FORMULATION DEVELOPMENT AND OPTIMIZATIONOF S-SEDDS TO IMPROVE SOLUBILITY AND BIOAVAILABILITY OF THE POOR WATER SOLUBLE DRUG BICTEGRAVIR SODIUM.

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Abstract: *The aim of the present study was to formulate, optimize and evaluate the solid self-emulsifying drug delivery systems (S-SEDDS) of Bictegravir Sodium by use of factorial designs to enhance the oral absorption of Bictegravir Sodium by improving its solubility, dissolution rate, and diffusion profile.SEDDS are the isotropic mixtures of oil, surfactant, co-surfactant and drug that form oil in water microemulsion when introduced into the aqueous phase under gentle agitation. Solubility of Bictegravir Sodium in different oils, surfactants, and co-surfactants was determined for the screening of excipients. Formulations were developed based on the optimum excipient combinations with the help of data obtained through the maximum micro emulsion region containing combinations of oil, surfactant, and co-surfactant. The optimum formulation of L-SEDDS contains Capmul MCM (16.58 mg/ml) was selected among the screened vehicles as an oil system with the highest solubilisation potential. As surfactants, PEG 400 was used as surfactants in about 80 (13.25 mg/ml) and Polyethylene glycol (17.48 mg/ml). Adsorbents including aerosil 200 and Neusilin US2 have been used in the optimised liquid SEDDS formulation (F12). Drop wise to the solid, adsorbent, and blended in a mortar and stick, optimized liquid SEDDS.The S-SEDDS was evaluated for different parameters. Overall, this study suggests that the dissolution and oral bioavailability of Bictegravir Sodium could be improved by S-SEDDS technology.*

Key Words: *Bictegravir Sodium, Solid self-emulsifying drug delivery systems (S-SEDDS).*

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INTRODUCTION

Bictegravir is a recently approved investigational drug that has been used in trials studying the treatment of HIV-1 and HIV-2 infection. It has been approved for HIV-1 monotherapy combined with 2 other antiretrovirals in a single tablet. A number of impediments to drug discovery and growth have been generated by the insoluble existence of prescription applicants. The aqueous solubility of this drug determines its degradation efficiency, a crucial factor in the bioavailability of medicines. As substance usually has a adsorption of low aqueous solubility, a reduction in the dissolution rate correlated with low solubility results in a low bioavailability of the orally administered pharmaceuticals. In some situations, improvements in dose may be necessary until blood medicine reaches the level of gastrointestinal sensitivity caused by oral administration, minimizing patient compliance. An incorrect distribution system makes a good medicinal product useless. Thus, a correct form of dose should be used.[1-4]

Because of their high solubility and increased degree and duration of lymph absorption, SEDDS are a suitable substitute. These novel formulations could, in principle, enable medicaments to remain in the gastrointestinal tract, thus growing the bioavailability and safe plasma profiles of insoluble agents in water-therapeutic agents.[5]

The compound was selected for Bictegravir Sodium. The BCS class II demonstrates poor solubility and hence low bioavailability. [6] This drug is of low importance. As a result, dosage forms are essential for these medicines that maximize their aqueous solubility and their bioavailability.

MATERIALS AND METHODS

Bictegravir Sodium was obtained from Solanki Enterprises, Pune. Oleic acid, Tween 20, Polyethylene glycol (PEG) was purchased from local suppliers.

Characterization of Bictegravir Sodium[7-11]

Description

The Bictegravir Sodium a sample was manually analyzed for the organoleptic properties like color, odor, texture, appearance.

Melting Point Determination

The Melting point of Bictegravir Sodium was identified by using melting point apparatus (Veego). The readings were taken in triplicate.

Fourier Transforms Infrared Spectrophotometry (FTIR)

A Fourier infrared spectrophotometer was used to identify the Bictegravir Sodiuminfrared spectrum (FTIR 4100 Jasco-Japan). The sample is prepared by melting and triturating the medicine into a glass mortar with potassium bromide (KBr). The sample was then put in the holder of the sample and scanned within the 4000-700cm -1 frequency range. The collected spectrum was compared with the I.R. spectrum.

Differential Scanning Calorimetry (DSC)

An alternative calorimetry thermograph (Make: Mettler Toledo, Japan, DSC 823e) fitted with a nitrogen cooling adapter was used to reported the differential scan calorimetry thermogram of Bictegravir Sodium. In a closed pierced aluminum pot between 100^{o}C and 400^{o}C, about 2 to 5mg of sample will be heated at 10^{o}C/min under nitrogen stream at a speed of 50ml/min.

X-ray Diffraction (XRD) Analysis

The XRD approach was used to assess the compound's composition and crystalline characteristics.Experiments is performed using an X-ray diffractometer (XRD) Cu-K radiation (Voltage 40 kV and the current 30mA). The scan angle could be adjusted from 20 to 500 degrees.

UV visible Spectroscopy

UV spectroscopy is a process in which monochromatic light passes, absorbs and then transmits a test solution; the light produced is detected by a detector and is recruited in a software system. Deuterium emits UV light while tungsten lamp emits visible light. UV light. In order to test themetal, chelate UV spectrum and ligand co-relationship, UV testing was used. In DMSO solvents and UV scans of ligands in methanol solvents the Shimadzu was used to record UV spectrum of metal chelates.

Preparation of standard stock solution

Bictegravir Sodium's UV spectrum was collected with a UV Visible spectrophotometer (Jasco, 630). In 100 ml methanol, 10 mg substance was weighed consistently and dissolved. A concentration of 100g/ml was achieved by diluting the stock solution.

The resultant solution is 2-12µg/ml Bictegravir Sodium levels. At the peak absorption maximum, the resulting wavelength was scanned from 200 to 400 nm.

Development of Calibration curve of the drug using UV visible spectrophotometer in methanol.

0.2- 1.2 ml samples were taken from the stock solution and mixed with methanol to obtain solutions with a concentration range between 2-12 g/ml. The sample solutions were prepared in triplicate using stock, and the absorbance was determined at a wavelength of 340 nm using a UV visible spectrophotometer.

Solubility of Drug of Bictegravir Sodium[12-17]

Aqueous solubility determination

Disposing a vial of sterile water with an amplitude of drugs was used to assess the solubility of narcotics in distilled water and to shake it in an orbital cord for 72 hours (REMI, Mumbai). The mixture was then centrifuged with filtration for 15 minutes at 3000 rpm. The filtrate was methanol-diluted and UV spectroscopy was used to measure its concentrations.

Solubility Study of Bictegravir Sodium in Oils, Surfactants and Co-surfactants

Bictegravir Sodium solubility was estimated for certain oils (Oleic acid; Castor oil; Olive oil; CRM 40, Tween 20, Tween 80 and Span 20: Capmul MCM; Isopropyl myristate; Aniseed oil) and co-surfactants. Solid oils Total oils were determined (PEG400, Transcutol HP, and Polyethylene glycol). The 2 mL solvent required and an excess of the medicament were filled into a vial. A UV spectrophotometer was used for separating, condensing and testing the mixture. All calculations have been performed three-fold.

Preliminary Screening of Surfactants for their Emulsification Ability

Various surfactants have been tested for their emulsification ability. A 1:1 ratio of tensile to oil was blended in a nutshell (300mg of the surfactants, Tween 20, Tween 80, and Span 20 were added to 300mg of the oily phase). With purified water the 50 mg mixture is dissolved and a UV spectrometer is used to calculate the percentage transmission at 340nm. Emulsions for turbidity or isolation of the process were also manually checked.

Preliminary Screening of Co-surfactants

In a 3:2:1 blend of crude, surfactant and co-surfactants, the preferred oily method and surfactant is used to test co-surfactants for emulsification (this results in a 1:1 ratio of oil to surfactant and co-surfactant). 100mg co-surfactant was produced and tested as before, 200mg co-surfactant, 300mg oils and mixtures.

Construction of Pseudo Ternary Phase Diagram

The pseudo ternary step diagram is a crucial and useful means for evaluating the scale and compartment of the microemulsion region. Using the titration technique, the pseudo ternary phase diagrams were developed. Add water to the uniform, liquid combination in a room temperature containing tar, surfactant and co-surfactant (water titration method). In a triangular form with three coordinates, you can see the pseudo ternary phase diagram (triangle). Each co-ordinate is a part of the system of microemulsion, i.e.

(1) Oil phase

(2) Surfactant Co-surfactant phase (Smix)

(3) Aqueous phase.

The pseudo-ternary step diagram determines the levels of components that lead to a broad variety of micro-emulsion life.

