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Research Article

Formulation and Evaluation of Lamivudine Nanosuspension

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Abstract

The present research aimed to develop & Evaluation of Lamivudine Nanosuspension. Lamivudine is a potent *in vitro* inhibitor of human immune deficiency virus belongs to the category of anti-retroviral drugs. The formulated Nanosuspension was subjected to various evaluation parameters like particle size, polydispersity index, zeta potential, drug content, viscosity, saturation solubility studies, *In vitro* release, treatment of kinetic data, and stability studies. The polydispersity ranged from 0.218 PDI to 0.331 PDI and zeta potential ranged from -1.60 mV to -4.79 mV are the important evaluation parameters are responsible for the stability of nanosuspensions. The Polydispersity index presents the quantity of particle size distribution ranges from 452.4 nm to 532.2 nm. In this result, LNSF4 shows spectacular drug content range of 86±1.8% to 97±2.5% it is the maximum drug content. The Brook field viscometer to determine the viscosity of Lamivudine Nano suspension of different formulations was found to be 44.4±2.1 cps to 87.7±1.4 cps. The general Nanosuspension formulations LNSF4 shows 98.64 % better controlled released in comparison with abundant formulation. In all the cases the best-fit model encounter uoto be peppas with 'n' value between 0.768 to 0.917. The 'n' value of formulation LNSF4 was 0.876 and suggesting so the drug was released by Zero-order kinetics. Acceleration stability studies intermediate storage condition has been changed from 30°C ± 2°C and 60% RH ± 5% Relative Humidity. After a 90 days study it revolves that there's no change in Drug content, *In vitro* drug release, and particle size.

Keywords: Lamivudine, Nanosuspension, Saturation solubility, Scanning Electron Microscopy, Stability study.

INTRODUCTION:

Nanosuspension is defined as a sub-micron colloidal system it's contains the poorly soluble drug, waver in a suitable dispersion medium stabilised by the surfactants. Nanosuspension usually consists of colloidal carriers like polymeric resins which are inert [1]. They help in the enhancement of drug solubility and thus bioavailability. Unlike microemulsions, there is no irritant in nature. Nanosuspension also imparts stability of your drug within the formulation. Nanosuspension can be prepared by different methods such as high-pressure homogenization and media milling. Lamivudine has been formulated i n many formulations but they do not overcome the main limitations like therapeutic effectiveness of Lamivudine [2] its dose-dependent toxicity, short biological half-life, and poor bioavailability, effectively so were developed the nanosuspension in high-pressure homogenizer technique. An effort has to conquer the problem with prepared Nanosuspension. The main objectives involved in the study are to extend the dose of medication of the drug to increase the rate plus extent of absorption of the drug to enhance the effectiveness in therapy.

MATERIALS AND METHODS:

Lamivudine was obtained as a gift sample from Hetero Pharmaceutical PVT Limited, Hyderabad, HPMC K-30, Eudragit S 100, Tween-80, PVP K-30, Poloxamer 188, Methanol was purchased from Himalaya pharmaceutical, Nellore and other ingredients used were of Analytical grade.

METHODOLOGY

Drug and excipients interaction (FTIR)

The compatibility between pure drug and polymers like HPMC K-30, PVP K-30, poloxamer-188, Eudragit S100 were detected by FTIR spectroscopy (Bruker Pvt. Ltd, Germany). The finely grounded powder was introduced into stainless steel die [3]. The powder was pressed in the die between polished steel anvils at a pressure of about 10t/in2. For liquid samples, a thin film of sample liquid is made on a pellet.

Preparation method of Nanosuspension

The drug used to be molten in methanol to prepare an organic solution and fixed Amount (Table 1). The polymers and surfactant are dissolved in a mentioned quantity of water it is the aqueous phase. The aqueous water is kept under a high- pressure homogenizer (Remi RQ- 127) at

room temperature. The organic solution is added drop-wise through a syringe to the aqueous solution. Under process of high-pressure homogenizer at a rotation speed of 100 Rpm up to 8 hours [4]. Nanosuspension was formed, Spectacular organic solution used to be gaseous at temperature (Table 1).

