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FORMULATION, OPTIMIZATION OF ITRACONAZOLE NANOSUSPENSION AND ITS DISSOLUTION PROFILE COMPARISON WITH MARKETED FORMULATION

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Abstract: A considerable portion (40%) of drugs fail full development, because of Poor water solubility of drug molecules, insufficient bioavailability, fluctuating plasma levels and high food dependency. Itraconazole is one of such agent, a poorly water soluble drug indicates insufficient bioavailability following oral administration. The purpose of the present study was to formulate and optimize Itraconazole nanosuspension. In the present work Itraconazole nanosuspension was prepared by pearl milling technique using zirconium oxide beads as a milling media. A series of polymers and surfactants were screened and finally HPC-EF as a polymer and SDS were finalized. To optimize formulation parameters, 3^3 factorial design was adopted. Effects of various process parameters like, stirring time and the ratio of the beads were optimized using final formulation. The optimized nanosuspension was lyophilized using mannitol (1:1 ratio) as a cryoprotectant. Nanosuspension was characterized by particle size and size distribution, drug content, scanning electron microscopy, differential scanning colorimetry and its dissolution profile was compared with marketed product. Optimized nanosuspension showed spherical shape with surface oriented surfactant molecules and a mean particle diameter of 475 nm. There was no significant change in crystalline nature after formulation and it was found to be chemically stable with high drug content. The in vitro dissolution profile of the optimized formulation compared to the pure drug and marketed formulation (Canditral Capsule) by using 0.1N Hydrochloric acid as release medium showed higher drug release.

Keywords: Nanosuspension, Itraconazole, Pearl milling technique, Optimization

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INTRODUCTION

Ample of new as well as existing drug substances are only very slightly soluble or even practically insoluble. A substantial portion (40%) of these drugs fail full development, because of Poor water solubility of drug molecules, insufficient bioavailability, fluctuating plasma levels and high food dependency[1-3]. Major efforts have been made for the drug. Hence, there is a growing need for a unique strategy that can tackle the formulation related problems associated with the delivery of hydrophobic drugs in order to improve their clinical efficacy and optimize their therapy with respect to pharmacoeconomics. [4]

The dissolution rate of poorly water soluble-drugs often becomes a rate-limiting step in their absorption from GI tract. [5-8] Various solubilization methods have been used to increase the drug solubility and dissolution properties, including the use of surfactant, water-soluble carriers, polymeric conjugates and solid dispersions. Recently nanonisation has become a popular approach since it is cost effective and technically simpler alternative, particularly for poorly soluble drugs, and yield a physically more stable product than liposome dispersions.With this technique, the drug, dispersed in water, is grounded by shear forces to particles with a mean diameter in the nanometer range (100-1000nm). The fineness of the dispersed particles causes them to dissolve more quickly owing to their higher dissolution pressure and leads to an increased saturation solubility. This may enhance the bioavailability of drugs compared with other microparticular systems. If in vivo dissolution rate of the drug particles is low enough, the drug nanosuspensions will have the passive targeting advantages of colloidal drug carriers[9-14]

For present study Itraconazole was selected as it is practically insoluble in water, but possesses high permeability, which makes it an excellent candidate for nanogrinding Itraconazole is an orally active triazole antimycotic agent, which is active against a broad spectrum of fungal species including *Cryptococcus*, *Candida*, *Aspergillus*, *Blastomyces* and *Histoplasma capsulatum* var. *capsulatum* . It is a weak basic drug which is soluble in lipids (n- Octanol/Water partition, 5.66 at pH of 8.1) and a pKa of 3.7. Itraconazole is ionized only at a low pH, such as gastric juice and as a result on oral administration, the gastric acidity is required for adequate dissolution.[15-19]

The aim of this work was to employ the nanosuspension technique to produce itraconazole nanoparticles by screening of series of polymers and surfactant for oral administration, enabling to enhance the saturation solubility, dissolution,oral absorption and the stability of itraconazole nanosuspension upon storage since it is also crucial. The optimized nanosuspension formulation was evaluated for in vitro dissolution profile in comparison to the pure drug and marketed formulation (Canditral Capsule).

