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Preparation and characterization of valsartan nanoparticles

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A B S T R A C T

Valsartan is an orally active, highly selective angiotensin II receptor blocker widely used to treat hypertension. However, its low oral bioavailability (only 23%) limits its effectiveness. This poor bioavailability is attributed to inadequate absorption in the gastrointestinal tract and rapid drug elimination. To enhance its oral bioavailability, solid lipid nanoparticles (SLNs) and polymeric nanoparticles (PNs) formulations of valsartan were developed. The SLNs formulation was prepared using glyceryl monostearate, while the PNs formulation utilized a pH-dependent polymer. Optimization of the formulations was carried out using Design Expert® software, considering five key response factors: drug content, drug entrapment efficiency, particle size, zeta potential, and polydispersity index. The optimized formulations underwent further characterization, including surface morphology analysis, in vitro drug release studies, cell line toxicity assessments, and pharmacokinetic evaluations. Toxicity studies confirmed that the optimized nanoformulations were non-toxic. Additionally, the relative bioavailability was determined by calculating the ratio of the area under the curve (AUC) of the valsartan SLNs or PNs formulation to that of standard valsartan. The values obtained were 4.07 for SLNs and 2.47 for PNs, demonstrating a significant improvement in bioavailability.

Keywords: valsartan, nanoformulations, bioavailability

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C O N T E N T S

1. Introduction

Over 90% of drugs are administered orally, with their absorption, bioavailability, and pharmacokinetic profile largely dependent on their solubility in aqueous media. Since 1995, more than 90% of approved drugs have exhibited poor solubility, and approximately 40% of newly identified active chemical entities (NCEs) from combinatorial screening programs also demonstrate low water solubility. Poor solubility presents a major challenge in achieving sufficient and reproducible bioavailability for oral drug delivery. Valsartan, a non-peptide angiotensin II antagonist used to treat hypertension, belongs to the Biopharmaceutics Classification System (BCS) Class II, characterized by low aqueous solubility and high permeability. As a result, its oral absorption is limited by its dissolution rate. Enhancing the dissolution velocity of Valsartan is crucial for improving its bioavailability. A bottom-up method is employed to develop drug nanocrystals, aiming to improve its dissolution profile. The specific objectives of this study include:

1. Fabrication of carrier-free Valsartan nanocrystals.

2. Screening of various polymeric stabilizers to ensure optimal stabilization.
3. Optimization of polymeric stabilizer levels.
4. In vitro dissolution studies to evaluate dissolution rate enhancement.
5. Physicochemical characterization of nanocrystals using analytical techniques such as SEM, DSC, FT-IR, and particle size analysis.
6. Development of an optimized nanocrystal formulation for Valsartan.

By implementing nanocrystal technology, this study seeks to improve the dissolution rate and bioavailability of Valsartan, making it a more effective oral therapeutic agent.

2. Materials and Methods

Valsartan has been procured from Lupin Ltd., Goa, India, the other chemicals used for the study were purchased from local chemicals provider of analytical grade.

Methodology:

Preformulation studies: Identification of drug using UV visible spectrophotometer: A standard stock solution of valsartan with 10 µg/mL concentration was prepared in phosphate buffer, pH 6.8 and was scanned in UV visible spectrophotometer (UV-1601PC, Shimadzu Corporation, Tokyo, Japan) under spectrum mode in the wavelength range from 200 to 400 nm against blank. Obtained λ_{max} was compared with reference wavelength value and also with certificate of analysis (COA) provided by drug supplier.

Identification of drug using Fourier Transformed Infrared (FTIR) spectroscopy:

An FTIR Spectrophotometer (FTIR 8300, Shimadzu Corporation, Tokyo, Japan) was used to identify the drug and spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}. Procedure consisted of dispensing a drug in potassium bromide (KBr) (2 mg drug sample in 200 mg KBr) and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. Pellet was placed in the light path and spectrum was obtained.

Identification of drug using Differential Scanning Calorimetry (DSC): A differential scanning calorimeter (DSC Plus with TA-60WS thermal analyzer, Shimadzu Corporation, Tokyo, Japan) was used to investigate the thermal behavior of drug. Drug sample (10 mg) was weighed in a standard open aluminum pan with an identical empty pan used as a reference. Heating was set to run from 20 to 250 ºC at a flow rate of 10 ºC/min for each sample. The inert atmosphere was maintained by purging nitrogen at a flow rate of 30 mL/min.

