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***In vivo* Absorption Study of Solid Dispersion Containing Atorvastatin Calcium in Human Volunteers**

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ABSTRACT

Objectives: Solid dispersion is a unique and promising approach for improving the oral absorption and oral bioavailability of the poorly water soluble drugs. Atorvastatin Calcium (ATV) is an anti-hyper lipidemic agent, It belongs to class II drugs according to the biopharmaceutical classification system (BCS), and undergoes extensive first-pass metabolism after oral absorption exhibiting 12% bioavailability. The Objective of the present study is to investigate the effect of formulating the hydrophobic drug (ATV) in a solid dispersion form on its bioavailability. **Methods:** ATV solid dispersion (ATV-SD) was prepared by microwave induced fusion method, containing poloxamer188 as a hydrophilic carrier. The resultant ATV-SD was compressed into tablets with other ingredients and was evaluated against pure ATV tablets, *in vitro* dissolution study was carried out to compare the dissolution profiles of the prepared ATV-SD against the pure form of ATV. *In vivo* study was conducted using healthy male volunteers (n = 6). A high performance liquid chromatography method was employed to determine the level of drug in human plasma. **Results:** *In vitro* dissolution study revealed an enhancement in the dissolution profile of ATV-SD. The study of pharmacokinetics parameters also showed an enhancement after oral administration of ATV-SD tablet when compared with pure ATV tablets, where, the AUC_{0-60h} and C_{max} were increased after intake of ATV-SD tablets orally compared with that of pure ATV tablets. **Conclusion:** All these could be demonstrated that ATV-SD would be prospective means for enhancing higher oral bioavailability of ATV.

Keywords: Atorvastatin; Bioavailability; Hyperlipidemia; Microwave; Poloxamer; Solid dispersion

INTRODUCTION

Most of developing countries represent a major proportion of global coronary heart disease burden. Hyperlipidemia is one of the risk factors reported for the development of coronary heart disease¹. Hyperlipidemia is a condition of elevation of lipids (fats) in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids

and triglycerides. It is also called hypercholesterolemia². The most widely used chemical agents among the drugs for the treatment of hyperlipidemia are the statins³. Atorvastatin (ATV) is the most preferred drug among statins for hyperlipidaemia, used to treat moderate to severe familial or nonfamilial hypercholesterolemia⁴. ATV is a 3-hydroxy-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor which catalyzes the conversion

of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol⁵.

ATV is also helpful in increasing the receptor of low density lipoprotein receptor on cell surface and decrease triglyceride levels in serum, meanwhile it can increase the level of high density lipoprotein (HDL)⁶. Development of promising delivery systems in order to enhance the oral delivery and bioavailability of hyperlipidaemia drugs is a must to reduce the mortality and the morbidity of hyperlipidaemia. Therefore, development of ATV formulation in presence of low solubility and oral bioavailability problems is challenging⁷. Several techniques are commonly used to improve dissolution and bioavailability of poorly water-soluble drugs, among those technologies used is solid dispersion (SD) technique.

Solid dispersion (SD) representing one of the most effective methods to enhance the dissolution profile of poorly soluble drugs⁸. The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug⁹. Their matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, in amorphous particles (clusters) or in crystalline particles¹⁰, so this method alter the solid state at the particle, or molecular level involves a physical change in the drug and is an attractive option for improving drug solubility¹¹. Numerous methods have been developed for preparation of solid dispersions, they deals with the challenge of mixing a carrier and a drug, preferably on a molecular level. Among those methods there is a promising one by using microwave irradiation¹².

Microwaves irradiation (MW) is a well-known method for heating and drying materials. Microwaves, with their ability to penetrate any substance, allow the production of heat in any point of the sample at the same time. This is due to the presence of molecules, characterized by a dipolar moment able to absorb microwave energy and convert it into heat. This phenomenon occurs when the microwave frequency is close to the resonance frequency of the polar molecules. The efficient heating of materials by microwaves depends on the capacity of a specific material to absorb microwave energy. Microwave energy has been employed to change the crystalline state of a drug, instead of conventional heating¹³.

The objective of this study is to prepare ATV-SD and evaluate the influence of the ATV-SD formulation on its oral bioavailability in human volunteers by measuring the pharmacokinetic parameters of ATV post administration of ATV-SD tablets and comparing it with pharmacokinetic parameters of pure ATV tablets.

