

Pre-Formulation Development of Lamivudine 300 mg and Tenofovir Disoproxil Fumarate (TDF) 300 mg Fixed Dose Combination Tablets

Prosper Tibalinda¹, Dickson Pius², Raphael Shedafa^{1,3}, Nelson Masota^{1,3}, Mary Temu², Eliangiringa Kaale^{1,3}

¹Pharm R&D Laboratory, School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania

²Department of Pharmaceutics and Pharmacy Practice, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania

³Medicinal Chemistry Department, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania

Email: ptibalinda@gmail.com

Received 12 April 2016; accepted 10 July 2016; published 13 July 2016

Copyright © 2016 by authors and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Introduction: In this study, physical and chemical characteristics of Lamivudine, Tenofovir Disoproxil Fumarate (TDF) and potential excipients were systematically followed and documented [1]. **Objective:** The objective of this scientific work was to carry out pre-formulation studies including compatibility studies on Lamivudine and Tenofovir Disoproxil Fumarate with their potential excipients prior a direct compression process [2]. **Methodology:** The interaction was studied in three set of environments namely uncontrolled room conditions for Zone VI b ($30^{\circ}\text{C} \pm 2^{\circ}\text{C}$), oven conditions in which the oven was set at 50°C and accelerated climatic conditions in which a climatic chamber was set at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \pm 5\%$ Relative Humidity (RH %). Sample preparation was done by mixing the amount of formulation excipients to active substances at a ratio of 1:10, whereas active substance to another active substance at a ratio of 1:1, active substance to coating materials at 1:4, coating materials to the whole set of excipients 1:4. The whole set of samples was geometrically mixed and triturated by mortar and pestle to very fine uniform powder to ensure homogeneity of the mixture. HPLC analytical method was used for simultaneous quantitative determination of lamivudine and tenofovir disoproxil fumarate. Transmittance of the mixture was determined by Near Infra-Red (NIR) technique. **Results:** The amount of Lamivudine as on day 0 was comparable to day 90 for in all tested conditions (Room, Oven and Climatic Chamber), whereas for Tenofovir Disoproxil Fumarate only the amount of the drug at Room ($30^{\circ}\text{C} \pm 2^{\circ}\text{C}$) was comparable to results on day 90. A significant drop of amount of Tenofovir Disoproxil Fumarate (TDF) exposed to moisture (Climatic chamber at $40^{\circ}\text{C} \pm$

2°C/75% ± 5% Relative Humidity (RH %)) and temperature of 50°C was observed. Colour change was observed for samples subjected to moisture (Climatic chamber at 40°C ± 2°C/75% ± 5% Relative Humidity (RH %)) and as well picked up in the NIR region 400 to 1500 cm⁻¹ (Finger print region) by a significant shift in Transmittance. Conclusion: It can be concluded that microcrystalline cellulose, cross linked sodium carboxymethyl cellulose, magnesium stearate and sodium carboxymethyl cellulose can be compressed together with Lamivudine and Tenofovir Disoproxil Fumarate (TDF) to produce a pharmaceutically acceptable solid dosage form, tablet. The produced tablets should be packed in moisture and light protective containers as Tenofovir Disoproxil Fumarate (TDF) has diester linkages which can be hydrolysed into the active drug Tenofovir in the presence of moisture.

Keywords

Compatibility, Interaction, Pre-Formulation, Lamivudine and Tenofovir Disoproxil Fumarate

1. Introduction

A generic fixed dose combinational product containing Lamivudine 300 mg/Tenofovir Disoproxil Fumarate 300 mg has never been produced in Tanzania and east Africa region as a whole though appears to be beneficial in TB/HIV co-infected patients. Most of the local manufacturers in developing countries like most of the countries in the sub-saharan regions have got no Research and Development (R&D) Laboratories, hence quite difficult to undertake formulation development of such kind which can lead into the drug formulation like Lamivudine-300 mg/Tenofovir Disoproxil Fumarate-300 mg. On the other hand, the drug formulations are very expensive and quite difficult for the typical third world pharmaceutical industries to take a step towards buying a full developed drug formulation. In any case, formulation development needs well trained and self motivated personnel, hence the pharmaceutical industries with the Pharmaceutical Research and Development laboratories are faced with the problem of man power which in most cases leads to the closure of the laboratories.

