

# Nanoparticulate Drug Delivery: Ezetimibe and Enteric-coated Atorvastatin in Hard Gelatin Capsules

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## Abstract

**Aims:** Atorvastatin (ATV) and ezetimibe (EZT), being BCS class II drugs, are used to treat dyslipidemia. In this study, the formulation of hard gelatin capsule containing a fixed dose combination of EZT and enteric-coated ATV enhances the rate of dissolution through solubility using nanoprecipitation method. **Materials and Methods:** ATV nanoparticles (NPs) and EZT NPs were prepared using polyvinyl pyrrolidone k-30 as an amorphous carrier in various drug to polymer ratios. The formulated NPs of both drugs were characterized for their particle size, zeta potential, and polydispersity index, and for optimized formulation entrapment efficiency and scanning electron microscopy (SEM) which were determined. AT V NPs were coated using ethyl cellulose and were characterized. Flow characteristics of enteric-coated ATV and EZT NPs were assessed and filled into empty hard gelatin capsules. The hard gelatin capsule containing powders of EZT and enteric-coated ATV NPs was also evaluated for weight variation, disintegration time, *in vitro* drug release, and drug content. **Results and Discussion:** The particle size for the formulation of ATV NP and EZT NP was found to be 470.5 nm and 469.2 nm, respectively. The morphology of the best formulation for both NPs, determined using SEM, indicates that they are spherical in shape. The *in vitro* release studies of enteric-coated ATV NPs show 5% release in an acidic pH for 2 h followed by 92.89% release in a basic pH at the end of 3 h before loading into hard gelatin capsules to prove that there was no release of ATV NPs in acidic medium. Hard gelatin capsules containing enteric-coated ATV NPs and EZT NPs showed drug release of 98% and 99% within 45 min. **Conclusion:** The hard gelatin capsule of enteric-coated ATV NPs and EZT NPs showed better drug release when compared to the marketed formulation.

**Key words:** Enteric-coated atorvastatin, dissolution, ezetimibe nanoparticle, hard gelatin capsule

## INTRODUCTION

Poor pharmacokinetics limits the bioavailability of the majority of drug candidates, especially in oral drug delivery, which is still the most common, popular, and preferred method of administration. Finding strategies to improve a drug's solubility without compromising its stability and therapeutic efficacy are one of the biggest challenges faced in the pharmaceutical industry because issues with inadequate bioavailability are frequently tied to the drug's poor solubility.<sup>[1]</sup>

Due to their small size and high surface area, nanoparticles (NPs) have various advantages over traditional drug delivery methods. Transporting intact NPs through the gastrointestinal mucosa is another approach that could provide significantly more bio-enhancement while increasing solubility is one way to increase bioavailability.<sup>[2]</sup>

NP is a form of colloidal drug delivery system consisting of particles with diameters ranging from 10 to 1000 nm which is used as one of the methods to enhance solubility.<sup>[3]</sup> The main benefits of NPs are increased bioavailability due to improved aqueous solubility, longer resistance time in body, targeting the drug to a specific location in the body (its site of action), and reduced toxicity.

This allows for the safe delivery of therapeutic medications, protection of non-target tissues, and cells from serious side effects thereby reduction in the drug dose.<sup>[3]</sup> The nanoprecipitation-homogenization method was carried out

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using a high-speed homogenizer. This technique relies in the precipitation of polymeric particles containing drugs from an organic solution in aqueous conditions.<sup>[2]</sup>

Atorvastatin (ATV), a BCS class II medication, has a meager 14% systemic bioavailability due to significant hepatic first-pass metabolism. ATV is metabolized by the CYP3A4 isoenzyme. ATV causes reduction in total cholesterol, low-density lipoproteins, and triglycerides, as well as rise in high-density lipoproteins.<sup>[4]</sup> The deterioration of ATV may be accelerated by several factors, including oxygen, humidity, acidity, and temperature.

Ezetimibe (EZT), a BCS class II medication, has a low water solubility and dissolution profile. EZT undergoes first-pass metabolism, enterohepatic recirculation, and P-glycoprotein efflux which reduce its bioavailability.<sup>[5]</sup> It reduces blood cholesterol by selectively limiting phytosterol and cholesterol absorption through the small intestine without disturbing the absorption of fat-soluble vitamins and minerals. Niemann-Pick C1-Like1 protein, involved in cholesterol transport, is the main target for EZT.