In pre-weighted vials, mixes were mixed with oil at ratios 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2 and 1:1 of the pre-weighed vials. Smix and olive oil had been combined at ratios 1:9, 1:8. Dropwise water was added to the resulting mixtures until a transparent or slightly bluish microemulsion was created that was readily flowable. An Emulsion is a somewhat less bluish-white or dazzling white emulsion device. Except in the ternary process map, the microemulsion zone boundaries, no other areas have been attempted. We have calculated the optimum microemulsion region at the required Smix value to prepare the liquid SEDDS and entered it in the design expert version 11 programme (Stat-Ease, Inc., Minneapolis, MN, trial version).

Table 1: Formulation and optimization using central composite design (CCD)

Composition of Bictegravir Sodium S-SEDDS formulations									
Ingredients mg/tab	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
SSEDDS	25	25	25	25	25	25	25	25	25

Crosscarmellose sodium (Ac-Di-Sol)	5	---	---	10	---	---	15	---	---
Crospovidone (PPXL)	---	---	5	---	---	10	---	---	15
Microcrystalline Cellulose	20	20	20	20	20	20	20	20	20
Povidone (PVPK-30)	10	10	10	10	10	10	10	10	10
Polacrillin Potassium	5	5	5	5	5	5	5	5	5
Saccharin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Magnesium Stearate	5	5	5	5	5	5	5	5	5

Composition of Bictegravir Sodium S-SEDDS formulations						
Ingredients mg/tab	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**
SSEDDS	25	25	25	25	25	25
Crosscarmellose sodium (Ac-Di-Sol)	5	---	---	10	---	---
Crospovidone (PPXL)	---	---	5	---	---	10
Microcrystalline Cellulose	20	20	20	20	20	20
Povidone (PVPK-30)	10	10	10	10	10	10
Polacrillin Potassium	5	5	5	5	5	5
Saccharin	0.5	0.5	0.5	0.5	0.5	0.5
Magnesium Stearate	5	5	5	5	5	5

Formulation and optimization using Central composite design (CCD)

Generally, the design and optimization of the formulations is the crucial step after QbD. In this study, the nanosponge formulations were designed using CCD design with the added center and axial points in the Response Surface Methodology (RSM) by Design Expert-13. Various concentrations of Crospovidone (PPXL) (A), Microcrystalline Cellulose (mg) (B) and Povidone (PVPK-30) (C) were employed as control variables (process parameters). Drug release (%) (Y1) and Entrapment efficiency (%) (Y2) were considered dependent variables. All the possible combinations of formulations were prepared by considering levels-1 and+1 for both controlled variables.

Table 2: DOE suggested batches

Formulation code	**Crospovidone (PPXL) (mg)**	**Microcrystalline Cellulose (mg)**	**Povidone (PVPK-30)**	**Drug release (%)**	**Entrapment Efficiency (EE %)**
B1	15	20	5	70.45	69
B2	10	23.409	7.5	75	72
B3	15	10	5	84.45	79.86
B4	5	10	5	92	82.45
B5	10	6.59104	7.5	88.45	86.34
B6	10	15	11.7045	93.14	87.63
B7	5	20	10	90.56	80.89
B8	5	10	10	92.7	89
B9	15	20	10	95	88
B10	1.59104	15	7.5	87	91
B11	15	10	10	88.45	92
B12	10	15	7.5	96	93
B13	10	15	3.29552	66	83.56
B14	5	20	5	79.84	77.15
B15	18.409	15	7.5	92.1	80.19

ANOVA for Linear model

Table 3: Response 1: Drug release

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	685.75	3	228.58	5.02	0.0197	significant
A-Crospovidone(PPXL)	4.89	1	4.89	0.1073	0.7493	
B-Microcrystaline cellulose	144.16	1	144.16	3.16	0.1029	
C-Povidone(PVPK)	536.71	1	536.71	11.78	0.0056	
Residual	501.19	11	45.56			
Cor Total	1186.94	14				

Factor coding is coded.
Sum of squares is Type III - Partial
The Model F-value of 5.02 implies the model is significant. There is only a 1.97% chance that an F-value this large could occur due to noise.
P-values less than 0.0500 indicate model terms are significant. In this case C is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 4: Fit Statistics

Std. Dev.	6.75	**R^2**	0.5777
Mean	86.08	**Adjusted R^2**	0.4626
C.V. %	7.84	**Predicted R^2**	0.2496
		Adeq Precision	6.0493

The Predicted R^2 of 0.2496 is not as close to the Adjusted R^2 of 0.4626 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.
Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 6.049 indicates an adequate signal. This model can be used to navigate the design space.
Final Equation in Terms of Coded Factors

Drug release	=
+86.08	
-0.5984	A
-3.25	B
+6.27	C

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.
Final Equation in Terms of Actual Factors

Drug release	=
+78.21288	
-0.119689	Crospovidone(PPXL)
-0.649785	Microcrystalline cellulose
+2.50757	Povidone(PVPK)

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

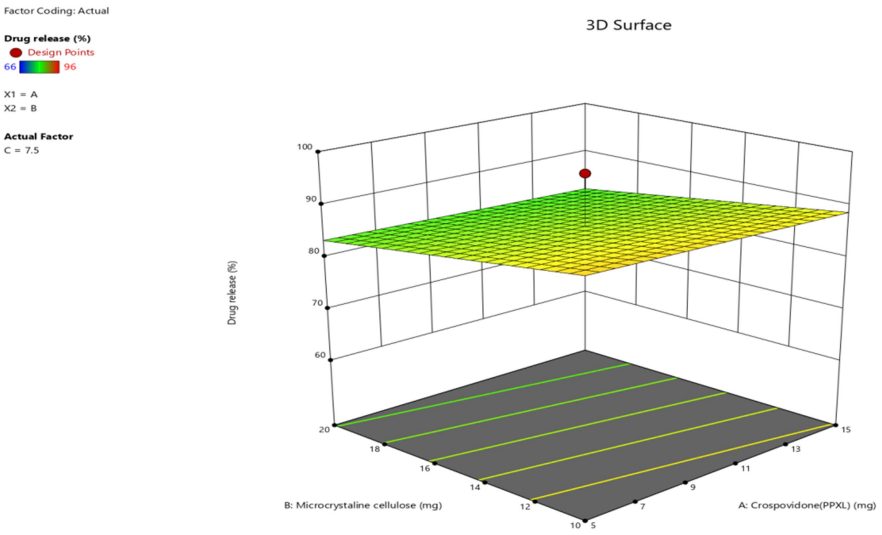


Figure 1: 3D surface graph

Preparation of Solid Self-Emulsifying Drug Delivery System (S-SEDDS) of Bictegravir Sodium:[18-21]

Adsorption to Solid Carriers

The addition of heavy carriers of the optimised liquid SEDDS solution resulted in the development of free-flowing powders. The strong carrier of adsorption consists of products with a vast area and a large degree of breakdown. Aerosil 200 Pharma (A1) and Neusilin US2 have been used as strong carriers (A2). up to 70% (w/w) of the material can be used by the carrier of choice. The method of conversion was to apply liquid formulations to the carriers, while continuously mixing with a blender. Until inclusion in a tablet formulation, the powder was dried and tested for different parameters. Use of the most efficient mixture of adsorbent and liquid SEDDS was developed for the final tablet formulation.

Adsorbent Selection for Optimized Liquid SEDDS Formulation

The adsorption of liquids into solid carriers led to the formulation in the optimised SEDDS solution of a free-flowing powder. Good carriers were used for adsorption products with a large area and acceptable decomposition properties. Aerosil 200 (A1) and Neusilin U.S.2 have been used as soil carriers (A2). The chosen carriers have 80 percent (w/w) of adsorption ability. In the transition phase liquid formulations is added to strong carriers when mixed continuously. 0.2 ml of optimised liquid SEDDS, that is F12 is used in the converting of liquid SEDDS into solid SEDDS. The following table summarises the adsorbents used for a free-flowing powder.

Pre-compression evaluation parameters of powder blend
The powder was pre-compressed until the compression in tablet form to make sure that the composition was simplified (F12). To confirm the precision of the final dose shape, pre-compression parameters are used.

1. Bulk density (BD)
Mass density in contrast with its bulk length corresponds to the mass of a material. The bulk density of a powder is mostly determined by the propagation, composition and inclination of the particles to adhere.

Procedure
1. Weigh 25 g of granules which are tamed and moved to a cylinder with a graduated 100 ml of 22 livres.
2. Level the powder cautiously and notice the obvious amount that has never settled it without compacting.
3. Use the following approach to calculate the evident bulk density in gm/ml.
Bulk density = weight of powder / Bulk volume.
$Db = M/V_0$
M = mass of the powder; V_0 = bulk volume of the powder.

