# *Atorvastatin Calcium nanoparticles using solvent-anti-solvent precipitation method*

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## ABSTRACT

Atorvastatin is used for lowering cholesterol. Atorvastatin calcium has a poor bioavailability of 12% after oral administration. To improvise bioavailability, nanoparticles had been designed by anti-solvent precipitation method using optimized 6 stabilizers of varying concentration of Poloxomer-188, HPMC-E5, PVP K-30, PVA, SLS, and Tween-80. The studies reviled, optimized AT37 batch nanoparticles had good drug loading capacity, good liquid state stability (30>) and average particle size was found to be after spray drying 133.5nm. Spray dryad particles were used for short term stability studies along with *in-vitro* and *in vivo* release studies. It was concluded that anti-solvent precipitation followed by spray drying of nanosuspension as a method can enhance the dissolution and oral bioavailability of poorly soluble drug like atorvastatin calcium.

**Keywords:** Atorvastatin calcium, spray dryad nanoparticles, lyophilisation, PVP K-30

## INTRODUCTION

The potential importance to intensify research on BCS class II & IV to improve bioavailability is the forbidden challenge to pharmaceutical scientists in recent time.[1] A various technique such as micronization, chemical modification, size reduction (nano-suspension, solid self-emulsification system, nanoemulsion), pH adjustment, solid dispersion, complexation, co-solvency, micellar solubilization, hydrography used extensively to improve aqueous solubility, poor bioavailability & dissolution rate.[2] Atorvastatin is a drug which is used to lower plasma cholesterol level by inhibiting Hydroxymethyl Glutaryl Coenzyme A (HMG-CoA) reductase. The absolute bioavailability of Atorvastatin (parent drug) is only ~12% and the systematic availability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic bioavailability is due to systemic clearance by gastrointestinal mucosa and

first-pass metabolism in the liver. It is very hydrophobic with Log P value of 5.7 and lower aqueous solubility 0.63mg/L. These factors contribute to its highly variable and poor bioavailability of 12% after oral administration. Nanoparticles prepared by anti-solvent precipitation method [3, 4] forms a submicron colloidal dispersion of nano-sized drug upon dilution with an aqueous medium or *in-vivo* administration. After much more screening in this study, 3 main stabilizers such as Poloximer-188, HPMC E-5, PVPK-30, were used. The final optimized batch was then spray dried and comparative studies were made with lyophilized nanoparticles. Saturation solubility of spray dried and lyophilized nanoparticles were almost 4 times greater than the plain drug. IR, DSC, PXRD studies concluded with good compatibility with drug and excipients. Two-month stability study also carried out at 25ºC, which concludes, optimized batch shows no significant degradation during a one month period. Further spray dried nanoparticles were evaluated by dissolution, where an increase in *In-vitro* dissolution profile for prepared nanosuspension was compared. Finally, Wistar albino male rat were used for In-vivo pharmacokinetic studies.

## MATERIALS AND METHOD

The Atorvastatin calcium obtained from Zydus Cadila Ltd., Ahmedabad, India. Povidone and D-Mannitol procured from S.D Fine Chemicals Ltd., Mumbai, India. Poloxamer 188 was purchased from Signet Chemicals Ltd., Mumbai, India. Methanol obtained from Merck Ltd., Mumbai, India. Ibuprofen procured from Wearable pharmaceuticals Ltd., Mehsana. HPLC grade Methanol procured from Merk Ltd., Mumbai, India. Finally, HPLC grade acetonitrile and water procured from Fine Chemicals Ltd., Mumbai India. All the chemicals and reagents were of analytical grade. Mansour Mansouri *et al.* [5] studied the characteristics of Ibuprofen nanoparticles by using solvent- anti-solvent precipitation method. He used PVP, SLS, and Tween-80 as stabilizers and the results were quite satisfactory. In this research, at 20000RPM (30minutes) using high-speed homogenizer (Kinematica, polytron. Pt 2500E) we prepared bluish nanosuspensions. The initial optimized batch was found to be AT26.This batch was then ultrasonicated (FS-5, Frontline limited, Mumbai, India) with a power input of 300W for 10-time length in small unit of bulk.

### Conversion of liquid nanosuspension to dry nanoparticles [6]

Different methods are available like lyophilization, spray drying, vacuum drying, evaporation by heating but we preferred lyophilization of all batches and Spray drying for only optimized batch. The equivalent weight of dry nanoparticle filled into the 0 size hard gelatine capsule.