MATERIALS AND METHODS

Materials

- Atorvastatin was kindly gifted sample from Sedico Pharmaceuticals, Egypt.
- Poloxamer 188, BASF SE, Germany.
- Croscarmellose, magnesium stearate and sodium lauryl sulphate, El-Nasr pharmaceutical chemicals Co., Egypt.
- Di Atorvastatin-D5, Sigma Aldrich, USA.
- All other reagents and chemicals (Acetonitrile, formic acid, ammonium formate and tertiary butyl methyl ether) were of analytical grade.

Methodology

Preparation of ATV-SD using microwave induced fusion method

An Accurately weight amount of ATV and poloxamer188 in ratio 1:8 (best drug : polymer ratio according to preliminary studies) were gently mixed for 5 minute using a mortar and a pestle. A fixed amount of this mixture (1g) was subjected to microwaves in domestic microwave (Sharp R-20MR(S)/R-20MT(S), Japan) for different times at a constant chosen power of 600 W. Only one porcelain plate at a time was placed inside the microwave in fixed place for a predetermined time interval. The porcelain plate was then placed at room temperature for solidification. Solid dispersions were collected and dried in dessicator for 24 hr, and then the product was pulverized using a mortar and pestle. The samples were stored in a closed screw-capped glass vials away from light and humidity until use¹⁴.

Preparation of ATV tablets

Pure ATV tablets and ATV-SD tablets were prepared by direct compression method using single punch machine 12 mm flat punch and using the formulae listed in **Table 1**. An amount of SD equivalent to 20 mg ATV was used. Active ingredients and excipients were sieved. ATV mixed well using mortar and pestle with calcium carbonate, cross-carmellose, avicell, magnesium stearate and sodium lauryl sulphate were they added sequentially to the mixture and mixed well. Finally it compressed using a single punch tablet machine (Single punch tableting press, EK0, Korsch, Germany) to produce flat faced tablet weighing 528 mg. The tablets produced undergo evaluation and the results were compared¹⁵.

Table 1. Composition of prepared tablets (amounts per one tablet)

Components	Tablets contain pure ATV (mg)	Tablets contain optimized ATV-SD (mg)
Pure ATV	20	-----
ATV-SD	-----	160**
Calcium carbonate	120	120
Avicell	360	220
Cross-carmellose	25	25
Magnesium stearate	2	2
Sodium lauryl sulphate	1	1

** (equivalent to 20 mg ATV)

***In vitro* dissolution study of prepared tablets**

In vitro dissolution studies of prepared tablets were carried out using USP dissolution test apparatus II (DIS 6000, Copley Scientific, UK) at 75 rpm in 900 ml of phosphate buffer (pH 6.8) as dissolution media, maintained at $37 \pm 0.5^\circ\text{C}$. Aliquot (5ml) was withdrawn at the specified time intervals 5, 10, 15, 20, 30, 45, 60, 90 and 120 min, filtered through syringe filter $0.45\mu\text{m}$ and analyzed spectrophotometrically at 241 nm. An equal volume of fresh medium, which was prewarmed at same condition, was replaced into the dissolution media after each sampling to maintain the constant volume throughout the test⁵.

***In vivo* study**

This study was carried out on six male volunteers after having provided their written informed consent. The subjects are aged between eighteen and fifty five years (18 – 55). They are within the accepted limits for their height and weight as defined by the body mass index range. They were non-smokers and were not abusive alcohol consumers. The subjects were required to abstain from taking any other drug for 15 days prior the start of the test. The subjects have a history of hypersensitivity and/or contraindications to the study drug and any related compounds were excluded.

Study design

The study was carried out to compare the bioavailability parameters of ATV from tablets of the ATV-SD as treatment A, to the tablets of pure ATV, as treatment B, following administration of single doses of 20 mg using two-treatment, two-period, randomized crossover design with a washout period of 7 days. The study protocol was approved by the ethics committee of faculty of pharmacy, Helwan University. The protocol complies with the declarations of Helsinki and Tokyo

for humans. After an overnight fast for twelve hours¹⁶, in the morning of the second day of each study period (Day 2), the study products were administered orally in a randomized fashion with 240 ml of water followed by a standardized breakfast that served after four hours from dosing. Drug administration to each subject was sequentially carried between subjects. Subjects were required to swallow the drug with the complete volume of water provided. Subjects were not allowed to have fluids one hour before dosing and one hour after, except for the 240 ml of water with the product on dosing. Otherwise, Subjects were allowed to drink water as desired. In each period, subject's vital signs were continually monitored.