In this study, oral enteric coated of ATV NPs and nanoparticulate EZT from hard gelatin capsule was formulated and assessed for quality parameters.

## MATERIALS AND METHODS

### Materials

ATV calcium and EZT were obtained as gift samples. Polyvinyl pyrrolidone (PVP K-30) (Loba chemie Pvt. Ltd, Mumbai), polyvinyl alcohol (PVA) (Loba chemie Pvt. Ltd, Mumbai), hydroxypropyl methylcellulose (HiMedia Laboratories Mumbai), Tween 80 (Loba chemie Pvt. Ltd, Mumbai), sodium lauryl sulfate (Reachem Laboratory Chemicals Pvt. Ltd, Chennai), methanol (Loba chemie Pvt. Ltd, Mumbai), ethyl cellulose (Loba chemie Pvt. Ltd, Mumbai), and PEG 4000 (HiMedia Laboratories Pvt. Ltd, Mumbai) were used.

### Methods

#### Preparation of ATV and EZT-loaded nanoparticle

ATV polymeric NPs were prepared by the nanoprecipitation technique using a high-speed homogenizer. ATV was dissolved in methanol, a water-miscible organic solvent, and kept in bath sonicator for 10 min. The organic phase was added drop wise into an aqueous phase containing different ratios of polymer and stabilizer, with homogenization at 15,000 rpm for 30 min resulting in the precipitation of NPs.<sup>[4]</sup> The ATV NPs were then lyophilized using lyophilizer (Lyodel freeze dryer).

Similarly, for EZT NPs, the organic phase was added to the aqueous phase containing different ratios of polymer with various stabilizers and homogenized (10,000 rpm for two cycles).<sup>[6,7]</sup> The ATV NPs were enteric-coated using 15% ethyl cellulose of core weight, PEG 400,020% w/w for polymer weight, and using Sudan Red dye in ethanol as solvent.

### Characterization of NPs

#### Particle size, polydispersity index (PDI), zeta potential

The zeta potential, particle size, and PDI of formulated NPs were determined using Malvern zetasizer. All the samples were suitably dispersed with colloidal dispersion in distilled water.<sup>[8,9]</sup>

#### Equivalent weight of ATV and EZT in preparation

To 10 mL volumetric flasks containing phosphate buffer, 10 mg of ATV NPsF8 and EZT NPsF8 were added in separate flask and then examined UV-Visible spectrophotometer at 247 nm and 231 nm, respectively. The amount of drug found in 10 mg NPs was determined separately.<sup>[10]</sup>

#### Entrapment efficiency

The entrapment efficiency for ATV NPsF8 and EZT NPs F8 was determined by centrifugation method. The samples were dissolved in methanol and kept for ultra-centrifugation at 15,000 rpm for 10 min and the unencapsulated ATV and EZT present in the supernatant liquid was determined at 247 nm and 231 nm UV-Visible spectrophotometrically.<sup>[11]</sup>

#### Scanning electron microscopy (SEM)

SEM was used to examine the surface morphology of polymeric NPs. The optimized formulation F8 was lyophilized and sprinkled on one side of the double adhesive stub. The NPs were observed at a voltage of 15–20 kV.

### Characterization of enteric-coated ATV NPs

#### Equivalent weight of ATV in enteric-coated NPs preparation

10 mg of enteric-coated ATV NPs F8 was dissolved in pH 6.8 phosphate buffer. The samples were diluted and analyzed in a UV-visible spectrophotometer at 247 nm. The amount of drug found in enteric-coated preparation was determined.<sup>[12-15]</sup>

#### In vitro drug release studies

Enteric-coated ATV NPs F8 was subjected to *in vitro* drug release using dissolution apparatus USP II (paddle) for evaluating its non-solubility in acidic pH. The dissolution medium used was 900 ml of 0.1 N HCl (pH 1.2), at 37 ± 0.5°C at 75 rpm for the first 2 h, followed by phosphate buffer (pH 6.8) with Tween 80 (0.2% w/v). Five milliliter samples

were taken, filtered and then replaced with fresh dissolution medium to maintain sink condition. The samples were appropriately diluted if necessary, and the percentage of drug release was measured using UV-Visible spectrophotometer at 247 nm.<sup>[12-15]</sup>

### Evaluation of micromeritic properties

The enteric-coated ATV NPs F8 and EZT NPs F8 were evaluated for angle of repose, bulk density, tapped density, Hausner's ratio, and compressibility index.<sup>[16,17]</sup>

### Filling of hard gelatin capsules

The enteric-coated ATV NPs F8 and EZT NPs F8 powder were filled into hard gelatin capsule [Table 3].