### Selection of stabilizer

The 60mg Atorvastatin were dissolved in 3 ml methanol and varying stabilizers such as Polox-188, HPMC-E5, PVK-30, PVA, SLS, and Tween-80 in different concentration were used to form nanosuspension. But AT1-AT29 batch has serious stability issues. Only AT26 (maintaining 20000 RPM for 10minutes) shows some good liquid state stability within 30 days. The following table1 shows the effect of increasing drug carrying capacity, with homogenization speed of 20000RPM and homogenization time of 30 minutes. AT37 showing good liquid state stability in nanosuspension.

## PREPARATION OF NANOSUSPENSION

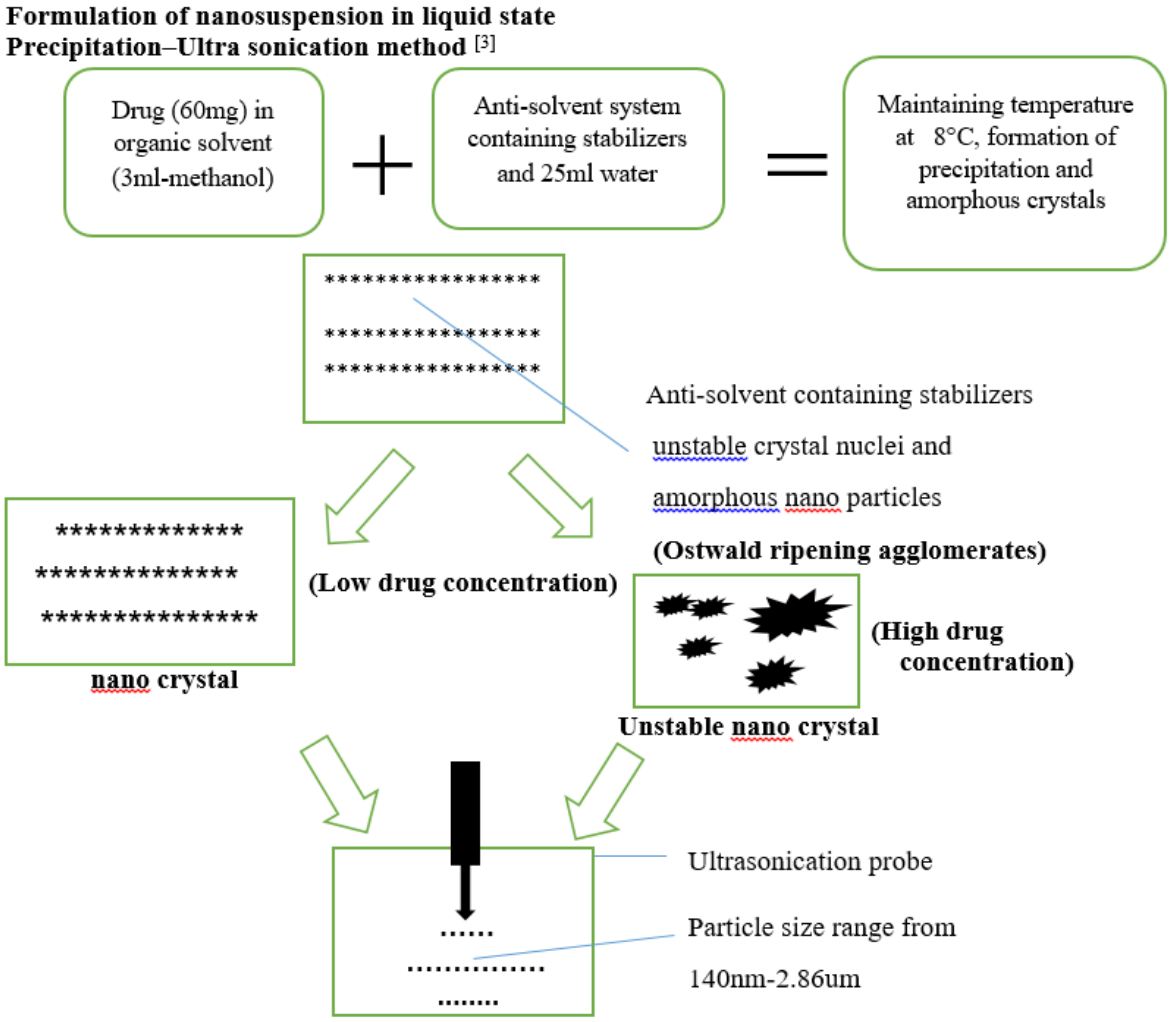



**Figure 1: Precipitation steps & ultra-sonication step diagram for Atorvastatin calcium nanosuspension**

AT37 batch nanosuspensions was precipitate out at 2-6ºC temperature and 20000RPM homogenization speed for 30 minutes. After homogenization, nanosuspension was subjected to probe sonication for 10 minutes. AT37 batch shown good liquid state stability and particle size of 124.9nm, while AT38 batch had only 1-day liquid state stability. So from the result of all batches finally AT37
batch has selected for the optimization of the formulation with the cooperation of spray dried nanoparticle and lyophilized nanoparticles.

**Lyophilisation of AT37 batch:** Along with the nanosuspension, D-mannitol 300% w/w of stabilizer which is used as a cryoprotectant, deep freezed at -45ºC for 8hr and then lyophilized. After lyophilisation, particle size was measured.
**Spray drying:** In this technique inlet temperature should be 120-130ºC, Outlet temperature 65-75ºC. Flow rate of solution maintained at 180ml/hr with air flow rate of 40-50$m^3$/h. After spray drying, particle size was measured.

**UV and HPLC analysis**

Using UV/Vis double beam spectrophotometer of Shimadzu Corporation, Japan (model number UV-1800) calibration studies of Atorvastatin calcium was performed. Atorvastatin calcium maximum absorbance observed at 245nm.

