Journal of Chemical Health Risks

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

Development and Characterization of Luliconazole Solid Dispersion by DOE Approach for Improved Topical Treatment of Fungal Infections

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(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS

Solid dispersion, Luliconazole, Anti-fungal, *Candida albicans* and *Aspergillus niger* etc.

ABSTRACT:

The objective of present research work was to develop solid dispersion of luliconazole (LNZ) by using different concentrations of drug and polymer ratio. The effect of different concentrations of polymers was determined on the drug content and, percentage cumulative drug release. Drug release followed zero-order models and mechanism of drug release are independent on the concentration of a drug. The optimized formulation LNZ-SD-7 is demonstrated particle size50.93 µm, cumulative drug release 99.35±2.54 and drug content of 98.24±2.18 %.Scanning Electron Microscopy reveled optimized LNZ-SD-7was spherical in shape having smooth surfaces. Antifungal studies conformed that LNZ-SDhave better efficacy in treating *Candida Albicans*having MIC-0.4µg $ml^{-1}$and MBC 0.8µg ml^{-1}compared to *A.Niger* having MIC*MIC*-1.6 µg $ml^{-1}$and MBC 3.2 µg ml^{-1} .Aslo LNZ-SD showed greter zone of inhibition on *Candida Albicans when compared to A.niger* having zone of inhibition 15 and 9 mm respectively.From the results we may conclude that LNZ-SDcan be used as competent alternative to treat fungal infection caused by *Candida albicans* as compared and *Aspergillus niger*. However; further *in vivo* studies are required to establish its efficacy against fungal infections.

Introduction

Fungal diseases affecting the skin and nails are the most prevalent in humans, impacting approximately 25% of the global population, or 1.7 billion people, with these infections.[1] The fungal infections that fall under these broad categories vary in terms of the traits of the causing organisms, their epidemiology, the clinical presentation, the method of diagnosis, and the guiding principles of treatment.[2] Opportunistic fungal infections are primarily caused by Candida species, and fluconazole or an echinocandin antifungal agent are typically used to treat bloodstream infections. Most invasive mold infections are caused by Aspergillus species; they are associated with a high mortality rate and primarily cause pulmonary and sinus infections in older adults.[3] When the mold form is spread and inhaled from the surroundings in those particular regions of the nation where these organisms flourish, the endemic fungi Histoplasma capsulatum, Coccidioides species, and Blastomyces dermatitidis cause infection.[4]

LNZ is a topical broad-spectrum antifungal drug, approved by the FDA (USA) in November 2013. LNZ has lower aqueous solubility that limits dermal bioavailability and acts as a barrier to topical delivery. The solubility of the drug in the lipid phase of stratum corneum also acts as a

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

rate-limiting step for permeation. Fungal infection involves epidermis, dermis as well as deeper layers of skin that require to customize the drug delivery in such a way which localize high drug concentrations at epidermis and dermis layers. However, the commercial topical formulation of LNZ (1%w/v cream LUZU®) is associated with lower skin permeation and shorter skin retention of drug. In recent years, the nano-carriers based topical formulation such as nanoparticle, nanoemulsion, lipid nanoparticle, nanocrystals etc. have gained a great importance as a potential drug carrier for topical delivery due to their unique advantages and great versatility as compared to conventional formulations.[5]

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Ample of approaches have been used to improve the solubility and dissolution rate of poorly water soluble drugs such as reduction of crystals size, conversion of drugs into prodrugs, use of amorphous forms, cosolvation and superdisintegrants, impregnating liquid drugs or drug solution in porous powders, using surface active self-emulsifying systems, micronization, formation of inclusion complexes with cyclodextrin, formation of amorphous drug, and formation of solid dispersion (SD) with hydrophilic carriers.[6]

Solid dispersions (SD) is now firmly established as a platform technology for the formulation of poorly soluble drugs.[7] SD is defined as dispersion of drug in an amorphous polymer matrix where the drug is preferably in the molecularly dispersed state.[8] These systems were defined as the dispersion of one or more active ingredients in an inert matrix in the solid state prepared by melting (fusion), solvent or melting solvent method with the goal of enhancing oral bioavailability.[9-10] SD attributed to faster carrier dissolution, releasing microcrystals or particles of drug. These SDs, which could be designated as first-generation SDs, were prepared using crystalline carriers. Crystalline carriers include urea and sugars, which were the first carriers to be employed in SDs.[11] They have the disadvantage of forming crystalline SDs, which were more thermodynamically stable and did not release the drug as quickly as the amorphous ones. Various methods were employed for preparation of SDs, namely, solvent evaporation method, melt extrusion, spray drying, coprecipitation, fusion method, melting method, supercritical fluid methods, etc.[12] The aim of the current study was to develop and characterize SD of LNZ (LNZ-SD) to improve solubility and antifungal efficacy.

Materials and Methods

Materials:

Luliconazole was procured from Clearsynth India Pvt. Ltd. Mumbai, India. PVP-K90, PEG-6000 were procured from Evonik Industries, Mumbai, India, PVA was procured from Loba Chem. Pvt. Ltd, Mumbai, India. Polaxomer-407 was obtained from Unique Chemical kolhapur. All chemicals and ingredients used were of analytical grade.

Drug-Excipient Compatibility study

FTIR Spectroscopy

Bruker, Alpha-II FTIR spectrophotometer was used to record the FTIR spectra of LNZ, PEG-600 and LNZ-SD in a range of 4000 to 650 cm^{-1} frequency· Drug sample and polymer sample mixed with potassium bromide and FTIR spectra were taken. Shifting or disappearance of drug peak was studied.[13]

Differential Scanning Calorimetry (DSC)

DSC is commonly used calorimetric techniques employed to characterize the physical state of drug and solubility in the complex. DSCof LNZ, PEG-600 and LNZ-SD were recorded using a differential scanning calorimeter and were compared. 5 mg of sample was sealed in aluminum pans which are flat bottomed and heated at a temperature range of

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

100-300 °C at a rate of 10 ° k/min using alumina as a reference standard. [14]

X-Ray Diffraction

For characterization of crystalline state, the X-ray diffraction pattern of LNZ alone and in combination with PEG-600 and LNZ-SD were determined using X-ray diffractometer with a copper target, at a current of 20 MA and a voltage of 40 kV. The rate of the scanning was 0.30 °C /min. [15]

Preparation of Solid Dispersions

Solid dispersion of Luliconazole was prepared by melting and solvent method. The composition is shown in table No:1. In melting method the drug and carrier P-407:PEG-6000 in 50:50,60:40 and 80:20 ratioswere mixed in 1:1, 1:2, and 1:3 ratios in a china dish and heated on a paraffin bath. The mixture was poured on a tile and cooled. The resulted solidified mass was dried pulverised and passed through sieve # 100. In solvent evaporation method, the drug and carrier P-407: PEG-6000 ratios were mixed in 1:1, 1:2 and 1:3 ratios in methanol (Table 1). Solvent was removed by evaporation under reduced pressure. The mass was pulverised and passed through sieve # 100. [16]

Evaluation of LNZ-SD

Solubility Studies

Phase and saturation solubility studies were conducted as per the method described by Higuchi *et al*. The saturation solubility of drug, physical mixtures, and SD in distilled water and 0.1N HCl was determined by adding an excess of drug, physical mixture, and SD to 50 mL distilled water and 0.1N HCl in a conical flask (Glassco, Delhi, India) and were shaken on rotary shaker (Remi Pvt.Ltd, Mumbai, India) for 72 h at 37°C ± 0.5°C. The saturated solutions were filtered through a 0.45-µm membrane filter (Membrane Filter India Pvt, Ltd. India), suitably diluted with water, 0.1N HCl, and analysed using Shimadzu UV-1900 Shimadzu, Japan) spectrophotometer at 295 nm.[17]

Surface morphology of LNZ-SD

Surface morphology and topography was best studied by using scanning electron microscopy (SEM). Gold palladium was used to coat the developed sample of LNZ-SD under an argon atmosphere at room temperature and surface morphology was studied with SEM. [18]

Particle size determination

Optical microscopy is used for the particle size determination of LNZ-SDdeveloped formulations. The Optical microscope was fitted with an ocular micrometer and a stage micrometer. The eyepiece micrometer was calibrated. 50 particles were measured randomly by optical microscope for diameter. [19]

Dug content

Prepared LNZ-SDwas assayed spectrophotometrically at 295 nm for the drug content at the maximum wavelength with proper dilution of formulations taking ethanol: water (1:1) ratio as blank. [20].

In vitro drug release studies

The invitro dissolution studies were done to compare the rate of dissolution of LNZ-SD with that of pure drug Luliconazole The test was performed in USP paddle apparatus using 900 ml phosphate buffer solution at pH 7.4 and temperature 37+ 2 ^{0}C. [21]

Antifungal Activity by Disc Diffusion Method

Brain Heart Infusion agar media is used and bring agar plates to room temperature before use.