Table 1: Formulation of Lamivudine Oral nanosuspension

Ingredient	LNSF1	LNSF2	LNSF3	LNSF4	LNSF5	LNSF6
Lamivudine	5gm	5gm	5gm	5gm	5gm	5gm
Poloxomer- 188	-	-	150mg	1500mg	-	-
HPMC k-30	150mg	1500mg			-	-
Eudragit S 100	-	-	-	-	150mg	1500mg
PVP k-30	3gm	3gm	3gm	3gm	3gm	3gm
Tween-80	1ml	1ml	1ml	1ml	1ml	1ml
Methanol	60ml	60ml	60ml	60ml	60ml	60ml
Water	100ml	100ml	100ml	100ml	100ml	100ml

Evaluation parameters

Scanning Electron Microscopy

The surface geomorphology of the freeze-dried nanosuspension used to be planned using SEM [5].

Particle size, polydispersity index measurement Zeta potential:

The particle size and its distribution were determined using Zetasizer Nano ZS using a process called Dynamic Light Scattering. The zeta potential of a particle is the overall charge that the particle acquires in a particular medium [6].

Drug content

0.5ml of each one preparation was utilized for dissolve in 10ml isotonic solution and kept overnight and also 10 mg of your drug were utilized for melted in ten ml of the isotonic solution and kept overnight [7]. From that, all preparations along with the drug were filtered dilutions made in the put concentration of one microgram per millimetre. These dilutions were estimated their content uniformity by reason a UV spectrophotometer at the wavelength 271nm.

Viscosity

The Nano suspension was performed by using the Brookfield viscometer set the spindle No - 60 at 100rpm [8].

Saturation Solubility Studies

The saturation solubility studies were done on both the unprocessed pure drug and different batches of the lyophilized Lamivudine Nanosuspension. By the way for 10 mg of the unprocessed pure drug compound and Nanosuspension equivalent to 10 mg were weighed and measured separately and introduced into a 25 ml Stoppard conical flask Containing 10 ml distilled water. The flasks were sealed and placed in a rotary shaker for 24 hrs at room temperature and equilibrated for 2 days [9]. The diluted samples were analyzed using a UV spectrophotometer at 271nm.

***In vitro* release study**

The entire formulations by the way of utilizing the USP category II dissolution equipment, Dissolution medium 900ml of 0.1N HCL rotating speed is 50 rpm Temperature kept constant at 37 ± 0.5°C sampling withdrawing time is followed 1 to 8 hours at programmed time interval aliquot samples (5ml) were collected and replenished by the same quantity of fresh medium[10]. The aliquot sample (5 ml) was filtered using the supporter of 0.45µm restricted membrane filter paper and the filtrate was to be diluted properly through the fresh medium and was predictable using UV-Vis spectrophotometer (model UV-2600plus) at wavelength 271 nm.

Treatment of dissolution data with a different kinetic model

The amount of drug released from Lamivudine Nanosuspension was analyzed by the way of the total value of point in time must be performed with a flexible model. For finding out the mechanism of drug release from the Lamivudine Nanosuspension, the dissolution data obtained from the above experiments were treated with the following different release kinetic models [11].

Zero order- $Q=Ko\ t$

First order- $\text{Log } Q= \text{Log } Qo-K1\ t/2.303$.

Higuchi- $Qt = KH\ t^{1/2}$

Korsmeyer - Peppas- $Qt/Q\alpha = K\ tn$

A plot of log (drug release) versus log t will be linear with a slope of n and the intercept gives the value of log k as shown in Table 2.

To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release vs. log time.

