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Original Article

ABIRATERONE ACETATE LOADED SOLID LIPID NANOPARTICLES FOR IMPROVED ORAL BIOAVAILABILITY: DESIGN OF EXPERIMENTS BASED FORMULATION OPTIMIZATION, *IN VITRO*, *EX-VIVO* AND *IN VIVO* CHARACTERIZATION

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ABSTRACT

Objective: Abiraterone acetate (AA), a BCS Class IV drug, demonstrates biopharmaceutical challenges like polymorphism, poor solubility (<0.5 µg/ml), inconsistent permeability, and low oral bioavailability (<10%) (Hence requires a high dose of 1000 mg/day). The current research's main objective is to improve oral bioavailability by manufacturing AA-loaded solid lipid nanoparticles (AA-SLNs).

Methods: SLNs were manufactured using hot homogenization followed by an ultra-sonication method. Initial screening of lipids (Glyceryl monostearate (GMS), Glyceryl Monooleate (GMO)), and surfactants (Tween 80 and Span 20) was done by mixture design. Based on statistical analysis, GMO and Tween 80 were selected for further optimization, and Central composite design (CCD) of experiments were done to optimize the composition using particle size, polydispersity index (PDI), encapsulation efficiency (EE), zeta potential, and cumulative % drug release as responses. Comparative *ex-vivo* and *in vivo* evaluations of optimized formulation were done with the pure drug and marketed formulation.

Results: Based on the statistical evaluation, GMO-4.4% and Tween 80-3.6% were optimized. Optimized AA-SLNs were found in a spherical shape with size of 286.7±12.6 nm, PDI of 0.138±0.015, EE of 94.0±1.0 %, and zeta potential of-25.0±1.0 mV. Drug release from optimized formulation was extended for 24 h, and *ex-vivo* permeability was increased by 2.5 and 1.42 times, whereas Relative Oral bioavailability was improved by 6.36 and 1.99 times compared to pure drug and marketed tablets, respectively.

Conclusion: The results concluded that AA-SLNs showed increased oral bioavailability compared to the pure drug and marketed formulation. Hence the dose of the formulation can be reduced to achieve the desired therapeutic effect.

Keywords: Abiraterone acetate, Solid lipid nanoparticles, Sustained release, BCS class IV drugs, Design of experiments

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INTRODUCTION

Abiraterone acetate is an acetyl ester of abiraterone, available as tablets under the brand name Zytiga. It is aCYP17 (17α-hydroxylase/C17,20-lyase) inhibitor. It is chemically known as (3β)-17-(3-pyridinyl) androsta-5,16-dien-3-yl acetate). The molecular formula of Abiraterone acetate is $C_{26}H_{33}NO_2$with a molecular weight of 391.55. Indicated in combination with prednisone for treating metastatic castration-resistant and metastatic high-risk castration-sensitive prostate cancer. Abiraterone inhibits the synthesis of dehydroepiandrosterone (DHEA) and androstenedione, precursors of testosterone. The pharmacokinetic data showed that the oral bioavailability of the tablet formulation is around 5% in the fasting state, and bioavailability is expected to increase in the fed state [1]. According to the clinical pharmacology and biopharmaceutics review, Abiraterone acetate has low solubility and low permeability (BCS Class IV molecule) [2, 3]. Nanoparticulate systems can increase the bioavailability of the abiraterone acetate.

Solid lipid nanoparticles belong to nano-particulate delivery systems, which are made up of lipids and surfactants. The drug and/or lipids can be solubilized in an appropriate solvent system, and formed particles are stabilized by surfactants [4]. SLNs can be used as controlled drug delivery vehicles, offering safety compared to polymeric nanoparticles [5,6]. The physicochemical characteristics of the SLNs are dependent on many variables, including the type and proportional amount of lipids and surfactants and the ratio of solid lipids to drugs in the formulation. The formulation design is the critical step of formulation optimization since the components significantly impact the physicochemical characteristics and release profiles [7, 8]. In recent times, the design of experiments (DOE) has been used to develop and optimize various dosage forms. DOE offers excellent advantages over the one variable-at-a-time approach. It provides information about the interaction between factors and their effect on the results [9, 10]. Response surface methodology (RSM) is a suitable technique to assess the relationships between the factors and responses to optimize the processes where multiple variables may influence the system attributes [11]; RSM requires lesser experimentation and generates estimates of the relative importance of different variables [12].

This study used the DOE methodology to screen the suitable lipid and surfactant for developing Abiraterone acetate solid lipid nanoparticles (AA-SLN). The optimization of the formulation was done by central composite design (CCD). The formulation optimization produced stable spherical SLNs with sustained delivery of the drug over 24 h. The permeability and oral bioavailability of the AA-SLNs is increased significantly compared to pure drug. Hence, the dosage can be reduced to achieve the desired therapeutic effect.

MATERIALS AND METHODS

Materials

A gift sample of Abiraterone acetate was obtained from Dr. Reddy's Laboratories Limited, India. BASF India provided a gift sample of Glyceryl Monostearate (GMS) Gattefosse SAS, France gifted Glyceryl Monooleate (GMO). Span 20 and Polysorbate 80 (Tween 80) were purchased from Croda India. Chloroform and Methanol were purchased from Sigma Aldrich, India. Analytical-grade reagents were used in this study.