HPLC analytical method development and validation

Instrumentation: HPLC system consisted of a Shimadzu Corporation, Kyoto, Japan, LC-2010CHT model connected to PDA (Photodiode array, model no. SPD-M20A PDA with 220-230V, Shimadzu Corporation, Kyoto, Japan) and dual wavelength UV detector, column oven and auto sampler (Shimadzu Corporation, Kyoto, Japan) was used in this study. Chromatograms were recorded by means of a computer and treated with the aid of LC solution 5.57 release. A Hyper Clone (Phenomenex®) C18 column (250 mm ×4.6mm id, 5µm, BDS 130 Å) was used to perform the separation, with a C18 Phenomenex®, 4 × 3.0 mm id, security guard column. Mobile phase was filtered with Millipore glass filter (Millipore filter cellulose nitrate grided with 0.22 µ size and 47 mm diameter) assembly attached with vacuum pump. Mobile phase was sonicated with Ultrasonic Cleaner – 15L (Equitron – Medica Instrument Mfg. Co., Mumbai, India). The pH of mobile phase or buffer was measured with Eutech Instruments pH 510 using a glass electrode Van London Co., USA. High pure water (18.2 MΩ.cm resistivity, Milli Q) was produced with a Direct-Q® 3 water purification system, Millipore Corporation (Billerica, MA, USA).

Preparation of buffer for mobile phase

20mM ammonium formate buffer was prepared by dissolving pre-calculated amount of ammonium formate in 1000 mL Milli Q water and formic acid was used to adjust the pH 3.0. The formic acid also helps to improve the peak characteristics. Prepared buffer was passed through 0.22 µm filter.

Preparation of mobile phase

Mobile phase used in the present study was a combination of buffer: acetonitrile in the ratio of 57:43. Ammonium formate was used in the preparation of mobile phase, because it is volatile in nature and doesn't get precipitated in column and HPLC system. Hence, the chances of development of high back pressure are less. The mobile phase was passed through the filter with 0.22 µm pore size and then sonicated for 15 min for degassing.

Optimization and development of HPLC method

In the present study, C18 silica packed HPLC column was used. A Hyper Clone (Phenomenex®) C18 column (250 mm × 4.6 mm id, 5µm, BDS 130 Å) was used to perform the separation, with a C18 Phenomenex®, 4 × 3.0 mm id, security guard column. In order to avoid column's high internal pressure, acetonitrile was used as an organic solvent, because of its low viscosity. Drug was detected in the samples with a UV detector (SPD-10A; Shimadzu, Japan). Injection volume 20 µL was used in. The study the composition of the mobile phase was also kept constant in the entire study such as buffer: acetonitrile in 57:43 ratio. Factorial design helps to study the effects caused by independent factors and interactions between those self-governing factors. In the present work, three independent factors were used such as flow rate (A), wavelength (B) and buffer, pH (C). Three factorial levels were used in the study and were coded as -1, 0 and +1 for low, medium and high, respectively. Total 27 experimental runs were suggested by the software for analyzing the interaction of each level on formulation characters and the peak area (R1), tailing factor (5%) (R2) and number of theoretical plate USP (NTP) (R3) were considered as response factors (dependent factors).

Validation of HPLC method

Developed and optimized method was validated as per the ICH Q2 (R1) guidelines for following parameters such as linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ) (ICH Harmonized Tripartite Guidance and Methodology Q2 (R1), 2005; Solanki et al, 2014).

System suitability:

For system suitability determination 6 µg/mL of valsartan was used as stock solution. Six replicate injections of this standard solution were analyzed with HPLC. From these

replicate injections, the acceptance criteria for peak area, tailing factor (5%), tailing factor (10%) and number of theoretical plate was studied.

Linearity:

Standard calibration curve was prepared with different concentrations from 0.1 to 12µg/mL. Three replicate injections of each concentration were analyzed for this study. The linear regression and correlation coefficient was found out from the graph between peak area and concentration.

