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Screening of Formulating and Processing Parameters for Ziprasidone Hydrochloride Nanosuspension Prepared by Nanoprecipitation-Ultrasonication Technique

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ABSTRACT:

Low oral bioavailability of poorly water-soluble dosage form poses a great challenge during formulation development. Poor water solubility and low dissolution rate are issues for the majority of upcoming and existing biologically active compounds. Ziprasidone Hydrochloride (ZH) is BCS class-II drug having low solubility and high permeability. The aim of the present investigation was to identify critical formulating and processing parameters which influences on quality of the nanosuspension. Nanosuspension formulation of a poorly soluble drug was developed using nanoprecipitation-ultrasonication technique. A total of 8 experiments were generated for screening 5 independent factors namely the amount of Ziprasidone Hydrochloride (mg) (X_1), amount of stabilizer (mg) (X_2), solvent to anti-solvent volume ratio (X_3), stirring speed (rpm) (X_4) and sonication time (min) (X_5). Mean particle size (nm) (Y_1) and Saturation solubility (µg/ml) (Y_2) were selected as dependent factors. The obtained results showed that nanosuspension prepared with the Poloxamer 407 has improved saturation solubility as compare to all other stabilizers. Result also revealed that concentration of drug and stirring speed were found to be promising formulating and processing parameters having prominent effect on quality of Ziprasidone Hydrochloride nanosuspension.

KEY WORDS: Ziprasidone Hydrochloride, Nanosuspension, Nanoprecipitation-ultrasonication, Plackett and Burman design.

INTRODUCTION:

In pharmaceutical field, formulation of poorly water-soluble drug has always been a challenging problem and it is a major issue for the development of new dosage form. Around 10% of the present drugs, 40% of the research drugs and 60% of drugs coming directly from synthesis have low solubility about 1–10 µg/ml.[1-3] If drug solubility cannot be improved, the drug cannot be absorbed through GI tract upon oral administration and cannot exert its pharmacological action on the target tissue.[4] It is due to the phospholipidic nature of cell membranes, thus certain degree of lipophilicity is required for those drug compounds, while in terms of permeability high lipophilicity is beneficial. In most of the cases it translates into poor aqueous solubility.[5]This creates delivery problems such as low oral bioavailability and erratic absorption. Drug solubility can be enhanced using traditional approaches such as co-solvents, salt formation, complexation, micronization or delivery through carriers like liposome, solid-dispersions.[6] However, in many cases they cannot solve the bioavailability problem. For

example, micronization of poorly soluble drugs has been applied for many years to improve dissolution velocity of poorly soluble drugs, but reducing the drug to micron size does not increase the saturation solubility of the drug, and at such a low saturation solubility, as generally observed in the BCS class II drugs, the increment in the dissolution characteristics does not help to a great extent, nanonization has been employed for treating the BCS class II drugs.

When the drug particle size being reduced to nanosized level, there is an increase in the saturation solubility assisted by improvement in dissolution characteristics, which could be attributed to the effective increase in the particle surface area, according to Ostwald–Freundlich equation and Noyes-Whitney equation. Ostwald–Freundlich equation expresses how particle size influences on saturation solubility (Cs), a compound-specific constant relying only on temperature in a given solvent. Accordingly, Cs of the drug increases substantially with a decrease of particle size.[2,7] Nanosuspensions have been emerged as a promising strategy for an efficient delivery of hydrophobic drugs because of their versatile features such as very small particle size.[8]

Ziprasidone Hydrochloride is categorized under an atypical antipsychotic agent. It is white or slightly pink powder, practically insoluble in water, slightly soluble in methanol and in methylene chloride having melting point 300°C. It is considered as BCS Class II drug having low solubility and high permeability. The absolute bioavailability of 20 mg dose under fed conditions is reported approximately 60%. Ziprasidone Hydrochloride is well absorbed from the gastrointestinal tract with peak plasma concentrations being reached 6 to 8 hours after oral dose. Ziprasidone Hydrochloride is extensively metabolized by aldehyde oxidase (about 66% of a dose) and by the cytochrome P450 iso-enzyme CYP3A4. It is excreted mainly as metabolites in the faeces (about 66%) and urine (about 20%); less than 5% of a dose appears as unchanged drug. 99% of drug is bound to plasma proteins. Terminal elimination is reported to about 7 hours and volume of distribution is 1.5 L/kg. Peak plasma concentration of Ziprasidone Hydrochloride is about 89ng/ml reaching 2 to 3 hours after oral dose.[9-14]