Design of experiment methodology

Development and optimization of the abiraterone acetate solid lipid nanoparticles (manufactured by hot homogenization followed by an ultra-sonication method) were carried out by the design of experiments methodology. Design-Expert-Software (Stat-Ease, Inc. Minneapolis, USA. Version 11.1.2.0) was used to screen the suitable lipid and surfactant, followed by optimization of the formulation.

The mixture design was employed for screening [13], and CCD was employed for formulation optimization.

Lipid and surfactants screening with mixture design

Design phase

Design-Expert-Software was employed to design and perform the statistical analysis of mixture design for screening lipids, surfactants, and their concentrations. Two lipids: Glyceryl Monostearate (GMS) and Glyceryl Monooleate (GMO), and Two surfactants: Polysorbate 80 (Tween 80) and Span 20, with concentration at two levels, were used. In this work, a two-level mixture design with four independent variables (two continuous, two nominal) was used to screen the main effects of four factors on dependent variables as particle size and encapsulation efficiency. Before proceeding with the execution of the trials, the model was evaluated for its power and suitability for evaluation using degrees of freedom values like lack of fit and pure error values. Based on the above-selected variables, a total of 15 experiments were conducted according to the design matrix (table 1) produced by Design-Expert-Software.

Preparation of abiraterone acetate solid lipid nanoparticles (AA-SLN)

The preparation of AA-SLN was prepared by hot homogenization followed by an ultra-sonication method [14]. Specified amounts of drug and lipids were dissolved in chloroform and then heated to 70 °C. These heated contents form the lipid phase of the formulation. The aqueous phase was prepared by dissolving a suitable quantity of surfactant in hot distilled water (70 °C). While maintaining the temperature at 70 °C, the lipid phase was added to the aqueous phase under homogenization using a Polytron homogenizer (Polytron PT 6100D-Kinematica AG, Switzerland) at 20000 rpm for 15 min to form oil-in-water (O/W) emulsion. After completion of homogenization, the emulsion was sonicated for 15 min using an ultrasonicator (Bransonic Ultrasonic bath, Model: CPX8800H-E, Branson Ultrasonics Corporation, USA) to produce the nano-emulsion. This nano-emulsion is rapidly brought down to room temperature and converted into AA-SLN by immersing the container in an ice bath. The above suspension was centrifuged, and the obtained pellet was washed with purified water. The AA-SLN suspension is filled into vials and lyophilized using a lyophilizer (FTS LyoStar™ 3 Freeze dryer, SP Scientific). The lyophilized vials were stored at 2-8 °C until further usage.

Determination of particle size, polydispersity index (PDI), and zeta potential

The particle size, PDI, and zeta potential of the prepared AA-SLN were measured by the Malvern Zeta sizer (Model: Nano ZS; Malvern Instruments, UK) at 25 °C. For each sample, analysis was performed in triplicate. Before measuring, solid lipid nanoparticles were diluted (1 in 100) with ultra-purified water, and the measurement was carried outat a detection angle of 173 degrees.

Determination of encapsulation efficiency (EE)

AA-SLNs equivalent to 250 mg drug were dissolved in 10 ml of methanol. A modified HPLC method [15] was used to estimate Abiraterone acetate content in the formulation. The drug content was measured using HPLC with a Hyperasil BDS C18 column (250 mm X 4 mm, 5 µm). Mixture of acetonitrile and 0.1% ortho-phosphoric acid at 20:80 ratio was used as the mobile phase. An isocratic method was used to analyze with 1 ml/min flow rate at a detector wavelength of 250 nm. The total run time of analysis is 15 min, and the retention time of 8.1 min. The encapsulation efficiency is calculated using the formula (Equation 1).

$$\text{Entrapment efficiency (\%)} = \frac{\text{Drug present in the sediment}}{\text{Theoretical quantity of drug present in the SLNs}} \text{ X } 100 \quad \text{(1)}$$

Analysis phase

After completion of the trials, the responses for the particle size and encapsulation efficiency were recorded in the design matrix. The relationship between factors and responses was analyzed using Design-Expert-Software. Analysis of variance (ANOVA) analysis was used to select the best-fit model based on F-value, and the validity of the model was confirmed by comparing the closeness of Adjusted R^2 and Predicted R^2 values and adequate precision value.

Optimization phase

For the optimization phase, constraints were used to finalize the type of lipid, surfactant, and suitable concentrations. Based on the outcome of the optimization and polynomial equations were solved to predict responses with the optimized composition. Three experiments were conducted with the selected lipid, surfactant, and their concentrations. The results of the experiments were compared with the predicted values to assess the model's precision.

Optimization of the solid lipid nanoparticles by CCD

After the finalization of the lipid and surfactant, further optimization of the formulation was conducted using a central composite design [16-18]. The same steps, i.e., The Design phase, Analysis Phase, Optimization, and Post analysis phase, were used to get the optimized formulations. Lipid concentration and surfactant concentrations were considered independent variables. These two variables were studied at 5 levels, i.e.,–α,-1, 0, 1, and+α. The coded values for the 5 levels of GMO concentration (%) are 3.59, 4.00, 5.00, 6.00, and 6.41. The coded values for the 5 levels for Tween 80 concentration (%) are 1.59, 2.00, 3.00, 4.00, and 4.41. The α value (1.41421) was generated by Design-Expert software to satisfy the design's orthogonality and rotatability. A total of 13 runs were performed per the design matrix (table 2) created by the design expert. ANOVA analysis was used to select the best-fit model based on F-value and p-value. The relationship between the independent and dependent variables can be predicted using a second-order polynomial equation (Equation 2).