Materials and methods:-

Materials :-

Itraconazole was a gift from Intas pharmaceutical limited (India). Zirconium oxide beads were gifted from Sun Pharmaceutical Industries Ltd. (India). Sodium dodecyl sulfate [SDS] (Crystal Chemicals Limited, Himmatnagar), Sodium docusate [DS] (Crystal Chemicals Limited, Himmatnagar), benzalkonium chloride [BK] (Crystal Chemicals Limited, Himmatnagar), Hydroxypropylcellulose [HPC-LF, HPC-EF] (Crystal Chemicals Limited, Himmatnagar) , Povidone [PVP] (Crystal Chemicals Limited, Himmatnagar), Poly Vinyl alcohol [PVA] (Chemdyes corporation, Rajkot), Hydroxypropylmethylcellulose [HPMC] (Hypromellose 2910, Methocel® E15 LV,Colorcon, Dow ChemicalsUK). All chemicals and solvents used were of analytical grade.

Preparation of nanosuspension:-

For nanogrinding of itraconazole, solutions of surfactant and polymer stabilizers in purified water were first prepared. Itraconazole was then dispersed in the stabilizer solution. Initial experiments were designed to screen most suitable surfactant and polymer stabilizers (Table 1). The resulting coarse pre-dispersion was comminuted using zirconium oxide beads (milling media) on a REMI Digital magnetic stirrer at higher speed. Zirconium oxide beads were used in the preparation of nanosuspension due to their low cost and easy availability for lab scale production of nanosuspension in comparison to silver beads. The screened and selected surfactant and stabilizer were used for further development(Table 2).

Optimization (Experimental design):-

For optimization 3^3 factorial design was employed using SAS 9.1 software. The optimized formulation was lyophilized using mannitol as a cryoprotectant (1:1 ratio). Lyophilized nanosuspension was used for further evaluation. [19,20]

Table 1: Itraconazole nanosuspension formulaions for screening of polymeric and surfactant stabilizers.

Itraconazole Concentration (%w/v)	Surfactant used and Concentration (%w/v)	Polymer used and Concentration (%w/v)
5	SDS, 0.05	HPC-EF, 1.25 HPMC, 1.25 PVP, 1.25 PVA, 1.25
10	SDS, 0.05	HPC-EF, 1.25 HPMC, 1.25 PVP, 1.25 PVA, 1.25
10	No surfactant SDS, 0.1 DS, 0.1 BK, 0.1	HPC-LF, 2.5

Table 2: Coded values parameters of Itraconazole nanosuspension formulation by 3^3 factorial designs.

Coded values	Actual values		
	X_1	X_2	X_3
(-1)	5	0.05	**1.25**
0	12.5	0.125	**3.125**
(+1)	**20**	**0.5**	**5**

Where, X_1 - Amount of Itraconazole (%w/w)

X_2 - % w/w of SDS

X_3 – Amount of HPC-EF (%W/W)

Table 3: Formula for preparation of nanosuspension using optimized polymer and surfactant

Batch No	Amount of miconazole (%W/W)	Amount of SDS (%W/W)	Amount of HPC-LF (%W/W)
F1	5	0.05	1.25
F2	5	0.05	3.125
F3	5	0.05	5
F4	5	0.125	1.25
F5	5	0.125	3.125
F6	5	0.125	5
F7	5	0.5	1.25
F8	5	0.5	3.125
F9	5	0.5	5
F10	12.5	0.05	1.25
F11	12.5	0.05	3.125
F12	12.5	0.05	5
F13	12.5	0.125	1.25
F14	12.5	0.125	3.125
F15	12.5	0.125	5
F16	12.5	0.5	1.25
F17	12.5	0.5	3.125
F18	12.5	0.5	5
F19	20	0.05	1.25
F20	20	0.05	3.125
F21	20	0.05	5
F22	20	0.125	1.25
F23	20	0.125	3.125
F24	20	0.125	5
F25	20	0.5	1.25
F26	20	0.5	3.125
F27	20	0.5	5

Particle size and Size distribution

The average particle diameter and size distribution of the prepared nanosuspension was determined by laser diffraction technique using Malvern particle size analyzer, SM 2000. Nanosuspension was added to the sample dispersion unit, and stirred at 2000 rpm with magnet in order to reduce the interparticulate aggregation and laser obscuration range was maintained between 10-20%. The mean particle size was measured by repeating the experiment in triplicates.