It is generally considered that compounds with very low aqueous solubility will show dissolution rate-limited absorption. Improvement of aqueous solubility in such case is a valuable goal to improve therapeutic efficacy. The dissolution rate is a function of the solubility and the surface area of the drug, thus, dissolution rate will increase if the solubility of the drug is increased, and it will also increase with an increase in the surface area of the drug.[15,16]

The aim of this work is to formulate the Ziprasidone Hydrochloride nanosuspension by nanoprecipitation-ultrasonication technique and to identify critical formulating and processing parameters which influences on quality of Ziprasidone Hydrochloride nanosuspension.

MATERIALS AND METHODS

Materials

Ziprasidone Hydrochloride was obtained as a gift sample from Amneal Pharmaceuticals, Ahmedabad, Gujarat, India. All stabilizers, solvents and chemicals used for the study were of analytical grade.

Selection of solvent and anti-solvent

The solubility of Ziprasidone Hydrochloride was studied in different solvents. About 50 mg of drug was added to 10 ml of solvent in specific gravity bottle. This amount was sufficient to obtain saturated solution. These specific gravity bottles were shaken at 100 RPM for 24 hours at 25ºC by keeping in a cryostatic constant temperature reciprocating shaker bath. The bottles were then opened and solutions were filtered with the help of Whatman filter paper. The absorbance of the solution was measured at λ_{max} 318 nm. This method was repeated for three times. Selection of good and poor solvent was done based upon solubility of drug.[17]

Preparation of Ziprasidone Hydrochloride nanosuspension [18]

Ziprasidone Hydrochloride nanosuspension was prepared by the nanoprecipitation–ultrasonication method. Ziprasidone Hydrochloride was dissolved in methanol by sonication for 5mins at room temperature. Different stabilizers were dissolved in water to obtain a series of anti-solvents. Both solutions were passed through a 0.45µm filter. The anti-solvent was cooled to below 3°C in an ice-water bath. Then, drug solution was quickly introduced by means of a syringe positioned with the needle directly into stabilizer solution into 40 ml of the pre-cooled anti-solvent at different stirring speed under overhead stirrer to allow the volatile solvent to evaporate at room temperature for 5 hours. After the precipitation

of anti-solvent, sample was immediately transferred to a test tube and was treated with an ultrasonic probe at different time lengths (in mins). The probe with a tip diameter of 6 mm was immersed in the liquid, resulting in the wave traveling downwards and reflecting upwards. Batch size for preparation of nanosuspension was taken 40 ml.

Selection of stabilizer

Different stabilizers like Polyvinyl Alcohol, PVP K-30, Sodium Lauryl Sulphate, Poloxamer 188 and Poloxamer 407 were screened by preparing nanosuspensions (Table1) and measuring their saturation solubility, mean particle size, poly dispersity index (PDI) and zeta potential.[19]

Plackett-Burman Design [20]

The Plackett-Burman design is suitably used to screen a large number of factors believed to be affecting important product characteristics or attributes, and is generally used during the initial phase of the study. The Plackett-Burman design, a fractional factorial design, is effective for measuring effects of processing and formulating factors.

