



ISSN: 0975-766X
Research Article

Available Online through
www.ijptonline.com

**DEVELOPMENT OF FIXED DOSE COMBINATION DISPERSIBLE TABLETS
CONTAINING STAVUDINE, LAMIVUDINE AND NEVIRAPINE FOR PAEDIATRIC
APPLICATIONS**

Y Padmavathi*¹, B Madhava Reddy¹, P Amareswar²

¹G. Pulla Reddy College of Pharmacy, Mehidipatnam, Hyderabad.

²University College of Technology, Osmania University, Hyderabad.

Email: pgundlapalli@yahoo.com

Received on 25-03-2011

Accepted on 10-04-2011

Abstract

In view of the lack of suitable paediatric antiretroviral formulations in the market, a fixed dose combination (FDC) dispersible tablet containing Stavudine, Lamivudine and Nevirapine was developed to allow administration of the correct weight-related dose in paediatric HIV patients as recommended by WHO. Dispersible tablets were prepared by direct compression method and formulated using different super disintegrants, Croscarmellose Sodium, Sodium Starch Glycolate and Crospovidone at different concentration levels. Effect of superdisintegrant on dispersion time, drug content and in vitro release has been studied. FDC tablets containing croscarmellose sodium showed excellent in vitro dispersion time and drug release as compared to other formulations. Differential Scanning Calorimetry (DSC) studies exhibited physicochemical compatibility between the three drugs and various excipients used in the tablet formulation. Stability studies were carried out as per ICH guidelines.

Keywords: Superdisintegrant, Dispersible tablet, fixed dose combination, Differential Scanning Calorimetry.

Introduction

Acquired Immuno deficiency syndrome (AIDS) is one of the most destructive epidemics, world has ever witnessed. Presently an estimated 33.3 million people were living with HIV infection of which 2.3 million were children living with HIV infection; of these 1.8 million children reside in sub-saharan Africa^[1]. Majority of children

were infected with Virus by mother-to-child transmission during pregnancy or birth, or via HIV-positive breast milk [2]. It was estimated that only 23% of pregnant HIV-positive women were receiving antiretrovirals (ARV) to reduce the risk of transmitting the virus to their infants [2]. It was reported that only 26% of HIV infected children below 14 years were receiving the treatment. Without treatment, ½ of all babies born with HIV infection die before their 2nd birthday. Therefore, numerous HIV positive children are in need of antiretroviral treatment (ART), which must start as soon as possible after birth, as in the absence of treatment, the acquired HIV infection can rapidly progress to a severe symptomatic disease and death [3]. However, the majority of these children are living in resource-limited settings, in which the availability of pediatric antiretroviral formulations is limited and where mortality by 2 years of age is above 50% due to late treatment. The epidemic is worst in developing countries, especially in sub-Saharan Africa where about 290,000 children under 15, died from AIDS in 2007 [4].

The most effective treatment for HIV-positive children is antiretroviral therapy. Quality-assured, ARV drugs in fixed-dose combinations (FDCs) or blister packs are mostly used in adults and older children [5]. Pediatric FDCs represent a new era in the treatment of children with HIV. The use of antiretroviral agents in the form of Fixed Dose Combinations (FDC) and once-daily dosing in both adults and children is an important consideration as this promotes better adherence and in turn limits the emergence of drug resistance and simplifies ARV storage and distribution logistics. WHO recommended first-line regimen for infants and children is two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) as the preferred option. These drugs prevent HIV replication by inhibition of the action of reverse transcriptase, the enzyme that HIV uses to make a DNA copy of its RNA. WHO recommended preferred first-line ARV regimens for infants and children are Zidovudine (AZT)+Lamivudine (3TC)+Nevirapine (NVP), Stavudine (d4T) + Lamivudine (3TC)+Nevirapine (NVP) and Abacavir (ABC) + Lamivudine (3TC)+Nevirapine (NVP) [6].