2. Tapped density (TD): It is the ratio of total mass of powder to the tapped volume of powder
Procedure
1. Weigh 25 gr of seven granules correctly with a sieve of 22 livres.
2. Transfer granules in a tap density testing device to the 100 mL graduated cylinder. The measurement tester was run for a fixed number of taps until the volume of the powder bed was lowered to a minimum.
Tapped density = Weigh of powder / Tapped volume
Dt = (*M)* / *(*Vt*)*
M = mass of the powder; Vt = tapped volume of the powder.

3. Carr's Index
It is a simple test to measure the mass density and total powder density as well as the speed of packaging. The index of Carr is determined as follows:

$$\text{Compressibility index} = 100 \times \frac{\text{density Tapped} - \text{Bulk denisty}}{\text{density Tapped}}$$

Table 5: Scale of Flowability as per USP.

Compressibility Index (%)	Flow Character
≤10	Excellent
11–15	Good
16–20	Fair
21–25	Passable
26–31	Poor
32–37	Very poor
>38	Very, very poor

4. Hausner's ratio

Hausner's ratio is a proxy for the ease at which powder flows. The following method is used to measure it:
Hausner's ratio = tapped density / bulk density**.**

Table 6: Relationship between flow character and Hausner ratio as per USP.

Flow Character	Hausner Ratio
Excellent	1.00–1.11
Good	1.12–1.18
Fair	1.19–1.25
Passable	1.26–1.34
Poor	1.35–1.45
Very poor	1.46–1.59
Very, very poor	>1.60

5. Angle of repose
Hausner's ratio is a proxy for the ease at which powder flows. The following method is used to measure it:
Tanθ = h/r
θ = tan-1 (h/r)
Where,
θ is the angle of repose, h is the height, r is the radius.

Procedure
1. The powder pile with funnel is formed around 2–4 cm across the top of the powder pile in order to minimize the effect of powder that falls on the nozzle edge.
2. The test sample was filled complete with a funnel, and was then freely transported through the aperture by weight.
3. A cone-shaped diagram was used to define the area of the pile and to calculate the flux potential of the granules. Furthermore, the height of the pile was calculated.
Determine the resting angle by evaluating the powder cone height and estimating the resting angle, a, using the following equation.
θ = tan-1 (h/r)

Table 7: Relationship between angle of repose (θ) and flow properties as per USP.

Flow Property	Angle of Repose (degrees)
Excellent	25–30
Good	31–35
Fair—aid not needed	36–40
Passable—may hang up	41–45
Poor—must agitate, vibrate	46–55
Very poor	56–65
Very, very poor	>66

Preparation of solid SEDDS[22-23]
Adsorbents including aerosil 200 and Neusilin US2 have been used in the optimised liquid SEDDS formulation (F12). Dropwise to the solid, adsorbent, and blended in a mortar and stick, optimised liquid SEDDS.

Solid State Characterization of S-SEDDS
FTIR

FTIR (JASCO) has been used to study the physical and chemical stability of Bictegravir Sodium and the excipients used for making S-SEDDS. With wavelengths of between 700 and 4000 cm-1 the infrared spectra were purchased utilizing the FTIR and KBr processing pellets. Correlations and contrasts were established between the Bictegravir Sodium and the S-SEDDS spectra.

Differential Scanning Colorimetry (DSC)
The pure drug and the optimised S-SEDDS were thermally studied using a differential calorimeter scanning (DSC). A heating intensity of 10°C/min has been used in an inert environment containing nitrogen at temperatures ranging from 100°C to 400°C.

Powder X-Ray Diffraction (PXRD) analysis
Powder X-ray diffraction (PXRD) study was studied by using an X-ray diffractometer with Cu-Kα radiation (Voltage 40 kV and the current 30 mA). The scanning angle ranged from 5 to 25^0 of 2θ.

Scanning electron microscope analysis
Using a Scan Electron Microscope, SEM micrographs of the S-SEDDS powder have been taken.

Formulation and development of solid self-emulsifying drug delivery system (S-SEDDS) tablets
For the S-SEDDS tablet formulation of Bictegravir Sodium. The S-SEDDS tablets have been made by direct compression using a number of super-disintegrants, including sodium starch glycolate, crosscarmellose sodium and crosspovidone. A 40# panel filtered all ingredients, including an S-SEDDS blend, super-decompressing agents, povidone, cellulose, mannitol, and polacrilin potassium. The final powder was compacted using a rotary tablet unit into devices.

Table 8: Formulation of Bictegravir Sodium tablet (S-SEDDS)

Composition of Bictegravir SodiumS-SEDDS formulations									
Ingredients mg/tab	**BSF1**	**BSF2**	**BSF3**	**BSF4**	**BSF5**	**BSF6**	**BSF7**	**BSF8**	**BSF9**
SSEDDS	25	25	25	25	25	25	25	25	25
Crosscarmellose sodium (Ac-Di-Sol)	5	---	---	10	---	---	15	---	---
Sodium Starch Glycolate	---	5	---	---	10	---	---	15	---
Crospovidone (PPXL)	---	---	5	---	---	10	---	---	15
Microcrystalline Cellulose	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Saccharin	50	50	50	50	50	50	50	50	50
Povidone (PVPK-12)	5	5	5	5	5	5	5	5	5
Doshion P-544 DS	5	5	5	5	5	5	5	5	5
Aspartame	1	1	1	1	1	1	1	1	1
Magnesium Stearate	5	5	5	5	5	5	5	5	5
Total	**200**	**200**	**200**	**200**	**200**	**200**	**200**	**200**	**200**

Evaluation of tablets
Thickness
Thickness of the tablets (n=3) was determined using a Vernier Caliper.

Hardness test
A Monsanto Hardness Tester (n=3) was used to assess the stiffness of the tablet. By touching the lower plunger with the tablet, a zero reading was taken. By spinning a threaded screw, the plunger was thrust against a source and shattered the tablet. The spring is squeezed by a pointer, which displays the sum of energy around a barrel gauge.

Friability test
The capacity of the tablets for packaging, handling and transport is determined by this test.

Procedure
1. A starting weight of 20 tablets is taken and inserted in the Friabilator for 4 minutes at a speed of 25 rpm.
2. The weight gap is observed and calculated as a percentage.
It should be preferably between 0.5 to 1.0%.
%Friability = [(W1-W2)/W1] × 100 ------Formula 6
Where, W1= weight of tablets before test, W2 = weight of tablets after test

Weight Variation test
Procedure -
1. Twenty tablets have been chosen, measured separately and together.
2. For calculating the average weight, the total weight was used.
3. Every tablet weight was then added to the overall weight to ensure it was kept within acceptable limits.
Not more than 2 tablets weighed more than 5.0 percent from the average weight of tablets containing more than 324 mg.
Average weight = weight of 20 tablets/20

$$\% \text{ Weight variation} = \frac{\text{Average waight - Weight of each tablet}}{\text{Average weight}} \times 100$$

……Formula 7

Drug content
The exact powder weight of a USP dissolution system with a weight of 50 rpm was dissolved at 37±0.5°C in a corresponding quantity of intestinal pH 6.8 buffer consisting of 2.5% SLS. After proper purification and dilution of the sample, a UV-Visible spectrometer calibrated to 340nm calculated the concentration of the compound.

***In-vitro*Drug Release Study**
Using the USP dissolving device type II, a quantitative in vitro release test was done at 50 rpm at 37±0.5°C in 900 cc of intestinal buffer pH 6.8 containing 2.5 percent SLS. At several points during the day, samples were obtained. At regular intervals, aliquots from 5 ml samples were obtained and analyzed using a UV spectrophotometer adjusted to 340 nm after filtering using 0.45 m pore-size membrane filters.

Stability Study
In order to optimize the wording in compliance with ICH standards, a stability assessment was carried out on Q1 A. (R2). A 400°C 20°C, RH 75%, humidity and temperature control accelerated stability analysis, with measurements measured at 1, 2, 3 and 6 months was carried out. Optimized formulation tablets have been packaged in bottle containers of HDPE (High Density Polyethylene) and put into a stabilisation chamber. Regular physical sample examinations were conducted, including time of disintegration, drug composition and pharmaceutical release.

Bioanalytical Method Development

A bioanalytical technique has been established for the formulated Bictegravir Sodium SSEDDS BSF7 formulation to determine different pharmacokinetic parameters after the ingestion of Bictegravir Sodium tablet.

