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Dissolution enhancement of Enzalutamide by solid dispersion approach: Development, characterization and ex-vivo intestinal absorption study

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***Abstract*---**Enzalutamide (ENZ) is a BCS class II, fast crystallizing, a hydrophobic compound that has solubility limited absorption in vivo. Ternary solid dispersion of Enzalutamide was prepared with water-soluble polymers Soluplus (SOL) and Poloxamer P 188 (POL) by solvent evaporation spray drying technique. Initially, different ratios of Soluplus: Poloxamer and solvents were used to prepare Ternary solid dispersions of Enzalutamide and evaluated for the dissolution enhancement property. The prepared solid dispersion was characterized by FTIR spectroscopy, Differential scanning calorimetry (DSC), X-ray diffraction (XRD) analysis, Scanning electron microscopy (SEM), Particle size distribution (PSD) by Malvern and Physico-chemical analysis. FTIR spectroscopy shows that there was no interaction between the ENZ and polymers. DSC and XRD analysis show that conversion to amorphous form of ENZ in ternary SD, which enhances the dissolution rate. *In vitro* dissolution studies and *Ex-vivo* intestinal absorption studies clearly show that the prepared solid dispersion enhanced the dissolution and *ex-vivo* intestinal absorption of ENZ compared with pure ENZ.

***Keywords*---**solid dispersion, dissolution enhancement, solvent evaporation, BCS II, spray drying, ternary solid dispersion.

Introduction

No more amount of the solute can dissolve in a given solvent at a specific temperature is called solubility. Dissolution is the process of mass transfer from the solid to the liquid phase in a given solvent. Under uniform conditions, the dissolution rate is defined as the volume of drug material entering the solution per unit time (Jain, S., 2015).The Biopharmaceutical Classification System (BCS) is focused on a drug substance's aqueous solubility and permeability through the intestine. The three major factors are considered in BCS are solubility, dissolution, and permeability (Bou-Chacra, N., 2017). Permeability/ Absorption are the property that determines the speed at which a dissolved drug passes through the intestinal wall and reaches the systemic circulation.Enzalutamide is a fast crystallizing, hydrophobic compound that has solubility limited absorption *in vivo*. Given the low aqueous solubility of this compound, it was of interest to evaluate amorphous formulations *in vitro* and *in vivo* (Qi, X., 2011).

Materials and Methods

Materials

Enzalutamide (ENZ) sample was gifted by Glenmark Pharmaceuticals Ltd, (Nashik, Maharashtra, India), while Soluplus® (SOL) a polyvinyl caprolactam–polyvinyl acetate–polyethene glycol graft copolymer and Poloxamer 188 (Kolliphor® P 188 Geismar) with a molecular weight of 7680–9510 gmol−1 and 80.5% of the poly-oxy-ethylene group were supplied by BASF Co. (Mumbai, India)(Nanaki, S., 2019). All the other materials and reagents were of analytical grade (Kumar, S., 2013).

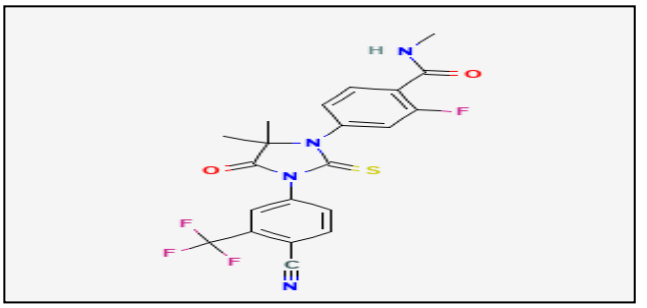

Figure 1: Molecular structure of Enzalutamide

Method of preparation of solid dispersion:

Drug-excipients compatibility study

The Drug-excipients compatibility studies were performed to check the interaction between the drug and selected excipients. Samples were kept at 40°C/75% RH condition for 1 month in closed glass vials and assay analysis was done to check the compatibility of ENZ & polymers. Samples were dissolved in 80% methanol and 20% purified water in volumetric flask (Łyszczarz, E., 2020).

Preparation of Physical mixture

A physical mixture (PM) of ENZ and polymers was prepared by a simple triturating method using mortar and pestle. The PM was sifted through an 80# sieve (Bolourchian, N., 2019).

