"FORMULATION AND EVALUATION OF DACLATASVIR NANOSUSPENSION"

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Abstract

Dacetasvir (DCV) is a potent antiviral agent used for the treatment of hepatitis C virus (HCV) infection. However, its poor aqueous solubility and low bioavailability pose challenges in achieving optimal therapeutic efficacy. In this study, we aimed to develop and evaluate a nanosuspension formulation of DCV to improve its solubility, dissolution rate, and bioavailability.The nanosuspension formulation was prepared using a top-down approach, employing a high-pressure homogenization technique. A combination of hydrophilic and hydrophobic stabilizers was incorporated to enhance the physical stability and prevent particle aggregation. The particle size, polydispersity index, zeta potential, and morphology of the nanosuspension were characterized using dynamic light scattering and transmission electron microscopy.In conclusion, the developed DCV nanosuspension showed promising results in terms of particle size reduction, enhanced dissolution rate, and improved bioavailability and stability. The optimized formulation (F6) shered particle size of PDI 0.32 And Drug release 96.54% contrast pure drug formulation which has 99.45% This nanosuspension formulation holds great potential for overcoming the solubility and bioavailability challenges associated with DCV, ultimately leading to improved therapeutic outcomes for patients with HCV infection.

Keywords: dacetasvir, nanosuspension, formulation, evaluation, hepatitis C virus (HCV), highpressure homogenization, nanoparticle characterization.

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INTRODUCTION

Daclatasvir is a direct- acting antiviral agent used to treat habitual Hepatitis C Virus(HCV) genotype 1 and 3 infections. It's retailed under the brand name DAKLINZA and is available as the hydrochloride swab in diurnal oral tablets. Hepatitis C is an contagious liver complaint caused by Hepatitis C Contagion infection(HCV). HCV is a single- stranded RNA contagion classified into nine distinct genotypes, with genotype 1 being the most current in the United States, affecting 72 percent of all habitual HCVpatients.The nonstructural 5A(NS5A) protein is a target for medicine development against hepatitis C contagion(HCV).still, NS5A has no given enzymatic functions, making it delicate to understand daclatasvir's mode of action(MOA) and to estimate its antiviral effectiveness.[1]

List to the N- boundary of the D1 sphere of NS5A prevents its commerce with host cell proteins and membranes, which is necessary for assembly of the replication complex. Daclatasvir disrupts the function of new HCV replication complexes by modulating the NS5A phosphorylation status 3. The most current critical NS5A amino acid negotiations that reduced vulnerability to daclatasvir remedy passed at position Q30(Q30H/ K/ R) and M28 in genotype 1a cases, and at position Y93H in genotype 3 cases.[2]

Introduction to Bioavailability

Bioavailability is the pace and degree to which an active component is absorbed from a drug product and becomes accessible at the site of action [2].Compared to the bioavailability statistics for a solution, suspension, or intravenous dosage form, the bioavailability data for a specific formulation provide an estimate of the relative proportion of the orally delivered dose that is absorbed into the systemic circulation.In addition, bioavailability studies give further relevant pharmacokinetic information on distribution, elimination, effects of nutrients on drug absorption, dosage proportionality, and linearity in the pharmacokinetics of the active and inactive moieties.Bioavailability of a medicine is governed mostly by the qualities of the dosage form, not by the physicochemical properties of the drug, which dictate absorption potential.Differences in bioavailability across formulations of a particular medication might have therapeutic importance; therefore, it is vital to determine if drug formulations are similar. Insufficient dissolving inside the gastrointestinal system, which is important for optimal oral bioavailability, is becoming an increasing concern with poorly water-soluble medications. [3].

Nanosuspension:

Still, pharmacokinetic examinations of BCS class – II specifics revealed that they had a low oral bioavailability, which may be a result of the drug's poor water solubility. Dissolution in arid mixes including an organic soap(4), product of ßcyclodextrin complexes(5), solid dispersion(6), and drug tar form(7) are some of the conventional medicinal styles for enhancing drug dissolution rates. A nanosuspension is truly finely dispersed solid medicine patches in an arid medium intended for oral, topical, parenteral, or pulmonary delivery. generally, the flyspeck size distribution of solid patches in nanosuspensions is lower than one micron, with an average flyspeck size ranging from 200 to 600 nm(8).