Sample collection and analysis

Blood samples (2ml) were collected in pre-labeled heparinized tubes through an indwelling cannula in the subject forearm in each period according to the following schedule: Before dosing (zero time) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 60 hrs post dosing. Collected blood samples were immediately centrifuged at 3500 rpm for 10 minutes. Supernatant plasma was transferred using into pre-labeled polypropylene tubes. Samples were analyzed according to a validated chromatographic method (LC-MS-MS). Analysis was carried on ATV concentrations in plasma using a linear range of (0.2-200) ng/ml for ATV.

Liquid chromatographic parameters:

Chromatographic elution was performed with a mobile phase consisting of 0.1% formic acid in water-acetonitrile (30:70, v/v) pumped through the column at a flow rate of 0.6 mL/ min. The column temperature was maintained at 40 °C. The injection volume was 5 μL , and the injector needle wash solvent was acetonitrile-water (50:50, v/v).

Table 2. Pharmacokinetic parameters of ATV in volunteers following administration of single oral dose of the Pure ATV tablets and ATV-SD tablets

PK parameter	ATV-SD tablets	pure ATV tablets
C_{max} (ng/ml)	15.43 ± 4.66	7.96 ± 1.74*
T_{max} (hr)	0.91 ± 0.31	2.33 ± 0.94**
AUC ₀₋₆₀ (ng.hr/mL)	87.38 ± 9.520	63.54 ± 2.94*
AUC _{0-∞} (ng.hr/mL)	90 ± 9.136	65.40 ± 3.29*
K (hr ⁻¹)	0.147 ± 0.025	0.15 ± 0.03
$T_{1/2}$ (hr)	4.84 ± 0.868	4.75 ± 1.20

Each value represents the mean ± standard deviation of six subjects

* $P < 0.05$ based on ANOVA, statistically significant difference between ATV-SD tablets and pure ATV tablets.

** $P < 0.05$ based on Wilcoxon signed rank test, statistical significant difference ATV-SD tablets and pure ATV tablets.

Mass spectrometric parameters:

System was operated in the positive ion mode for the detection of ATV and internal standard (Di-Atorvastatin-D5), The drying gas flow was set at 9.0 L/min with nebulizer pressure of 40 psi and temperature at 350 °C. Multiple reaction monitoring (MRM) transitions measured at positive mode at 556.7 → 396.7 m/z for ATV and 561.7 → 401.7 m/z for the internal standard. The capillary voltage was set at +4000 V for both ATV and internal standard. The sheath gas flow was set at 12.0 L/min and temperature at 350 °C with nozzle voltage of 2000 V. Collision energy was set at 10.0 V while fragmentor voltage was set at 135 V. Quantitation of the analytes in human plasma was based on the peak area ratio of cited drugs versus internal standard. Data acquisition and treatment were carried out using XCalibur® Software.

Pharmacokinetic Analysis

ATV pharmacokinetics parameters were determined by non-compartmental kinetics¹⁷ using Kinetica® 5.1 computer program. Peak plasma concentration (C_{max}) and the time to peak concentration (T_{max}) were obtained directly from the individual plasma concentration versus time curve. The area under the plasma concentration–time curve from zero to the last measurable plasma concentration at time t (AUC_{0-t}) was calculated using linear trapezoidal rule. The area under the curve from zero to infinity, AUC_{0-∞} was calculated as well.

Statistical Analysis

An analysis of variance (ANOVA) was performed for untransformed data for the pharmacokinetic parameters C_{max} , AUC_{0-t} and AUC_{0-∞}

using the software SPSS 11.0 (SPSS Inc., Chicago, USA). The values of T_{max} were analyzed using Wilcoxon signed rank test for the paired samples. A statistically significant difference was considered at P value <0.05.