Preparation of Ternary solid dispersion by Spray drying technique

SOL was dissolved in a combination of solvent Acetone (90%) and Dichloromethane (10%) under slow stirring using a mechanical stirrer followed by the addition of POL. After getting a clear solution of polymers, ENZ was added slowly to it slowly and stirred till to get a clear solution. Table 1 comprises composition of solid dispersions prepared by spray drying technique. This solution was spray dried using spray dryer (Lab model: SprayMate, Manufacture by: JISL, Mumbai, India) with below parameters (Table 2). Spray dried powder obtained then milled through Lab Comill (Manufacture by: Bowman & Archer, Mumbai, India) using 0.5 mm screen at speed of 500 RPM. The milled powder was then dried in an oven at 60°C for 24 hrs to remove the solvents used. Finally, dried powder was passed through ASTM # 60 sieve (Maniruzzaman, M., 2013).

Table 1: Composition of the prepared ENZ binary and ternary solid dispersions

Sample	Ratio	Technique	Dissolution enhancement (Initial)	Dissolution enhancement (after 1 month at 40°C/75% RH)
Binary SDs				
SOL/ ENZ	2:1	Spray drying	Yes	Significant decrease in dissolution was observed as compared to initial
SOL/ ENZ	4:1	Spray drying	Yes	
POL/ ENZ	0.5:1	Spray drying	Yes	
POL/ ENZ	1:1	Spray drying	Yes	
Ternary SDs				
SOL/POL/ENZ	2:0.5:1	Spray drying	Yes	No significant decrease in dissolution was observed as compared to initial
SOL/POL/ENZ	2:1:1	Spray drying	Yes	
SOL/POL/ENZ	4:0.5:1	Spray drying	Yes	
SOL/POL/ENZ	4:1:1	Spray drying	Yes	

Table 2: Spray dryer process parameters

Sr. No.	Parameter	Set value
1.	Aspirator speed	2000 RPM
2.	Inlet temperature	40°C ± 5°C
3.	Outlet temperature	35°C ± 5°C
4.	Atomization pressure	2.0 kg/cm^2
5.	Nozzle diameter	1.0 mm
6.	Feed pump speed	10 RPM
7.	Spray rate	10 g/min
8.	% Solid in solution	5 % w/w

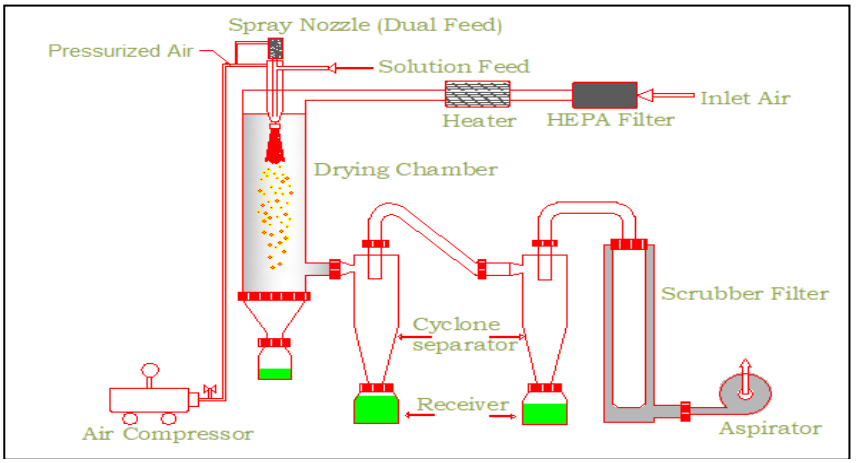

Figure 2: Schematic diagram of Spray dryer

Characterization of Solid dispersion:

Evaluation of drug content and *percent yield* of solid dispersion

The amount of ENZ drug within the solid dispersions prepared was determined by UV – vis spectrophotometer (Jasco V-630, Tokyo, Japan). An accurately weighed 40mg equivalent of SDs were dissolved in 100 ml volumetric flask containing 80 ml of methanol and 20 ml purified water. This mixture was stirred continuously to get the solution. This solution was filtered using 0.22µm filter and analysed after suitable dilution using UV–vis spectrophotometer by measuring absorbance at 236 nm (Paudel, A., 2013). The percent yield of SD was calculated using below formula.

% yield = (Practical yield / Theoretical yield) X 100

Fourier transform-infrared (FT-IR) spectroscopy

To check the compatibility of drug and polymers, infrared spectra of ENZ, SOL/POL and prepared SD were obtained using FTIR spectrophotometer (FTIR-8400; Shimadzu, Asia Pacific Pvt. Ltd. Singapore).

Differential scanning calorimetry (DSC) analysis

DSC Thermogram of ENZ, SOL/POL and SD were obtained using a differential scanning calorimeter (Mettler Toledo, Switzerland) with a heating rate of 10°C/minute from 30°C to 350°C in a nitrogen atmosphere.

