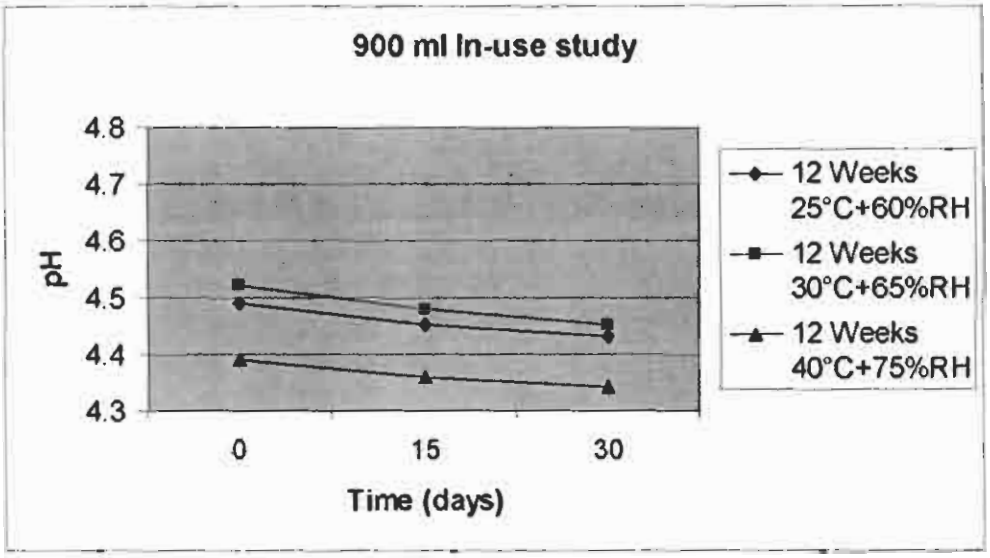


Figure A.3.5: Graphic representation of the pH results obtained for the 900 ml ARV suspension during 12 week in-use stability testing.

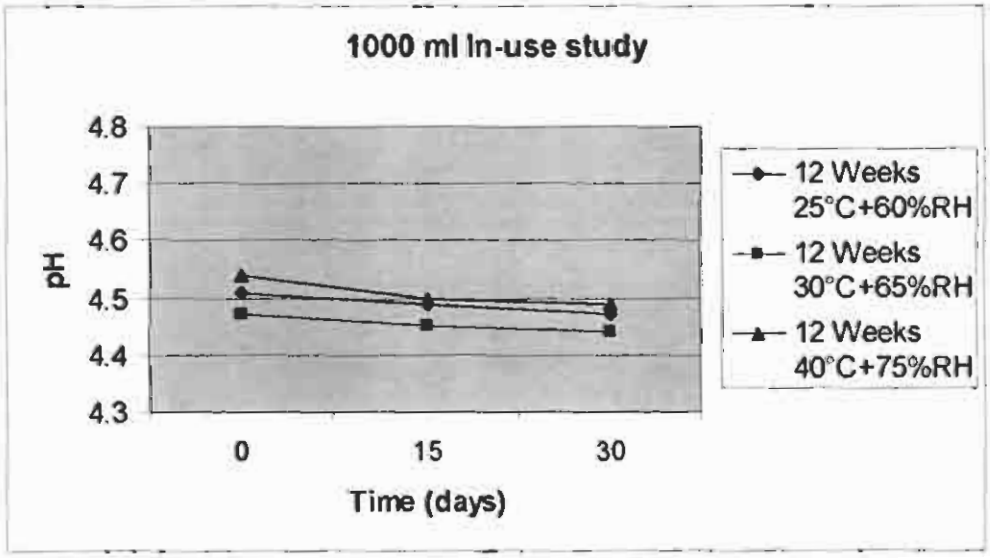


Figure A.3.6: Graphic representation of the pH results obtained for the 1000 ml ARV suspension during 12 week in-use stability testing.

ANNEXURE B

Relative density: Initial in-use stability study

- **150 ml** **B2**
- **300 ml** **B2**
- **420 ml** **B3**
- **600 ml** **B3**
- **900 ml** **B4**
- **1000 ml** **B4**

Relative density: Accelerated stability study

- **150 ml** **B5**
- **300 ml** **B5**
- **420 ml** **B6**
- **600 ml** **B6**
- **900 ml** **B7**
- **1000 ml** **B7**

Relative density: 12 Week in-use stability study

- **150 ml** **B8**
- **300 ml** **B8**
- **420 ml** **B9**
- **600 ml** **B9**
- **900 ml** **B10**
- **1000 ml** **B10**

B.1: RELATIVE DENSITY: INITIAL IN-USE STUDY

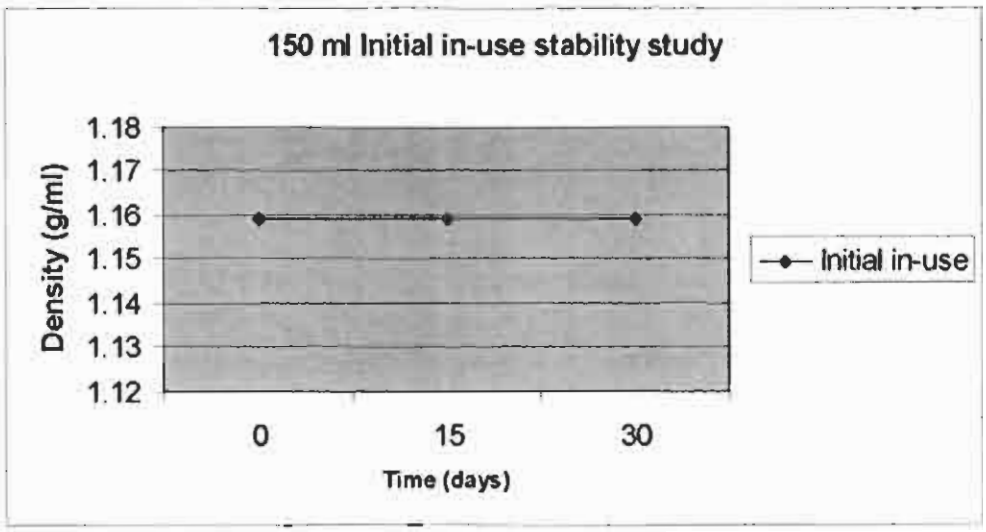


Figure B.1.1: Graphic representation for the relative density results obtained for the 150 ml reconstituted suspension during the initial in-use stability testing.

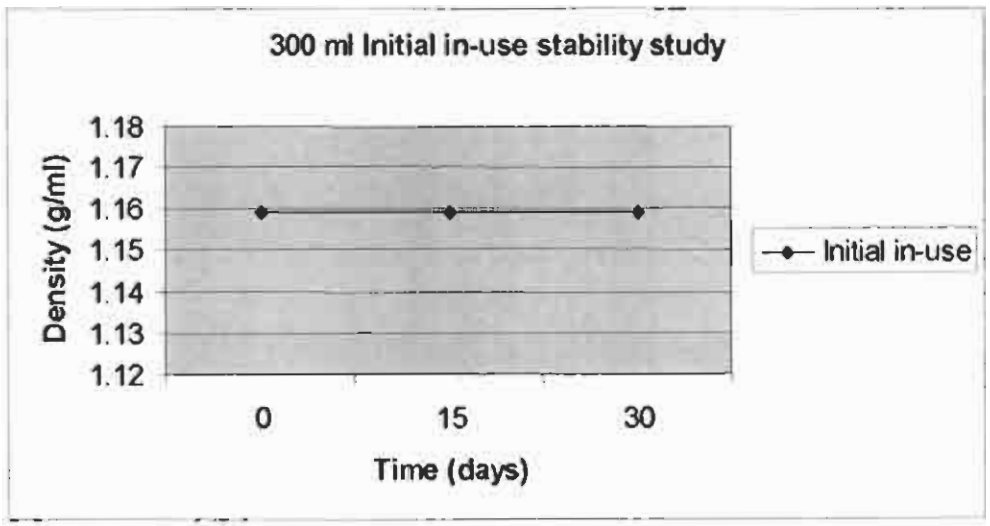


Figure B.1.2: Graphic representation for the relative density results obtained for the 300 ml reconstituted suspension during the initial in-use stability testing.

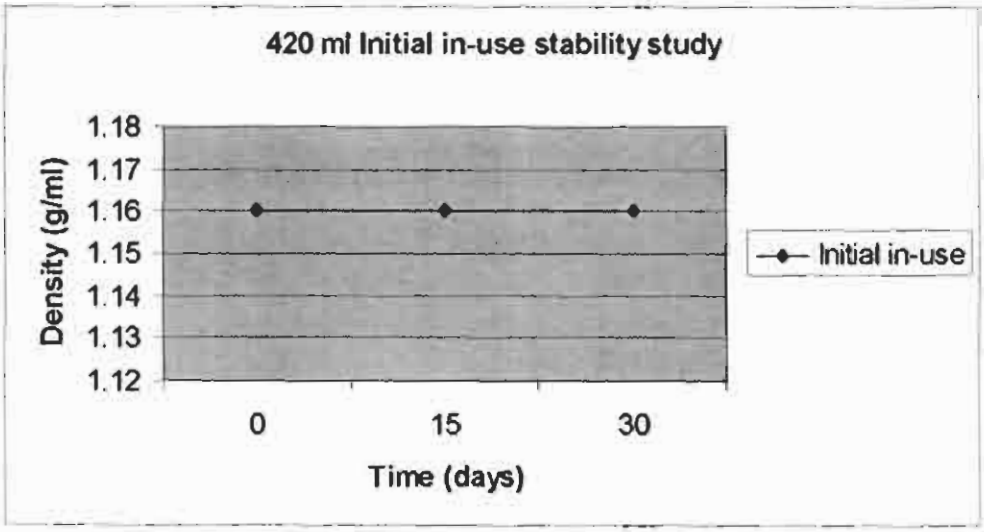


Figure B.1.3: Graphic representation for the relative density results obtained for the 420 ml reconstituted suspension during the initial in-use stability testing.

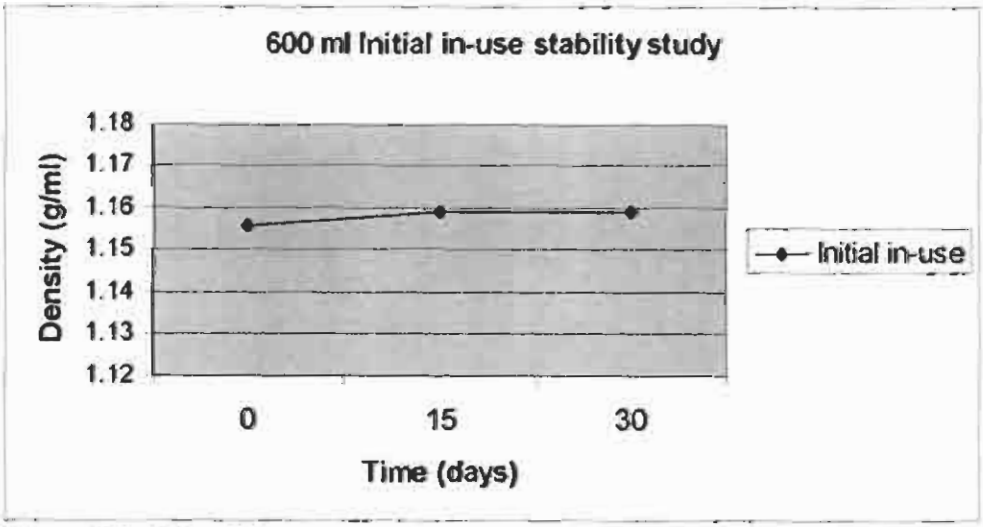


Figure B.1.4: Graphic representation for the relative density results obtained for the 600 ml reconstituted suspension during the initial in-use stability testing.

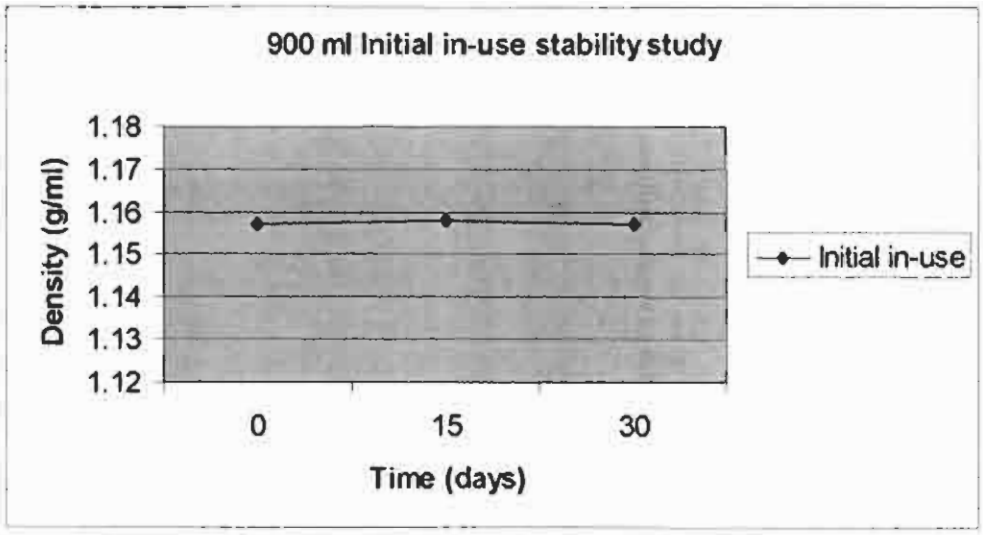


Figure B.1.5: Graphic representation for the relative density results obtained for the 900 ml reconstituted suspension during the initial in-use stability testing.

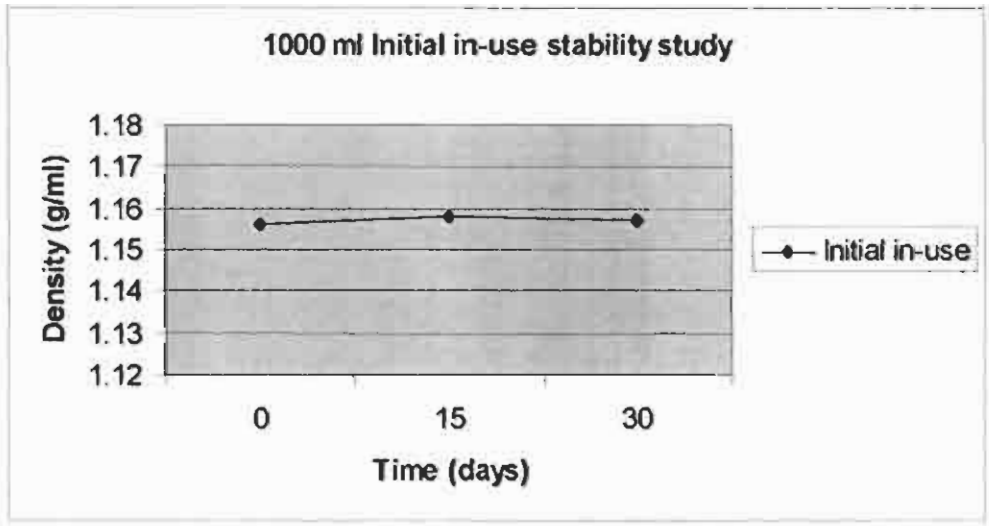


Figure B.1.6: Graphic representation for the relative density results obtained for the 1000 ml reconstituted suspension during the initial in-use stability testing.

B.2: RELATIVE DENSITY: STABILITY STUDY

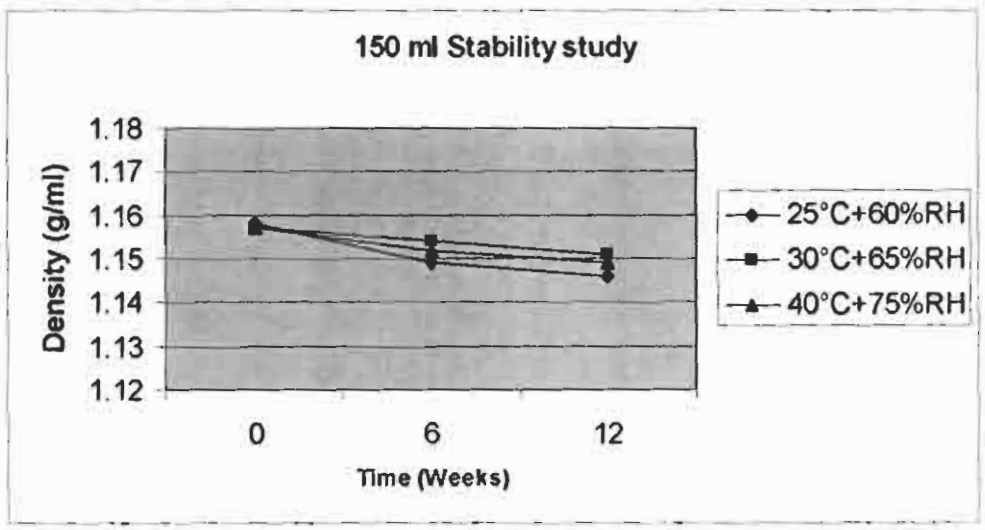


Figure B.2.1: Graphic representation for the relative density results obtained for the 150 ml reconstituted suspension during the accelerated stability testing.

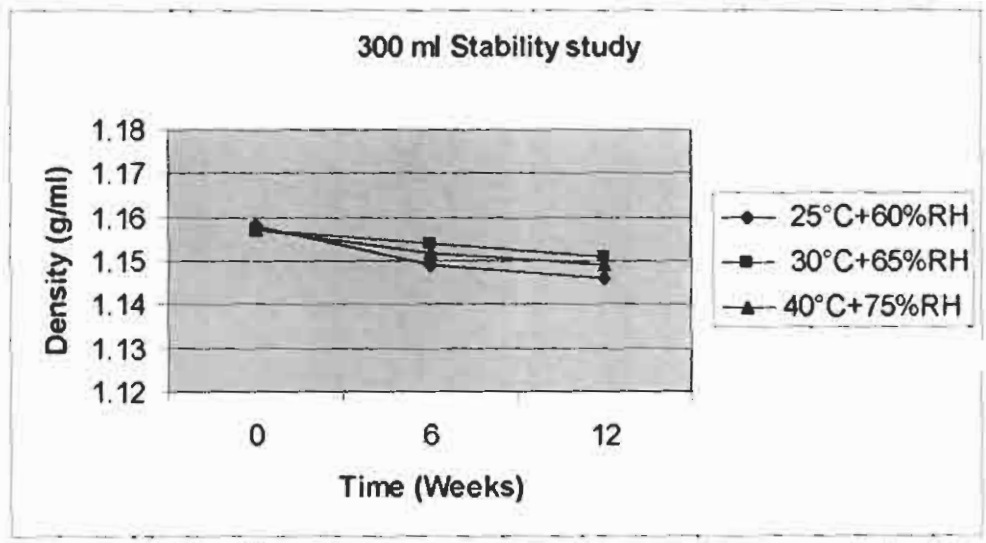


Figure B.2.2: Graphic representation for the relative density results obtained for the 300 ml reconstituted suspension during the accelerated stability testing.

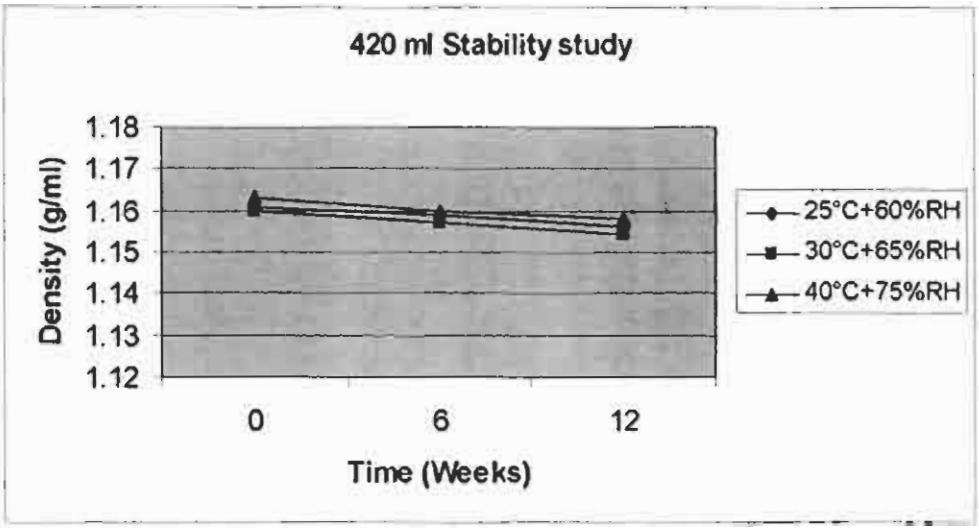


Figure B.2.3: Graphic representation for the relative density results obtained for the 420ml reconstituted suspension during the accelerated stability testing.

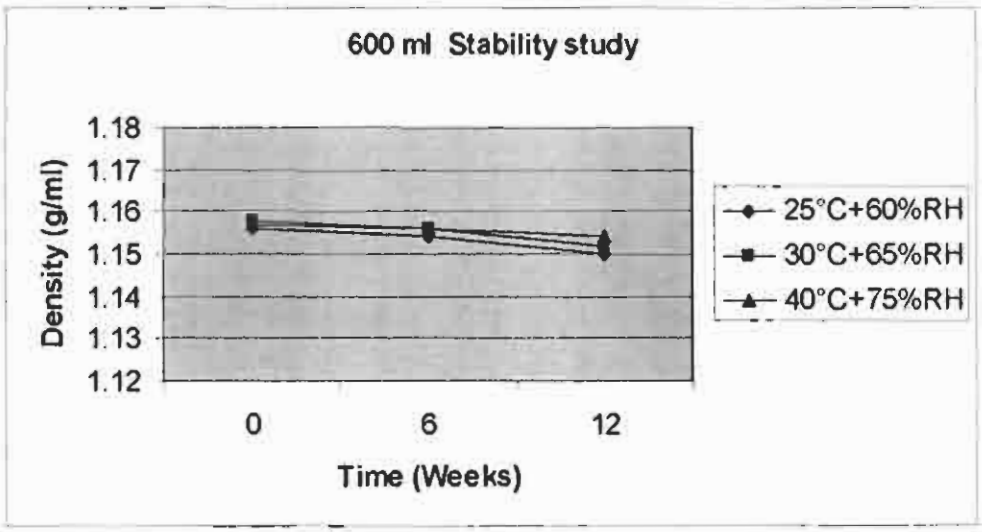


Figure B.2.4: Graphic representation for the relative density results obtained for the 600 ml reconstituted suspension during the accelerated stability testing.

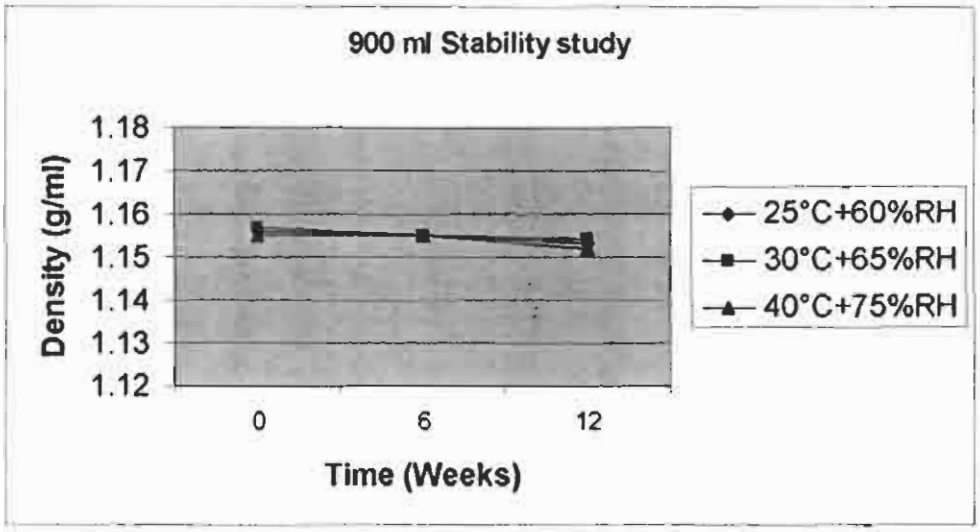


Figure B.2.5: Graphic representation for the relative density results obtained for the 900 ml reconstituted suspension during the accelerated stability testing.

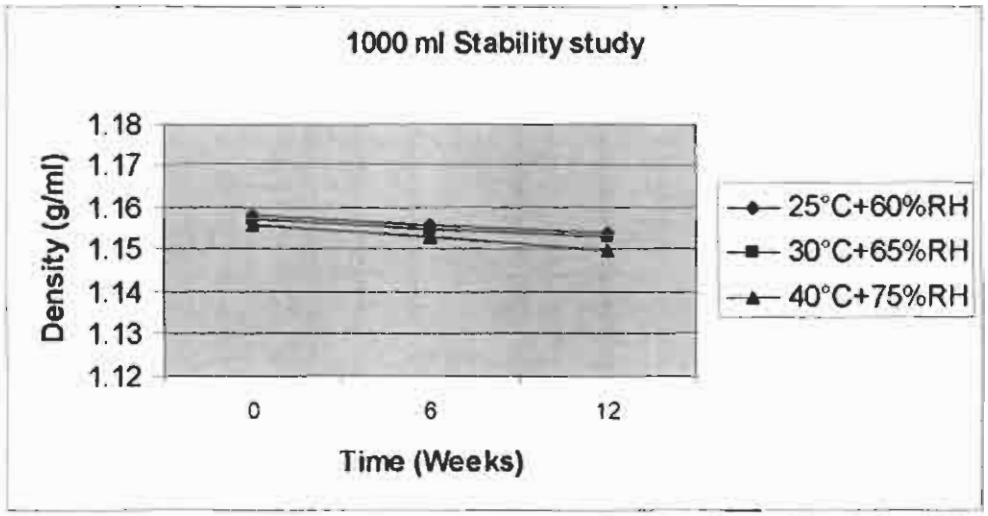


Figure B.2.6: Graphic representation for the relative density results obtained for the 1000 ml reconstituted suspension during the accelerated stability testing.

B.3: RELATIVE DENSITY: 12 WEEK IN-USE STABILITY STUDY

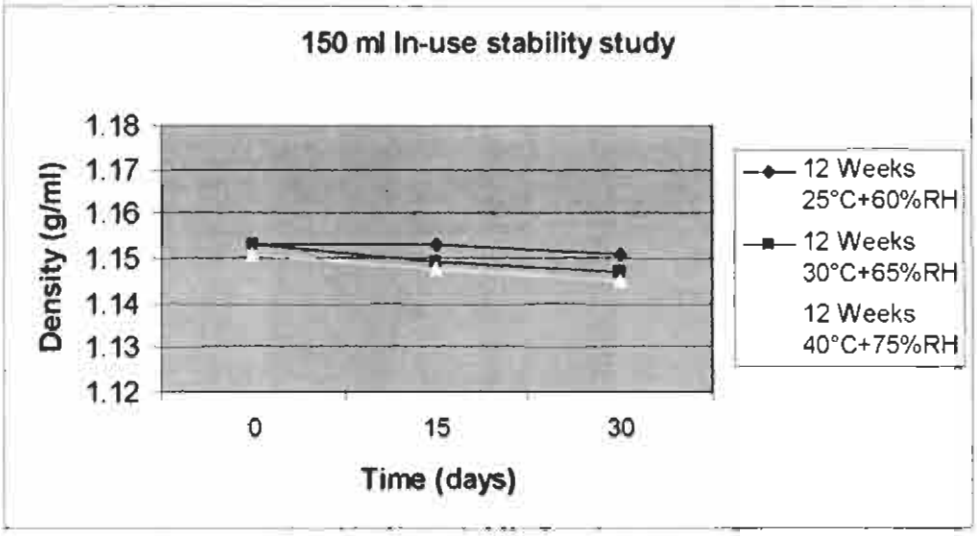


Figure B.3.1: Graphic representation for the relative density results obtained for the 150 ml reconstituted suspension during the 12 week in-use stability testing.

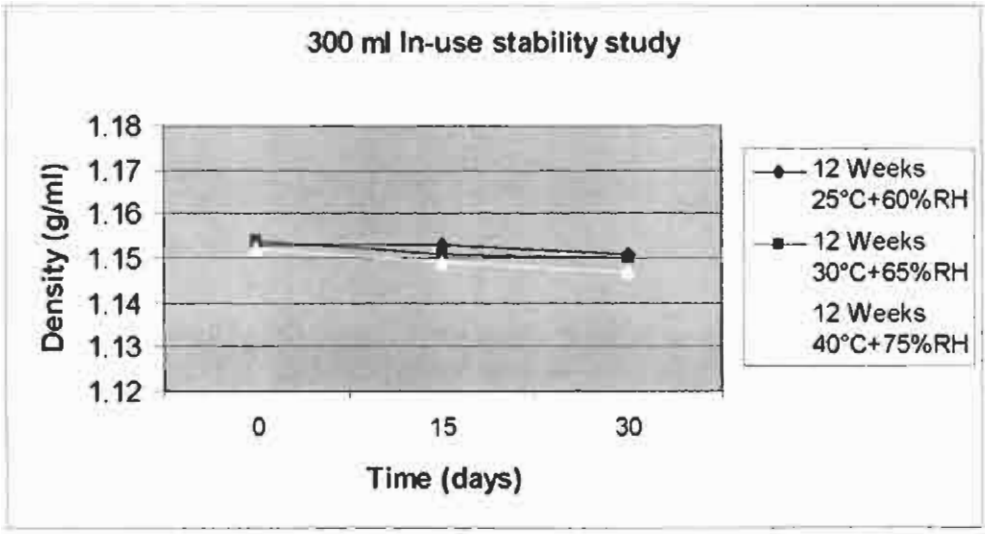


Figure B.3.2: Graphic representation for the relative density results obtained for the 300 ml reconstituted suspension during the 12 week in-use stability testing.