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_{11}X_1^2+\beta_{22}X_2^2++\beta_{12}X_1X_2 \quad \text{Equation (2)}$$

Where Y represents response value, $\beta_0$represents intercept, β_1 and β_2 are linear coefficients, β_{11} and β_{22} are squared coefficients, β_{12}represent interaction coefficient, and X_1 and X_2 are independent variables. Design-expert-software (Stat-Ease, Inc. Version 11.1.2.0).) was used to do statistical analysis. The fitness of the polynomial equation was evaluated using the fit statistics, i.e., Closeness between adjusted R^2 and Predicted R^2 (Should be less than 0.2), and Adequate precision value should be more than 4. During the analysis phase, the interaction between the factors and responses was evaluated by RSM plots. The optimization phase was performed to select the optimal points based on the RSM plots analysis and constraints given for the responses. The model's precision was evaluated by performing the experiments in triplicate with optimized composition and comparing the results with predicted values.

***In vitro* release studies**

In vitro release studies of the prepared SLNs were performed using USP dissolution apparatus IV/Flow through cell apparatus (Model: SotaxCE7 smart with CP7 pump Make: Sotax Ag, Switzerland) [19, 20]. Dissolution was conducted using 22.6 ml cells. Sandwich-type model sample preparation was followed for dissolution analysis. Sandwich-type model sample preparation consists of placing a ruby ball with 5 mm diameter at the bottom opening of the cell. On top of the ruby ball, glass beads having 1 mm diameter were added to decrease the central pulse of the jet fluid coming from the pump. On top of the glass beads, a sample equivalent to 250 mg of the drug (For AA-SLN and API, direct powder samples were added, in the case of tablet formulation, tablets were triturated and powder was taken) was added, followed by placing of the glass beads till complete loading of the sample cell. Whatman grade Glass microfiber filters (GF/F, diameter 25 mm; pore size 0.7 µ) were used in the filter head to prevent the undissolved particles' escape from the sample cell [21]. 900 ml of dissolution medium (0.25% SLS in 0.0056M Phosphate buffer, pH 4.5) was recirculated at a flow rate of 8 ml/min. Dissolution was performed in closed operating mode. The sample cells and dissolution medium temperature was maintained at 37±0.5 °C. Sampling (10 ml) was done at specified intervals, fresh dissolution medium was added to the reservoir, and samples were analyzed for drug content in the sample. The data was fitted into various release order models to evaluate the release kinetics and mechanism.

Ex-vivo permeation studies

BCS class IV drug permeability was expected to increase due to the presence of lipids in the formulation. The *ex-vivo* permeability studies were conducted for AA-SLN, Pure drug, and marketed formulations (Abiraterone acetate Tablets 250 mg) using the goat intestinal membrane. Freshly excised, thick male goat intestinal membranes were collected from a local animal slaughterhouse without causing any damage to the epidermal layer. The collected intestinal membrane was tagged to one end of an open-ended glass tube. Then it was submerged vertically in 50 ml of diffusion medium (0.05 M Sodium Phosphate Buffer with 0.05% SLS, pH 6.8) so that the membrane was immersed 1-2 mm deep into the diffusion medium [22-24]. Solid lipid nanoparticles (1% CMC), drug suspension(1% CMC), and tablets (triturated and dispersed in 1% CMC) equivalent to 100 mg dose were taken into the donor compartment containing 10 ml of 0.05 M Sodium Phosphate Buffer with 0.05% SLS, pH 6.8. On a magnetic stirrer (Remi, India), the diffusion medium was continuously stirred at 75 RPM while maintaining the temperature at 37±0.5 °C

In vivo pharmacokinetic study

The Institutional Animal Ethics Committee approved the protocol for the comparative *in vivo* pharmacokinetic evaluation of the optimized formulation using the pure drug and the commercial formulation (Zytiga) in healthy male Wistar rats procured from Mahaveera Enterprises Pvt Ltd, Hyderabad, Telangana, India(Approval Number: VPC/IAEC/2022/1, Date: 29/01/2022). The animals were kept in metal cages with unrestricted movement, given a standard laboratory meal, and given access to water. The formula below was used to calculate the necessary animal dose [25].

$$\text{Animaldose}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{HumanDose}\left(\frac{\text{mg}}{\text{kg}}\right) * \frac{\text{HumanKmValue}}{\text{AnimalKmValue}}$$

Km value of Rat is 7

Km value of Human is 37

Each formulation was made by dispersing the weight equivalent of the medication in a 1% Sodium CMC Suspension. All formulations were given to the animals via an oral cannula. A three-group open labelled, randomized, balanced, single-dose parallel trial design was used, with each group consisting of six healthy male Wistar rats with a body weight range of 200–250g. As described below, one formulation was used to treat each group.