Precision:

Precision is a study which is based upon the intra-day precision and inter-day precision. Precision of developed method was determined by performing repeatability (six times of each concentration) of three distinct concentrations two times in a day (i.e. morning and evening) whereas the intermediate precision study was performed by repeating three distinct concentrations on two different days. Intra-day and inter-day precision was performed by using three unusual concentrations such as 1, 6 and 12 µg/mL. Peak area was measured and percent relative standard deviation (% RSD) was calculated (Rajput and Sonanis, 2011).

Accuracy:

Accuracy of developed method was studied by determining recovery values. Term accuracy means the value which is near to the value of reference. For the determination of accuracy, standard drug in the range of 75, 100 and 125% of sample's concentration (2µg/mL) were mixed with sample solution, which produces the final concentration 3, 4 and 5µg/mL. Percent recovery (Sagirli et al., 2007) of the added standard drug to the assay samples was calculated with following formula:

$$\text{Percent recovery} = [(C_C - C_f)/C_S] \times 100$$

Where, C_C is concentration of analyte presents in the combination of standard and test; C_f is concentration of analyte in nano-formulation and C_S is the concentration of standard analyte used in combination.

Limit of detection and limit of quantitation

Sigma method was used for limit of detection (LOD) (Awotwe-Otoo et al., 2012). This method is based upon slope and least standard deviation obtained from the response. Formula for the calculation of LOD used was; LOD = 3.3σ/SP, where σ is means standard deviation value in response and SP is slope obtained from linearity. Similarly, limit of quantitation (LOQ) was also calculated from the response of valsartan. Formula for calculation of LOQ used was; LOQ = 10σ/SP, where σ is a least standard deviation value in response and SP is slope obtained from linearity.

3. Results and Discussion

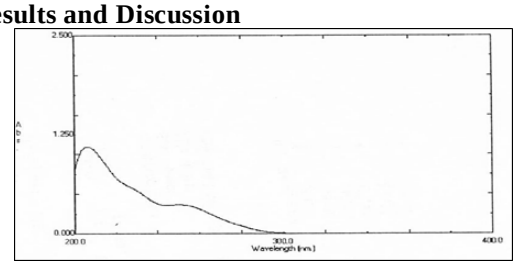

Fig.1. UV Scan of valsartan in buffer, pH 6.8

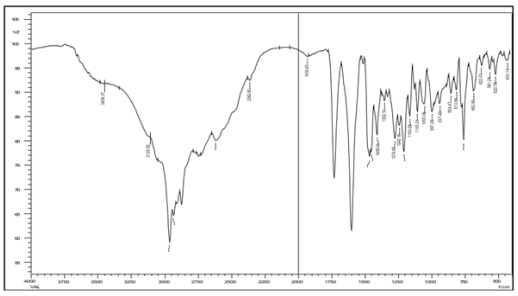

Fig.2. FTIR spectra of valsartan

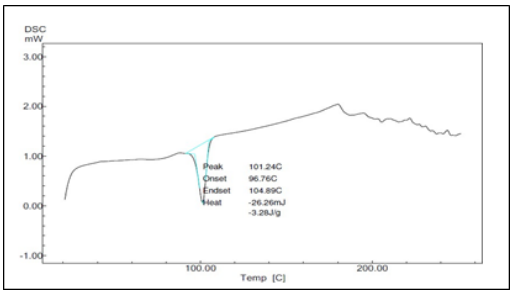

Fig.3. DSC thermogram of valsartan

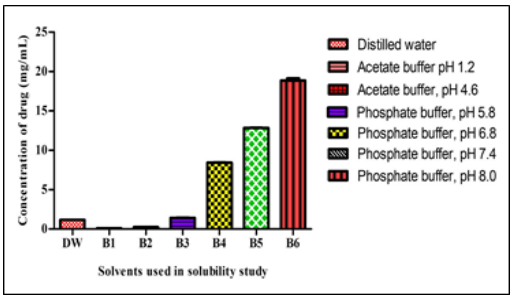

Fig.4. Saturated solubility study of valsartan in water and buffers

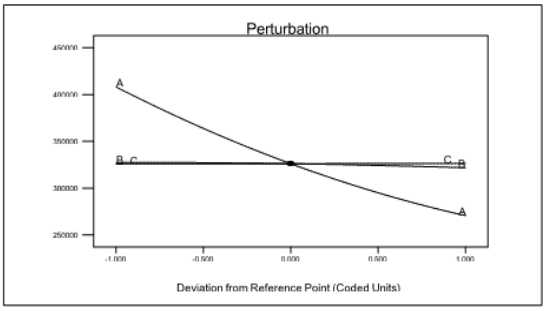

Fig.5. Perturbation plot for the effect of flow rate (A), wavelength (B) and pH of buffer (C) on peak area (R1) in HPLC analytical method of valsartan