Using a loop or swab, transfer the colonies to the plates. Visually adjust turbidity with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardize the suspension with a photometric device.Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum. Swab entire surface of agar plate three times, rotating plates approximately 60 º between streaking to ensure even distribution. Avoid hitting sides of the Petri plate and creating aerosols. Allow the inoculated plate to stand for at least 3 minutes but no longer than 15 min. before making

Journal of Chemical Health Risks

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

wells. Prepare the stock solution weighing 10 mg of the compound and dissolve it in 1ml of DMSO.Take a hollow tube of 5 mm diameter, heat it. Press it on above inoculated Agar plate and remove it immediately by making a well in the plate. Likewise, make five well on each plate. Incubate plates within 15 min of compound application. Invert plates, and stack them no more than five high. Incubate for 18-24 h at 37 ºC in the incubator. Read plates only if the lawn of growth is confluent or nearly confluent. Measure the diameter of the inhibition zone to the nearest whole millimeter by holding the measuring device. [22]

MIC Test

9 dilutions of each drug have to be done with BHI for MIC. In the initial tube 20 microliter of drug was added into the 380microliter of BHI broth. For dilutions 200microliter of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10-1 dilution. From 10-1 diluted tube 200microliter was transferred to second tube to make 10-2 dilution. The serial dilution was repeated up to 10-9 dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth. In each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated for 24 hours and observed for turbidity. [23]

Stability study

Following ICH recommendations Q1A (R2), a stability study has been performed. It was kept in a stability chamber at a temperature of 40 ± 2 °C and 75 ± 5% RH for 90 days. Different parameters like drug content (%), cumulative drug release (% CDR) were examined at specified time intervals (1, 2 and 3 months). [24]

Results ad Discussion

3.1. Solubility Studies

Solubility studies were performed to identify suitable vehicles with maximum potential to soubise the drug and having good miscibility with each other which helps in minimizing the final volume of SD. The results of saturation solubility studies are given in Figure 1. The solubility of pure LNZin water and in 0.1N HCl was found to be 0.78 ± 0.05 mg/mL and 1.12 ± 0.36 mg/mL, respectively. The solubility LNZ-SDusing P-407, PEG-6000and PVPK-90 in the ratio (1:1, 1:2, and 1:3) was 37.54 ± 2.85 mg/mL, 25.54 ±2.12µg/mL, and 18.25 ± 1.58 mg/mL, respectively, in water and 44.38 ± 3.15 mg/mL, 31.87 ± 3.58 mg/mL, and 25.19 ± 2.91 mg/mL, respectively, in HCl. These results revealed that solubility of LNZ was enhanced with SD using P-407:PEG-6000 ratio at 80:20 [25]

Compatibilities Studies:

LNZ, P-407 and LNZ-SD were analyzed by infrared spectroscopy for knowing purity and to characterize the probable structural modification of the drug sample. The sample was analyzed in the region of 4000 and 400 cm-1. FTIR of LNZ(Fig.1A) shows specific peaks related to specific structural features as follows 3257.7 &3116.1 cm-1 (Aromatic C-H Stretch), 2922.2 (C-H aliphatic stretch), 2527 & 2614cm-1 (S-H Stretch), 2199.08 (C N Stretch), 1994.1,1904.7 &1736.4 (C=C Alkene Stretch), 1647.5 cm-1 (C=N Stretch), 1599.0 cm-1 (C=C Aromatic Stretch) and 887.1& 1196.5 cm-1 (C-Cl Stretch)whose presence resembled the structure of LNZ.The values are near or equal to values mentioned in standard structure of luliconazole. FTIR spectra of LNZ-SD(Fig.1C) formulation characteristic peaks as follows, 3306.1 cm-1 (Aromatic C-H Stretch),2922.2 (C-H aliphatic stretch), 2340.1 cm-1 (S-H Stretch), 2117.1 (C N Stretch), 1849.3 &1736.9 cm-1(C=C Alkene Stretch), 1502.1 cm-1 (C=N Stretch), 1461.1 cm-1 (C=C Aromatic Stretch) and 872.2& 1140.6 cm-1 (C-Cl Stretch).It revealed that the fundamental peaks of the LNZ are retained in the SD formulation. Results showed that there exist no chemical interaction between LNZ and excipients used and were found to be compatible. [26]

Journal of Chemical Health Risks

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

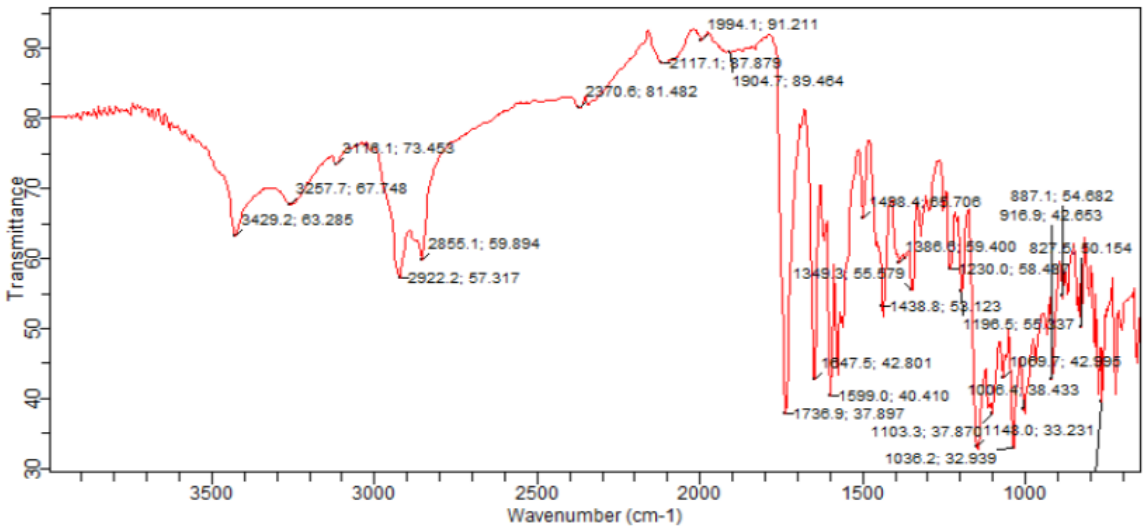

Fig.1A: FTIR Spectra of Luliconazole

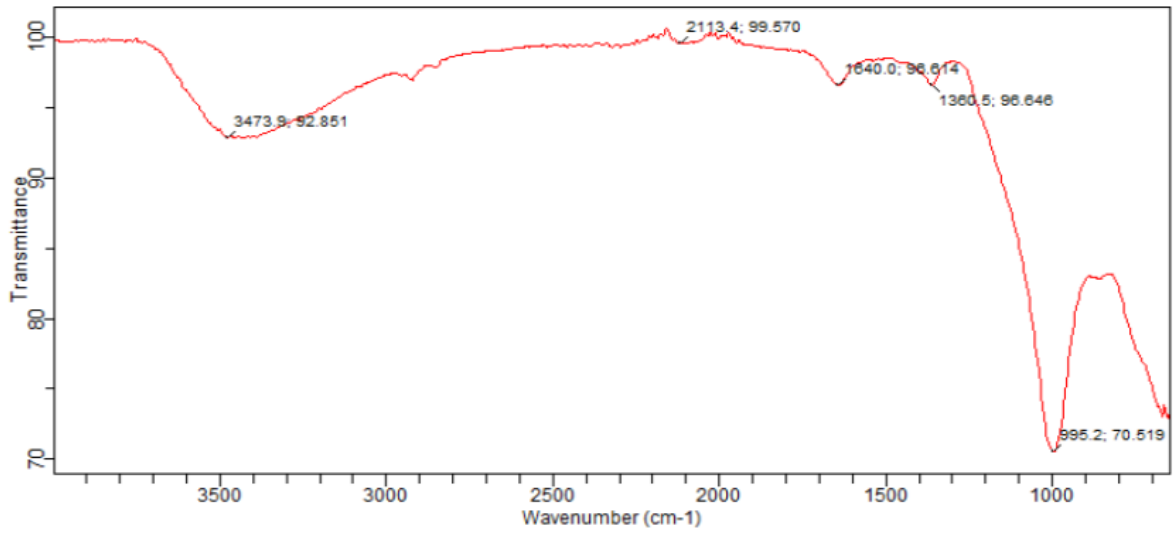

Fig.1B: FTIR Spectra of P-407

Journal of Chemical Health Risks

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

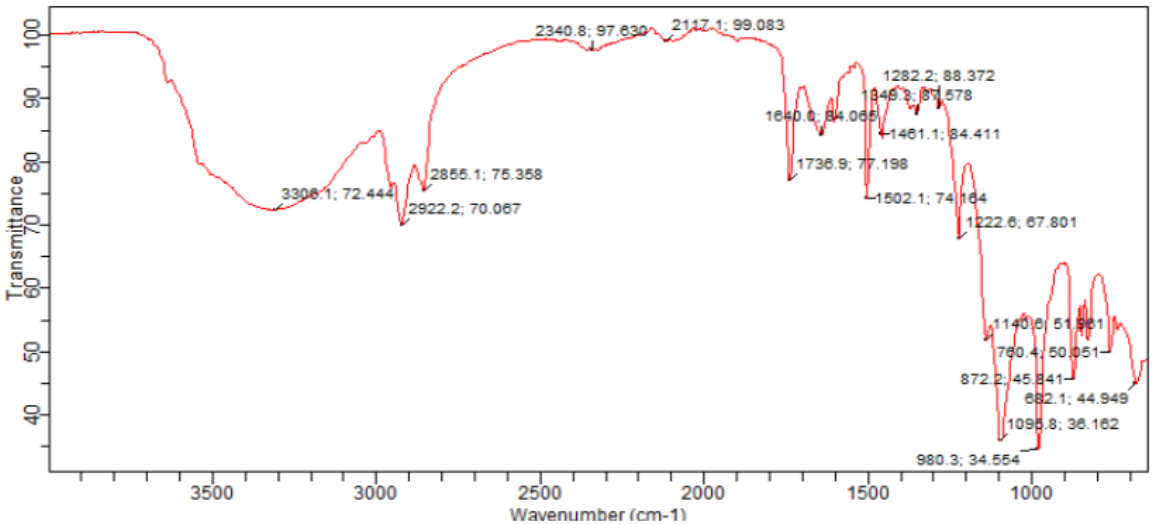

Fig.1C: FTIR Spectra of Optimized LNZ-SD-7

DSC Studies

To evaluate the samples' melting behavior and crystallinity, the DSC investigation was performed. The DSC thermograms of LNZ, P-407 and LNZ-SD are shown in Fig.2. The DSC thermogram of plain LNZ (Fig.2A), showed peaks corresponding to LNZ at 152.74 °C andP-407 (Fig.2B) showed peaks at 92.54°C. On the other hand, the DSC thermogram of LNZ-SD (Fig.2C), an endothermic peak observed at 97.24°C which might be corresponds to P-407. This indicates that the LNZ incorporated in the solid dispersion in a non-crystalline state which signifies that LNZ might be molecularly dispersed in SD or in changed to its amorphous state. Additionally, it also suggests complete entrapment of LNZ within the lipid matrix of the SD