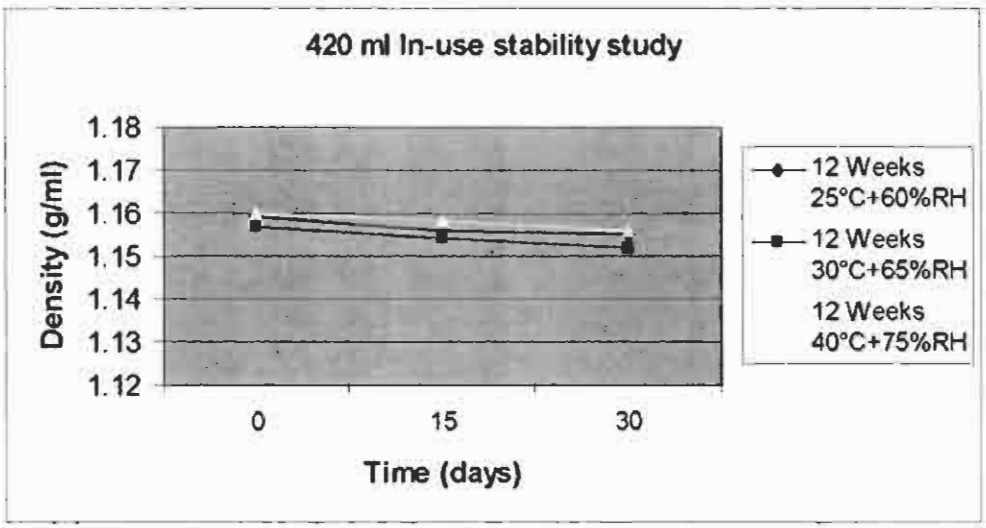


Figure B.3.3: Graphic representation for the relative density results obtained for the 420 ml reconstituted suspension during the 12 week in-use stability testing.

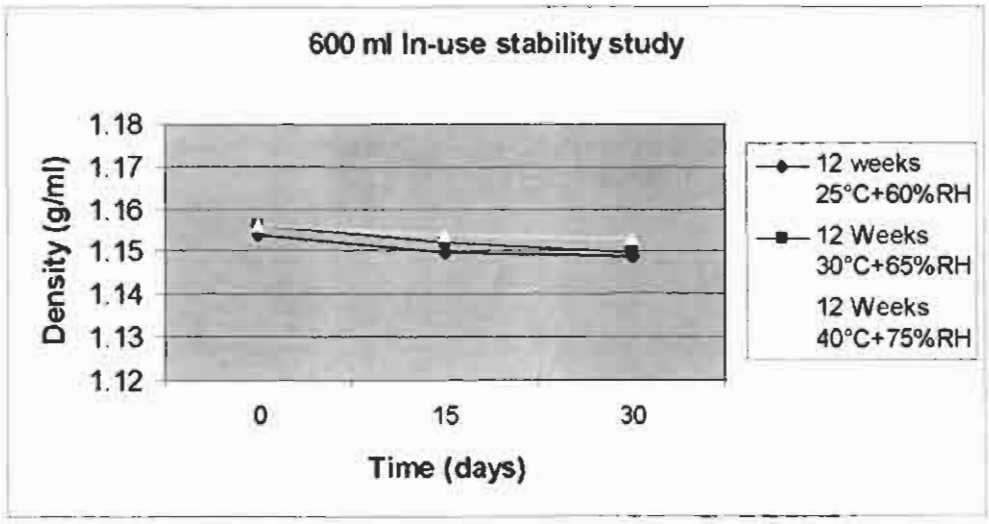


Figure B.3.4: Graphic representation for the relative density results obtained for the 600 ml reconstituted suspension during the 12 week in-use stability testing.

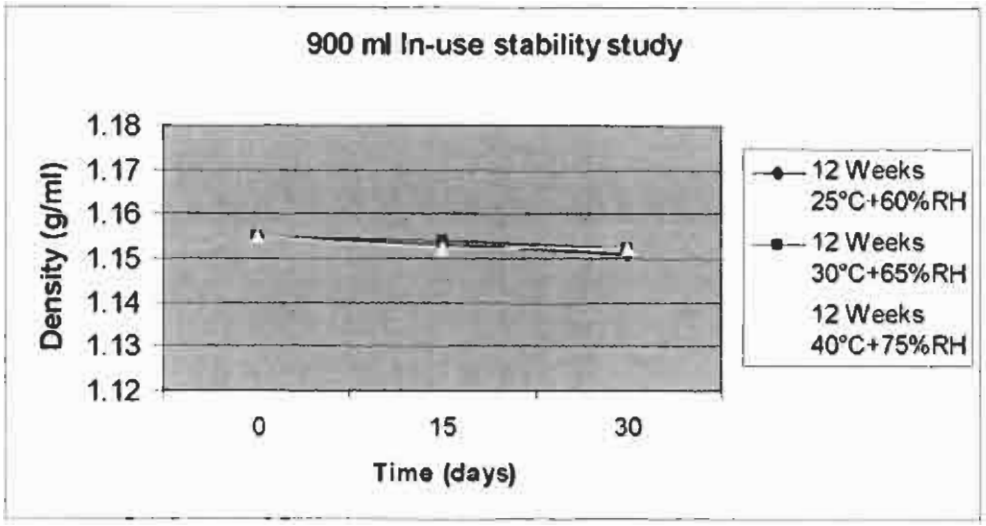


Figure B.3.5: Graphic representation for the relative density results obtained for the 900 ml reconstituted suspension during the 12 week in-use stability testing.

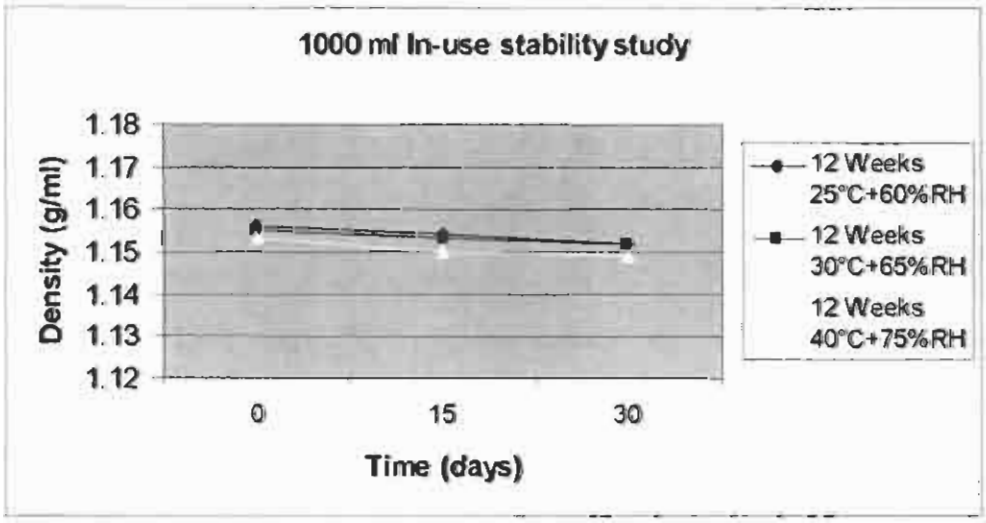


Figure B.3.6: Graphic representation for the relative density results obtained for the 1000 ml reconstituted suspension during the 12 week in-use stability testing.

ANNEXURE C

Viscosity: Initial in-use stability study

- **150 ml** C2
- **300 ml** C2
- **420 ml** C3
- **600 ml** C3
- **900 ml** C4
- **1000 ml** C4

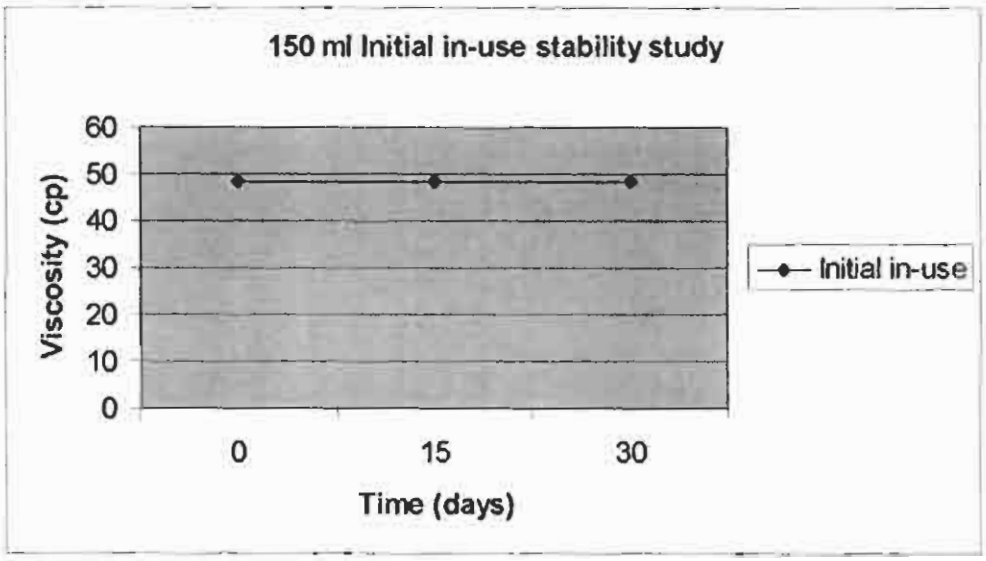
Viscosity: Accelerated stability study

- **150 ml** C5
- **300 ml** C5
- **420 ml** C6
- **600 ml** C6
- **900 ml** C7
- **1000 ml** C7

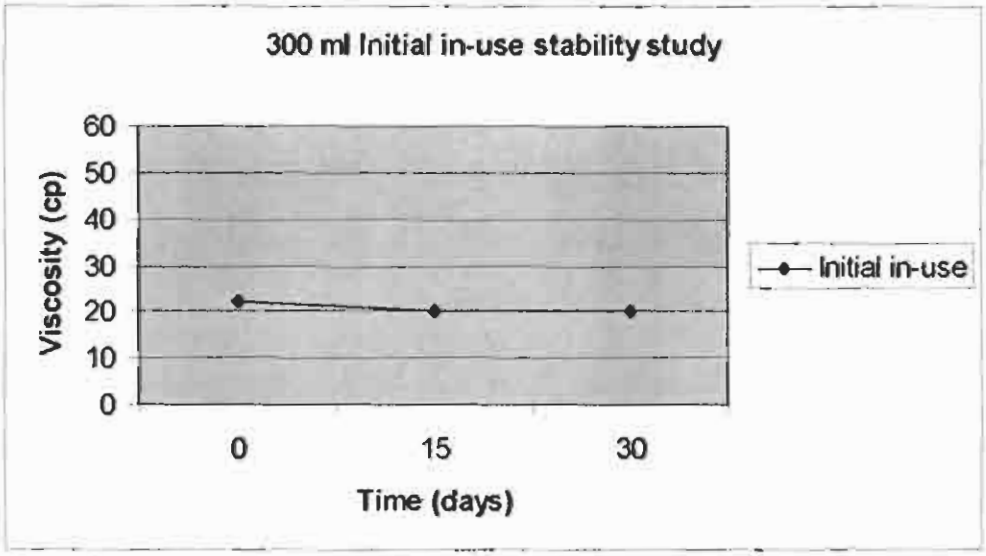
Viscosity: 12 Weeks in-use stability study

- **150 ml** C8
- **300 ml** C8
- **420 ml** C9
- **600 ml** C9
- **900 ml** C10
- **1000 ml** C10

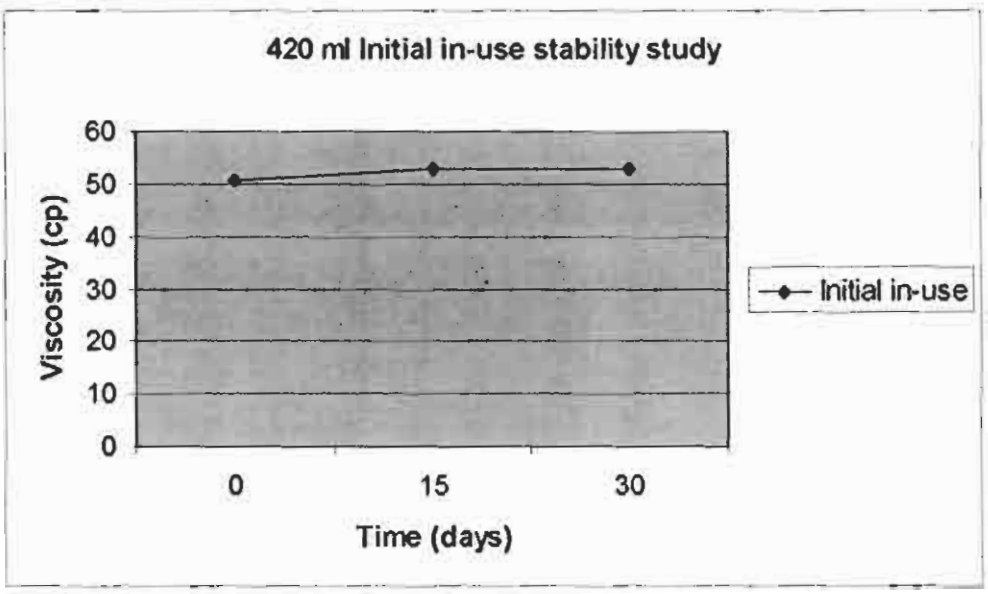
C.1: VISCOSITY: INITIAL IN-USE STABILITY STUDY



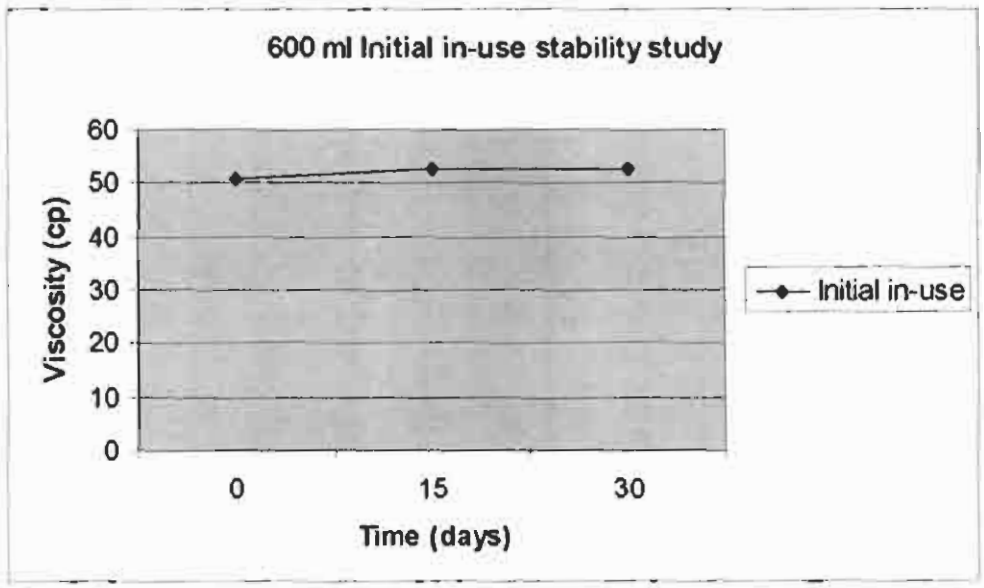
C.1.1: Graphic representation of the viscosity results of the 150 ml ARV reconstituted suspension during the initial in-use stability test period.



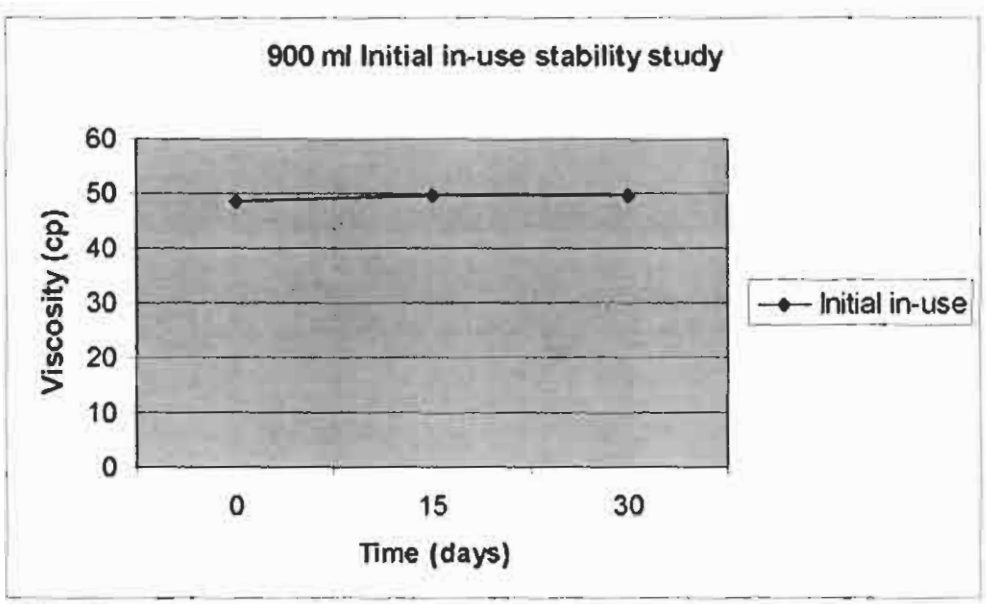
C.1.2: Graphic representation of the viscosity results of the 300 ml ARV reconstituted suspension during the initial in-use stability test period.



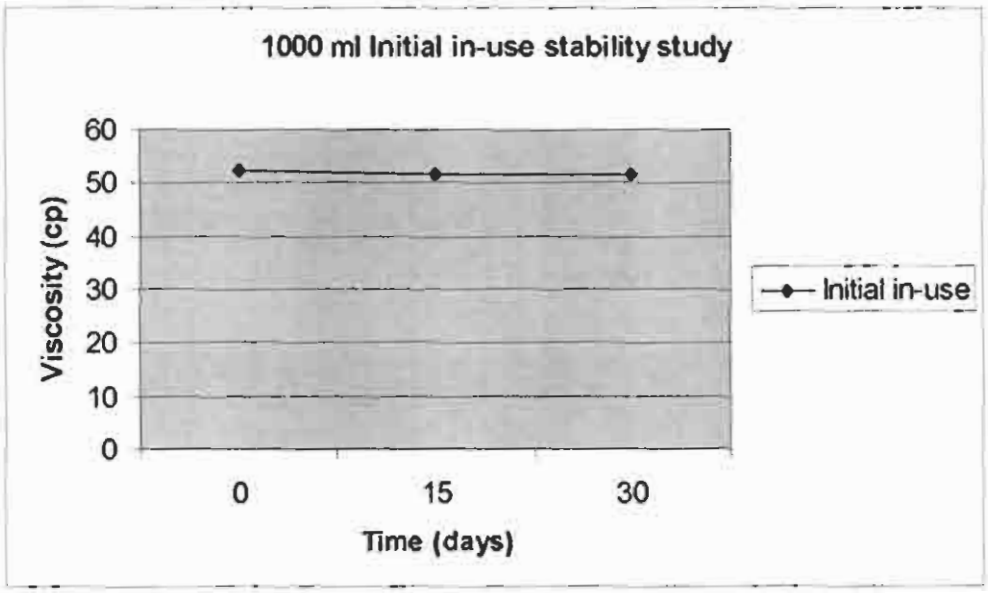
C.1.3: Graphic representation of the viscosity results of the 420 ml ARV reconstituted suspension during the initial in-use stability test period.



C.1.4: Graphic representation of the viscosity results of the 600 ml ARV reconstituted suspension during the initial in-use stability test period.

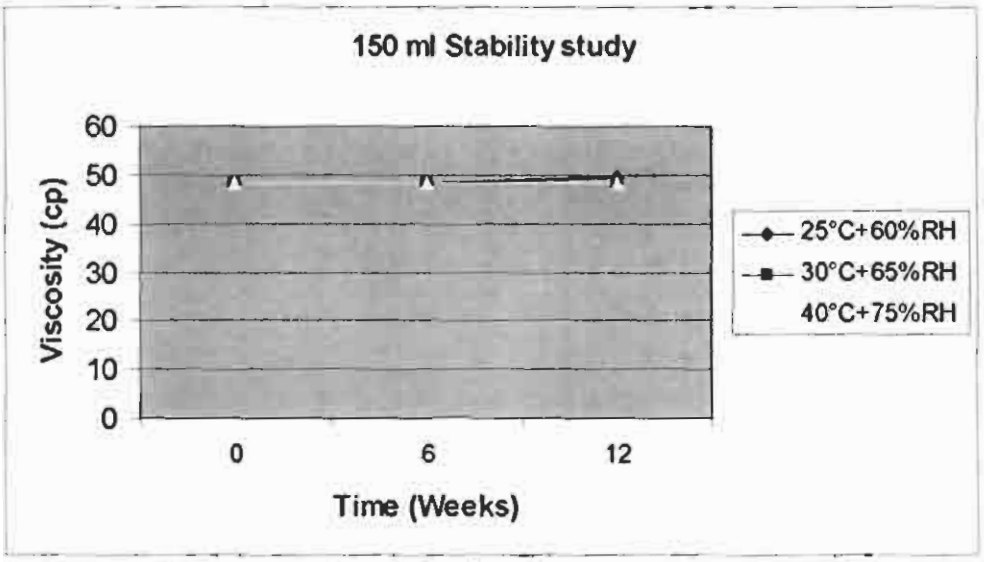


C.1.5: Graphic representation of the viscosity results of the 900 ml ARV reconstituted suspension during the initial in-use stability test period.

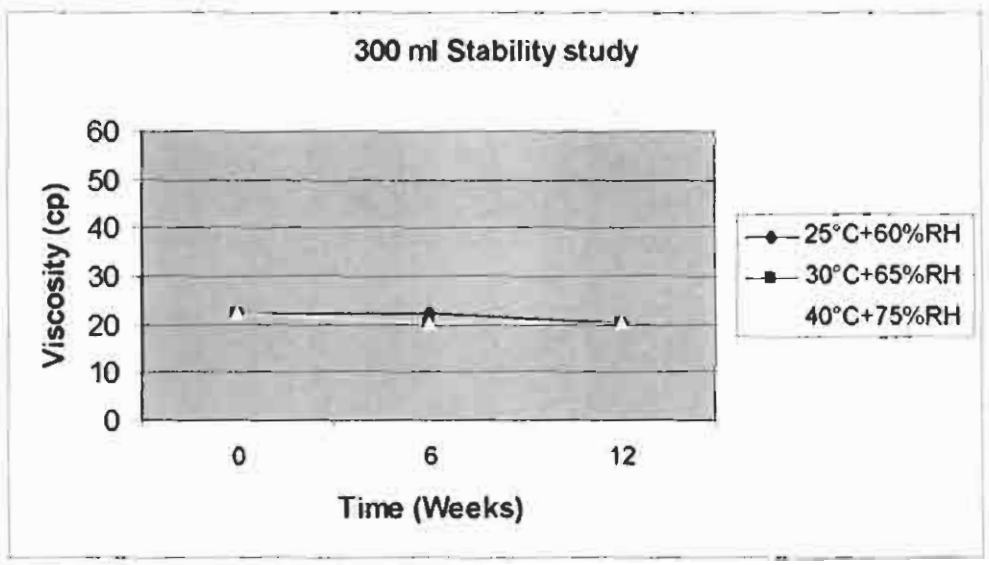


C.1.6: Graphic representation of the viscosity results of the 1000 ml ARV reconstituted suspension during the initial in-use stability test period.

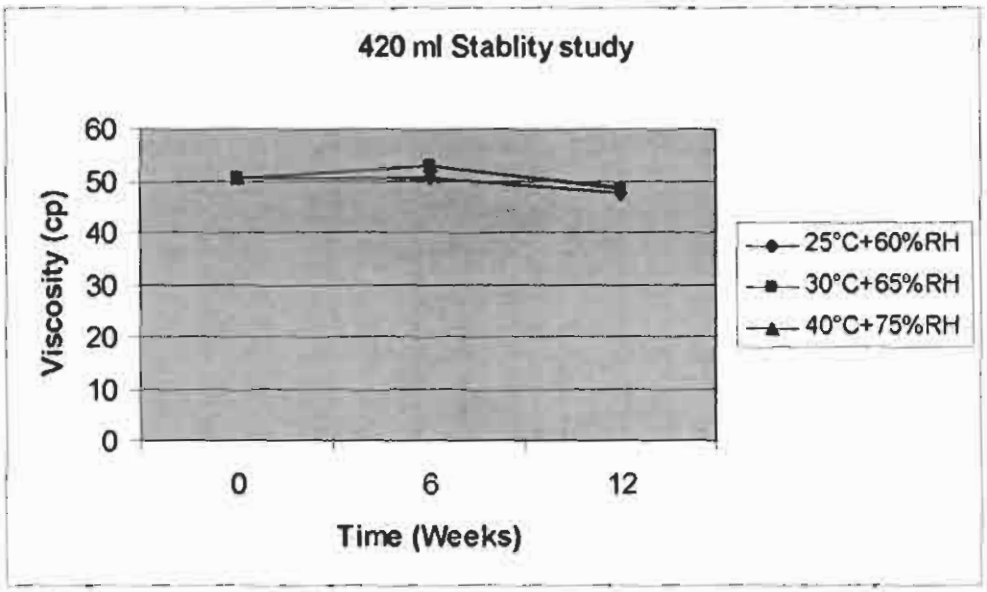
C.2: VISCOSITY: STABILITY STUDY



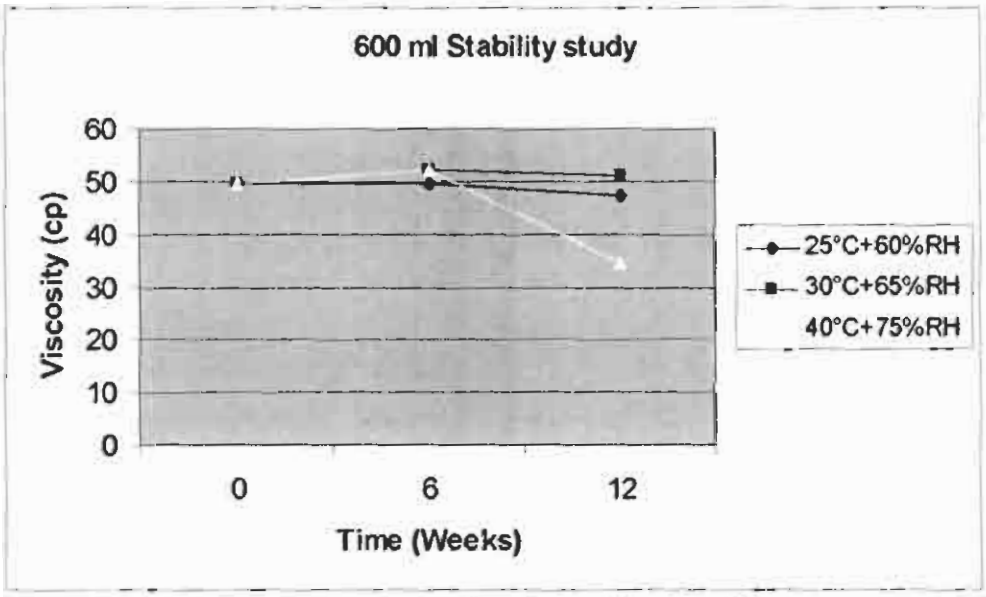
C.2.1: Graphic representation of the viscosity results of the 150 ml ARV reconstituted suspension during the accelerated stability test period.



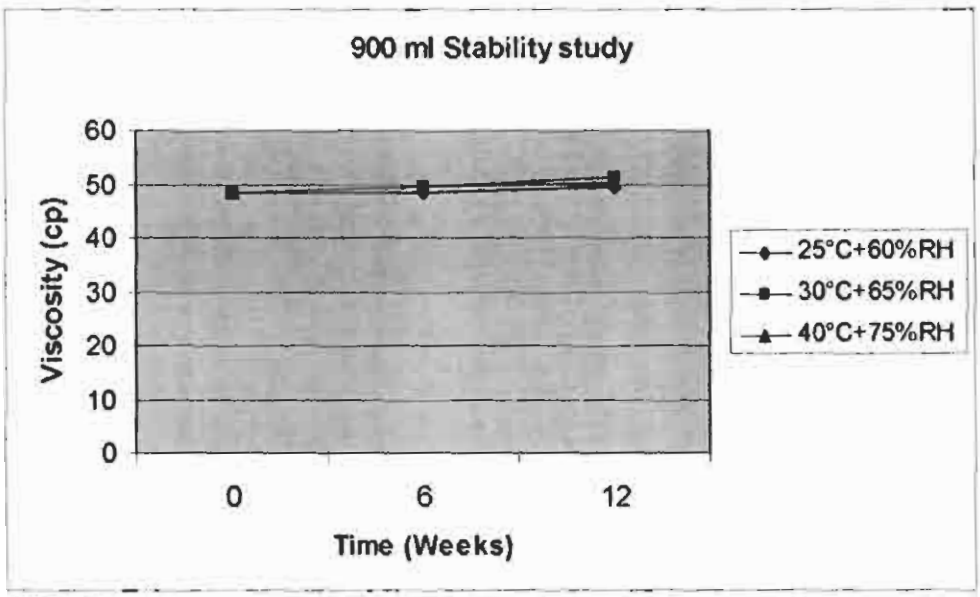
C.2.2: Graphic representation of the viscosity results of the 300 ml ARV reconstituted suspension during the accelerated stability test period.



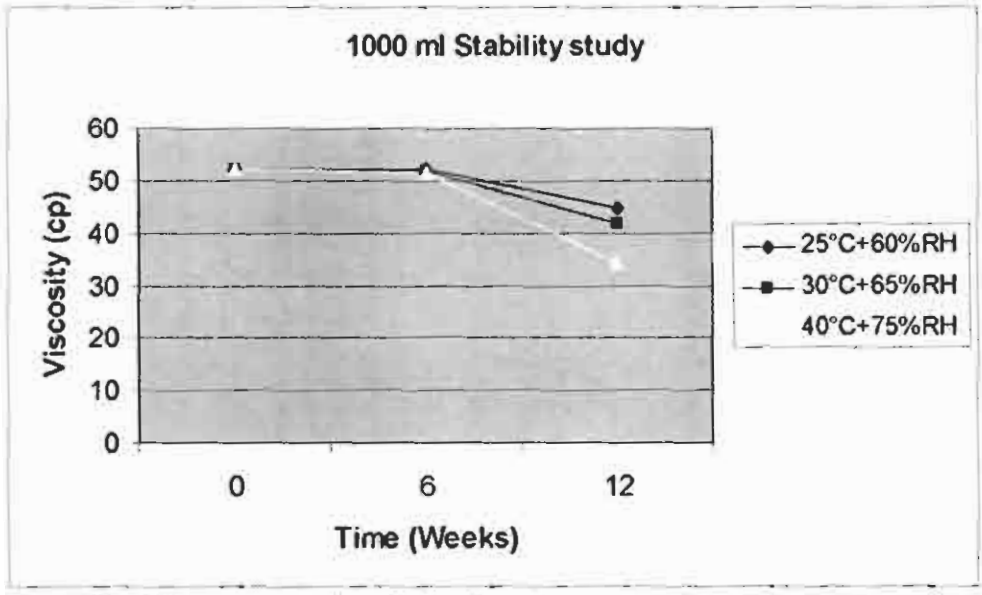
C.2.3: Graphic representation of the viscosity results of the 420 ml ARV reconstituted suspension during the accelerated stability test period.