RESULTS AND DISCUSSION

Formulation of ATV containing solid dispersion

ATV-SD were prepared by microwave melting technique containing polymer (poloxamer 188). Water-soluble amphiphilic surfactants have been widely used to prevent drug precipitation and increase the aqueous solubility of poorly water-soluble drugs¹⁸. For example, Poloxamers their greater hydrophilicity has been exploited in pharmaceutical formulations for solubilization of poorly water-soluble drugs¹⁹. They are formulated with drugs to form a drug/polymer solid solution or solid dispersion causing pore formation to improve dissolution and bioavailability²⁰. Poloxamer consists of an ethylene oxide hydrophilic and polypropylene oxide hydrophobic core blocks arranged in a tri-block structure resulting in an amphiphilic structure. They have low melting point, making them suitable carriers for the melt technique by microwave²¹.

In vitro dissolution study of prepared tablets

Comparison between % of ATV dissolved from tablets contain pure drug and tablets contain optimized formula is carried out, the results are graphically represented in **Figure 1**. It shows an enhancement in percentage of ATV dissolved from ATV-SD tablets compared to pure ATV tablets. percentage of ATV dissolved reached 100 % in ATV-SD tablets after 10 minutes compared to pure ATV

tablets, where, it reached only 65.3 ± 3.18 after 10 minutes. Maximum percentage of ATV dissolved from pure ATV tablets was 89.15 ± 0.62 after 120 min. The enhancement of dissolution rates of ATV from ATV-SD might be due to either the reduction of particle size caused by the formation of a solid solution of the drug in the carrier in which the drug was molecularly dispersed in almost solubilized form within the solid dispersion matrix,²² or due to the fact that the carriers used affected the crystallinity of the drug, and converted a great portion of its crystals to the to the amorphous form which represents the a more solubilized form²³.

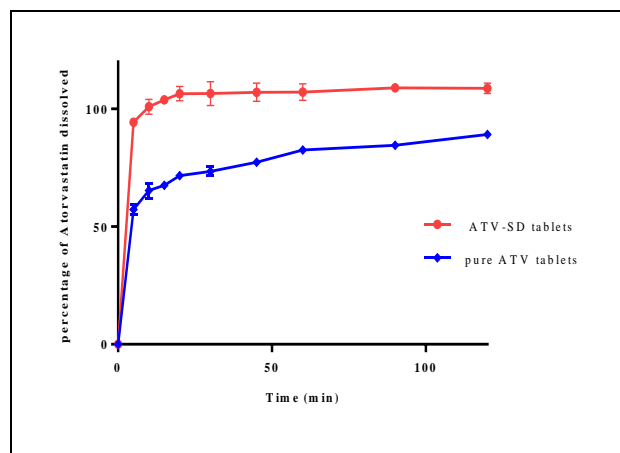


Figure 1. Dissolution profile of tablets of pure Atorvastatin Versus tablets of ATV-SD in 900 ml phosphate buffer (pH 6.8) (mean \pm SD; n=3).

In vivo study

The mean plasma profiles of the two tablets formulations of six volunteers are shown in **Figure 2** and the corresponding pharmacokinetic parameters are given in Table (2). Compared to pure ATV The mean peak plasma concentration (C_{max}) of ATV-SD tablets was increased from 7.96 ng/mL to 15.43 ng/ml, this result indicated that ATV-SD significantly increase the C_{max} of ATV. Moreover, the T_{max} decreased from 2.3 hr to 0.5 hr in ATV-SD tablets which indicates a shorter time to reach maximum concentration in subjects treated with ATV-SD tablets. A clear change was observed in the AUC_{0-60h} and $AUC_{0-\infty}$ between the different formulae. In ATV-SD tablets the mean $AUC_{(0-60)}$ was found to be increased from 63.54 ng.hr/ ml to 92.35 ng.hr/ml, in addition the $AUC_{(0-\infty)}$ was increased from 65.40 ng.hr/ ml to 95.73 ng.hr/ml , which indicated that the pharmacological activities of ATV may be increased²⁴. The mean elimination half-life ($T_{1/2}$) and elimination rate constant (K) were 4.68 hr and $0.15hr^{-1}$, respectively in ATV-SD tablets . Regarding

the pure ATV tablets , it was observable that the mean elimination half-life ($T_{1/2}$) and elimination rate constant (K) were 4.75 h and $0.15 hr^{-1}$, respectively with no remarkable difference from ATV-SD tablets.