HPLC studies of Atorvastatin calcium were performed in human plasma, were 10µg/ml Ibuprofen considered as an internal standard. Reverse phase-HPLC instrument equipped with a UV-Visible detector and a photodiode array detector (Shimadzu, LC-2010CHT, Japan) with auto sampler were used. In the column, Phenomenex (Torrance, CA) C18 column (250 mm × 4.6 mm id, 5 µm particle size) were used. LC-solution software was used for experimental output.

**Table 1: Effect of drug carrying capacity**

| Batch No. | Amount of Drug(mg) in 3ml methanol | Amount of Stabilizer(mg) in 25ml Dist. Water | Result | Liquid State stability of Nanosuspension in Day(s) |
|---|---|---|---|---|
| AT 2 | 60 | 12.5 mg polox-188 | Nano suspension | 5 |
| AT 30 | 120 | 25 mg polox-188 | Nano suspension | 2 |
| AT 31 | 240 | 60 mg polox-188 | Aggregation of drug | - |
| AT 26 | 60 | 75 mg PVP K-30 | Nano suspension | 30> |
| AT 32 | 120 | 150 mg PVP K-30 | Nano suspension | 30> |
| AT 33 | 240 | 300 mg PVP K-30 | Nano suspension | 30> |
| AT 34 | 500 | 625 mg PVP K-30 | Nano suspension | 30> |
| AT 35 | 1000 | 1250 mg PVP K-30 | Nano suspension | 2hr only |
| AT 36 | 750 | 937.5 mg PVP K-30 | Nano suspension | 12hr only |
| **AT 37** | **625** | **781.25 mg PVP K-30** | **Nano suspension** | **30>** |
| AT 38 | 675 | 843.75 mg PVP K-30 | Nano suspension | 1 |

**EVALUATIONS OF NANOPARTICLES**

**Particle size**

The average diameter of Atorvastatin calcium dry nano suspensions was determined by Dynamic Light Scattering (DLS) (Zetatrac, micro track, India) at room temperature. The samples were adequately diluted with deionized water and placed in a cell. The average particle size was measured after performing the experiment in triplicates.

**Saturation Solubility studies**
Weighed the amount of Atorvastatin calcium (pure drug) and nanoparticles (obtained from spray drying and lyophilization of batch AT37) equivalent to 20mg of the drug were separately introduced into 25 ml stoppered conical flasks containing 10 ml of distilled water. The sealed flasks were agitated on a rotary shaker for 24 h at 37°C and equilibrated for 1 days. An aliquot was passed through a 0.22µm membrane filter (Millipore Corporation) and the filtrate was suitably diluted and analyzed on a UV Spectrophotometer at 245 nm.