Group I (N=6): Pure drug oral suspension

Group II (N=6): Optimized formulation

Group III (N=6): Marketed formulation (Zytiga)

All the animals fasted for one night prior to the required dose. A standard diet was given the morning after a zero-hour blood sample (blank) was obtained. Following administration of the appropriate dose (51 mg/Kg equivalent), 0.5 ml of blood samples from each animal and group were taken from the retro-orbital vein at intervals of 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 96h. Immediately following collection, the plasma was separated by centrifugation at 5000 rpm for 30 min. The approved UPLC-MS/MS method was used to assess the drug concentration in plasma samples [26].

The pharmacokinetic parameters of all administered formulations, including peak plasma concentration (C_{max}), time needed to reach the maximum plasma concentration (t_{max}), area under the curve (AUC), biological half-life ($t_{1/2}$), absorption rate constant (K_a), and mean residence time (MRT), were calculated using the data by Kinetica™ 2000 software (Inna Phase Corporation, U. S. A.) using a non-compartmental approach. Compared to the pure drug and the commercial formulation, the improved formulation's percent relative bioavailability was also calculated.

Surface morphology

Transmission Electron Microscopy (TEM) was used to evaluate the shape of the prepared AA-SLNs [27]. Samples were analysed using the established procedures; particles were deposited onto the carbon-coated grids, and images were recorded using Hitachi, H-7500 transmission electron microscope (Hitachi High Technologies, Tokyo, Japan).

Stability studies

Stability studies of the solid lipid nanoparticles were conducted at 2-8 °C and 25±2 °C/60±5% RH. Assay, particle size, PDI, zeta potential, and cumulative % drug release were the key product attributes to evaluate the stability.

RESULTS

Lipid and surfactants screening with mixture design

In total, 15 experimental runs were generated by Design-Expert-Software. The design matrix was summarized in table 1. The trials were performed per the trial order, and the responses were summarized in table 1. A linear model was selected as the best fit for both responses based on the statistical analysis, F-value and p-value. The ANOVA analysis showed that all the models were statistically significant (p-value less than 0.001) with an insignificant Lack of Fit (p-value more than 0.05). Best-fit models were selected for the optimization phase. The optimization tool was employed using the following constraints, Lipid concentration (%): 2-4; Surfactant concentration (%): 1-2; Lipid type: GMO–GMS; Surfactant type: Tween 80-Span 20; Particle Size (nm): 100-500 and Encapsulation efficiency (%): 75-100. Based on the outcome of the optimization tool, Lipid: GMO; Surfactant: Tween 80, Lipid concentration: 4%; Surfactant concentration: 2%.

Table 1: Mixture design: formulation and physicochemical evaluation of abiraterone acetate solid lipid nanoparticles

Run	Lipid (%)	Surfactant (%)	Lipid	Surfactant	Particle size (nm)*	Encapsulation efficiency (%)*
1	2.34	2.00	GMO	Tween 80	216.5±4.3	88.65±1.4
2	4.00	2.00	GMS	Tween 80	296.1±3.5	88.24±2.8
3	2.00	1.00	GMO	Tween 80	195.3±5.6	75.62±2.4
4	2.00	1.17	GMS	Span 20	350.3±6.3	45.68±1.9
5	4.00	1.00	GMS	Span 20	371.6±3.8	68.54±5.3
6	4.00	2.00	GMO	Span 20	390.3±6.8	71.65±3.2
7	2.00	2.00	GMS	Span 20	335.5±4.5	64.21±2.7
8	4.00	2.00	GMO	Span 20	388.9±6.1	73.14±1.7
9	2.00	2.00	GMS	Span 20	315.9±5.2	58.65±0.9
10	2.79	1.52	GMS	Tween 80	238.6±4.8	79.65±2.9
11	3.69	1.00	GMO	Tween 80	290.2±3.0	78.35±2.5
12	2.00	1.00	GMO	Tween 80	218.3±5.4	69.54±3.2
13	3.06	1.43	GMO	Span 20	338.1±4.8	65.68±6.3
14	4.00	1.00	GMS	Span 20	379.9±7.8	63.41±7.4
15	4.00	2.00	GMS	Tween 80	298.1±3.2	89.35±2.9

%: Percentage, GMO: Glyceryl Monooleate, GMS: Glyceryl Monostearate, nm: Nanometer *:Data expressed as mean±SD (n=3)

Optimization of solid lipid nanoparticles by central composite design (CCD)

After selecting the type of lipid, surfactant, and concentration from the screening study, a central composite design(CCD) was generated using 2 independent variables i.e., GMO concentration and Tween 80 concentration; and 5 dependent variables i.e., particle size, PDI, EE, zeta potential and cumulative % drug release at 24 h. A total of 13 trials were generated per the CCD design matrix, and the results were summarized in table 2. Statistical analysis (ANOVA) was performed using Design-Expert-Software. The best models were selected based on the F-value and p-value. According to the ANOVA results, a quadratic model, linear model, quadratic model, linear model, and quadratic model were found to be the best fit for particle size, PDI, EE, Zeta potential, and cumulative % drug release at 24 h, respectively. All the above models were statistically significant ($p<0.05$) with an insignificant Lack of Fit ($P>0.05$). The relationship between the factors and responses were shown in table 3. RSM plots were plotted for each dependent variable to evaluate the interaction between each independent variable. RSM plots for each response are depicted in fig. 1A-E. An optimization tool was used to optimize the formulation based on the constraints. The constraints were GMO concentration (%): in the range of 4 to 6; Tween 80 concentration (%): in the range of 2 to 4; Particle size (nm): to minimize (150-350); PDI: to minimize (less than 0.25); EE (%): to maximize (75-100); Zeta potential(mV): between-30 to+30; and Cumulative % drug release at 24 h: to maximize (85-100). The overlay of the constraints is depicted in fig. 1F. GMO concentration of 4.4% and Tween 80 of 3.8% was selected as the optimized composition for Abiraterone acetate SLNs. Three trials were performed with the optimized formulation and analysed for the responses. The results of the optimized formulations were as follows: Particle Size of 286.7±12.6 nm (fig. 2A), PDI of 0.138±0.015, EE of 94.0±1.0 %, the zeta potential of-25.0±1.0 mV (fig. 2B), and the cumulative % drug release after 24 h was 98.7±0.6%. The results were compared with the Predicted values achieved by the polynomial equations. All the results were within the 95% confidence interval, confirming the model's validity and precision.