For a pharmaceutical scientist to undertake such a step in pharmaceutical formulation development he/she has to pass through a crucial step of undertaking pre-formulation studies [3]. A pre-formulation begins with literature search of similar type of compounds to provide and understand of the degradation process, any adverse conditions relevant to the drug, solubility and hence bioavailability, pharmacokinetics and formulation of similar compound and toxicity [4]. Hence a successful pre-formulation study will influence selection of the drug candidate itself, selection of formulation components, API & drug product manufacturing processes, determination of the most appropriate container closure system, and development of analytical methods [5]. Pre-formulation studies strengthen the scientific foundation of the guidance, provide regulatory relief and conserve resources in the drug development and evaluation process, improve public safety standards, enhance product quality, facilitate the implementation of new technologies, facilitate policy development and regulatory decision making [6]. Pre-formulation studies give directions for development of formulation in choice of drug form, excipients, composition, physical structure, helps in adjustment of pharmacokinetic and biopharmaceutical properties, support for process development of drug substance support for PAT (Process Analytical Technology) (critical process parameters), produce necessary and useful data for development of analytical methods [7].

2. Procedure

2.1. Sample Preparation

The preparation of samples for compatibility considered the amount of active ingredients and excipients in the specific ratios by mixing excipients to active substances at a ratio of 1:10, active substance to active substance at a ratio of 1:1, active substance to coating materials at 1:4, coating materials to the whole set of excipients 1:4 [8]. The samples were geometrically mixed and triturated by mortar and pestle to very fine uniform powder to ensure homogeneity of the mixture [9] [10].

2.2. Materials

Acetonitrile HPLC grade was procured from Carlo Elba Spain through financial support from MUHAS/SIDA

capacity building programme. Water was in-house prepared at MUHAS Pharm R&D Laboratory. Lamivudine and Tenofovir Disoproxil Fumarate were purchased from Desano, China through financial support from MUHAS/SIDA capacity building programme. All excipients, Microcrystalline Cellulose [8], Hydroxypropyl Methyl Cellulose (HPMC), Cross Carmellose Sodium, Crospovidone, Magnesium Stearate were donated by MUHAS Pharm R&D Laboratory.

2.3. Sample Analysis

2.3.1. Tested Variables

A total of two variables were tested in this study, this include Assay and Identity of the prepared mixtures. All these variables were compared as on day 0 and day 90.

2.3.2. Chromatographic Technique, HPLC

The validated in-house HPLC analytical method was used for quantitative determination (assay) of the amount of drug substance in the mixture. The chromatographic conditions were set with mobile phase-Acetonitrile: Water-55:45% v/v, flow rate-1 ml/min, column-C18, 4.6 mm × 250 mm × 5 µm temperature: ambient temperature, analysis (development) time: 20 minutes, detector: 252 nm DAD (Diode Array Detector).

2.3.3. Near Infra-Red

Transmittance of the mixture was determined by Near Infra-Red (NIR) technique.

3. Results and Discussion

It can be seen clearly in **Table 1** that the amount of active substance as on day 0 was comparable to day 90 for Lamivudine in all tested conditions (Room, Oven and Climatic Chamber), hence no significant change of Lamivudine was observed whereas for Tenofovir Disoproxil Fumarate only the amount of the drug at Room ($30^{\circ}\text{C} \pm 2^{\circ}\text{C}$) was comparable to day 90. There was a slight colour change of the materials of which the containers were opened and subjected to accelerated conditions at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \pm 5\%$ Relative Humidity (RH %). The observed colour change as per **Table 2** was clearly depicted by the Near Infra-Red spectra at 400 to 1500 cm^{-1} (Fingerprint region) as indicated in **Figures 1-4**. The results indicated a promising compatibility of Lamivudine and Tenofovir Disoproxil Fumarate (TDF), however, the preparation process for a final formulation should be restricted from light, moisture uptake and high temperature above 30°C .

