ORIGINAL ARTICLE

Development and characterization of a novel nanosuspension based drug delivery system of valsartan: A poorly soluble drug

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The purpose of the present study was to formulate valsartan nanosuspension to increase the aqueous solubility and to improve its oral bioavailability. Valsartan is a poorly water-soluble drug which results in its insufficient bioavailability. Low oral bioavailability of poorly water-soluble drugs poses a great challenge during drug development. Poorly water-soluble drugs are difficult to develop as drug products, using conventional formulation techniques. Valsartan nanosuspension was prepared by pearl milling technique using zirconium oxide beads as a milling media and poloxamer 407 (Lutrol F 127) as a stabilizer. Effects of various process parameters like, stirring time and the ratio of the beads were optimized by keeping drug: Surfactant as a constant initially and then optimized process parameters were used to optimize the formulation. The optimized formulation of nanosuspension was used as granulating fluid as well as spray dried onto the mannitol (Pearlitol SD 200) and formulated into tablets. The nanosuspension was characterized by particle size, size distribution analysis, and differential scanning calorimetry. The nanosuspension was evaluated for drug content, drug release by *in vitro* dissolution studies and stability. The nanosuspension with 0.3% poloxamer showed improved solubility and dissolution rate. The *in vitro* dissolution profile of valsartan nanosuspension performed in 6.8 pH phosphate buffer as medium showed complete release.

Key words: *Nanosusupension, pearl milling, poloxamer, valsartan*

INTRODUCTION

Valsartan, chemically is N-valeryl-N ([2-[1H-tetrazol-5-yl] biphenyl-4-yl] methyl) valine has an empirical formula of $C_{24}H_{29}N_5O_3$ and a molecular weight of 435.5 g/mol. Valsartan is a nonpeptide, orally active, and specific angiotensin II antagonist acting on the angiotensin II type 1 (AT_1) receptor subtype. Valsartan is a new potent, highly selective, and orally active antihypertensive drug belonging to the family of AT_1 receptor antagonists. Valsartan has much greater affinity (about 20,000-fold) for the AT_1 receptor than for the AT_2 receptor, thereby relaxing blood vessels and causing them to widen, which lowers blood pressure and improves blood flow.[1]

Peak plasma concentrations of valsartan will be reached in 2-4 h after dosing. The amount absorbed varies widely. The mean absolute bioavailability is about 25%, and steady state volume of distribution is low (17 L). After oral dosing, 83% of the dose is excreted in the feces and 13% in the urine, mainly as unchanged compound. Solubility of valsartan is low which is about 0.16 mg/ml in water. The partition coefficient of valsartan is 0.033. The solubility of valsartan increases comparatively in the pH range of 4-8 and the lipophilicity decreases in the same rate. As a result, the permeability of drug decreases accounting for its decreased bioavailability across the gastrointestinal tract.[2]

It is estimated that more than one-third of the compounds being developed by the pharmaceutical industry are poorly water-soluble. Design and

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formulation of a different dosage form requires consideration of the physical, chemical, and biological characteristics of all the drug substances and pharmaceutical ingredients which are to be used in fabricating the product. An important property of a drug substance is solubility, especially aqueous system solubility. The solubility/dissolution behavior of a drug is the key factor to its oral bioavailability. Oral bioavailability of poorly water-soluble drugs depends on their dissolution rate and extent at the absorption site. In recent years, it has been estimated that up to 40% of the new drugs discovered by the pharmaceutical industry are poorly soluble or lipophilic compounds. Many procedures have been investigated to enhance dissolution properties and thus, oral bioavailability of drugs with very low aqueous solubility. Conventional approaches include the use of co-solvents, salt formation, pH adjustment, emulsions and micellar dispersions, micronization, and complexation with cyclodextrin. An alternative to such methods is nanonization of drug particles. The reduced particle size within the nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of increased saturation solubility as described by Freundlich-Ostwald equation.[3]

Nanosuspension, a carrier-free colloidal drug delivery system, consists essentially of pure drug nanoparticles (100-1000 nm) and a minimum amount of surface active agent required for stabilization. By definition, drug nanocrystals are nanoparticles composed of 100% drug without any matrix material, with a mean diameter below 1000 nm. The dispersion medium can be water, aqueous solutions or nonaqueous media. Surfactants and/or polymeric stabilizers are used for the stabilization of these systems. Nanonization of drug powders increases the surface of the particles, leading to an increase in dissolution velocity. Another important aspect is the increase in saturation solubility. In addition, the distance of diffusion on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient. The increased concentration gradient leads to much higher increase in the dissolution velocity as well.[4]