X-ray diffraction (XRD) analysis

Powder XRD patterns of ATR, NG, and SD were recorded using a diffractometer (PW 1140, Mettler Toledo, Columbus, OH, USA) and Cu-Kαradiation. The diffractometer was run at a scanning speed of 2°/mm and a chart speed of 2°/2 cm per 2θ.

Powder physical properties and Particle size distribution (PSD) analysis

Powder physical properties such as bulk density (BD) and tapped density (TD) were carried out on lab Tapped density apparatus (Electrolab, Mumbai), by using below formulae

Bulk density (BD) = Weight of sample in g ÷ Volume of sample in ml

Tapped density (TD) = Weight of sample in g ÷ Tapped volume of sample in ml

$$\text{Compressibility index (CI)} = 100\left(1 - \frac{TD}{BD}\right)$$

Hausner ratio (HR) = TD ÷ BD

The prepared SD particle size distribution (PSD) was carried out using Malvern master sizer 2000S (Malvern Instruments Ltd. UK). Around 50 mg of SD powder was taken in 25 mL beaker and 2-3 drops of Sunflower oil was added to make a uniform paste. 25ml Sunflower oil was added to it and sonicate it for 30 seconds (PCI Analytics, India). The sample was transferred into the master sizer tank until stabilisation of obscuration level was achieved. D10, D50, D90 PSD value were calculated.

***In vitro* drug release (dissolution) study**

In vitro drug release (dissolution) study of pure ENZ and prepared SD were performed using USP type II paddle apparatus (Electrolab dissolution tester, Mumbai) in 900ml 0.1N HCL with 1% SLS at 37°C ± 1°C, run at 75 RPM. Aliquots (5.0 mL) were withdrawn at appropriate time intervals and replaced with 5.0mL of fresh dissolution medium to maintain the sink condition. The aliquots were filtrated and the ENZ absorption was determined spectrophotometrically at 239nm for 0.1N HCL with 1% SLS. The equation obtained by calibration curve was used to determine the ENZ concentration. The ENZ *In vitro* drug release test was made in sextuplicate.

Ex-vivo intestinal drug absorption study

Ex-vivo intestinal drug absorption study was performed using everted intestine method and modified apparatus and USP type II dissolution test apparatus according to the method reported (Pandit, A. P., 2019, Tekade. A 2014). Goat intestine was procured from a local slaughter house. The small intestine was taken for the study. The lumen was rinsed with saline solution to remove the unwanted particles from the intestine. A segment of the intestine (6 cm) was taken and transferred to oxygenated saline solution. The intestine was everted using a modified glass rod and then clamped to arm B of modified apparatus. The total volume of the absorption compartment was 55 ml. The apparatus was then placed in dissolution apparatus containing 1000 ml of 0.1N HCL with 1% SLS as a dissolution medium at 37°C±0.5°C. The pure drug ENZ and optimized solid dispersion was transferred to the dissolution medium and apparatus was rotated at a speed of 75 rpm. The amount of drug diffused from dissolution medium (mucosal side) to the absorption compartment side was measured by withdrawing

5 ml sample from absorption compartment (arm B) at 5, 10, 20, 30, 40, 50, 60, 90 and 120 min and analyzed UV- Vis spectrophotometrically at 239 nm. The experiment was carried out in triplicate (n = 3)

Result and discussion

Drug excipients compatibility study

Drug excipients compatibility study was performed to check the compatibility of drug ENZ & SOL/POL polymers selected for the preparation of SD. Samples kept at 40°C/75% RH condition for 1 month in closed glass vials, were analysed for assay using UV-vis spectrophotometric method having ʎmax = 239nm. The result shows that there is no significant change in the assay value (showed in table 3) at initial & 1 month samples kept at 40°C/75% RH condition.

Table 3: Chemical analysis (Assay) of Drug excipients compatibility study

Sr. No.	Particular	Ratio	Initial	1 month (40°C/75% RH)
			% Assay	% Assay
1	API ENZ	NA	100.00 (2.35)	99.06 (0.03)
2	API ENZ + Soluplus	1:5	100.85 (0.31)	100.86 (0.03)
3	API ENZ + Kolliphor P 188 (Poloxamer 188)	1:5	100.76 (1.73)	99.01 (0.11)
4	API ENZ + Soluplus: Kolliphor P 188 (Poloxamer 188)	1:5	97.24 (1.67)	97.75 (0.81)

Evaluation of drug content and percent yield of solid dispersion

The drug content in all binary & ternary SD was found in the range of 97.26% to 99.88% (as shown in table 4). The % drug content for all SDs were found within the pharmacopoeial limit, which indicate the uniform distribution of drug ENZ in the solid dispersion prepared by spray drying. The % yield of the prepared SDs were calculated using below formula. The yields of all SDs prepared were in the range of 80.10% to 86.10%, which is due to the small batch size. This yield can be increased during the scaleup batches.