***In-vitro* dissolution study**[7]
The dissolution profiles of the plain drug, a physical mixture of drug & polymer, Spray dried nano suspension & lyophilized nanosuspension of optimized batch AT37 were determined in a dissolution tester (TDT-06P Tablet Dissolution Rate Test App Electro. Lab, Mumbai) by USP apparatus I (basket) in 900ml phosphate buffer pH6.8. The dissolution media were maintained at 37±0.5°C with a basket rotation speed at 75 RPM. The amount of drug used was equivalent to 20 mg, filled in a capsule. At specified time intervals (5, 10, 15, 20, 30 minutes) 10ml of dissolution media were withdrawn and replaced with an equal volume of the fresh medium to maintain at 37°C to maintain a constant total volume. Samples were filtered through a 0.22µm nylon membrane filter (Millipore, Bedford, MA) and assayed for drug content spectrophotometrically at 245nm using UV-1800, Shimadzu Corporation, and Japan UV/Vis double beam spectrophotometer. The cumulative percentage of the drug dissolved in the preparations was calculated using calibration equations. Dissolution tests were performed in three vessels per formulation (n = 3).

**Differential scanning calorimetry (DSC)**
DSC scans of the powdered sample of Atorvastatin calcium and final optimized formulation (Spray dried nanoparticles of AT 37 Batch) were recorded using DSC-Shimadzu 60 with TDA trend line software. The thermal traces were obtained by heating from 50°C to 300°C at a heating rate of 10°C under inert N2 dynamic atmosphere (100ml. min-1) in open crucibles. Aluminum pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

**Powder X-ray diffraction (PXRD)**
PXRD diffractograms of the pure drug and spray dried Atorvastatin calcium nano suspensions were recorded in Start lab. The 2thetascan range was 0 to 60 theta.

***In vivo* bioavailability studies**
By using male Wister rats, *In vivo* bioavailability studies was performed. As per animal house registration number 197/99/CPCSEA, total nine rats were taken for this study. Atorvastatin was administered orally using 1mL of 0.2% (w/v) methyl cellulose aqueous suspensions containing raw Atorvastatin calcium (equivalent to 25 mg/kg body weight as atorvastatin), spray dried Atorvastatin calcium nanoparticles and suspensions containing without drug respectively. HPLC analysis was done on a plasma sample with respect to Ibuprofen as an internal standard. The *in-vivo* study was carried out three times on interval of the week to reduce the variation. Pharmacokinetic parameters $C_{max}$, $t_{max}$ and $AUC_{0\text{-}12h}$ were calculated using software graph pad prism 5.

**Short-term Stability study** [8]
During stability study, optimized formulations were placed in glass vial fitted with aluminum foil and make a pinhole in it. The formulation was subjected to stability studies as per ICH (The International Conference on Harmonization) guidelines for a period of 1 month at room temperature (25ºC). The samples subjected to stability studies were then analyzed at 2-week intervals. The dried Nanoparticles was evaluated by comparisons of % Drug content and $Q_{5min}$.

## RESULT AND DISCUSSION

**Particle size determination**
The average particle size (nm) of the lyophilised AT37 batch was found to be 141.7nm. The average particle size after reconstitution of spray dried powder of AT37 batch was found to be 133.5nm.

**Saturation Solubility study**
The dry powder obtained after spray drying and lyophilization of AT 37 batch and the pure drug were subjected to saturation solubility study. Weighed the amount of Atorvastatin calcium (pure drug) and nanoparticles equivalent to 20mg of the drug were separately introduced into 25 ml stoppered conical flasks containing 10 ml of distilled water. The sealed flasks were agitated on a rotary shaker for 24 h at 37° C and equilibrated for one days.

**Table 2: Saturation Solubility study**

| Product | Lyophilized | Spray dried | Pure drug |
|---|---|---|---|
| Saturated Solubility (µg/ml) | 516.4 | 528.1 | 131.9 |
| Average particle size (After reconstitution) | 141.7nm | 133.5nm | - |

***In vitro* dissolution study**
From this result, it can be concluded that spray dried nanoparticle was considered as an optimized formulation as it gives quickest and highest release rate. Even, spray drying is commercially viable and economic method as compared to lyophilisation. Further studies carried out with spray dried nanoparticle of AT 37 batch.