By review of literature five factors were selected to affect the formulation of Ziprasidone Hydrochloride nanosuspension. To identify which factor has its prominent effect on quality, stability as well as efficacy of the nanosuspension, this design was used. A total of 8 experiments were generated for screening of five independent factors namely Amount of Ziprasidone Hydrochloride in mg (X_1), Amount of Poloxamer 407 in mg (X_2), Solvent: Anti-solvent volume ratio (X_3), Stirring Speed in rpm (X_4) and Sonication Time in min (X_5). Saturation Solubility in µg/ml (Y_1) and Mean Particle Size in nm (Y_2) were selected as dependent factors. The coded and uncoded values of different independent factors are as shown in below table 2.

For evaluation of above batches of nanosuspensions, saturation solubility study and mean particle size were selected as evaluation parameters. Net effect of individual factor was calculated from the value of saturation solubility as well as mean particle size value from following equations,

$$\textbf{Effect of } X_1 = [(Y_1+Y_4+Y_6+Y_7)-(Y_2+Y_3+Y_5+Y_8)]/8$$
$$\textbf{Effect of } X_2 = [(Y_1+Y_2+Y_5+Y_7)-(Y_3+Y_4+Y_6+Y_8)]/8$$
$$\textbf{Effect of } X_3 = [(Y_1+Y_2+Y_3+Y_6)-(Y_4+Y_5+Y_7+Y_8)]/8$$
$$\textbf{Effect of } X_4 = [(Y_2+Y_3+Y_4+Y_7)-(Y_1+Y_5+Y_6+Y_8)]/8$$
$$\textbf{Effect of } X_5 = [(Y_1+Y_3+Y_4+Y_5)-(Y_2+Y_6+Y_7+Y_8)]/8$$

After getting net effect of individual parameters two key parameters were identified which had maximum effect on product characteristics. These two parameters can be selected for product optimization by factorial design and other three parameters can be optimized by trial and error method.

Evaluation of Nanosuspensions

Saturation solubility

The saturation solubility of prepared nanosuspension was performed by filling it in a vial and kept for 48 hrs stirring with the help of magnetic stirrer at 100 RPM to ensure saturation. Then 2 ml of nanosuspension was filled in eppendorf tube and centrifuged at 10,000 rpm for 30 minutes. Supernatant was filtered through 0.2µm syringe filter and analyzed by UV-visible spectrophotometer [UV-1800, Shimadzu, Japan] at 318 nm after suitable dilution with 0.05M Sodium Phosphate Buffer , pH 7.5 + 2%w/w SDS which was used as blank. Each sample was analyzed in triplicate. By using the calibration curve, saturation solubility was calculated.[21]

Particle size and PDI

Mean particle size and size distribution (polydispersity index) of the prepared nanosuspension was determined by using Zetasizer [Zetatrac, Microtrac, Japan] which follows principle of light diffraction, also called Photon correlation spectroscopy (PCS). Prior to the measurement, the samples were appropriately diluted with water to a suitable scattering intensity and re-dispersed by shaking before measurement. [22]

Zeta potential

The Zeta potential is a measure of the electric charge at the surface of the particles, indicating the physical stability of colloidal systems. The zeta potential values higher than |30mV| indicate long-term electrostatic stability of aqueous dispersions. In this study, the Zeta Potential was assessed by determining the electrophoretic mobility of the particles using Zetasizer [Zetatrac, Microtrac, Japan]. [22]

RESULT AND DISCUSSION

Selection of solvent and anti-solvent

Selection of solvent and anti-solvent was performed on the basis of solubility of Ziprasidone Hydrochloride in different solvents and their combinations. Results showed that drug has highest solubility (2.443mg/ml) in methanol and least solubility (0.022 mg/ml) in water, so they were selected as solvent and anti-solvent respectively.

Selection of stabilizer

Different stabilizers like Polyvinyl Alcohol, PVP K-30, Sodium Lauryl Sulphate, Poloxamer 188 and Poloxamer 407 were used to prepare nanosuspensions and subjected for measurement of their saturation solubility, mean particle size, poly dispersity index (PDI) and zeta potential.