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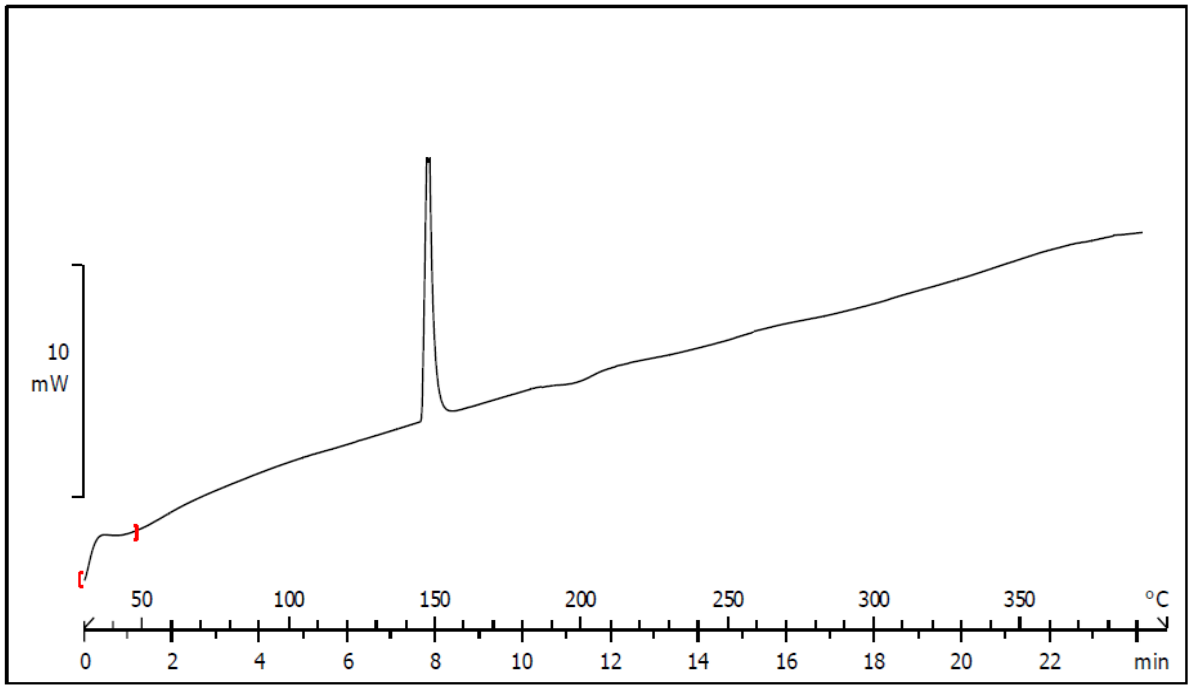

Fig.2A: DSC Thermogram of Luliconazole

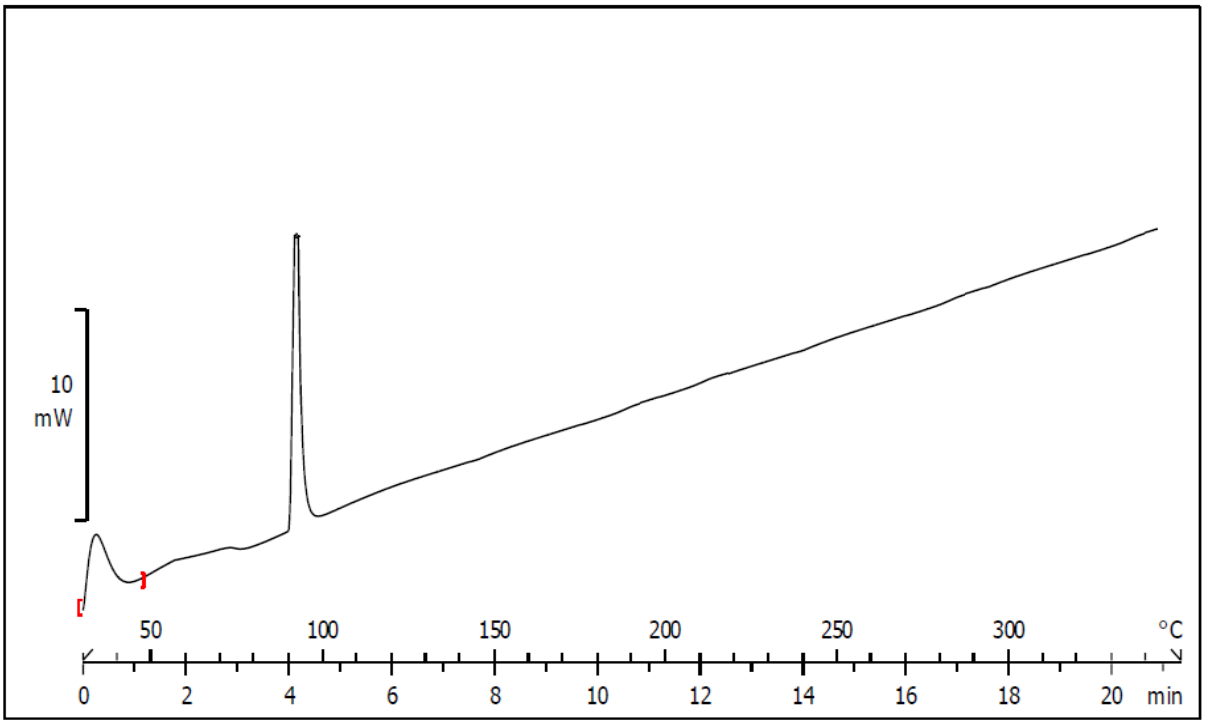

Fig.2B: DSC Thermogram of P-407

Journal of Chemical Health Risks

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

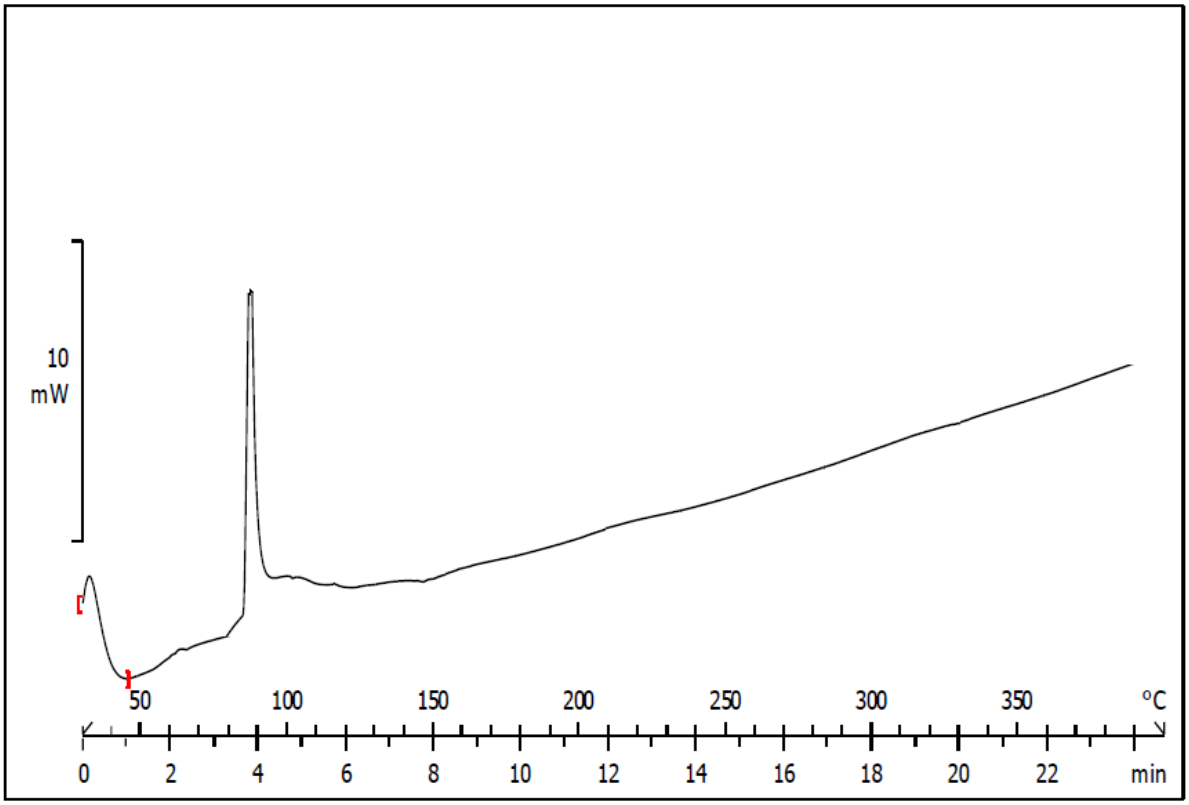

Fig.2C: DSC Thermogram of Optimized LNZ-SD-7

XRD Studies:

X-ray diffraction studies were carried out to confirm the physical state of LNZ-SDin comparison to LNZand P-407. It is clear that the diffractogram of the LNZ (Fig.3A)exhibited characteristic intensity sharp peak reflections, indicating its crystalline nature. However, these characteristic peaks disappeared in the X-ray diffraction pattern of LNZ-SD(Fig.3C). Results conformed molecular dispensability ofLNZ in to solid dispersion from crystalline to amorphous form. [27]