Drug Content

Assay was carried out by taking 10 mg of lyophilized powder (weigh equivalent to 1.25 mg of drug), dissolved in 0.4 ml of tetrahydrofuran in 50 ml dry volumetric flask and then volume was made up using 0.1 N HCl. Then 4 ml of the solution was taken to 10 ml dry volumetric flask, and volume adjusted with 0.1 N HCl. The absorbance at 255.0 nm wavelength was taken using SHIMADZU UV-VISIBLE spectrophotometer UV-1601 and the drug content was calculated accordingly (20).

In vitro dissolution profile

In vitro dissolution study was carried out using USP dissolution test apparatus-I (basket assembly). The dissolution was performed using 500 ml of 0.1N HCl and 900 ml phosphate buffer solution (PBS) having pH 6.8 as dissolution mediums maintained at 37 ± 0.5°C and 100 rpm for pure itraconzole, lyophilized itraconazole nanosuspension formulation and marketed formulation (Canditral capsule). Samples (5ml) were withdrawn at regular intervals of 5 min for 60 min and replaced with fresh dissolution medium. Samples taken were filtered through 0.2µ filter paper and assayed spectrophotometrically on SHIMADZU UV-VISIBLE spectrophotometer UV-1601 at 255.0 nm wavelength. Dissolution for each formulation was performed in triplicates and mean of absorbance was employed to calculate cumulative percent of drug release (21).

Result and Discussion:

Excipient screening for Itraconazole nanogrinding

To select a suitable polymeric excipient, itraconazole (5 and 10%, w/w) was nanoground using SDS (0.05 and 0.1%, w/w) and different types of polymers (1.25 and 2.5%, w/w) (Table 1; Figs. 1 and 2).The higher concentrations of Itraconazole and excipients promoted particle size reduction (Fig. 1 *versus* Fig. 2). The polymeric stabilizers HPMC and HPC were found to be highly effective for nanogrinding Itraconazole, whereas PVA/SDS and PVP/SDS were ineffective (Figs. 1 and 2). The reasons for the inefficiency of PVA/SDS and PVP/SDS remain unknown. Nonetheless, microscopic observation of unground Itraconazole dispersions revealed large

aggregates in PVP/SDS and PVP/SDS solutions, but well dispersed particles with the other excipients, as illustrated exemplarily for the PVP/SDS and HPC-LF/SDS formulations in Fig. 2.

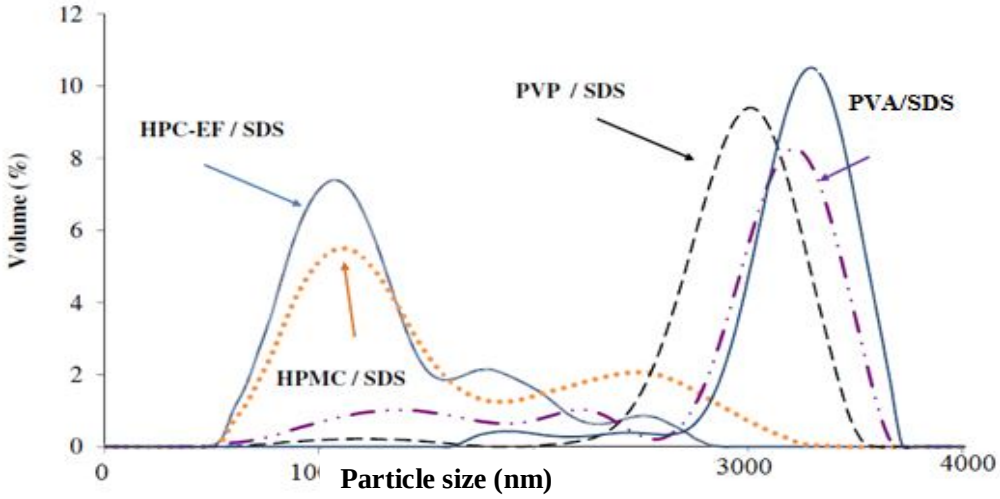


Figure 1: Effect of the type of polymeric excipient on the efficiency of Itraconazole nanogrinding in terms of volume distributions of particle sizes. The formulations contained 5% (w/w) Itraconazole, 1.25% (w/w) polymer, and 0.05% (w/w) SDS. SDS was not present when PVP/SDS was used as polymeric stabilizer.