Table 2: Central composite design: formulation and physicochemical evaluation of abiraterone acetate solid lipid nanoparticles

Std	Run	GMO (%)	Tween 80 (%)	Particle size (nm)*	PDI	EE (%)*	ZP (mV)	%DR 24
1	5	4.00	2.00	301.65±6.3	0.17±0.005	87.88±2.2	-23.65±0.8	72±0.8
2	8	6.00	2.00	545.65±4.7	0.25±0.011	92.36±0.9	-28.54±1.1	85±1.6
3	11	4.00	4.00	299.47±7.2	0.11±0.007	92.35±1.4	-22.65±0.9	88±0.8
4	1	6.00	4.00	524.31±9.1	0.23±0.009	94.56±1.7	-28.47±1.2	98±1.5
5	2	3.59	3.00	289.65±5.5	0.09±0.021	85.64±2.0	-21.54±1.7	84±1.0
6	6	6.41	3.00	615.35±5.2	0.26±0.015	90.35±1.9	-27.97±1.0	97±0.8
7	4	5.00	1.59	488.14±7.9	0.20±0.010	91.24±1.5	-24.65±0.5	65±0.8
8	12	5.00	4.41	368.74±8.4	0.19±0.009	96.54±1.3	-25.33±1.1	99±1.4
9	7	5.00	3.00	295.87±5.5	0.18±0.011	93.54±1.5	-26.41±0.9	95±1.0
10	9	5.00	3.00	314.65±5.1	0.16±0.012	92.3±1.4	-28.65±1.2	97±1.2
11	13	5.00	3.00	325.14±3.8	0.19±0.008	91.54±1.6	-24.15±1.1	91±1.5
12	10	5.00	3.00	300.21±6.2	0.16±0.015	94.14±0.9	-24.95±0.6	93±1.4
13	3	5.00	3.00	335.33±4.8	0.21±0.011	92.41±1.0	-24.65±1.0	95±1.0

%: Percentage, GMO: Glyceryl Monooleate, nm: Nanometer, PDI: Polydispersity Index, EE: Encapsulation Efficiency, ZP: Zeta potential, mV: millivolts, %DR 24: Cumulative % drug release at 24 h, *:Data expressed as mean±SD (n=3) for drug release n=6

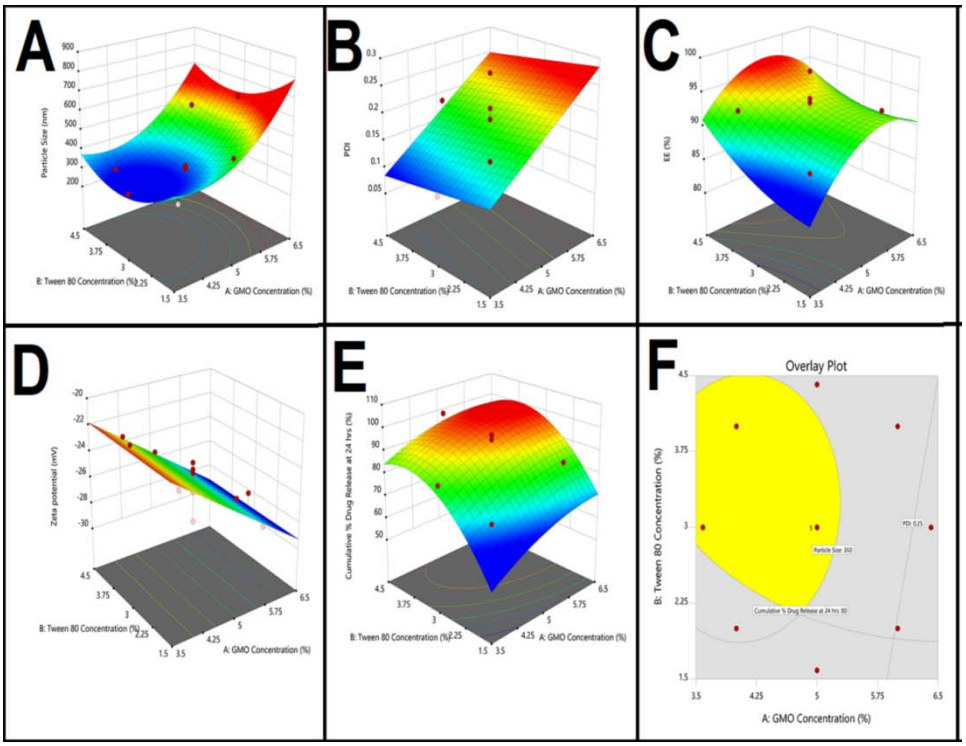

Fig. 1: Response surface plots of: (a) particle size, (b) PDI, (c) encapsulation efficiency, (d) zeta potential (e) drug release at 24 h and (f) overlay of constraints for optimization of SLN composition

