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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR THE DETERMINATION OF LAMIVUDINE FROM DRUG LOADED NANOPARTICLE PHARMACEUTICAL FORMULATION

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Abstract:

Lamivudine nanoparticles were prepared by Ionotropic pregelation method by optimizing the parameters like polymer concentration, stirring time and stirring speed. The optimized lyophilized nanoparticles were compressed into a tablet by direct compression method by varying the type and concentration of cushioning agent and disintegrant. MCC was showing more drug release compared to other cushioning agents. The prepared tablets were evaluated for pre compression and post compression properties. Among all the formulations, dissolution studies F8 formulation produced sustained release in comparison with the marketed Tablet and released 2.57% of drug in acidic PH (1.2) and 96.22% of drug in intestinal PH (6.8).The dissolution samples of F8 and marketed lamivudine tablet were estimated by the developed RPHPLC method by using Inertsil ODS 3V column,C18(150mm x4.6mm) 5µm, an isocratic mobile phase consists of Mixed phosphate buffer and ACN (60 : 40) at PH 4 with UV detection at 273nm where Lamivudine was eluted at 2.4 min. The developed method was validated according to ICH guidelines. The release kinetics of F8 formulation was found to be first order with Anomalous transport.Stability studies of F8 formulation showed good results.

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INTRODUCTION:

Lamivudine [1] is an active antiretroviral drug comes under nucleoside reverse transcriptase inhibitor (NRTI) with activity against both Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV).It is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.

Tablet is the most preferred oral formulation used but for treating chronic diseases, they have to be administered many times a day and as a result shows disadvantages, so nanoparticulate drug delivery sytem can overcome these problems. Lamivudine drug used for the treatement of hepatitis B (HBV) is administered many times due to its moderate half life [1]. Various analytical methods for the estimation of the drug have been found in the literature such as UV-spectrophotometric method [2], HPLC methods for the estimation in tablets and HPLC method of the drug and its metabolite in biological fluids [3]. But HPLC method for determination of lamivudine in any novel drug delivery system has not been reported in any scientific literature. The aim of the present work is to formulate the developed lamivudine nanoparticles into a tablet by direct compression method for sustained release action and to develop a simple, precise, accurate and validated RP-HPLC method for the estimation of lamivudine in nanoparticle tablets and also in commercially available tablets [4-7]. The results of the analysis were validated by statistical methods and recovery studies according to ICH guidelines [8].

Keywords: Lamivudine Nanoparticles, Direct compression method, RPHPLC and Validation**.**

Experimental Details:

Lamivudine was obtained as a gift sample from Cipla Pharmaceuticals. Commercial Lamivudine 100 mg tablet was procured from local Pharmacy. Potassium Dihydrogen Phosphate, Dipotassium hydrogen orthophosphate was purchased from N.R Chemicals**.** MCC and Dicalcium Phosphate, Talc were purchased from Finar Reagents. HPLC grade Methanol and Acetonitrile were procured from Merk India and all other chemicals used were of analytical grade.

Instrumentation and Chromatography

The HPLC system consisted of a Shimadzu LC-2010 with UV-detector. The software used for data acquisition is lab solutions. Separation was achieved using a Inertsil ODS 3V column, C18(150x4.6 ID) 5µm **.**The isocratic mobile phase pumped at a flow rate of 1 mL/min consisted of Mixed phosphate buffer and ACN (60 : 40) at PH 4 and UV detection at 273nm.Lamivudine was eluted at 2.4 min.The mobile phase was filtered through a 0.45 µm filter and degassed by sonication for 15 min. The injection volume was 10 µL. All separations were performed at room temperature.

METHOD:

Preparation of Lamivudine loaded Nanoparticle tablets:

Lamivudine loaded Nanoparticle tablets were prepared by direct compression method after freeze drying of optimized formulation of nanoparticles i.e.,NP2 that gave best in vitro dissolution profile in 24hrs in comparison with other nanoparticle formulations[9]. Amount of lyophilised powder taken was 280mg equivalent to 100mg of Lamivudine. All ingredients were collected and weighed accurately. Lamivudine[10] was mixed with required amount of excipients as in Table 1, 2 and 3. Drug with excipients were sifted and passed through sieve #40 and then after pre blending all ingredients in mortar for 15minutes, then magnesium stearate and talc were added and blended for 5-6 minutes, lubricated powder was compressed into round, concave tablets under 11mm punch of tablet punching machine.

Evaluation Parameters:

Pre-Formulation studies:

Fourier Transform Infra Red Spectroscopy:

Compatibility of drug with excipients was determined by carrying out FTIR studies. Samples studied were pure drug and physical mixtures of lamivudine, MCC, CCS and PVPK-30. All these samples were grounded and mixed thoroughly with potassium bromide, at 1:100 (sample: potassium bromide) weight ratio. The FTIR spectrum was obtained by using Fourier Transform Infrared spectrophotometer BRUKER, FTIR(8400 S Shimadzu) using KBr Pellet method. KBr pellet obtained was scanned in the range of 400-4000cm-1. The characteristic peaks are recorded for pure drug and blend used for nanoparticle tablet.

Differential Scanning Calorimetric:

The thermal behaviour of pure drug lamivudine and physical mixtures of lamivudine, MCC, CCS and PVPK-30, was examined by using DSC 60, Shimadzu. Sample of about 5mg is placed in aluminium pan and analysed at a scanning temperature range from 40 to 250^0c at the heating rate of 10^0c/min under constant purging of Nitrogen at 20ml/min.

Pre-Compression studies [10-12]

Angle of Repose

Angle of repose was determined by using funnel method. The blend was poured through funnel that can be raised vertically until a maximum cone height (h) was obtained. Radius of the heap (r) was measured and angle of repose was calculated using the formula.

$$\emptyset = \tan^{-1}\frac{h}{r}$$

Where, θ is the angle of repose, h is height of pile; r is radius of the base of pile.

Bulk Density

Apparent bulk density (ρb) was determined by pouring the blend into a graduated cylinder. Bulk density is the ratio of weight of powder (*M*) and bulk volume (*Vb*)

Tapped Density

The graduated cylinder containing known mass of blend was placed under mechanical Tapper apparatus ,which is operated for a fixed number of taps until blend has reached a minimum volume. The minimum volume (*Vt*) occupied in the cylinder and weight (M) of the blend was measured. The tapped density (ρb) is the ratio of weight (M) of the blend and minimum volume (*Vt*) occupied in the cylinder.