C.2.4: Graphic representation of the viscosity results of the 600 ml ARV reconstituted suspension during the accelerated stability test period.

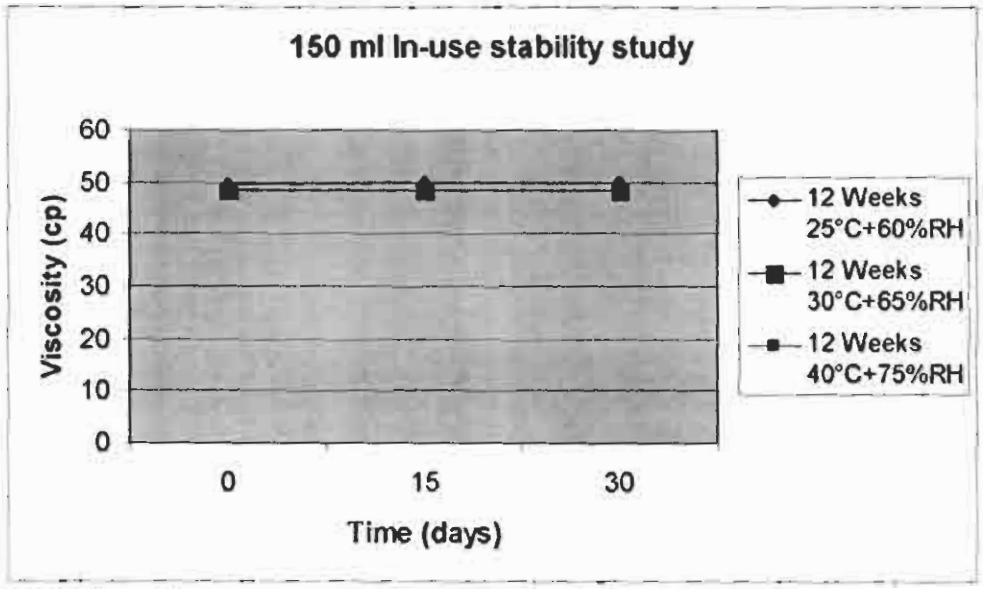


C.2.5: Graphic representation of the viscosity results of the 900 ml ARV reconstituted suspension during the accelerated stability test period.

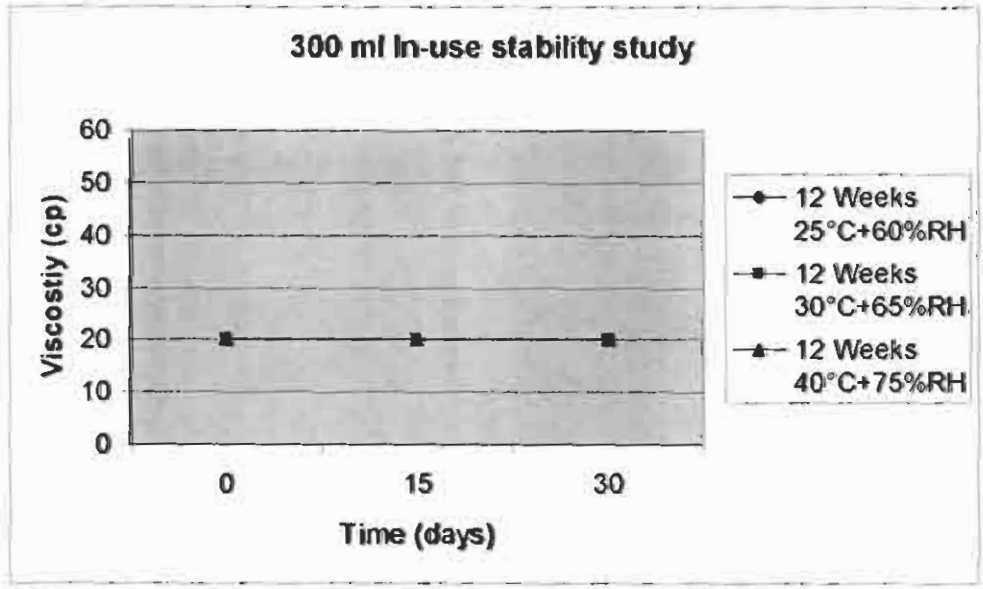


C.2.6: Graphic representation of the viscosity results of the 1000 ml ARV reconstituted suspension during the accelerated stability test period.

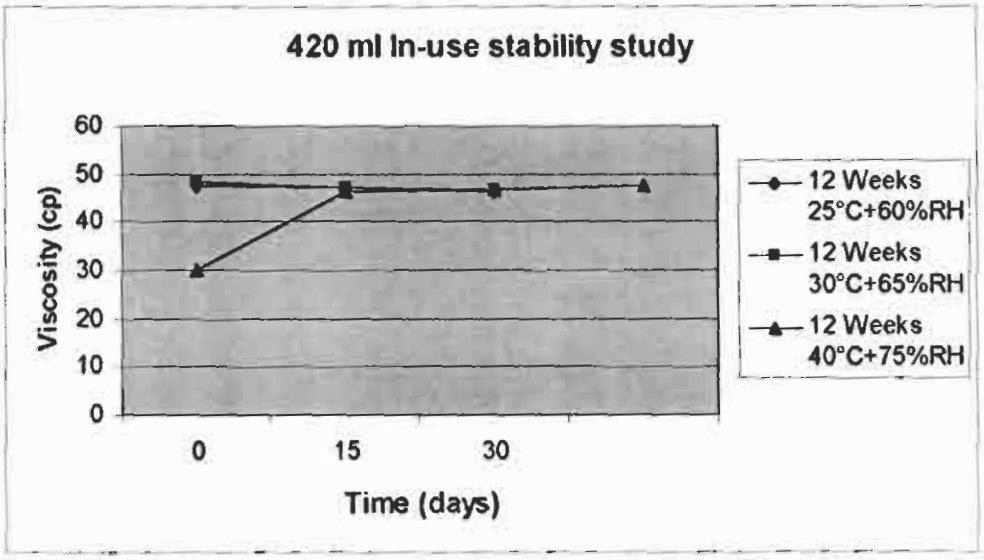
C.3: VISCOSITY: IN-USE STABILITY STUDY (12 WEEKS)



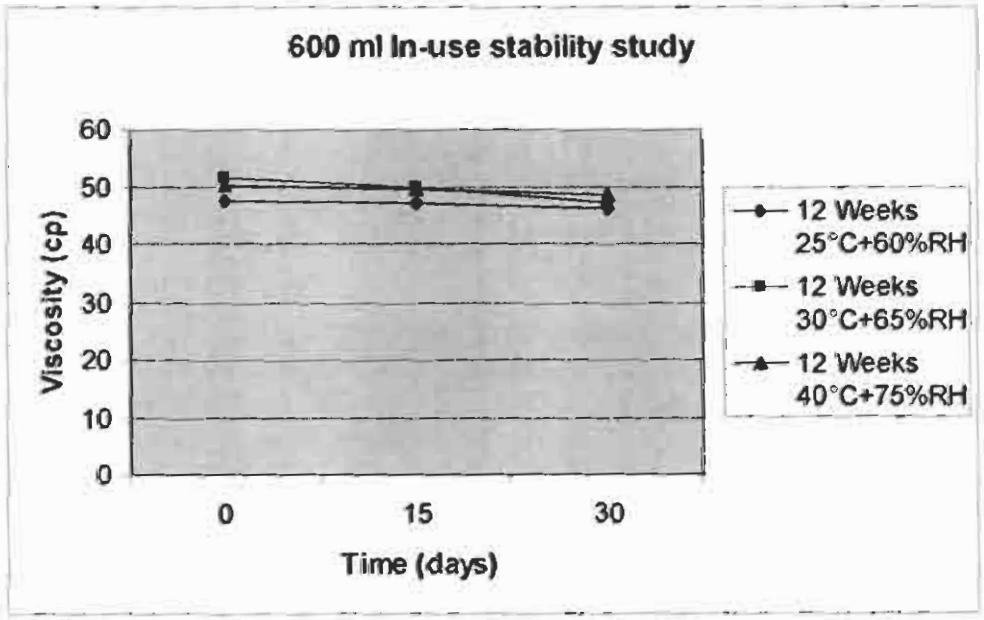
C.3.1: Graphic representation of the viscosity results of the 150 ml ARV reconstituted suspension during the 12 week in-use stability test period.



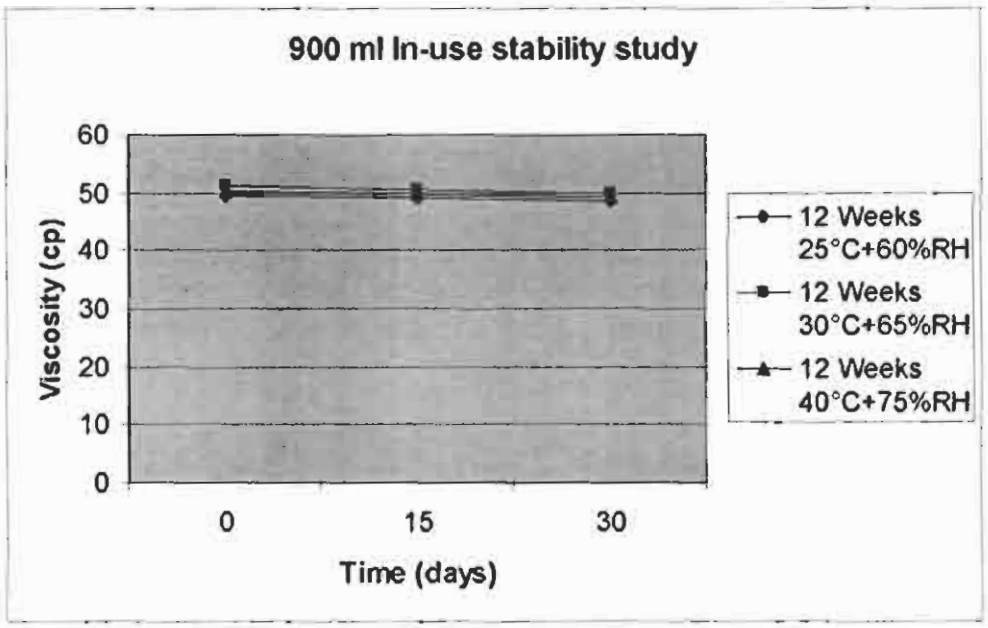
C.3.2: Graphic representation of the viscosity results of the 300 ml ARV reconstituted suspension during the 12 week in-use stability test period.



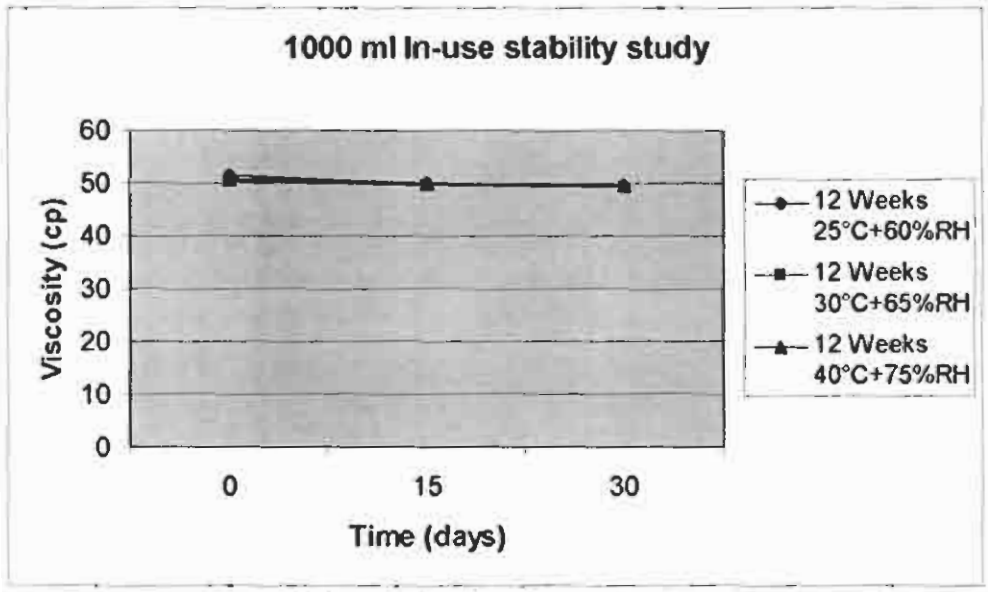
C.3.3: Graphic representation of the viscosity results of the 420 ml ARV reconstituted suspension during the 12 week in-use stability test period.



C.3.4: Graphic representation of the viscosity results of the 600 ml ARV reconstituted suspension during the 12 week in-use stability test period.



C.3.5: Graphic representation of the viscosity results of the 900 ml ARV reconstituted suspension during the 12 week in-use stability test period



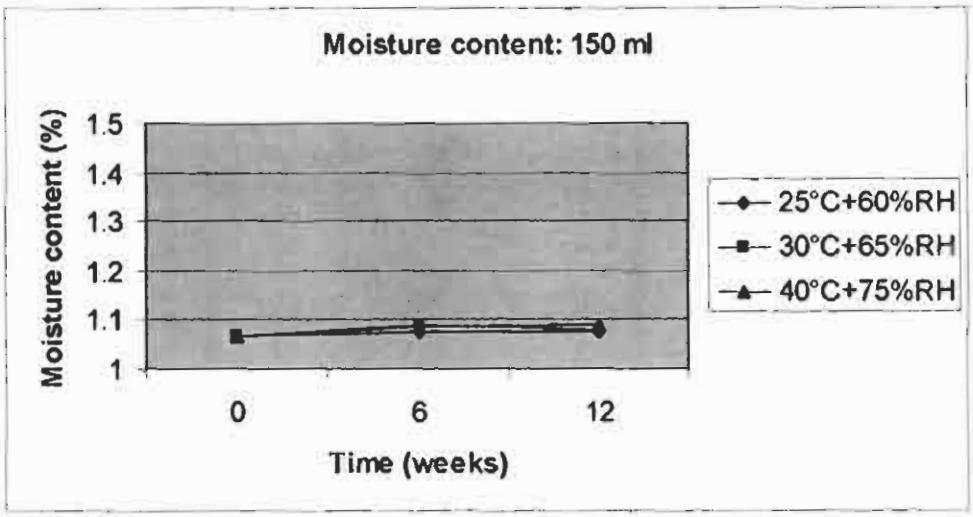
C.3.6: Graphic representation of the viscosity results of the 1000 ml ARV reconstituted suspension during the 12 week in-use stability test period.

ANNEXURE D

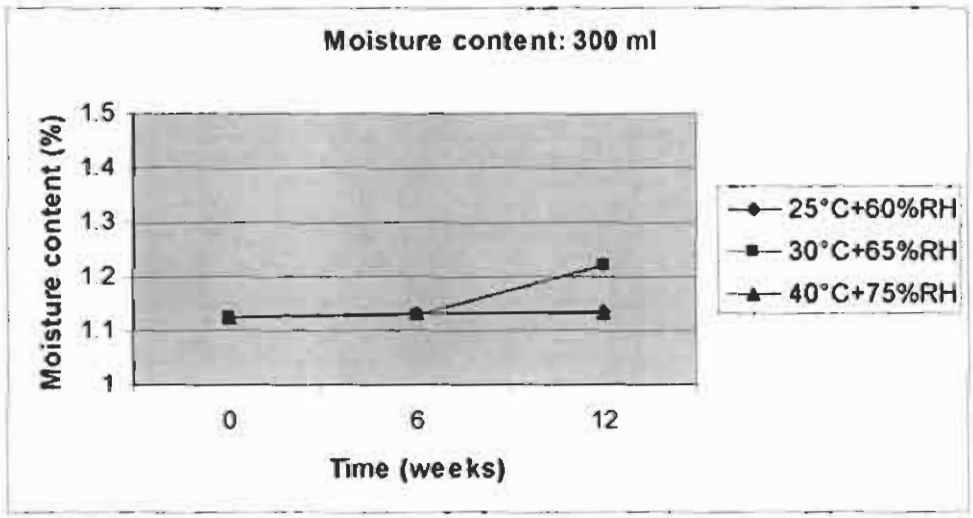
Moisture content: Accelerated stability study

- **150 ml** **D2**
- **300 ml** **D2**
- **420 ml** **D3**
- **600 ml** **D3**
- **900 ml** **D4**
- **1000 ml** **D4**

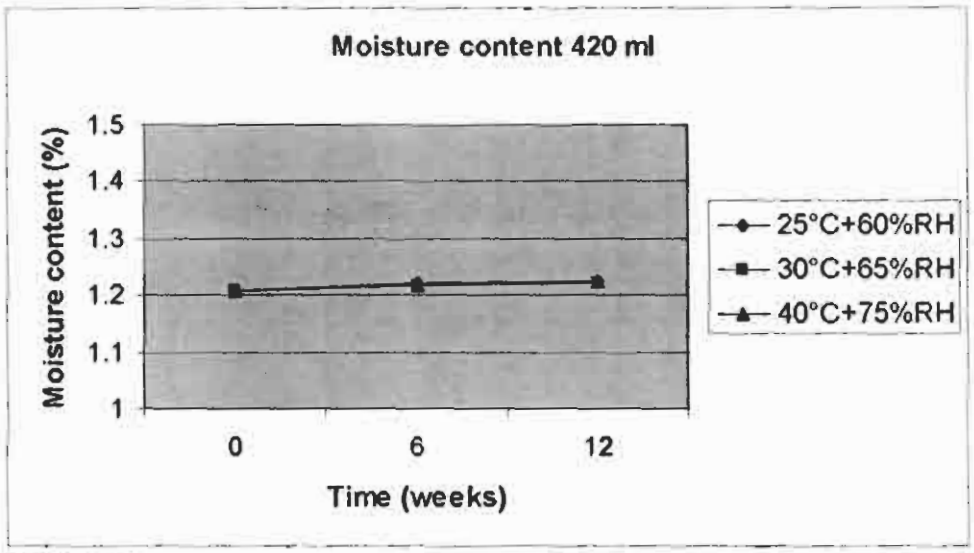
D.1: MOISTURE CONTENT: STABILITY STUDY



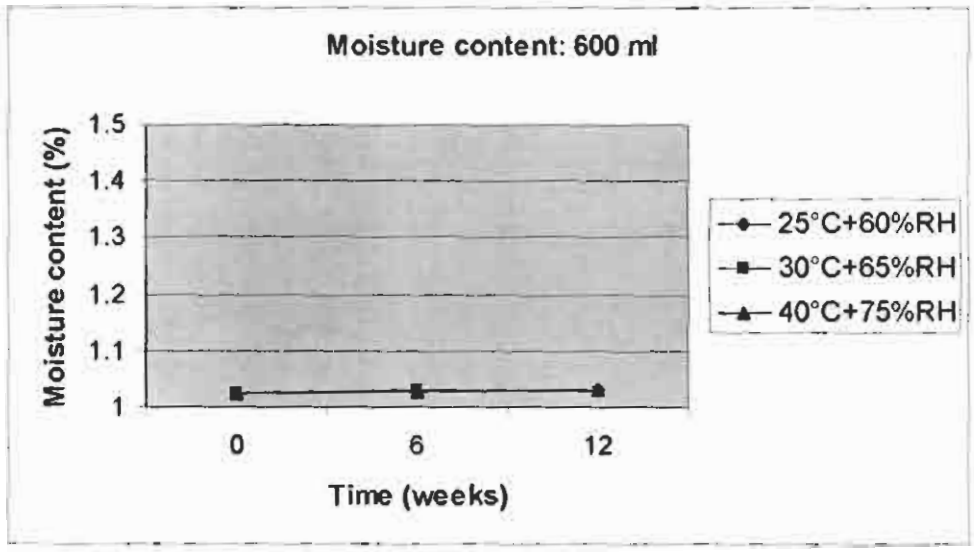
D.1.1: Moisture content of the 150 ml ARV powder for suspension during the accelerated stability study.



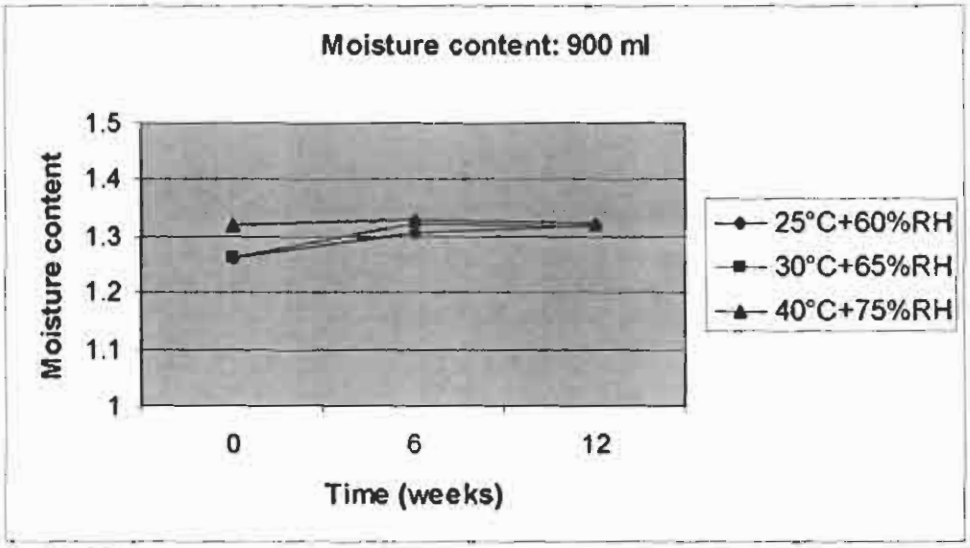
D.1.2: Moisture content of the 300 ml ARV powder for suspension during the accelerated stability study.



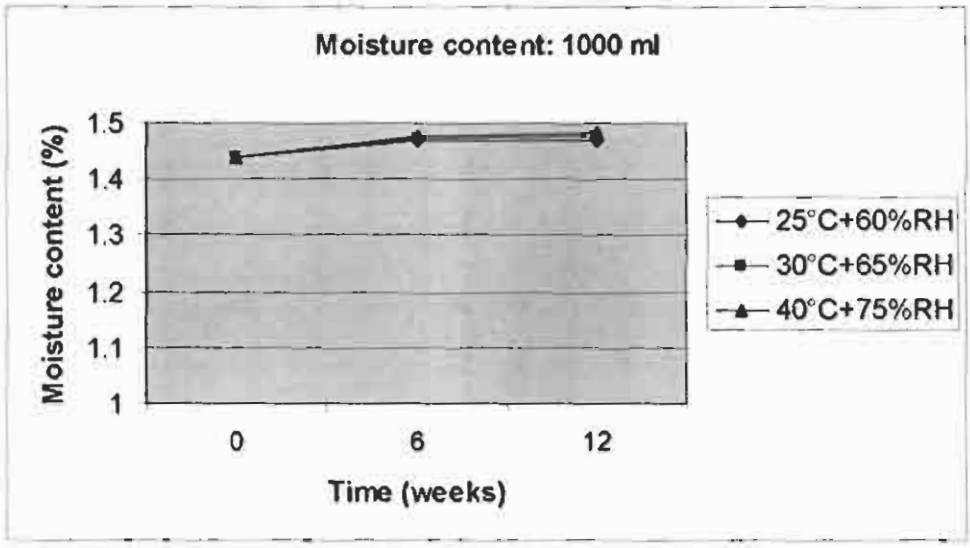
D.1.3: Moisture content of the 420 ml ARV powder for suspension during the accelerated stability study.



D.1.4: Moisture content of the 600 ml ARV powder for suspension during the accelerated stability study.



D.1.5: Moisture content of the 900 ml ARV powder for suspension during the accelerated stability study.



D.1.6: Moisture content of the 1000 ml ARV powder for suspension during the accelerated stability study.

ANNEXURE E

Lamivudine assay: 150 ml	**E2**
Lamivudine assay: 300 ml	**E2**
Lamivudine assay: 420 ml	**E3**
Lamivudine assay: 600 ml	**E3**
Lamivudine assay: 900 ml	**E4**
Lamivudine assay: 1000 ml	**E4**
Zidovudine assay: 300ml	**E5**
Zidovudine assay: 420ml	**E5**
Zidovudine assay: 600ml	**E6**
Zidovudine assay: 900ml	**E6**
Zidovudine assay: 1000ml	**E7**
Nevirapine assay: 150ml	**E7**
Nevirapine assay: 300ml	**E8**
Nevirapine assay: 420ml	**E8**
Nevirapine assay: 600ml	**E9**
Nevirapine assay: 900ml	**E9**
Nevirapine assay: 1000ml	**E10**

E.1: LAMIVUDINE

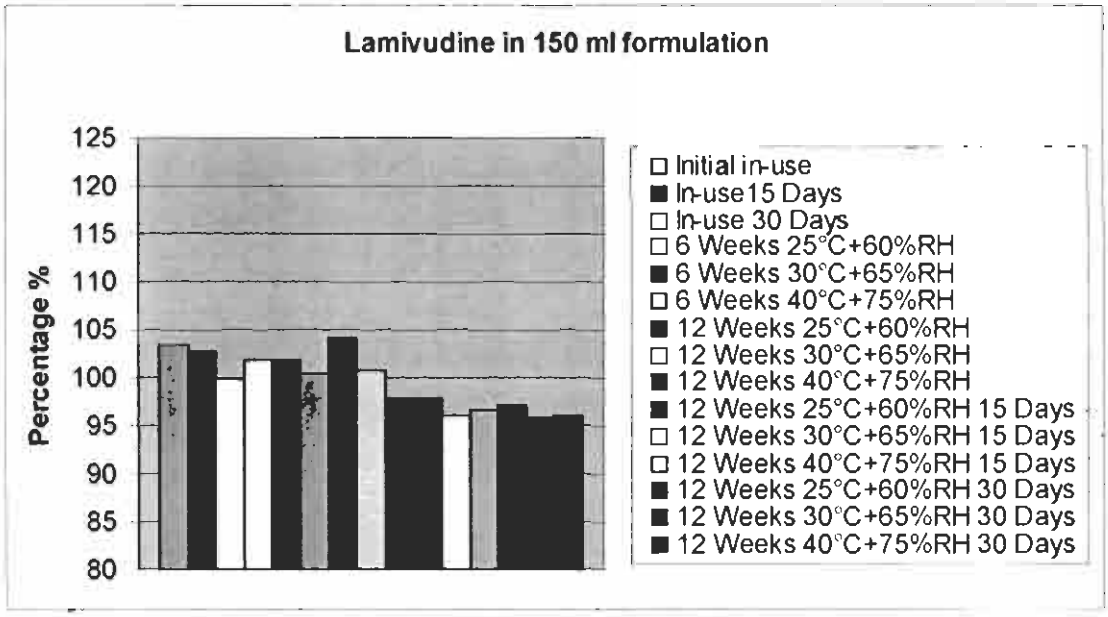


Figure E.1.1: Assay concentrations for the lamivudine 150 ml suspension formulation, during in-use and accelerated stability testing.

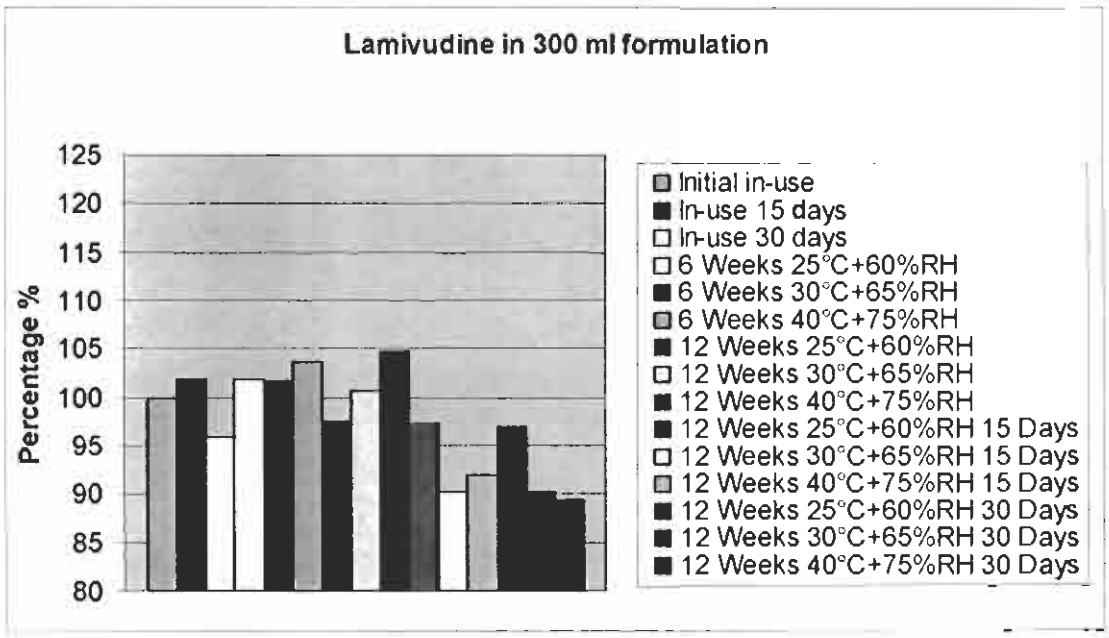


Figure E.1.2: Assay concentrations for the lamivudine 300 ml suspension formulation, during in-use and accelerated stability testing.