Table 2: Diffusion exponent values indicating drug release mechanism

S.No.	Diffusion exponent value (n)	Drug release mechanism
1	< 0.45	Fickian release
2	0.45 to 0.89	non-Fickian transport
3	0.89	Case II transport
4	> 0.89	Super case II transport

Stability Study of Nanosuspension

The stability studies for Nanosuspension have performed storage conditions for 90 days as follows Acceleration stability studies intermediate storage condition has been changed from 30°C ± 2°C and 60% RH ± 5% RH. The optimized batch LNSF4 Nanosuspension was utilized for each condition; the particle size, Drug content, and *In-vitro* dissolution are the most vital specification for the activity & physical stability [16].

RESULT AND DISCUSSION

PREFORMULATION STUDIES

Drug and excipients interaction study

Drug polymer compatibility studies were performed by the FTIR method. The spectra of Lamivudine, HPMC K30, PVP K-30, poloxamer- 188, and Eudragit S100 alone and prepared formulations of drug with the above polymers were measured and interpreted. The IR spectra were depicted as Figures 1 to 6 and interpreted values are tabulated in Table. 3. The IR spectra of drug and polymer alone and prepared formulations show no significant interaction between drug and polymer.

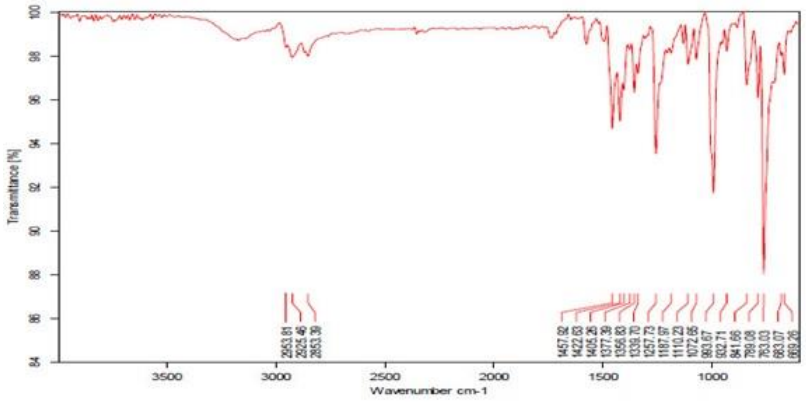

Figure 1: FTIR Spectrum of Lamivudine

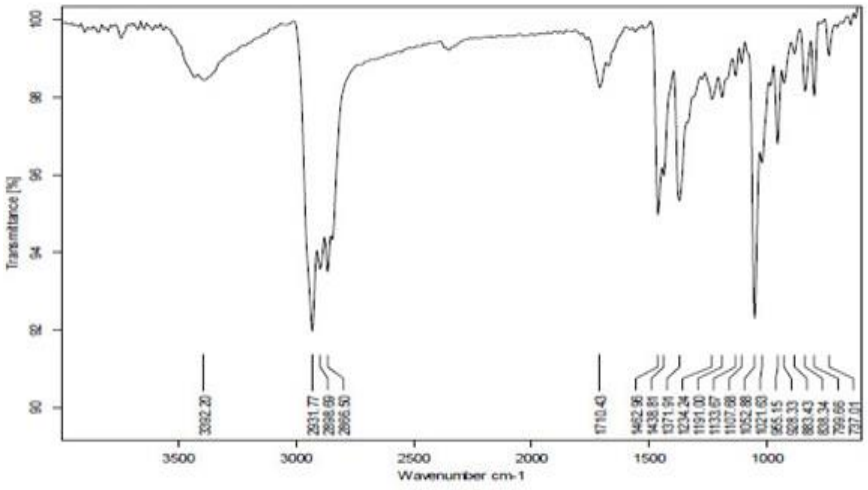

Figure 2: FTIR Spectrum of Poloxamer 188

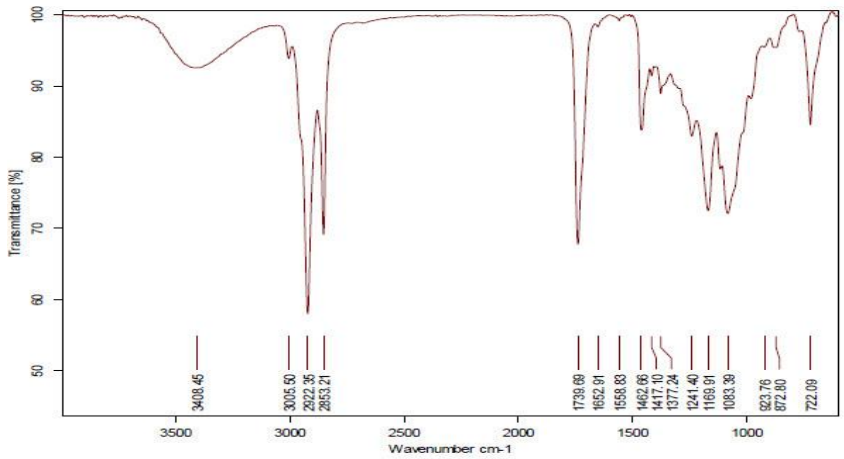

Figure 3: FTIR Spectrum of Eudragit S 100

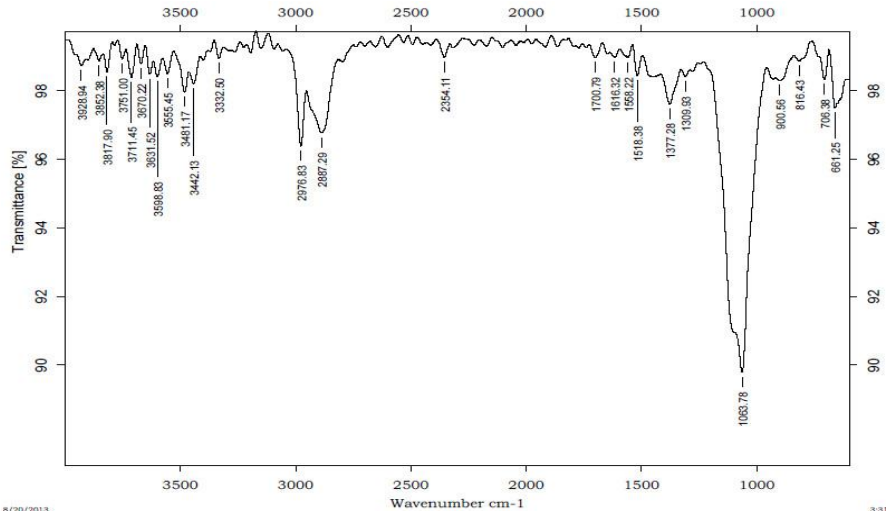

Figure 4: FTIR spectra of HPMC K 100

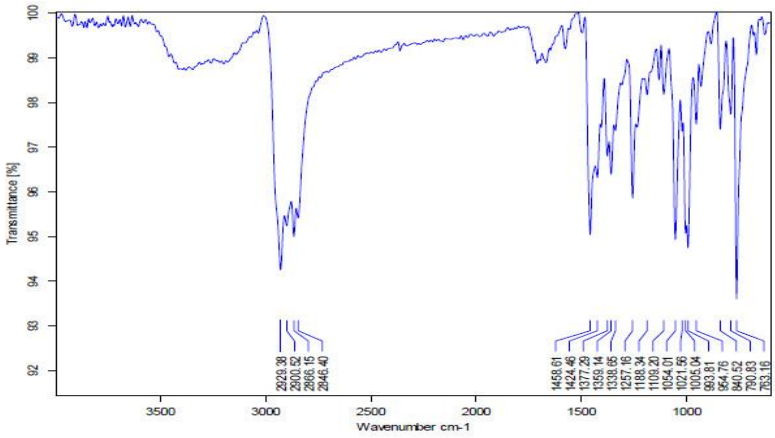

Figure 5: FTIR spectra of PVP K30

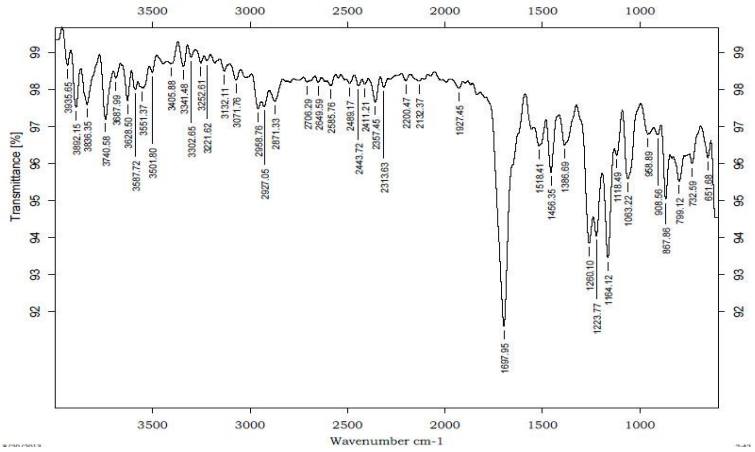

Figure 6: FTIR Spectrum of Lamivudine + Poloxamer + Eudragit + PVP K30 & Tween 80

Table 3: FTIR Spectrum Interpretation obtained for drug along with excipients

Functional Group	Literature Value	Pure Drug	Poloxamer 188	Eudragit S 100	HPMC K 100	PVP K30	Mixture
N-H stretch	3500-3180	3171.36	3392.20	3408.45	3711.45	3740.58	2929.38
Alkyl C-H Stretch	2950 - 2850	2868.62	2868.62	2922.35	2887.29	2927.05	2929.38
C=O stretch	1810-1775	1714.21	1710.43	1739.69	1700.79	1697.95	1658.61
C-H bend (para)	850-800	840.62	883.45	872.80	816.43	867.86	840.52

Scanning Electron Microscopy (SEM)

The shape and surface morphology of freeze-dried optimized nanosuspension (LNSF4) was observed by the SEM. The SEM images of optimized Nanosuspension at 2000× Magnification LNSF4 show a 20 µm spherical shape of Nanosuspension as shown in figure 7.