$$\% \text{ yield} = (\text{Practical yield} / \text{Theoretical yield}) \times 100$$

Table 4: Drug content and % yield of the SD prepared by spray drying

Batch No.	Sample	Ratio	Technique	% Yield	% Drug content
	Binary SDs				
B-001	SOL/ ENZ	2:1	Spray drying	80.10%	98.21%
B-002	SOL/ ENZ	4:1	Spray drying	82.56%	97.26%
B-003	POL/ ENZ	0.5:1	Spray drying	82.20%	99.45%
B-004	POL/ ENZ	1:1	Spray drying	83.04%	97.61%
	Ternary SDs				
T-001	SOL/POL/ENZ	2:0.5:1	Spray drying	85.12%	99.88%
T-002	SOL/POL/ENZ	2:1:1	Spray drying	82.88%	99.01%
T-003	SOL/POL/ENZ	4:0.5:1	Spray drying	86.10%	98.55%
T-004	SOL/POL/ENZ	4:1:1	Spray drying	84.21%	99.51%

Fourier transform-infrared (FT-IR) spectroscopy

FTIR spectra of drug ENZ and selected ternary SD (SOL/POL/ENZ ratio 2:1:1) were presented in figure 3 Principle peaks were observed at wavenumbers 3431.36, 3084.18, 2968.45, 2241.28, 1764.87, 1516.05, 1298.09, 1143.79, 742.59 cm^{-1}. Principle peaks of drug ENZ also appear in the prepared SD (figure4), which indicates that there is no interaction between the drug ENZ and polymers SOL/POL selected for the preparation of SD.

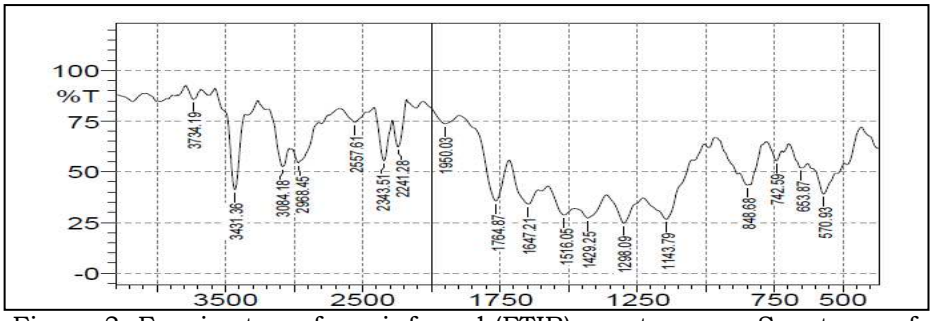

Figure 3: Fourier-transform infrared (FTIR) spectroscopy Spectrum of enzalutamide drug

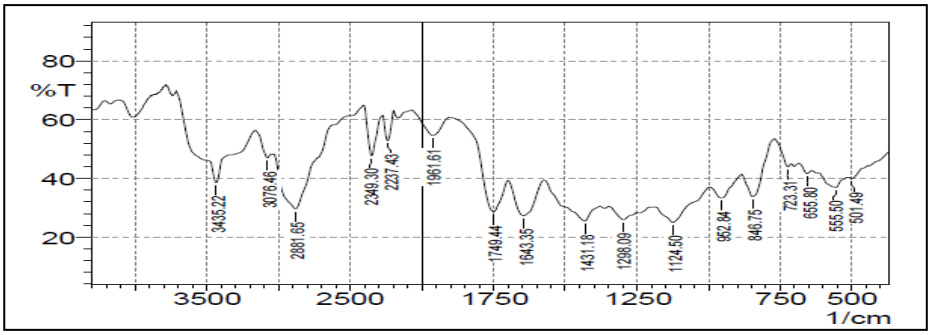

Figure 4: Fourier-transform infrared (FTIR) spectroscopy spectrum of SD prepared by spray drying

Differential scanning calorimetry (DSC) analysis

DSC study was performed to evaluate the thermal properties of the pure drug ENZ and the prepared SDs. Drug ENZ shows the melting endotherm at a temperature of 202.01°C. In the physical mixture of drug ENZ and polymers SOL/POL (1:4:1), shows melting endotherm at a temperature of 54.01° of polymers SOL/POL and subsequently at a temperature of 199.24°C of drug ENZ. In regards to selected SD (T-004), DSC thermogram showed only one endothermic peak at a temperature of 60.24°. No drug ENZ DSC thermal peaks were observed in the selected SD, which indicates that in the prepared SD, drug ENZ is probably dispersed in amorphous phase.