Table 1. Drug content of samples subjected into pre-formulation studies as from day 0 to 90. The acceptance limits for assay 95% - 105% (In-house specifications).

s/n	Details	Day 0		Day 90					
		L %	T %	Room ($30^{\circ}\text{C} \pm 2^{\circ}\text{C}$)		Climatic ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \pm 5\%$)		Oven 50°C	
		L %	T %	L %	T %	L %	T %	L %	T %
1	Lamivudine-Tenofovir Disoproxil Fumarate Alone	101	99	100.1	98.5	99.2	80.2	99.1	93.3
2	Lamivudine-Tenofovir Disoproxil Fumarate-Crospovidone.	104.1	105.3	104.0	103.2	100.3	87.3	99.3	90.1
3	Lamivudine-Tenofovir Disoproxil Fumarate-Magnesium Stearate	101.2	103.4	100.2	102.3	101.2	90.3	100.2	90.5
4	Lamivudine-Tenofovir Disoproxil Fumarate-Carboxymethylcellulose	100.4	100.2	99.5	99.7	100.1	92.2	100.1	92.2
5	Lamivudine alone	99.8	-	99.7	-	99.2	-	98.2	-
6	Tenofovir Disoproxil Fumarate Alone	-	101.9	-	101.9	-	87.1	-	90.1
7	Lamivudine-Tenofovir Disoproxil Fumarate-All Excipients	105.1	106.5	102.1	102.5	103.1	88.5	100.1	90.5

Table 2. Appearance of pre-formulation samples as on day 0 compared to day 90.

s/n	Details	Day 0		Day 90	
			Room 30 ± 2°C	Climatic 40°C ± 2°C/75% ± 5% Relative Humidity (RH %)	Oven 50°C
1	Lamivudine-Tenofovir Disoproxil Fumarate Alone	white	white	off-white	white
2	Lamivudine-Tenofovir Disoproxil Fumarate-Crospovidone.	white	white	off-white	white
3	Lamivudine-Tenofovir Disoproxil Fumarate-Magnesium Stearate	white	white	off-white	white
4	Lamivudine-Tenofovir Disoproxil Fumarate -Carboxymethylcellulose	white	white	off-white	white
5	Lamivudine Alone	white	white	off-white	white
6	Tenofovir Disoproxil Fumarate Alone	white	white	yellowish white	white
7	Lamivudine-Tenofovir Disoproxil Fumarate-All Excipients	white	white	off-white	white