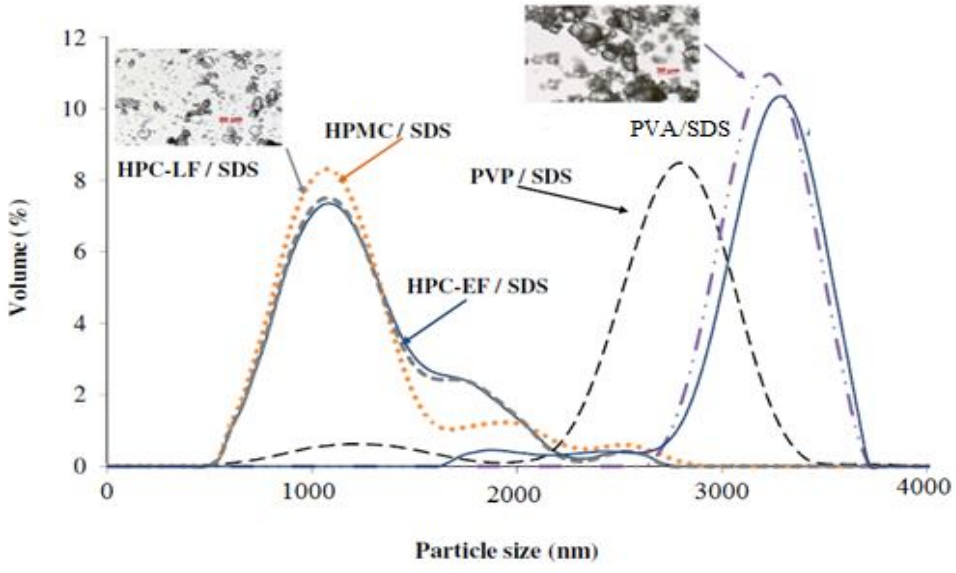


Figure 2: Effect of the type of polymeric excipient on the efficiency of Itraconazole volume distributions of particle sizes. Micrographs (insets) illustrate two extreme cases of native drug particles in the aqueous medium before Itraconazole, 2.5% (w/w) polymer, and 0.1% (w/w) SDS.

To make a choice of a suitable surfactant, Itraconazole (10%, w/w) was minced using HPC-LF (2.5%, w/w) and different types of surfactants (0.1%, w/w) (Table 1), since HPC-LF was found to be a highly effective polymeric excipient for nanogrinding of Itraconazole in the previous experiment. While all surfactants facilitated nanogrinding and yielded nanoparticles with similar d50 values (d50 of 455 to 575 nm). SDS was the most effective in minimizing the large particle size fraction of the bimodal size distribution . Here again, the extent of particle size reduction seemed to be predetermined by the degree of dispersion of unground Itraconazole in the different stabilizer solutions, From the initial screening batches using HPC-LF & SDS were formulated using 3^3 factorial design of experiments as shown in table 2 & 3. The optimization was carried out using DOE SAS 9.1 Software. The data obtained are given here.