Journal of Chemical Health Risks

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

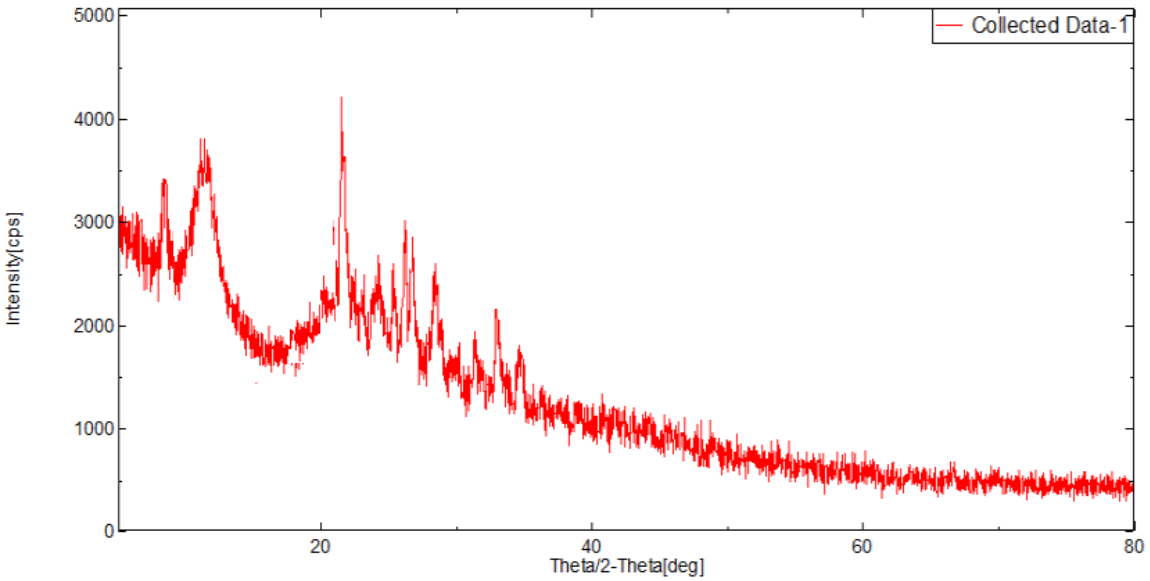

Fig.3A: XRD Spectra of LNZ

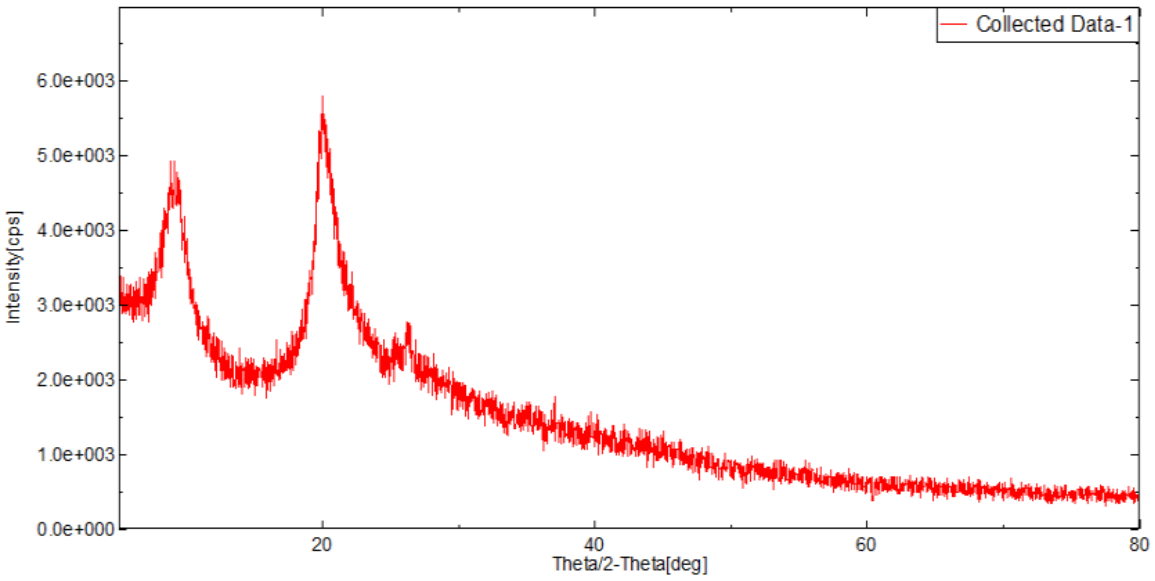

Fig 3B: XRD Spectra of P-407

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

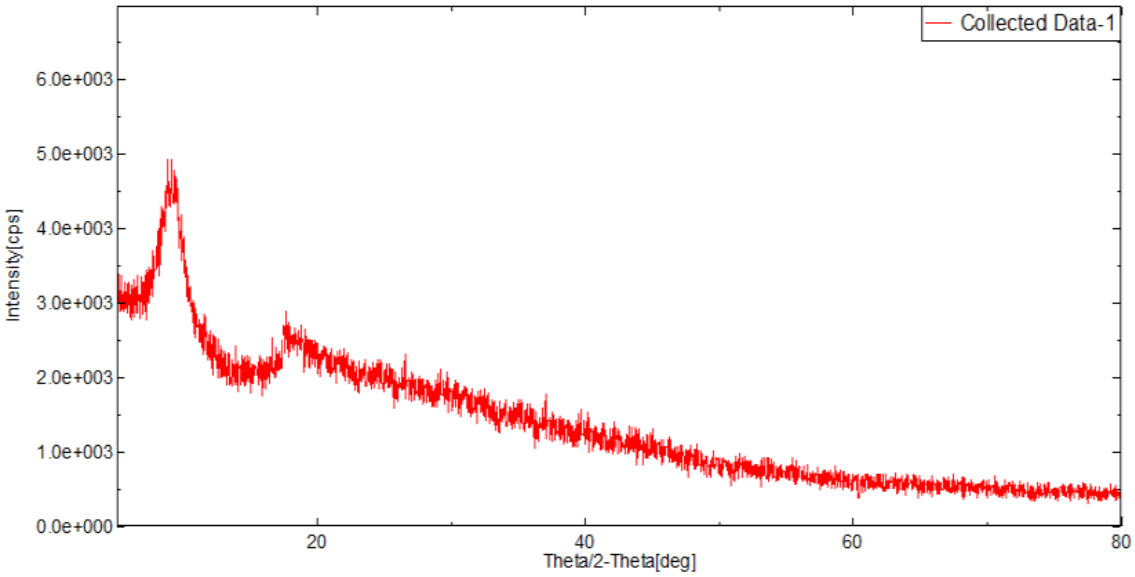

Fig 3C: XRD Spectra of Optimized LNZ-SD-7

Experimental design

A two-factor, three level (3^2) full factorial design was employed for the optimization of Luliconazole solid dispersions (LNZ-SD) using Design Expert® software (Version 7.0.0, Stat-Ease Inc., USA). The effect of two independent variables namely polymer ratio (X_1) and drug to polymer ratio (X_2) on the two dependent variables viz., percentage cumulative drug release, and percentage drug contentwere studied at three levels each i.e., low(-1), medium (0) and high (+1). A total of 9 experimental runs generated with levels as per the Design Expert® software are shown in Table 1. All the prepared formulations as per the design were investigated for percentage cumulative drug release (Y_1)and drug content (Y_2) which was designated as response or dependent variables. The observed responses were simultaneously fitted into various mathematical models (i.e. linear, two factor interaction (2FI), quadratic and cubic). Analysis of variance (ANOVA) was used to determine the statistical significance of the generated model and model terms. The 3D- response surface plots, 2D-contour plots and perturbation graphs generated by the Design Expert® software were used to understand the relationship between the independent and dependent or response variables.[28]

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

Table 1. Full factorial Design matrix summarizing the levels, factors, and responses of 09 runs for optimization of Luliconazole Solid Dispersion (LNZ-SD)

Run	Block	Factor 1 Polymer	Factor 2 (X2) (Drug: Polymer Ratio)	Cumulative Drug Release (Y1)	Drug Content (Y2)
1	LNZ-SD-1	50:50 (-1)	1:1 (-1)	79.51±3.15	89.23±3.57
2	LNZ-SD-2	60:40 (0)	1:1 (-1)	77.35±4.85	85.45±3.57
3	LNZ-SD-3	80:20 (1)	1:1 (-1)	74.12±3.25	87.75±2.19
4	LNZ-SD -4	50:50 (-1)	1:2 (0)	87.65±2.12	92.45±3.78
5	LNZ-SD-5	60:40 (0)	1:2 (0)	84.31±3.45	88.75±3.52
6	LNZ-SD-6	80:20 (1)	1:2 (0)	82.42±3.72	91.84±2.54
7	LNZ-SD-7	50:50 (-1)	1:3 (1)	99.35±2.54	98.24±2.18
8	LNZ-SD-8	60:40 (0)	1:3 (1)	92.12±2.85	93.14±2.74
9	LNZ-SD-9	80:20 (1)	1:3 (1)	94.15±2.12	94.25±2.37

Factor	**Levels used, actual (coded)**		
	Low (-1)	Medium (0)	High (+1)
Independent variables			
Factor 1 (X_1) (P-407: PEG-6000)	50:50	60:40	80:20
Factor 2 (X_2) (Drug: Polymer)	1:1	1:2	1:3
Dependent variables			
Drug Release (Y_1)	**Maximize**		
Drug content (Y_2)	**Maximize**		

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

Fitting of data into the model:

In order to find out the best fit model, all the observed responses obtained for 9 formulations were concurrently fit into different mathematical models using Design-Expert® software. The selection of best fit model was based on the high values of multiple correlation coefficients (R^2), adjusted R^2, and predicted R^2 values and low values of standard deviation (SD), coefficients of variation (% CV), and predicted residual sums of the square (PRESS) (Table 2). The PRESS value indicates how well the model fits the data. The PRESS value for the chosen model should be small in comparison to the other models. The best fit model for all the three responses was quadratic model.