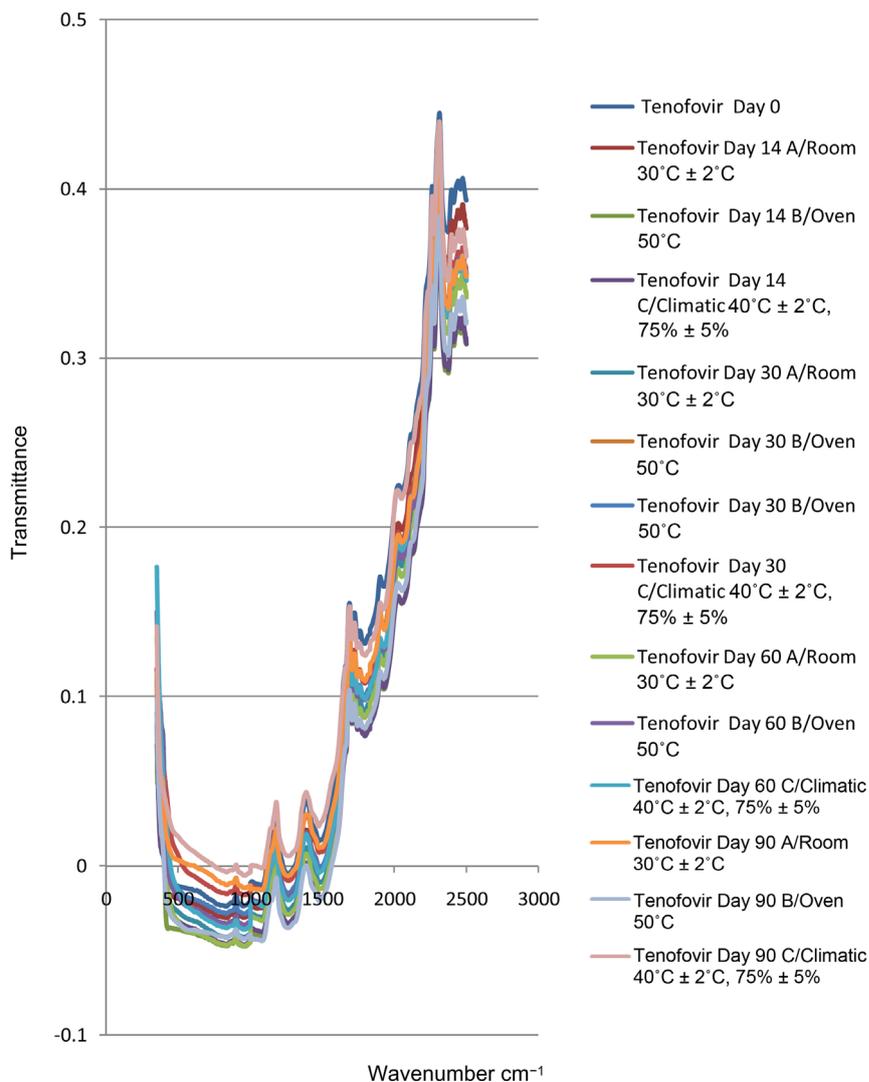


Figure 1. An overlay plot of Tenofovir disoproxil fumarate alone subjected at 40°C ± 2°C/75% ± 5%-Climatic chamber, 50°C-Oven and uncontrolled room conditions with 30°C ± 2°C, from Day 0 to Day 90.

