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QUALITY BY DESIGN APPROACH FOR DEVELOPMENT OF AZITHROMYCIN ORAL RECONSTITUTABLE SUSPENSION AND ITS COMPARISON WITH MARKETED PRODUCT

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ABSTRACT

The aim of this work was to develop stable suspension containing Azithromycin was suitable for large scale manufacturing. The product developed by QbD was found comparable with marketed product. The present investigation concerns with the evaluation of the effect of formulation variables on viscosity and phase separation volume in developing stable reconstitution suspension containing azithromycin by applying Quality by Design. The quality by design (QbD) approach was applied for optimizing the formulation of Azithromycin dry powder for oral suspension using Design-Expert Software (version 10.0 state ease, USA).A Central Composite Design (CCD) was employed in formulating the suspension containing Xanthan Gum (XG) and Hydroxy Propyl Cellulose (HPC). To optimize this formulation a Quality Target Product Profile (QTTP) was established in which Critical Quality Attributes (CQAs) such as viscosity and phase separation volume quantified. As Critical Process Parameters (CPP) that was evaluated for their effect on the CQAs the percentage of XG and the percentage of HPC were chosen.CCD was used to evaluate the effects of the CPPs on the CQAs of the final product. The main effect and interaction terms were evaluated by quadratic model to predict formulation with the desired viscosity and phase separation volume. The concentration of suspending agent and its quadratic term were found to be significantly effective for all the response variables.

KEYWORDS: Azithromycin, Reconstitution suspension, Quality by Design, Design of Experiment.

INTRODUCTION

Oral drug delivery has gained a higher scope and popularity and has been widely employed for the systemic delivery of drugs among all pharmaceutical products available. The advantages of oral dosage form are high level of acceptance due to its ease of administration, patient compliance and stability of formulation.[1]

The Reconstitution suspensions are dry mixtures that require the addition of water at the time of administration.[2] The choice of reconstituted formulation is when the drug stability is a major concern. Oral suspension contains drug, colorants, flavors, sweeteners, stabilizing, suspending and preserving agents that may be required to enhance the stability of the formulation. Reconstitution suspension shows improved bioavailability as compared to tablets and capsules as it is in the dispersed state at the time of administration. The studies have concluded that the suspension for reconstitution is stable for 24 hours. After reconstitution of formulations are to be administered in a specific period as indicated in the label.[3]

Azithromycin is one of the world's bestselling antibiotics, and is derived from erythromycin. Azithromycin is used to treat certain bacterial infections, most often those causing middle ear infection, tonsillitis, throat infections, laryngitis, bronchitis, pneumonia, typhoid and sinusitis.[4]

It is white in color, bitter in taste, crystalline powder, having low water solubility.[5] Although the drug is formulated in the form of tablet to mask the bitter taste; administration of the formulation to children has been a problem. Currently, there is no pharmaceutical alternative to circumvent the compliance problem for azithromycin and thus, taste masking is necessary to achieve an improved patient compliance especially in case of pediatric. In view of these difficulties it would be desirable to develop a reconstitutable suspension with a

high degree of stability and good taste masking characteristics.[6]

According to ICH Q8 guidelines, QbD is defined as; a systematic approach to development that begins with predefined objectives emphasizes product, process understanding and process control, based on sound science and quality risk management.[7] It means the design development and manufacturing process of formulation should be predefined product quality. It gives an idea about how product and process variables influence on product quality. USFDA agreed to include QbD in Pharmaceutical cGMP 21st Century- A risk based approach which includes ICH Q8 (Pharmaceutical Development), Q9 (Quality Risk Management) and Q10 (Pharmaceutical Quality System) guidelines. QbD consist of elements like QTTP, CQAs, CMAs, CPPs and Control strategy.[8]

The design of the experiment has been widely used in pharmaceutical field to study the effect of formulation variables and their interaction on dependent (response) variables. In the present study, a CCD was used and different independent variables include XG (X1) and HPC (X2). The formulation variables and their ranges were chosen from the knowledge acquired from the preliminary studies. Dependent (response) variables evaluated for the viscosity and phase separation volume. All the response variables were fitted to a quadratic model and analysis was carried out to get a relationship between the dependent and the independent variables. The purpose of this work was to develop a stable QbD based Azithromycin suspension suitable for large scale manufacturing and furthers its marketing.