Carr's Index or Compressibility Index

The compressibility index of the granules was determined by using values of bulk density and tapped density, calculated by using the following formula

C.I(%)= Tapped density-Bulk Density

_____*100

Tapped density

It should be 5-15, indicates free flowing.

Hausner Ratio

Hausner ratio is an indirect index of ease of powder flow. It is the ratio of tapped density and bulk density.Lower Hausner ratio (< 1.25) indicates better flow properties than higher ones (>1.25).

Post-compression parameters for formulated tablets.

Weight variation

Twenty tablets from each formulation were selected at random and average weight was determined. Then the individual tablets were weighed and were compared with average weight.

% Deviation=(X-X*/X) x100

X-Actual wt of the tablet

X*-Average wt of the tablet

Hardness

The hardness of the tablet from each formulation was determined using Pfizer hardness tester. The force required to break the tablet is measured in Kilograms.

Friability

Friability of the tablets was determined using Roche Friabilator. This device subjects the tablets to the combined effect of abrasion and shock in a plastic chamber revolving at 25 rpm and dropping the tablets at a height of 6 inches in each revolution. Pre weighed sample of tablets was placed in the friabilator and were subjected to 100 revolutions. Tablets were dedusted and reweighed. The friability (f) is given by the formula.

Friability (f) = (1 – W)/Wo*100

Where, W_0 is weight of the tablets before the test and

W is the weight of the tablet after the test.

Thickness

The thickness of the tablet was carried out using Digital Vernier caliper. Ten tablets were used for the above test from each batch and results were expressed in millimeter.

Drug content

Weigh accurately a quantity of tablet powder equivalent to 100mg of Lamivudine, extraction was carried out using 6.8pH buffer. The solution was filtered through 0.45µm membrane filter and then absorbance was measured at 279nm spectrophotometrically after suitable dilution against appropriate blank and Calculate the drug content.

In-vitro dissolution studies

The release rate of formulated tablets were determined as per United States Pharmacopoeia (USP) dissolution testing apparatus I(Basket Method). The dissolution test was performed using 900ml of 0.1N HCL for 2 hours and then replaced with 6.8pH buffer for the rest of the study, at 37 ± 0.5°C and 100 rpm. The samples were taken at pre-determined time intervals with replacement of equal volume of dissolution medium. After filtration the samples were analyzed using UV spectroscopy at 279 nm.

HPLC METHOD DEVELOPMENT FOR LAMIVUDINE NANOPARTICLE TABLETS

Preparation of Standard solution

10mg of lamivudine was weighed and transferred in to 100ml volumetric flask and dissolved in mobile phase and then dilute up to the mark with methanol to give 100 µg /ml of solution.

Method validation [13]:

The method was validated according to the guidelines set on the International Conference on Harmonisation (ICH) for the validation of analytical procedures. The parameters which were used to validate the method of analysis were linearity,specificity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and System suitability**.**

System suitability:

This is an integral part of method validation and ensures the adequate performance of the chromatographic system.It is assessed by six injections of the drug at a concentration of 100µg/ml and checked for the number of theoretical plates, %RSD for the peak area and retention time for lamivudine peaks.

Linearity:

It is the ability of a method to give results that are directly proportional to the concentration of analyte in the samples. Standard solutions of drug 5,8,10,12,15 ml are taken and diluting to 10ml with mobile phase to give 50,80,100,120 and 150µg/ml.

Accuracy:

Accuracy was performed by recovery studies by standard addition method by adding known quantities of pure drug into pre analysed samples at 50%, 100% and 150% levels in triplicate. Recovery was calculated for marketed formulation and nanoparticle Tablet using Regression line equation.

Precision:

The Precision of the analytical method was done under two categories system precision and Method precision.

System precision:

It was performed by analyzing a standard solution of Lamivudine at working concentration 100µg/ml six times. The %RSD was calculated for peak area and Rt.

Method precision:

It was performed by analyzing a standard solution of Lamivudine by injecting 10µl of 100µg/ml concentrations six times. The %RSD was calculated for peak area and Rt.

Preparation of sample solutions for assay.

For the analysis of the dosage form, Weigh a quantity of crushed tablet powder equivalent to 100mg of lamivudine in 100 ml volumetric flask and add 70mL of mobile phase then sonicated for 30min and make up volume with mobile phase. Pipette 1 ml of the clear solution in to 10 mL volumetric flask and make up volume with mobile phase.Weigh a quantity of crushed nanoparticle tablet powder equivalent to 100mg of lamivudine in 100 mL volumetric flask and add 70mL of mobile phase then sonicated it for 30min and make up volume with mobile phase. Pipette 1 mL of the clear solution in to 10 mL volumetric flask and make up volume with mobile phase. Filter both the solution through 0.45µm filter paper. The resulting solutions with six replicate injections were determined by HPLC using calibration curve.

Specificity:

It was performed by interference check by injecting placebo to determine whether any peaks in the placebo are coeluting with the drug peak.

Limit of Detection and Limit of Quantification.

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value**.**The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Stability Studies of lamividine nanoparticle loaded tablets:

The optimized tablets were subjected to stability studies at $40^0C \pm 2^0C$ and 75%±5%RH and they were evaluated for their physical characteristics drug content and in-vitro drug release profiles over a period of 3 months.

RESULTS AND DISCUSSION:

Pre-Compression studies:

Fourier Transform Infra Red Spectroscopy:

The Characteristic peaks of lamivudine pure drug were at 3199.76 cm^{-1}(-OH- stretching), 3075.75 (=C-H stretching), 2840.38(C-H stretching), 3327.93cm^{-1} (-NH-stretching), 1651.81 cm^{-1} (-C=O Stretching in amide), 1160.19 cm^{-1} (C-O-C–Stretching) and 1496.56 cm^{-1}(CH_2 Aromatic bending) as shown in Figure 1.

In the spectrum of physical mixtures of lamivudine, MCC, CCS and PVPK-30, peaks were obtained at

3405.46 cm^{-1}(-OH- stretching), 2929.43 (=C-H stretching), 2881.95(-C-H stretching), 3057.28cm^{-1} (-NH-stretching) 1625.87 cm^{-1}(-C=O Stretching in amide), 1187.53 cm^{-1} (C-O-C–Stretching) and 1484.26 cm^{-1}(CH_2 Aromatic bending) as shown in Figure 2.This indicates that no interaction was occurred between the drug and the excipients.

Differential Scanning calorimetry.
The characteristic peaks due to pure drug lamivudine and physical mixtures of lamivudine, MCC, Crosscarmellose sodium and PVP-K 30, are at 149.2^{o}c & 147.2^{o}c respectively by DSC are shown in Figure 3 and 4.This indicates that no interaction was occurred between the drug and the excipients.