Table 3: Mathematical modeling: Relationship between the dependent and independent variables

Response	Polynomial equation
Particle Size (nm)	314.24+116.18*A-24.05*B-4.79*AB+63.46* A^2+51.42*B^2
PDI	0.1846+0.0551*A-0.0118*B
%EE	92.79+1.67*A+1.77*B-0.5675*AB-2.18* A^2+0.7633*B^2
Zeta potential (mV)	-25.51-2.48*A+0.0135*B
Cumulative % Drug release %	94.20+5.17*A+9.64*B-0.75*AB-1.98* A^2-6.22*B^2

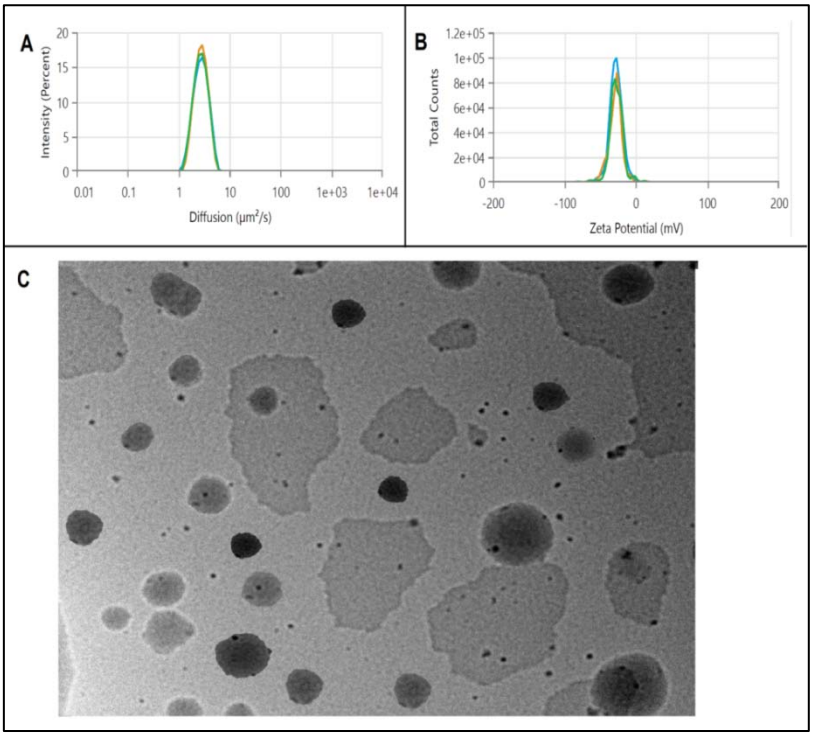

Fig. 2: Characterization of SLNs. (a) histogram of particle size, (b) histogram of zeta potential, Abiraterone acetate tablets and Abiraterone acetate drug, and (c) transmission electron microscopic image (Magnification: 100000x) of Abiraterone acetate SLNs

In vitro release studies

In vitro release study data of the optimized formulation, as such API and abiraterone acetate tablets, was depicted in fig. 3. Compared to the tablets and API, the dissolution was slow in the solid lipid nanoparticles. Around 50% of the release was observed in the 2 h with SLNs. In comparison, nearly 100% release was observed in 30 min for API and tablet formulation. DDsolver was used to fit the data into various mathematical models. The rate constants and correlation coefficient confirmed that the drug release followed the first-order release kinetics. Based on the n-value of 0.317, calculated from the Korsmeyer-Peppas equation, the drug release mechanism was found to be followed Fickian diffusion.

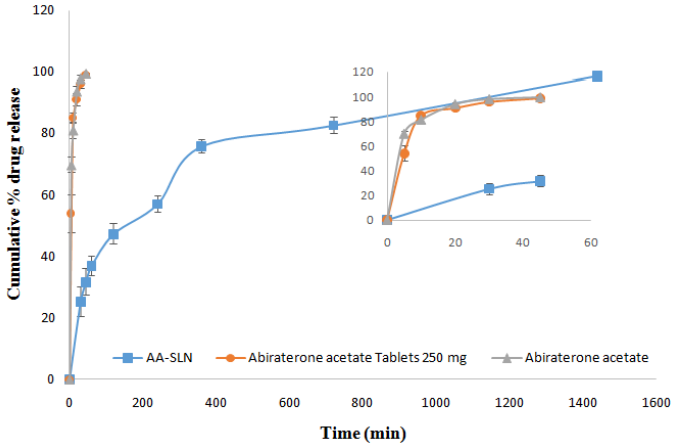

Fig. 3: *In vitro* release data of Abiraterone acetate SLNs, Abiraterone acetate tablets, and abiraterone acetate drug (Data expressed as mean±SD; n=6)

Ex-vivo permeation studies

Permeability studies were conducted on solid lipid nanoparticles, drug, and tablet formulations. The results of the permeation study were summarized in fig. 4. The results indicate that the permeability of the solid lipid nanoparticles was increased by 2.5 and 1.42 times compared to the pure drug and tablet formulations, respectively. More than 94.5+2.4 % of the drug was diffused within 12 h from solid lipid nanoparticles, whereas 66.6±1.9% and only 37.5±2.3% of abiraterone acetate were diffused from tablets and drug suspension, respectively.