Particle size, poly dispersivity index measurement Zeta potential

The polydispersity ranged from 0.218 PDI to 0.331 PDI and zeta potential ranged from -1.60 mV to -4.79 mV. The particulate distribution ranges from 452.4 nm to 532.2 nm as shown in Table 4.

Drug content

In this result LNSF4 ranges from 86±1.8% to 97±2.5% it is the maximum drug content (Table 4).

Viscosity

The Lamivudine Nanosuspension of different formulations was found to be 44.4±2.1 cps to 87.7±1.4 cps and as shown in table 4. They show the result within satisfactory limits.

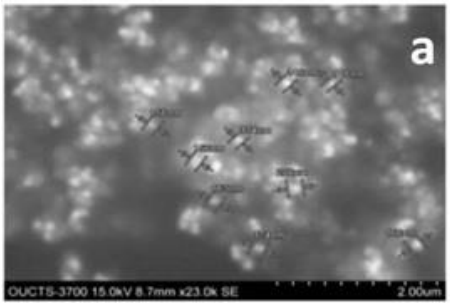

Figure 7: Scanning Electron microscopy image of optimized formulation

Table 4: Particle size and zeta potential of Lamivudine nanosuspensions

Formulation	Particle size (nm)	PDI	ZP (mV)	Drug content (%)	Viscosity (cps)
LNSF1	523.2	0.293	-1.60	95±1.3	44.4±2.1
LNSF2	435.2	0.218	-2.93	94±2.6	47.3±1.3
LNSF3	364.9	0.288	-2.79	92±2.8	54.5±1.2
LNSF4	532.2	0.283	-4.79	97±2.5	87.7±1.4
LNSF5	342.0	0.278	-3.26	86±1.8	64.9±3.8
LNSF6	452.4	0.331	-4.10	89±1.7	67.2±13

Saturation Solubility

This is due to the decrease in particle size when compared to pure dug according to the Ostwald-Freundlich equation. The fold increase in the solubility of the drug is depicted in Table 5.