MATERIALS AND METHODS

Materials

The Innovator Azithromycin dry powder for oral suspension 200mg/5ml were purchased from Singapore. For preparation of dry powder for oral suspension, Azithromycin dihydrate (USP) was obtained from SAVA Healthcare Pvt.Ltd (Gujarat), and all other excipients (lactose, castor sugar, HPC, XG, sodium phosphate, menthol, aerosil, sucralose, flavor peppermint dry powder) from SAVA Healthcare Pvt.Ltd (Chinchwad, Pune, India).

Equipments

Fluidized bed dryer (Bectochem) was used for drying of granules, Double cone blender (Karnavati) was used for proper mixing, rapid mixing granulator (Karnavati) was used for wet granulation, Moisture analyzer (Sartorius, MA35) was used for moisture determination, Viscometer (Brookfield, DV-II+Pro) was used for viscosity measurement, HPLC (2998, Waters) was used for assay and dissolution determination, Infrared spectrophotometer (Jasco, 4100) was used for drug excipients compatibility study.

METHODS

Identification of elements of QbD parameter

Quality Target Product Profile (QTTP)

Based on the pharmaceutical requirement and physicochemical characteristics of the innovator, a QTTP was defined for generic azithromycin dry powder for oral suspension 200 mg/5 ml shown in Table no. 1.[9]

Table No 1: QTTP for dry powder for oral suspension.

QTPP Elements		Target	Justification
Dosage form		Solid	Pharmaceutical equivalence requirement: same dosage form.
Dosage design		Dry powder for oral suspension	Design needed to meet label claims.
Route of administration		Oral	Pharmaceutical equivalence requirement: same route of administration.
Dosage strength		200mg/5ml	Pharmaceutical equivalence requirement
Stability		At least 24-months shelf-life at room temperature	Equivalent to or better than marketed formulation shelf-life.
Drug product quality attributes	Physical Attributes	Pharmaceutical equivalence requirement: Must meet the same compendia or other applicable (quality) standards (i.e., identity, assay, purity, and quality).	
	Identification		
	Assay		
	Content Uniformity		
	Dissolution		
	Water Content		
Container closure system		Container closure system qualified as suitable for this drug product.	Needed to achieve the target shelf-life and during shipping.

Quality Risk Assessment of CMAs and CPPs with CQAs

It is a linking material attributes and process parameters to drug product CQAs. A main objective of risk assessment in pharmaceutical development is to identify which material attributes and process parameters affect the drug product CQAs, to understand and predict sources of variability in the manufacturing process so that an appropriate control strategy can be implemented to ensure that the CQAs are within the desired requirements. The relative risk that each attributes present was ranked as high, medium and low shown in Table no. 2.

The risk assessment for drug substance attributes on the drug product CQAs (as a Critical drug substance attributes), formulation variables on drug product CQAs and manufacturing process variable (as a Critical Process Parameters) are shown in Table no. 3, 4 and 5.

Table No. 2: Table for risk ranking.

Level	Action to be taken
Low	Broadly acceptable risk. No further investigation is needed.
Medium	Risk is acceptable. Further investigation may be needed in order to reduce the risk.
High	Risk is unacceptable. Further investigation is needed to reduce the risk.

Table No. 3: Potential impact of drug substance attributes on drug product CQAs.