Improvement of aqueous solubility in such case is a valuable goal to improve therapeutic efficacy. The dissolution rate is a function of the solubility and the surface area of the drug. Thus, dissolution rate will increase if the solubility of the drug is increased and it will also increase with an increase in the surface area of the drug.[5] In recent years, much attention has been focused on drug nanosuspension for the improvement of bioavailability of water insoluble drugs. The aim of this study was to employ the nanosuspension technique to produce valsartan nanosuspension for oral administration and enabling to enhance the saturation solubility, dissolution, and oral absorption of valsartan. The optimized nanosuspension formulation was evaluated though various *in vitro* parameters.

MATERIALS AND METHODS

Materials

Valsartan was obtained from Mylan Laboratories Ltd., Lutrol F 127 (poloxamer 407) was procured from BASF Ltd., and all other excipients, solvents, and reagents of analytical grade were procured from commercial sources.

Solubility studies of valsartan

Solubility of valsartan in different buffers was determined by shake flask method. An excess amount of valsartan was added to each volumetric flask containing the selected vehicle and mixed thoroughly. The volumetric flasks were then fixed onto a water bath shaker and shaken for 24 h at 25°C. Samples were removed after a specified time and filtered through 0.22 µm syringe driven membrane filter unit. The filtrates were then analyzed by ultraviolet (UV) spectrophotometer at 248 nm to evaluate the amount of drug dissolved. The solubility of valsartan in various solvents is given in Table 1.

Preparation of nanosuspension

Valsartan nanosuspension was prepared by dispersing the valsartan powder into an aqueous solution containing various concentration of surfactant that is poloxamer 407 (Lutrol F 127). This solution was then passed through colloidal mill to obtain a homogeneous predispersion. This resulting coarse predispersion was comminuted using zirconium beads (which act as milling media) with the help of magnetic stirrer. Zirconium oxide beads were used in the preparation of nanosuspension due to their low cost and easy availability for lab scale production of nanosuspension in comparison to silver beads. The compositions of various valsartan nano suspensions are given in Table 2.

Various parameters like the effect of stirring time and ratio of zirconium oxide beads were optimized by keeping the drug: Surfactant: Milling media volume as constant initially, then the optimized conditions of stirring time and ratio of different size of zirconium oxide beads were used throughout the study to optimize the concentration of poloxamer 407 and volume of milling media to achieve minimum particle size. The stirring was

Table 1: Solubility profile of valsartan in various solvents

Solvent	Solubility (mg/ml)
Water	0.16
0.1N HCl	0.08
4.5 sodium acetate buffer	0.92
pH 6.8 buffer	1.32

HCl: Hydrochloric acid

Table 2: Formulation of valsartan nanosuspension

	F1	F2	F3	F5	F5
Drug (mg)	80	80	80	80	80
Poloxamer (mg)	8	16	24	32	40
Water (ml)	1	1	1	1	1

continued for 24 h at 750 rpm for the preparation of optimized nanosuspension formulation. The formulation variables employed for optimizing nanosuspension are given in Table 3.

Characterization of valsartan nanosuspension

Microscopy test

The samples (before and after nano-nization) were visualized by using Optical Microscope (Leica, DFC 295) at 40X zoom. The optical microscopic image of valsartan suspension and nanosuspension at 40X are given in Figure 1.

Particle size distribution

Particle size of the formulated nanosuspension was analyzed by Photon Correlation Spectroscopy using Malvern particle size analyzer (Zetasizer) equipped with DTS software. The Zetasizer system determines the size by measuring the Brownian motion of the particles in a sample using Dynamic Light Scattering. The samples were measured after appropriate dilution with bi-distilled water (millipore). The reading was carried out at 90° angle with respect to the incident beam. The zeta potential was measured by a laser Doppler anemometer coupled with the same instrument. The analysis of the samples was carried out in triplicate for accuracy. The particle size and distribution pattern of valsartan nanosuspension is shown in Figure 2.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was performed using a DSC Calorimeter (Mettler Toledo, Schwerzenbach, Switzerland). Analysis was performed under a nitrogen purge (20 ml/min). The samples (about 3 mg) were weighed accurately, placed in aluminum pans, and then sealed with a pinhole-pierced cover. Heating curves were recorded at a scan rate of 10°C/min from 25°C to 250°C, and an empty pan was used as reference.[6] The DSC thermograms of pure drug, poloxamer, and optimized formulation are shown in Figure 3.