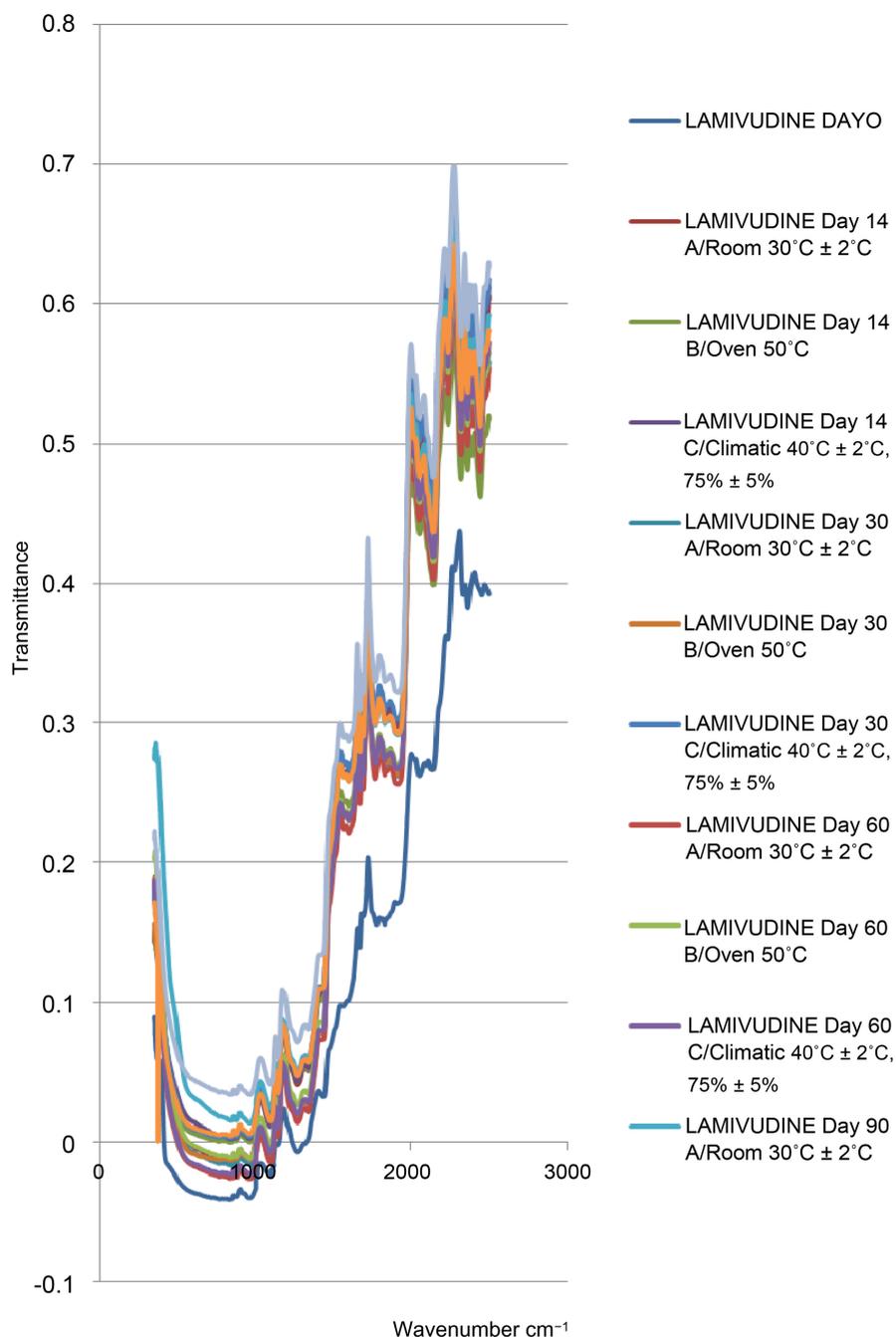


Figure 2. Overlay plot of Lamivudine alone subjected at 40°C ± 2°C/75% ± 5%-Climatic chamber, 50°C-Oven and Room uncontrolled room conditions with 30°C ± 2°C, from Day 0 to Day 90.

Regarding the obtained results, it could be clearly noted that excipients including Microcrystalline cellulose, Cross Linked Sodium carboxymethylcellulose, Magnesium Stearate and sodium carboxymethylcellulose could be safely applied into a final formulation of Lamivudine and Tenofovir Disoproxil Fumarate Fixed Dose Combination (FDC). These findings were obtained and verified by first exposing the active substances alone to the harsh conditions as it could be evidenced in **Figures 1-3**.

The effect of excipients on the active substances, Lamivudine and Tenofovir Disoproxil Fumarate (TDF) was followed by exposing the drug substances to harsh conditions when well mixed together with the potential