The individual $AUC_{0-\infty}$ values for the ATV-SD tablets were compared to those for the pure ATV tablets to determine the relative bioavailability. The mean relative bioavailability of the ATV-SD tablets to the pure ATV tablets was $137.65 \pm 15.8 \%$. This result indicated that 37.15 % increase in the oral bioavailability of ATV was achieved by the solid dispersion formulation.

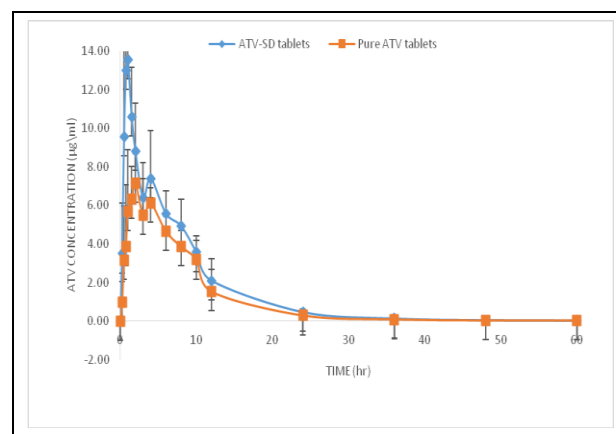


Figure 2. Mean plasma concentration–time curves of Atorvastatin (\pm SD) in human volunteers (n=6) after oral administration of as single oral dose of ATV-SD Tablets and pure ATV tablets.

CONCLUSION

According to the previously mentioned results, the ATV-SD tablets offered higher bioavailability compared to pure ATV tablets due to higher areas under the curve, and peak plasma concentration (C_{max}) and lower time to peak plasma concentration (T_{max}). This confirming that formulation of ATV in solid dispersion formula using microwave technique resulting in a higher bioavailability for ATV if compared to its pure form. Its therefore reasonable to point out that ATV-SD could be an effective method for increasing the oral bioavailability of Atorvastatin .

Conflict of Interest

The authors declare that they don't have any kind of conflict of interest.