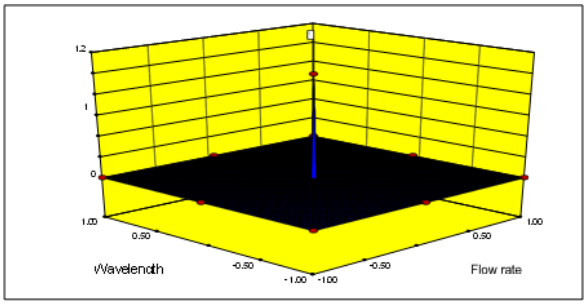

Fig.6. 3D surface response plot of desirability for optimization of factors for HPLC analytical method.

Table 1. System suitability (6 µg/mL concentration) of HPLC analytical method of valsartan

S. No.	Parameters	Results	
		Mean ± SD (n=6)	% RSD
1	Peak area	363052.300 ±1245.4620	0.343
2	Tailing factor (5%)	1.114 ± 0.0067	0.606
3	Tailing factor (10%)	1.092 ± 0.0039	0.361
4	Number of Theoretical Plate	13153.500 ± 49.6659	0.378

Table 2. Intra-day and inter-day precision of HPLC analytical method for valsartan

S. No.	Conc. (µg/mL)	Intra-day Precision		Inter-day Precision	
		Mean area ± SD (n=6)	% RSD	Mean area ± SD (n=6)	% RSD
1	1.0	60148.08 ± 512.65	0.85	59316.92 ± 809.18	1.36
2	6.0	360614.70 ± 3150.57	0.87	357362.60 ± 2292.19	0.64
3	12.0	637475.00 ± 4276.53	0.67	636835.40 ± 5108.63	0.80

Table 3. Linearity of HPLC bioanalytical method of valsartan in rat plasma and tissues

S. No.	Rat Plasma/Other Tissues	Correlation Coefficient (r^2)	Linear Regression Equation
1	Plasma	0.999	y = 0.0004x + 0.0116
2	Brain	0.998	y = 0.0002x + 0.0102
3	Intestines	0.997	y = 0.0002x + 0.0106
4	Heart	0.989	y = 0.0002x + 0.0096
5	Kidneys	0.999	y = 0.0002x + 0.0040
6	Liver	0.999	y = 0.0001x + 0.0157
7	Lungs	0.999	y = 0.0002x + 0.0071
8	Spleen	0.997	y = 0.0002x + 0.0111
9	Stomach	0.998	y = 0.0002x + 0.0114

Discussion

Valsartan solid lipid nanoparticles and polymeric nanoparticles were successfully prepared by modified constant temperature ultra sonication emulsion method and emulsion solvent evaporation method, respectively. Following conclusions have been drawn from the present study:

Preformulation studies

Procured drug sample is pure as per the UV visible spectrophotometer, FTIR and DSC studies and the results also complies with COA. Saturated solubility study results of drug indicates that the valsartan has pH dependent solubility. As the pH increases, drug solubility also increases, which also reflects the main reason for its low oral bioavailability. It clearly indicates that there is a need to increase the solubility of drug in gastrointestinal tract. HPLC analytical method was successfully developed and optimized through DoE and data was studied using Design Expert®, v.9.0.6.2 software. Significant effect of independent factors was also analyzed using ANOVA and the effect was also reported in the form of perturbation plots. Design of experiments provide efficient tools for the optimization of variable factors for HPLC analytical method development. Further, the method was validated as per the ICH guidelines. Obtained results, revealed that the present method is novel, simple, accurate, precise, economical and robust, and can be used for the analysis of valsartan in nanoformulations. HPLC bioanalytical method was also successfully developed using rat plasma and all other tissues. Results obtained for the linearity confirms that the method can be used for the analysis of drug in plasma and tissues.