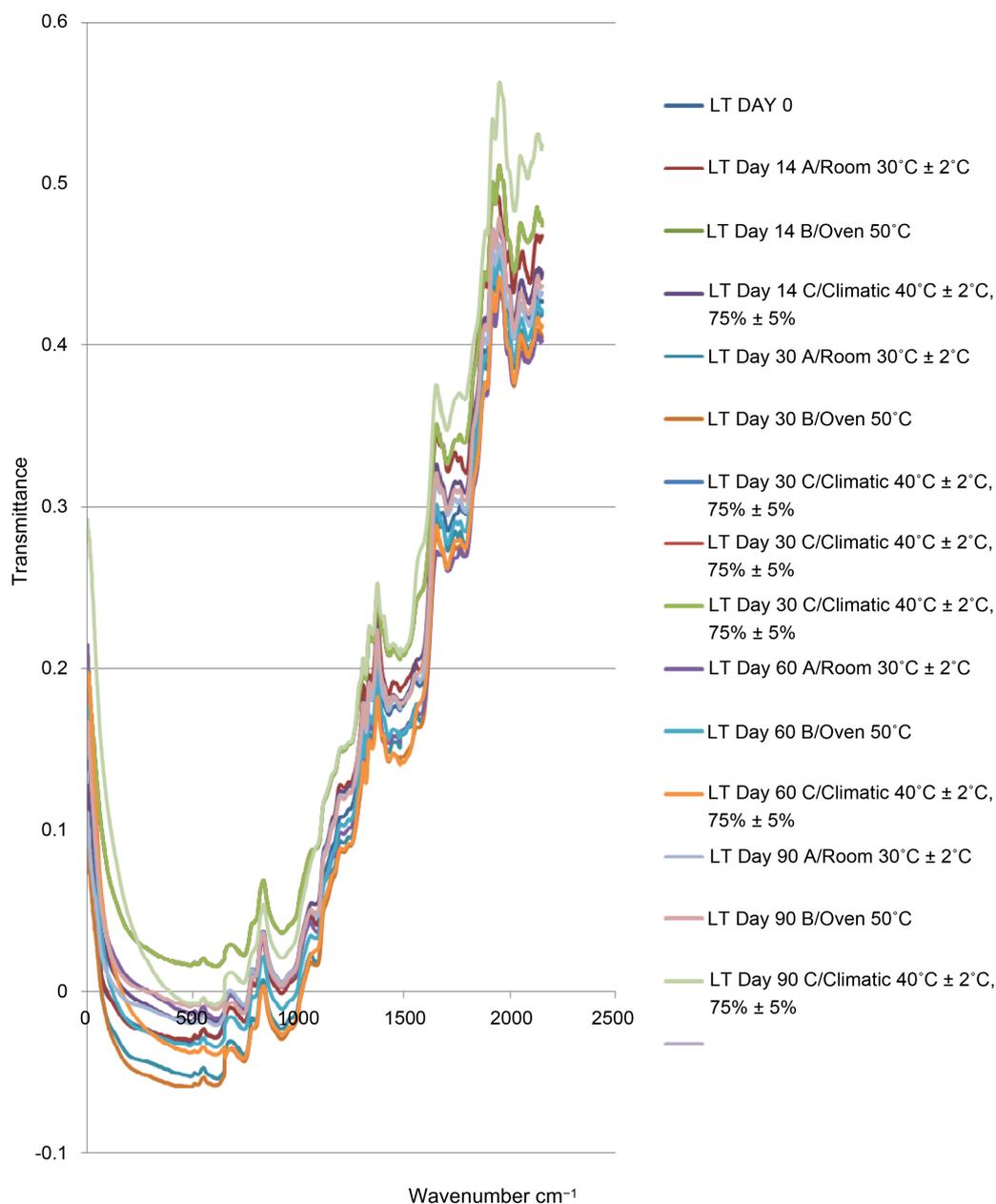


Figure 3. An overlay plot of Lamivudine and Tenofovir mixture subjected at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \pm 5\%$ -Climatic chamber, 50°C -Oven and Room uncontrolled room conditions with $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$, from Day 0 to Day 90.

excipients (**Figure 4**). As in **Figure 4**, Lamivudine, Tenofovir Disoproxil Fumarate and Magnesium Stearate were mixed together and the mixture was exposed to harsh condition to allow for plausible basic hydrolysis reaction between Magnesium which is basic in nature and TDF. Generally there was no formation of new substance as indicated by **Figure 4**. This is confirmed by having identical spectra for day 0 and 90.