REFERENCES

1. Haq, H.; Kiyani, A. M. The prevalence of hyperlipidemia in patients suffering from

- coronary artery disease. *Pakistan Heart J.* **2012**, 32 (3).
- Pandit, A. P.; Chavan, T. T.; Khandelwal, K. R. Enhancement of solubility, dissolution rate and bioavailability of atorvastatin using solid lipid: in vitro and in vivo characterization. *J. Pharm. Invest.* **2015**, 45 (6), 503-513.
 - Mohammad, S.; Arshad, U.; Abbass, N.; Parvez, I.; Abbas, G.; Mahmood, W. Bioequivalence Study of Atorvastatin Tablets in Healthy Pakistani Volunteers. *Thérapie* **2015**, 70 (4), 329-335.
 - Sarker, M. S.; Barman, R. K.; Ali, M. A.; Noguchi, S.; Iwao, Y.; Itai, S.; Wahed, M. I. I. Formulation Development and In-Vivo Evaluation of Atorvastatin Calcium Solid Dispersion in Streptozotocin Induced Diabetic Mice. *Pharmacol. Pharm.* **2018**, 9 (09), 395.
 - Sharma, M.; Garg, R.; Gupta, G. Formulation and evaluation of solid dispersion of atorvastatin calcium. *J. Pharm. Sci. Innov.* **2013**, 2 (4).
 - Jahangiri, A.; Barzegar-Jalali, M.; Garjani, A.; Javadzadeh, Y.; Hamishehkar, H.; Asadpour-Zeynali, K.; Adibkia, K. Evaluation of physicochemical properties and in vivo efficiency of atorvastatin calcium/ezetimibe solid dispersions. *Eur. J. Pharm. Sci.* **2016**, 82, 21-30.
 - Choudhary, A.; Rana, A. C.; Aggarwal, G.; Kumar, V.; Zakir, F. Development and characterization of an atorvastatin solid dispersion formulation using skimmed milk for improved oral bioavailability. *Acta Pharmaceutica Sinica B* **2012**, 2 (4), 421-428.
 - Dong, W.; Su, X.; Xu, M.; Hu, M.; Sun, Y.; Zhang, P. Preparation, characterization, and in vitro/vivo evaluation of polymer-assisting formulation of atorvastatin calcium based on solid dispersion technique. *Asian J. Pharm.Sci.* **2018**, 13 (6), 546-554.
 - Taral Mayur, N. Solubility Enhancement of Atorvastatin Calcium by Using Microwave Assisted Solid Dispersion Preparation Method. *Int. J.* **2015**, 4 (1), 51-56.
 - Lakade, S. H.; Bhalekar, M. Different types of method for modified dosage form for enhancement of dissolution rate through solid dispersion. *Int. J. Pharma. Stud. Res* **2010**, 1, 54-63.
 - Shamsuddin, M. F.; Ansari, S. H.; Ali, J. Atorvastatin solid dispersion for bioavailability enhancement. *J. Adv. Pharm. Tech. Res.* **2016**, 7 (1), 22.
 - Alshehri, S.; Shakeel, F.; Ibrahim, M.; Elzayat, E.; Altamimi, M.; Shazly, G.; Mohsin, K.; Alkholief, M.; Alsulays, B.; Alshetaili, A. Influence of the microwave technology on solid dispersions of mefenamic acid and flufenamic acid. *PloS one* **2017**, 12 (7), e0182011.
 - Moneghini, M.; Bellich, B.; Baxa, P.; Princivalle, F. Microwave generated solid dispersions containing ibuprofen. *Int. J. Pharm.* **2008**, 361 (1-2), 125-130.
 - Maurya, D.; Belgamwar, V.; Tekade, A. Microwave induced solubility enhancement of poorly water soluble atorvastatin calcium. *J. Pharm. Pharmacol.* **2010**, 62 (11), 1599-1606.
 - Ahjel, S. W.; Lupuleasa, D. Enhancement of solubility and dissolution rate of different forms of atorvastatin calcium in direct compression tablet formulas. *Farmacia* **2009**, 57 (3), 290-300.
 - Food; Administration, D. Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products—general considerations. *Food and Drug Administration, Washington, DC* **2003**.
 - Shargel, L.; Andrew, B. Applied Biopharmaceutics and Pharmacokinetics. **2005**.
 - Dai, W.-G.; Dong, L. C.; Li, S.; Deng, Z. Combination of Pluronic/Vitamin E TPGS as a potential inhibitor of drug precipitation. *Int. J. Pharm.* **2008**, 355 (1-2), 31-37.
 - Nepal, P. R.; Han, H.-K.; Choi, H.-K. Enhancement of solubility and dissolution of Coenzyme Q10 using solid dispersion formulation. *Int. J. Pharm.* **2010**, 383 (1-2), 147-153.
 - Reddy, R.; Khalil, S. A.; Gouda, M. W. Effect of dioctyl sodium sulfosuccinate and poloxamer 188 on dissolution and intestinal absorption of sulfadiazine and sulfisoxazole in rats. *J. Pharm. Sci.* **1976**, 65 (1), 115-118.
 - Jahan, R.; Islam, M. S.; Tanwir, A.; Chowdhury, J. A. In vitro dissolution study of atorvastatin binary solid dispersion. *J. Adv. Pharm. Tech. Res.* **2013**, 4 (1), 18.
 - Lu, T.; Sun, Y.; Ding, D.; Zhang, Q.; Fan, R.; He, Z.; Wang, J. Study on enhanced dissolution of azilsartan-loaded solid dispersion, prepared by combining wet milling and spray-drying technologies. *AAPS Pharm. Sci. Tech.* **2017**, 18 (2), 473-480.
 - Abdelbary, A.; El-Gazayerly, O. N.; Fahmy, R. H.; Salem, L. H. Influence of Various Polymers on the Improvement of Etodolac Solubility and Dissolution Rate via. Solid Dispersion Technique. *Inventi Impact Pharm. Tech.* **2013**.
 - Sun, S.; Wang, R.; Fan, J.; Zhang, G.; Zhang, H. Effects of Danshen tablets on pharmacokinetics of atorvastatin calcium in rats and its potential mechanism. *Pharm. Biol.* **2018**, 56 (1), 104-108.