Experimental animals

The experiment in vivo, which was carried out by an Institute animal ethical committee (IAEC) in the In Vivo Biotech, Hyderabad, was carried out according to the guidelines defined for the purposes of the animal monitoring and surveillance (CPCSEA).

The study was carried out with white rats 150-200g. Rats have been acquired from local suppliers and is housed in the. The enclosure held a constant temperature of 25°C.

Animals required

a. Species / Common name	Rat
b. Age / Weight / Size	150 ± 200 gm
c. Gender	Male
d. No. to be used	16
e. Purpose of animal use	*In-vivo* study of solid self emulsifying drug delivery system

Reagents and chemicals

Bictegravir Sodium, Bictegravir Sodium-IS, Acetonitrile, Methanol, buffer (0.1% o-phthaldialdehyde), Na_2EDTA solution.

Instruments

The method was developed using a quaternary pump and UV/VIS detector from Jasco PU-2085 Plus. The HPLC architecture was developed with the Chromatopro platform. Average particle size 5 mm column Hypersil ODS C18 HPLC tests were performed (250 mm, 4.6 mm).

Mobile Phase

Acetonitrile was used in the moving phase: 0.1% (60:40 v/v) o-phthaldialdehyde. At a flow rate of 1 mL/min and sample size 20 µL the eluent was monitored with the UV detector at 340nm The membrane filter was used for filtration of 0.45 mm.

Table 9: HPLC System Parameters

Column	c18
Mobile Phase Ratio	60:40%
mobile phase	acetonitrile: 0.1% o-phthaldialdehyde
Ph	2.2
flow rate	1ml/min
Wavelength	340nm
Drug	Bictegravir sodium
Chromatographic analysis method	Isocratic
Sample Size	20µl
Retention Time	3.31 min

Selection of Mobile Phase

The mobile process for the bioanalytical method is examined with extractions of different solvents. A 0.1% o-phthaldialdehyde is a compound of methanol, acetonitrile and acetonitrile used as a solvent extractor.

Preparation of rat plasma sample preparation

1. The fluid-fluid extraction technique was used to separate Bictegravir Sodium from rat plasma.
2. Samples of the blood were obtained after administering Bictegravir Sodium to rats (n = 4 per population, 150–200 gm).
3. Samples for blood (100 mL) were gathered and vortexed in Na2EDTA fluid-listed polypropylene tubes of pre-dose 0.15, 0.30, 1, 2, 4, 8, 12, 20 and 24 h at about 10 minutes before a centrifuge and at 20°C in pre-dose 0.15, 0.30, 1, 2, 4, 8, 12, 20 and 24 h.
4. The supernatant was moved from each sample to a labelled vial and evaporated until it was dry completely at 40°C.
5. These samples were reconstituted in acetonitrile of 500 ml and vortexed shortly before the auto sampler bottles were moved.

Preparation of standard stock solution

1. Weigh around 10 mg in a 100 mL volumetric flake of Bictegravir Sodium IS.
2. Sonication for 15 minutes, raising to the required degree and filtering through a membrane filter of 0.22 was achieved. 2.
3. Bictegravir Sodium was generated utilizing the filtrate at a standard stock solution at 100g/mL.

Preparation of Sample solution

1. 20 quick-dissolving tablets are accurately weighed.
2) Measurement and switch to a 100mL volumetric flask of the required powdered 25mg Bictegravir Sodium capsule.
3. Handheld 80 mL procedure was applied to this and 15 minutes of sonication.
4. The final volume has been raised to 100mL utilizing the mobile shape, and the solvent has been filtered using the 0.22 membrane filter.
5. More filtrate has been diluted to a sodium level of 100g/mL of Bictegravir sodium.
6. We estimated the whole area below the curve (AUC) according to the time with the Linear Trapezoidal Rules.
7. The findings determine R^2 and the regression equation.

Linearity

The linearity was calculated by preparing a 100 mL normal solution of Bictegravir Sodium. Solution concentrations of 0.8-1.8 mg/ml were obtained through a series of dilutions. Peak areas were used to map calibration curves against concentration following injection.

RESULTS AND DISCUSSION

Preformulation Studies of Drugs and Excipients

Description

The powder of blend was found to be white to off-white to yellow solid.

Melting point determination

The melting point is then used in the formulation analysis as a characterization parameter. With a reported 130°C, Bictegravir Sodium melts at 128°C - 130°C.

FT-IR Spectra of Bictegravir Sodium

Identification of pure drug was carried out by Fourier Transform Infra-red Spectrophotometry (Shimadzu 8400s).

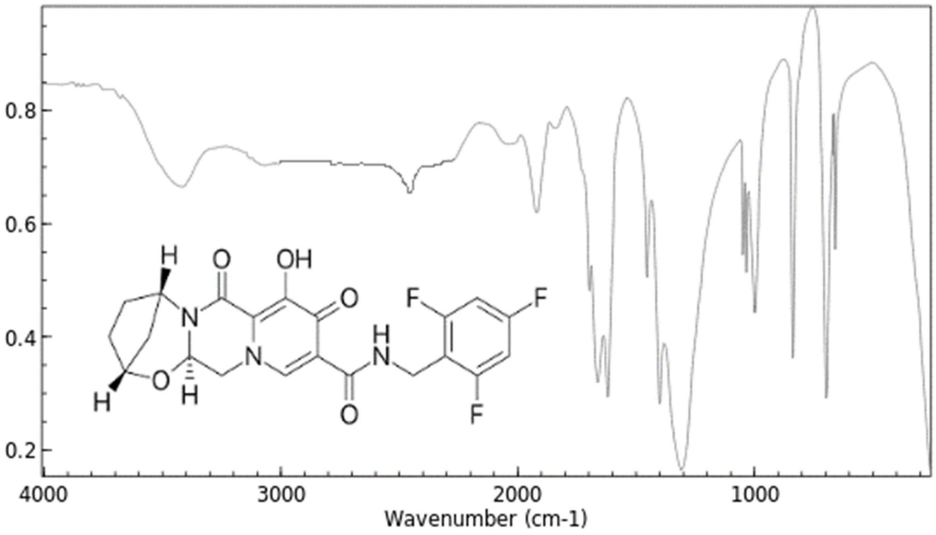


Figure 2: FTIR spectra of pure drug Bictegravir Sodium

The pure drug Bictegravir Sodium exhibited sharp peak at 3397 cm−1indicating the presence of C-N stretching, C≡C stretching, C-O-C stretching, C-H bending, and Ar-H bending. C=O stretching at1548cm−1.

DSC Study

DSC is an unbelievably useful tool to detect all electrical processes quantitatively. At 129.99°C, Bictegravir Sodium displayed a sharp endothermic plateau that matched its melting point.

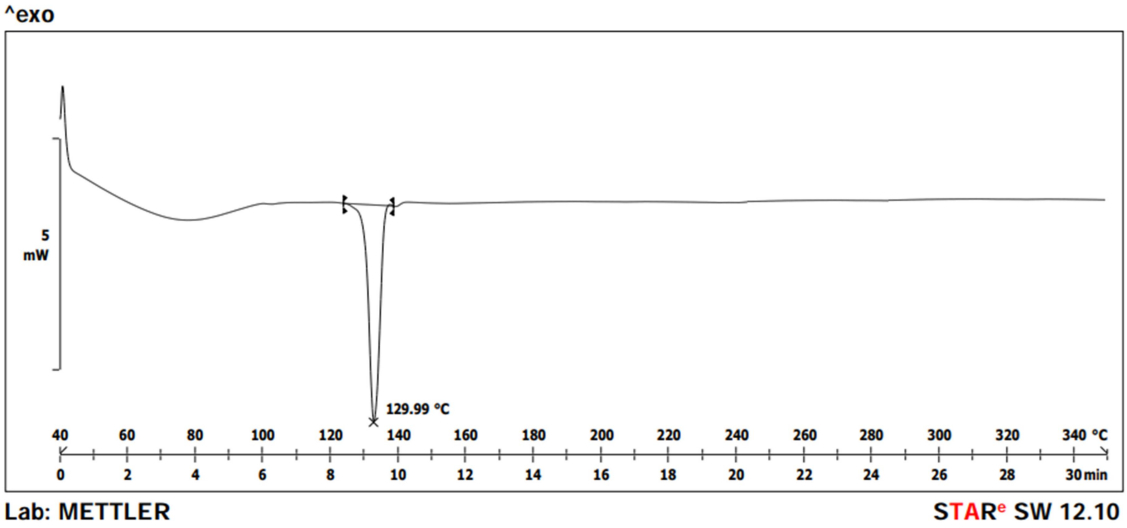


Figure 3: DSC spectra of pure drug Bictegravir Sodium

X-ray Diffraction Study

An XRD spectrum of pure drug is depicted in Figure. The diffraction pattern has shown numerous distinctive sharp peaks with high relative intensity at different. Positions and this finding demonstrated the high crystalline nature of pure Bictegravir Sodium.

