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Design and development of solid nanoparticulate dosage forms of telmisartan for bioavailability enhancement by integration of experimental design and principal component analysis

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ABSTRACT

Aim of the present investigation was to develop nanoparticulate solid oral dosage forms of a poorly water soluble antihypertensive agent, telmisartan (TLM) by converting the optimized batch of drug loaded nanosuspensions into a tablet dosage form using lyophilization technique. The TLM loaded nanosuspensions were optimized by implementation of 3^2 full factorial design along with principal component analysis (PCA) with concentration of stabilizer and amount of milling agents as factors. The optimized batch of TLM loaded nanosuspension exhibited a mean particle size of 334.67 ± 10.43 nm. The results of various instrumental techniques illustrated retention of drug crystallinity after milling and lyophilization. The results of in vitro drug release study of tablets containing drug nanocrystals revealed remarkable improvement in the dissolution rate as compared to the marketed tablet (Sartel® 20). The results of in vivo pharmacokinetic study on Wister rats revealed 1.5-fold enhancement in oral bioavailability for tablets containing TLM nanocrystals against the marketed tablets. The present study proposed nanosuspension as a suitable approach for developing nanosized solid oral dosage forms of poorly water soluble drugs like telmisartan using design of experiment and principal component analysis as two important paradigms of quality by design technique.

1. Introduction

Oral drug delivery is a preferred route for drug administration as it avoids pain and risk of infection associated with parenteral administration and thereby leads to greater patient compliance. However, oral administration of many drugs results in lower absorption which is generally attributed to poor aqueous solubility, poor membrane permeation and efflux by P-glycoprotein (P-gp) [1]. Aqueous solubility of an orally administered drug is a critical determinant since the drug becomes available for absorption at specific sites within the GI tract only after its dissolution in GI fluid. Typically, these drugs have low oral bioavailability, erratic absorption, large inter and intrasubject variability and lack of dose proportionality [2,3]. Telmisartan (TLM) is the most widely prescribed selective antagonists of angiotensin II type-1 receptor (AT_1R) for the treatment of essential hypertension [4,5]. However, high lipophilicity and practical insolubility in water renders it a class II drug in the biopharmaceutical classification system which might be the reason for its slow or incomplete dissolution in the GI tract along with poor oral bioavailability (42%). Solubility enhancement approaches such as solid dispersion [6,7] and inclusion complexation [8] have already been investigated for the improvement of oral efficacy of TLM. However, none of these approaches offered an adequate improvement in therapeutic potential due to the limitations of the dosage form itself.

Nanotechnology is a technique which reduces the particle size of drug molecules down to the sub-micron range. This is a prevalent practice in the pharmaceutical field especially for the delivery of poorly water soluble drugs. Nanosuspensions are colloidal dispersions of nanosized drug particles (nanocrystals) stabilized by surfactants. The high phase stability of nanocrystals offers a potential benefit against the most commonly employed formulation technologies for poorly soluble drugs. Apart from this, drug nanocrystals not only increase dissolution velocity but also increase saturation solubility and hence they can improve the oral absorption of poorly soluble drugs, more efficiently [9–11]. The Quality by Design (QbD) paradigm underlying pharmaceutical drug product development relies on multivariate data, both from formulation and the process in order to explain the multi-factorial relationship between formulation variables, process variables and drug product attributes [12]. Design of experiments (DoE), risk assessment, principal component analysis (PCA) and process analytical technology (PAT) are the major tools that can be used in the QbD process as and when necessary [13]. The majority of scientists now routinely use DoE in order to reduce costs, improve quality within timelines to obtain robust products and processes. In light of these, the aim of the present investigation was to design and develop novel nanoparticulate solid oral dosage forms of TLM using design of experiment and PCA.

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2. Materials and methods

2.1. Materials

Telmisartan was obtained as a gift sample from Torrent Research Center, Gandhinagar, India. The materials; hydroxy propyl methyl cellulose (HPMC) E5 and hydroxy propyl cellulose (HPC) (klucel LF) were donated by Colorcon, Goa, India. Zirconia and glass milling beads were purchased from BioSpec Inc., Bartlesville, OK, USA. Cryoprotectants such as glucose, mannitol, trehalose and sucrose were purchased from S.D. Fine Chem, Mumbai, India. Stabilizers such as poloxamer 188, poloxamer 407, polyvinyl alcohol (PVA), polyvinyl pyrrolidine (PVP) K30 and sodium lauryl sulfate (SLS) were purchased from Himedia Labs, Mumbai, India. Microcrystalline cellulose (MCC) PH 200, sodium starch glycollate (SSG), magnesium stearate and talc were procured from Loba Chem, Mumbai, India. Acetonitrile and methanol used in the present study were of high performance liquid chromatography (HPLC) grade. Double distilled water was used throughout the study.