Table 2. Regression analysis results obtained for various responses Y_1 (% cumulative drug release) and Y_2 (drug content) of LNZ-SD for fitting to different models

Models	SD	R^2	Adjusted R^2	Predicted R^2	PRESS	CV (%)	Remark
Response (Y_1)							
Linear	1.74	0.9673	0.9563	0.9204	44.36	2.04	Suggested
2FI	1.91	0.9673	0.9476	0.8138	103.83	2.23	
Quadratic	1.80	0.9827	0.9537	0.7952	114.21	2.10	
Cubic	0.88	0.9986	0.9888	0.7459	141.67	1.03	
Response (Y_2)							
Linear	1.98	0.8027	0.7369	0.5753	50.72	2.17	
2FI	2.10	0.8158	0.7053	0.2648	87.81	2.30	
Quadratic	0.72	0.9870	0.9653	0.8433	18.71	0.79	Suggested
Cubic	0.21	0.9996	0.9969	0.9295	8.42	0.24	

SD: standard deviation, R^2: multiple correlation coefficient, 2FI:two factor interaction, PRESS:predicted residual sum of square, CV:coefficient of variation.

Effect of independent variables on % cumulative drug release (Y_1) of LNZ-SD:

The % cumulative drug release (Y1) of LNZ-SD formulations are shown in table 1. The % cumulative drug release values were found in the range of 74.12 to 99.35 %.The effect of the independentvariables on the % cumulative drug release can be described bythe following linear equation.

$$Y_1 = + 85.66 - 2.64X_1 + 9.11X_2 \quad (1)$$

Where Y_1 is % cumulative drug release, X_1 is the ratio of polymers and X_2 is the ratio of drug to polymer. The equation indicates that ratio of polymers has a negative effect and ratio of drug to polymer has a positive effect on the % cumulative drug release. This demonstrates that % cumulative drug release of LNZ-SD decreases with increase in the ratio of polymers while it increases with increase in the ratio of drug to polymer. The high coefficient value of X_2 specify that theratio of drug to polymer has more prominent effect on the % cumulative drug release of LNZ-SD compared to that of ratio of polymers. The ANOVA results for the %cumulative drug release data are shown in Table 3. The Model F-value of 88.61 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. A p value (Prob > F) less than 0.05 signifies that the model parameter is statistically significant. In this case, X_1, and X_2 parameters are significant. The "Pred R-Squared" of 0.9204 is in reasonable agreement with the "Adj R-Squared" of 0.9563."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 23.320 indicates an adequate signal. This model can be used to navigate the design space. (Table 2).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

The effects of the independent variables onthe % cumulative drug release are represented by (Figure 4(A)) and their corresponding 2D- contourplots (Figure. 4(B)). It is noticeable from the graphs and the plots that the %cumulative drug releasedecreases with increase in the ratio of polymers while it increases with increase in the ratio of drug to polymer. The perturbation plot (Figure 4(C))for % cumulative drug release also supported these observations.The perturbation graph exhibits a steep slope for factor B (ratio of drug to polymer) compared to factor A (ratio of polymers) representing that the ratio of drug to polymer was a significant factor controlling the % cumulative drug release.[29]

Table 3. ANOVA results for various responses of LNZ-SD

Source	Responses					
	Y_1 (% Cumulative drug release)			Y_2 (drug content %)		
	F-value	p-value Prob > F	Adequacy precision	F-value	p-value Prob > F	Adequacy precision
Model	88.61	< 0.0001	23.32	45.55	0.0050	21.37
X_1	13.71	< 0.0001		11.90	0.0409	
X_2	163.52	< 0.0001		173.31	0.0009	
X_1X_2				3.04	0.1794	
X_1^2				39.07	0.0083	
X_2^2				0.42	0.5628	

X_1, and X_2 are coded terms for independent variables; X_1X_2 interaction terms; X_1^2 and X_2^2 are quadratic terms

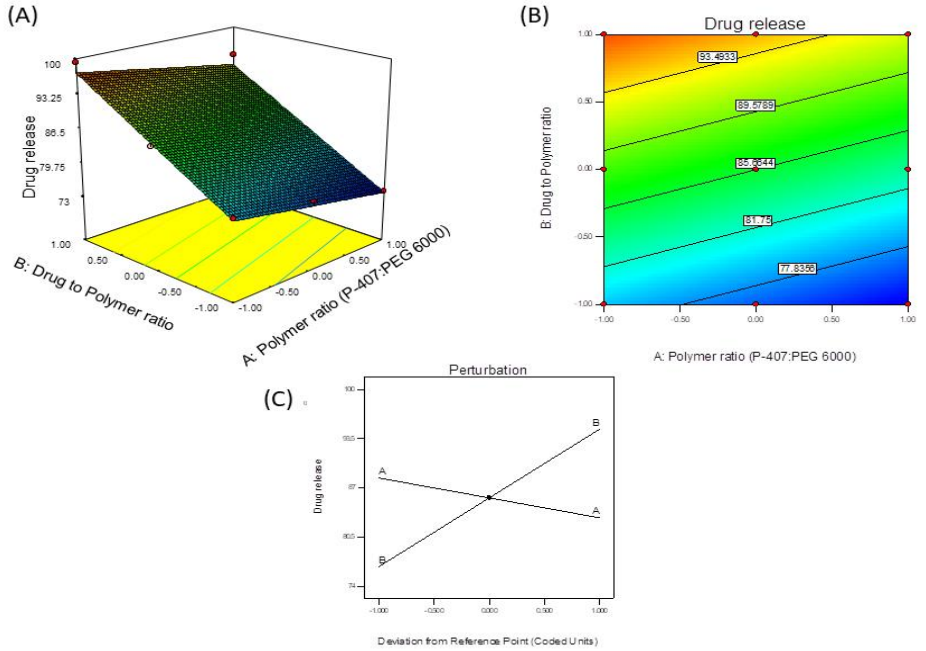

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Figure 4. 3D-Response surface (A), 2D-contour (B) and perturbation plots (C) showing the effect of ratio of polymers and the ratio of drug to polymer on % cumulative drug release (Y1) of LNZ-SD

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Effect of independent variables on % drug content (Y_2) of LNZ-SD:

The % drug content (Y2) of LNZ-SD formulations are shown in table 1. The % drug content values were found in the range of 85.45 to 98.24 %. The effect of the independent variables on the % drug content can be described by the following quadratic equation.

$$Y_2 = + 88.89 - 1.01\ X_1 + 3.87\ X_2 - 0.63X_1X_2 + 3.18\ X_1^2 + 0.33\ X_2^2 \quad (2)$$

Where Y_2 is % drug content, X_1 is the ratio of polymers and X_2 is the ratio of drug to polymer. The equation indicates that ratio of polymers has a negative effect and ratio of drug to polymer has a positive effect on the % drug content. This demonstrates that % drug content of LNZ-SD decreases with increase in the ratio of polymers while it increases with increase in the ratio of drug to polymer. The high coefficient value of X_2 specify that the ratio of drug to polymer has more prominent effect on the % drug content of LNZ-SD compared to that of ratio of polymers. The ANOVA results for the % drug content data are shown in Table 3. The Model F-value of 45.55 implies the model is significant. There is only a 0.50% chance that a "Model F-Value" this large could occur due to noise. A p value (Prob > F) less than 0.05 signifies that the model parameter is statistically significant. In this case, X_1, X_2 and X_1^2parameters are significant. The "Pred R-Squared" of 0.8433 is in reasonable agreement with the "Adj R-Squared" of 0.9653. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 21.371 indicates an adequate signal. This model can be used to navigate the design space (Table 2).

The effects of the independent variables on the % drug content are represented by (Figure 5(A)) and their corresponding 2D- contour plots (Figure. 5(B)). It is noticeable from the graphs and the plots that the % drug content decreases with increase in the ratio of polymers while it increases with increase in the ratio of drug to polymer. The perturbation plot (Figure 5(C)) for % drug content also supported these observations. The perturbation graph exhibits a steep slope for factor B (ratio of drug to polymer) compared to factor A (ratio of polymers) representing that the ratio of drug to polymer was a significant factor controlling the % drug content.[30-31]

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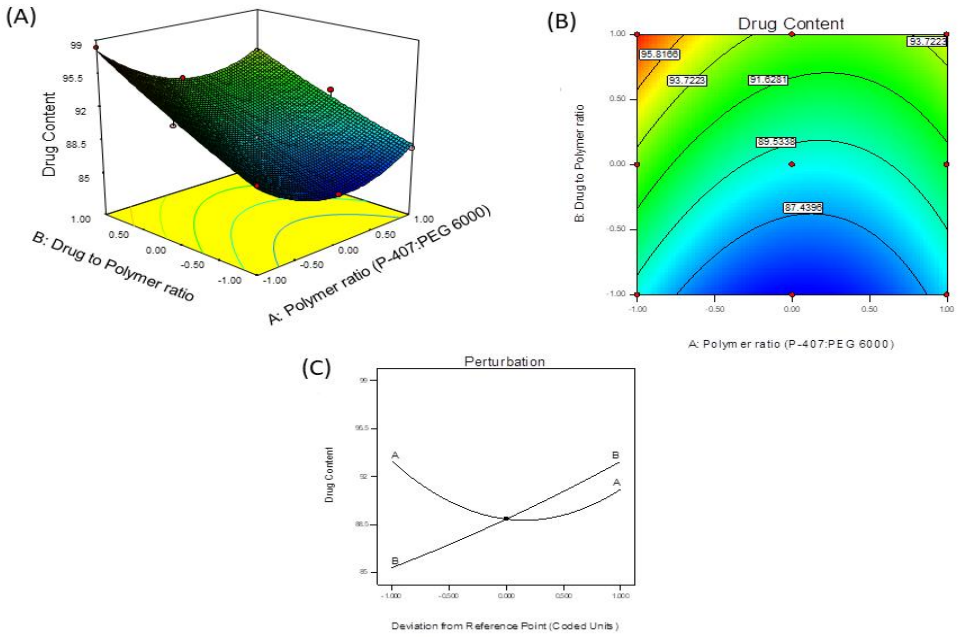

Figure 5. 3D-Response surface (A), 2D-contour (B) and perturbation plots (C) showing the effect of ratio of polymers and the ratio of drug to polymer on % drug content (Y1) of LNZ-SD.