Drug Product CQAs	Drug Substance Attributes								
	Solid state form	Particle Size	Crystallinity	Bulk Density	Polymorphism	Solubility	Impurities	Assay	LOD
Appearance	High	Low	Medium	Low	Low	Low	Low	Low	Low
Dissolution	Low	High	Low	Low	Low	High	Low	Low	Low
Assay	Low	Low	Low	Low	Low	Low	Low	Medium	Medium
Blend uniformity	Low	High	Low	Low	Low	Low	Low	Low	Low
Deliverable volume	Medium	Low	Low	Low	Low	Low	Low	Low	Low
Powder Flow	Low	High	Low	High	Low	Low	Low	Low	Low
Viscosity	Low	Low	Low	Low	Low	Low	Low	Low	Low
pH	Low	Low	High	Low	Low	Low	Low	Low	Low
Sedimentation	Low	Medium	Low	Low	Low	Low	Low	Low	Low
Redispersibility	Low	Medium	Low	Low	Low	Low	Low	Low	Low
Stability	Low	Low	High	Low	High	Low	High	Low	Low

**Based upon the physicochemical properties of the drug substance, the initial risk assessment of drug substance attributes on drug product CQAs was determined.*

Table No. 4: Potential impact of formulation variables on drug product CQA's.

Drug Product CQAs	Formulation Variables					
	Sucrose	Sodium Phosphate	HPC	XG	Color	Flavors
Appearance	High	Low	low	Medium	High	Low
Assay	Medium	Low	Low	Low	Low	Low
Dissolution	Low	Low	Low	Low	Low	Low
Blend uniformity	Low	Low	Low	Low	Low	Low
Moisture absorption	High	Low	Low	High	Low	Low
Viscosity & Pourability	High	Low	High	High	Low	High
Microbial limit	Low	Low	High	High	Low	High
Suspendability	Low	Low	High	High	Low	High

**Risk assessment was conducted to determine the potential impact of the excipients on final product quality.*

Table No. 5: Potential impact of manufacturing process variables on drug product CQA's.

Drug Product CQAs	Manufacturing process variables						
	Sugar Drying	Dry-Mixing	Granulation	Drying	Sifting (Milling)	Blending	Filling
Appearance	Medium	Low	Low	low	Low	Low	Low
Assay	Low	High	Medium	Low	Medium	High	Low
Dissolution	Low	Low	Medium	Low	Low	Low	Low
Blend uniformity	Low	High	Low	Low	Low	High	High
Moisture absorption	Medium	Low	Low	Medium	Low	Low	Low

**Risk assessment was conducted to determine the potential impact of the manufacturing process variables on final product quality.*

Formulation development of suspension

Use of Central Composite Design

A CCD is the most commonly used response surface design experiment. CCD are a factorial or fractional factorial design with center points, were added to detect any curvature effect.

CCD is an experimental design technique in which the factors involved and their relative importance can be assessed. In the present study, a full factorial design and three center points were added to detect any curvature effect for the estimation of pure error was chosen.[10] CCD was employed containing two factors evaluated at three levels and experimental trials were performed at all possible combinations. The levels, low (-1), medium (0), high (+1) of the two independent variables(X1, X2) were selected 2, 4, 6 and 0.1, 0.3, 0.5 respectively. X1, X2 are XG and HPC respectively and Y1, Y2 are viscosity and phase separation volume.

Drug –excipients compatibility study

The first stage of formulation development usually involves excipients compatibility studies to select excipients that are physically compatible with the API. Azithromycin dihydrate was mixed with excipients in different ratios. These mixtures were filled in glass vials and packed properly and exposed to 60°C for closed condition and 30^0C ± 2^0 C/75%RH ± 5% RH,40°C ± 2^0/75% RH ± 5% RH for an open and closed condition for period of 4 weeks. FTIR (Jasco, 4100) was used for spectrum determination.[11]

Development of Azithromycin reconstitutable suspension

Azithromycin dihydrate, lactose supertab 11SD, and sucrose (in a small amount) sifted through #40 sieve and remaining quantity of sucrose co-sifted with sucralose through #40sieve. The above dry mix was added into rapid mixing granulator and blending was carried out for 10 min. The accurately weighed quantity of HPC was socked in a required amount of water to get a clear solution and this solution was used as binder for the granulation. The wet damp mass was prepared by adding binder into preblended dry mix in RMG. The wet mass was formed into granules and dried in the fluidized bed dryer (FBD) and further passed through #20 sieve after drying and flow properties of granules were checked. In these granules XG, sodium phosphate, flavor peppermint, menthol, colloidal silicone dioxide, sucralose and castor sugar (which were previously sifted through the #40 sieve) were added. Then final blending was carried out in a double cone blender for 15 minutes at 25 RPM.[12] Trial batches are shown in Table 6.