2.2. Formulation and development of drug loaded nanosuspensions

2.2.1. Preparation of drug loaded nanosuspensions

A wide mouth glass vial (outside diameter of 2.5 cm, inside diameter of 2.2 cm and inside depth of 5.2 cm with total volume 10 mL) was exploited to mimic a media milling machine. The total volume of the slurry (drug, stabilizer and water) was 5 mL which was considered as the batch size [14]. Subsequently each batch was charged with milling agents and exposed to stirring at fixed speed using a magnetic stirrer (Remi Laboratory Instruments, Mumbai, India) for preset time periods. At the end of the process each system was filtered through a membrane filter (0.45 μm) and the milled suspension (filtrate) was combined with rinsing of beads and vessel. The samples were stored at refrigerated conditions (2–8 °C) in screw capped glass vials until further use [15–17].

2.2.2. Preparation of drug loaded coarse suspensions

Drug powder was grounded in a mortar for 10 min and dispersed in 1% w/w of stabilizer solutions with a drug load of 5% w/w. The obtained suspension was sonicated in an ultrasonic water bath (Frontline FS-4, Mumbai, India) for 20 min and stored at refrigerated conditions (2–8 °C) in screw capped glass vials until further use [18].

2.2.3. Preliminary optimization of formulation parameters

TLM loaded nanosuspensions were optimized exclusively for factors like type of milling agents, type of stabilizer, concentration of drug, size of milling agents, ratio of drug to stabilizer, amount of milling media, stirring time and stirring speed as contributing factors by one variable at one time (OVAT) approach, keeping others constant [19,20]. Each batch was repeated thrice for the confirmation of reproducibility.

2.2.4. Experimental design

Based on preliminary trials the outcome of critical factors like amount of milling agent and drug to stabilizer ratio on the properties of TLM loaded nanosuspensions were evaluated by implementing 3^2 full factorial design along with principal component analysis (PCA) [21,22]. Critical responses were identified by PCA using a trial version of Unscrambler® 10.2 (CAMO AS, Norway, Switzerland). The data for all experimental design batches of drug loaded nanosuspensions were utilized to construct loading plot, scoring plot, agglomerative hierarchy cluster analysis (AHCA) plot, correlation loading plot and scree plot by PCA [23,24]. The experimental design consisted of a total of 9 runs (TLM-NS-F1 to TLM-NS-F9) and each of them was formulated in triplicates in order to estimate reproducibility of the model (Table 1). A second order quadratic model incorporating interactive and polynomial terms was used to evaluate the responses.

Table 1
Design layout of 3^2 full factorial design batches for TLM loaded nanosuspensions.

Batch code	Transformed values	
	X_1[a]	X_2[b]
TLM-NS-F1	−1	−1
TLM-NS-F2	−1	0
TLM-NS-F3	−1	1
TLM-NS-F4	0	−1
TLM-NS-F5	0	0
TLM-NS-F6	0	1
TLM-NS-F7	1	−1
TLM-NS-F8	1	0
TLM-NS-F9	1	1
Coded values	Actual values	
	X_1[a]	X_2[b]
−1	0.5	1
0	0.75	3
1	1	5

[a] X_1 — Concentration of stabilizer (% w/v).
[b] X_2 — Amount of milling agents (g).

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2 \quad (1)$$

where, Y_i was dependent variable, b_0 was arithmetic mean of nine runs and b_i was the estimated coefficient for factor X_i. The main effects (X_1, X_2 and X_3) signify average result of altering one factor at a time from its lowest to highest value whereas the interaction terms (X_1X_2, X_2X_3 and X_1X_3) prompt change in responses when two factors were simultaneously altered. The polynomial terms (X_1^2, X_2^2 and X_3^2) were added to investigate nonlinearity of the model [25]. Data were further analyzed by Microsoft Excel® version 2010 (Microsoft Corporation, Washington, USA) for regression analysis. Analysis of variance (ANOVA) study was used to assure nonsignificant difference between the developed full model and the reduced model. Contour, response surface and perturbation plots were generated to study response variations against independent variables using Statistica® 8 (StatSoft Inc. Oklahoma, USA) and Design Expert® 8.0.7.1 (Stat-Ease, Inc. Minneapolis, USA) software. Additionally, the composition of the optimized (check point) batch was derived by constructing overlay plots. The percentage relative error of each response was calculated using the following equation in order to judge validity of the model [26].

$$\%\text{Relative Error} = \frac{|\text{Predicted value-Experimental value}|}{\text{Predicted value}} \times 100. \quad (2)$$

2.2.5. Evaluation parameters for drug loaded nanosuspensions

2.2.5.1. Particle size and size distribution. The particle size and its distribution (polydispersibility index — PI) were measured for all batches of TLM loaded nanosuspensions by particle size analyzer (Zetatrac, U2552, New York, USA). For each sample a drop of nanosuspension (about 50 μL) was diluted with 5 mL purified water. All samples were subjected to a brief period of sonication (15–30 s) prior to size analysis with the intention to disperse any aggregates if present. The particle size and PI were recorded in triplicates at 25 °C [27,28].

2.2.5.2. Saturation solubility. For all experimental design batches of TLM loaded nanosuspensions, saturation solubilities were determined by shake-flask method. Surplus amount of samples were added individually to volumetric flasks (250 mL), each containing 100 mL of distilled