MATERIALSANDMETHODS: [9-16]

Preformulation:

1.Authentication of Drug:

- Authentication of Drug by IR: The obtained drug sample was subjected to IR and Spectrum of sample was compared with standard spectrum of Drug. Excipient compatibility study of physical mixture of drug & excipient was performed and analysed using FTIR.[9]
- Authentication of Drug by Melting point: Melting of the drug was obtained by using melting point apparatus.

2. **Determination of Wavelength:** Wavelength of the drug was determined by dissolving the drug in the 0.1 N HCl and analysing it using UV Visible spectrophotometer from 200-800 nm.
3. **Construction of Calibration Curve of drug:** Calibration of curve was constructed by dissolving the drug in 0.1 N HCl in different concentration ranges and respective absorbance was obtained and plotted a graph of concentration Vs absorbance.[10]

2. Preparation of Nanosuspension:

The drug was dissolved in the methanol to prepare an organic solution. The polymer and the surfactant were dissolved in the sufficient quantity of water to prepare an aqueous phase. The aqueous phase was kept under a high pressure homogenizer at room temperature. The organic solution is added drop wise through a syringe to the aqueous solution. The rotation speed of homogenizer was maintained at 11000 rpm for 2 hrs. Nanosuspension was formed; organic phase i.e. methanol got evaporated as the temperature of the solution rose due to homogenization.[11]

3. Evaluation of Nanosuspension:

a) Appearance:

The Appearance of the nanosuspension was observed visually.

b) Redispersibility:

Nanosuspension was stored in vials and determined by tipping the vial bottle over and down with hand till the deposition was slightly dispersed in the waterless phase and the number of times listed was noted.12]

C) Viscosity:

The Nanosuspension was achieved by utilizing the Brookfield viscometer set the spindle No- 60 at 100rpm.

d) Saturation solubility study:

To determine the saturation solubility of the nanosuspension, it was placed in a vial and stirred with a magnetic stirrer at 100 revolutions per minute for 48 hours to guarantee saturation. The nanosuspension was then transferred to an Eppendorf tube and centrifuged at 10,000 RPM for 30 minutes. The supernatant was filtered through a 0.2m syringe filter and examined using a UV-visible spectrophotometer [UV-1800, Shimadzu, Japan] at max of the drug after appropriate dilution with dissolving fluid that served as a blank.[13] Assay:

Assay:

An aliquot (1ml) of the prepared nanosuspension equivalent 2 mg of the drug was diluted in 100 ml methanol and filtered with a 0.2 µm filter. Total drug content was determined by UV spectrophotometer at λmax of the drug. Similar concentration of standard solution was prepared and the absorbance of standard solution was used to calculate % Assay by using following formula;

$$\% \text{ Assay} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

Particle Size:

The mean particle size of the generated nanosuspension was measured using Zetasizer, which is based on the light diffraction principle and is also known as Photon Correlation Spectroscopy (PCS). Prior to measurement, the samples were diluted with water to the required scattering intensity and re-dispersed by shaking.[14]

Zeta Potential:

The Zeta potential is a measurement of the electric charge at the particle's surface, which indicates the physical stability of colloidal systems. aqueous dispersions with zeta potential levels exceeding |30mV| are electrostatically stable over the long term. In this investigation, the Zeta Potential was determined by measuring the electrophoretic mobility of the particles with Zetasizer.[15]

PowderX-ray Diffraction:

PXRD diffractograms of optimized nanosuspension were recorded using analytical XRDGoniometer using K-beta filter.

In-vitro Drug Release:

i) All formulations use USP category II dissolving apparatus, Dissolution medium 900ml of 0.1N HCL at 50 rpm. Temperature was maintained at 37 0.5 °C. Sampling was performed every 0.5 to 8 hours at a predetermined time interval. Five millilitre aliquot samples were obtained

4. Stability Study of the Optimized Nanosuspension:

The optimized nanosuspension was subjected to stability study at 40°C and 70% Relative Humidity for 3 months in stability chamber. The samples were evaluated for drug content every month.[16]

RESULT

1. Pre-formulation Studies:

a.Authentication of Daclatasvir Drug by IR Spectrum:

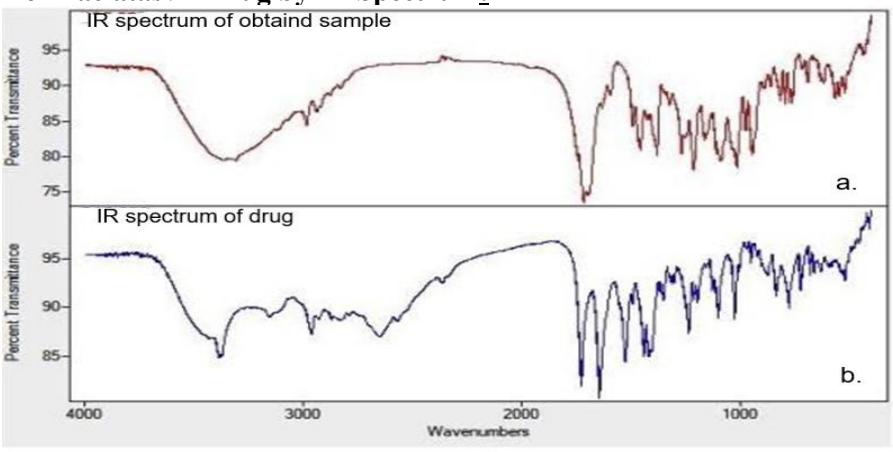

Fig-1: FTIR spectrum of a) physical mixture b) pure drug

The Obtained sample of Daclatasvir was subjected to FT-IR Analysis. The Figure a depicts the IR Spectrum of the obtained Daclatasvir and it was compared with Standard Spectrum from Indian Pharmacopoeia 2018 and it matched. In Figure b the physical mixture of drug and excipients showed compatibility between drugs and excipients. The major peaks of Daclatasvir were observed at 3234 for –OH group, 3370 for –N-H group, 2948 for –C-H group, 1688.8 for –C=O group and 1437 for –C=C group.

b.Authentication of Drug by Melting point:
The Melting Point of the obtained sample was found to 157.3°C. It was found to be within the range of the standard Daclatasvir. Hence, it was confirmed the obtained Daclatasvir is pure.

Preparation of Nanosuspension:
Nanosuspension of Daclatasvir. The prepared formulations were kept at room temperature for 24 hours for stabilization in an amber coloured container

Table No. 2. Formulation for Nanosuspension

Sr No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Daclatasvir (mg)	5	5	5	5	5	5	5	5	5
2	HPMC E15 (mg)	70	80	90	-	-	-	40	50	60
3	PVPK90 F (mg)	-	-	-	70	80	90	60	50	50
4	Sodium Lauryl Sodium (mg)	300	200	100	500	300	200	500	300	200
5	Methanol (ml)	8	8	8	8	8	8	8	8	8
6	Water (ml) (q.s. upto)	40	40	40	40	40	40	40	40	40

a. Appearance:
The appearance of the prepared formulations was observed visually. All the formulations appeared translucent of the nanoparticles of the Daclatasvir suspended all over the formulation. Table 3 shows the results for the appearance.Other stabilizers showed good stabilization as the nanosuspension did not sediment during the time of visual observation.

Table No. 3: Results of Nanosuspension

Batches	Appearance	Redispersibility	Viscosity (cps)	Saturation Solubility (mg/ml)	(%) Drug Content
F1	Translucent	Fast	0.521	0.52	91.24
F2	Translucent	Very Fast	0.756	0.35	92.66
F3	Translucent	Medium	0.984	0.74	87.25
F4	Translucent	Medium	0.765	0.65	97.24
F5	Translucent	Very Fast	0.513	0.58	102.55
F6	Translucent	Very Fast	0.412	0.96	99.54
F7	Translucent	Medium	0.621	0.65	86.19
F8	Translucent	Very Fast	0.743	0.71	94.34
F9	Translucent	Very Fast	0.963	0.82	98.74

b. Redispersibility:
Nanosuspension formulations F2, F5, F6, F8, and F9 exhibit excellent redispersibility, which means that when they are shaken or agitated after settling, they disperse very quickly. This property is highly desirable for nanosuspensions, especially in pharmaceutical and biotechnological applications, where stable and rapid redispersion is crucial for drug delivery and therapeutic effectiveness.