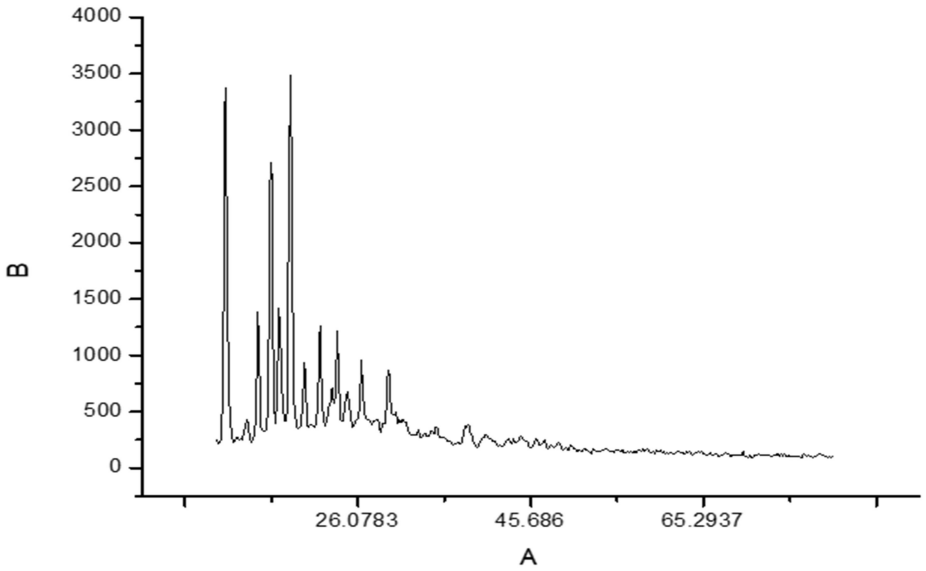


Figure 4: X ray diffraction graph of pure drug Bictegravir Sodium

Development of Calibration curve of the drug using UV visible spectrophotometer in methanol.
The calibration curve was based on methanol within a concentration range of 2-12 g/ml. The solution for the sample was formulated in three cases with a material, and the UV spectrophotometer at a wavelength of 340nm was used to test its absorption. The results are summarized in the table. In order to create a standard Bictegravir Sodium calibration curve, concentration vs. absorbent values were used.

Table 10: Concentration Vs Absorbance values for development of calibration curve for Bictegravir Sodium in methanol (mean± SD, n=3)

Sr. No	Concentration (µg/ml)	Absorbance (nm)
1	2	0.021±0.26
2	4	0.044±0.89
3	6	0.064±1.02
4	8	0.085±0.58
5	10	0.104± 0.63
6	12	0.126±0.18

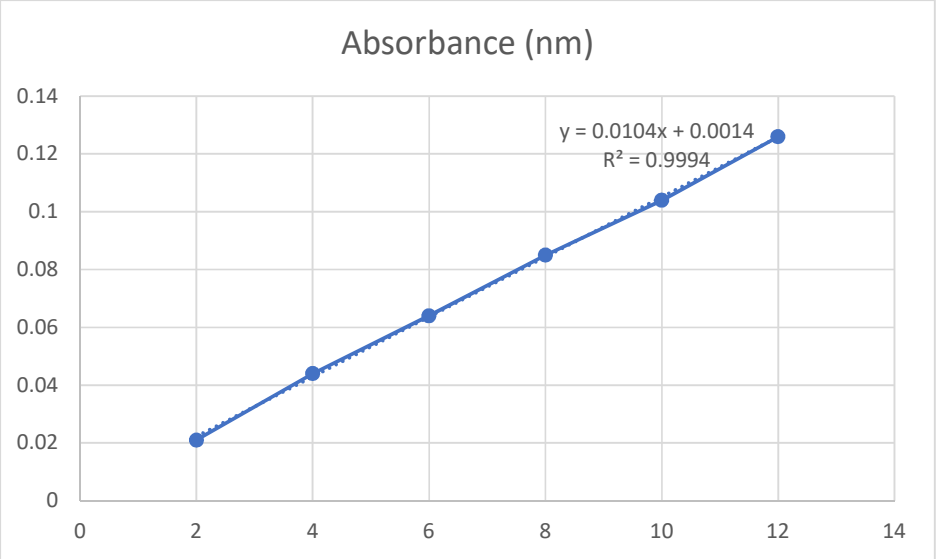


Figure 5:Calibration curve of Bictegravir Sodium in methanol

Solubility determination of Bictegravir Sodium

Solubility study

As most formulas are precipitated until solutions are in situ, the solubility of a drug in excipients is essential to the consistency of the formulation. The whole Bictegravir Sodium formulation ingredients must be soluble in the SEEDS to ensure the effectiveness of Bictegravir Sodium SEDDS loaded. The below table summarises the solubility in various oils, surfactants and co-surfactants of Bictegravir Sodium. Capmul MCM (16.58 mg/ml) was selected among the screened vehicles as an oil system with the highest solubilisation potential. As surfactants, PEG 400 was used as surfactants in about 80 (13.25 mg/ml) and Polyethylene glycol (17.48 mg/ml).

Table 11: Saturation solubility of Bictegravir Sodium in different oil

Sr. No	Oil	Solubility(mg/ml)
1	Castor oil	1.20± 0.89
2	Aniseed oil	14.78 ±0.18
3	Oleic acid	4.15± 1.05
4	Capmul MCM	16.58 ± 0.63
5	Olive oil	2.41 ±0.78
6	Isopropyl Myristate	9.59± 0.25

Data are expressed as (Mean ± SD n=3)

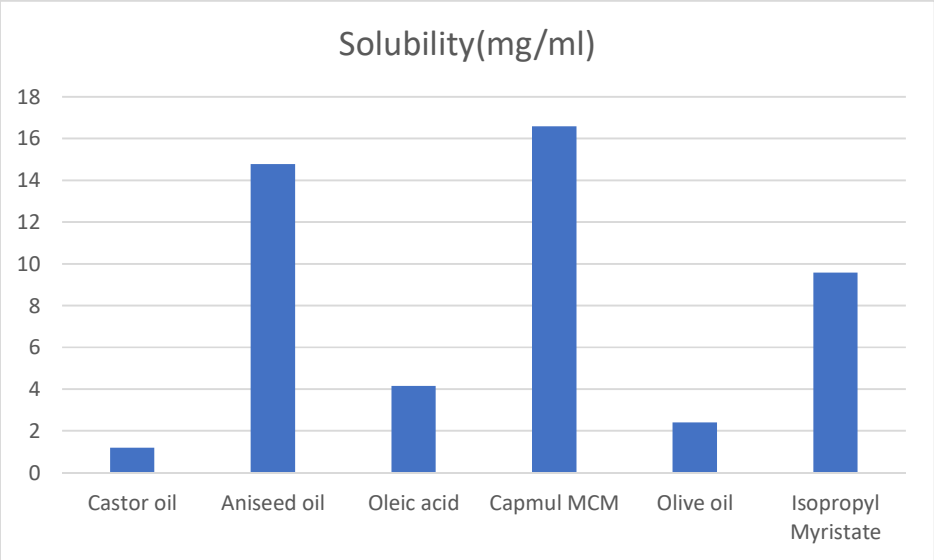
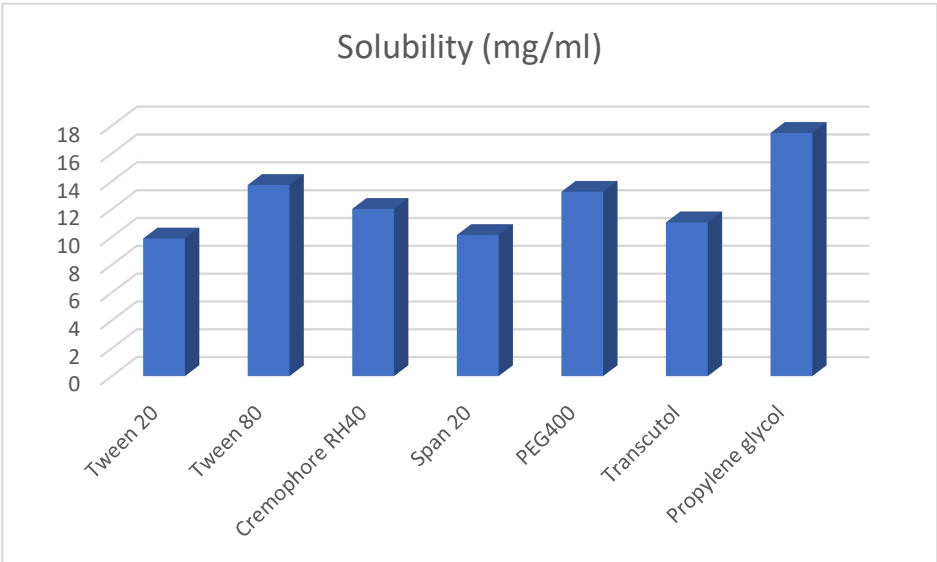