In terms of the active pharmaceutical ingredients used to fight HIV/AIDS, children depend on the same ARVs as adults. But the formulations of these ARVs differ for children. They require smaller volumes of the active

ingredient, they require products that are easy to administer, and they require adjustments in the amount taken based on patient weight or body surface area. The available formulations of ARVs for pediatric use syrups, suspensions solutions and tablets. Liquid formulations have problems with storage, transportation, adherence. If split and use adult formulation, risk of toxicity and resistance, uneven dosing of the medicament^[7]. Patients often need a dozen bottles of suspension per month and dosing accurate quantities of multiple liquid formulations twice a day can be difficult for parents. Children liquids may contain preservatives, alcohol etc not suitable for children. Liquids are difficult to formulate and also to mask the bitter taste of actives. Moreover, since these children have to be treated life-long with multiple drugs the administration of several formulations might lead to poor therapy adherence. Tablet suit children for better adherence to ARV treatment as per WHO criteria. Easily breakable or dispersible tablets help clinician to adjust to accurate dose by weight band in children.

Until recently there are no FDC formulations for children. Many children have been treated with broken halves of adult FDCs, which cause risk of under or over dosing.

In this perspective the aim of this study was to develop a new FDC dispersible tablet of Stavudine, Lamivudine and Nevirapine for pediatric use allowing easy oral administration.

Materials and Methods

Materials

Stavudine, Lamivudine and Nevirapine (Matrix laboratories, Hyderabad), Sodium Starch glycolate, Croscarmellose Sodium and Crospovidone, Avicel PH102 (Dr.Reddys Laboratories, Hyderabad) were obtained. Magnesium Stearate, Aerosil, Sodium Dihydrogen ortho phosphate, Orthophosphoric acid, Acetonitrile HPLC grade and Methanol HPLC grade were purchased from S.d fine chemicals Ltd.

Tablet formulation

Dispersible tablets containing fixed dose combination of Stavudine, Lamivudine and Nevirapine were prepared by direct compression method using superdisintegrants. All the ingredients without magnesium stearate and Aerosol were sifted through the sieve #40 and admixed for about 15 minutes to make a uniform blend. Magnesium stearate

and aerosol were passed through sieve # 60 and mixed with the above blend for sufficient time, usually 5-7 minutes.

The prepared powder blend was evaluated for various parameters like bulk density, tapped density, Angle of repose, Compressibility index and Hausner ratio. The mixed powder blend was compressed using a ten-station Rotary punch tableting machine (Rimek, Multi press 1, ten station) using 11.9 mm flat punches set ^[8, 9, 10].

Evaluation of FDC dispersible tablets

The prepared tablets were evaluated for hardness, weight variation, friability, thickness and disintegration time. The mass uniformity was calculated according to European pharmacopoeia. Twenty tablets were selected, they were weighed individually, average weight and standard deviation were calculated ^[11]. Hardness or tablet crushing strength (F_c), the force required to break a tablet in a diametric compression, was measured using Monsanto tablet hardness tester. Friability of the tablets was determined using automated tablet Friabilator (Electrolab). Pre-weighed and dedusted sample of tablets was placed in the drum of the friabilator. After 100 times rotations they were removed from the drum and accurately weighed ^[12]. Disintegration time was determined via USP method using tablet disintegration tester (Electrolab) using 900 ml of distilled water without disk at room temperature.

Drug content

The content of three drugs in the FDC dispersible tablets was determined using a validated RP-HPLC method ^[13]. The tablets were weighed individually and dissolved in 100 ml of mobile phase in a volumetric flask. The mobile phase was composed of phosphate buffer (20 mM sodium dihydrogen phosphate containing 8mM 1-octanesulphonic acid sodium salt): acetonitrile (4:1, v/v) with pH adjusted to 3.5 using phosphoric acid. The concentration of three drugs Stavudine, Lamivudine and Nevirapine was calculated using calibration curves constructed using standard solutions containing d4T in the range of 1-10 µg/ml, 3TC in the range of 4-40 µg/ml and nevirapine in the range of 7 – 70 µg/ml. The calibration curve was constructed by plotting peak area and drug concentration. The HPLC system used (Schimadzu) consisted of pump (Lc-10AT vp) and UV detector (SPD – 10A vp). The separation of three drugs was done on RP C18 column (4.6mm × 250 mm, Phenomenix , particle size 5 µm), the volume of injection loop was 20 µl. The flow rate was 1.5 ml/min and the effluents were monitored at 265 nm. The sample solution was

suitably diluted and used for the analysis. Twenty microlitres of standard and sample solutions were injected, respectively, under the specified conditions and scans were recorded.