### Evaluation of hard gelatin capsules

#### Weight variation

Ten capsules were selected randomly. The capsules were weighed individually and the contents were removed completely as possible, and then, the empty shells were weighed. The net weight was determined by subtracting the weight of the shells from the weight of the intact capsule. The procedure was repeated for all other capsules. The percentage deviation from the average weight of each capsule was determined.<sup>[18]</sup>

#### Disintegration time

Disintegration time was determined using the disintegration apparatus. One capsule was placed in each of the six tubes of the apparatus and the assembly was suspended in buffer. Disks were added to each tube, the temperature was maintained at  $37 \pm 2^\circ\text{C}$ , and the assembly was operated.

#### Drug content

Drug content was estimated by taking enteric-coated ATV NPs and EZT NPs equivalent to 10 mg and dissolved in 100 mL of pH 6.8 phosphate buffer, diluted, filtered, and analyzed UV-Visible spectrophotometrically at 247 nm and 231 nm, respectively.<sup>[19]</sup>

#### In vitro drug release

Using dissolution apparatus USP II (paddle), the release rate of ATV and EZT from hard gelatin capsules was calculated. The dissolution medium used was pH 6.8 phosphate buffer 900 mL with tween 80 (2% w/v) and 5 mL samples were taken for 15 min, 30 min, and 45 min and replaced with fresh medium to keep the sink condition. The aliquots were examined UV-Visible spectrophotometrically at 247 nm and 231 nm, respectively, after filtering using Whatman filter paper.<sup>[20,21]</sup>

## RESULTS AND DISCUSSION

### Characterization of ATV NPs and EZT NPs

#### Particle size, PDI, and zeta potential

The particle size and PDI for ATV NPs (F8) were found to be 470.5 nm and 0.562, respectively [Figure 1]. The zeta potential for ATV NPs (F8) was found to be  $-27.7$  mV [Figure 2]. The particle size and PDI for EZT NP (F8) were found to be 462.9 nm and 0.656, respectively [Figure 3]. The zeta potential for EZT NP (F8) was found to be  $-37.30$  mV [Figure 4], [Tables 1 and 2] and proves the suitability of both the drugs for NPs.

#### Entrapment efficiency

Entrapment efficiency for ATV NPs and EZT NPs (F8) was found to be good with 86.54% and 63.68%, respectively.

#### Equivalent weight of ATV and EZT in NPs preparation

10 mg of ATV NPs and EZT NPs were dissolved in pH 6.8 phosphate buffer and absorbance was noted in UV-visible