Figure 6: Saturation solubility of Bictegravir Sodium in different oils

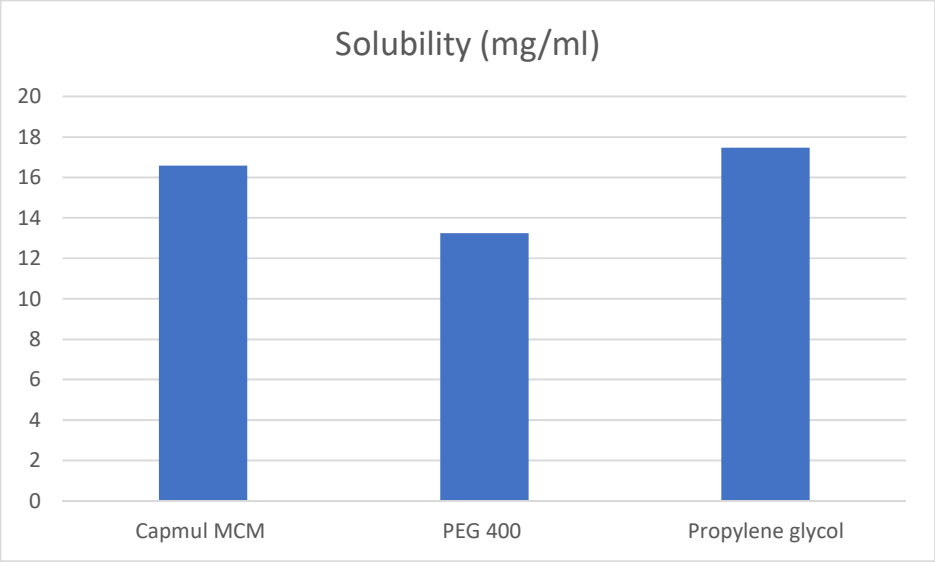
Table 12: Saturation solubility of Bictegravir Sodium in surfactant and co-surfactant

Sr. No.	Excipients	Solubility (mg/ml)
1	Tween 20	9.89± 0.10
2	Tween 80	13.74±1.18
3	Cremophore RH40	12.02± 0.21
4	Span 20	10.14± 0.10
5	PEG400	13.25± 0.31
6	Transcutol	11.05 ± 1.01
7	Propylene glycol	17.48 ± 0.26

Data are expressed as mean± SD (n=3)



(A)



(B)

Figure 7: Saturation solubility of Bictegravir Sodium in oils (A), surfactants (B), co-surfactants

Preliminary screening of surfactants for their emulsification ability

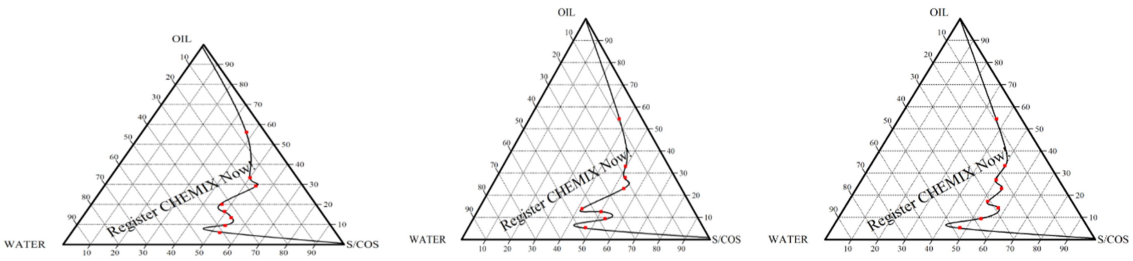
Once stirred, it was found that the correctly cooked SEDDS were dispersed in seconds. Two 20 were of the highest transmitted, followed by Tween 80 and Cremophore RH 40 emulsification performance. The maximum transmission was between 80, and the lowest transmission was between 20.

Preliminary screening of co-surfactants

capacity to emulsify the oily stage was evaluated by tensile substances and cosurfactants. Capmul MCM, Tween 80 and Propylene glycol were selected as the oily phase, based on preliminary screening results.

Construction of pseudo ternary phase diagram

Using a 1-1, 1-2, and 1-3 surfactant (Tween80) and co-surfactant (Capmul MCM) oils, a quasi-ternary step diagram was constructed (Propylene glycol).The shaded area denotes the emulsification zone. It was found that the pureness, toughness and self-emulsification range for a mixture of 10% to 20% oil and 80% to 90% surfactants were more serious.



A **B** **C**

Figure 8: Pseudo ternary phase diagram for different Smix ratio

Formulation of Solid SEDDS [S-SEDDS]

SEDDS increases the in vitro potency of insoluble in water compounds, making it an ideal courier for medicines like Class II and IV of Biopharmaceuticals (BCS). In this research, the objective was to assess the consequences for solubility and bioavailability of Bictegravir Sodium as an antiretroviral medication of self-emulsifying formulations.

Optimization of S-SEDDS

Two particular adsorbents (Aerosil 200 and Neusilin US2) were employed to turn liquid SEDDS into free flow powder. For optimised liquid SEDDS to free flow powder, only 100 mg of Neusilin US2 adsorbents are needed, while 120 mg of Aerosil 200 adsorbents are required. The findings of the powder features analysis are summarised in table. The results indicate the superior flow properties of Neusilin US2 in powdered form. A vitro dissolution test was carried out using capsules packaging these two specific adsorbents with free flow powders.

Table 13: Adsorbent selection.

Formulation	Adsorbent	Amount of Liquid SEDDS (ml)	Amount of adsorbent required to get free flow powder (mg)
A1	Aerosil 200	0.2	100
A2	Neusilin US2	0.2	80

Evaluation of S-SEDDS tablets of Bictegravir Sodium

Table numberbelow summarises tablet testing requirements for BSF1-BSF9 batches. Both were found to be acceptable and under the provisions of the Dolutegravir S-SEDDS pill. For batch BSF7, the average time required is 22 seconds. This batch disintegrated the quickest, along with the other samples. The in-vitro release studies for batches BSF1 to BSF9, as seen in Figures. Batch BSF7 was then considered to be an improved batch and used for further testing.

Table 14: Evaluation parameters for S-SEDDS tablets of Bictegravir Sodium

Factorial design used 3^3 for preparation of solid SEDDS						
Formulation code	**Hardness**	**Thickness**	**Uniformity of Weight**	**Friability**	**Disintegration time**	**Drug Content**
	(kg/cm2)	**(mm)**	**(mg)**	**(%)**	**(sec.)**	**(%)***
BSF1	3.56	2.66	149.78	0.04	65	98
BSF2	4.01	2.89	150.5	0.12	42	98
BSF3	3.95	3.03	148.8	0.07	38	98
BSF4	4.04	2.95	149.5	0.06	45	97
BSF5	4.03	2.95	149.8	0.39	72	98
BSF6	3.96	2.81	149.5	0.19	66	97
BSF7	3.63	3.10	141.12	0.04	22	99.25
BSF8	3.90	2.67	150.2	0.08	42	98
BSF9	3.97	2.75	120.22	0.40	27	99.85

DSC studies

DSC thermostatics for pure Bictegravir Sodium and stable SEDDS as seen in Figures. A sharp endothermic pitch at 355^0C in pure drug content shows the compound's strongly crystalline activity. If

there is no dosage peak in solid SEDDs, the medication would be contained in a dissolved molecular state.