**Differential scanning calorimetry (DSC) analysis**
In DSC curves of Plain drug particles, a sharp endotherm at 158.65$^{O}$C might be due to the melting point of atorvastatin calcium were observed. The DSC curve of Spray dried nanoparticles, an endotherm at 154$^{O}$C observed the shifting of the endotherm from 158.65$^{O}$C to 154$^{O}$C. This Indicates that the crystalline nature of the drug has been transformed to amorphous nature and it might be due to the presence of the stabilizer as its melting point is 1500C.

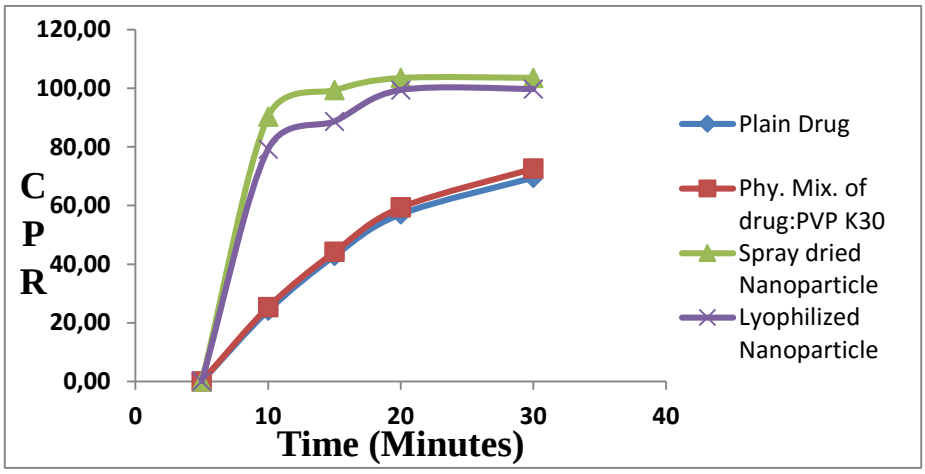



**Figure 2: In vitro dissolution profile of nanoparticle of AT 37 batch**

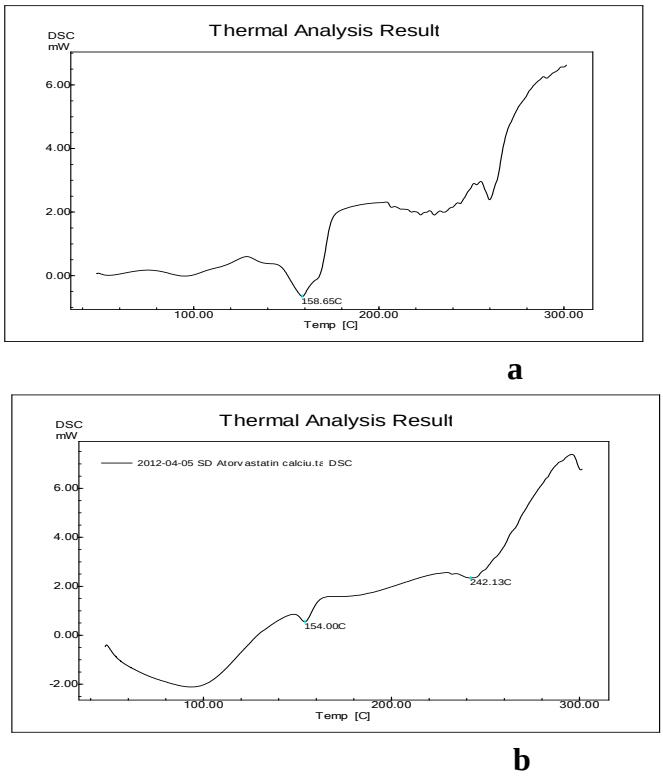
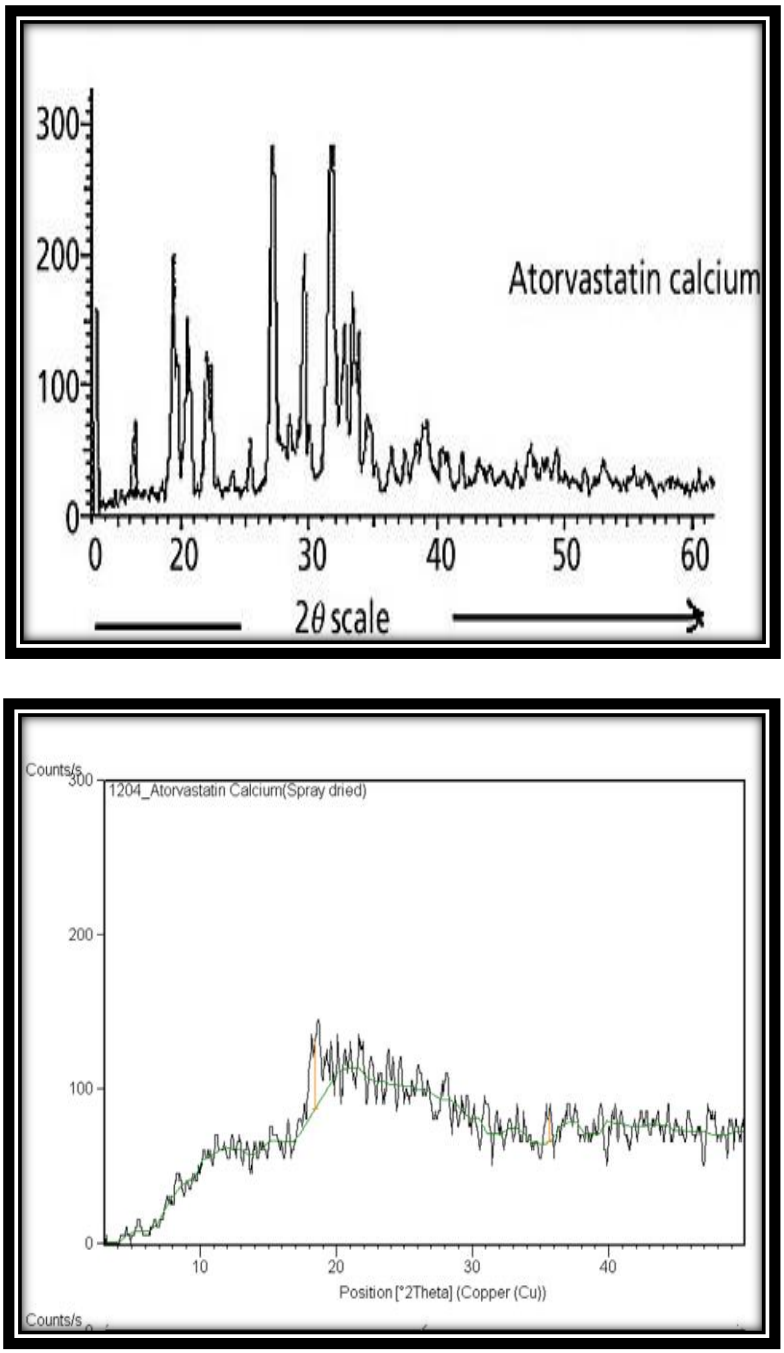



**Figure 3: DSC of a) DSC of plain Atorvastatin calcium and b) Spray dried nanoparticle of Atorvastatin calcium-AT37 batch**

**Powder X-ray diffraction (PXRD)**

The diffraction pattern of the plain drug showed characteristic high-intensity diffraction peaks at 8.96, 10.05, 16.83, 19.26, 22.54, 23.08, 23.57, and 28.74 of 2theta. However, no characteristic diffraction peaks corresponding to plane Atorvastatin calcium were observed in spray dried nanoparticle. Therefore, Atorvastatin calcium nanoparticles existed as an amorphous form.




**a** **b**

**Figure 4: XRD of a) Atorvastatin calcium b) Spray dried nanoparticles of Atorvastatin calcium AT37 batch**

***In vivo* bioavailability studies** [9] *In vivo* pharmacokinetic study was performed as described in earlier section. The obtained results were shown in the graphical presentation in Figure 5.

On the basis of this data, we calculated Area under Curve (AUC), $t_{max}$ and $C_{max}$ using Graph pad prism V5 software and we found out the results shown in Table3.