Table 4: Formulation parameters optimization for the preparation of itraconazole nanosuspension

Batch No	X1	X2	X3	Y1	Y2
F1	5	0.05	1.25	1120	1.025
F2	5	0.05	3.125	974	0.874
F3	5	0.05	5	969.2	0.861
F4	5	0.125	1.25	963.2	0.842
F5	5	0.125	3.125	884.6	0.813
F6	5	0.125	5	872.5	0.792
F7	5	0.5	1.25	856	0.781
F8	5	0.5	3.125	845.9	0.776
F9	5	0.5	5	822.9	0.725
F10	12.5	0.05	1.25	829.3	0.738
F11	12.5	0.05	3.125	785.6	0.713
F12	12.5	0.05	5	765.9	0.701
F13	12.5	0.125	1.25	772.8	0.696
F14	12.5	0.125	3.125	746.3	0.687
F15	12.5	0.125	5	712.5	0.675
F16	12.5	0.5	1.25	705.3	0.662
F17	12.5	0.5	3.125	692.5	0.650
F18	12.5	0.5	5	679.6	0.641

F19	20	0.05	1.25	678.4	0.628
F20	20	0.05	3.125	651.3	0.619
F21	20	0.05	5	642.5	0.607
F22	20	0.125	1.25	586.2	0.576
F23	20	0.125	3.125	542.3	0.546
F24	20	0.125	5	523.7	0.540
F25	20	0.5	1.25	490.7	0.502
F26	20	0.5	3.125	478.5	0.482
F27	20	0.5	5	459.7	0.432

Where, X_1 - Amount of Itraconazole (%w/w) Y_1- Particle size (nm)

X_2 - % w/w of SDS Y_2- Polydispersity index

X_3 – Amount of HPC-LF (%W/W)

Optimization:-

- **Fit details for Y1:-**

Table 5 : ANOVA for Y1

		MASTER MODEL					PREDECTIVE MODEL			
Source	DF	SS	MS	F	Pr > F	DF	SS	MS	F	Pr > F
X1	1	55453	55453	767.01	0.0001	1	58861	58861	737.53	0.0001
X2	1	10658	10658	147.42	0.0001	1	10658	10658	133.54	0.0001
X3	1	13112.	13112.	18.136	0.0005	1	17013.	17013.	21.318	0.0001
X1*X1	1	6	6	0.0082	0.9284					
X1*X	1	18.969	18.969	0.0262	0.87322					
X1*X	1	1756.9	1756.9	2.4301	0.13744					
X2*X	1	21796.	21796.	30.148	0.0001	1	21796.	21796.	27.3116	0.000
X2*X	1	2342.6	2342.6	3.2402	0.08962					
X3*X	1	1142.6	1142.6	1.5804	0.22569					
Mod	9	71853	79837.	110.42	0.0001	4	71326	17831	223.4324	0.000
Error	17	12290.	722.97			22	17557.	798.08		
Total	26	73082				26	73082			

Table 6: Fit Statistics for Y1

		MASTER MODEL	PREDICTIVE MODEL
Mean		742.6444	742.6444
R-square		98.32%	97.60%
Adj.	R-square	97.43%	97.16%
RMSE		26.88826	28.25033
CV		3.62061	3.804018

Predictive Model for Y1

Coded Levels(-1,1):

Y1 = 630.8222 - 180.8333*X1 - 76.95*X2 - 30.74444*X3 + 116.25*X2*X2

Uncoded Levels:

Y1 = 1251.159 - 24.11111*X1 - 1604.963*X2 - 16.39704*X3 + 2296.296*X2*X2

Table 7: Effect Estimates for Y1

		Master Model				Predictive		
Term	Estimate	Std Err	T	Pr > |t|	Estimate	Std Err	T	Pr > |
X1	-181.0941	6.53886	-27.695	0.0001	-	6.65866	-	0.00
X2	-76.95	6.33762	-12.1418	0.0001	-76.95	6.65866	-	0.00
X3	-27.84677	6.53886	-4.25865	0.00053	-	6.65866	-	0.000
X1*X	-1	10.9770	-0.0911	0.92847				
X1*X	-1.173387	7.24391	-0.16198	0.87322				
X1*X	12.1	7.76197	1.55888	0.13744				
X2*X	116.25	21.1718	5.49078	0.0001	116.25	22.2443	5.2260	0.00
X2*X	13.03951	7.24391	1.80006	0.08962				
X3*X	13.8	10.9770	1.25716	0.22569				
3		9	4	2				