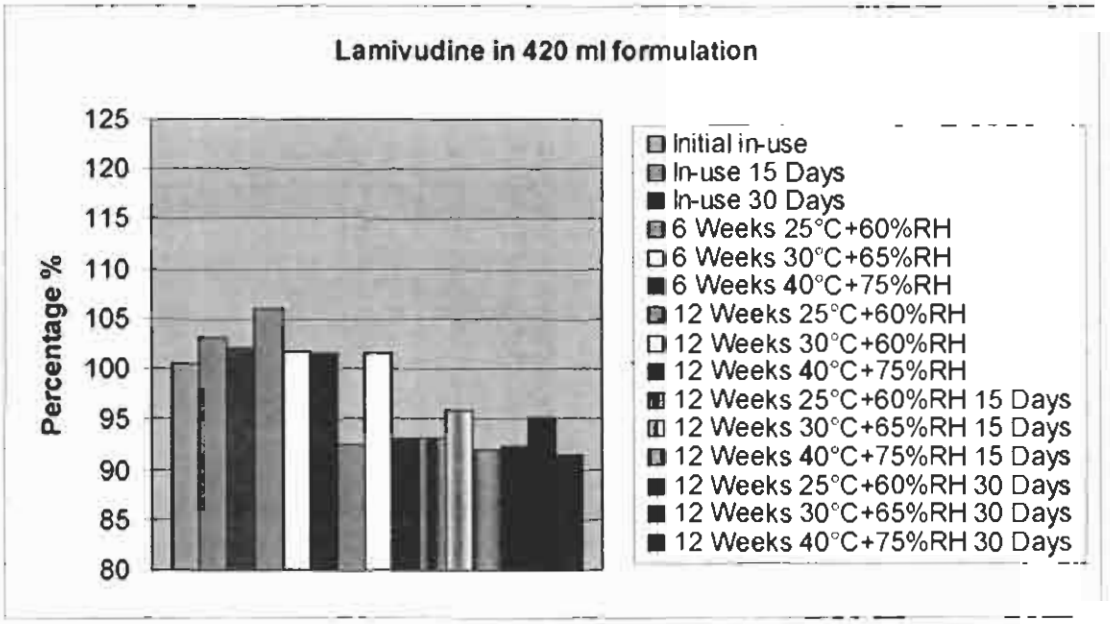


Figure E.1.3: Assay concentrations for the lamivudine 420 ml suspension formulation, during in-use and accelerated stability testing.

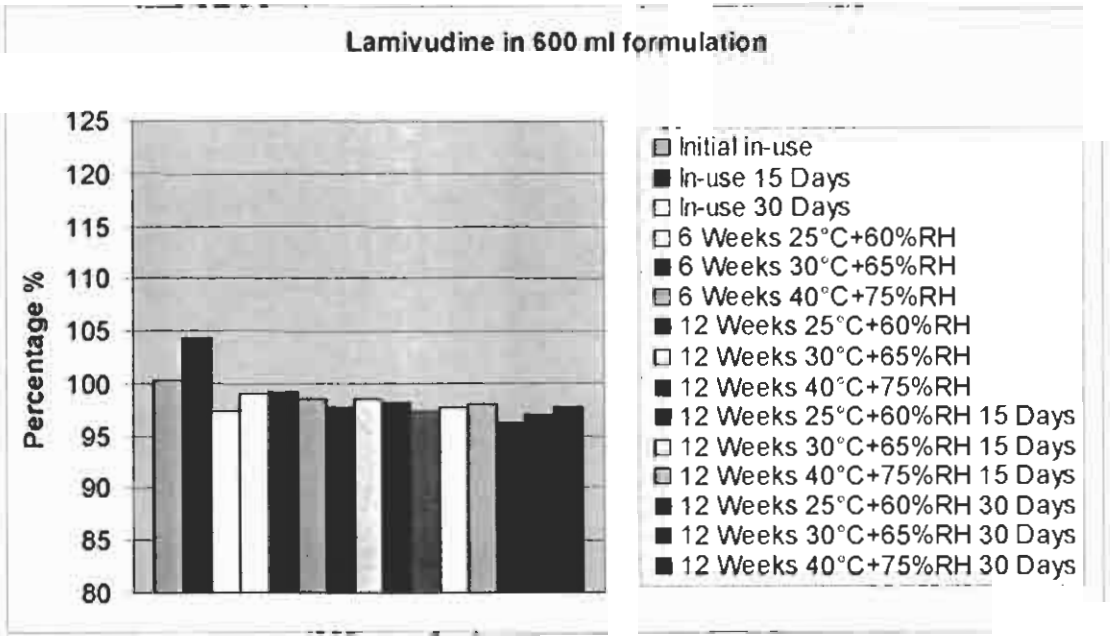


Figure E.1.4: Assay concentrations for the lamivudine 600 ml suspension formulation, during in-use and accelerated stability testing.

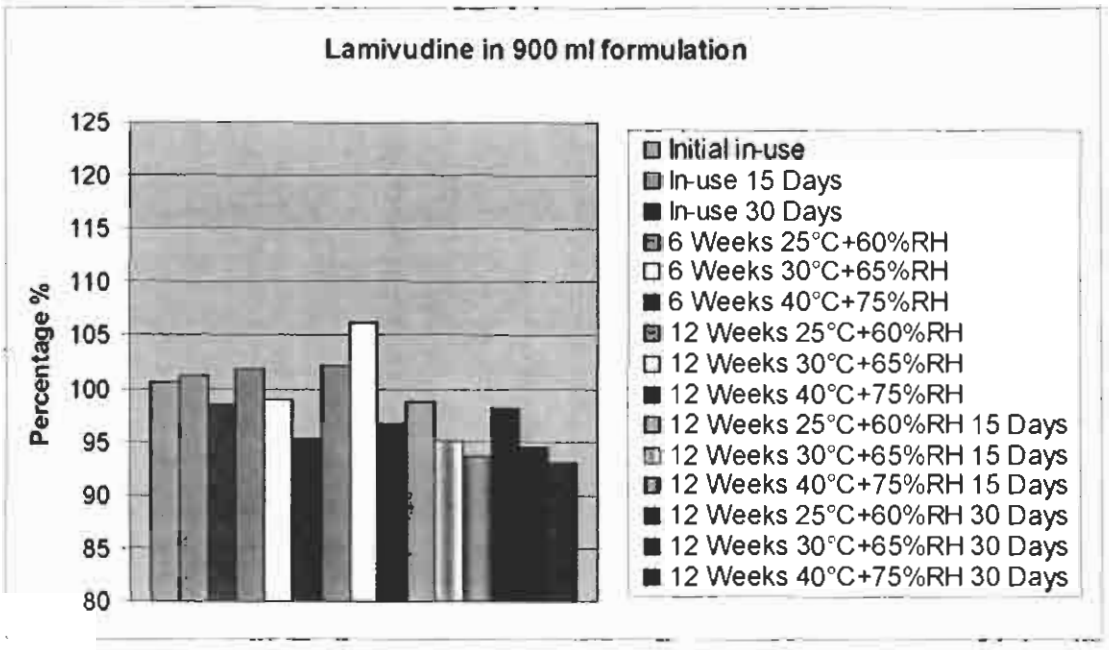


Figure E.1.5: Assay concentrations for the lamivudine 900 ml suspension formulation, during in-use and accelerated stability testing.

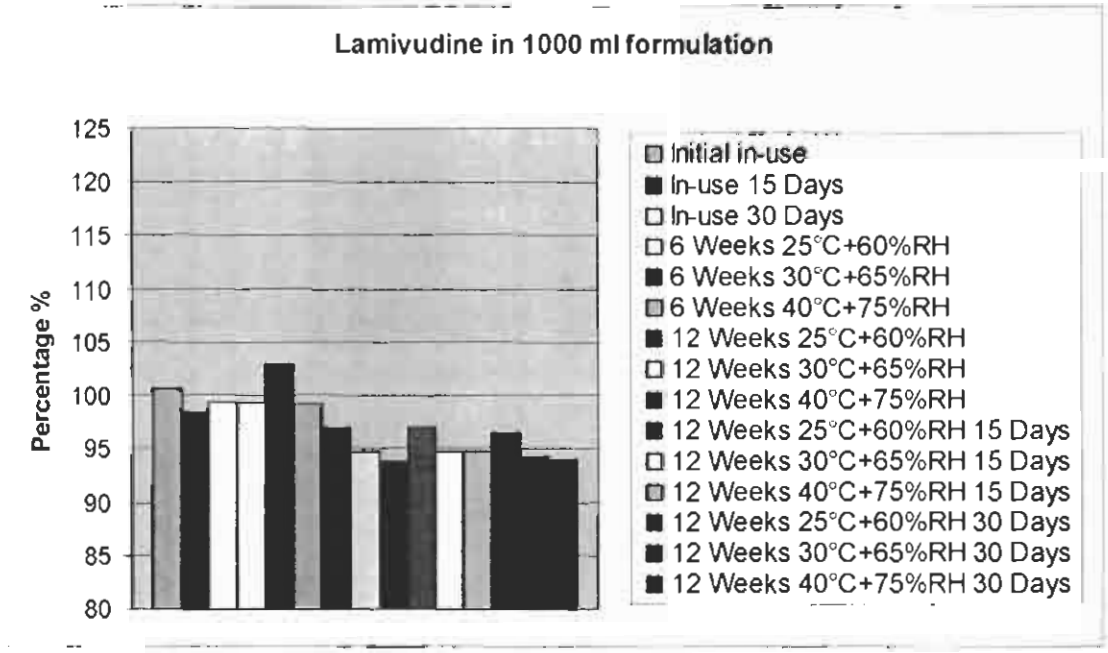


Figure E.1.6: Assay concentrations for the lamivudine 1000 ml suspension formulation, during in-use and accelerated stability testing.

E.2: ZIDOVUDINE

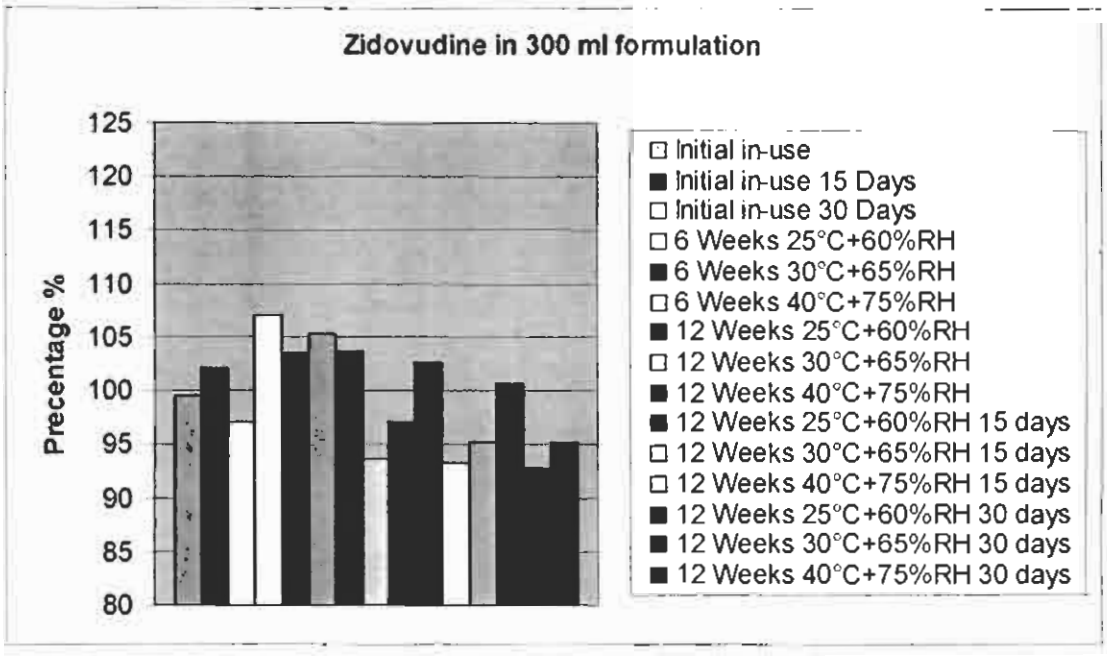


Figure E.2.1: Assay concentrations for the zidovudine 300 ml suspension formulation, during in-use and accelerated stability testing.

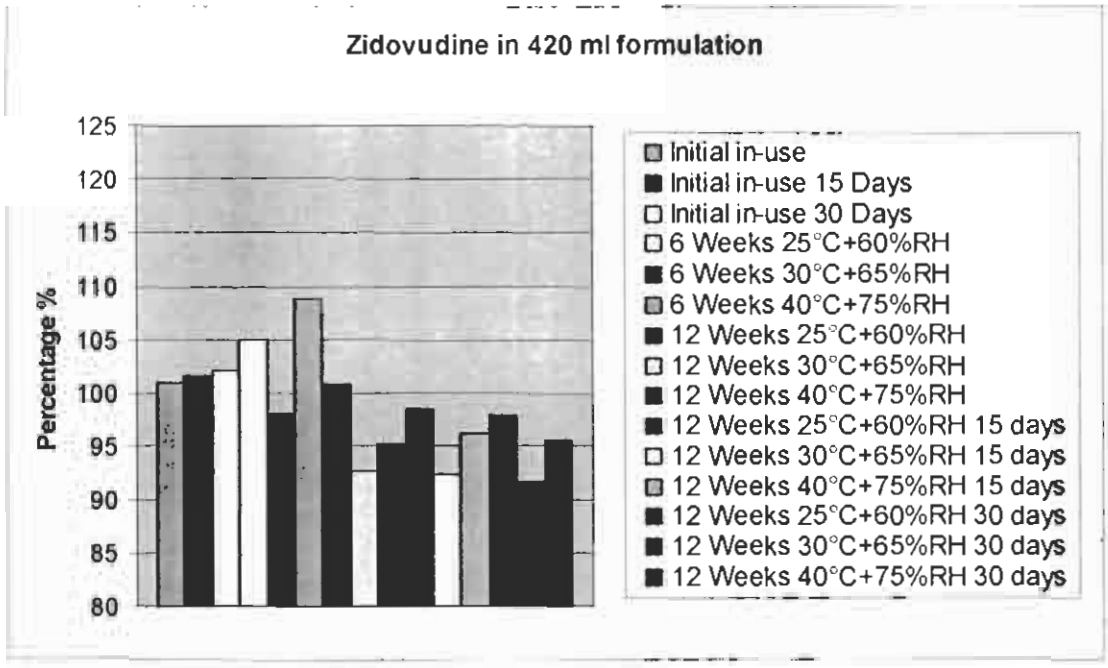


Figure E.2.2: Assay concentrations for the zidovudine 420 ml suspension formulation, during in-use and accelerated stability testing.

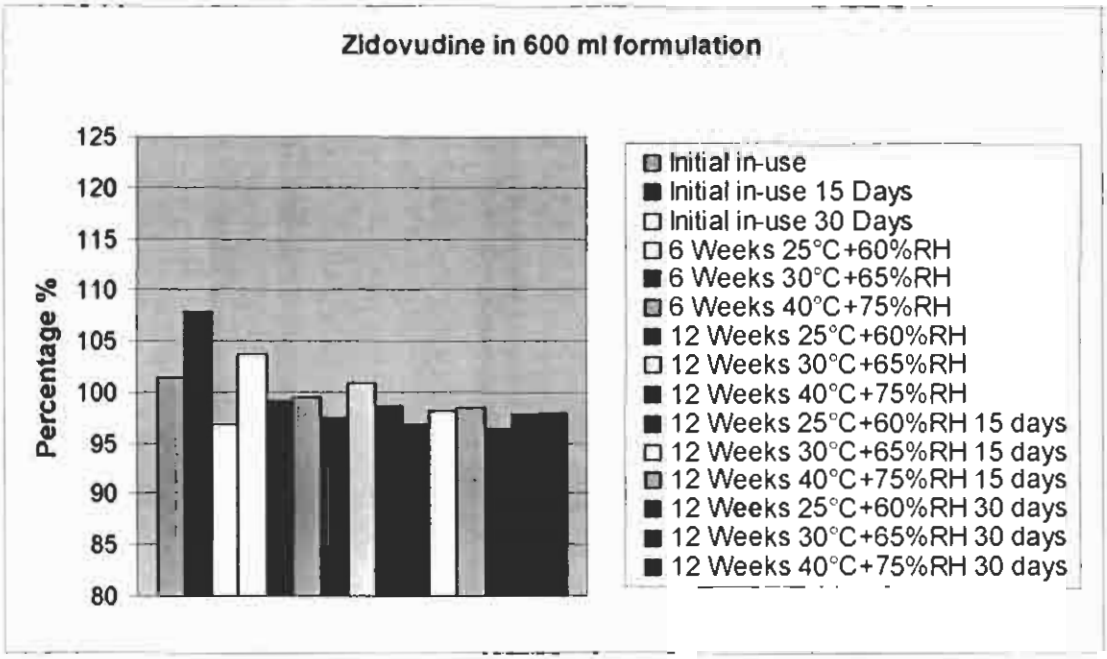


Figure E.2.3: Assay concentrations for the zidovudine 600 ml suspension formulation, during in-use and accelerated stability testing.

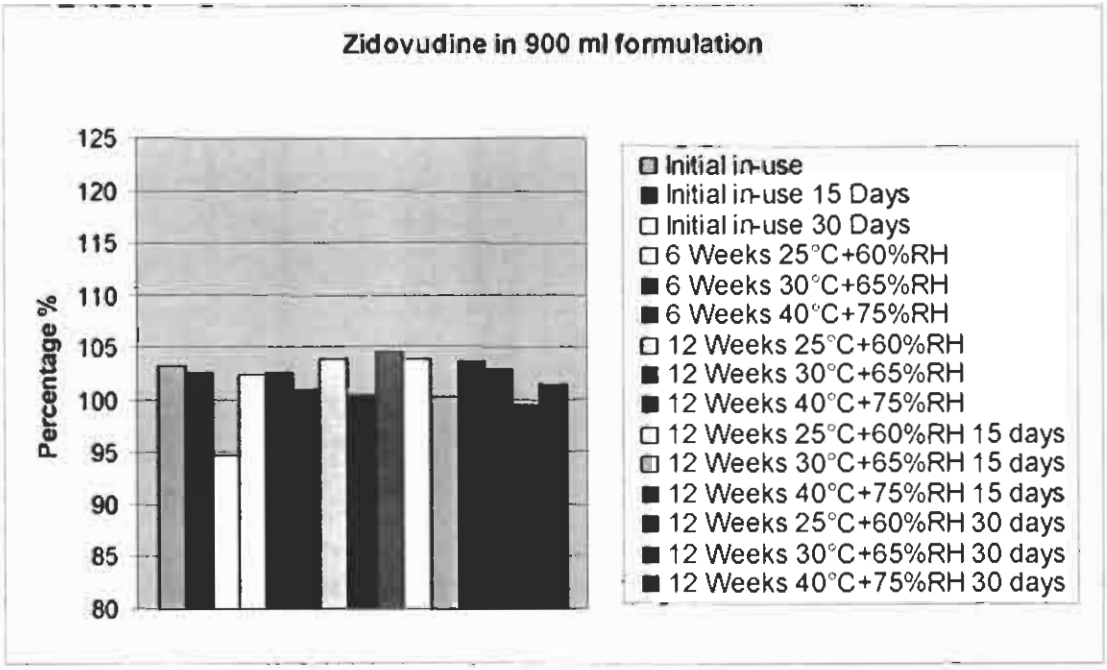


Figure E.2.4: Assay concentrations for the zidovudine 900 ml suspension formulation, during in-use and accelerated stability testing.

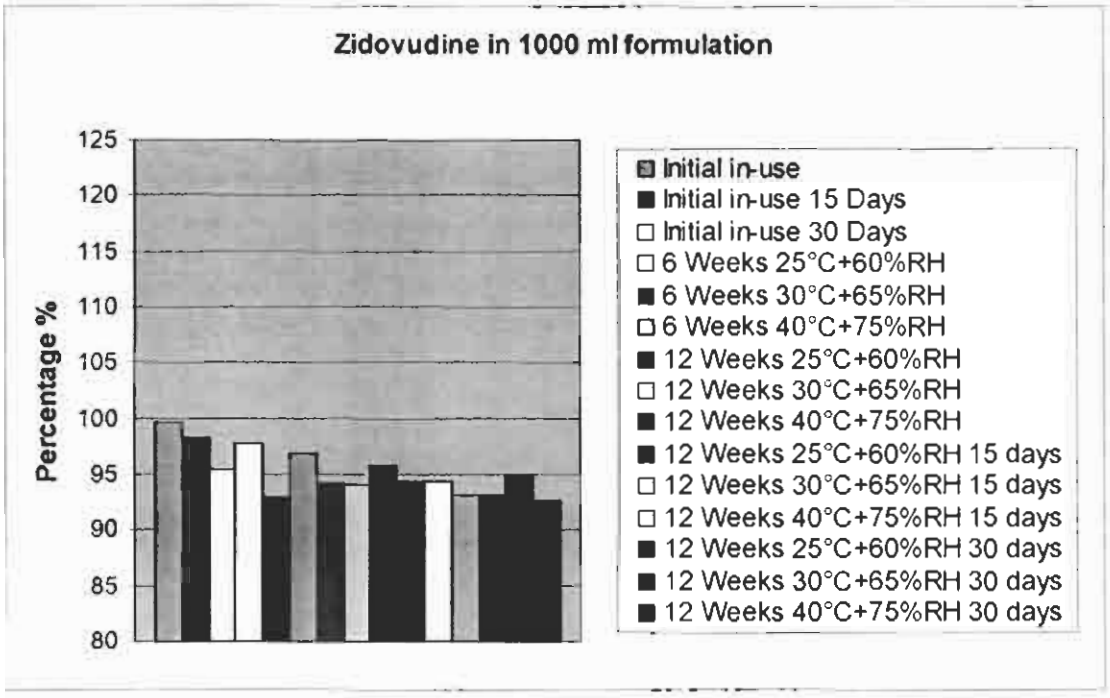


Figure E.2.5: Assay concentrations for the zidovudine 1000 ml suspension formulation, during in-use and accelerated stability testing.

E.3: NEVIRAPINE

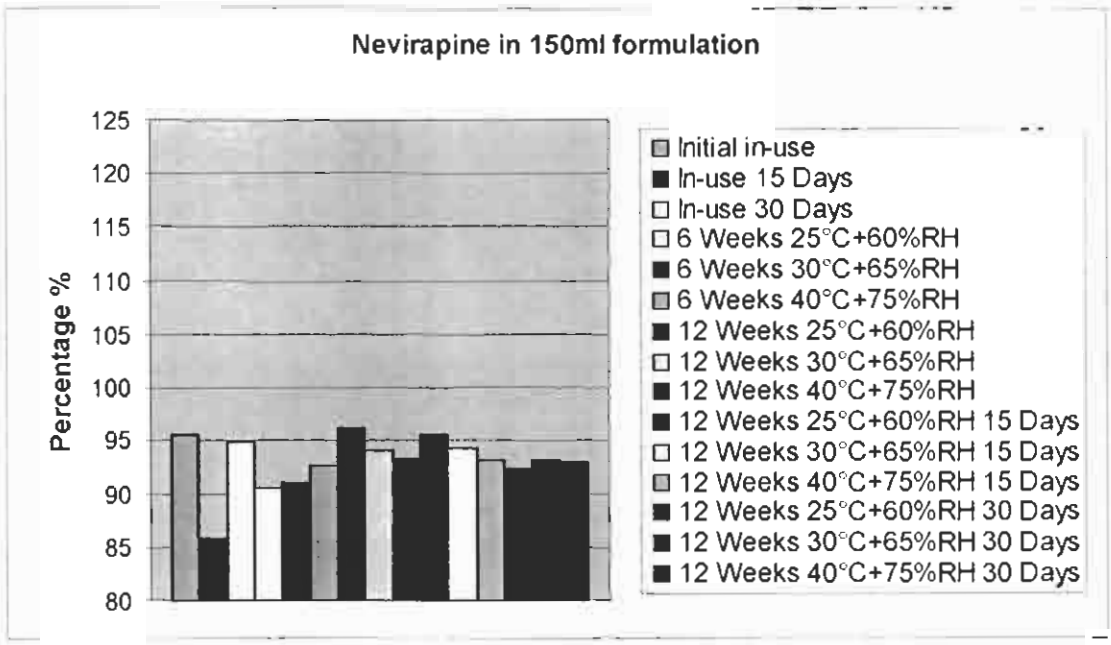


Figure E.3.1: Assay concentrations for the nevirapine 150 ml suspension formulation, during in-use and accelerated stability testing.

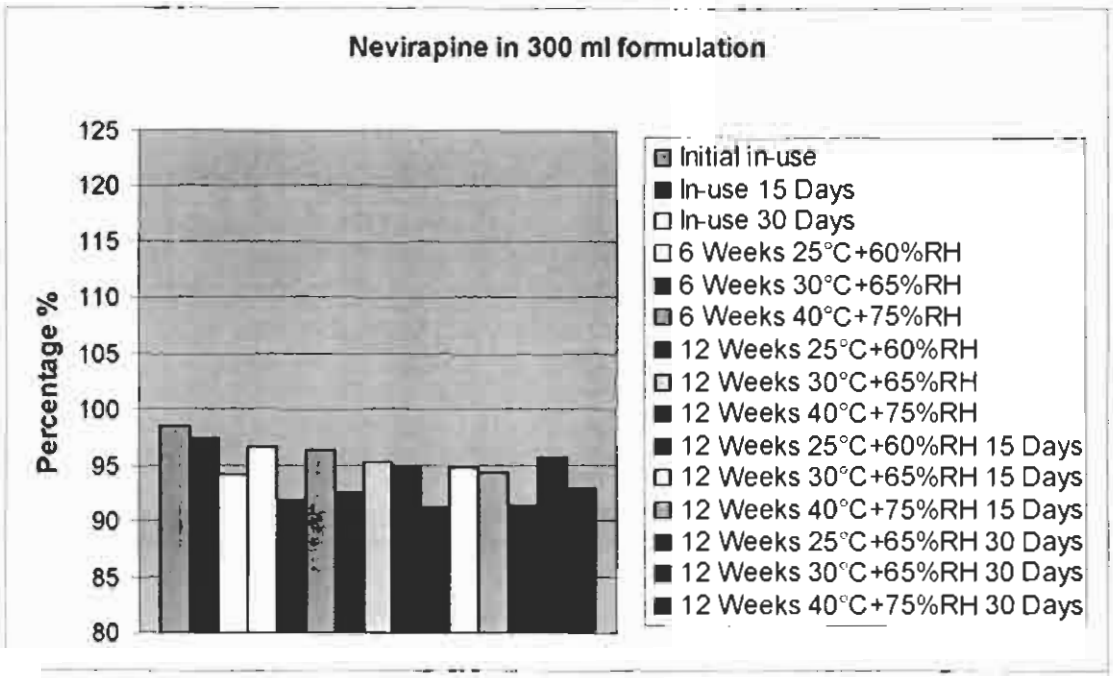


Figure E.3.2: Assay concentrations for the nevirapine 300 ml suspension formulation, during in-use and accelerated stability testing.

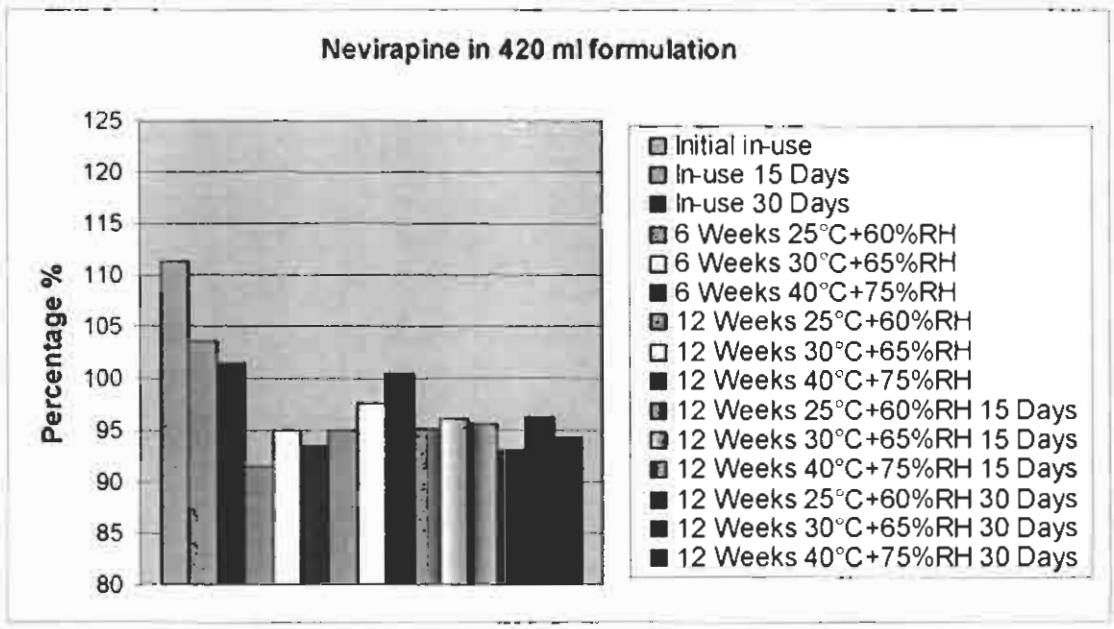


Figure E.3.3: Assay concentrations for the nevirapine 420 ml suspension formulation, during in-use and accelerated stability testing.

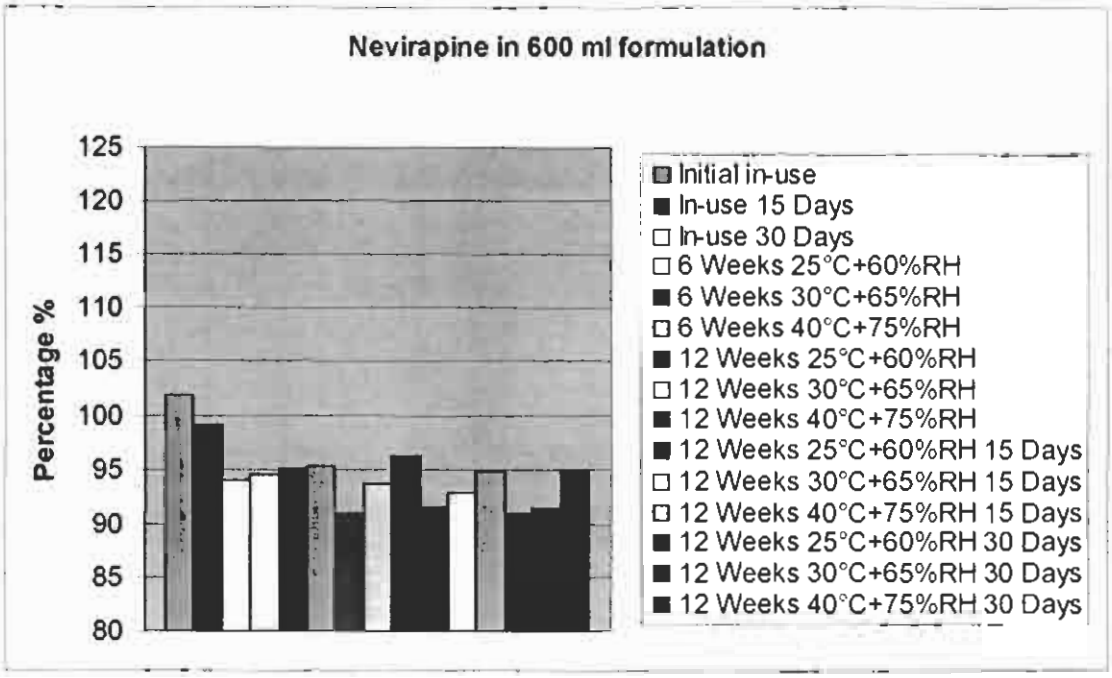


Figure E.3.4: Assay concentrations for the nevirapine 600 ml suspension formulation, during in-use and accelerated stability testing.