The fast redispersibility of formulations F2, F5, F6, F8, and F9 indicates their stability and homogeneity. When these formulations settle over time, perhaps due to gravity or other factors, they quickly and uniformly disperse back into the liquid phase when subjected to shaking or agitation. This property is particularly valuable for pharmaceutical formulations as it ensures that the drug remains evenly distributed and available for effective delivery upon administration.

a. Viscosity:
The viscosity results are mentioned in Table No. 3 The Viscosity of the formulation F5 and F6 were found to 0.5133 and 0.412 cps which was lower compared than other batches.A lower viscosity means that the fluid flows more easily, while a higher viscosity suggests that the fluid is thicker and flows less easily. In the context of nanosuspensions, viscosity plays a crucial role in their behavior and performance.

b. Saturation solubility study:
The Saturation solubility of the formulation batches F3, F6 and F9 were found to be 0.74, 0.96 and 0.82 mg/ml. The Saturation solubility of the different formulations is given in Table No.3 The improvement in the saturation solubility is due to the reduction in the particle size and subsequent increase in the surface area. Hence, it can be

assumed that this increase in saturation solubility will increase the bioavailability.

c. Drug Content:

During the medication process there was no any medicine loss step involved, so theoretically the expression was considered as being 100 medicine content. Table 3, demonstrates the medicine content for each nanosuspension expression prepared. The medicine content of different phrasings is given in tableno. 3. phrasings F4, F5, F6 and F9 showed the medicine content between the ranges of 97- 103, whereas rest of the phrasings had medicine content of lower that 95. Hence, these batches were named for the farther studies of patches size and zetapotential.According to the data of Table 3, it can be seen that all the medicine content were within respectable limit.

Table No.4: Results for Particle size and Zeta Potential

Batches	Zeta Potential (mV)	Particle Size (nm)
F1	18.25	450.22
F2	20.35	322.14
F3	21.24	312.98
F4	17.25	354.21
F5	13.45	215.22
F6	14.52	194.35
F7	16.84	542.55
F8	19.25	421.65
F9	17.74	326.69

d. Polydispersity index (PDI)

The PDI valu shows the particle size distribution of the formulation. PDI ranges between 0 (monodisperse) to 1 (veryheterodisperse), and value less than 0.4 is the optimumvalue. The mean particle size, PDI, and % EE of the optimizedformulationwas 194.35 nm, 0.322, and 99.22%, respectively.These nanoparticles tend to form coalescence. Surfacecharge, zeta potential measurements give an indicationabout the coalescence behavior of the Nanosuspension. They showed a pronounced shift towards negative values,14.52 mV. The higher negative value of zeta potentialdemonstrates the stability of behavior with respect to aggregation, it will not form coalescence[17]