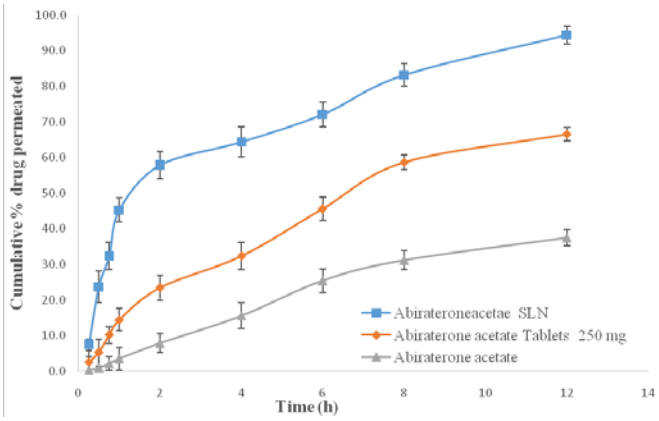

Fig. 4: *Ex-vivo* permeation data of Abiraterone acetate SLNs, Abiraterone acetate tablets, and abiraterone acetate drug (Data expressed as mean±SD; n=6)

In vivo pharmacokinetic study

The plasma concentrations of all the formulations at each specified time point from all the animals were estimated using the UPLC MS/MS method in an *in vivo* pharmacokinetic investigation. Fig. 5 depicts the graph created by plotting the time on the X-axis and the associated average plasma concentration on the Y-axis. Using Kinetica™ 2000, all potential pharmacokinetic parameters were calculated and shown in table 4. Relative bioavailability of the optimized formulation against pure drug and commercial formulations is also presented in table 4.

Table 4: Pharmacokinetic parameters of abiraterone acetate pure drug, marketed formulation (Zytiga Tablets 250 mg), and SLNs

S. No.	Parameters	Units	Pure drug	Marketed formulation	SLNs
1	t1/2	h	49.20	24.00	30.38
2	Tmax	h	6.00	6.00	3.00
3	Cmax	ng/ml	40.65	95.34	750.46
4	AUC 0-t	ng/mlh	1042.74	3802.87	7950.49
5	AUC 0-inf_obs	ng/mlh	1295.45	4145.31	8237.16
Relative Bioavailability with Pure Drug				3.20	6.36
Relative Bioavailability with Marketed					1.99

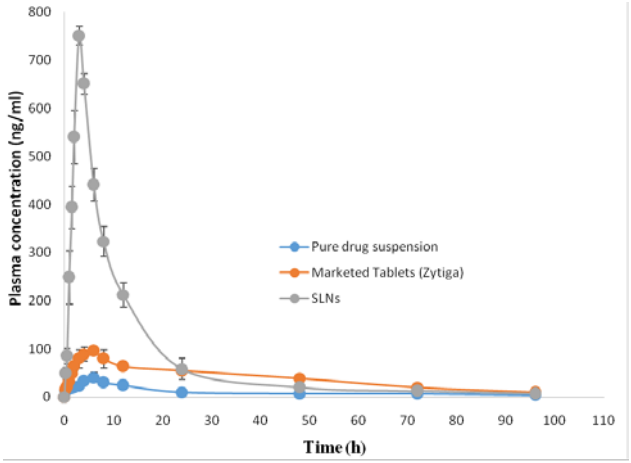

Fig. 5: Pharmacokinetic profiles of abiraterone acetate pure drug suspension, marketed tablets (Zytiga), and SLNs after oral administration (Data expressed as mean±SD; n=6)

The C_{max} of the pure drug was found to be 40.65 ng/ml, and the C_{max} of the marketed formulation was found to be 95.34 ng/ml, whereas the C_{max} of the optimised formulation was found to be higher than the pure drug and the marketed formulation (750.46 ng/ml). Higher C_{max} shows that the optimised formulation significantly increased the rate of absorption.

Additionally, it is clear from the outcomes that the optimised formulations' bioavailability has greatly increased compared to the pure medication and commercial formulations (6.36 times against pure drug and 1.99 times against marketed). Therefore, the Optimized AA-SLN formulation is the best and most promising formulation to enhance the bioavailability,

lengthening residence time, and consequently, duration of action.

Surface morphology

TEM images (fig. 2C) of optimized formulation showed that the particles manufactured were spherical and the particles' size was below 350 nm.

Stability studies

Stability studies results (table 5) showed that AA-SLNs were found to be more stable at 2-8 °C compared to 25±2 °C/60±5% RH. The assay was decreased by less than 5% after 6 mo of storage in the 25±2 °C/60±5% RH stability condition; this might be due to the change in the solidification behaviour of lipids at 25 °C compared to the 2-8 °C. The other parameters were well within acceptable limits.