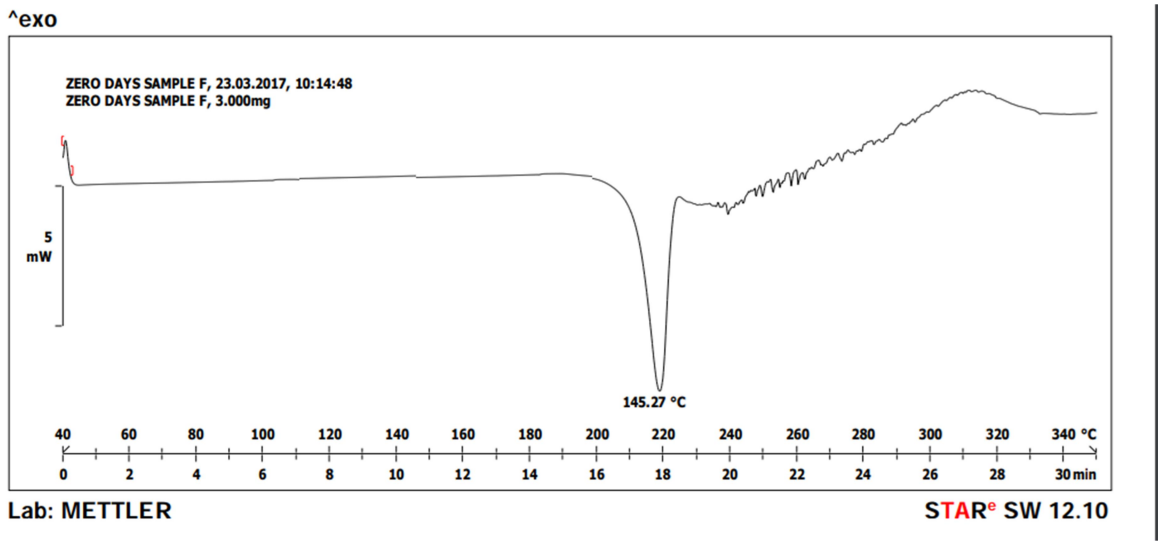


Figure 9: DSC of Optimized BSF7 formulation

X-ray diffraction (XRD) analysis

In comparison, we used nickel buffer, CuK () radiation, 20 mA current and 0.2-inch receiving slot in the XRD pure medication and optimized mixture (f7). The XRD sodium study showed several sharp summits of 10 to 30 degrees at an angle. In the XRD range of the optimized formulation, only one sharp peak at 20–30° was noticeable. In the SEEDS for Bictegravir Sodium-optimized formulation, sharp peaks referring to the surfactant and cosurfactant were observed at 20 to 30°. When the SEEDS optimized surfactant and cosurfactant mix was used, the crystallinity of the pure drug was considerably decreased. The XRD trends are seen in Figure 33.

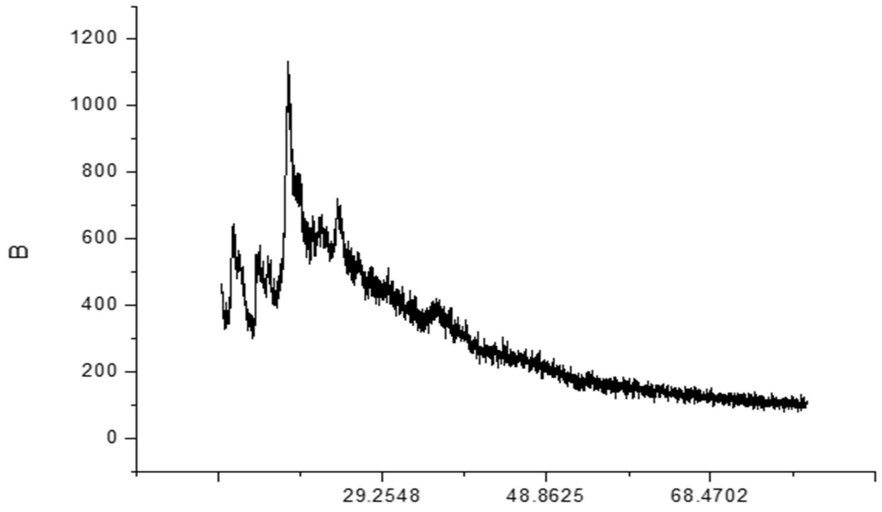


Figure 10: X-ray diffraction diffractograms: Optimized Formulation (BSF7)

SEM analysis

As Figurereveals, the anatomy of solid SEDDS is shown in electron scanning microscopy. In view of the physical mixture used during the formula procedure to support adsorption, the partly covering of the Neusilin US2 is clear.

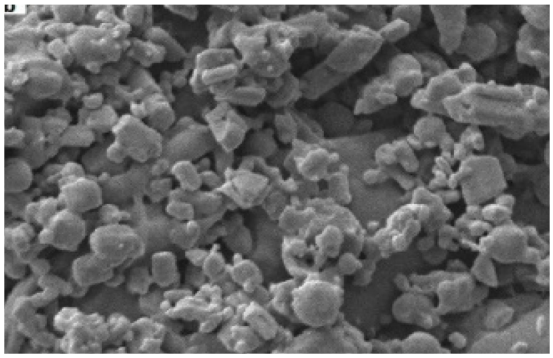


Figure 11:SEM of Optimized solid SEDDS F7 Formulation

***In vitro* dissolution study**

In vitro dissolution study

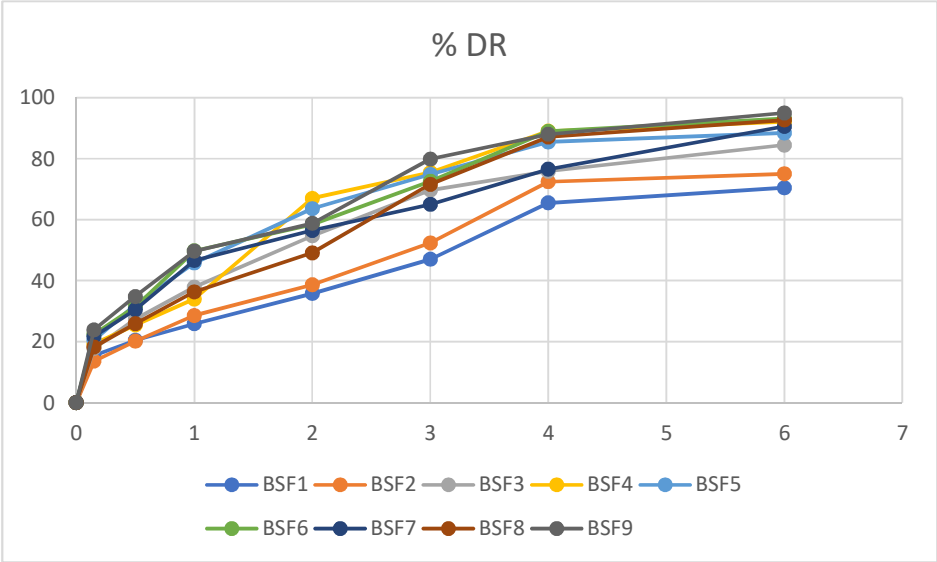


Figure 12: *In-vitro* drug release profile of formulations BSF1-BSF9

Accelerated stability studies

On optimised batch T5 (40°±2°C/75 per cent RH), a six-month rapid stability study was performed. The tablets-maintained shape, decay time, globular scale, zeta potential, drug consistency and release properties. The figures are entered in the chart.

Table 15: Evaluation parameters of formulation BSF7 during stability study

Time	Parameters	
(In Months)	Disintegration time (sec.)	Drug content (%)

1 M	25.1±1.520	99.81±0.720
2 M	26.5.150	98.48±0.500
3 M	26.2±1.5	96.59±1.500
6 M	29.4±570	96.12±0.800

Mean ± S.D., n=3

Bioanalytical Method Development
Mobile Phase
Acetonitrile:0.1 percent o-phthaldialdehyde (60:40 v/v) was used as the mobile step. On a 10 L sample, the eluent was tracked using a UV detector set to 340 nm and a flow rate of 1 mL/min. The filtering procedure was carried out using a 0.45 mm membrane buffer.

9.10.2. Selection of Mobile Phase-
In an optimum solvent for a bioanalytical technique the mobile extraction approach was applied. Methanol, acetonitrile, and 0.1% acetonitrile are used as solvents for extraction.

Extraction of Bictegravir Sodium with different concentrations.