Zeta potential measurement

Zeta potential of the suspension is measured by Malvern Zetasizer. The zetasizer mainly consists of the laser which is used to provide a light source to illuminate the particles within the sample. For zeta potential measurements, this light splits to provide an incident and reference beam. The incident laser beam passes through the center of the sample cell, and the scattered light at an angle of about 130 is detected. Zetasizer software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potential is calculated.[7] The zeta potential of optimized nanosuspension formulation is shown in Figure 4.

Table 3: Optimization of formulation variables for formulation of valsartan nanosuspension

Concentration of drug (mg)	Concentration of poloxamer (mg)	Volume of milling media (Zirconium oxide beads)	Polydispersity index
80	8	40	0.655
80	8	50	0.412
80	8	60	0.324

X-ray diffraction

X-ray diffraction (XRD) analysis was carried out for the pure drug, poloxamer, and optimized nanosuspension using XRD (Brucker AXS, model D8 advanced, Germany) with Cu line as a source of radiation. Standard runs were taken using 40 kV voltage, 40 mA current, and scanning rate of 0.02°/min over a 2θ range of 5-50°. The powder X-ray diffraction (PXRD)

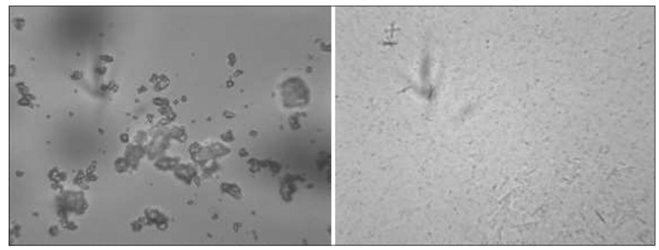

Figure 1: Optical microscopic image of Valsartan suspension and nanosuspension at X40

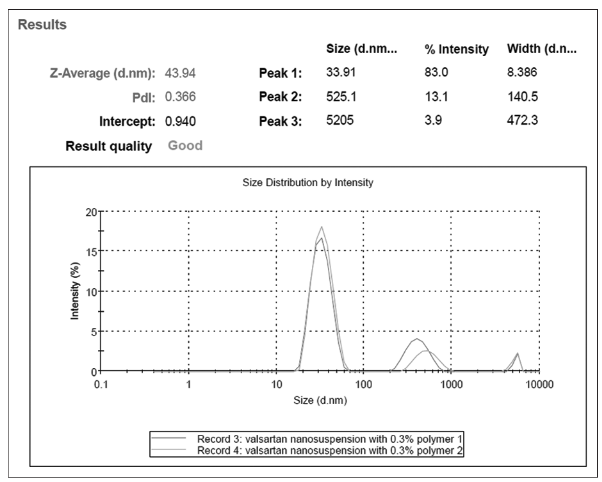

Figure 2: Particle size distribution by Malvern particle size analyzer

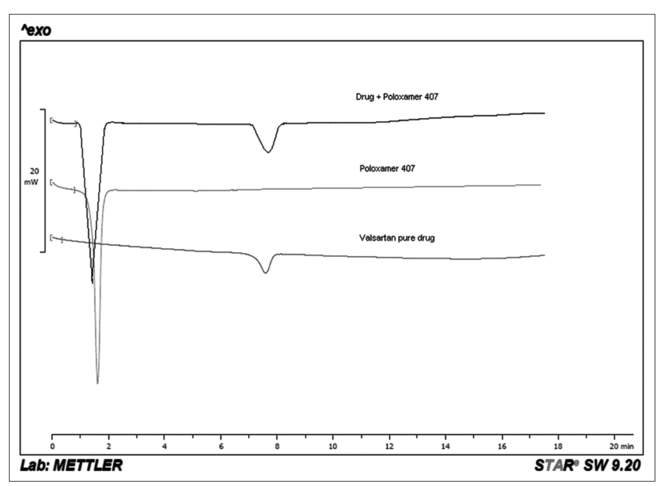

Figure 3: Differential scanning calorimetry thermogram of pure drug, poloxamer and nanosuspension

patterns of pure drug, poloxamer, and optimized formulation are shown in Figures 5-7.

Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) spectra were recorded for valsartan, polymer (poloxamer), and optimized formulation using KBr pellet technique. The pellets were prepared using KBr hydraulic press under hydraulic pressure of 150 kg/cm^2. The spectra were scanned over 3600-400 cm^{-1} at ambient temperature with a resolution of 4 cm^{-1}, using FT-IR 2500 apparatus and spectra were recorded. The FT-IR spectra of pure drug and optimized nanosuspension are given in Figures 8 and 9.

Evaluation of valsartan nanosuspension

Dissolution rate studies on valsartan nanosuspension

In vitro dissolution study was performed using USP dissolution test apparatus-II (paddle assembly). The dissolution was performed using 900 ml of 0.1N HCl and 900 ml phosphate buffer solution of pH 6.8 as dissolution mediums maintained at 37°C ± 0.5°C and 50 rpm for valsartan pure drug and nanosuspension. Five milliliters of samples were withdrawn at regular intervals of 5 min for 60 min and replaced with fresh dissolution medium. Samples were filtered through 0.4 μ polyvinylidene difluoride filter and assayed spectrophotometrically on Shimadzu UV-visible spectrophotometer UV 2450 at 248 nm wavelength. Dissolution for each formulation was performed in triplicates and mean of absorbance was used to calculate cumulative percent of drug release.[8-10] The dissolution profiles of valsartan pure drug and nanosuspension are shown in Figure 10.

RESULTS AND DISCUSSION

The aim of the present study was to formulate valsartan nanosuspension to increase the aqueous solubility and to improve its oral bioavailability. Valsartan nanosuspension was prepared by pearl milling technique using zirconium oxide beads as a milling media and poloxamer 407 (Lutrol F 127) as a stabilizer. The solubility study of pure drug was carried out by shake flask method. The solubility of valsartan in water is only 0.16 mg/ml which categorizes it into very slightly soluble drug. The solubility of valsartan in 0.1N HCl is found to be least which accounts for its low bioavailability and fluctuated plasma levels in the

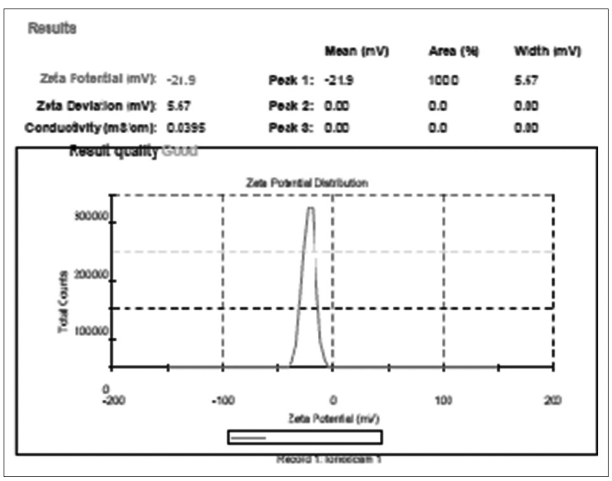

Figure 4: Zeta Potential by Malvern Zetasizer

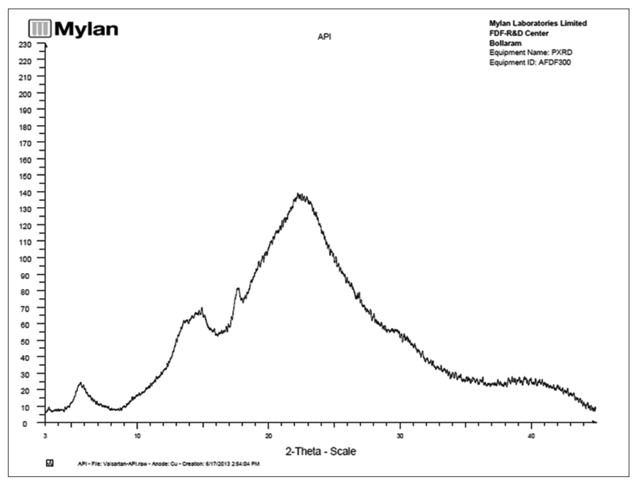

Figure 5: Powder X-ray diffraction pattern of pure drug (Valsartan)