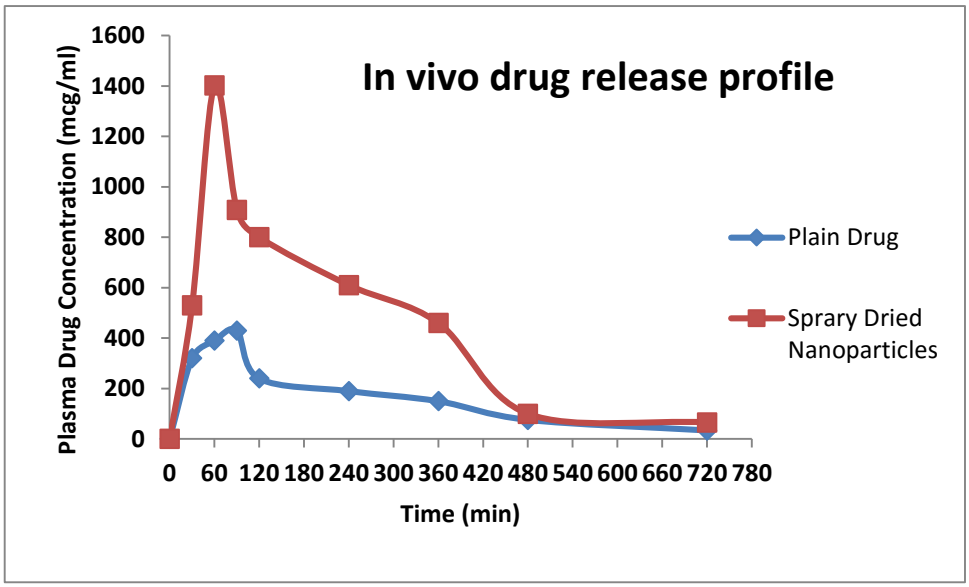



**Figure 5: *In vivo* Plasma drug concentration profile**

**Table 3: Results of pharmacokinetic parameters**

| Parameters | Plain drug | Spray dried Atorvastatin calcium nanoparticle |
|---|---|---|
| AUC | 1134.7±170.5 | 3298.5± 502.7 |
| $T_{max}$ (minute) | 90 | 60 |
| $C_{max}$ (µg/ml) | 430 ±10 | 1403 ± 24 |

The result of in vivo study showed that the AUC of spray dried nanoparticles in almost 3 times greater than the pure drug. Also, $t_{max}$ of spray dried nanoparticles in 60 minutes compare to a plain drug that was 90 minutes and $C_{max}$ of spray dried nanoparticles is higher than the plain drug.

**Short-term Stability Study**

Stability study of the final optimized formulation was performed for 2 months at room temperature (25ºC) and refrigeration temperature (0-4°C) and evaluated by % drug content. Stability study data at the one-month interval by comparing drug content and Q5min are shown in Table 4. There were minored changes.

**Table 4: Short time stability data**

| Parameters | Initial | After 1 month | After 2 month |
|---|---|---|---|
| Drug content (%) | 99.34 | 101.32 | 98.30 |
| Q 10 min (%) | 90.30 | 90.74 | 90.26 |

## CONCLUSION AND DISCUSSION

In this research, Atorvastatin calcium nanoparticles were successfully prepared by the solvent-anti-solvent precipitation method, using PVP K-30 as a stabilizer followed by spray drying of nanosuspension was evaluated for its physicochemical properties. The

saturation solubility of the spray dried optimized AT37 batch nanoparticles was showing excellent saturation solubility, compare to lyophilised nanoparticle and pure drug. The physicochemical characterization showed that crystalline Atorvastatin was converted to an amorphous form, as in DSC studies, spray dried AT37 shows sifting of the endotherm from 158.65$^{O}$C to 154$^{O}$C. Spray dried nanoparticles also exhibit enhanced dissolution rate due to their amorphous nature, in comparison with crystalline Atorvastatin. The increase in the drug dissolution rate and solubility had a significant impact in the oral bioavailability of the drug. *In vivo* bioavailability studies in male Wister rats confirmed the rapid increase of plasma drug concentration in initial hours compare to the plain drug, due to this effect, overall AUC increased, $T_{max}$ decreased and $C_{max}$ increased, compared to the plain drug of Atorvastatin calcium.The stability studies of nanoparticles for 2 months shown an excellent drug content profile. Overall the study demonstrated the usefulness of the solvent-anti-solvent precipitation followed by spray drying of nanosuspension as a method of enhancing the dissolution and oral bioavailability of poorly soluble drug like Atorvastatin calcium.

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