Pre-Compression studies:
Bulk density and Tapped density values were in the 0.361±0.016 to 0.375±0.047 and 0.401±0.017 to 0.427±0.014 gm/cm^3 respectively. This indicates good flow properties of the granules. Angle of Repose for the granules were found in the range of 25.26^0±0.35 to 27.49^0 ±0.47 indicates that blend is free flowing. Carr's Index was in between 9.98±0.17 and 12.71±0.74 indicates that all batches of powder blend shows good compressibility. Hausner's Ratio for the different batches of the granules was in the range of 1.11±0.04 to 1.14±0.47, it also indicates good flow properties of the granules. All the values are within the accepatable limits and shown in table 4.

Post-Compression studies:
The weight variation was in the range of 397.56±1.47 to 399.47±1.08 mg(limits-5% deviation).The hardness was in the range of 5.14±0.49 to 5.94±0.17Kg/cm2(Limits-4-6).The % friability was in the range of 0.26±0.16 to 0.49±0.26 and was within limits(0.5-1).The drug content was in the range of 98.24-98.96 % all the parameters are within the acceptable range. All the values are shown in table 5.

***In-vitro* dissolution studies**
All the formulations were subjected for the invitro dissolution studies as per the United States Pharmacopoeia (USP) dissolution testing apparatus I(Basket Method).The cumulative % drug release was calculated by UV spectroscopy, the results of F1,F2,F3,F4 with different cushioning agents and F5,F6 with different concentrations of MCC, F7 and F8 with different concentrations of disintegrant were found to be 69.25, 63.12, 65.49, 67.63, 68.35,73.65 and 72.84 and 96.22 respectively as shown in the Table 6,7 and 8.Among all the formulations F8 showed maximum release.The results of dissolution profile obtained for the invitro drug release were shown in Figure 5,6 and 7.

HPLC Method development:
Lamivudine was separated by using a Inertsil ODS 3V column, C18 (150x4.6 ID) 5µm**.**The mobile phase pumped at a flow rate of 1 ml/min consisted of Mixed phosphate buffer and ACN (60 : 40) at PH 4 by isocratic elution and UV detection at 273nm.Lamivudine was eluted at 2.4 min and shown in Figure 8.The chromatograms of Lamivudine extracted from Marketed formulation and drug loaded nanoparticle tablet were shown in Figure 9 and 10.

System suitability:
The number of theoretical plates were more than 2000 and it indicates the efficiency of the column. The %RSD for the peak area was less than 2% and less than 1% for retention time .Tailing factor was less than 2% and shown in Table 9.

Linearity:
Calibration curve was plotted by taking concentration on x axis and peak area on y axis and correlation coefficient was found to be 0.999. The Peak area of the drug was linear in the range of 50-150µg/ml and Linearity data and graph of Lamivudine were shown in Table 10 and Figure 11 respectively.

Accuracy:
Recoveries of pure drug from solutions of marketed formulation and nanoparticle tablet were in the range of 99.6-101.6% and 101.1-101.9% respectively using calibration curve and were shown in Table 11 and 12.

Precision:
Method precision:
The %Relative Standard Deviation for peak area and Rt was less than 2 and 1.5 respectively and shown in Table 13.

System precision:
The % Relative Standard Deviation for peak area and Rt was less than 2 and 1.5 respectively and shown in Table 14.

Application of the HPLC method to invitro drug release studies:
The samples withdrawn from pre-selected time intervals from invitro dissolution studies of marketed tablets and formulated nanoparticle tablets were filtered and injected into HPLC. The amount of

lamivudine obtained from the drug release was determined and shown in Table 15 and Figure 12.

Assay of marketed tablets and nanoparticle tablets:
Sample solutions of marketed tablets and nanoparticle tablets were injected six times and the amount of lamivudine was found to be 100.69-101 % and 101.23-101.52% respectively and data is shown in Table 15.

LOD and LOQ:
Limit of Detection and Limit of Quantification was found to be 0.0209 and 0.063 µg /ml.

Specificity:
There was no interference of peaks in placebo with the drug peak and it indicates that the method was specific and chromatogram was shown in Figure 13.

Drug release and Release Kinetics:
The in vitro release pattern of marketed tablets and F8 nanoparticle tablets revealed that the drug release was 96.62% in 24 hours and 98.92 % in 12 hrs respectively. To determine the release model the results of in vitro drug release study of optimized formulation were substituted in equations of zero order, first order, higuchi model and Korsmeyer - peppas model and the results are shown in Figure 14 and Table 16 . Among them first order model showed a high R^2 Value of 0.989, and the plot is linear to Higuchi indicates the release of drug followed first order release Kinetics. To understand the mechanism of drug release, Korsmeyer - peppas equation is applied and it showed good linearity and the release exponent is found to be 0.891. According to this model, if the value of n is >0.85, indicates that the release mechanism is Anomalous transport and shown in Table 17.

Stability Studies of Lamividine Nanoparticle loaded Tablets:
The optimized tablets were subjected to stability studies at $40^0C \pm 2^0C$ and 75%±5%RH the product were evaluated for their physical characteristics like drug content and in-vitro drug release profiles over a period of 3 months and there is no much variation of the parameters and shown in Table 18.

CONCLUSION:

The developed RPHPLC method provides simple, precise, sensitive and reproducible quantitative method for routine analysis of lamivudine in conventional dosage form such as tablets, as well as in novel drug delivery system like nanoparticle tablet. The retention time less than 3 min make this method unique and suitable for analyzing a number of samples in a short period of time. The accuracy and precision were checked by recovery study in both tablets and nanoparticles. High percentage of recovery ensures the method to be free from interference of excipients used in the formulation. The proposed HPLC method was also applied to the determination of lamivudine in release rate studies from nanoparticle tablet. The release kinetics of lamivudine from nanoparticle tablet was in agreement with First order model ensuring the sustained release of the drug from nanoparticles.