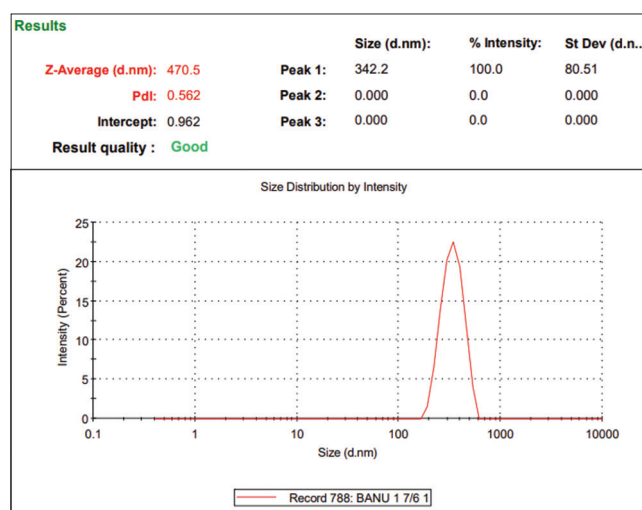


Figure 1: Particle size and polydispersity index of atorvastatin nanoparticle

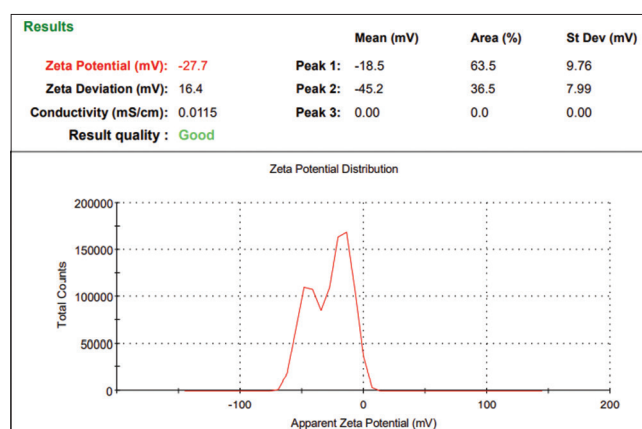


Figure 2: Zeta potential of atorvastatin nanoparticle

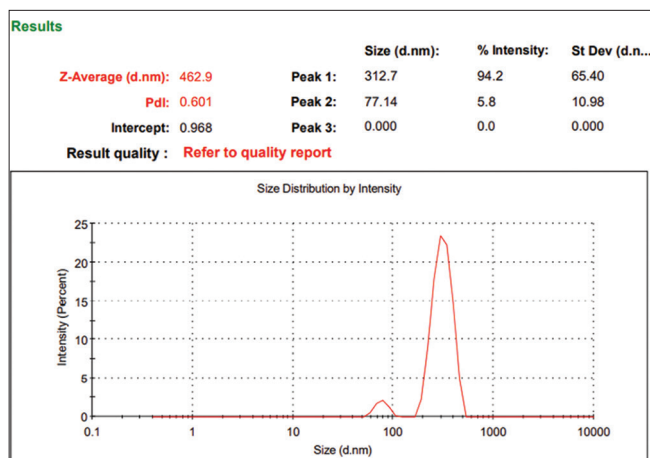


Figure 3: Particle size and polydispersity index of ezetimibe nanoparticle

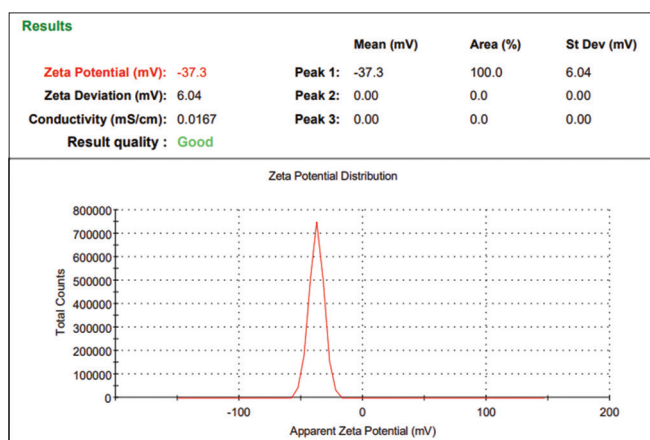


Figure 4: Zeta potential of ezetimibe nanoparticle

spectrophotometer at 247 nm and 231 nm, respectively, and found that 10 mg of ATV and EZT were present in 10.64 mg and 16.97 mg of preparation, respectively.

### Morphological evaluation by SEM

The morphological evaluation was determined for lyophilized NPs of ATV and EZT (F8), which was found to be spherical in shape [Figures 5 and 6].

### Characterization of enteric-coated ATV NPs

#### Equivalent weight of ATV in preparation

10 mg of enteric-coated ATV NPs was dissolved in pH 6.8 phosphate buffer and absorbance, which was noted in UV-visible spectrophotometer at 247 nm after suitable dilution and found that 10 mg of ATV was present in 41.32 mg of enteric-coated preparation.

#### In vitro drug release study for enteric-coated ATV NP

After 2 h, 5% drug release was seen at pH 1.2, followed by 92.89% release at pH 6.8 after 3 h, as shown in Figure 7 which indicates drug release of ATV only in intestinal pH.