Table 4 shows results of preliminary trial batches for selection of stabilizer. From table 4 result revealed highest solubility (45.58 ± 1.62µg/ml) and lowest mean particle size (210.4 ± 5.9nm) with Poloxamer 407. This stabilizer also showed narrow range of particle size distribution by showing least value of PDI (0.40 ±0.03) among all stabilizers. Batch ZF5 had zeta potential 32.53mV, so proving long-term electrostatic stability of aqueous dispersions.

Plackett-Burman design

Plackett-Burman design was applied to screen various formulating as well as processing parameters that could provide high saturation solubility and low mean particle size. As shown in table 2 five independent variables were selected viz. amount of Ziprasidone Hydrochloride (mg) (X_1), Amount of Poloxamer 407 (mg) (X_2), Solvent: Anti-solvent Volume Ratio (X_3), Stirring Speed (RPM) (X_4) and Sonication Time (Min) (X_5). Saturation solubility (µg/ml) (Y_1) and mean particle size (nm) (Y_2) were selected as dependent factors.

As shown in table 5 the selected response parameters showed a wide variation suggesting that the independent parameters has a significant effect on the dependent parameters chosen.

Net effect (Coefficient) of individual factors were calculated from the value of saturation solubility as well as mean particle size value from equations.

From the Pareto chart as shown in Figure 1 and Figure 2, it is cleared that amount of Ziprasidone Hydrochloride (X_1) and Stirring speed (X_4) showed highest effect on saturation solubility and mean particle size. Amount of Ziprasidone Hydrochloride (X_1) and Stirring speed (X_4) were combinedly responsible for almost 70% cumulative effect to the quality of the products.

CONCLUSION

The obtained results showed that nanosuspension prepared with the Poloxamer 407 has improved saturation solubility as compare to all other stabilizers. Result also revealed that stirring speed as well as concentration of drug were found to be promising formulating parameters having prominent effect on quality of Ziprasidone Hydrochloride nanosuspension.

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FIGURES

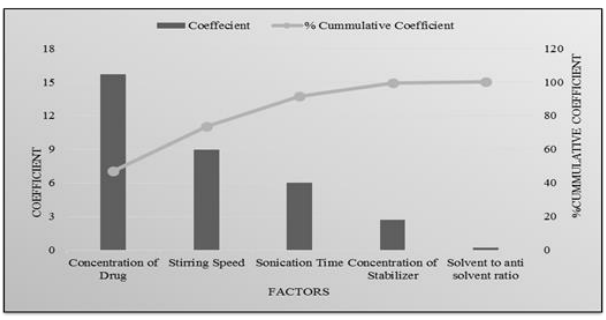

Figure 1: Pareto chart of effect on saturation solubility

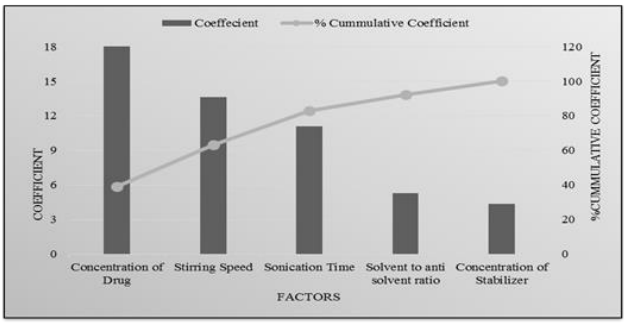

Figure 2: Pareto chart of effect on particle size

TABELS

Table 3: Results of selection of solvents

Drug	Solvents	Solubility* (mg/ml) (Mean ± SD)
Ziprasidone Hydrochloride	Water	0.022 ± 0.0013
	Methanol	2.443 ± 0.052
	Alcohol	0.375 ± 0.033
	Iso-propanol	0.082 ± 0.0023
	N-Butanol	0.120 ± 0.010
	Alcohol:2-Propanol	0.138 ± 0.025
	Alcohol: Butanol (1:1)	0.158 ± 0.034
	Ethyl Acetate	0.044 ± 0.018
	Dichloromethane	0.047 ± 0.0016