***In vitro* drug release:**

In vitro drug release of Stavudine, Lamivudine and Nevirapine from the prepared FDC formulations was determined using USP Dissolution testing apparatus II, Paddle type¹². (Scientific Tablet Dissolution test apparatus, DA-60). The dissolution test was performed using 900 ml of 0.1 N Hydrochloric acid at $37 \pm 0.5^{\circ}\text{C}$. The speed of rotation of paddle was set at 50 rpm (FDA, US food and drug administration, Dissolution methods). At a predetermined time interval (5 min); 5 ml samples were withdrawn, filtered through Whatmann filter paper. The samples were analysed by validated RP_HPLC method (Modified S. Anbazhagan et al, 2005) as defined above for drug content. Drug release for the three drugs was calculated from standard curves.

Drug excipient compatibility by DSC

The DSC profile of pure and physical mixtures of Stavudine, Lamivudine, Nevirapine and excipients used in the formulation were recorded on (Schimadzu, DSC-60) in order to assess the compatibility of excipient used in the formulation with respect to the drugs. The drug excipient ratios were selected on the basis of their ratios in the experimental design and thermal behaviors were studied under normal conditions with perforated and sealed aluminum pans with a nitrogen gas flow of 50 ml /min. The samples were heated at $10^{\circ}\text{C} / \text{min}$ over a temperature range of 40°C to 250°C . The reference sample used in all the determinations was alumina with a weight of 2 mg.

Stability studies:

Stability studies were carried out as per ICH Q1A stability testing guidelines^[14]. The optimized formulations were stored in aluminium capped clear glass vials and subjected to a storage condition of $40^{\circ}\text{C} \pm 2 / 75\% \text{RH} \pm 5$ for 6 months in stability chamber (Cintex humidity oven). The samples were withdrawn at time intervals of 0, 1, 2, 3, and 6 months and evaluated for percentage drug content of three drugs using High performance liquid chromatograph. (Shimadzu).

Results and Discussion

Before formulation, preformulation study was carried out by comparing FTIR spectra of pure Stavudine, Lamivudine and Nevirapine in its physical mixture with super disintegrants using Fourier Transform Infrared Spectrophotometer (FTIR-8400S Shimadzu). There was no considerable difference in their spectra. It was observed that the drugs remained intact in the presence of the superdisintegrants.

DSC experiments were carried in order to characterize the physical state of the drugs in formulation. The thermograms of pure drugs exhibit the single isothermic peaks at around 168.5⁰c Stavudine, 177.4⁰c lamivudine and 245.4⁰c for Nevirapine. In thermogram of physical mixture of drugs with excipients, the drugs peak were shifted to lower temperature with reduced intensity which may be due to baseline shift. Baseline shifts are caused by changes in sample weight or specific heat of the sample.

The prepared FDC tablets containing Stavudine, Lamivudine and Nevirapine were designed for pediatric applications. The tablets were formulated using commonly used excipients for tableting purposes (**Table 1**). The values of pre-compression parameters evaluated were within prescribed limits and indicated good free flowing property.

TableNo-1: Composition of optimized fixed dose combination tablet containing Stavudine, lamivudine and Nevirapine.

Ingredient	Amount per Tablet(mg)
Stavudine	10
Lamivudine	40
Nevirapine	70
Croscarmellose sodium	18
Avicel PH102	311.1
Magnesium Stearate	0.45
Aerosil	0.45

Ten formulations of FDC tablets were prepared by direct compression method and evaluated for weight variation, hardness, friability, wetting time, drug content and in vitro disintegration are shown in **Table 2**. In all the formulations, hardness test indicated good mechanical strength, friability is less than 1%, indicated that tablets had a good mechanical resistance. The tablets were subjected for evaluation of in vitro disintegration time. The tablets prepared with croscarmellose sodium have disintegrated in 20 secs. All other formulations prepared were showed disintegration time between the ranges of 30 – 91 sec. The drug content of all formulations was to be in the range (95% - 98%).