➢ **Fit details for Y2:-**

Table 8: ANOVA for Y2

	MASTER MODEL					PREDICTIVE MODEL				
Sour	D F	SS	MS	F	Pr > F	D F	SS	MS	F	Pr > F
X1	1	0.3425	0.3425	311.83	0.0001	1	0.3643	0.3643	384.47	0.0001
X2	1	0.0690	0.0690	62.879	0.0001	1	0.0690	0.0690	72.879	0.0001
X3	1	0.0112	0.0112	10.239	0.0052	1	0.0125	0.0125	13.282	0.0014
X1*X	1	0.0001	0.0001	0.1652	0.6894					
X1*X	1	7.025E-	7.025E-	0.0006	0.9801					
X1*X	1	0.0017	0.0017	1.5513	0.2298					
X2*X	1	0.0108	0.0108	9.8333	0.0060	1	0.0108	0.0108	11.397	0.0027
X2*X	1	0.0001	0.0001	0.1086	0.7456					
X3*X	1	0.0001	0.0001	0.1553	0.6983					
Mod	9	0.4483	0.0498	45.348	0.0001	4	0.4461	0.1115	117.68	0.0001
Error	1	0.0186	0.0010			2	0.0208	0.0009		
Total	2 6	0.4669 83				2 6	0.4669 83			

Table 9: Fit Statistics for Y2

		MASTER MODEL	PREDICTIVE MODEL
Mean		0.688444	0.688444
R-square		96.00%	95.54%
Adj.	R-square	93.88%	94.72%
RMSE		0.033142	0.030785
CV		4.814106	4.471648

Predictive Model for Y2

Coded Levels(-1,1):

Y2 = 0.608 - 0.142278*X1 - 0.061944*X2 - 0.026444*X3 + 0.081833*X2*X2

Uncoded Levels:

Y2 = 1.087158 - 0.01897*X1 - 1.164362*X2 - 0.014104*X3 + 1.616461*X2*X2

Table 10: Effect Estimates for Y2

	MASTER MODEL				PREDICTIVE MODEL			
Term	Estimate	Std Err	t	Pr > |t|	Estimate	Std Err	t	Pr > |
X1	-0.142328	0.00806	-17.659	0.0001	-0.142278	0.00725	-19.6082	0.00
X2	-0.061944	0.00781	-7.92965	0.0001	-0.061944	0.00725	-8.53694	0.00
X3	-0.02579	0.00806	-3.19987	0.00524	-0.026444	0.00725	-3.64447	0.001
X1*X	0.0055	0.01353	0.40649	0.68945				
X1*X	-0.000226	0.00892	-0.02529	0.98011				
X1*X	0.011916	0.00956	1.24554	0.22982				
X2*X	0.081833	0.02609	3.13581	0.00602	0.081833	0.02424	3.37596	0.002
X2*X	0.002943	0.00892	0.32966	0.74567				
X3*X	0.005333	0.01353	0.39417	0.69835				

Table 11: Check point batch data: -

Coded			Uncoded			Predicted		Actual	
X1	X2	X3	X1	X2	X3	Y1	Y2	Y1	Y2
0.5	0.5	0.5	16.52	0.3125	4.0625	515.4	0.516	502.3	0.498
-0.5	-0.5	-0.5	8.75	0.0875	2.187	881.5	0.801	895.3	0.786

The check point batches were again treated with chi^2 test to find out whether there is a significant difference or not for Y1 and Y2.

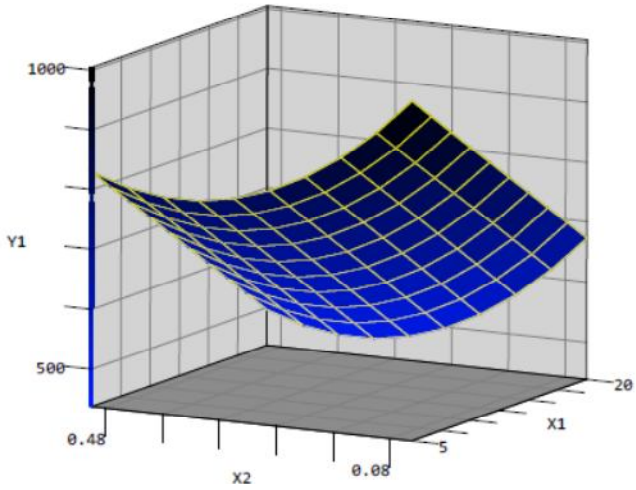
Table 12: Resultant P value obtained from chi^2 test

Response	P Value obtained from chi^2 test
Y1	0.458724
Y2	0.97595029

The data clearly stated no significant difference since both the values are far higher than 0.05.