4. Conclusion

It was generally found that Microcrystalline cellulose, Sodium carboxymethyl cellulose cross linked, Magnesium Stearate and sodium carboxymethyl cellulose can be compressed together with Lamivudine and Tenofovir Disoproxil Fumarate (TDF) to produce a pharmaceutically acceptable solid dosage form, tablet. The produced

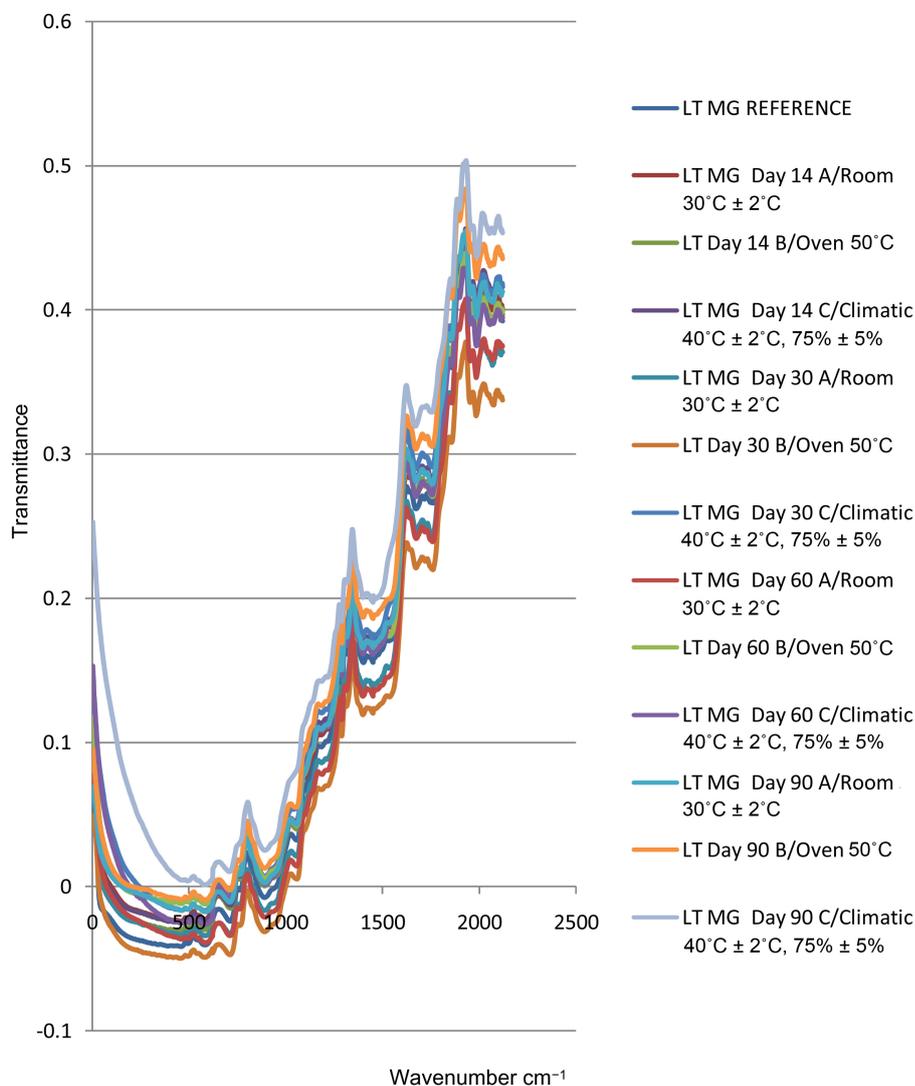


Figure 4. An overlay plot of Lamivudine/Tenofovir/Magnesium Stearate mixture subjected at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \pm 5\%$ -Climatic chamber, 50°C -Oven and uncontrolled room conditions with $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$, from Day 0 to Day 90.

tablets should be protected from moisture, high temperature above 30°C and oxidation by light as they are found to affect Tenofovir Disoproxil Fumarate (TDF) [11].

Acknowledgements

We would like to appreciate SIDA Surec and MUHAS for the financial support to procure reagents and materials for this study. Also we acknowledge the technical contribution from members of staff at MUHAS Pharmaceutical Research and Development Laboratory (MUHAS Pharm R&D), this includes Ms. Ruth Ng'wananogu and Ms. Bertha Francis, Mr. Edson Lutta and Mr. Mhando Maro.