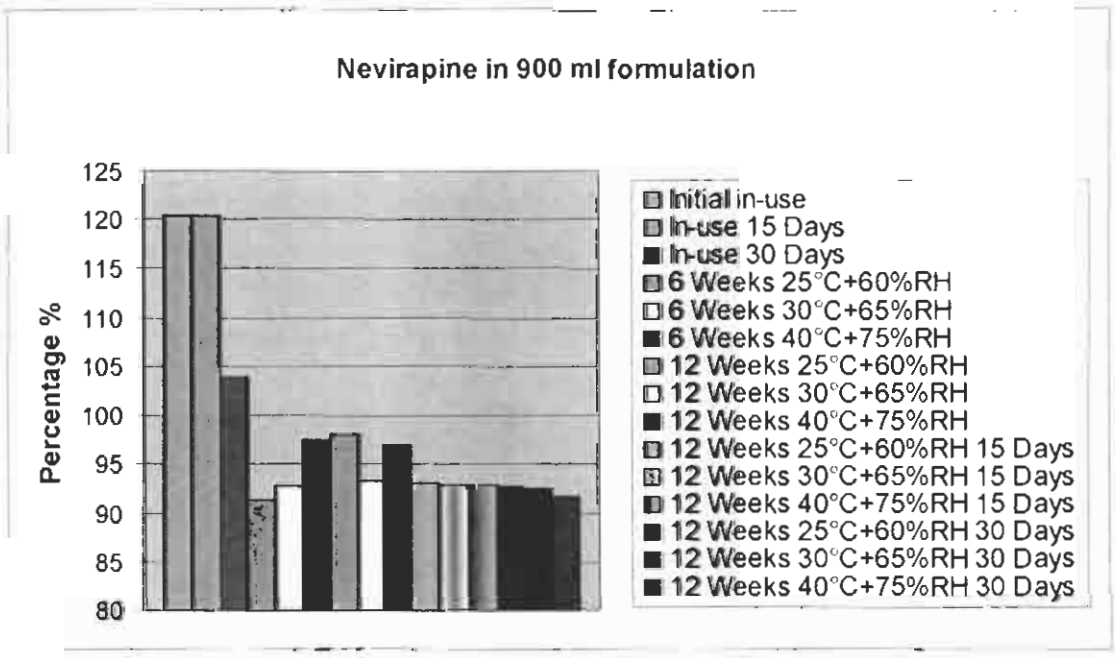


Figure E.3.5: Assay concentrations for the nevirapine 900 ml suspension formulation, during in-use and accelerated stability testing.

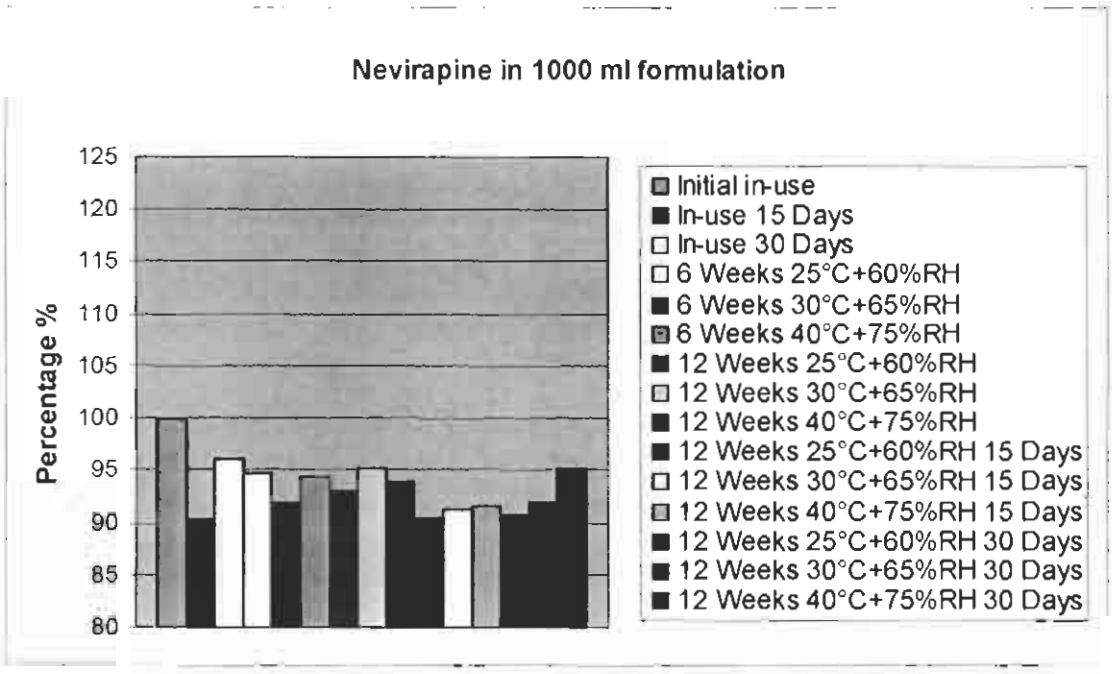


Figure E.3.6: Assay concentrations for the nevirapine 1000 ml suspension formulation, during in-use and accelerated stability testing.

ANNEXURE F

Sodium methyl hydroxybenzoate assay: 150 ml **F2**

Sodium methyl hydroxybenzoate assay: 300 ml **F2**

Sodium methyl hydroxybenzoate assay: 420 ml **F3**

Sodium methyl hydroxybenzoate assay: 600 ml **F3**

Sodium methyl hydroxybenzoate assay: 900 ml **F4**

Sodium methyl hydroxybenzoate assay: 1000 ml **F4**

Sodium propyl hydroxybenzoate assay: 150 ml **F5**

Sodium propyl hydroxybenzoate assay: 300 ml **F5**

Sodium propyl hydroxybenzoate assay: 420 ml **F6**

Sodium propyl hydroxybenzoate assay: 600 ml **F6**

Sodium propyl hydroxybenzoate assay: 900 ml **F7**

Sodium propyl hydroxybenzoate assay: 1000 ml **F7**

F1: SODIUM METHYL HYDROXYBENZOATE

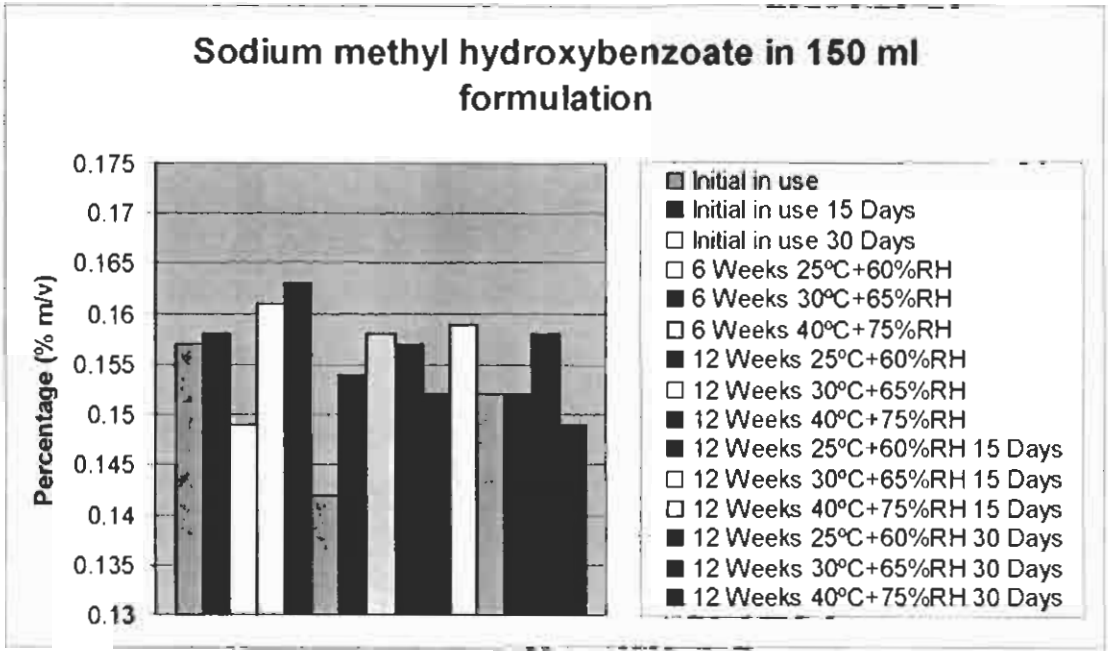


Figure F.1.1: Assay concentrations for the sodium methyl hydroxybenzoate 150 ml suspension formulation, during in-use and accelerated stability testing.

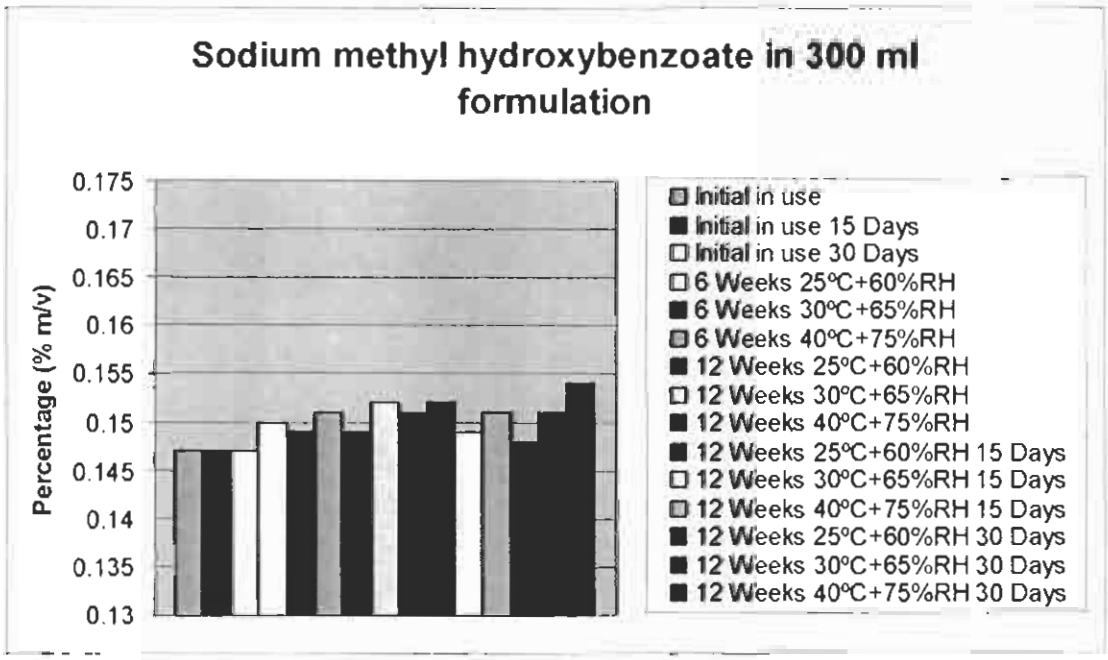


Figure F.1.2: Assay concentrations for the sodium methyl hydroxybenzoate 300 ml suspension formulation, during in-use and accelerated stability testing.

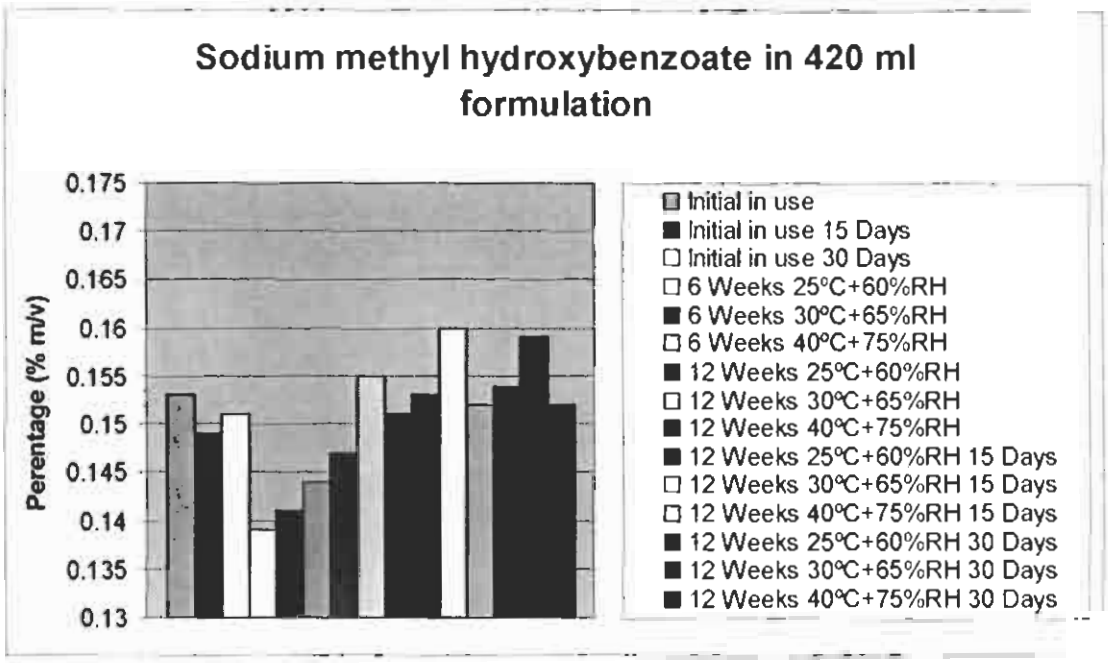


Figure F.1.3: Assay concentrations for the sodium methyl hydroxybenzoate 420 ml suspension formulation, during in-use and accelerated stability testing.

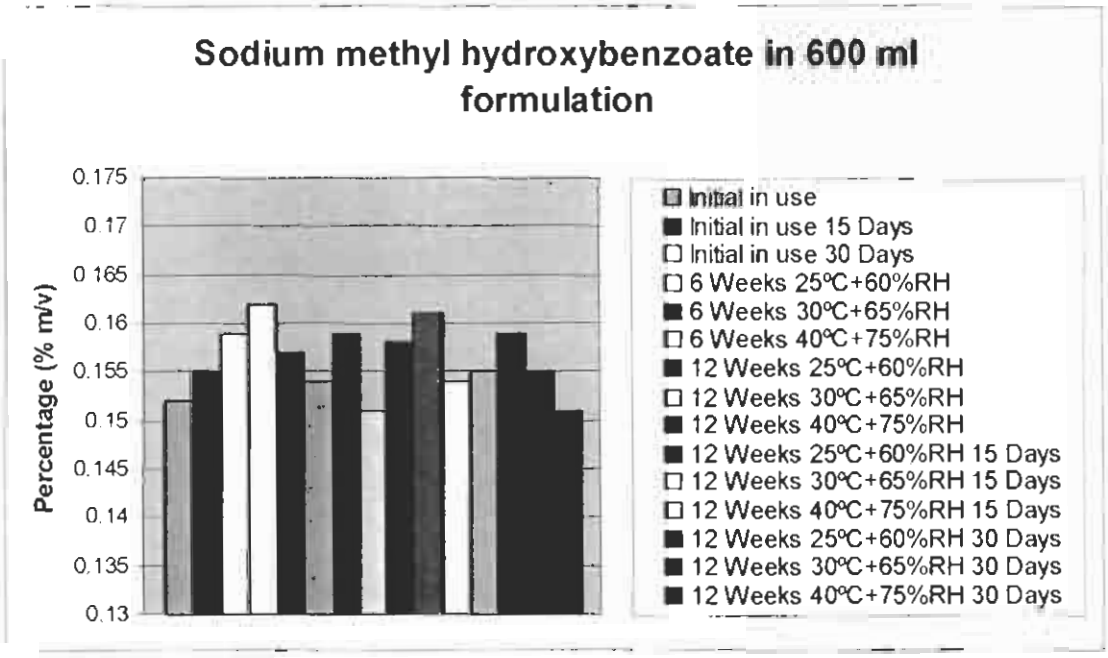


Figure F.1.4: Assay concentrations for the sodium methyl hydroxybenzoate 600 ml suspension formulation, during in-use and accelerated stability testing.

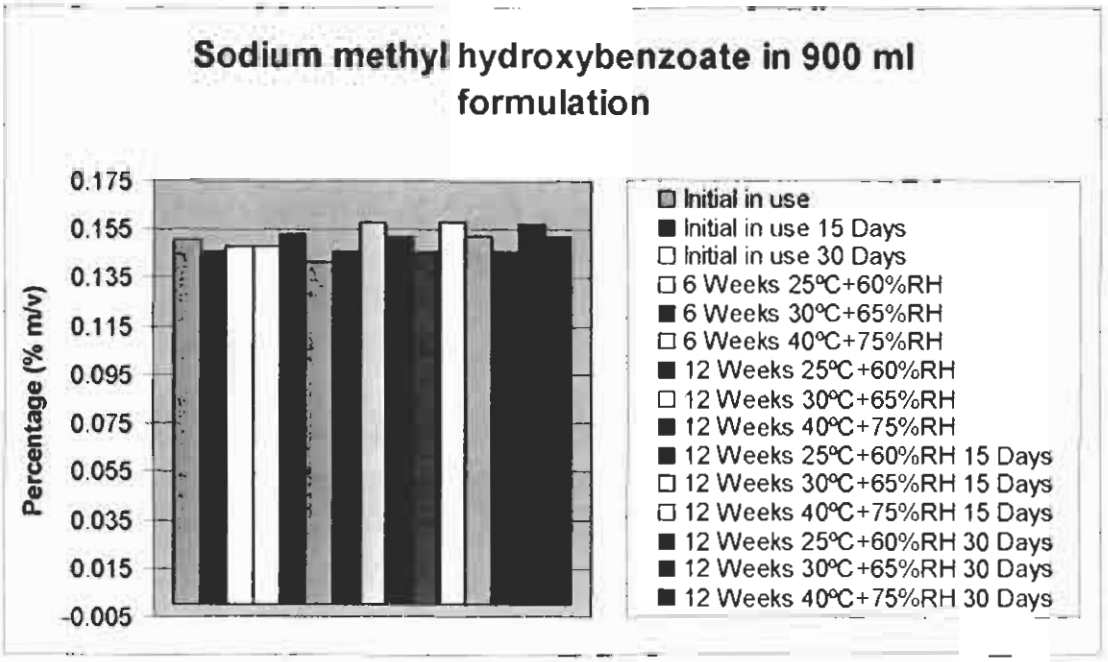


Figure F.1.5: Assay concentrations for the sodium methyl hydroxybenzoate 900 ml suspension formulation, during in-use and accelerated stability testing.

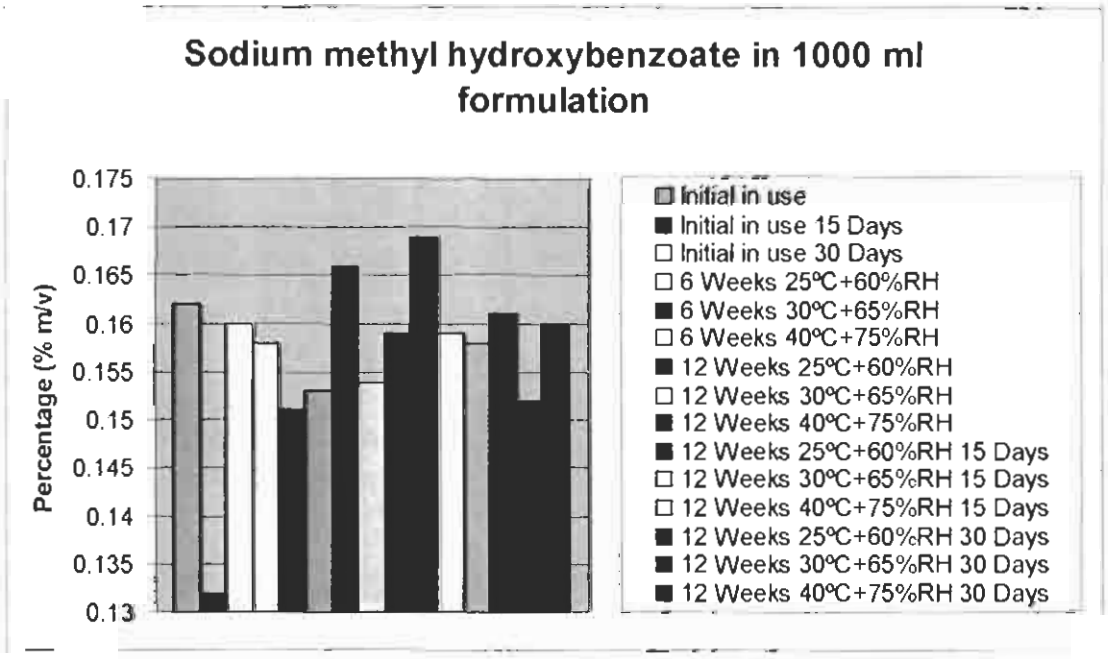


Figure F.1.6: Assay concentrations for the sodium methyl hydroxybenzoate 1000 ml suspension formulation, during in-use and accelerated stability testing.

B2: SODIUM PROPYL HYDROXYBENZOATE

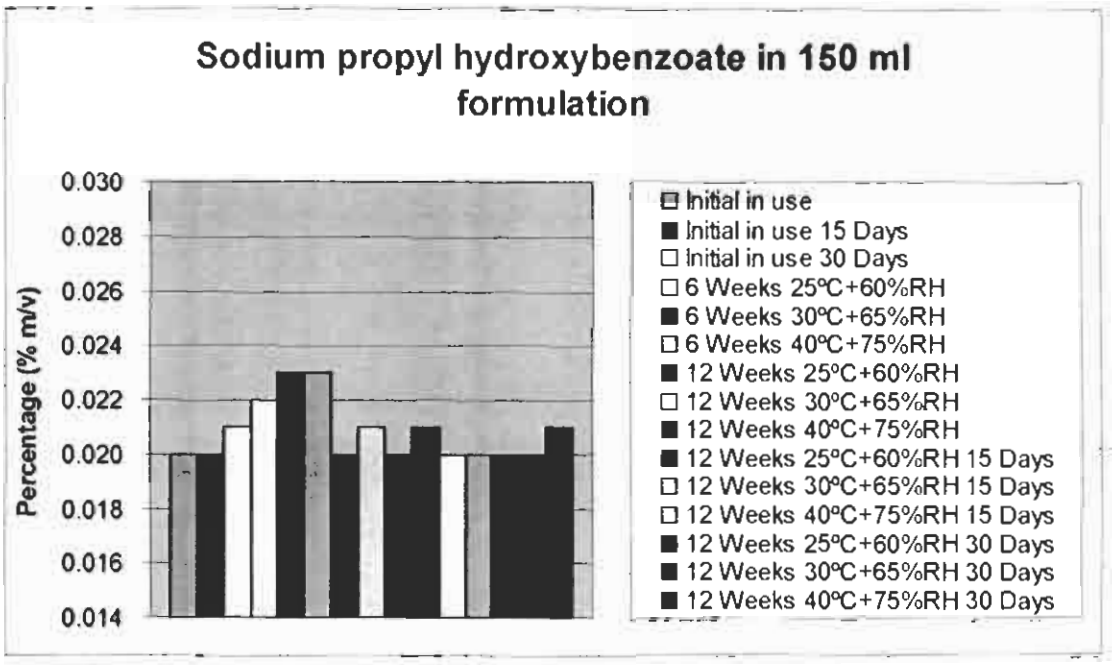


Figure F.2.1: Assay concentrations for the sodium propyl hydroxybenzoate 150 ml suspension formulation, during in-use and accelerated stability testing.

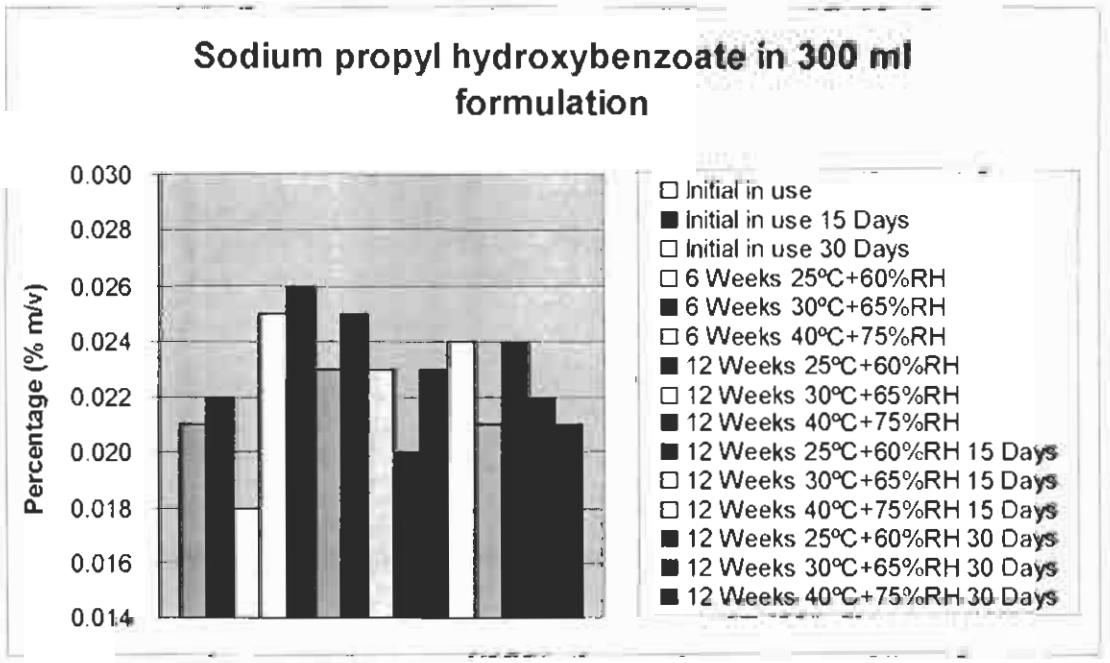


Figure F.2.2: Assay concentrations for the sodium propyl hydroxybenzoate 300 ml suspension formulation, during in-use and accelerated stability testing.

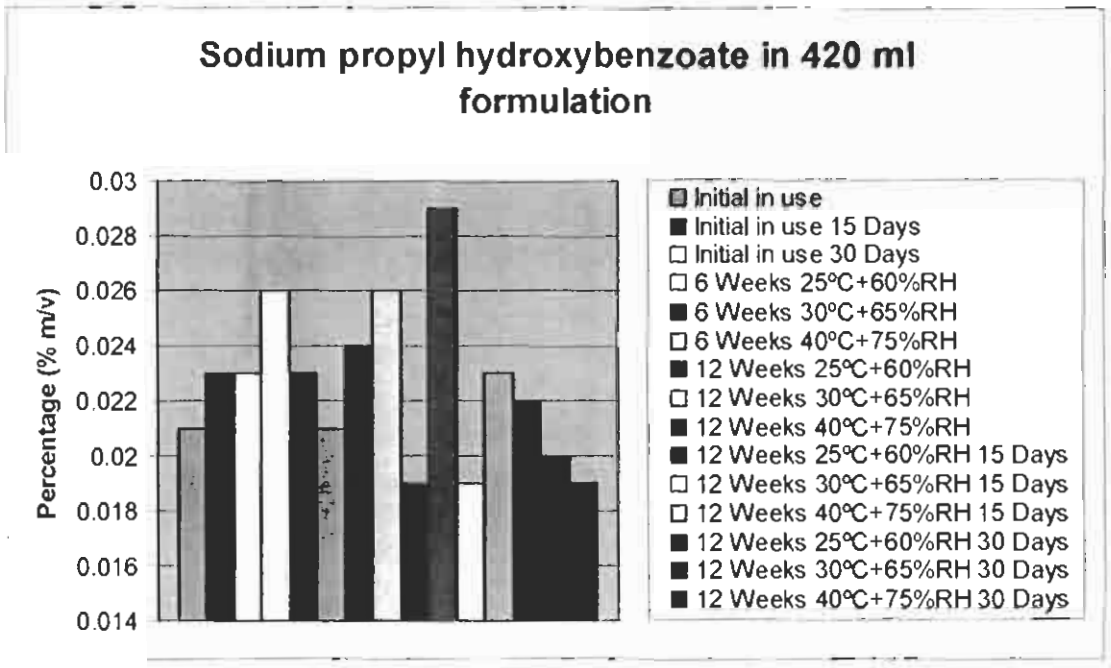


Figure F.2.3: Assay concentrations for the sodium propyl hydroxybenzoate 420 ml suspension formulation, during in-use and accelerated stability testing.