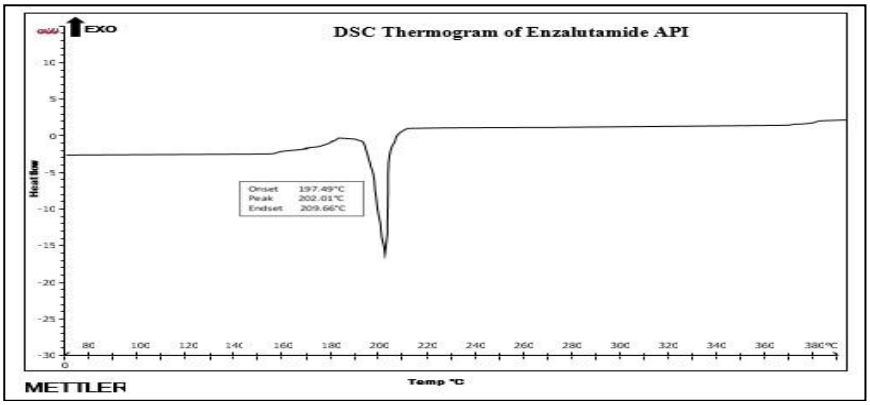

Figure 5: DSC Thermogram of Enzalutamide API

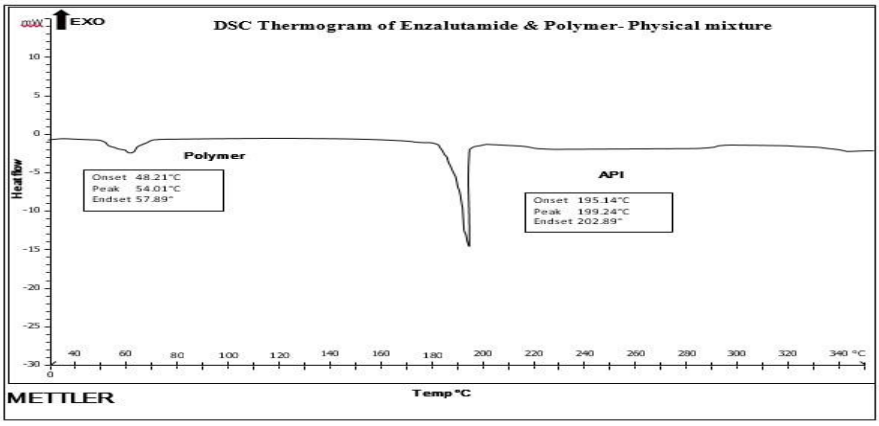

Figure 6: DSC Thermogram of Enzalutamide & polymers physical mixture

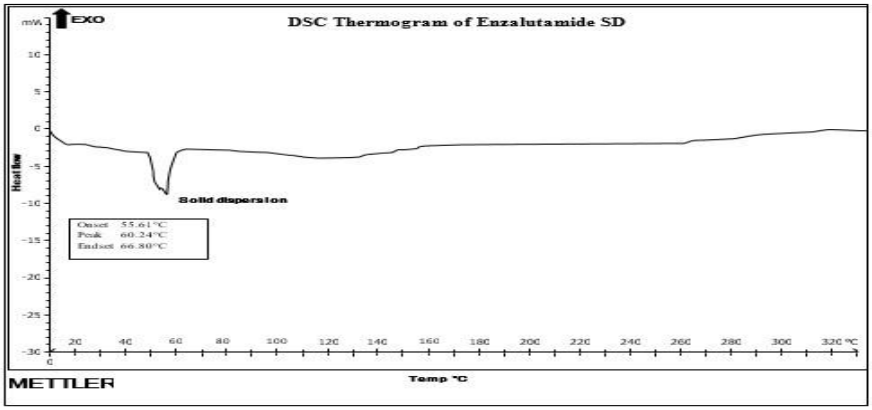

Figure 7: DSC Thermogram of Enzalutamide SD prepared by spray drying

X-ray diffraction (XRD) analysis

DSC analysis of selected SD (T-004) showed no drug ENZ melting peak endotherm, indicating that the API probably in amorphous form. Below figure8 shows p-XRD analysis of drug ENZ, polymers, physical mixture and selected SD. P-XRD of drug ENZ diffractogram appears to be crystalline nature having characteristic peaks.

Polymers SOL/POL mixture shows amorphous nature of SOL and also shows characteristic peaks of POL at 19.5° and 23.6° which indicates that crystalline nature of POL. In the physical mixture of drug ENZ:SOL:POL (1:4:1 ratio) shows characteristic peaks of drug ENZ and POL, which indicates that no conversion of crystalline form of drug ENZ to amorphous form. In the selected SD having ratio of ENZ:SOL:POL as 1:4:1, shows that disappearance of characteristic peaks of drug ENZ which is due to amorphous form of ENZ in SD. This amorphous SD shows enhancement in the dissolution of ENZ(Dahiya, S., 2022).