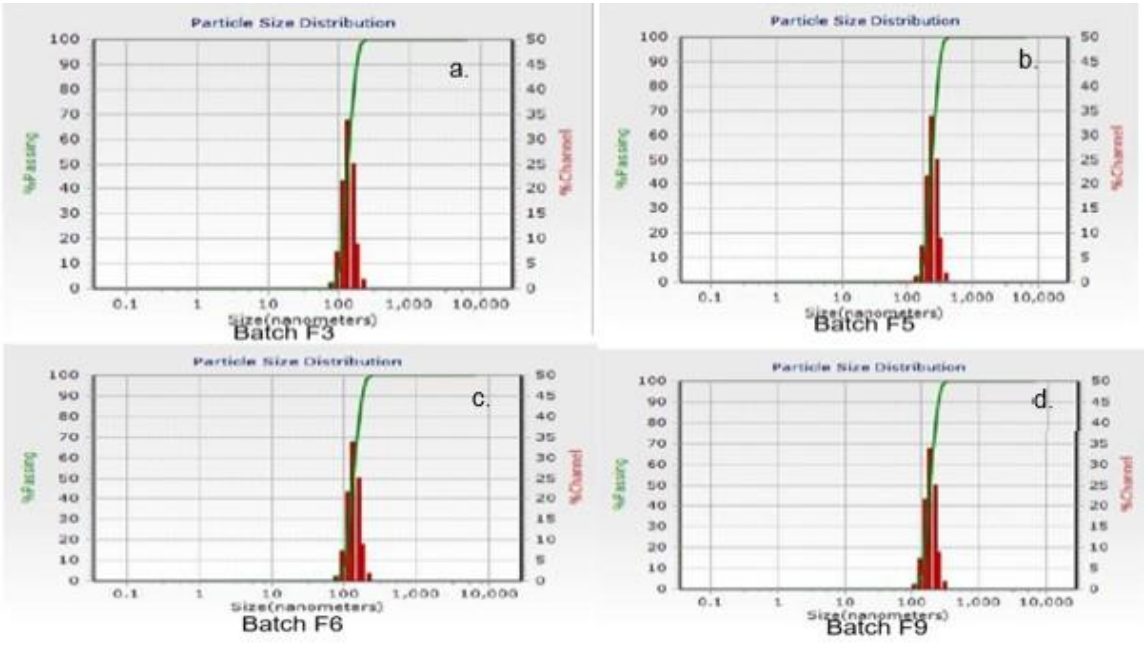

Fig-4 Particle size of F3(a),F5(b),F6(c) and F9(d) batch

e. Particle Size:

The Particle size of different batches is mentioned in table no.4. Figures 4 depict the particle size of the various formulations. The Particle size measurement was carried out using a zetasizer. The lower the particles size the more the surface area and better bioavailability. Batch F6 showed the least particle size of 194.35 nm.Overall, the particle size of 194.35 nm observed in Batch F6 indicates a promising nanosuspension formulation with potential advantages in terms of surface area, bioavailability, and drug delivery efficiency. It could be a promising candidate for further development and evaluation as a drug delivery system.

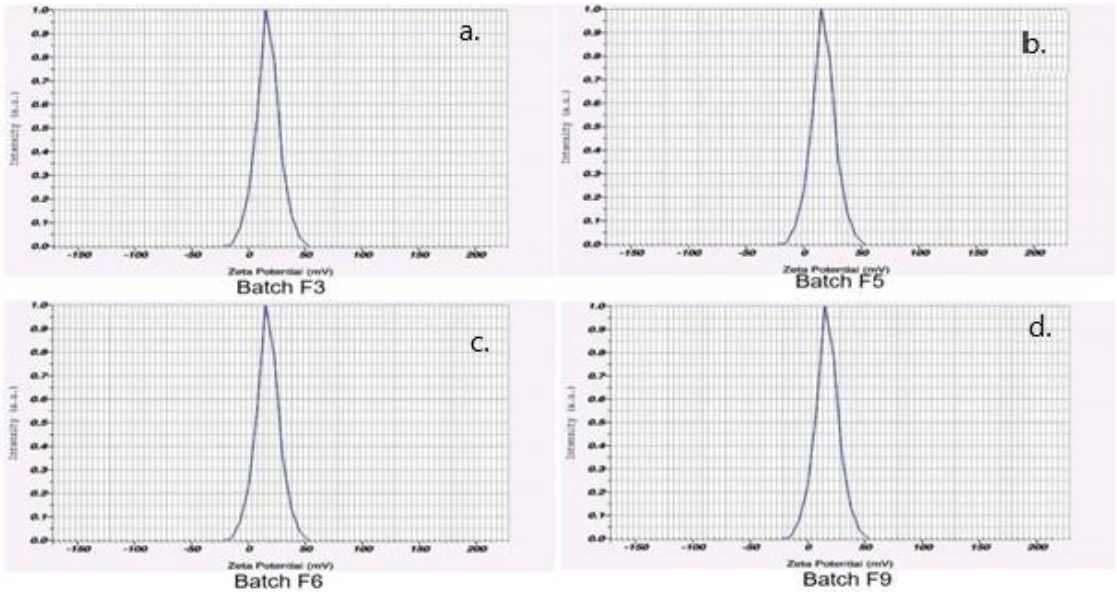

Fig-5 Zeta Potenial of F3(a),F5(b),F6(c) and F9(d) batch

f. Zeta potential:

The Zeta potential of different formulations is mentioned in table no. 4 . The zeta potential shows the particle charge possessed. Figures show the Zeta Potential graphs of different formulations. The zeta potential of all the formulations was found to be positive showing good stability.A positive zeta potential indicates that the particles possess a net positive charge on their surface. This positive charge leads to electrostatic repulsion among the particles, preventing them from coming too close to each other and thus inhibiting aggregation or flocculation. As a result, nanosuspensions with positive zeta potential tend to exhibit good stability and are less likely to undergo particle aggregation or settling over time.

g. X-Ray Diffraction:

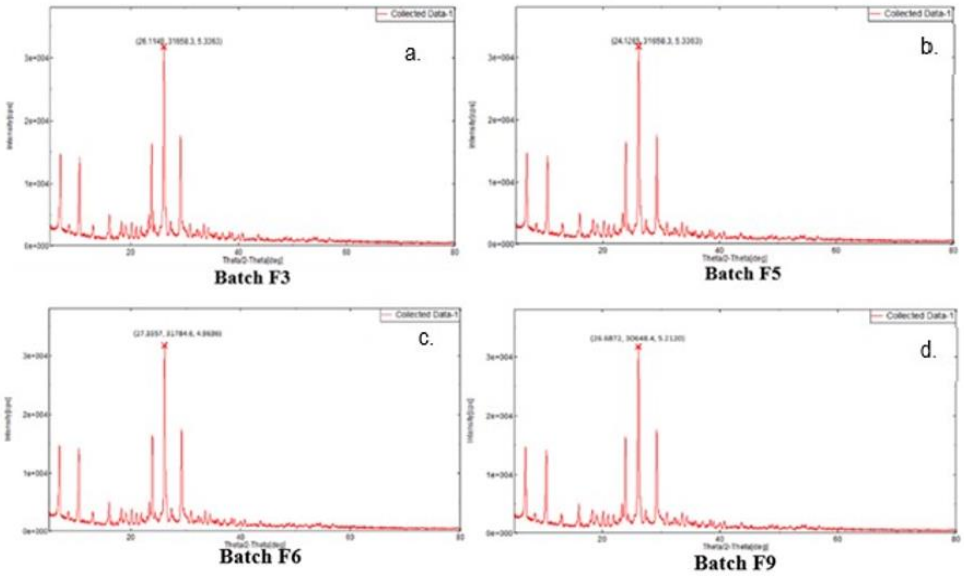

Fig-6.P-XRD of F3(a),F5(b),F6(c) and F9(d) batch

Figure 6 depicts P- XRD of the Nanosuspension displaying sharp peaks at around 26 Theta/ 2-Theta(deg) which are characteristics of crystallinecompound.PXRD was used to research the physical nature of the medicine in the nanosuspensions. From the PXRD graphs, it was observed that the crystallanity of the medicine was changed in the nanosuspensions(Figure 6). The peaks attained for pure medicine was veritably clear and sharp and the intensity of the peaks was veritably high when analogized with peaks of ezetimibe nanosuspensions. Reduction in the peak intensity indicates the change in crystal clear structure. From this, we can conclude that there was reduction in the crystallanity and change into unformed structures upon fabricating into ezetimibe nanosuspensions. Nanosuspensions of ezetimibe weren't preliminarily devlped.

h.In-Vitro Drug release:

The formulations F3, F5, F6 and F9 were subjected to in-vitro drug release. The Drug release study was performed for 8 hours. The Study showed that the batch F3 showed 96.45% of drug release overthe period of the 8 hours.F5, F6 and F9showed the drug release of 85.24%, 96.45% and 75.82% respectively. In-vitro drug release studies are essential preliminary assessments for nanosuspensions to predict their behavior in the human body and guide further formulation development and optimization before proceeding to in vivo studies and clinical trials.

Table no.5 Results for Drug Release Studies

Time (hrs.)	Pure Drug	F3	F5	F6	F9
0	0	0	0	0	0
0.5	3.44	7.54	8.22	10.25	7.41
1	8.47	14.36	15.74	19.35	13.55
2	12.63	19.35	21.36	28.47	18.69
3	16.52	26.87	28.59	39.81	24.32
4	21.68	38.74	42.65	47.62	36.57
5	27.65	45.68	54.84	59.31	47.24
6	33.74	56.78	62.57	72.08	56.56
7	42.98	64.27	74.25	85.94	62.74
8	47.55	72.96	85.24	96.45	75.82