Table 16: % Recovery with Methanol.

concentration	Auc	Con Found	Recovery %
50	301242	301242.2238	86.0735695
50	302564	302564.2238	86.4513032
50	304125	304125.2238	86.8973261

Table 17: % Recovery with acetonitrile: 0.1% o-phthaldialdehyde.

concentration	Auc	Con Found	Recovery %
50	324520	324520.2238	92.7247687
50	332457	332457.2238	94.9925996
50	324151	324151.2238	92.6193347

Table 18:% Recovery with Acetonitrile.

concentration	Auc	Con Found	Recovery %
50	274515	274515.2238	78.436891
50	283485	283485.2238	80.99988
50	274581	274581.2238	78.4557492

Consequently, the maximum extraction of acetonitrile representing 0.1 per cent of the mobile phase was selected at 50.0-150.0 ng/mL.
An isocratic elution chromatographic study was performed with the mobile step filtered into 0,45 membrane filters paper with a buffer to acetonitrile ratio of 60:40 v/v (0.1 per cent o-phthaldialdehyde). Mobile phase flow rhythms were 1.0 ml per minute when the eluent was 265 nm wavelength. In a 10-minute run, 10 litres was recovered.

Linearity
The correlation coefficient values of these three analytes were 0.999.

Table 19: Linearity concentration Vs Area Under Curve.

Concentration mg/ml	AUC
10	75415
30	230415
50	401578
100	751474
130	984785
150	1121514

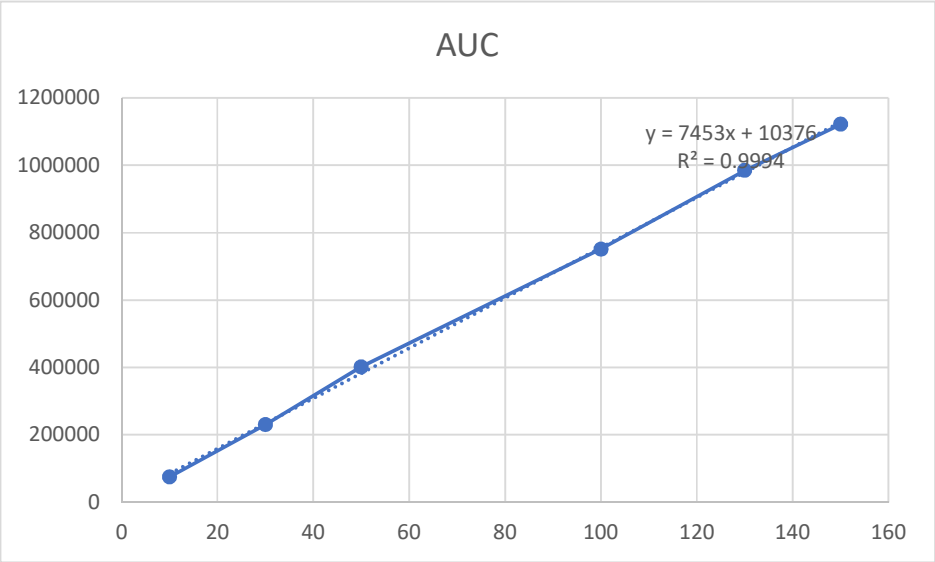


Figure 13: Linearity graph.

Pharmacokinetic data analysis

Plasma concentration *vs.* time data of Bictegravir Sodium was analyzed by Pk solver version 2.0 to derive various pharmacokinetic parameters, *viz.*, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, C_{max}, t_{max} and $t½$.

Formulation	BSF7
AUC Calculation Method	Linear Trapezoidal

Table 20: Summary Table- Input Variable

Time	Conc	ln(C)	AUC	AUMC	R	R_adj
0	0		0	0		
0.15	5.45	1.69561561	0.40875	0.0613125		
0.3	8.9	2.18605128	1.485	0.322875		
1	13.56	2.60712428	9.346	6.003375		
2	15.23	2.72326717	23.741	28.013375	-0.9914656	0.97875493
4	10.23	2.32532458	49.201	99.393375	-0.9942395	0.98468293
8	7.24	1.97962121	84.141	297.073375	-0.9912868	0.97397431
12	6.32	1.84371921	111.261	564.593375	-0.9997865	0.99914625
20	2.98	1.0919233	148.461	1106.35338		

24	2.11	0.74668795	158.641	1326.83338		

Table 21: Calculation Results

Parameter	Unit	Value
Lambda_z	1/h	0.091784303
t1/2	h	7.551914219
Tmax	h	2
Cmax	µg/ml	15.23
Tlag	h	0
Clast_obs/Cmax		0.138542351
AUC 0-t	µg/ml*h	158.641
AUC 0-inf_obs	µg/ml*h	181.6296804
AUC 0-t/0-inf_obs		0.873431036
AUMC 0-inf_obs	µg/ml*h^2	2129.025888
MRT 0-inf_obs	h	11.72179504
Vz/F_obs	(mg)/(µg/ml)	1.49963227
Cl/F_obs	(mg)/(µg/ml)/h	0.137642702

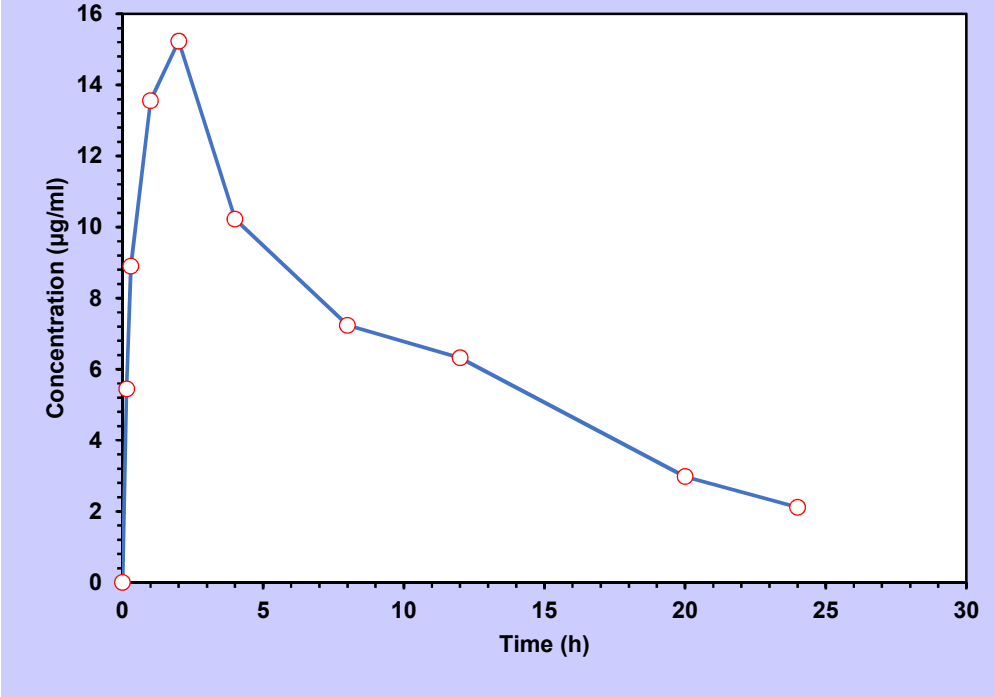


Figure 14:Time in (min) Vs Concentration (µg/ml)

CONCLUSION

The purpose of this research was to develop and assess solid self-emulsifying drug delivery systems (SSEDDS) for Bictegravir Sodium in pediatric and geriatric populations. The following conclusions have been taken from the findings and discussion of this research.

Based on their solubilizing potential for Bictegravir Sodium, the optimum liquid SEDDS of Bictegravir Sodium included Capmul CMC as an oil, Tween 80 surfactant, and Propylene glycol as a cosurfactant. The optimized liquid SEDDS of Bictegravir Sodium were further tested using TEM, DSC, FTIR, XR-D, and stability techniques. These studies supported the formulation and development of stabilized liquid SEDDS of Bictegravir Sodium with enhanced solubility. The optimal S-SEDDS of Bictegravir Sodium was synthesized by wet granulation of liquid SEDDS onto fine ultralight porous granules Neusilin-US2®, which had a larger surface area adsorption of liquid SEDDS than any other adsorbent investigated.
Other excipients such as diluents, disintegrants, glidants, flavor masking agents, sweeteners, and lubricants were used to manufacture the S-SEDDS into tablets.
Tablets of solid SEDDS were prepared and tested using a set of experiments (32 factorial design). Further studies on the optimized Solid SEDDS of Bictegravir Sodium included SEM, XR-D, stability, and bioavailability in rats, which verified the formulation and production of stabilized solid SEDDS tablets Bictegravir Sodium with increased solubility.
As a result, it can be stated that solid SEDDS is a beneficial approach for increasing Bictegravir Sodium's solubility and bioavailability.

CONFLICT OF INTEREST

None. Declared by authors

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