Table 5: Stability data of abiraterone acetate solid lipid nanoparticles

Time points (Months)	2-8 °C				25±2 °C/60±5% RH			
	Assay (%)	Particle size (nm)	PDI	Zeta potential (mV)	Assay (%)	Particle size (nm)	PDI	Zeta potential (mV)
0	99.6±0.9	282±3	0.14±0.016	-25.0±1.0	99.6±0.9	282±3	0.14±0.016	-25.0±1.0
1	98.9±0.8	275±5	0.15±0.012	-24.5±1.5	98.5±1.0	290±4	0.14±0.022	-24.9±1.0
2	98.4±1.1	294±5	0.13±0.009	-24.6±1.1	97.9±0.9	289±7	0.15±0.019	-23.4±1.2
3	97.6±0.7	294±3	0.15±0.016	-24.2±1.3	97.6±0.7	303±6	0.16±0.009	-23.5±2.3
6	97.4±0.9	298±6	0.14±0.014	-24.6±1.3	96.9±1.1	289±5	0.13±0.014	-23.6±0.9

%Percentage, nm: Nanometer, PDI: Polydispersity Index, mV: millivolts, *Data expressed as mean±SD (n=3) for drug release n=6

DISCUSSION

The design of the experiment methodology was employed to screen the type of lipids, surfactant, and their concentrations for developing Abiraterone acetate solid lipid nanoparticles. Particle size and encapsulation efficiency were considered responses during the screening stage. The results and polynomial equations derived from the Design-Expert-Software show that nanoparticles size was more with GMS than the GMO, irrespective of the surfactant used. This phenomenon might be due to the differences in the melting point of the lipids. The melting point of the GMS (55-60 ℃) was more when compared to the GMO (35 ℃); due to this lower melting temperature, the formation of the lipid crystals starts early in the GMO formulation during the homogenization process; which could have led to smaller particle size nanoparticles with GMO. This is in agreement with the findings of Ekambaram and Abdul 2011 [28].

It was also observed that the particle size of SLNs prepared with Span 20 was larger when compared to SLNs prepared with Tween 80, which can be attributed to the hydrophilic-lipophilic balance (HLB) value of the surfactant. The HLB value of Span 20 (8.6) was less compared to Tween 80 (15); as the expected emulsion to be formed during formulation is oil in water, a surfactant with higher HLB value (more hydrophilic surfactant like Tween 80) will have more potential to reduce the interfacial tension when compared to the surfactant with lower HLB value (more lipophilic surfactant like Span 20). Thus more particle size reduction might have happened with same level of energy with high reduction in the interfacial tension in case of the formulation prepared with Tween 80; hence, the particle size was less with Tween 80. Similarly, as the zeta potential and entrapment efficiency are directly linked to the interfacial tension and particle size of the SLNs, high EE and high zeta potential was perceived with formulation prepared in combination with GMO and Tween 80 when compared to GMS and Span 20. These results are in-line and in agreement with the studies conducted and reported by Ekambaram and Abdul 2011 [28].

The polynomial equations in table 3 show the interaction between the factors and responses. The negative coefficients in the equations indicate that the independent variable shows a negative effect on the response, and apositive coefficient indicates the positive effect on the response. A higher GMO quantity increases in the viscosity of the formulation, which requires higher energy and stirring times to get the required particle size and PDI. Thus, larger particle sizes and PDI were observed. Higher GMO quantity ensures the availability of the lipid to entrap the drug; thus, higher EE was observed.

As discussed above, as Tween 80 is having higher HLB value and more hydrophilicity, it requires lower energy to reduce the interfacial tension and this phenomenon is directly proportional to its concentration. Hence, an increase in the Tween 80 concentration in the formulation has led to a decrease in Particle size, PDI, increase in zeta potential and EE [29-31]. And it is established fact that the dissolution is inversely proportional to the particle size of any nanoparticle. Hence, it was noticed that the formulations prepared with high concentrations of Tween 80 using GMO as the lipid has shown faster drug release when compared to the others during the *in vitro* release study, a biphasic release pattern was observed for AA-SLNs. This biphasic release pattern is very common with SLNs and also reported in previous studies [31-33] This initial rapid release of the drug is could be attributed due to the weekly bounded drug on the surface of the SLNs. In the later stages, controlled drug release was observed due to drug entrapment in the lipid matrix. The drug release followed first-order release kinetics and the release mechanism followed Fickian diffusion. *Ex-vivo* permeability showed that the permeability of the drug from AA-SLNs was more than tablets and as such drug. This phenomenon is due to the presence of lipids in the composition, which increases the formulation lipophilicity and thus, permeability of the drug through the membranes. The increased permeability of the drug results in the increased bioavailability of the Solid Lipid Nanoparticles. Another factor attributed to the increased bioavailability os due to the presence of Tween 80 in the formulation, which enhances the solubility of the drug [34-37].

CONCLUSION

Abiraterone acetate SLNs were prepared by hot homogenization followed by an ultra-sonication method by applying DOE methodologies for screening and optimization. The optimized formulation showed increased permeability and sustained release compared to the tablet dosage form. Based on the above observations, the optimized formulation of Abiraterone acetate SLNs can reduce the required dose and increase the bioavailability.

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AUTHORS CONTRIBUTIONS

All the authors have been contributed equally.

CONFLICTING OF INTERESTS

The authors declare that there is no conflict of interests.

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