Table No-2: Evaluation of FDC dispersible tablets.

Formulation Code	Uniformity of Weight mg	Hardness Kg/Cm2	Friability %	Disintegration Sec
F1	450.66 ± 2.08	3.1	0.42	41.33 ± 1.15
F2	449.33 ± 1.52	3.0	0.36	31.00 ± 1.0
F3	446.66 ± 1.53	3.2	0.39	52.33 ± 2.5
F4	448.66 ± 1.15	3.1	0.50	79.66 ± 1.15
F5	449.66 ± 0.57	3.1	0.37	30.33 ± 0.57
F6	450.33 ± 0.57	3.5	0.80	90.33 ± 1.52
F7	449.66 ± 0.57	3.5	0.70	91.33 ± 1.15
F8	450.66 ± 2.08	3.0	0.38	20.33 ± 0.57
F9	448.66 ± 1.15	3.1	0.40	35.00 ± 1.0
F10	449.00 ± 1.0	3.1	0.42	39.33 ± 1.15

In vitro drug release was tested using USP paddle type apparatus. The formulations containing croscarmellose sodium as superdisintegrant showed good drug release of all the three drugs, when compared to formulations containing sodium starch glycolate and crospovidone. The in vitro drug release profiles were summarized in Fig. Showed that the drug release was fast and 92.8, 92.7 and 90.5 of Stavudine, Lamivudine and Nevirapine respectively, was released from the prepared FDC dispersible tablets after 30 minutes. According to USP pending monograph not

less than 75% of the labeled amount of Stavudine, lamivudine and Nevirapine are dissolved. The formulations showed enhanced dissolution rate as compared to pure drugs. The preparation process in direct compressible tablets includes co-grinding of all the excipients before compression, resulting in increase in the solubility due to the reduction in the effective particle size of the drug molecules.

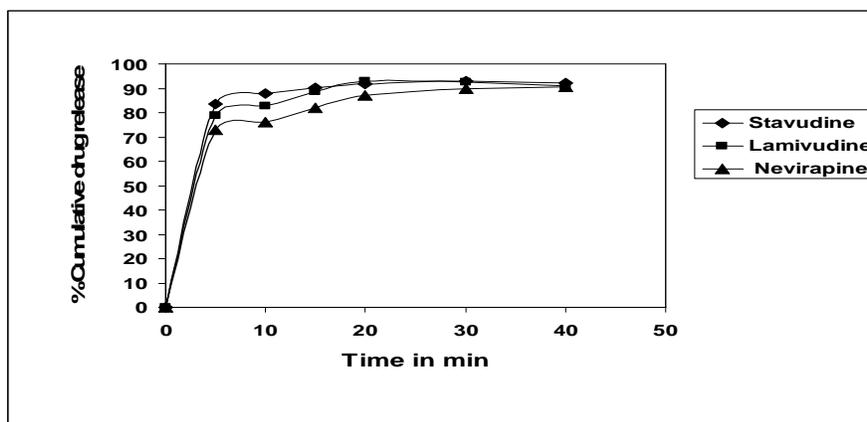


Figure – I: Dissolution profile of formulation prepared with CCS at 4% level.