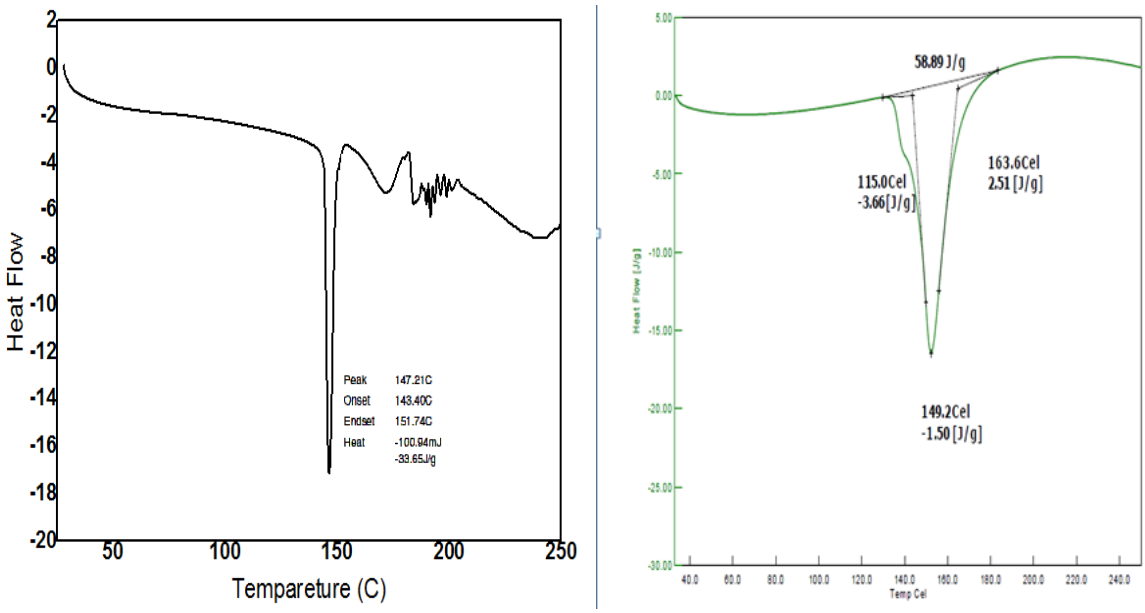


Fig 3 DSC of Pure Drug

Fig 4 DSC of physical mixture of Lamivudine, MCC,CCS and PVP K-30

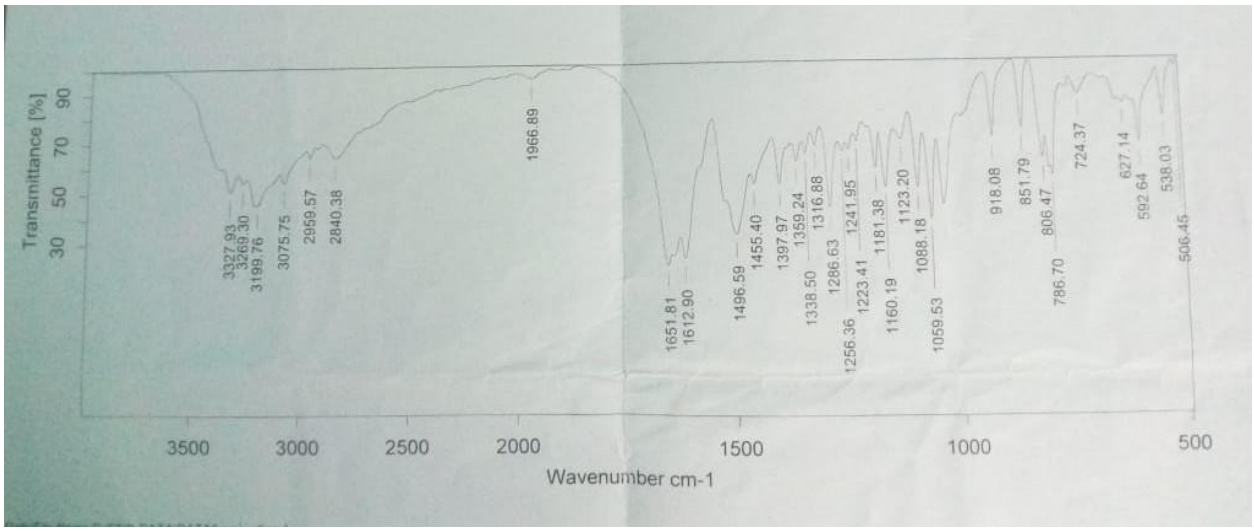


Figure1 FTIR Spectrum of pure drug:

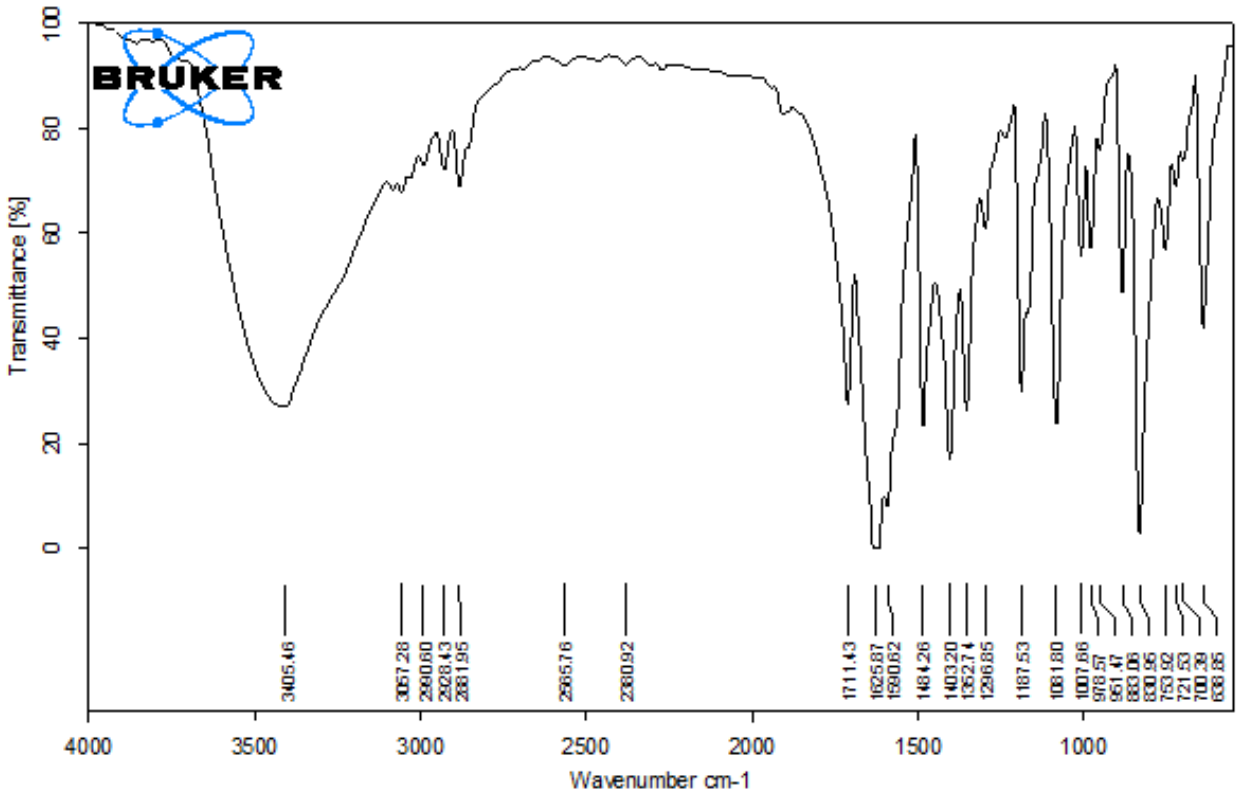


Figure 2 FTIR spectrum of physical mixture of Lamivudine, MCC, CCS and PVP K-30

Table 1: Formulation of Lamivudine Nanoparticle tablets using different Cushioning agents

Ingredients (mg)	F1	F2	F3	F4
Lamivudine	280	280	280	280
MCC	80	-	-	-
Lactose	-	80	-	-
Dibasic Calcium Phosphate	-	-	80	-
Starch	-	-	-	80
PVP K- 30	20	20	20	20
Talc	10	10	10	10
Mg.stearate	10	10	10	10
Total weight	400	400	400	400

Table 2:Formulation of Lamivudine Nanoparticle tablets using different concentration of MCC

Ingredients (mg)	F5	F6
Lamivudine	280	280
MCC	70	90
Talc	10	10
Mg.stearate	10	10
PVP K- 30	30	10
Total weight	400	400

Table 3:Formulation of Lamivudine Nanoparticle tablets using different concentration of disintegrant

Ingredients(mg)	F7	F8
Lamivudine	280	280
MCC	89	88
CCS	1	2
Talc	10	10
Mg.Stearate	10	10
PVP K -30	10	10
Total weight	400	400

Table 4: Precompression parameters of lamivudine tablets

Batches	Micrometric properties of powder blend				
	Angle of Repose (θ)*	**Tapped Density(g/ml)***	**Bulk Density (g/ml)***	**Carr's Index. (%)***	**Hausner's Ratio***
F1	25.59±0.16	0.403±0.021	0.361±0.016	10.42±0.16	1.11±0.04
F2	26.17±0.47	0.417±0.049	0.364±0.017	12.71±0.74	1.14±0.47
F3	25.26±0.35	0.409±0.075	0.362±0.065	11.49±0.65	1.13±0.14
F4	27.49±0.47	0.417±0.036	0.375±0.047	10.07±0.45	1.11±0.32
F5	27.26±0.15	0.412±0.014	0.365±0.059	11.41±0.11	1.12±0.12
F6	26.65±0.25	0.427±0.014	0.374±0.017	12.41±0.25	1.14±0.15
F7	25.47±0.36	0.415±0.026	0.369±0.014	11.08±0.64	1.12±0.65
F8	26.15±0.47	0.401±0.017	0.361±0.026	9.98±0.17	1.11±0.47

Values are expressed as mean±SD,*n=3

Table 5: Postcompression parameters of lamivudine tablets:

Formula	Weight Variation [a](mg)	Hardness[b](kg/ cm^2)	Thickness[b](mm)	Friability[b](%)	Drug content[c](%)
F1	398.14±1.25	5.53±0.03	3.19±0.63	0.29±0.26	98.61±1.26
F2	397.62±2.01	5.74±0.14	3.15±0.26	0.26±0.16	98.01±1.41
F3	398.95±1.54	5.94±0.17	3.26±0.17	0.36±0.24	98.80±10.1
F4	399.47±1.08	5.64±0.26	3.14±0.26	0.41±0.14	98.82±0.95
F5	398.65±1.91	5.25±0.14	3.48±0.14	0.52±0.23	98.24±0.81
F6	399.15±1.32	5.14±0.49	3.49±0.26	0.45±0.14	98.23±1.34
F7	399.47±2.12	5.54±0.85	3.47±0.14	0.49±0.26	98.84±0.92
F8	397.56±1.47	5.56±0.65	3.15±0.16	0.41±0.10	98.96±0.74

a-weight variation=20,b-hardness,friability,thickness=10,c-assay=6

Table 6 Cumulative percentage drug release of Lamivudine tablets using different Cushioning agents:

Time(hrs)	F1	F2	F3	F4
0	0	0	0	0
0.5	6.16±0.26	3.25±0.23	4.12±0.32	4.99±0.21
2	13.72±0.65	9.62±0.15	9.16±0.26	11.26±0.13
4	21.26±0.48	16.45±0.32	17.87±0.48	23.14±0.45
8	35.14±0.95	30.56±0.56	29.68±0.11	36.59±0.84
12	47.36±0.65	41.65±.0.44	42.89±0.23	48.71±0.56
16	58.95±0.54	52.16±0.54	54.12±0.16	57.14±0.54
24	69.25±0.36	63.12±0.48	65.49±0.28	67.63±0.32

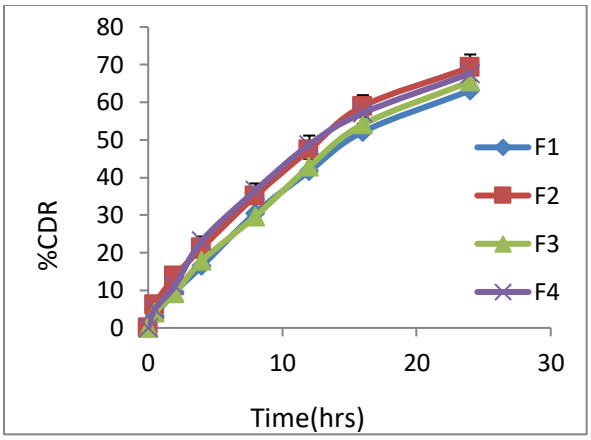


Fig 5: Dissolution profile of Lamivudine tablets using different Cushioning agents.