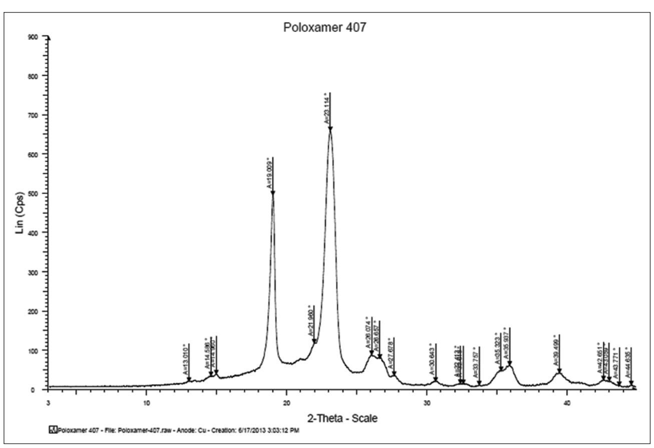

Figure 6: Powder X-ray diffraction pattern of poloxamer

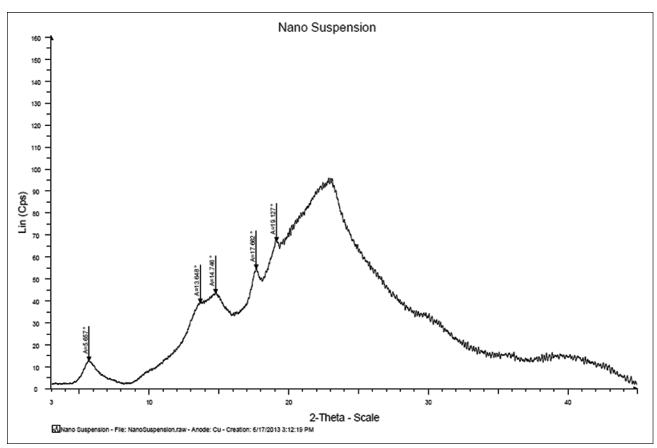

Figure 7: Powder X-ray diffraction pattern of optimized nanosuspension

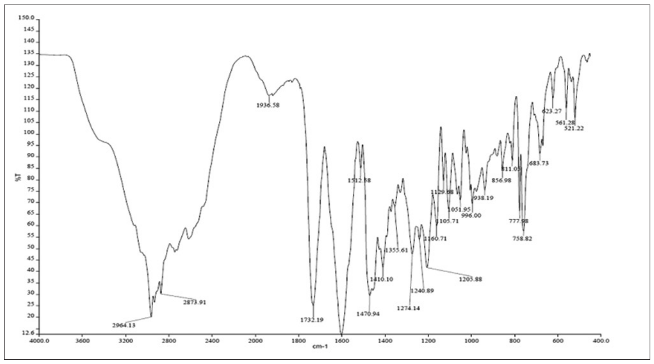

Figure 8: Fourier transform infrared spectroscopy spectra of pure drug

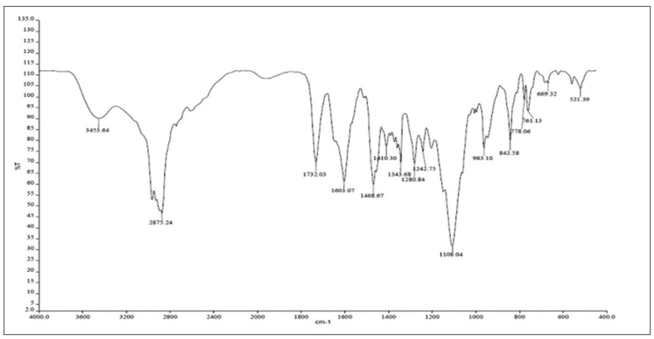

Figure 9: Fourier transform infrared spectroscopy spectra of optimized valsartan nanosuspension

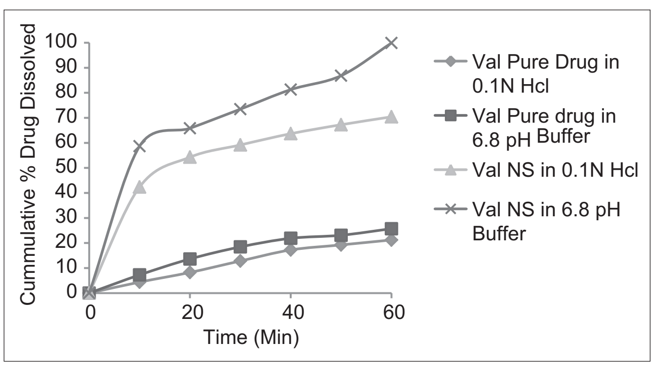

Figure 10: Dissolution profile Valsartan pure drug and optimized nanosuspension in 0.1N HCl and 6.8 pH phophate buffer