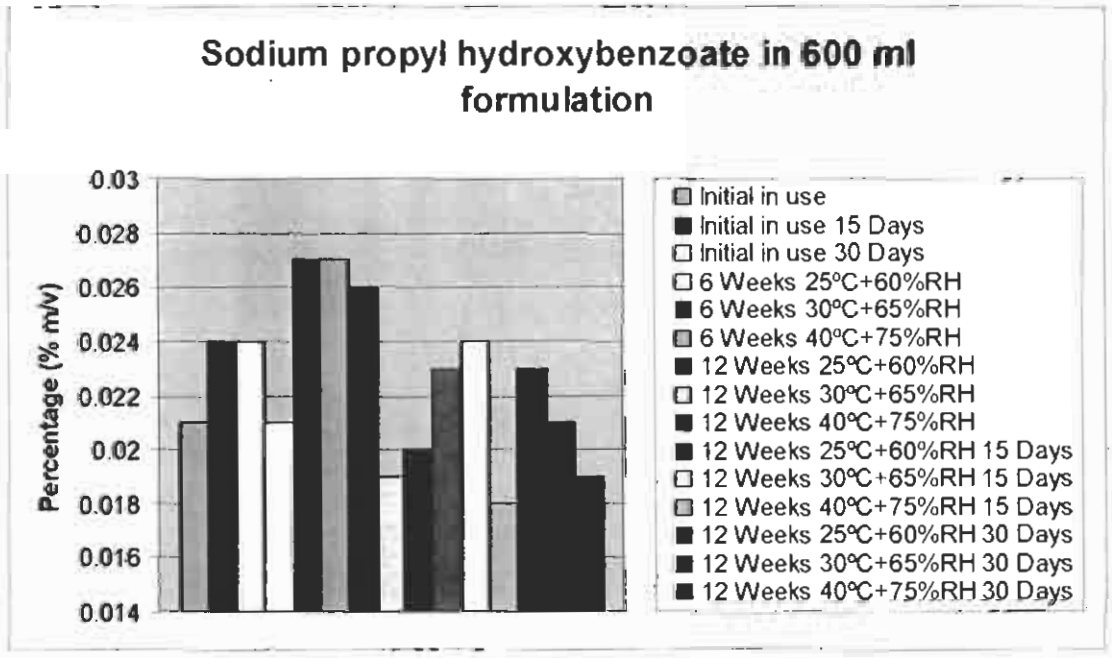


Figure F.2.4: Assay concentrations for the sodium propyl hydroxyhenzoate 600 ml suspension formulation, during in-use and accelerated stability testing.

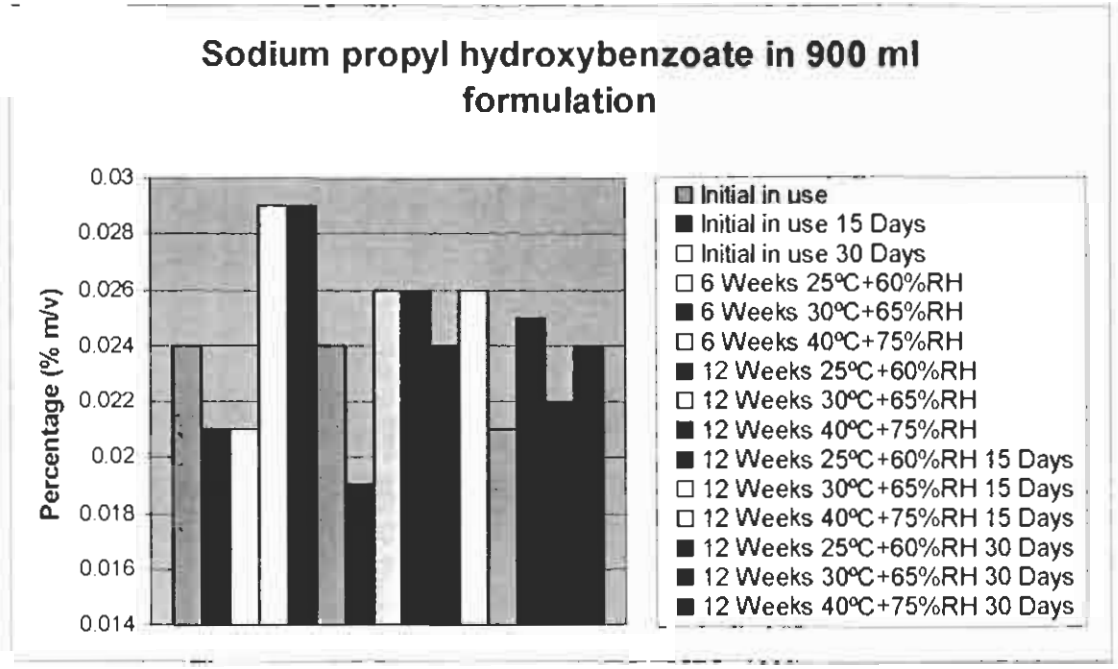


Figure F.2.5: Assay concentrations for the sodium propyl hydroxybenzoate 900 ml suspension formulation, during in-use and accelerated stability testing.

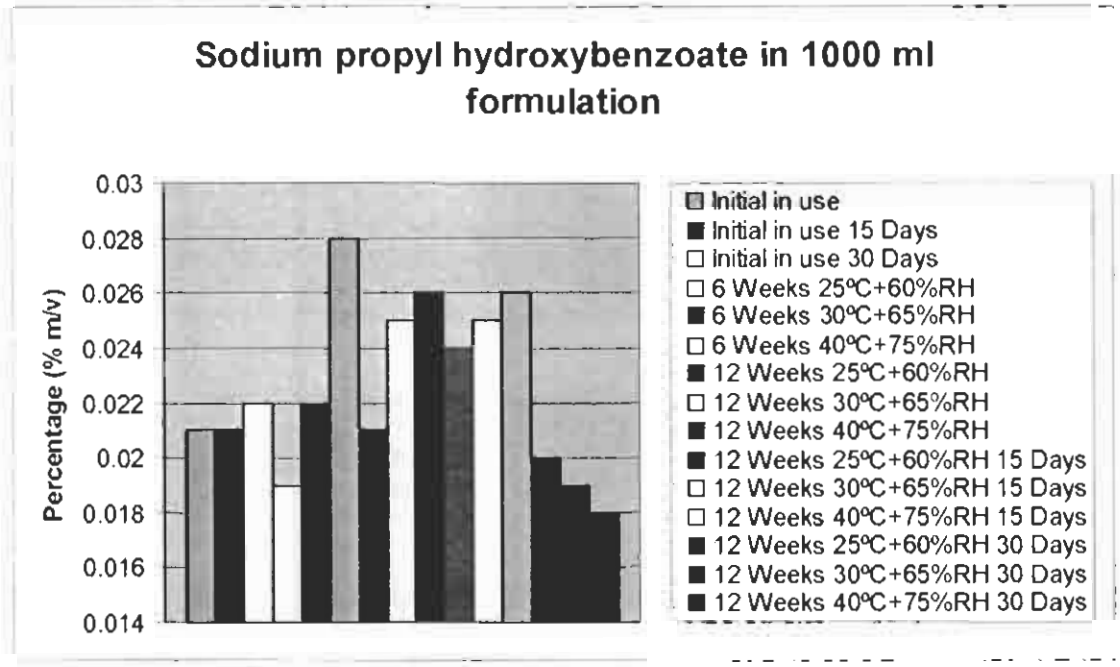


Figure F.2.6: Assay concentrations for the sodium propyl hydroxybenzoate 1000 ml suspension formulation, during in-use and accelerated stability testing.

ANNEXURE G

VALIDATION OF AN ASSAY FOR THE SIMULTANEOUS DETERMINATION OF LAMIVUDINE, ZIDOVUDINE, NEVIRAPINE, SODIUM METHYL HYDROXYBENZOATE AND SODIUM PROPYL HYDROXYBENZOATE IN SUSPENSION

SUMMARY

Table G1: Summary of the validation results for the simultaneous determination of lamivudine, zidovudine, nevirapine, sodium methyl hydroxybenzaote and sodium propyl hydroxybenzoate in suspension.

Tests	Results
Specificity	Complies
Range	Lamivudine 0.9 – 348 µg /ml Zidovudine 0.9 – 349 µg /ml Nevirapine 0.9 – 350 µg /ml Sodium propyl hydroxybenzoate 0.1 – 50 µg /ml Sodium methyl hydroxybenzoate 0.05 – 206 µg /ml Cytosine 0.1 – 202.7 µg /ml Thymine 0.1 – 208.9 µg /ml
Linearity	Lamivudine $r^2 = 0.9991$ Zidovudine $r^2 = 0.9992$ Nevirapine $r^2 = 0.9999$ Sodium propyl hydroxybenzoate $r^2 = 0.9999$ Sodium methyl hydroxybenzoate $r^2 = 0.9991$ Cytosine $r^2 = 0.9990$ Thymine $r^2 = 0.9997$
Accuracy	Lamivudine 101.5% Zidovudine 101.6% Nevirapine 100.1% Sodium propyl hydroxybenzoate 100.3% Sodium methyl hydroxybenzoate 100.4%
Precision	Lamivudine RSD 1.29% Zidovudine RSD 1.25% Nevirapine RSD 1.47% Sodium propyl hydroxybenzoate RSD 1.59% Sodium methyl hydroxybenzoate RSD 0.78%
Ruggedness	Complies
Robustness	Complies

1. ORIGIN OF METHOD

The method was developed and validated by Dr. J.L. du Preez at the Analytical Technology Laboratory (ATL) at the North-West University.

2. CHROMATOGRAPHIC CONDITIONS

- **Analytical Instrument:** HP1100 series HPLC equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software or equivalent.

- **Column:** Luna C18 (2) 150x4.6 mm, 5µm (Phenomenex, Torrance, CA).

- **Mobile phase:** Acetonitrile (solvent B), water with 0.2% triethylamine, pH adjusted to 7.0 with phosphoric acid or ammonium hydroxide (solvent A).

- **Gradient table:**

Time	Solvent A	Solvent B
0.00	92	8
1.500	35	65
10.00	35	65
15.00	92	8

- **Flow Rate:** 1.0 ml/min.
- **Injection Volume :** 10 µl.
- **Detection:** UV at 270 nm, 16 nm bandwidth.
- **Retention time:** ± 1.7 minutes for cytosine
  ± 2.3 minutes for thymine
  ± 3.7 minutes for lamivudine
  ± 6.5 minutes for zidovudine
  ± 7.7 minutes for nevirapine
  ± 8.2 minutes for sodium methyl hydroxybenzoate
  ± 9.9 minutes for sodium propyl hydroxybenzoate
- **Run time:** 15 minutes.

3. SAMPLE PREPARATION

1. Place a sterile 5 ml surgical syringe on an analytical balance and tare.
2. Accurately weigh 5 ml of the 150, 300, 420 and 600 ml suspensions into separate 250 ml volumetric flasks.
3. Accurately weigh 3 ml of the 900 ml suspension and 4 ml of the 1000 ml suspension into separate 250 ml volumetric flasks.
4. Add about 150 ml methanol to each flask, remove the stoppers and put the flasks in an ultrasonic bath, containing luke warm water and sonicate for 20 minutes.
5. Make sure that all the suspension has dissolved. If solid particles are visible, shake the flask and sonicate it again until completely dissolved.
6. Allow the flasks to cool to room temperature and fill each flask to 250 ml with distilled water.
7. Filter the solutions into auto sampler vials and analyse.

4. STANDARD PREPARATION

1. Place a clean dry weighing boat on an analytical balance and tare.
2. Accurately weigh 71.38 mg zidovudine, 35.65 mg lamivudine and 71.29 mg nevirapine separately, each in its own weighing boat.
3. Place the three accurately weighed APIs together in a 250 ml volumetric flask.
4. Place a clean dry weighing boat on an analytical balance and tare.
5. Accurately weigh 21 mg thymine, 75 mg sodium methyl hydroxybenzoate and 10 mg sodium propyl hydroxybenzoate in separate weighing boats.
6. Add the three abovementioned substances together in a 100 ml volumetric flask.
7. Place a 200 ml volumetric flask on an analytical balance and tare.
8. Accurately weigh 14 mg cytosine into the flask.
9. Add 150 ml methanol to the 200 ml flask and put the flask in an ultrasonic bath, containing luke warm water and sonicate for 20 minutes.
10. Allow the flask to cool to room temperature and fill to volume with distilled water.
11. With a 10 ml pipette, extract 10 ml of the abovementioned solution into the 100 ml flask.
12. Add 50 ml methanol to the 100 ml flask and put the flask in an ultrasonic bath, containing luke warm water and sonicate for 20 minutes.

13. Allow to cool to room temperature and fill to volume with distilled water.
14. Using a 10 ml pipette, transfer 10 ml of the abovementioned solution into the 250 ml flask.
15. Add 200 ml methanol to the 250 ml flask and put the flask in an ultrasonic bath, containing luke warm water and sonicate for 20 minutes.
16. Allow to cool to room temperature and fill to volume with distilled water.
17. This is the final solution which will be used as a standard.

5. CALCULATIONS

The compliance range for lamivudine, zidovudine , nevirapine, sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate is set at 90 – 110%.

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

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

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

$$\frac{SAR \times (\text{mass of std. mg}) \times (\text{std. Potency}) \times wt/ml}{STR \times \text{mass of sample (g)} \times 100} = \text{mg sodium methyl hydroxybenzoate /ml}$$

$$\frac{SAR \times (\text{mass of std. mg}) \times (\text{std. Potency}) \times wt/ml}{STR \times \text{mass of sample (g)} \times 100} = \text{mg sodium propyl hydroxybenzoate /ml}$$

Where:

SAR = sample peak area

STR = standard peak area

Wt/ml = weight per ml of the oral suspension in g

6. VALIDATION TEST PROCEDURE AND ACCEPTANCE CRITERIA

6.1 SPECIFICITY

METHOD:

1. Prepare a sample from a placebo of the suspension that does not contain the various actives which are being tested with this method.
2. Dilute 4 x 10 ml standard solution with water, 1 ml of 1M hydrochloric acid, 1 ml of 1M sodium hydroxide and 0.5 ml 10% hydrogen peroxide respectively.
3. Store these solutions overnight at 70°C to degrade.
4. Filter these samples into an auto sampler vial and analyse to determine whether any additional peaks were formed.

ACCEPTANCE CRITERIA

- The placebo should not contain any peaks that will interfere with the determination of the actives.
- Extra peaks formed in the stressed samples should be discernible from those of the active components.

6.2 LINEARITY

Standard Preparation

1. Prepare a standard solution as described under standard preparation.
2. Inject variable volumes into the chromatograph to obtain standards from 75-125% of the expected sample concentration.

ACCEPTANCE CRITERIA

- Linear regression analysis should yield a regression coefficient (r squared) of ≥ 0.99.

6.3 ACCURACY

1. Spike known amounts of active at concentrations of approximately 80%, 100% and 120% of the expected sample concentration.
2. Inject into the chromatograph in duplicate.

ACCEPTANCE CRITERIA

- Recovery must be between 98-102%.

6.4 PRECISION

6.4.1 INTRA DAY PRESCISION (REPEATABILITY)

1. Measure approximately 3 x 8 ml (80%), 3 x 10 ml (100%) and 3 x 12 ml (120%) of the suspension into 100 ml volumetric flasks and fill to volume with methanol.
2. Prepare a single standard at 100% of the expected sample concentration as described in the method.
3. Inject into the chromatograph in duplicate.

6.4.2 INTER DAY PRECISION

1. Analyse the same homogeneous sample in triplicate as described above for intra-day precision (at 100% of the sample concentration) on two more occasions to determine the between-day variability of the method. On one occasion (day 3) a different analyst should perform the analysis on a different set of equipment.

ACCEPTANCE CRITERIA

- Repeatability must be better than 2% (n=9).
- Inter-day precision must be better than 5% (n=9).

6.5 RUGGEDNESS

6.5.1 STABILITY OF THE SAMPLE SOLUTIONS

1. Prepare a sample as directed under sample preparation in the method.
2. Inject the sample into the chromatograph.
3. Leave the sample in the auto sampler tray and reanalyse over a period to determine the stability of the sample.

ACCEPTANCE CRITERIA

- Sample solutions should not be used for a period longer than it takes to degrade by 2%, and in the case of degradation, special precautions should be followed to compensate for the loss.

6.5.2 SYSTEM REPEATABILITY

1. Inject a sample six times consecutively in order to test the repeatability of the peak area as well as the retention time.

ACCEPTANCE CRITERIA

- The peak and retention times should have an RSD of 2% or less.

6.6 ROBUSTNESS

1. Make deliberate changes to the chromatographic conditions to determine the method's tolerance towards changes.
2. Change the flow rate, wavelength, injection volume, acetonitrile concentration and use a similar column from a different manufacturer.

ACCEPTANCE CRITERIA

- The method should be able to tolerate a 5% variance in the chromatographic conditions.

6.7 SYSTEM AND METHOD PERFORMANCE CHARACTERISTICS (SYSTEM SUITABILITY)

1. Calculate the chromatographic performance characteristics of the separation, like retention time, USP peak tailing factor, capacity factor, and resolution between peaks and repeatability of multiple injections.
2. Use the data obtained to set realistic performance limits that should be met before the analysis can be performed.

ACCEPTANCE CRITERIA

- RSD of 2 injections not more than 2%
- The column must have more than 3000 theoretical plates for all the API and preservative peaks.
- The resolution between subsequent peaks must be greater than 2.

7. VALIDATION RESULTS

7.1 SPECIFICITY

Placebo and standard solutions were prepared as described in paragraph 6.1.

All the samples were analysed at 270 nm.

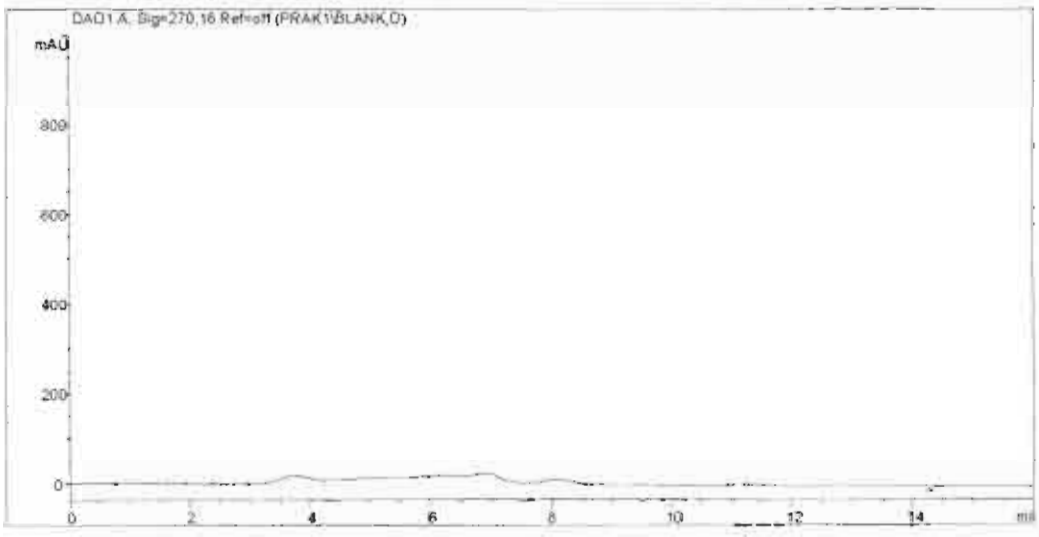


Figure G1: HPLC chromatogram of a placebo sample.

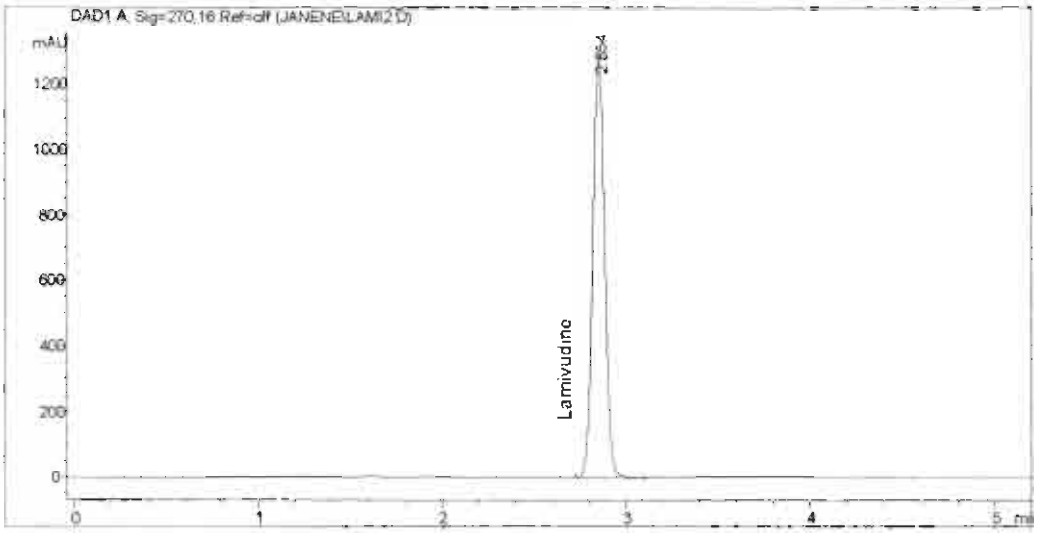


Figure G2: HPLC chromatogram of a standard solution of lamivudine.

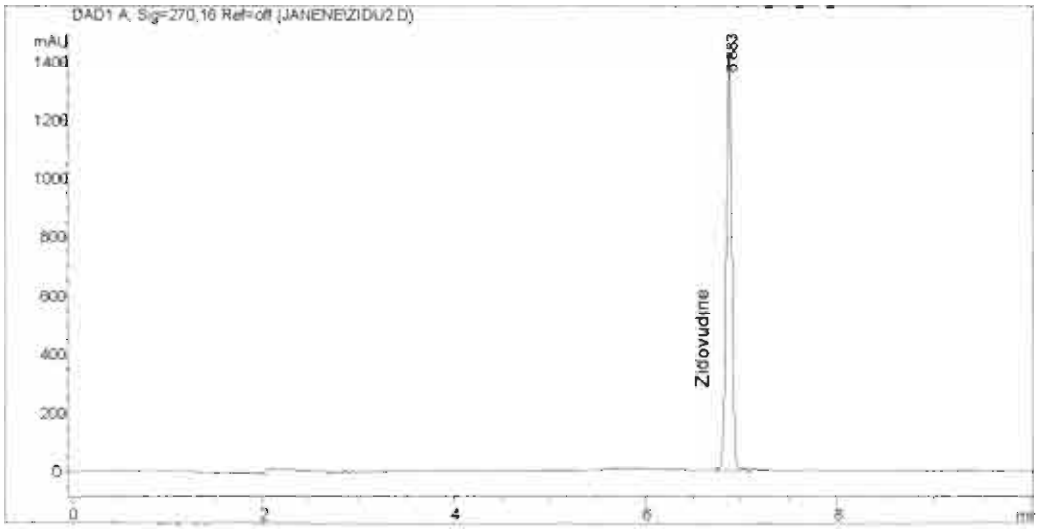


Figure G3: HPLC chromatogram of a standard solution of zidovudine.

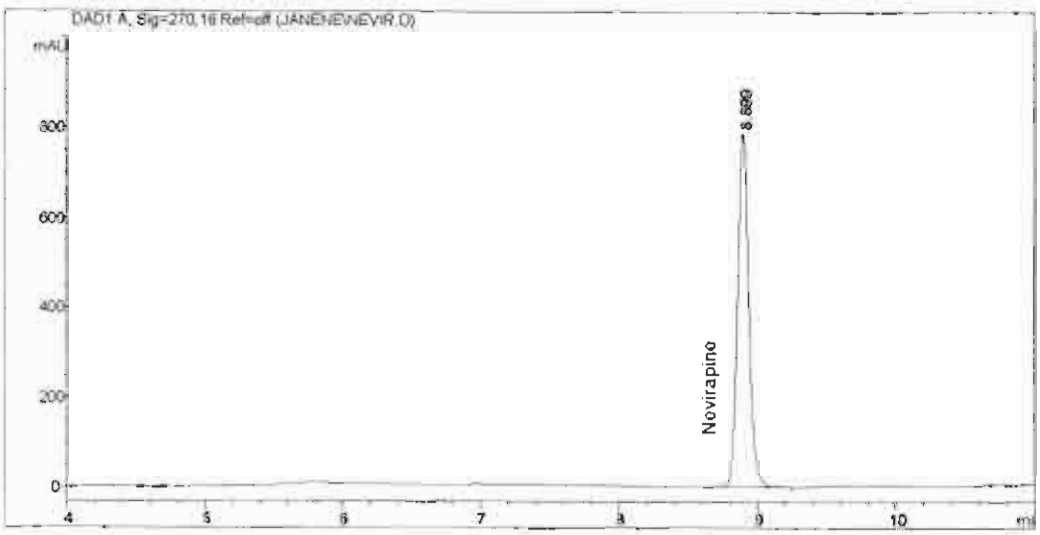


Figure G4: HPLC chromatogram of a standard solution of nevirapine.

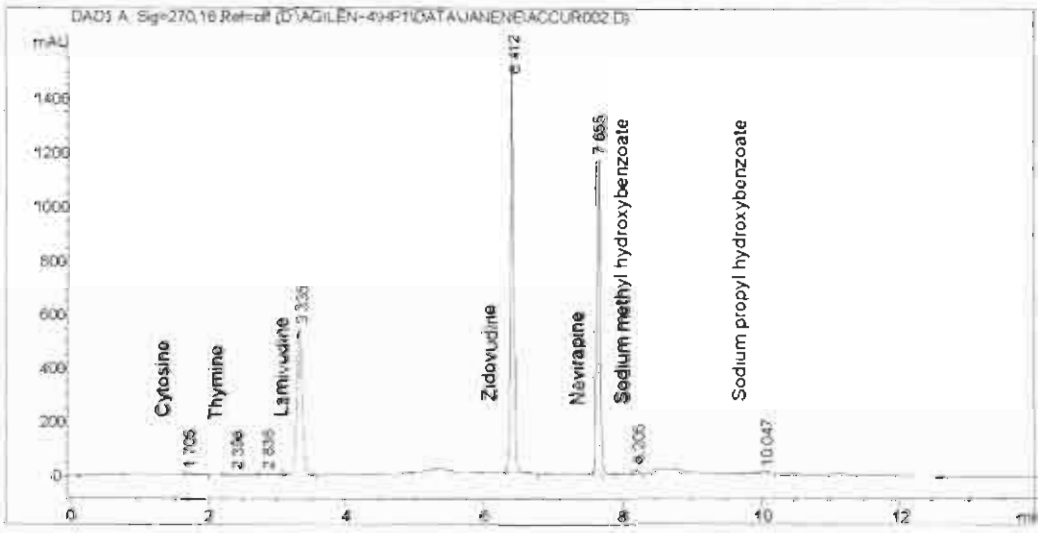


Figure G5: HPLC chromatogram of a standard solution of the reconstituted ARV powder for suspension.

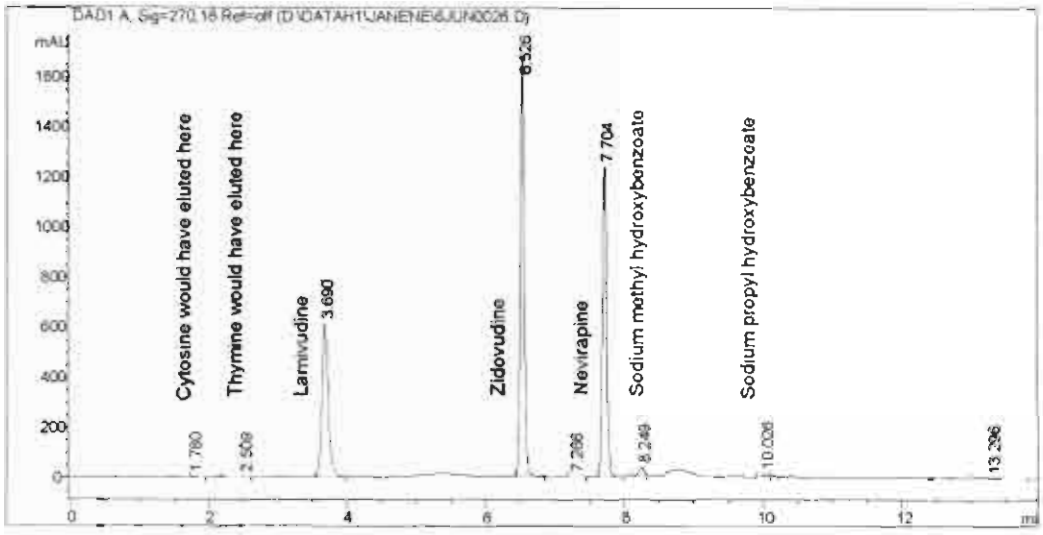


Figure G6: HPLC chromatogram of the 300 ml ARV formulation.

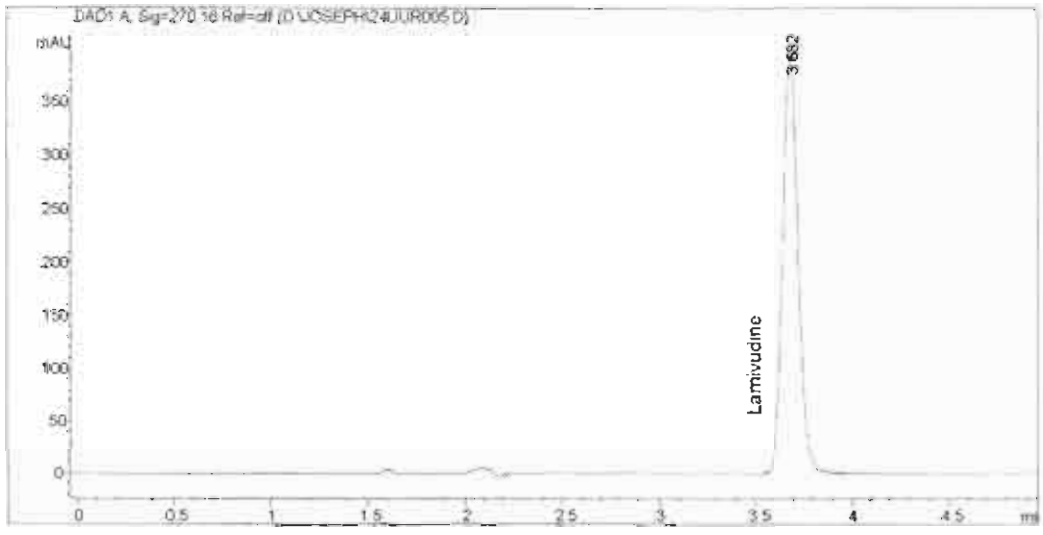


Figure G7: HPLC chromatogram of lamivudine stressed in water at 60°C for 24 hours.