Table 7 Cumulative percentage drug release using different concentration of MCC

Time(hrs)	F5	F6
0	0	0
0.5	5.45±0.15	2.56±0.43
2	11.69±0.49	13.75±0.14
4	22.74±0.15	24.49±0.48
8	31.95±0.26	36.47±0.12
12	43.45±0.54	49.69±0.32
16	55.67±0.69	61.14±0.54
24	68.35±0.58	73.65±0.56

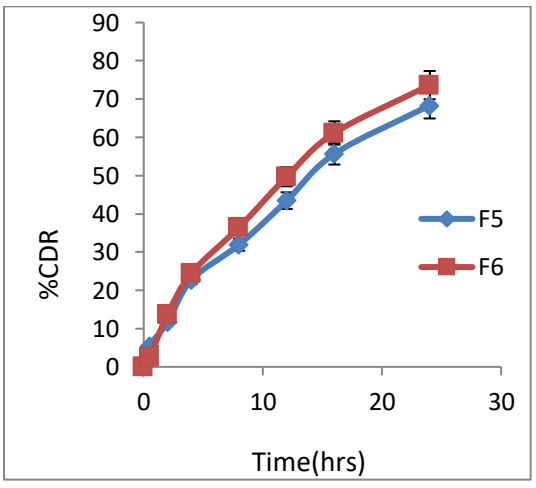


Fig 6: Dissolution profile of Lamivudine tablets using different concentrations of MCC

Table 8 Cumulative percentage drug release using different concentration of disintegrant

Time(hrs)	F7	F8
0.5	0.26±0.32	3.23±0.16
2	15.57±0.14	15.72±0.11
4	22.42±0.15	29.69±0.68
8	36.54±0.36	41.02±0.45
12	52.57±0.65	55.36±0.65
16	68.62±0.32	69.54±0.35
24	72.84±0.14	96.22±0.56

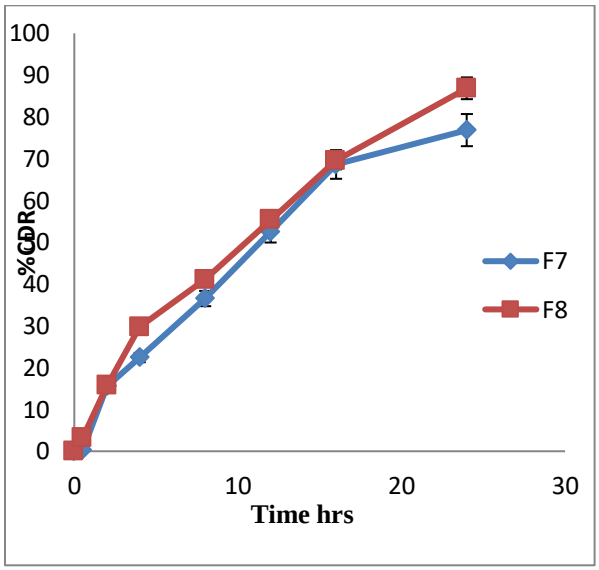
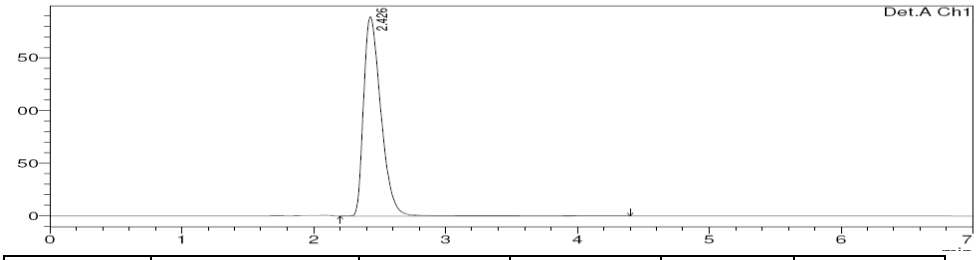


Fig 7: Dissolution profile of Lamivudine tablets using different concentrations of disintegrant



Retention time	Theoretical plates/meter	Area	Tailing factor	Height	Area %
2.426	10263.153	1731259	1.537	189138	100.000
		1731259		189138	100.000