Preparation and characterization of solid lipid nanoparticles:

From the lipid screening study, it was found that the GMS has high drug intake in comparison of the other lipid. FTIR and DSC study of physical mixtures of drug and excipients expressed that there is no chemical interaction between drug and excipients. Drug content, drug entrapment, particle size, polydispersity index and zeta potential results conclude that the present modified constant temperature ultrasonication emulsion method is suitable for the preparation of solid lipid nanoparticles. A nanoformulation was optimized using DoE and data was analyzed using Design Expert®, v.9.0.6.2 software. Significant effect of independent factors was also studied using ANOVA. The software suggestion were compared with actual obtained results and the validation results concludes that the formulation (VSNP8) can be used for further studies. TEM study results shows that the particles are almost spherical and are covered with soft surfactant layers. These will have high solubility and permeability. Accelerated stability results confirms that the formulation is stable at least for 30 days at both 30 ± 2 °C / 65 ± 5 RH and 40 ± 2 °C / 65 ± 5 RH. DSC and FTIR results indicates the presence of α-form and β-form of polymorphs of GMS, which may further enhance the solubility and permeability of drug. In vitro drug release

studies confirms the significant change in drug release pattern of formulation in comparison of standard. It also confirms the high solubility and drug release from SLN formulation in all dissolution medium. Further the study confirms the first order based drug release from formulation. In vitro cytotoxicity and animal toxicity result confirms that the SLN formulation is not having any serious side effect and is safe.

Pharmacokinetics study of valsartan loaded solid lipid nanoparticles confirms that the SLN formulation can be used an alternative, as it increases the oral bioavailability of valsartan by more than 4 folds. Biodistibution study of SLNs formulation indicates that the major site of absorption of this formulation is gastrointestinal tract (intestines and stomach), it can be distributed into the other organs such as heart, liver, brain, lungs and kidneys due to its high partition coefficient, higher concentration of drug in liver indicates that liver is a major site for absorption of this formulation and concentration of drug in the intestines and kidneys confirms the elimination of this formulation is through feces and urine.

Preparation and characterization of polymeric nanoparticles: FTIR of physical mixtures of drug and excipients confirms that there is no chemical interaction between drug and excipients. Drug content, drug entrapment, particle size, polydispersity index and zeta potential results conclude that the present emulsion solvent evaporation method is suitable for the preparation of polymeric nanoparticles.

A nanoformulation was optimized using DoE and data was analyzed using Design Expert®, v.9.0.6.2 software. Significant effect of independent factors was also studied using ANOVA. The software suggestion were compared with actual obtained results and the validation results concludes that the formulation (PVNP3) can be used for further studies. AFM study results confirms that the particles are spherical and smooth. It was expected that the particles will have high solubility and permeability. Accelerated stability results certifies that the polymeric nanoformulation is stable at least for 15 days at both 30 ±2°C/65 ± 5RH and 40 ± 2°C /65 ± 5 RH. FTIR results confirms the absence of chemical interaction and absence of structural modifications. In vitro drug release studies confirms the significant change in drug release pattern of formulation in comparison of standard. It also confirms the high solubility and drug release from polymeric nanoformulation in pH 6.8. The study also confirms the polymer has pH dependent solubility. Further the study confirms the first order based drug release from nanoformulation in pH 6.8.

4. Conclusion

This study concludes that the expected site for absorption of valsartan polymeric nanoformulation is intestines. In vitro cytotoxicity result endorses that the polymeric nanoformulation is safe. Pharmacokinetics study of valsartan loaded polymeric nanoparticles indicates that this formulation can be used an alternative as it increases the oral bioavailability of valsartan by approximately 2.5 folds. Biodistibution study of polymeric nanoformulation confirms that the major site of absorption of this formulation is gastrointestinal tract (intestines and stomach), it can be distributed into the other organs such as heart, liver and kidneys, higher concentration of drug in liver indicates that liver is a major site for absorption of this formulation and concentration of drug in the intestines and kidneys confirms the elimination of this formulation is through feces and urine.

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