Table 4. Validation of optimized formulation

Response	Predicted value	Observed value	Prediction error (%)
% CDR	97.40	98.24	-0.86
% Drug content	97.91	99.12	-1.23

% Prediction error = Predicted value – observed value X 100

Predicted value

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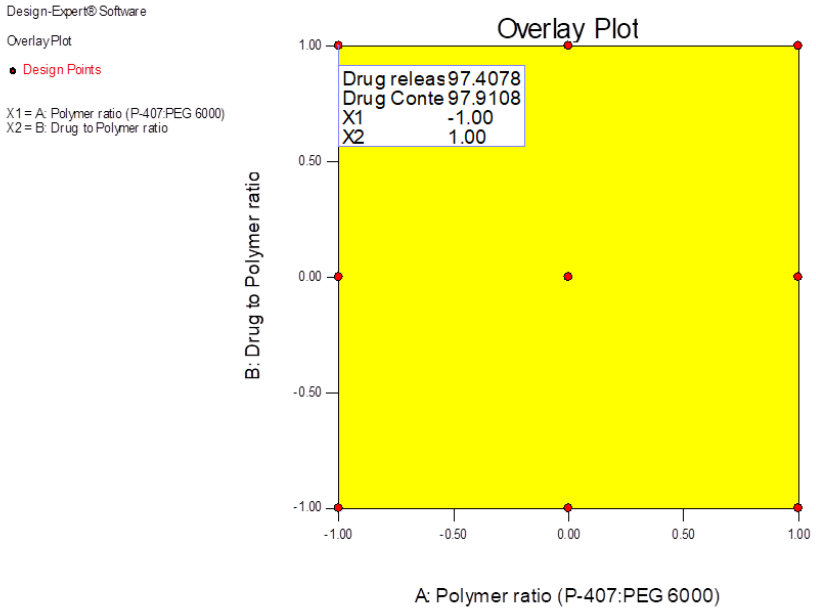

Figure 5D: The overlay plot displaying the optimized LNZ-SD formulation in the design space and their predicted response values.

Scanning Electron Microscopy (SEM) of LNZ-SD

Scanning electron microscopy was used to determine surface morphology. SEM of **LNZ-SD**formulation showed that the surface was smooth with devoid cracks having spherical in shape. The SEM of **LNZ-SD** shown in Fig.6

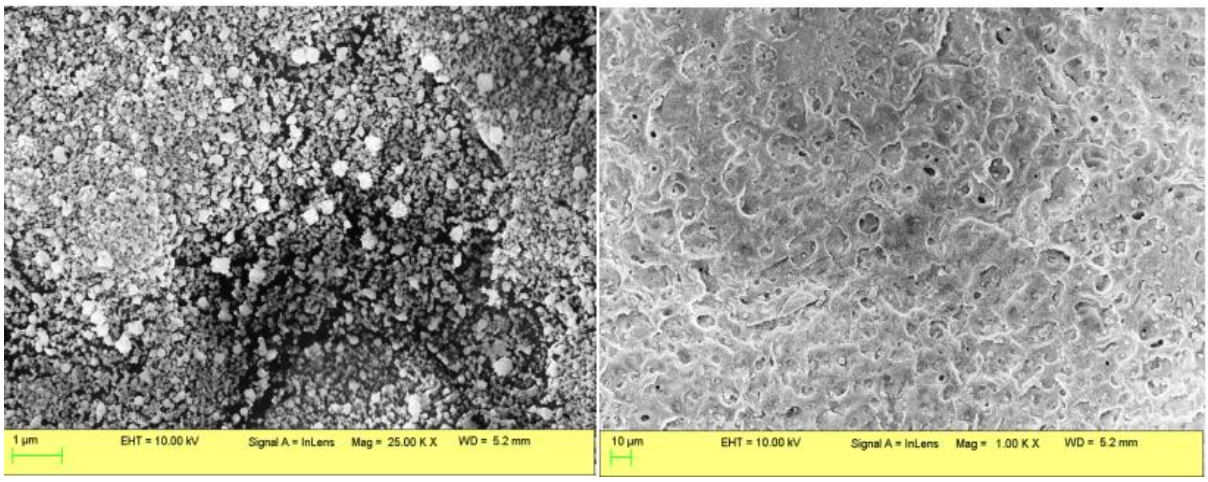

Fig. 6: SEM of LNZ-SD Optimized formulation

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

Particle Size and Zeta Potential

Particle size and zeta potential of Optimized **LNZ-SD**-7 formulation was found to be 50.93µm and -18.8 mVhaving PDI 0. 567.Results were shown in Fig.7.

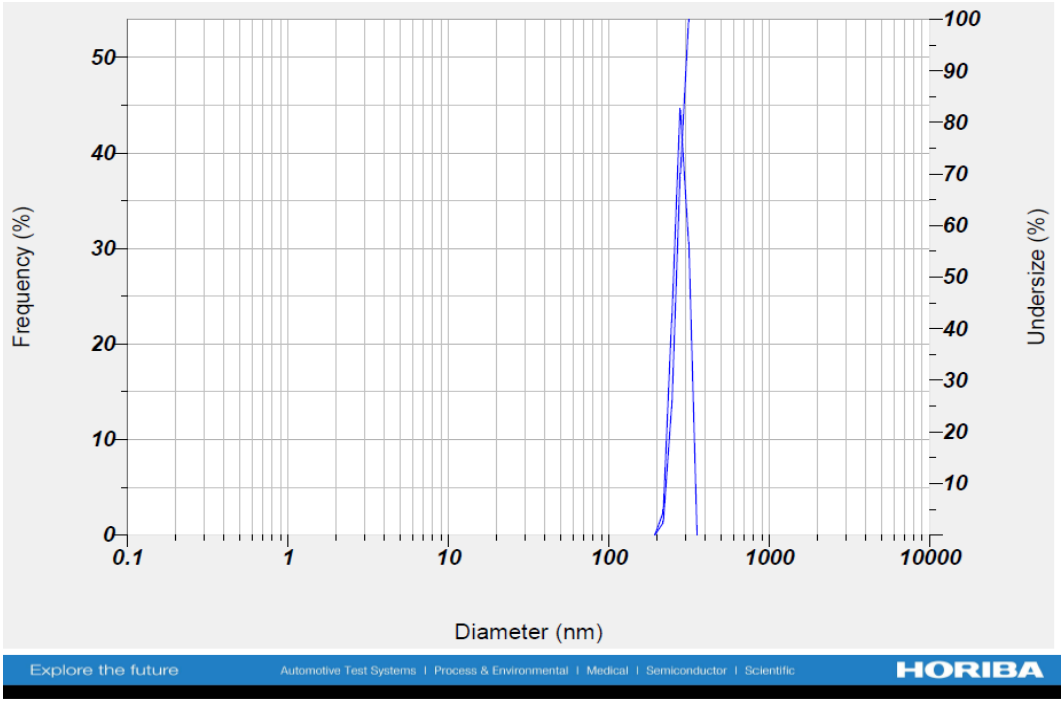

Fig. 7A: Particle size of LNZ-SD Optimized formulation

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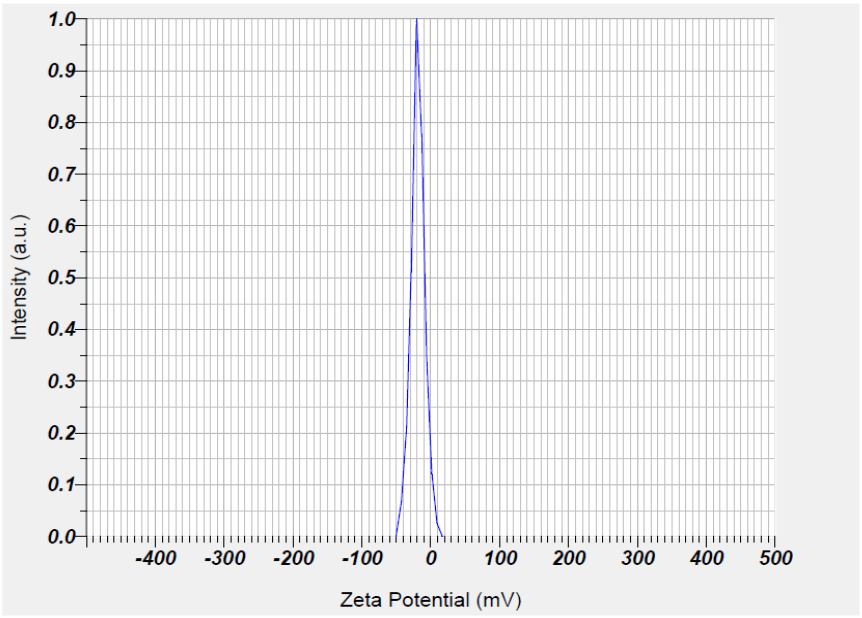

Fig. 7B: Zeta Potential ofLNZ-SD Optimized formulation

Drug Release profile:

In-vitro dissolution of **LNZ** was studied using phosphate buffer (pH 6.8) as dissolution medium. Enhanced dissolution rate of **LNZ** was attained in all cases. Dissolution rate of the prepared SD significantly exceeded the plain drug LNZ (Table 1). Plain drug showed significantly sluggish and incomplete dissolution (34.68±2.57%) within the total dissolution time (one hour (h)). However, dissolution of all systems almost reached completion at the end of dissolution time. The maximum and minimum drug dissolved were found to be for LNZ-SD-7 and NSD-SD-2 99.35±2.54and 74.12±3.25 respectively after 60 minutes. Results showed that the highest dissolution was achieved from systems prepared at 1:3 (w/w) D:P ratio, whereas the least dissolution was obtained from systems prepared at 1:1 (w/w) D:P ratio. Also results revealed that P-407 based SD systems displayed superior dissolution rates compared to other prepared systems. Improved dissolution rate of LNZ within the prepared systems can be attributed to several factors including incorporation of strongly hydrophilic polymers to enhance drug's wettability. Enhanced wetting properties of hydrophobic LNZ resulted in localized enhancement of its solubility within the diffusion layer surrounding drug particles. Similar results were obtained by El-nawawy et al. in their study on olmesartan solid dispersions. Finally, amorphous form of LNZ requires low energy to be dissolved where, reduced particle size increased surface area of drug particles subjected to dissolution medium. All these factors could contribute to enhanced dissolution profiles of LNZ within the prepared LNZ-SD systems. [32-33]