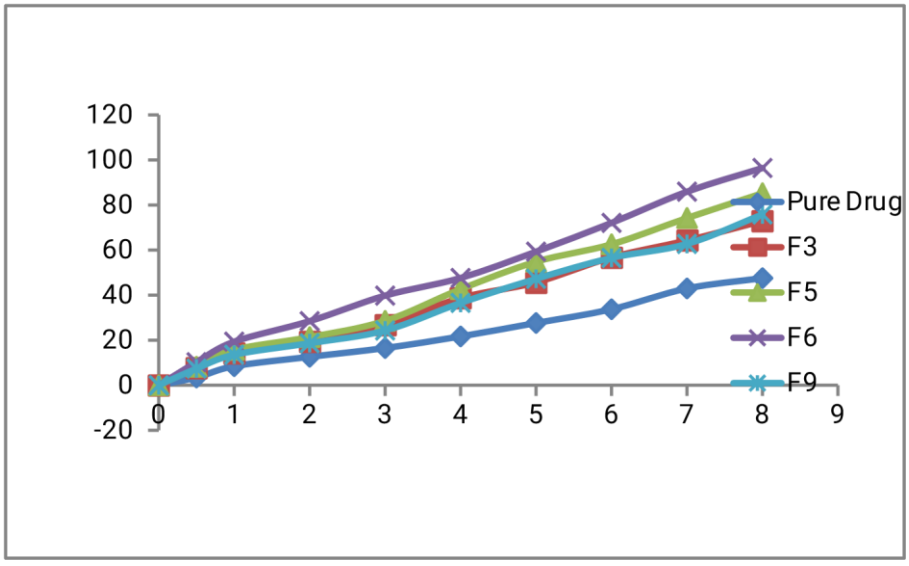

Fig- 7:-Drug Release Studies

I. Stability Studies:

The Stability study was performed for 3 months at 40℃ and 70% Relative Humidity and at time interval of every month drug content of the formulation F6 was performed. The results for stability study are mentioned in Table no.6

Table no.6 :-Results of Stability Study

Sr. No.	Time interval	% Assay
1	0 day	100.32
2	1 month	99.58
3	2 month	98.74
4	3 month	99.22

Based on the results, we can conclude that the formulation F6 is stable over the three-month period at 40℃ and 70% Relative Humidity. The drug content (% assay) remained within an acceptable range throughout the study, indicating that the formulation is robust and can withstand the specified storage conditions. However, it's essential to keep in mind that this conclusion is based on the data presented in Table no.6, and other aspects of stability testing should also be considered before making a final determination.

Summary:

The DACLATASVIR has a poor bioavailability and hence was selected for the formulation of Nanosuspension. The DACLATASVIR was obtained from Aadhaar Life Sciences Pvt. Ltd. as gift sample.

As preformulation studies, the obtained sample was authenticated by FT-IR and melting point. After authentication, the DACLATASVIR sample was subjected to wavelength determination and standard calibration curve was constructed using UV -Visible Spectrophotometer.

The DACLATASVIR Nanosuspensions were prepared by using HPMCE15 and PVPK90F as Polymer, Sodium Lauryl Suphate was used as Surfactant, Methanol was used as organic solvent, and water was used as aqueous solvent. The Nanosuspensions were formulated using High Pressure Homogenization technique.

The Prepared Nanosuspensions was evaluated for different parameters like Appearance, Redispersibiility, Viscosity, Saturation solubility, and Drug content. From the results it was found that formulation F3, F5, F6 and F9 were having better characteristics as compared to other formulations.

Further, these formulations were subjected to evaluations like Particle size, Zeta Potential and In-vitro drug release. Particle Size of the F6 batch was found to be 194.35 nm and zeta potential was found to be 14.52 where the positive charge showed that it can be stable formulation. The Drug release of batch F3 showed 96.34% of drug release over the period of 8 hours.

Batch F6 was further subject to stability study for 3 months at 40℃ and 70% Relative Humidity and was evaluated for Drug content at different time intervals.

From the stability data it was found that the formulation was stable for 3 months.

Conclusion:

The DACLATASVIR is used for the treatment of the Hypertension. The drug has a poor oral bioavailability of approximately <10%. Hence, an attempt was made to formulate a Nanosuspension of DACLATASVIR using High Pressure Homogenization technique. Different polymers like HPMCE15 and PVPK90 F was used. The formulated Nanosuspension was evaluated for different parameters like Appearance, Redispersibiility, Viscosity, Saturation solubility, Drug content, Particle Size, Zeta Potential and In-vitro drug release studies. From the evaluation parameters, Batch F6 was selected as an optimized batch and subjected to Stability study for 3 months and it was found to be stable. Therefore, it can be concluded that Nanosuspension can be a potential candidate for the delivery of DACLATASVIR for the treatment of hypertension.

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