Table 5: Saturation Solubility of pure drug

Medium	Pure drug concentration (Mean SD)					
	LNSF1	LNSF2	LNSF3	LNSF4	LNSF5	LNSF6
Distilled water	2.90 ± 0.53	1.50 ± 0.43	3.50 ± 0.23	2.50 ± 0.13	1.60 ± 0.42	2.40 ± 0.62
pH 1.2 buffer + 2.2% w/v SLS	3223 ± 25.12	2836 ± 30.43	2234 ± 29.61	2435 ± 23.47	2653 ± 21.56	2826 ± 45.18
Phosphate buffer pH 7.4	3.52 ± 1.22	2.22 ± 1.32	2.42 ± 1.41	3.25 ± 1.83	2.31 ± 1.43	3.24 ± 1.54
2.2% w/v SLS in water	3910 ± 31.40	3420 ± 32.50	3430 ± 33.30	2630 ± 28.10	2840 ± 30.50	3989 ± 30.30

***In-vitro* Drug Release Studies**

The dissolution kinetic profiles of nanosuspension formulations, LNSF4 shows 98.64 % better controlled released compared to other formulations (Tables 6 & Figures 8).

Table 6: *In-vitro* drug release of Lamivudine Nanosuspension

Time (Hrs)	% Drug Release					
	LNSF 1	LNSF 2	LNSF 3	LNSF 4	LNSF 5	LNSF 6
0	0	0	0	0	0	0
1	10.00	12.12	23.51	12.21	13.63	12.82
2	25.32	29.62	32.38	21.62	22.84	22.47
3	36.83	42.73	42.57	30.38	39.29	32.61
4	48.02	53.62	51.41	49.92	47.21	53.04
5	57.52	62.81	60.83	62.26	56.05	64.07
6	71.27	73.01	70. 46	71.43	75.02	73.92
7	83.45	84.07	82.00	82.73	84.26	81.05
8	96.03	97.12	90.75	98.64	92.01	94.53

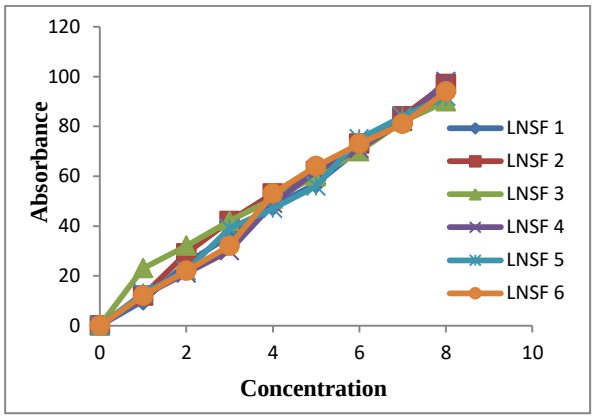

Figure 8: *In-vitro* drug release Profiles of Formulations LNSF 1- LNSF 6

Release kinetics

The kinetic versions chosen were Zero order, First order, Higuchi Matrix, and Korsemayer Peppas best formulation LNSF4. The regression coefficient values for all these models.

In all the cases the best-fit model was found to be peppas with 'n' value between 0.768 to 0.917. The 'n' value of formulation LNSF4 was 0.876 and suggesting that the drug was released by Zero-order kinetics as shown in Table 7.