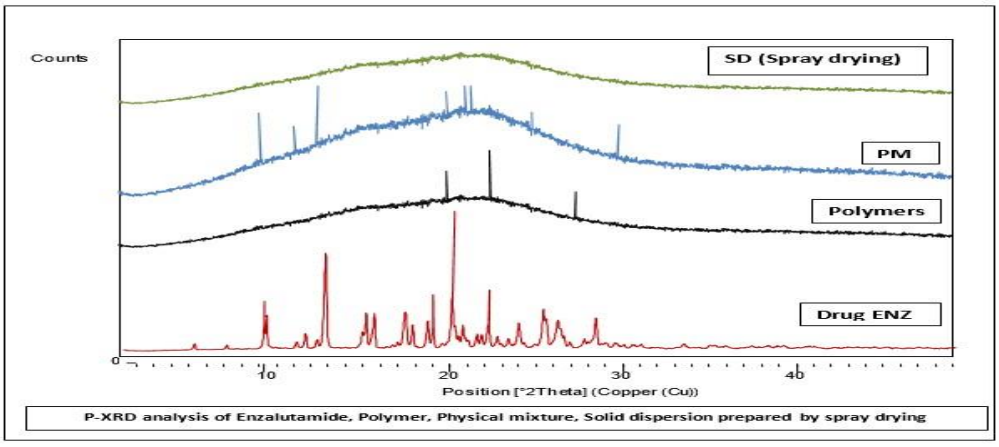

Figure 8: P-XRD diffractogram of ENZ, Polymers, physical mixture, SD prepared by spray drying

Powder physical properties and Particle size distribution (PSD) analysis

Powder physical properties such as bulk density (BD), tapped density (TD, compressibility index (CI), hausner ratio (HR) and Particle size distribution (PSD) carried out and the observed values tabulated in the below table/graph (Paudwal, G., 2019).

Table 5: Spray dried powder physical properties and particle size distribution (PSD)

Sr. No.	Powder Property	Observed value
1.	Bulk density (BD)	0.450 g/ml
2.	Tapped density (TD)	0.625 g/ml
3.	Compressibility index (CI)	28.000 %
4.	Hausner ratio (HR)	1.389
5.	Particle size distribution (PSD)	
6.	D10	2.927 µm
7.	D50	7.792 µm
8.	D90	16.853 µm

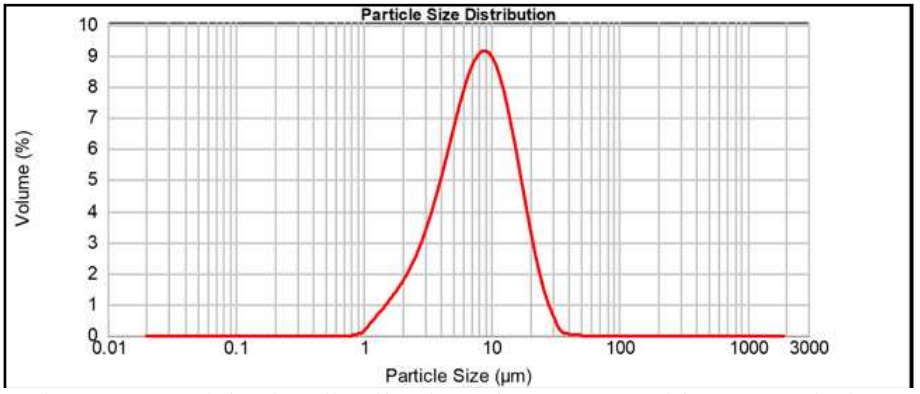

Figure 9: Particle size distribution of SD prepared by Spray drying

***In vitro* drug release (dissolution) study**

The decrease in the binary SD dissolution may be due to the recrystallization of ENZ. Figure 10 shows the mean dissolution profile of ENZ and Selected ternary SD. As expected pure drug ENZ shows slow dissolution rate in 0.1 N HCL with 1% SLS as dissolution medium, due to its low aqueous solubility in 0.1N HCl with 1% SLS. The selected ternary SD (T-004) prepared by spray-drying technique shows a drastic increase in the dissolution rate in 0.1N HCl with 1% SLS, this is due to the amorphous nature of drug ENZ in the selected ternary SD. Also, initial burst effect in the dissolution is due to the presence of POL in the SD (Kundu, R., 2022).