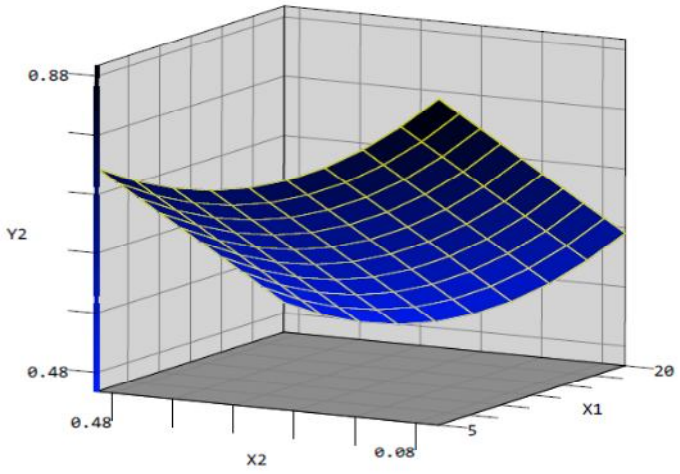
Optimization:-

Figure 3: Surface Plot for Y1:-



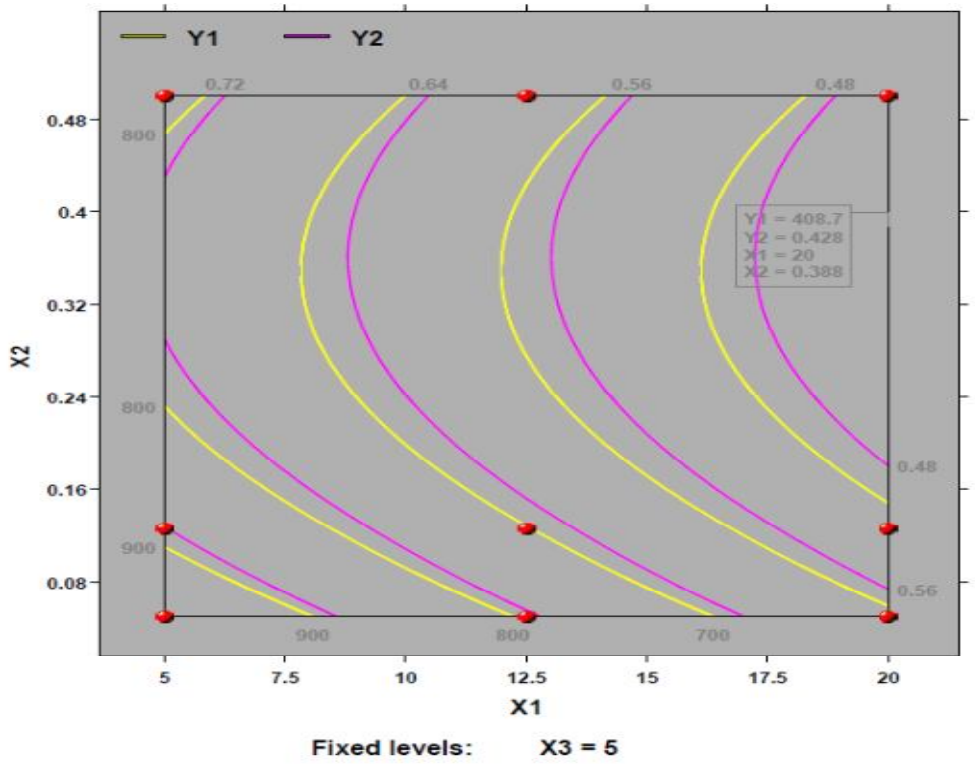
Fixed levels: X3 = 3.125

Figure 4: Surface Plot for Y2:-



Fixed levels: X3 = 3.125

Figure 5: Contour plot for optimization:-



From the contour plot and numerical optimization tool of SAS 9.1 the optimized formula was as below.