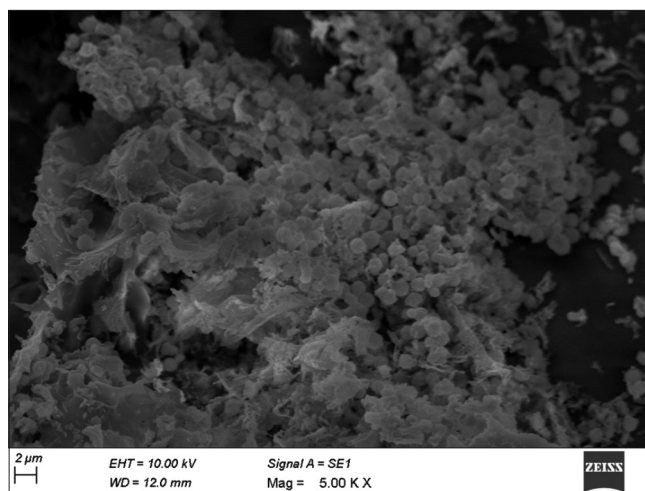


Figure 5: SEM images of atorvastatin nanoparticle

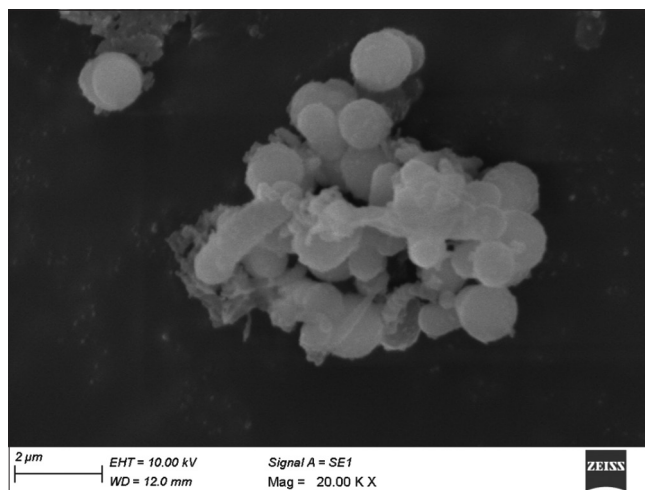


Figure 6: SEM images of ezetimibe nanoparticle

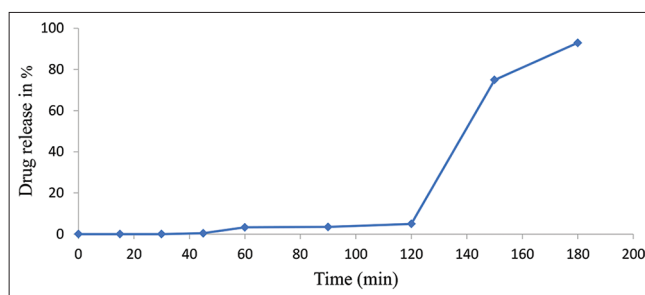


Figure 7: In vitro release study of enteric-coated atorvastatin nanoparticle

### Micromeritic properties of the enteric-coated ATV NPs and EZT NPs powder

Bulk density and tapped density were found to be 0.35 g/cc and 0.37 g/cc, respectively. The compressibility index and Hausner's ratio were found to be 5.405% and 1.057, respectively. The angle of repose was found to be



**Table 1: Formulation of atorvastatin nanoparticles**

Formulation code	ATV (%)	PVP K-30(%)	PVA (%)	Particle size (nm)	PDI	Zeta potential (mV)
F1	1	0.5	0.5	417.9	0.230	-0.958
F2	1	0.75	0.25	592.7	0.392	-2.79
F3	1	0.25	0.75	523	0.664	-3.27
F4	1	1	1	518	0.546	-3.22
F5	1	0.5	1.5	833	0.982	-5.33
F6	1	1.5	0.5	655.1	0.696	-0.161
F7	1	1.75	0.25	515.2	0.639	-0.926
F8	1	2	0.5	470.5	0.562	-27.7
F9	1	2	1	539.1	0.749	-21.9

ATV: Atorvastatin, PVPK-30: polyvinyl pyrrolidone-30, PVA: Polyvinyl alcohol, PDI: Polydispersity index

**Table 2: Formulation of ezetimibe nanoparticles**

Formulation code	EZT (%)	PVPK-30 (%)	Stabilizer (%)	Particle size (nm)	PDI	Zeta potential (mV)
F1	1	1	1	1088	0.592	-0.674
F2	1	2	0.5	1373	0.242	-7.4
F3	1	2	1	1199	0.417	-4.96
F4	1	3	0.5	952.2	0.021	-3.14
F5	1	2	0.5	1051	0.799	-5
F6	1	3	0.5	1102	0.467	-7.81
F7	1	0.5	0.5	752	0.648	-29.1
F8	1	1	0.5	469.2	0.656	-37.30
F9	1	2	0.5	693	0.886	-53.2
F10	1	1	0.5	239.3	0.875	-21.5