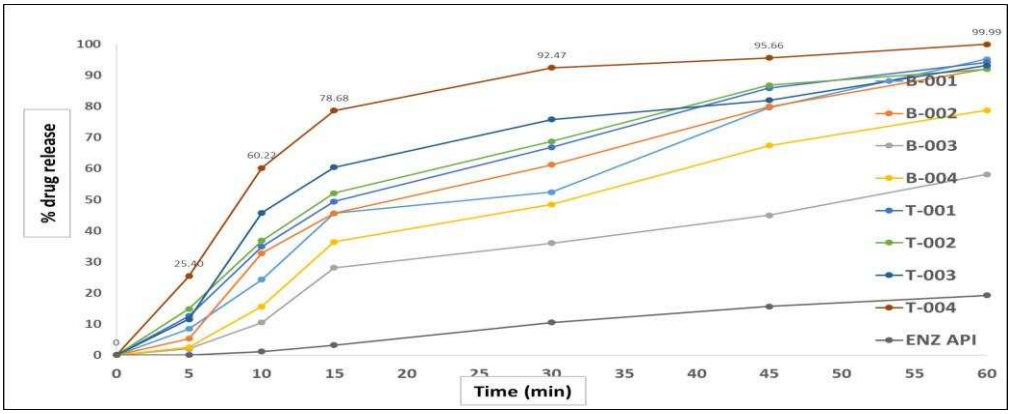

Figure 10: Initial Dissolution profile of Binary and Ternary SDs in 0.1N HCL with 1% SLS

***Ex-vivo* intestinal drug absorption study**

Ex-vivo intestinal drug absorption study was performed to forecast the dissolution and absorption rate of pure drug ENZ and the selected ternary SD (T-004) prepared by spray drying technique through everted goat intestine. Pure drug ENZ showed 19.25% drug absorption, while selected SD (T-004) showed 97.99 % drug absorption in 60 min (figure 11). The rate of drug absorption in SD was significantly increased as compared to pure drug. The enhancement in the absorption rate is due to the amorphous form of the drug in the SD and also due to the water-soluble SOL-POL which increases the wettability and ultimately the drug dissolution(Nousheen, L., 2022).

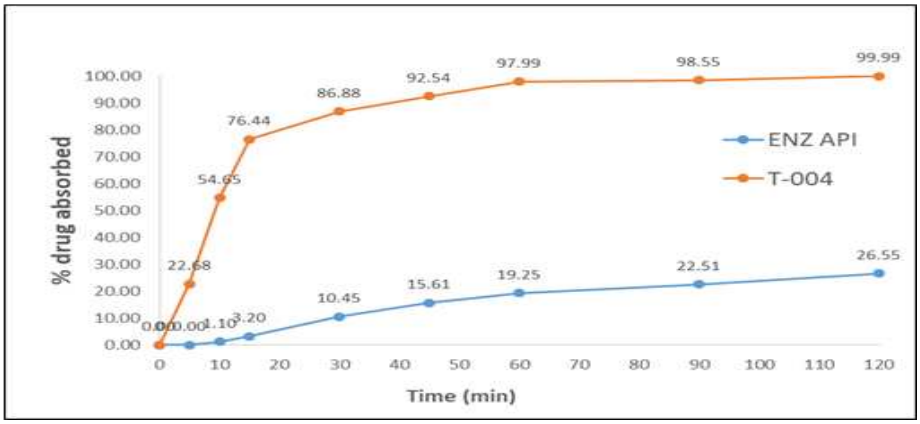

Figure 11: Ex-vivo intestinal drug ENZ & SD (T-004) absorption study

Stability study

All binary and ternary SDs were kept for stability study at 40°C/75% RH in a closed HDPE container. Analysis of all SDs done at 1month for assay and dissolution profile. The stability of the selected SD was continued for 3 months and analysed for assay and dissolution profile(Chiou, W. L., 1971).

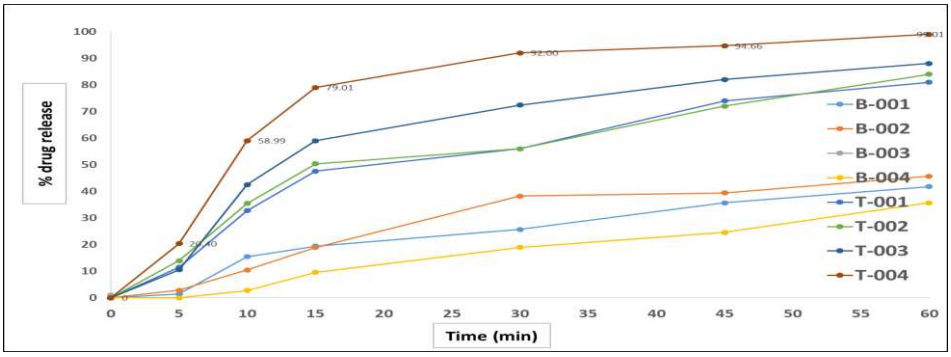

Figure 12: Dissolution profile of binary and Ternary SDs (1-month at 40°/75% RH)