Drug Content

The mean percent of drug content ranged from 85.45±3.57 to 98.24±2.18 indicating as drug polymer ratio increases drug content also increases. Results are listed in Table 1. It means the drug and polymer has positive impact of drug content and vice versa. The maximum and minimum drug content were recorded by and NSD-SD-7 and NSD-SD-2 98.24±2.18 and 85.45±3.57 respectively. [34]

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JCHR (2023) 13(5), 646-671 | ISSN:2251-6727

Antifungal Activity of SD and LNZ-SD

Table 5: Minimum Inhibitory Concertation of SD and LNZ-SD

Candida albicans	**100 µlml**	**50 µl/ml**	**25 µl/ml**	**12.5 µlml**	**6.25 µl/ml**	**3.12 µl/ml**	**1.6 µl/ml**	**0.8 µl/ml**	**0.4 µl/ml**	**0.2 µl/ml**
LNZ	S	S	S	S	R	R	R	R	R	**R**
LNZ-SD	S	S	S	S	S	S	S	S	S	**R**
A. Niger										
LNZ	S	S	S	R	R	R	R	R	R	**R**
LNZ-SD	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**

Table 6: Results of Minimum Bactericidal concentration of SD and LNZ-SD

Candida Albicans	**100 µl/ml**	**50µl/ ml**	**25µl/ ml**	**12.5 µl/ml**	**6.25 µl/ml**	**3.12 µl/ml**	**1.6 µl/ml**	**0.8 µl/ml**	**0.4 µl/ml**	**0.2 µl/ml**
LNZ	NG	NG	NG	21	27	38	53	74	90	**98**
LNZ-SD	NG	NG	NG	NG	NG	NG	NG	NG	53	**97**
A.Niger										
LNZ	NG	NG	52	32	43	57	63	78	84	**99**
LNZ-SD	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**51**	**62**	**79**	**96**

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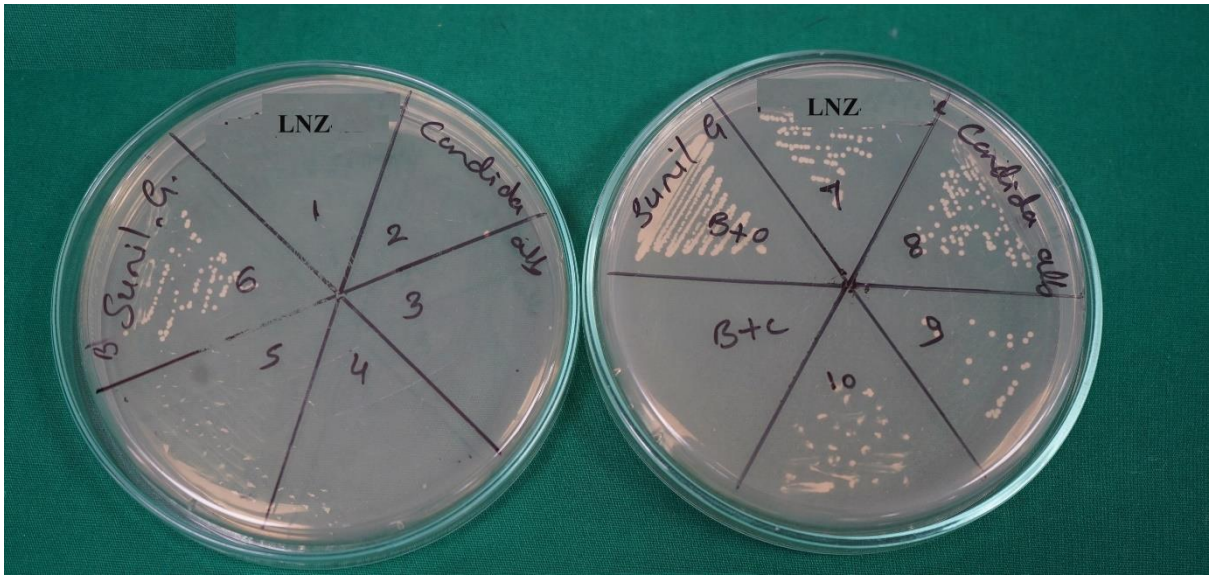

Fig.8A: Antifungal Activity of LNZ *on Candida albicans*

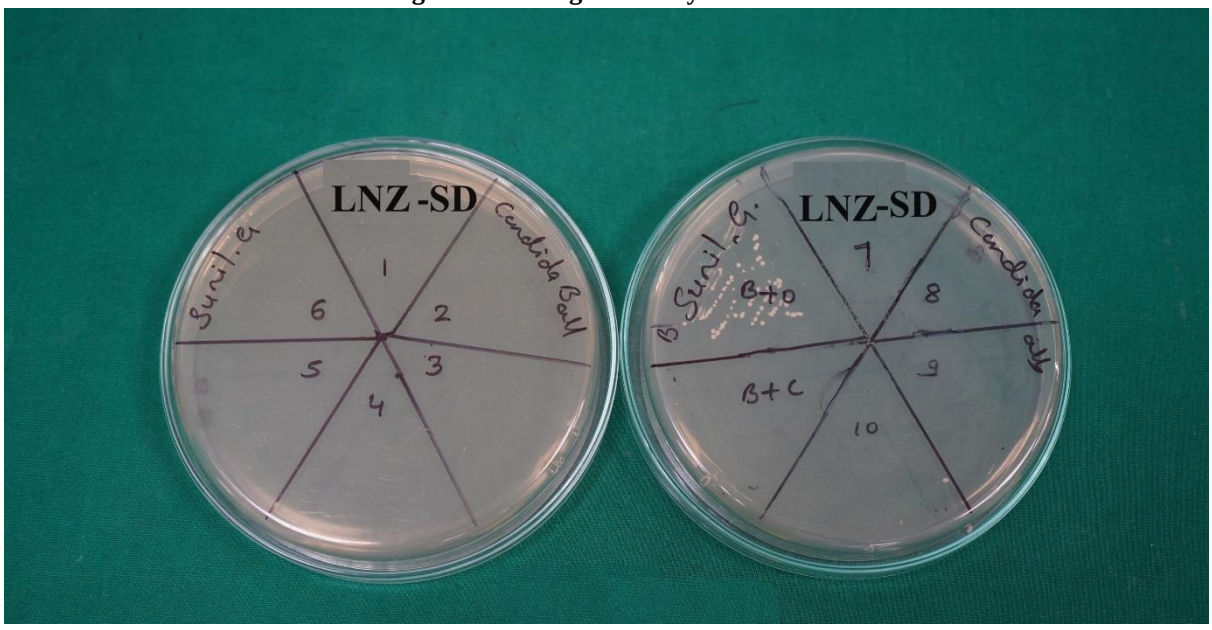

Fig.8B: Antifungal Activity of LNZ-SD *on Candida albicans*

MIC Results (Tube Dilution Method)

Minimum inhibitory concentration required to inhibit visual growth of an microorganism by drug is called MIC.MIC results showed that both organisms are sensitive at very low concentration of **LNZ-SD** when compared to pure LNZ.[35-36]Antifungal studies conformed that LNZ-SDhave better efficacy in treating *Candida albicans* having *MIC*-0.4µg $ml^{-1}$and MBC 0.8µg ml^{-1}compared to A.Niger having MIC*MIC*-1.6 µg $ml^{-1}$and MBC 3.2 µg ml^{-1}

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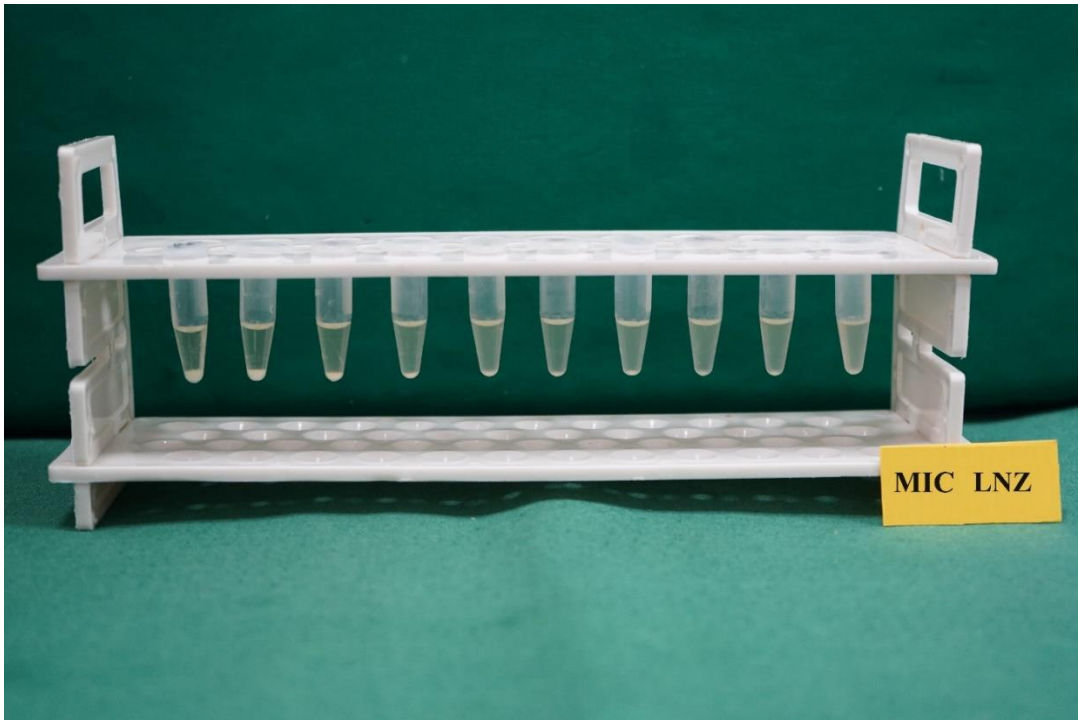