Table 7: Release Order Kinetics for formulation LNSF1 to LNSF6

Formulation code	Zero order (r^2)	First order (r^2)	Higuchi model (r^2)	Korsmeyer-Peppas model	
				(r^2)	n
LNSF1	0.984	0.773	0.962	0.988	0.781
LNSF2	0.964	0.842	0.984	0.993	0.794
LNSF3	0.985	0.858	0.958	0.983	0.768
LNSF4	0.994	0.998	0.896	0.996	0.876
LNSF5	0.980	0.885	0.964	0.978	0.892
LNSF6	0.986	0.834	0.961	0.98	0.793

Stability studies

Stability studies are done for best formulation (LNSF4) as per ICH guideline as follows Acceleration stability studies intermediate storage condition has been changed from 30°C ± 2°C and 60% RH ± 5% RH. It focuses that there's no change in Drug content, *In vitro* drug release, and particle size but it was within the acceptable limit as shown in Table 8 & 9 & Figure 9.

Table 8: Stability study for formulation LNSF4 in Acceleration stability studies

Period	30°C ± 2°C and 60% RH ± 5% RH	
	% Drug content	Particle size
15 Days	98±1.4	542.1
30 Days	98±1.9	543.0
60 Days	98±2.1	544.2
90 Days	98±2.4	546.3

Table 9: *In vitro* studies of formulation LNSF4 Acceleration stability studies

Time in hrs	Cumulative percentage drug release 30°C ± 2°C and 60% RH ± 5% RH			
	15 Days	30 Days	60 Days	90 Days
1	12.21	12.24	13.24	14.24
2	21.62	21.73	22.73	24.73
3	30.38	30.40	31.40	33.40
4	49.92	49.94	50.94	53.94
5	62.26	62.29	64.29	66.29
6	71.43	71.48	73.48	77.48
7	82.73	82.78	83.78	86.78
8	98.64	98.68	98.70	99.70

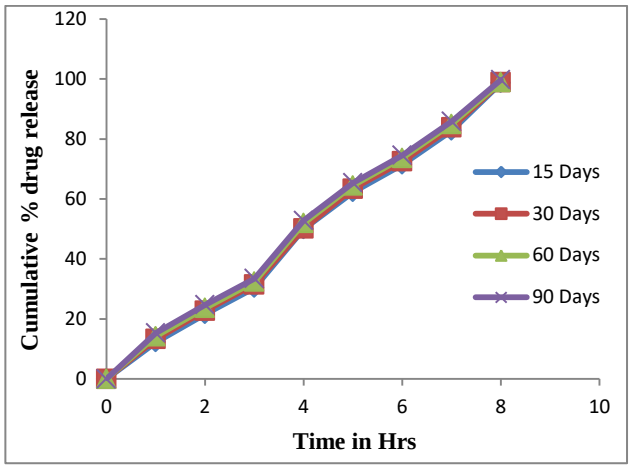

Figure 9: *In vitro* studies of formulation LNSF4 Acceleration stability studies

CONCLUSION

From the experimental result, it might be complete so the evaluation of your suspension, unconcealed that the following parameters for the customized formulation LNSF4 are as follows saturation solubility, % Drug content 97±2.5%, average particle size 532.2 nm, polydispersity index 0.283, Zeta potential -4.79mv. The poloxamer-188 used a polymer in LNSF4 has shown effective cumulative drug waiver equal 8 hours in comparison to any other formulations. The Release order kinetic study shown in the LNSF4 revealed that the exponent "n" value is within the limits. It indicated that the release mechanism for LNSF4 may be a diffusion mechanism followed by the non fickian transport so the LNSF4 was chosen as first formulation. These are an indication of the stability going from the Nanosuspension. The stability studies indicate for which the Nanosuspension is more stable in acceleration stability studies. From the above studies, it is evident that a promising drug delivery system of the Lamivudine nanosuspensions.

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Conflict of interest

No conflict of interest in this study.

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