References

- [1] Preechagoon, D., Sumyai, V., Tontisirin, K., Aumpon, S. and Pongjanyakul, T. (2005) Formulation Development and Stability Testing of Oral Morphine Solution Utilizing Preformulation Approach. *Journal of Pharmacy & Pharmaceutical Sciences*, **8**, 362-369.
- [2] Gopinath, R. and Naidu, R.A.S. (2011) Pharmaceutical Preformulation Studies—Current Review. *International Jour-*

- nal of Pharmaceutical and Biological Archive*, **2**, 1391-400.
- [3] Article, R. (2012) Available through Online Review Article IMPORTANCE OF Preformulation Studies in Designing. *Formulations for Sustained Release Dosage Forms*, **4**, 2311-2331.
- [4] Pérez, P., Suñé-Negre, J.M., Miñarro, M., Roig, M., Fuster, R., García-Montoya, E., et al. (2006) A New Expert Systems (SeDeM Diagram) for Control Batch Powder Formulation and Preformulation Drug Products. *European Journal of Pharmaceutics and Biopharmaceutics*, **64**, 351-359. <http://dx.doi.org/10.1016/j.ejpb.2006.06.008>
- [5] Kalinkova, G.N. (1999) Studies of Beneficial Interactions between Active Medicaments and Excipients in Pharmaceutical Formulations. *International Journal of Pharmaceutics*, **187**, 1-15. [http://dx.doi.org/10.1016/S0378-5173\(99\)00176-3](http://dx.doi.org/10.1016/S0378-5173(99)00176-3)
- [6] Capková, Z., Vitková, Z., Tilandyová, J. and Cizmárik, J. (2005) Preformulation Studies of Potential Drug XIX M: Partition Coefficient. *Česká a Slovenská Farmacie*, **54**, 75-77.
- [7] Du, S. and Deng, Y. (2006) Studies on the Encapsulation of Oxymatrine into Liposomes by Ethanol Injection and pH Gradient Method. *Drug Development and Industrial Pharmacy*, **32**, 791-797. <http://dx.doi.org/10.1080/03639040600760556>
- [8] Heng, P.W.S., Liew, C.V. and Soh, J.L.P. (2004) Pre-Formulation Studies on Moisture Absorption in Microcrystalline Cellulose Using Differential Thermo-Gravimetric Analysis. *Chemical and Pharmaceutical Bulletin (Tokyo)*, **52**, 384-390. <http://dx.doi.org/10.1248/cpb.52.384>
- [9] Salnikova, M.S., Joshi, S.B., Rytting, J.H., Warny, M. and Middaugh, C.R. (2008) Preformulation Studies of Clostridium Difficile Toxoids A and B. *Journal of Pharmaceutical Sciences*, **97**, 4194-4207. <http://dx.doi.org/10.1002/jps.21300>
- [10] Gupta, M. and Saini, T. (2009) Preformulation Parameters Characterization to Design, Development and Formulation of Vancomycin Hydrochloride Tablets for Pseudomembranous Colitis. *International Journal of Research and Development in Pharmacy & Life Sciences*, **9**, 1-7. <http://www.ijprd.com/>
- [11] Heidemann, D.R. and Jarosz, P.J. (1991) Preformulation Studies Involving Moisture Uptake in Solid Dosage Forms. *Pharmaceutical Research*, **8**, 292-297. <http://dx.doi.org/10.1023/A:1015877011807>



Scientific Research Publishing

Submit or recommend next manuscript to SCIRP and we will provide best service for you:

Accepting pre-submission inquiries through Email, Facebook, LinkedIn, Twitter, etc
A wide selection of journals (inclusive of 9 subjects, more than 200 journals)
Providing a 24-hour high-quality service
User-friendly online submission system
Fair and swift peer-review system
Efficient typesetting and proofreading procedure
Display of the result of downloads and visits, as well as the number of cited articles
Maximum dissemination of your research work

Submit your manuscript at: <http://papersubmission.scirp.org/>