Fig.9A: Antifungal Activity of LNZ *on Candida albicans* by Tube Dilution Method

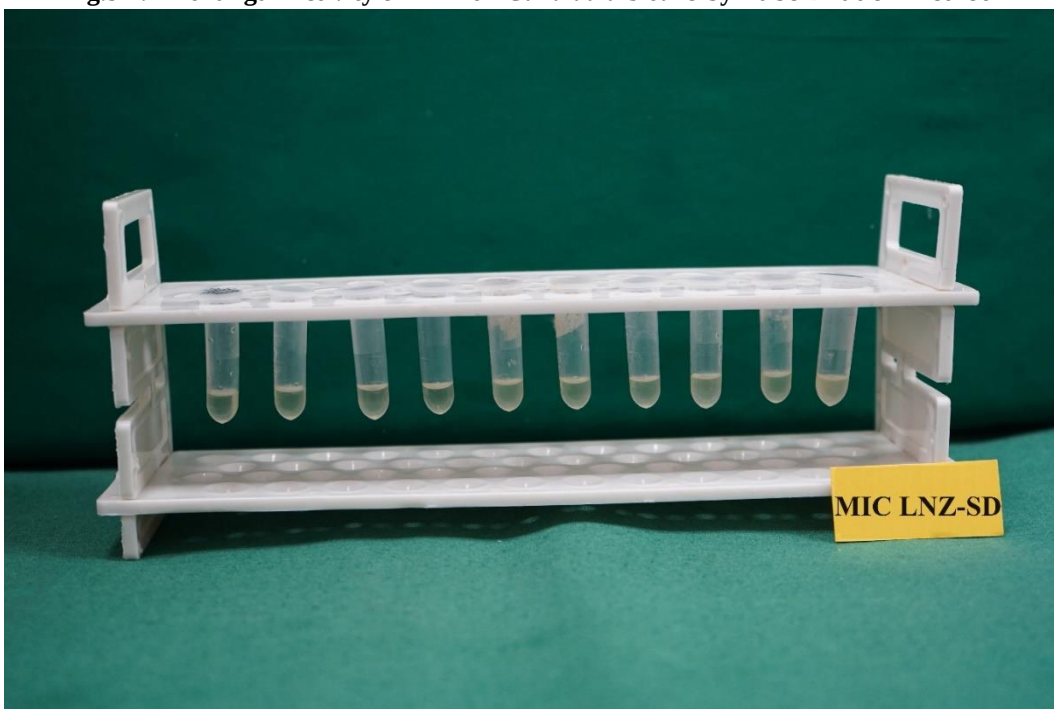

Fig.9B: Antifungal Activity of LNZ-SD on *A.Niger* by Tube Dilution Method

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Antifungal activity by Disc Diffusion Method: In-Vitro antifungal activity of LNZ and optimized LNZ-SD formulation carried out by disc diffusion method. Fungus species used for biological evaluation were *Candida albicans* and *A.niger*. Antifungal activity of LNZ and LNZ-SD was shown in Table 7 and Fig.10Results showed that LNZ-SD showed greater zone of inhibition on *C. albicans* (15mm) as compared to *A.niger*(9 mm) [37-38]

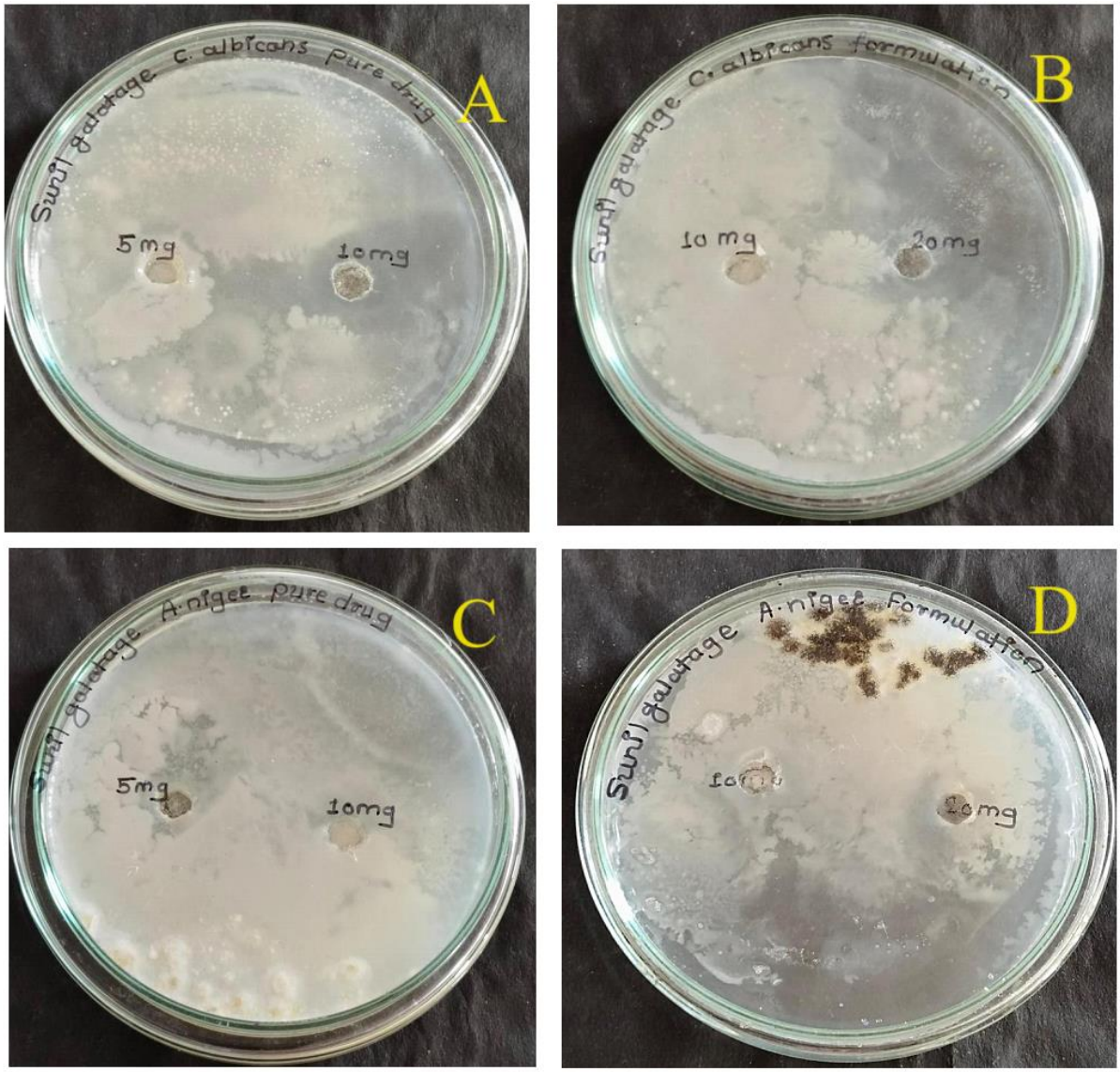

Fig. 10: Antifungal activity of (*A)* LNZ *on C. albicans.* (B) LNZ-SD on *C.albicans* (C) LNZ *on A.* niger. (D) LNZ-SD *on A. niger* by Zone of Inhibition Method

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Table 7: Antifungal activity of LNZ and LNZ-SD (Zone of Inhibition Method)

Sr.No	Sample	Concentration (mg/ml)	Zone of inhibition in mm *C. albicans*	Zone of inhibition in mm *niger*
1	**LNZ**	5	01	02
2		10	02	04
3	**LNZ-DSD powder equivalent to 5& 10 mg LNZ**	10	12	06
4		20	15	09

Stability Study

Stability studies were conducted on optimized formulation. The sample was stability studies conducted for 90 days. To determine how storage conditions affected drug content and cumulative drug releaseof **LNZ-SD**, stability study was conducted.[39-40] The properties of LNZ-SDdid not change significantly ($p>0.5$) during storage. Results were shown in table 8.

Table 8: Accelerated Stability Study Data of optimized LNZ-SD

Parameter	At temp. 40°C ±0.5°Cand RH75+5%			
	Initial	**1st Month**	**2nd Month**	**3rd Month**
% CDR	99.54±0.67	99.51+0.87	99.45±0.74	99.35±0.81
% Drug Content	98.51±1.54	98.37+1.12	98.24 ±1.28	98.20±1.37

Conclusions

From the results it is concluded that LNZ-SDwas prepared by using different polymers such as P-407: PEG-6000 ration from 50:50 to 80:20 increases which enhances solubility and cumulative drug release of LNZ in the form of LNZ-SD. The in-vitro drug release study indicates that the release of LNZ from LNZ-SDformulations containing a varying concentration of polymer was inversely proportional. The higher release rate was found from prepared LNZ-SDfrom the lower concentration of polymer. SEM revealed that the surface of LNZ-SDwas smooth and with ideal surface morphology. In vitro antifungal studies conformed that LNZ-SDhave better efficacy in treating *Candida albicans* as compared to *Aspergillus niger*. From the results we may conclude that formulations LNZ-SD-*7* of luliconazole solid dispersion can be used as competent alternative to treat fungal infection caused by *Candida albicans* as compared and *Aspergillus niger*. However; further *in vivo* studies are required to establish its efficacy against fungal infections.

CONFLICT OF INTEREST:

All authors declare that they have no competing interests

ACKNOWLEDGMENT

The authors are thankful to B. R. Nahata College of Pharmacy, Faculty of Pharmacy, Mandsaur University, Mandsaur. Authors are also thankful to Diya Labs Mumbai

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for Characterization of formulations and Biocyte Sangli (BIRD) for antifungal activity.

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