Table 13: Optimized formulation with responses

Factors	Value
X1	20
X2	0.3875
X3	5
Y1 (Predicted)	409.8
Y2 (Predicted)	0.429
Y1 (Actual)	401.5
Y2 (Actual)	0.417

Assay: The percentage drug content was estimated in triplicate sample and was found to be 99.42±0.2 % for the optimized formulation. Which were within specified limits according to monograph. [19]

In vitro dissolution study

Drug release from pure drug, marketed formulation (Canditral capsule) and optimized nanosuspension formulation was determined by dissolution study. The amount of drug released from the optimized nanosuspension formulation was 90% within 10 min in comparison to amount of 10% and 17% for pure drug and marketed formulation (Canditral capsule) respectively (Figure 6) in 0.1N HCl (pH 1.2). The increase in accessible surface area to the dissolution medium and hydrophilic surfactant coating on the particle surfaces may be considered as the core reason for approximately six fold increase in dissolution rate in optimized formulation.

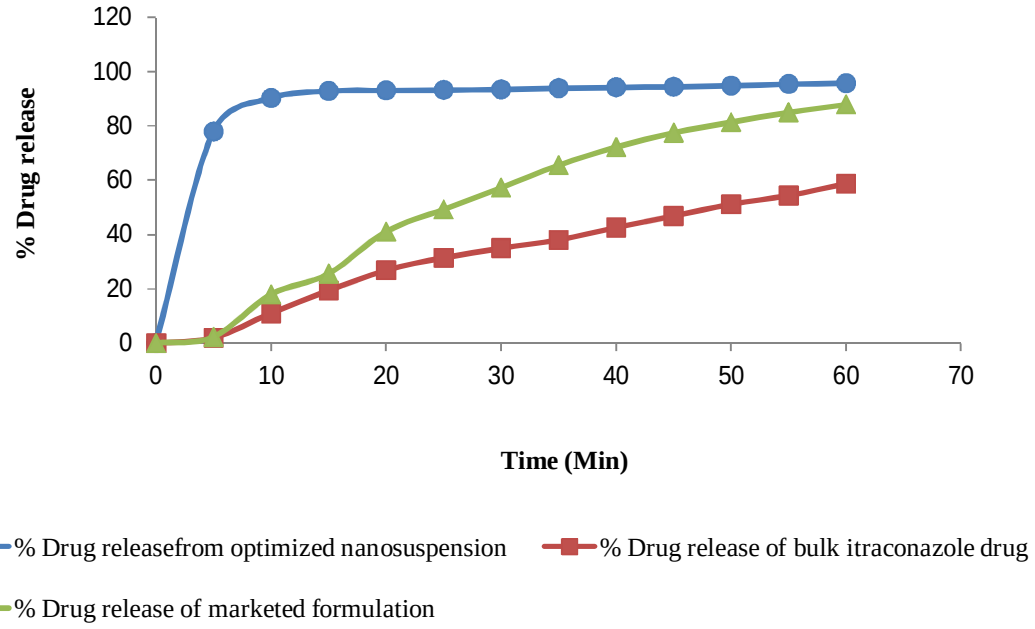


Figure 6: Dissolution profile for nanosuspension formulation (circle), pure drug (square), and marketed formulation (triangle) [mean ± SD (n=3)] in 0.1 N HCl.

CONCLUSION:-

It may be concluded from the results of this study that nanocrystalline suspensions of poorly soluble drugs such as itraconazole can be easily prepared and lyophilized for extended period of time and represent a promising new drug formulation for oral drug delivery for treatment of

fungal infection. Dissolution study in 0.1N HCl indicates that nanosuspension formulation gives higher drug release compared to the pure drug and marketed formulation. Moreover, nanosuspensions represent a promising alternative to current delivery systems aspiring to improve the biopharmaceutic performance of drugs with low water solubility.

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