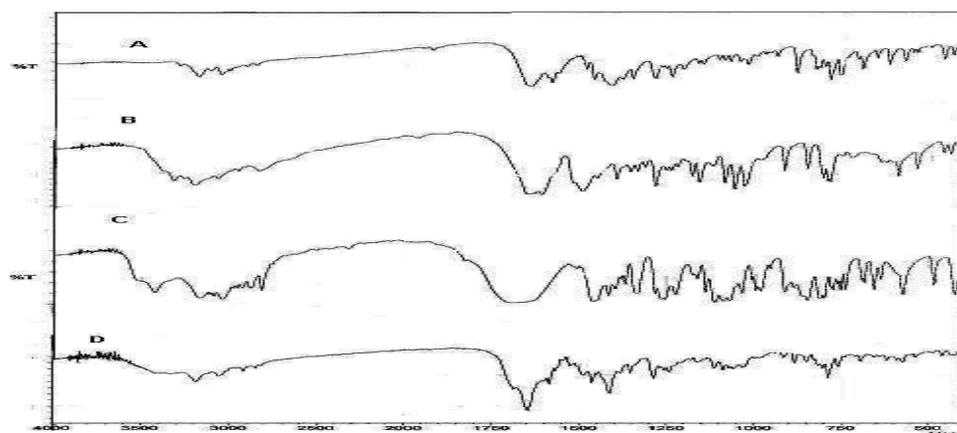


Figure – II IR spectra of Tablet mixture (A), Stavudine (B), Lamivudine (C), Nevirapine (D)

Conclusion

The prepared FDC dispersible tablets were acceptable for use in pediatric HIV patients of ages 1 -8 years. By combining three of the most commonly used first line drugs (Stavudine, Lamivudine and Nevirapine) into one tablet form, dosing for children becomes greatly simplified.

References

1. Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO) 2009. AIDS epidemic update, December 2009. Available at www.unaids.org.
2. Rouzioux, C., Costagliola, D., Burgard, M., 1995. Estimated timing of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission by use of a Markov model. The HIV Infection in Newborns French Collaborative Study Group. *Am. J. Epidemiol.* 142, 1330–1337
3. American Academy of Pediatrics, 2007. Increasing Antiretroviral Drug Access for children with HIV infection, Committee on Pediatric AIDS, Section on International Child Health. *Pediatrics* 119, 838–845.
4. Newell, M.L., Coovadia, H., Cortina Borja, M., et al., 2004. Mortality of Infected infants born to HIV-infected mothers in Africa: a pooled analysis. *Lancet* 364, 1236–1243.
5. World Health Organisation, 2006. Paediatric guideline on Antiretroviral therapy of HIV infection in infants and children in resource-limited settings: towards universal access. 25–36. Available at: www.who.int/hiv/pub/guidelines/WHOPaediatric.pdf.
6. WHO model formulary for children 2010, page no.
7. Menson, E.N., Walker, A.S., Sharland, M., Wells, C., et al., 2006. Underdosing of antiretrovirals in UK and Irish children with HIV as an example of problems in prescribing medicines in children 1997–2005: cohort study. *Brit. Med. J.* 332, 1183–1187.
8. E. Kayitarea, C. Vervaet b, J.D. Ntawukulilyayoa, B. Seminegaa, Van Bortel c, J.P.Remonb. Development of fixed dose combination tablets containing zidovudine and Lamivudine for paediatric applications. *International Journal of Pharmaceutics* 370 (2009) 41–46.
9. Milind P Wagh*, Chetan P Yewale, Santosh U Zate, Paresh I Kothawade, Ganesh H Mahale. Formulation and Evaluation of Fast Dispersible Tablets of Aceclofenac Using Different Superdisintegrant. *International Journal of Pharmacy and Pharmaceutical Sciences*. Vol 2, Suppl.

10. Rowe RC, Sheskey PJ, Weeler PJ, editors. Handbook of pharmaceutical excipients. 4th edition. London and Washington DC: The Pharmaceutical Press; 2003.
11. European Pharmacopoeia, 1997. Uniformity of mass of single-dose preparations, 3rd Edition, pp. 133–134.
12. The United States Pharmacopoeia – 24, National formulary NF-19, 2000, 2148, 1942 – 43.
13. S. Anbazhagan, N. Indumathy, P. Hanmugapandiyan, S. K. Sridhar. Simultaneous quantification of Stavudine, lamivudine and Nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets. Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 801–804.
14. ICH Q1A Stability testing of existing drug and drug products. Available at [http:// www.ichguidelines.com](http://www.ichguidelines.com).

Corresponding Author:

Y.Padmavathi*,

Associate Professor,

G.Pulla Reddy College Of Pharmacy,

Mehdipatnam, Hyderabad.