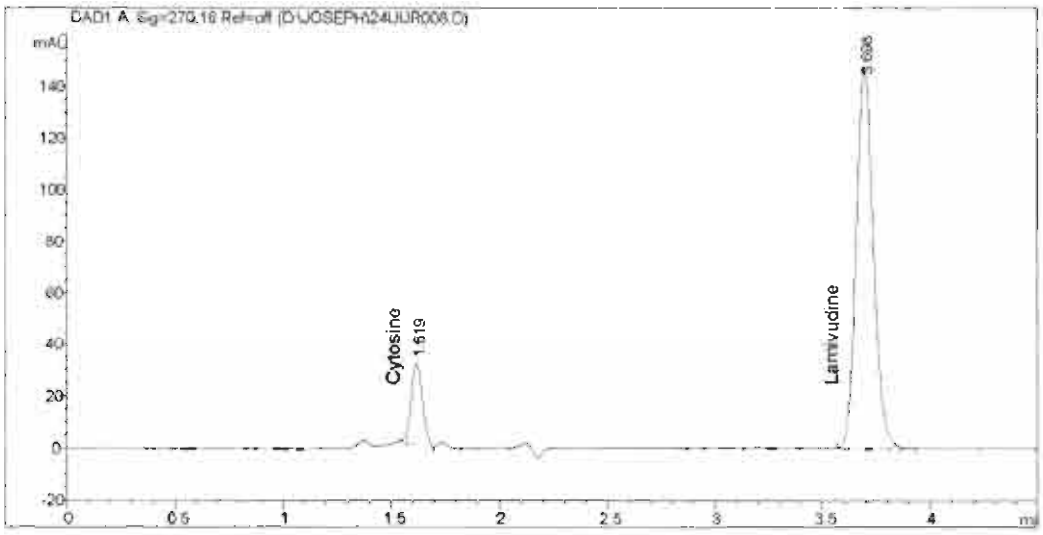


Figure G8: HPLC chromatogram of lamivudine stressed in 1 M sodium hydroxide at 60°C for 24 hours.

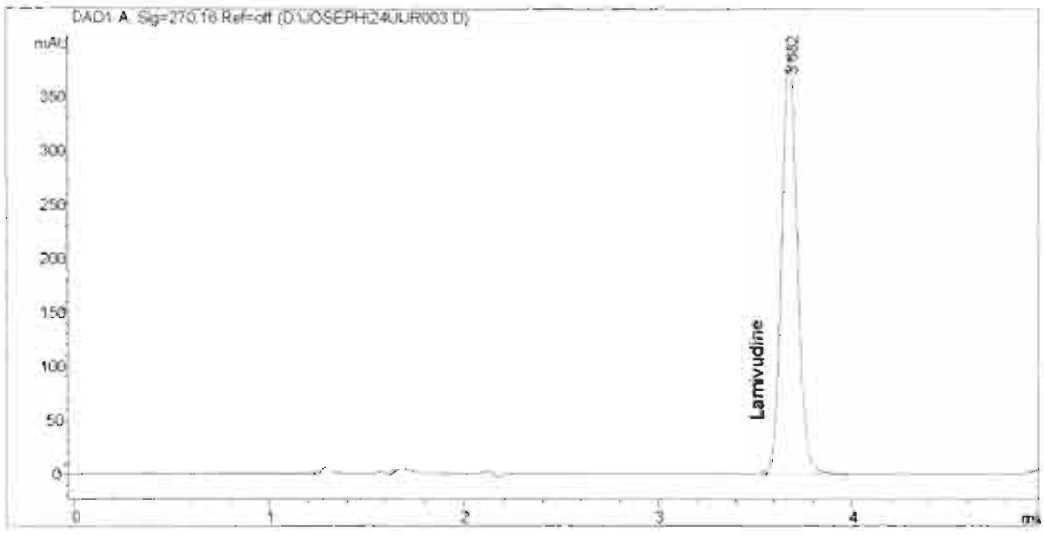


Figure G9: HPLC chromatogram of lamivudine stressed in 1 M hydrochloric acid at 60°C for 24 hours.

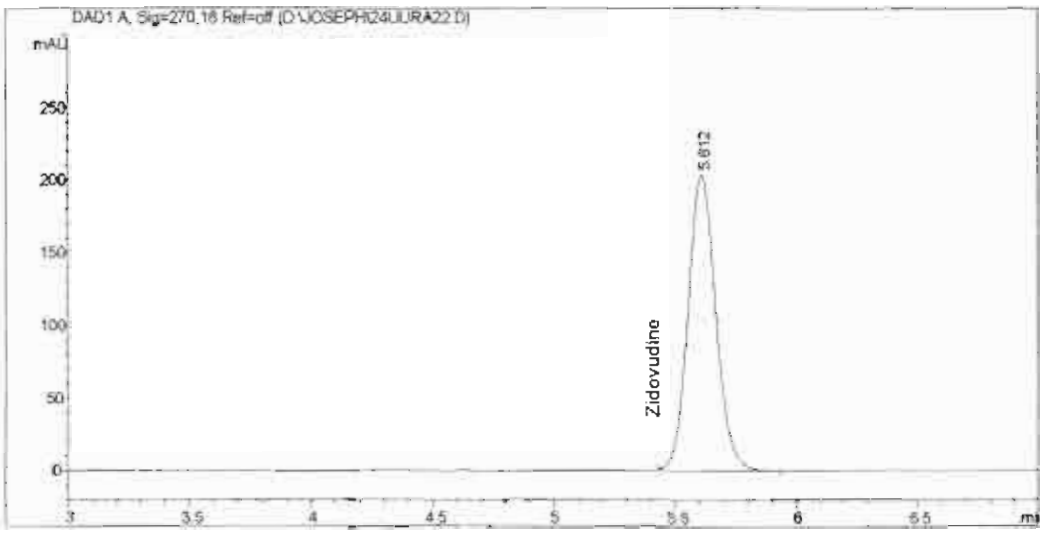


Figure G10: HPLC chromatogram of zidovudine stressed in water at 60°C for 24 hours.

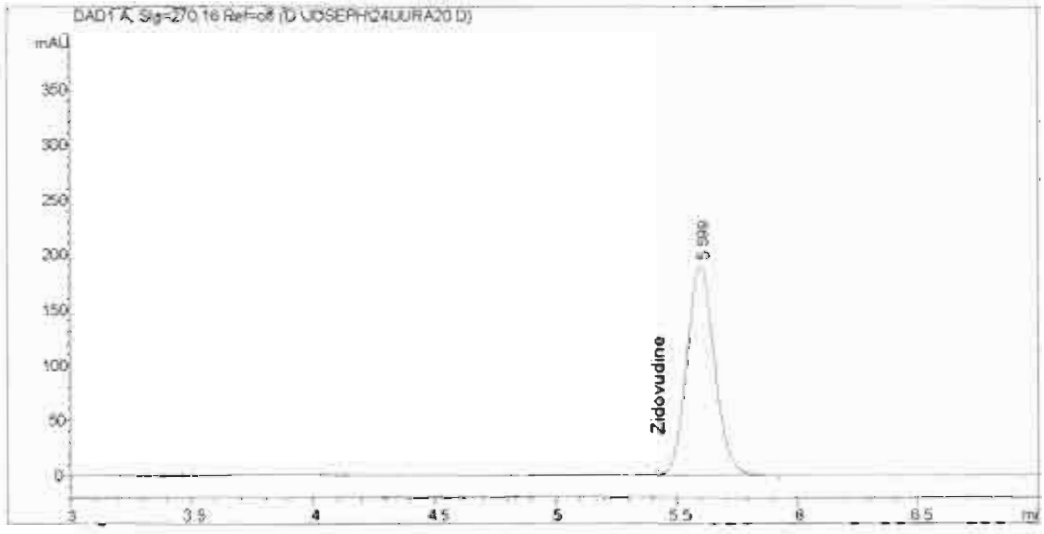


Figure G11: HPLC chromatogram of zidovudine stressed in 0.1 M sodium hydroxide at 60°C for 24 hours.

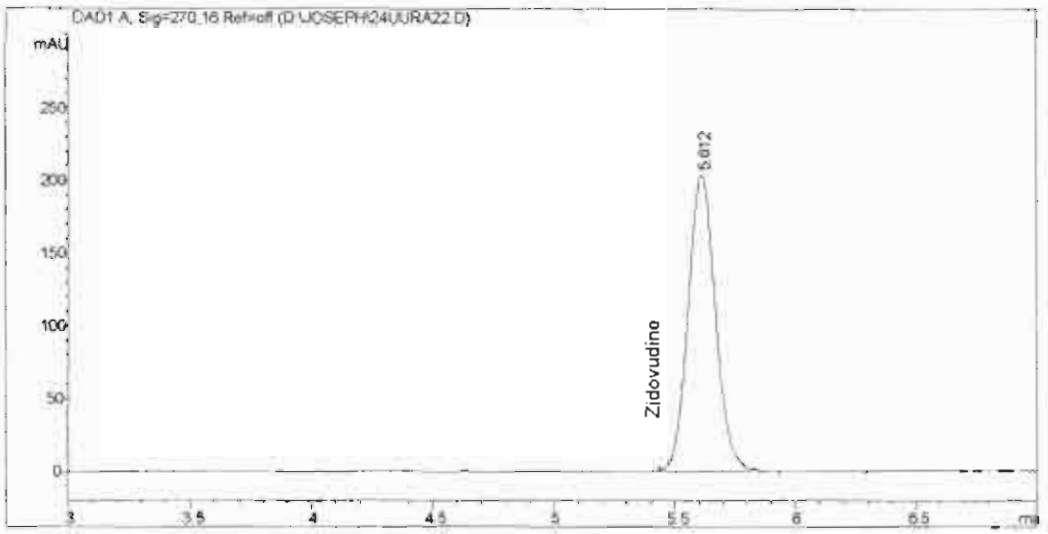


Figure G12: HPLC chromatogram of zidovudine stressed in 0.1 M hydrochloric acid at 60°C for 24 hours.

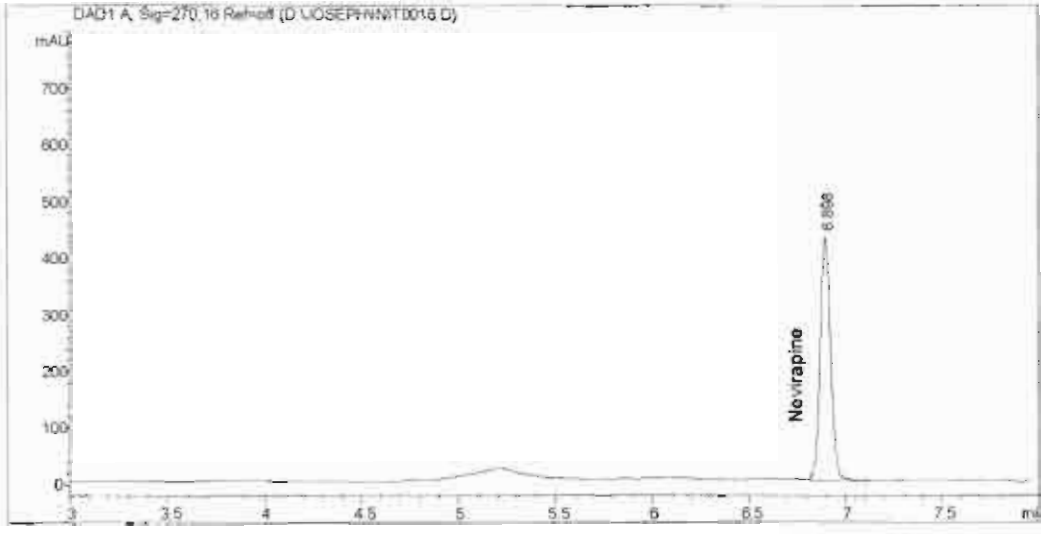


Figure G13: HPLC chromatogram of nevirapine stressed in water at 60°C for 24 hours.

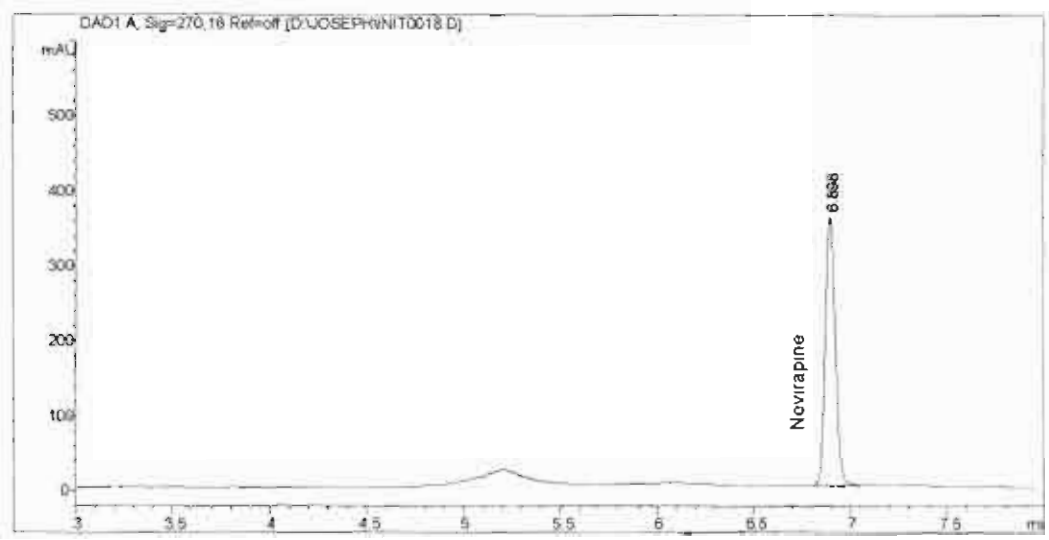


Figure G14: HPLC chromatogram of nevirapine stressed in 0.1 M sodium hydroxide at 60°C for 24 hours.

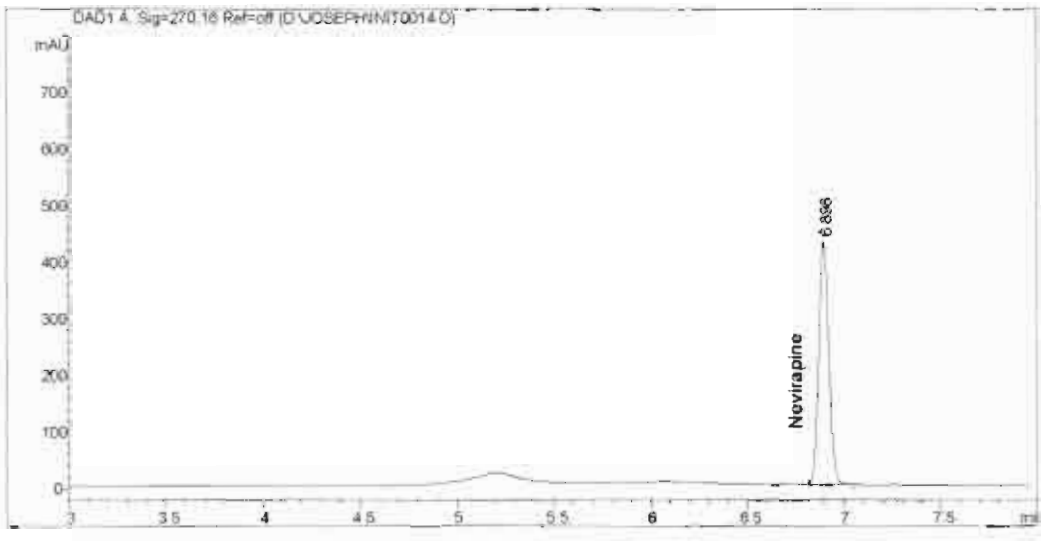


Figure G15: HPLC chromatogram of nevirapine stressed in 0.1 M hydrochloric acid at 60°C for 24 hours.

7.1.1 CONCLUSION

None of the ingredients in the placebo interfered with the lamivudine, zidovudine or nevirapine peaks. Although the degradation process formed extra peaks, it did not interfere with the simultaneous detection of lamivudine, zidovudine, nevirapine, sodium propyl hydroxybenzoate and sodium methyl hydroxybenzoate. Peak purity was preformed and all peaks were found to be satisfactory.

7.2 LINEARITY AND RANGE

Wider ranges of the three APIs and the two preservatives were determined because the specific method was used to determine the assay, as well as the dissolution values of the ARV reconstituted powder for suspension.

7.2.1 LAMIVUDINE

Table G2: **Peak area and concentration for lamivudine.**

Concentration	Peak area		
µg/ml	Area 1	Area 2	Mean
0.9	23	24	23
1.7	47	47	47
3.5	93	94	94
5.2	137	140	139
7.0	187	187	187
8.7	234	234	234
3.5	79	78	78
7.0	155	155	155
13.9	310	310	310
20.9	466	466	466
27.9	622	662	642
34.8	779	779	779
34.8	787	777	782
69.6	1555	1552	1554
139.3	3111	3099	3105
208.9	4670	4655	4663
278.6	6182	6153	6168
348.2	7557	7786	7672

Table G 3: Regression parameters for lamivudine.

REGRESSION PARAMETERS			
R squared	0.999114	Lower 95%	Upper 95%
Intercept	-4.37997	-47.2294	38.46948
Slope	22.13184	21.78249	22.481192

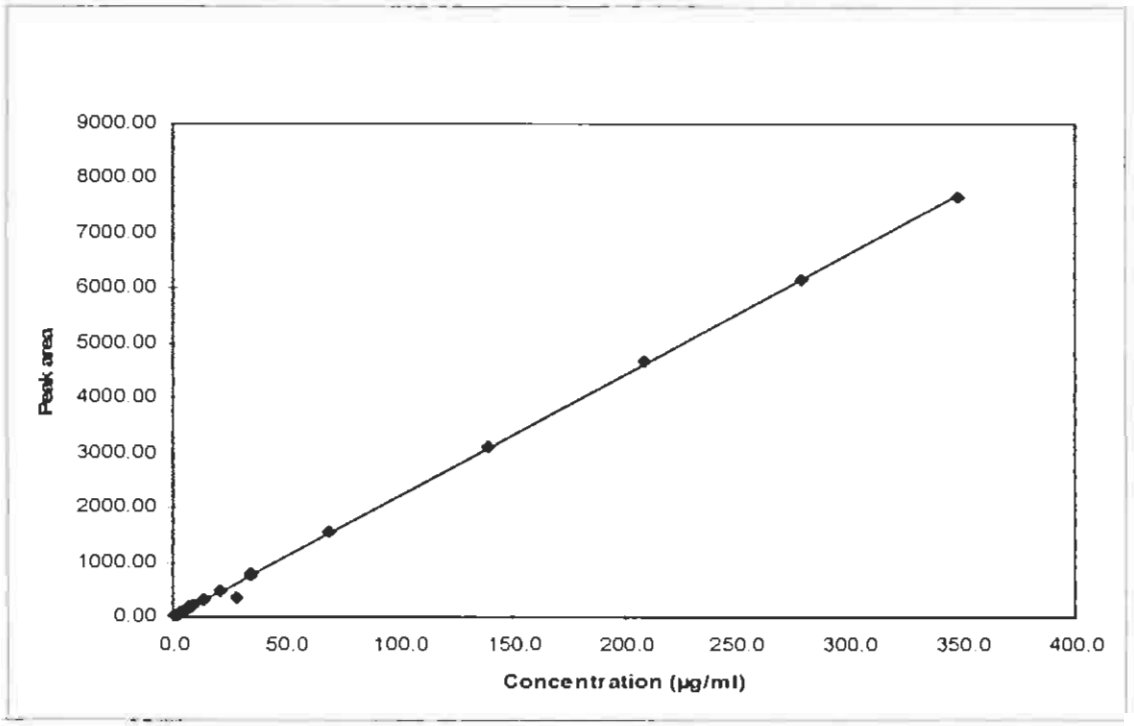


Figure G16: The linear regression curve for lamivudine.

7.2.2 ZIDOVUDINE

Table G4: Peak area and concentration for zidovudine.

Concentration	Peak area		
µg/ml	Area 1	Area 2	Mean
0.9	19	21	20
1.7	40	39	40
3.5	81	82	81
5.3	120	121	120
7.0	162	156	159
8.5	199	199	199
3.5	66	65	65
7.0	130	131	131
14.0	264	262	263
21.0	395	393	394
28.0	523	536	530
35.0	667	672	670
35.0	676	679	677
70.0	1352	1359	1355
139.9	2684	2688	2686
209.9	3987	3985	3986
279.8	5211	5207	5209
349.8	6235	6366	6301

Table G 5: Regression parameters for zidovudiue.

REGRESSION PARAMETERS			
R squared	0.999241	Lower 95%	Upper 95%
Intercept	28.50334	-4.51478	61.521452
Slope	18.34325	18.07529	18.61122

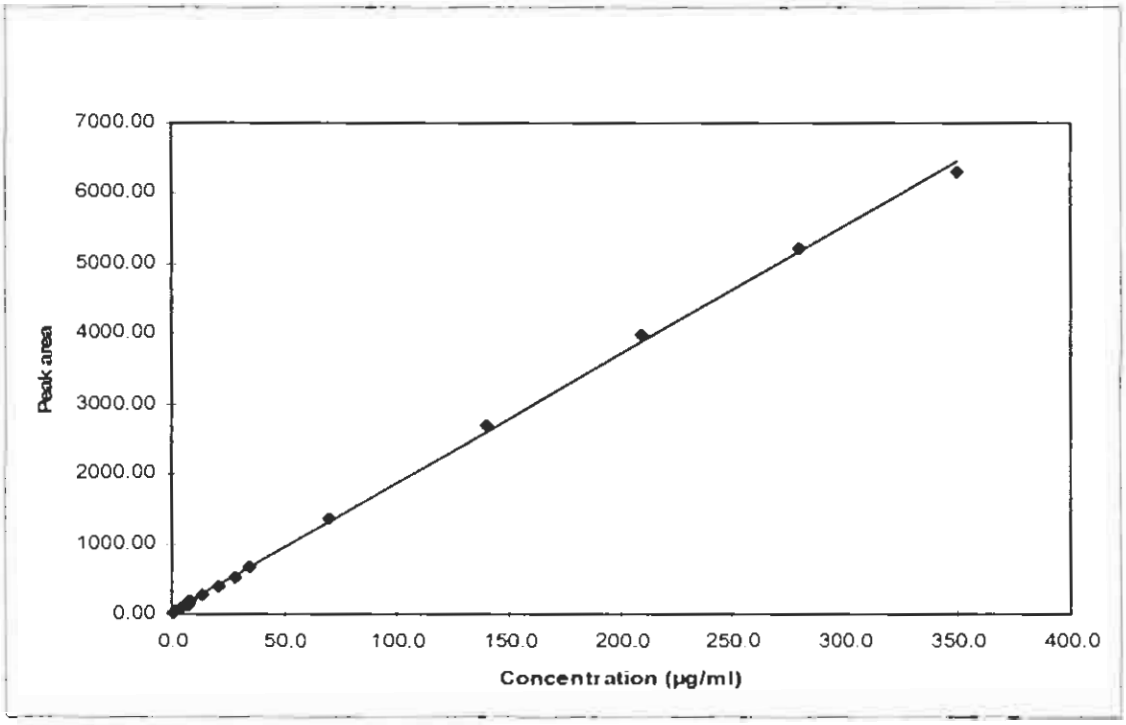


Figure G17: The linear regression curve for zidovudine.

7.2.3 NEVIRAPINE

Table G 6: Peak area and concentration for nevirapine.

Concentration	Peak area		
µg/ml	Area 1	Area 2	Mean
0.9	15	16	16
1.8	31	30	30
3.5	61	61	61
5.3	91	92	91
7.0	122	122	122
8.8	153	152	152
3.5	52	51	52
7.0	101	102	102
14.0	205	205	205
21.0	307	307	307
28.0	412	412	412
35.1	516	516	516
35.1	522	526	524
70.1	1035	1042	1038
140.3	2072	2072	2072
210.4	3104	3101	3103
280.5	4113	4099	4106
350.6	5059	5162	5110

Table G7: Regression parameters for nevirapine.

REGRESSION PARAMETRS			
R squared	0.999953	Lower 95%	Upper 95%
Intercept	8.955198	2.424111	15.486286
Slope	14.60493	14.55205	14.657814

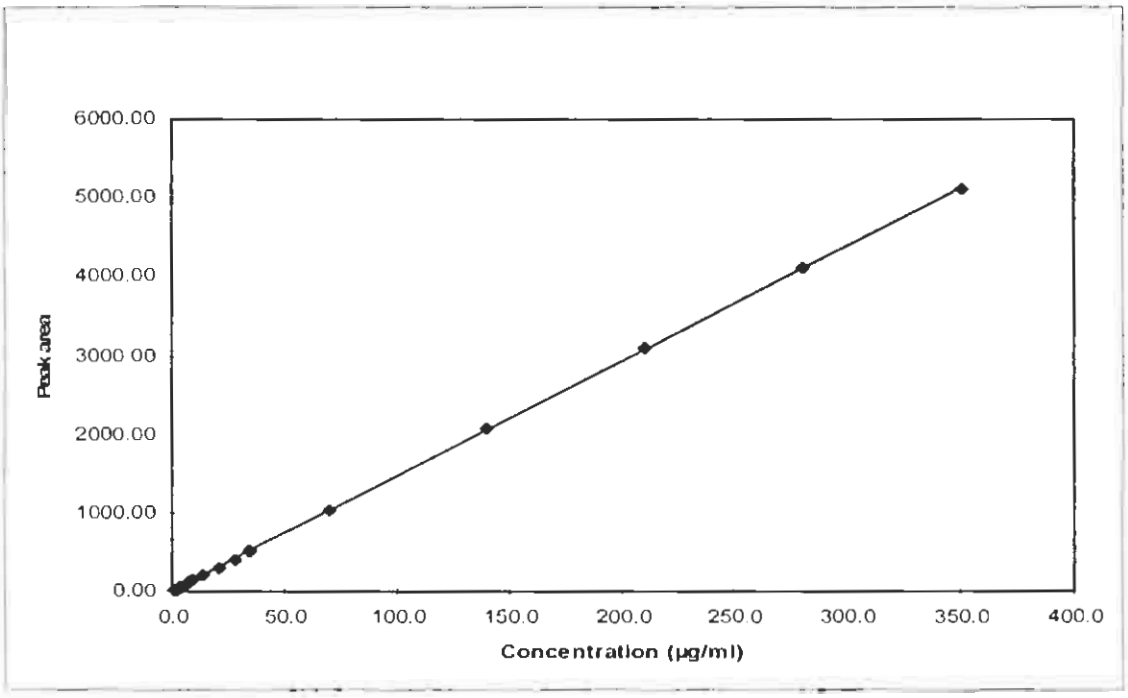


Figure G18: The linear regression curve for nevirapine.

7.2.4 SODIUM METHYL HYDROXYBENZOATE

Table G8: Peak area and concentration for sodium methyl hydroxybenzoate.

Concentration	Peak area		
µg/ml	Area 1	Area 2	Mean
0.05	16	17	16
0.10	36	33	34
0.21	62	60	61
0.31	92	90	91
0.41	121	124	122
0.52	174	149	161
2.06	53	52	52
4.13	102	103	103
8.25	205	206	206
12.38	303	309	306
16.50	412	411	412
20.63	510	512	511
20.63	517	527	522
41.26	1049	1045	1047
82.52	2090	2092	2091
123.78	3122	3129	3125
165.04	4142	4113	4128
206.30	5048	5114	5081

Table G9: Regression parameters for sodium methyl hydroxybenzoate.

REGRESSION PARAMETERS			
R squared	0.999197	Lower 95%	Upper 95%
Intercept	39.718223	12.853399	66.583047
Slope	24.614284	24.244488	24.984081

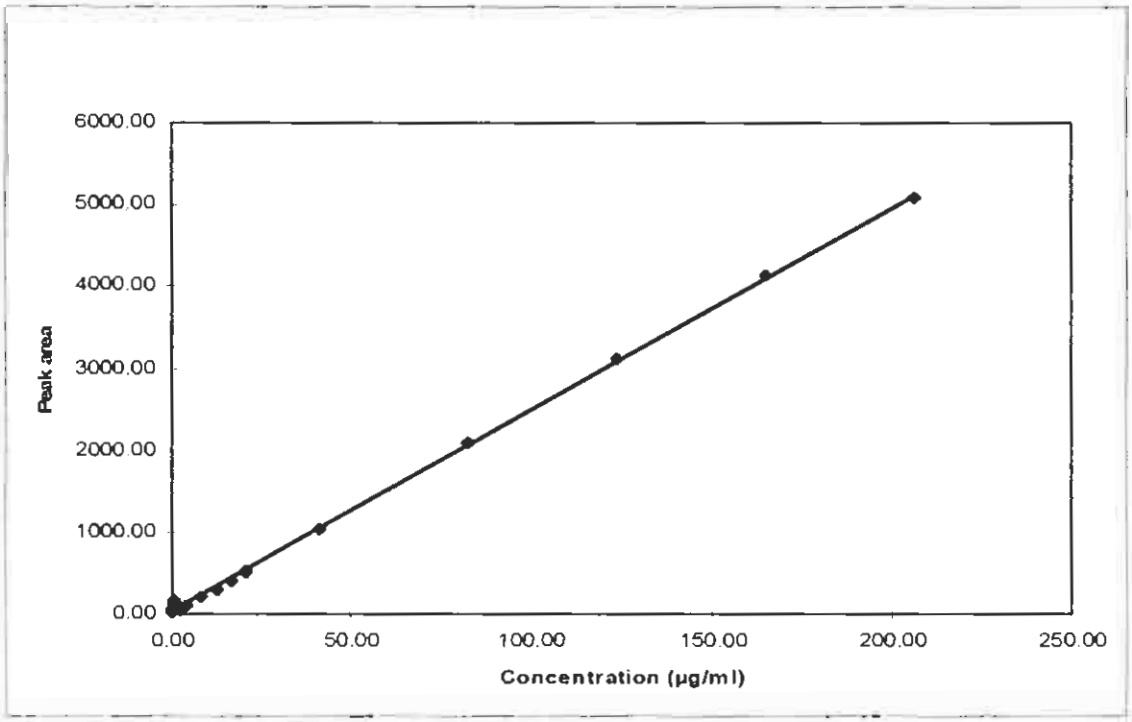


Figure G19: The linear regression curve for sodium methyl hydroxybenzoate.

7.2.5 SODIUM PROPYL HYDROXYBENZOATE

Table G10: Peak area and concentration for sodium propyl hydroxybenzoate.

Concentration	Peak area		
µg/ml	Area 1	Area 2	Mean
0.1	7	7	7
0.3	9	10	10
0.5	16	16	16
0.8	21	20	21
1.0	22	26	24
1.3	31	29	30
0.5	15	13	14
1.0	23	23	23
2.0	40	40	40
3.0	61	61	61
4.0	76	80	78
5.0	100	100	100
5.0	101	103	102
10.1	200	203	201
20.1	410	408	409
30.2	614	616	615
40.2	821	821	821
50.3	1026	1026	1026

Table G11: Regression parameters for sodium propyl hydroxybenzoate.