gastrointestinal tract. Hence, by reducing the particle size of the drug to nanometer range increases the surface area which corresponds to increased dissolution velocity and enhanced bioavailability. The solubility of valsartan in various solvents was given in Table 1. Valsartan nanosuspension was prepared by dispersing the valsartan powder into an aqueous solution containing various concentration of surfactant that is poloxamer 407 (Lutrol F 127).The compositions of various valsartan nanosuspensions and formulation variables employed are given in Tables 2 and 3. The samples (before and after nano-nization) were visualized using Optical Microscope (LEICA, DFC 295) at 40X zoom. The optical microscopic image of valsartan suspension and nanosuspension at 40X is given in Figure 1. The particle size of nanosuspension plays a vital role in drug release, hence, is an important parameter to be determined. The particle size of input active phamaceutical ingredients was analyzed by Malvern mastersizer and the particle size (d90) was found to be 25 μ. The particle size distribution of the optimized nanosuspension formulation was analyzed by Malvern particle sizer (Zetasizer). The particle size of the optimized nanosuspension was found to be 43 nm. The particle size and distribution of valsartan nanosuspension are shown in Figure 2. DSC was performed to investigate the effect of surfactant on valsartan nanosuspension. Pure valsartan powder showed melting exotherm at 117°C, which corresponds to its melting point. From thermograms, it was concluded that the drug and the surfactant do not interact with each other. The DSC thermograms of pure drug, poloxamer, and optimized formulation are shown in Figure 3. Zeta potential analysis was performed to get information about the surface properties of nanoparticles. Zeta potential is an important parameter for prediction of stability of nanosuspension. The zeta potential of optimized nanosuspension formulation was - 21.9 mV showing excellent stability. The zeta potential of optimized nanosuspension formulation is shown in Figure 4. The XRD analysis was performed for drug, poloxamer, and optimized nanosuspension. The X-ray Diffractogram of valsartan has sharp peaks at diffraction angles (2θ) 13.04° and 19.12° showing a typical crystalline pattern. However, all major characteristic peaks were appearing in the diffractogram of optimized nanosuspensions. Moreover, the relative intensity and 2θ angle of these peaks remains practically unchanged. Thus, it is clear that from PXRD data it was observed that there is no conversion into amorphous form and the valsartan remains still in its original crystalline nature. The XRD data reveal that there was no change in molecular form of the drug upon nano-nization. The PXRD patterns of pure drug, poloxamer, and optimized formulation are shown in Figures 5-7. The FT-IR spectra, it was observed that model drug showed a band at 2964.13 cm^{-1} which is due to C-H stretching superimposed upon O-H stretching indicating the presence of phenyl groups and a band due to C-H stretching at 2873.91 cm^{-1}. The FT-IR spectra of drug and polymer showed no interaction as the peaks characteristic for model drug, 2875.24 cm^{-1} and polymer, 1108.04 cm^{-1} were distinct and separate. The FT-IR spectra of pure drug and optimized nanosuspension are given in Figures 8 and 9.

Dissolution studies were performed for pure valsartan drug and optimized nanosuspension. The amount of drug released from the optimized nanosuspension formulation was about 70.41% and 99.89% in 0.1N HCl and 6.8 pH buffer. The increase in accessible surface area to the dissolution medium and hydrophilic surfactant coating on the particle surfaces may be the reason for the increase in dissolution rate and extent. The dissolution profiles of valsartan pure drug and nanosuspension are shown in Figures 10 and the *in vitro* dissolution parameters were shown in Table 4.

Table 4: *In vitro* dissolution parameters for valsartan nanosuspension

Parameters	Valsartan Pure drug	Valsartan nanosuspension
T_{50} (min)	>60	8.51
T_{90} (min)	>60	51.81
DE_{30}%	13.11	65.99
K (min^{-1})	0.0048	0.0366
R^2	0.941	0.938

CONCLUSION

From the results, it may be concluded that formulation of poorly soluble drugs like valsartan into nanosuspension proves to be beneficial and represents a promising new drug formulation for oral drug delivery. Consequently, nanosuspensions represent a promising alternative to current delivery systems aiming to improve the biopharmaceutical performance of drugs with low-water solubility. The media milling process proved to be a successful approach for the formulation of valsartan nanosuspension. The process resulted in poloxamer stabilized valsartan nanosuspension with a mean particle size of 43 nm. These findings indicated that nanosuspension technology was suitable for increasing the aqueous solubility and to improve its oral bioavailability of poorly soluble drugs like valsartan.

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