Figure 8 Chromatogram for lamivudine standard

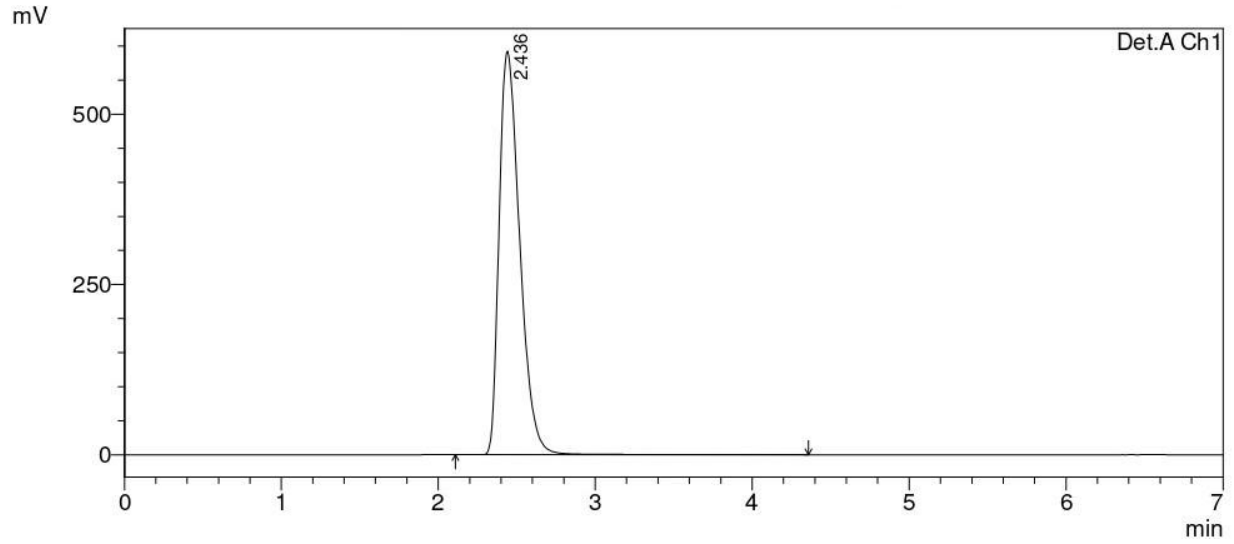


Figure 9 chromatogram for lamivudine extracted from Marketed Formulation

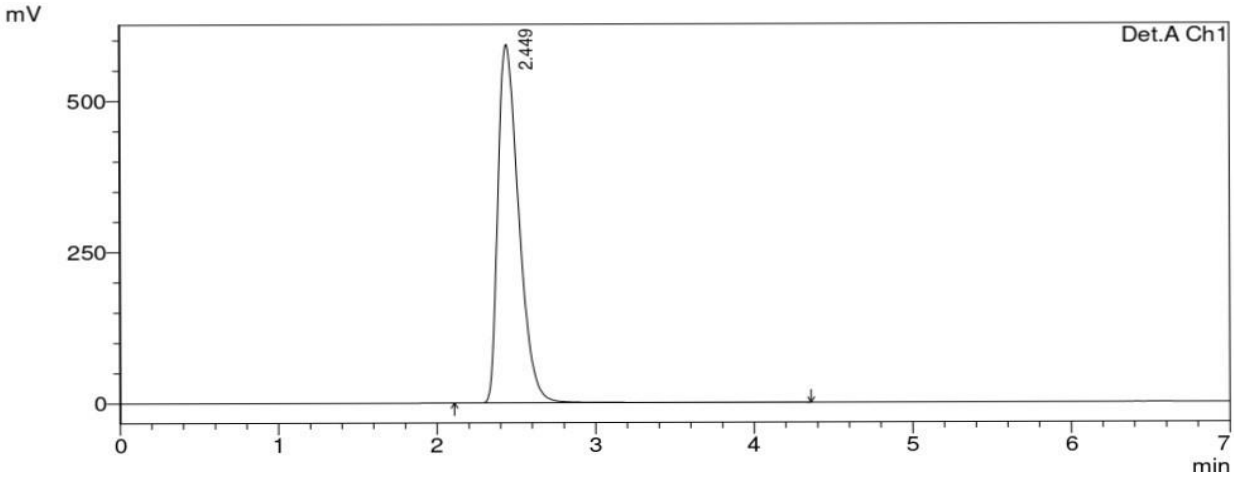


Figure 10 chromatogram for lamivudine extracted from NP tablet Formulation

Table 9 System suitability data for Lamivudine

S.No.	RT	Peak Area
1	2.416	1727842
2	2.417	1720774
3	2.416	1725494
4	2.418	1728126
5	2.419	1722379
6	2.417	1727823
Avg	2.417	1725406.333
SD	0.001	3155.956
%RSD	0.048	0.183
Theoretical plates	10260	>2000
Tailing Factor	1.53	<2

Table 10 Linearity Data

S.No	Conc	Area
1	50	578034
2	80	1134547
3	100	1728925
4	120	2257388
5	150	2814101

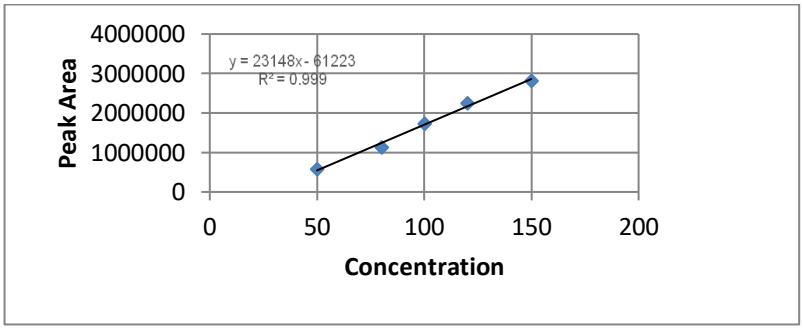


Figure 11 Linearity range for Lamivudine

Table 11 Recovery data for marketed tablets

% Level	Peak Area	Amount of sample taken(µg)	Amount of standard spiked(µg)	% Recovery	
50%	2621968	100	50	99.6	
50%	2613257	100	50	99.78	Mean =99.83
50%	2620392	100	50	100.13	%RSD= 0.269/99.83 =0.269
100%	3476191	100	100	100.78	
100%	3473853	100	100	100.8	
100%	3475361	100	100	100.79	%RSD=0.009
150%	4325467	100	150	101.5	
150%	4320817	100	150	101.5	
150%	4320249	100	150	101.6	%RSD=0.056

Table 12 Recovery data for Nanoparticle tablets:

% Level	Area	Amount of sample taken(µg)	Amount of standard spiked(µg)	% Recovery	
50%	2631208	100	50	100.17	
50%	2625497	100	50	100.48	Mean =100.276
50%	2623632	100	50	100.18	%RSD= 0.176/100.27 =0.175
100%	3482431	100	100	101.1	
100%	3482093	100	100	101.2	
100%	3483201	100	100	101.28	%RSD=0.089
150%	4331707	100	150	101.8	
150%	4329057	100	150	101.8	
150%	4327609	100	150	101.9	%RSD=0.056

Table 13: Method precision for Lamivudine

S.No.	RT	Peak Area
1	2.419	1728661
2	2.419	1722379
3	2.417	1723820
4	2.418	1728126
5	2.417	1722272
Avg	2.417	1727823
SD	2.4178	1725513.5
%RSD	0.000983	3008.86

Table 14 System precision for Lamivudine

S.No.	RT	Peak Area
1	2.417	1731191
2	2.416	1727842
3	2.417	1726965
4	2.416	1725494
5	2.417	1721872
Avg	2.417	1720774
SD	2.4167	1725689.67
%RSD	0.00052	3880.73

Table 15 Assay results of commercially available tablets and optimized nanoparticles.

S.No.	Drug content in tablet (%)	Drug content in nanoparticles tablet(%)
1	100.98	101.52
2	100.69	101.40
3	101.04	101.23
4	100.93	101.29
5	100.87	101.34
6	100.53	101..2
Avg	100.84	101.356
SD	0.193	0.111
%RSD	0.191	0.110

Table 16 Cumulative percentage drug release of optimized Lamivudine NP tablets & marketed Lamivudine tablets

Time(hrs)	F8	Marketed formulation
0.5	2.57	13.2
2	18.64	26.3
4	27.98	57.3
8	41.65	76.94
12	61.99	98.92
16	77.48	
20	84.96	
24	96.62	