* Indicates average of three readings

Table 1: Selection of stabilizer

Formulation Code	Stabilizers	Stabilizers (Mg)	Drug (Mg)	Stirring Speed (RPM)	Stirring Time (Hrs)	Sonication Time (Min)	Solvent: Anti-solvent Volume Ratio
ZF1	Poly Vinyl Alcohol	30	10	1000	5	20	1:8
ZF2	PVP K-30	30					
ZF3	Sodium Lauryl Sulphate	4					
ZF4	Poloxamer 188	30					
ZF5	Poloxamer 407	30					

Table 2: Coded and uncoded value of Plackett - Burman design

Batch Code	Amount of Ziprasidone Hydrochloride (mg) X_1		Amount of Poloxamer 407 (mg) X_2		Solvent : Anti-solvent Volume Ratio X_3		Stirring Speed (RPM) X_4		Sonication Time (Min) X_5	
ZF6	+	20	+	50	+	1:8	-	800	+	30
ZF7	-	10	+	50	+	1:8	+	1200	-	10
ZF8	-	10	-	30	+	1:8	+	1200	+	30
ZF9	+	20	-	30	-	1:5	+	1200	+	30
ZF10	-	10	+	50	-	1:5	-	800	+	30
ZF11	+	20	-	30	+	1:8	-	800	-	10
ZF12	+	20	+	50	-	1:5	+	1200	-	10
ZF13	-	10	-	30	-	1:5	-	800	-	10

Table 4: Results of selection of stabilizer

Batch Code	Stabilizer Used	Saturation Solubility* (µg/ml)	Mean Particle Size* (nm)	PDI*	Zeta Potential* (mV)
ZF1	Polyvinyl Alcohol	29.64 ± 2.78	336.8 ± 5.1	1.27 ± 0.15	-21.56 ± 1.12
ZF2	PVP K-30	42.49 ± 1.08	234.0 ± 4.7	0.84 ± 0.11	27.85 ± 0.32
ZF3	Sodium Lauryl Sulphate	34.03 ± 0.81	333.5 ± 7.3	1.09 ± 0.05	-29.11 ± 0. 72
ZF4	Poloxamer 188	31.27 ± 1.74	318.0 ± 6.8	0.75 ± 0.06	-31.41 ± 1.15
ZF5	Poloxamer 407	45.58 ± 1.62	210.4 ± 5.9	0.40 ±0.03	32.53 ± 0.90

* Indicates average of three readings

Table 5: Layout and observed responses of Plackett-Burman design batches

Batch Code	Ziprasidone Hydrochloride (mg) X_1	Poloxamer 407 (mg) X_2	Solvent: Anti-solvent Volume	Stirring Speed (RPM) X_4	Sonication Time (Min) X_5	Saturation Solubility (µg/ml) Y_1	Mean Particle Size (nm)
ZF6	20 (+)	50 (+)	1:8 (+)	800 (-)	30 (+)	89.46	325.5
ZF7	10 (-)	50 (+)	1:8 (+)	1200 (+)	10 (-)	45.01	395.2
ZF8	10 (-)	30 (-)	1:8 (+)	1200 (+)	30 (+)	95.74	318
ZF9	20 (+)	30(-)	1:5 (-)	1200 (+)	30 (+)	82.69	348.7
ZF10	10 (-)	50 (+)	1:5 (-)	800 (-)	30 (+)	63.92	378.3
ZF11	20 (+)	30 (-)	1:8 (+)	800 (-)	10 (-)	78.33	354.9
ZF12	20 (+)	50 (+)	1:5 (-)	1200 (+)	10 (-)	120.11	298.4
ZF13	10 (-)	30 (-)	1:5 (-)	800 (-)	10 (-)	40.28	410.5

Table 6: Coefficient Values of dependent variables

Factors	Coefficient from Saturation	Coefficient from
Concentration of Drug	**15.71**	**21.81**
Concentration of	2.68	4.34
Solvent to anti solvent	0.19	5.29
Stirring Speed (X4)	**8.95**	**13.61**
Sonication Time (X5)	6.01	11.06