REGRESSION PARAMETERS			
R squared	0.999907	Lower 95%	Upper 95%
Intercept	1.807741	-0.033412	3.6488947
Slope	20.32149	20.217579	20.425404

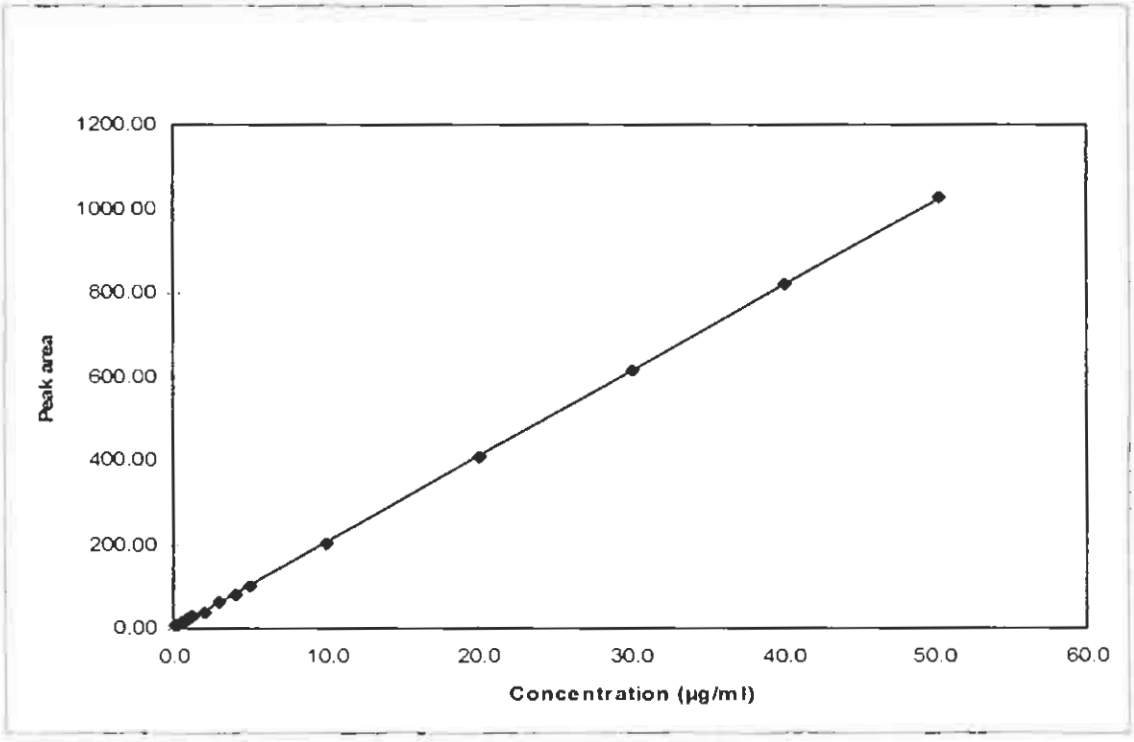


Figure G20: The linear regression curve for sodium propyl hydroxybenzoate.

7.2.6 CONCLUSION

This method is linear over the concentration range of 0.9-348.8 µg/ml for lamivudine, 0.9-349.8 µg/ml for zidovudine, 0.9-350.6 µg/ml for nevirapine, 0.1-50.3 µg/ml for sodium propyl hydroxybenzoate and 0.05-206.3 µg/ml for sodium methyl hydroxybenzoate. This method meets the criteria for acceptance and is therefore suitable for single point calibration.

7.2.7 CYTOSINE

Table G12: Peak area and concentration for cytosine.

Concentration µg/ml	Peak area		
	Area 1	Area 2	Mean
0.1	10.14	10.60	10.37
0.2	16.23	16.24	20.82
0.4	21.47	20.17	26.53
0.8	26.79	26.26	50.16
1.6	50.53	49.78	96.73
3.3	97.70	95.76	185.48
6.5	184.94	186.01	371.88
13.1	371.99	371.76	371.87
26.1	728.45	731.33	729.89
52.2	1533.62	1536.56	1535.09
104.5	3024.10	3006.57	3015.34
208.9	5891.49	5865.59	5878.54

Table G13: Regression parameters for cytosine.

REGRESSION PARAMETERS			
R squared	0.999031	Lower 95%	Upper 95%
Intercept	49.63720418	6.842477126	92.43193123
Slope	28.00677254	27.39219495	28.62135013

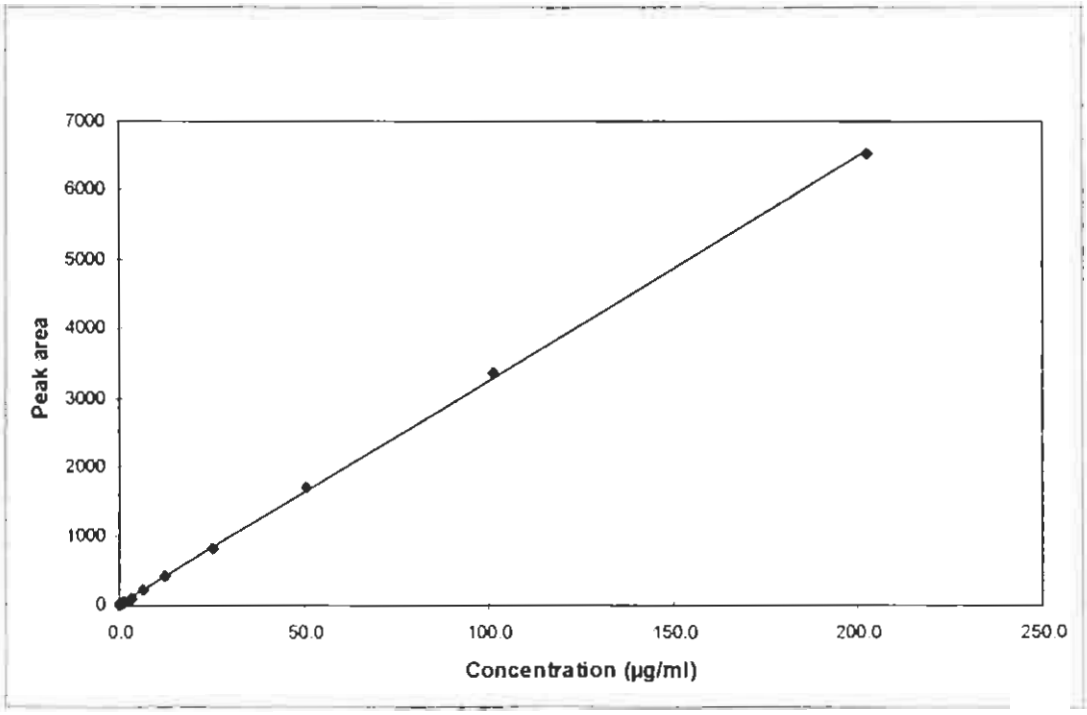


Figure G22: The linear regression curve for thymine.

7.2.10 CONCLUSION

This method is linear over the concentration range 0.1 – 202.7 µg/ ml. According to the USP 28, 2005 p. 2048, a preparation should not contain more than the equivalent of 3.0% m/m, with respect to zidovudine, of thymine. The limit of quantification and the limit of detection of this method were based on baseline noise. The limit of quantification of this method is 0.1 µg/ ml (0.1%) of thymine, and the limit of detection is 0.03 µg/ ml. The method is suitable for single point calibration.

7.2.9 THYMINE

Table G14: Peak area and concentration for thymine.

Concentration µg/ml	Peak area		
	Area 1	Area 2	Mean
0.1	6	6	6
0.2	16	15	16
0.3	22	20	21
0.7	27	26	26
1.5	52	51	52
3.1	106	103	104
6.3	211	210	210
12.6	417	414	416
25.3	823	828	825
50.6	1708	1702	1705
101.3	3356	3356	3356
202.7	6522	6524	6523

Table G15: Regression parameters for thymine.

REGRESSION PARAMETERS			
R squared	0.999766	Lower 95%	Upper 95%
Intercept	14.67263522	-8.883529426	38.22879986
Slope	32.32889817	31.9801949	32.67760144

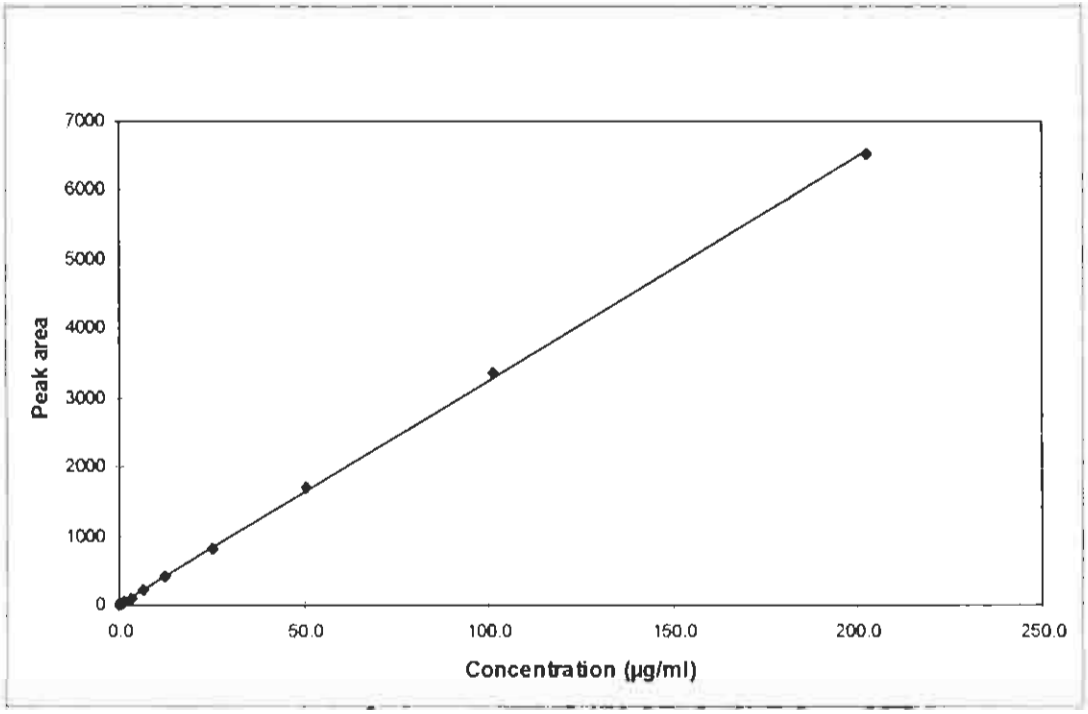


Figure G22: The linear regression curve for thymine.

7.2.10 CONCLUSION

This method is linear over the concentration range 0.1 – 202.7 µg/ ml. According to the USP 28, 2005 p. 2048, a preparation should not contain more than the equivalent of 3.0% m/m, with respect to zidovudine, of thymine. The limit of quantification and the limit of detection of this method were based on baseline noise. The limit of quantification of this method is 0.1 µg/ ml (0.1%) of thymine, and the limit of detection is 0.03 µg/ ml. The method is suitable for single point calibration.

7.3 ACCURACY

7.3.1 LAMIVUDINE

Table G16: Percentages lamivudine recovered.

Conc. spiked µg/ml	Area 1	Area 2	Mean	Recovery µg/ml	Recovered %
90.7	2032	2038	2035	92	102
90.7	2028	2031	2030	92	101
90.7	2037	2043	2040	92	102
113.4	2540	2543	2542	115	101
113.4	2540	2542	2541	115	101
113.4	2542	2537	2539	115	101
141.8	3194	3190	3192	144	102
141.8	3185	3184	3184	144	102
141.8	3187	3191	3189	144	102

Table G17: Confidence intervals for lamivudine.

Statistical analysis	
Mean %	101.5
SD	1.6
% RSD	1.6
95% confidence intervals	
Lower limit	97.2
Upper limit	101.8
Estimated median	101.4
Confidence	0.6

7.3.2 ZIDOVUDINE

Table G18: Percentages zidovudine recovered.

Conc. spiked µg/ml	Area 1	Area 2	Mean	Recovery µg/ml	Recovery %
189.0	3567	3564	3566	193	102
189.0	3548	3437	3493	189	100
189.0	3551	3542	3547	192	101
238.0	4490	4488	4489	243	102
238.0	4485	4480	4483	243	102
238.0	4488	4484	4486	243	102
298.0	5594	5596	5595	303	102
298.0	5578	5574	5576	302	101
298.0	5578	5587	5583	303	102

Table G19: Confidence intervals for zidovudine.

Statistical analysis	
Mean %	101.6
SD	0.68
% RSD	0.67
95% confidence intervals	
Lower limit	99.3
Upper limit	100.5
Estimated median	99.7
Confidence	0.5

7.3.3 NEVIRAPINE

Table G20: Percentages nevirapine recovered.

Conc. spiked µg/ml	Area 1	Area 2	Mean	Recovery µg/ml	Recovery %
187.0	2766	2754	2760	188	101
187.0	2759	2748	2754	188	100
187.0	2742	2739	2741	187	100
236.0	3459	3456	3457	236	100
236.0	3410	3407	3409	233	99
236.0	3447	3445	3446	235	100
295.0	4356	4349	4352	297	101
295.0	4332	4334	4333	296	100
295.0	4313	4318	4315	295	100

Table G21: Confidence intervals for nevirapine.

Statistical analysis	
Mean %	100.1
SD	0.81
% RSD	0.84
95% confidence intervals	
Lower limit	99.3
Upper limit	100.5
Estimated median	99.0
Confidence	0.5

7.3.4 SODIUM PROPYL HYDROXYBENZOATE

Table G22: Percentages sodium propyl hydroxybenzoate recovered.

Conc. spiked µg/ml	Area 2	Area 2	Mean	Recovery µg/ml	Recovery %
38.6	791	798	795	39	101
38.6	784	781	783	38	100
38.6	779	784	782	38	99
49.0	998	993	996	49	100
49.0	989	994	992	49	101
49.0	995	992	994	49	100
57.9	1206	1198	1202	59	102
57.9	1194	1139	1167	57	99
57.9	1216	1190	1203	59	102

Table G23: Confidence intervals for sodium propyl hydroxybenzoate.

Statistical analysis	
Mean	100.4
SD	1.0
% RSD	1.0
95% confidence intervals	
Lower limit	100.4
Upper limit	102.1
Estimated median	101.0
Confidence Level (95.0%)	0.6

7.3.5 SODIUM METHYL HYDROXYBENZOATE

Table G24: Percentages sodium methyl hydroxybenzoate recovered.

Conc. spiked µg/ml	Area 1	Area 2	Mean	Recovery µg/ml	Recovery %
50.39	1291	1298	1295	51	101
50.39	1302	1294	1298	51	101
50.39	1286	1280	1283	51	100
64.41	1649	1641	1645	65	101
64.41	1629	1622	1626	64	100
64.41	1601	1611	1606	64	99
73.48	1864	1872	1868	74	101
73.48	1842	1840	1841	73	100
73.48	1854	1859	1857	74	100

Table G25: Confidence intervals for sodium methyl hydroxybenzoate.

Statistical analysis	
Mean	100.3
SD	0.8
% RSD	0.7
95% confidence intervals	
Lower limit	100.7
Upper limit	101.8
Estimated median	100.2
Confidence Level (95.0%)	0.7

7.3.6 CONCLUSION

The method yielded a mean recovery of 101.5% for lamivudine, 105.4% for zidovudine, 103.5% for nevirapine, 103.8% for sodium propyl hydroxybenzoate and 103.1% for sodium methyl hydroxybenzoate.

7.4 PRECISION

7.4.1 INTRA DAY PRECISION

7.4.1.1 LAMIVUDINE

Table G26: Intermediate (intra-day) precision results for lamivudine.

Mass (mg)	Area 1	Area 2	Mean	Concentration µg/ml	Recovered %
4.890	3672	3705	3688	53.4	102.4
4.823	3819	3830	3824	55.3	107.7
5.412	4181	4177	4179	60.5	104.8
5.815	4490	4488	4489	65.0	104.8
5.828	4490	4476	4483	64.9	104.4
5.855	4519	4511	4515	65.3	104.7
6.443	5031	5024	5028	72.8	106.0
6.446	5045	5032	5039	72.9	106.1
6.607	5103	5100	5102	73.8	104.8

Mean	64.87	105.09
SD	7.07	1.35
RSD %	10.90	1.29

7.4.1.2 ZIDOVUDINE

Table G27: Intermediate (intra-day) precision results for zidovudine

Mass (mg)	Area 1	Area 2	Mean	Concentration µg/ml	Recovered %
9.451	7213	7296	7255	105.0	104.2
9.647	7367	7381	7374	106.7	103.8
10.613	8229	8365	8297	120.1	106.2
11.464	8954	8993	8974	129.9	106.3
11.691	8982	8951	8967	129.8	104.1
11.903	9012	8995	9004	130.3	102.7
12.789	10039	10078	10059	145.6	106.8
12.968	10086	10049	10068	145.7	105.4
13.121	10231	10257	10244	148.2	106.0

Mean	129.02	105.06
SD	15.20	1.31
RSD %	11.78	1.25

7.4.1.3 NEVIRAPINE

Table G28: Intermediate (intra-day) precision results for nevirapine.

Mass (mg)	Area 1	Area 2	Mean	Concentration µg/ml	Recovered %
9.813	7456	7591	7524	108.9	104.1
9.742	7342	7295	7319	105.9	102.0
11.106	8349	8406	8378	121.2	102.4
11.598	8974	8874	8924	129.1	104.5
11.924	8893	8758	8826	127.7	100.5
11.651	8991	8769	8880	128.5	103.5
12.639	9331	9416	9374	135.6	100.7
12.917	9858	9873	9866	142.8	103.7
13.486	10462	10381	10422	150.8	104.9

Mean	127.84	102.93
SD	13.74	1.52
RSD %	10.74	1.47

7.4.1.4 SODIUM PROPYL HYDROXYBENZOATE

Table G29: Intermediate (intra-day) precision results for sodium propyl hydroxybenzoate.

Mass (mg)	Area 1	Area 2	Mean	Concentration µg/ml	Recovery %
0.122	89	91	90	1.3	100.4
0.121	91	89	90	1.3	100.6
0.138	105	108	107	1.5	104.8
0.140	109	106	108	1.6	104.6
0.145	107	108	108	1.6	100.8
0.146	113	117	115	1.7	107.2
0.157	124	121	122	1.8	105.9
0.161	126	132	129	1.9	108.3
0.168	130	135	133	1.9	107.1

Mean	1.61	104.42
SD	0.21	1.61
RSD %	12.91	1.59

7.4.1.5 SODIUM METHYL HYDROXYBENZOATE

Table G30: Intermediate (intra-day) precision results for sodium methyl hydroxybenzoate.

Mass (mg)	Area 1	Area 2	Mean	Concentration µg/ml	Recovery %
0.920	738	735	737	10.7	108.7
0.913	738	733	736	10.6	109.4
1.040	826	825	826	11.9	107.8
1.089	877	871	874	12.6	109.0
1.118	891	871	881	12.7	107.0
1.092	867	864	866	12.5	107.6
1.185	960	955	957	13.9	109.7
1.211	967	972	970	14.0	108.7
1.265	1002	1008	1005	14.5	107.9

Mean	12.62	108.42
SD	1.31	0.84
RSD %	10.38	0.78

7.4.1.6 CONCLUSION

Precision was satisfactory with a RSD of 1.29% for lamivudine, 1.25% for zidovudine, 1.47% for nevirapine, 1.59% for sodium propyl hydroxybenzoate and 0.78% for sodium methyl hydroxybenzoate.

7.4.2 INTER DAY PRECISION

7.4.2.1 LAMIVUDINE

Table G31: Inter-day precision for lamivudine

	Day 1	Day 2	Day 3	Between days
	98.6	97.2	95.0	
	97.8	96.4	95.6	
	97.7	97.1	95.3	
Mean	98.03	96.90	95.30	96.74
SD	0.40	0.36	0.24	1.12
RSD %	0.41	0.37	0.26	1.16

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Day 1	3	294.100	98.033	0.243
Day 2	3	290.700	96.900	0.190
Day 3	3	285.900	95.300	0.090

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between days	11.316	2.0	5.658	32.433	0.001
Within days	1.047	6.0	0.174		
Total	12.362	8.0			

7.4.2.2 ZIDOVUDINE

Table G32: Inter-day precision for zidovudine.

	Day 1	Day 2	Day 3	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

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Day 1	3	310.010	103.337	0.597
Day 2	3	313.442	104.481	0.450
Day 3	3	309.204	103.068	0.174

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between days	3.376	2.0	1.688	4.146	0.074
Within days	2.443	6.0	0.407		
Total	5.819	8.0			

7.4.2.3 NEVIRAPINE

Table G33: Inter-day precision for nevirapine

	Day 1	**Day 2**	**Day 3**	**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

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Day 1	3	303.602	101.201	0.489
Day 2	3	299.600	99.867	0.863
Day 3	3	296.700	98.900	0.490

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between days	8.007	2.0	4.003	6.520	0.031
Within days	3.684	6.0	0.614		

Total	11.691	8.0

7.4.2.4 SODIUM PROPYL HYDROXYBENZOATE

Table G34: Inter-day precision for sodium propyl hydroxybenzoate.

	Day 1	Day 2	Day 3	Between days
	102.6	101.5	101.8	
	103.7	102.8	103.1	
	101.1	104.6	102.4	
Mean	102.46	102.98	102.43	102.63
SD	1.05	1.26	0.53	0.25
RSD %	1.03	1.22	0.52	0.25

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Day 1	3	307.4	102.40	0.172
Day 2	3	308.9	102.96	0.129
Day 3	3	307.3	102.43	0.330

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between days	3.313	2.0	1.657	7.877	0.021
Within days	1.262	6.0	0.210		
Total	4.575	8.0			

7.4.2.5 SODIUM METHYL HYDROXYBENZOATE

Table G35: Inter-day precision for sodium methyl hydroxybenzoate.

	Day 1	Day 2	Day 3	Between days
	108.6	105.2	104.2	
	106.2	105.8	103.8	
	106.9	104.3	103.2	
Mean	107.23	105.10	103.74	105.36
SD	1.01	0.62	0.40	1.44
RSD %	0.94	0.59	0.38	1.36

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Day 1	3	321.70	107.23	0.172
Day 2	3	315.30	105.10	0.129
Day 3	3	311.21	103.73	0.330

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between days	4.201	2.0	2.319	6.814	0.027
Within days	2.087	6.0	0.426		
Total	6.812	8.0			

7.4.2.6 CONCLUSION

Precision was satisfactory with RSD's of less than 4%. The repeatability is still within acceptable limits, and the assay should perform well, even when executed by other personnel in a different laboratory.

7.5 RUGGEDNESS

7.5.1 STABILITY OF SAMPLE SOLUTIONS

7.5.1.1 LAMIVUDINE

Table G36: **Ruggedness for lamivudine.**

Time (hours)	Peak Area	%
0	3152	100.0
1	3180	100.9
2	3178	100.8
4	3186	101.1
6	3198	101.5
8	3203	101.6
10	3189	101.2
12	3155	100.1

Mean	3181	100.9
SD	17.22	0.54

RSD %	0.54	0.54

7.5.1.2 ZIDOVUDINE

Table G37: Ruggedness for zidovudine.

Time (hours)	Peak Area	%
0	5197	100.0
1	5293	101.8
2	5246	100.9
4	525	101.0
6	5227	100.6
8	5261	101.2
10	5283	101.7
12	5223	100.5

Mean	5248	101.0
SD	29.75	0.57
RSD %	0.57	0.57

7.5.1.3 NEVIRAPINE

Table G38: Ruggedness for nevirapine.

Time (hours)	Peak Area	%
0	4076	100.0
1	4075	100.0
2	4079	100.1
4	4094	100.4
6	4099	100.6
8	4087	100.3
10	4081	100.1
12	4092	100.4

Mean	4086	100.2
SD	8.32	0.20
RSD %	0.20	0.20

7.5.1.4 SODIUM PROPYL HYDROXYBENZOATE

Table G39: Ruggedness for sodium propyl hydroxybenzoate.

Time (hours)	Peak Area	%
0	150	100.0
1	150	100.0
2	150	100.1
4	150	100.1
6	150	100.0
8	150	100.0
10	150	99.80
12	150	99.72

Mean	150.82	99.96
SD	6.21	0.114
RSD %	0.114	0.114

7.5.1.5 SODIUM METHYL HYDROXYBENZOATE

Table G40: Ruggedness for sodium methyl hydroxybenzoate.

Time (hours)	Peak Area	%
0	779	100.0
1	779	100.0
2	779	100.0
4	778	99.99
6	778	99.99
8	778	99.99
10	778	99.99
12	778	99.99

Mean	778.92	100.2
SD	4.27	0.36
RSD %	0.36	0.36

7.5.1.6 CONCLUSION

Sample solutions containing lamivudine, zidovudine, nevirapine, sodium propyl hydroxybenzoate and sodium methyl hydroxybenzoate are stable for a period of 12 hours.

7.5.2 SYSTEM REPEATABILITY

7.5.2.1 LAMIVUDINE

Table G41: System repeatability for lamivudine.

Area	Retention time (minutes)
3169	3.249
3206	3.217
3171	3.220
3145	3.240
3190	3.240
3155	3.290

Mean	3173.1	3.243
SD	20.34	0.024
RSD %	0.64	0.741

7.5.2.2 CONCLUSION

The system performance was satisfactory for lamivudine with RSD values of 0.64% for peak area and 0.741% for retention time respectively.

7.5.2.3 ZIDOVUDINE

Table G42: System repeatability for zidovudine.

Area	Retention time (minutes)
5197	6.390
5293	6.400
5246	6.380
5250	6.393
5227	6.390
5261	6.400

Mean	5246.1	6.392
SD	29.45	0.007
RSD %	0.56	0.107

7.5.2.4 CONCLUSION

The system performance was satisfactory for zidovudine with RSD values of 0.56% for peak area and 0.107% for retention time respectively.

7.5.2.5 NEVIRAPINE

Table G43: System repeatability for nevirapine.

Area	Retention time (minutes)
4086	7.623
4086	7.621
4089	7.617
4082	7.624
4082	7.620
4094	7.624

Mean	4086.9	7.622
SD	4.38	0.002
RSD %	0.11	0.033

7.5.2.6 CONCLUSION

The system performance was satisfactory for nevirapine with RSD values of 0.11% for peak area and 0.03% for retention time respectively.

7.5.2.7 SODIUM PROPYL HYDROXYBENZOATE

Table G44: System repeatability for sodium propyl hydroxybenzoate.

	Area	Retention time (minutes)
	150	10.61
	150	10.62
	150	10.64
	150	10.69
	150	10.66
	150	10.65
Mean	150.39	10.62
SD	2.67	0.06
RSD %	0.14	0.06

7.5.2.8 CONCLUSION

The system performance was satisfactory for sodium propyl hydroxybenzoate with RSD values of 0.14% for peak area and 0.06% for retention time respectively.

7.5.2.9 SODIUM METHYL HYDROXYBENZOATE

Table G45: System repeatability for sodium methyl hydroxybenzoate.

	Area	Retention time (minutes)
	776	8.99
	776	9.01
	775	8.97
	776	8.99
	776	8.97
	776	9.02
Mean	776.40	8.99

SD	2.36	0.02
RSD %	0.34	0.02

7.5.2.10 CONCLUSION

The system performance was satisfactory for sodium methyl hydroxybenzoate with RSD values of 0.34% for peak area and 0.02% for retention time respectively.

7.5 ROBUSTNESS

Changes were made to the chromatographic conditions to determine the robustness of the method.

The following changes were made and found to be acceptable:

Column: Luna C18-2 column, 150 x 4.6 mm, 5 µm, 100 Å pores, 17.8 % carbon load, endcapped, Phenomenex, Torrance, CA, and Lichrospher 100-5 RP – 18ec cartridge column, 125 x 4.0 mm, 5 µm, 100 Å pores, 21.5% carbon load, endcapped, Machery – Nagel, Düren, Germany were found to be suitable.

Mobile phase: Acetonitrile/water with 0.2% triethylamine, pH adjusted to between 6.90 – 7.10 with phosphoric acid or ammonium hydroxide is still suitable.

Flow rate: 0.8 – 1.2 ml/minute is still suitable.

Wavelength: The wavelength can be altered by ± 2nm without any ill effect.

8. CHROMATOGRAPHIC PERFORMANCE PARAMETERS

REFERENCE: USP 28, 2005.

Retention time (minutes):

- Lamivudine 3.335
- Zidovudine 6.412
- Nevirapine 7.655

- Sodium methyl hydroxybenzoate 8.205
- Sodium propyl hydroxybenzoate 10.047
- Cytosine 1.705
- Thymine 2.398

Number of theoretical plates (N) plates/column (tangent method)

- Lamivudine 7775
- Zidovudine 83526
- Nevirapine 110169
- Sodium methyl hydroxybenzoate 1114585
- Sodium propyl hydroxybenzoate 25771
- Cytosine 1599
- Thymine 860

USP Tailing factor (T):

- Lamivudine 0.982
- Zidovudine 1.029
- Nevirapine 1.058
- Sodium methyl hydroxybenzoate 0.895
- Sodium propyl hydroxybenzoate 1.139
- Cytosine 2.437
- Thymine 1.013

Capacity factor (k')

- Lamivudine 0.561
- Zidovudine 2.002
- Nevirapine 2.583
- Sodium methyl hydroxybenzoate 2.841
- Sodium propyl hydroxybenzoate 3.703
- Cytosine 0.000
- Thymine 0.122

Resolution between peaks (R):

- Cytosine – Thymine 2.785
- Thymine – Lamivudine 3.919
- Lamivudine – Zidovudine 5.128
- Zidovudine – Nevirapine 25.639
- Nevirapine - Sodium methyl hydroxybenzoate 5.128
- Sodium methyl hydroxybenzoate - Sodium propyl hydroxybenzoate 10.605

9. SYSTEM SUITABILITY PARAMETERS

- Make duplicate injections of standard solution.
- Calculate the relative standard deviation of the peak areas obtained.
- Calculate the number of theoretical plates for the paraben peaks.

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 3000 theoretical plates for all the peaks.
3. The resolution between subsequent peaks must be greater than 2.

10. CONCLUSION

The method preformed well and should be suitable to analyse lamivudine, zidovudine, nevirapine, sodium propyl hydroxybenzoate and sodium methyl hydroxybenzoate simultaneously in suspension for stability testing, quality control and batch release purposes. No interference was encountered from stressed samples or known degradation products.

The method can therefore be regarded as being stability indicating.