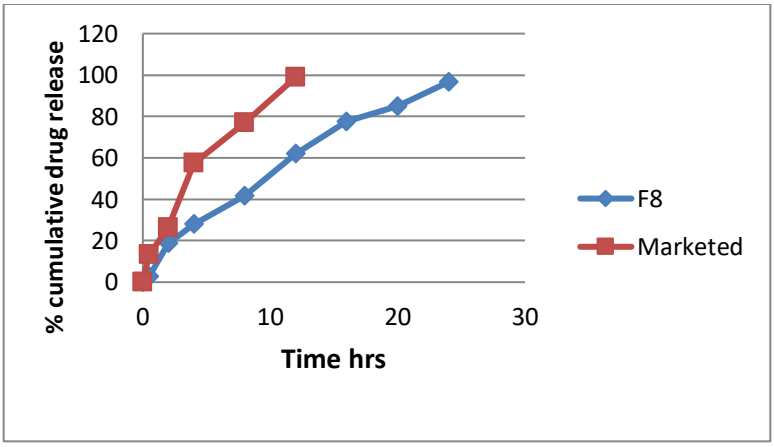


Figure 12 Dissolution profile of marketed and nanoparticle Lamivudine tablets

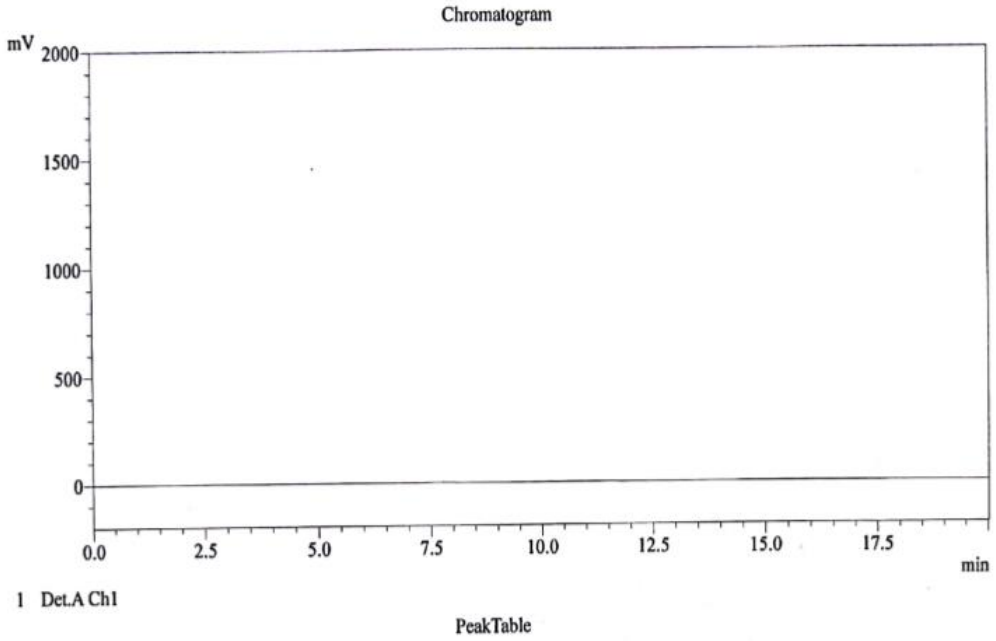
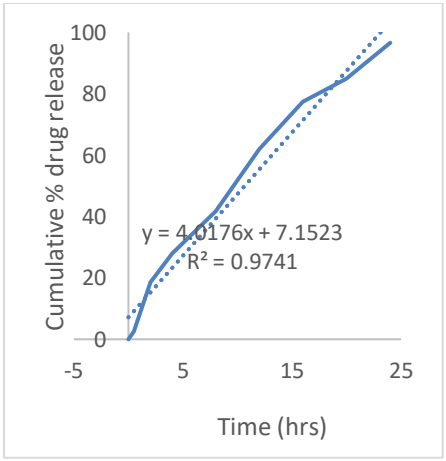
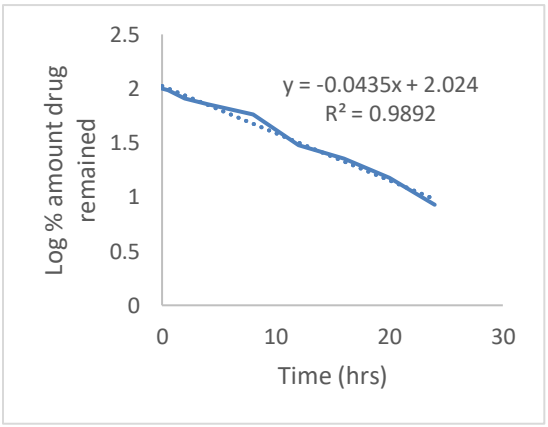


Figure 13 specificity

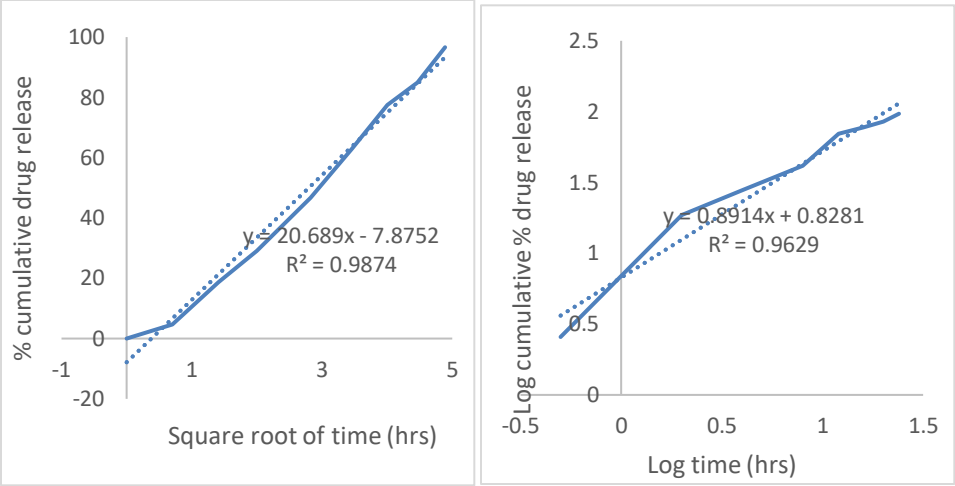
Figure 14 Kinetic assessment of dissolution data for F8 Formulation by HPLC



Zero order



First order



Higuchi Plot **Korsmeyer Peppas Plot**

Figure 14 Kinetic assessment of dissolution data for F8 Formulation by HPLC

Table 17 Model dependent kinetics for F8 optimised formulation

Formulation	R2(ZERO ORDER)	R2(FIRST ORDER)	R2(Higuchi model)	R2(KP MODEL)	N(RELEASE RATE CONSTANT)
F8	0.974	0.989	0.987	0.962	0.891

Table 19 Stability Studies of Lamividine Nanoparticle loaded Tablets

Parameter	F8 Initial	F8 1st Month	F8 2nd Month	F8 3rd Month
Drug content	98.96±0.74	98.84±0.54	98.73±0.46	98.56±0.4
%Drug release after 24 hrs	96.62±0.56	96.61±0.08	96.58±0.32	96.28±0.12

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