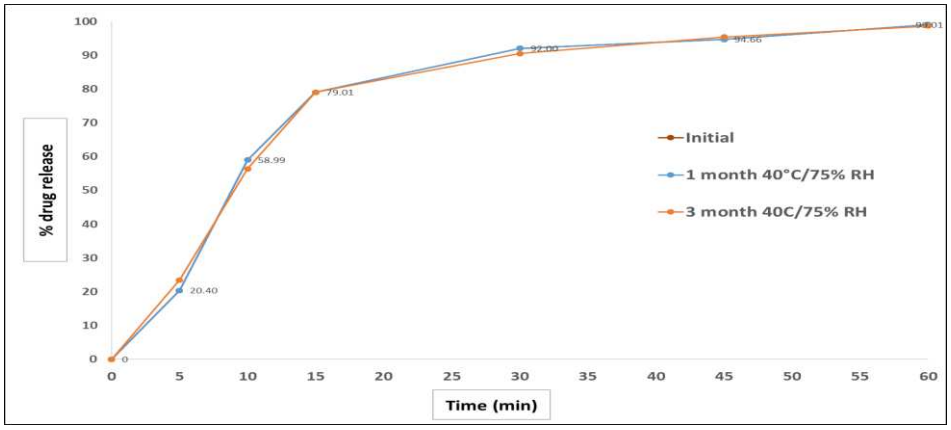

Figure 13: Dissolution profile of Ternary SD T-004 (3-month at 40°/75% RH)

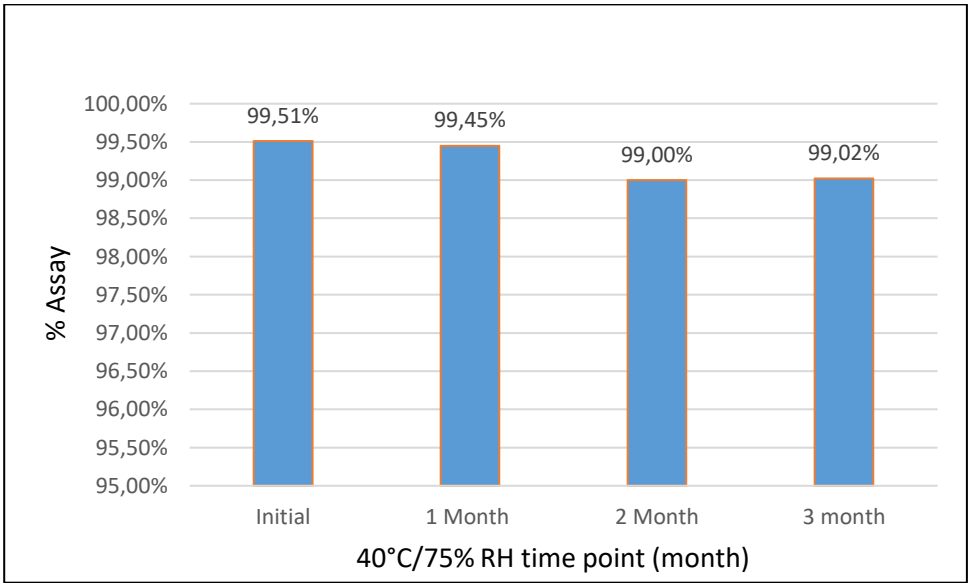

Figure 14: % Assay of Ternary SD T-004 (upto 3-month at 40°/75% RH)

Conclusion

In the present study ENZ ternary SD was prepared successfully using SOL & POL using spray drying technique. The dissolution and absorption rate of ENZ was increased many folds as in ternary SD prepared using SOL/POL as compared to the pure drug ENZ. The DSC & XRD study confirmed the conversion of the crystalline drug into an amorphous form in the ternary SD. *In vitro* drug release and *ex-vivo* intestinal drug absorption study suggest the rapid absorption of ENZ as compared to pure ENZ. From this study, it was concluded that ternary SD prepared from Soluplus (SOL) and Poloxamer P 188 (POL) by spray drying technique is the right approach for dissolution and absorption enhancement of poorly soluble drugs such as Enzalutamide (ENZ). This formulated SD can be used in the preparation of the oral solid dosage form such as capsules, tablets, etc.

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Conflict of interest: The authors report no conflicts of interest regarding this research article.

Ethical clearance: Not applicable

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