EZT: Ezetimibe, PVP K-30: Polyvinyl pyrrolidone k-30, PDI: Polydispersity index, F1, F2, F3: PVA act as a stabilizer; F4, F5, F6: HPMC act as stabilizer, F7, F8, F9: SLS act as stabilizer; F10: Tween-80 act as stabilizer

**Table 3: Composition of hard gelatin capsules**

S. No.	Ingredients	For 1 capsule (%)	For 20 capsules (%)
1	Enteric-coated ATV nanoparticle	41.32 mg	826.4 mg
2	EZT nanoparticle	16.97 mg	339.4 mg
3	Talc	1	1
4	Magnesium stearate	2	2

\*41.32 mg F8 is equivalent to 10 mg ATV, \*16.97 mg F8 is equivalent to 10 mg EZT

28.76. The enteric-coated ATV NPs and EZT NPs powder were found to have excellent flow characteristics.

### Evaluation of hard gelatin capsules

#### Weight variation

One capsule deviates from the limit, as shown in Table 4, while the others were found to be in the acceptance range.

**Table 4: Weight variation for hard gelatin capsules**

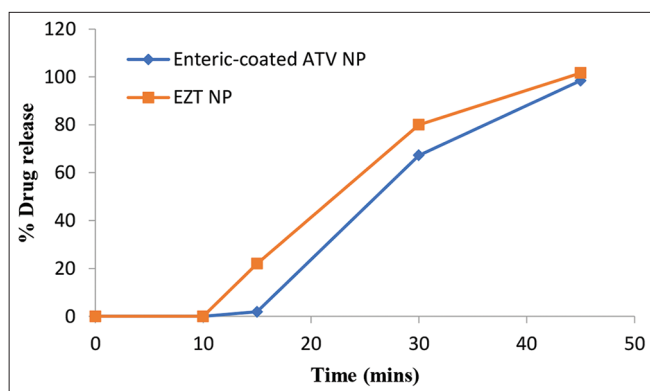
S. No.	Individual weight (mg)	Percentage deviation
1	137.7	-0.44
2	134.4	1.96
3	123	10.27
4	128.1	6.55
5	138.3	-0.882
6	138.4	-0.955
7	137.9	-0.590
8	136.5	0.430
9	138.6	-1.101
10	136.3	0.576

#### Disintegration time

The enteric-coated ATV NPs and EZT NPs of hard gelatin capsules disintegrated in 7 min and 25 s and pass the test.

#### Drug content

Drug content for enteric-coated ATV NPs and EZT NPs hard gelatin capsule was found to be 96.34% and 88.56%, respectively, and passes the test.



**Figure 8:** *In vitro* drug release study for atorvastatin and ezetimibe hard gelatin capsule

### ***In vitro* drug release studies**

The release rate of enteric-coated ATV NPs and EZT NPs of hard gelatin capsule shows release of 98% and 99% in 45 min, as shown in Figure 8. Whereas marketed tablet shows only 55% release for ATZ and 50% for EZT<sup>[22,23]</sup> in 45 min which proves NPs are having enhanced rate of dissolution.

## **CONCLUSION**

The objective of the present investigation has been achieved by formulating a hard gelatin capsule containing a fixed dose combination of enteric-coated ATV NPs and EZT NPs. The formulation F8 was selected as the best formulation as it shows better particle size (ATV NPs and EZT NPs were 470.5 nm and 462.9 nm) and zeta potential (ATV NPs and EZT NPs were -27.7 mV and -37.3 mV) with PDI (ATV NPs and EZT NPs were 0.562 and 0.601). This formulation F8 was analyzed for entrapment efficiency and SEM. ATV NPs were enteric-coated and characterized. The flow characteristics of enteric-coated ATV NPs and EZT NPs are assessed. Based on the above studies, it may be concluded that the enteric-coated ATV NPs and EZT NPs of hard gelatin capsules showed better drug release, when compared to marketed formulation, it shows 1.7-fold increase in ATV NPs and 1.8 fold increase in EZT NPs Which may further evaluated for its *in vivo* behavior.

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