Table No.6: Composition of Reconstitutable suspension (30ml).

Sr.No.	Ingredients	Quantity per bottle												
	Batch no.	1	2	3	4	5	6	7	8	9	10	11	12	13
	HPC concentration	4	6	4	4	4	4	2	4	2	6	4	6	2
	XG concentration	0.3	0.3	0.5	0.3	0.1	0.3	0.1	0.3	0.5	0.1	0.3	0.5	0.3
	dry mix													
1	Azithromycin dihydrate	1.258	1.258	1.258	1.258	1.258	1.258	1.258	1.258	1.258	1.258	1.258	1.258	1.258
2	lactose supertab 11 SD	2.25	2.06	2.1	2.25	2.07	2.25	2.28	2.25	2.29	2.07	2.25	2.1	2.1
3	Sucrose	17	16.12	16.56	17	16.56	17	17	17	17	16.12	17	16.12	17
	binder													
4	HPC	0.44	1.32	0.88	0.44	0.88	0.44	0.44	0.44	0.44	1.32	0.44	1.32	0.44
5	purified water	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s
	blending													
6	XG	0.02	0.06	0.1	0.02	0.02	0.02	0.02	0.02	0.11	0.02	0.02	0.11	0.06
7	sodium phosphate	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
8	Flav.pappermint	0.17	0.03	0.03	0.17	0.03	0.17	0.17	0.17	0.03	0.03	0.17	0.03	0.03
9	Menthol	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
10	Aerosil	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
11	sucralose	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
12	sucrose	0.4	0.67	0.63	0.4	0.7	0.4	0.4	0.4	0.4	0.7	0.4	0.63	0.67
	Total (g)	22.03	22.01	22.05	22.03	22.01	22.04	22.06	22.03	22.02	22.01	22.04	22.07	22.05

Micromeretic studies

The formulated powder batches for oral suspension were evaluated for their bulk density, tapped density, Carr's index and Hausner's ratio.

Evaluation of the design

The formulated factorial batches exhibited Quadratic model for each response. The relation between the independent and dependent variables were also investigated. The quality of a model was evaluated by using ANOVA.

ANOVA analysis of the model

The ANOVA-test of a model, the coefficient of the equation, was calculated by Design of Experiment software. If the coefficient's variance $p<0.05$, it could be conclude that the corresponding terms had significant value within the equation.

Model validation

The process was optimized for the responses like viscosity and phase separation volume. The results clarified an optimum setting for the azithromycin oral suspension. To verify the reproducibility, a formulation

with 0.3% XG concentration and 4% HPC concentration formulated and evaluated for response study.[1]

Analysis of suspension

Description

The formulated suspensions were analyzed for color and taste.

Assay

Mobile phase preparation

A 5.4 g of dipotassium hydrogen phosphate (K_2HPO_4) was added in 1000ml of water and sonicated for 5-10 minutes to get a completely clear solution. The pH 8.0 was adjusted with octadecylphosphonic acid and used as buffer in preparation of mobile phase with the acetonitrile in ratio of 30:70% v/v.[13] Mobile phase itself used as diluents for the dilutions of solutions and injected the solutions into HPLC system (waters; 2998).

DISSOLUTION

Preparation of buffer pH6.8 as dissolution media

A 6.8g of potassium hydrogen phosphate was dissolved in 1000ml of distilled water and sonicated to get clear solution and the pH was adjusted to 6.8.

To carry out the dissolution studies, 900 ml of buffer pH 6.8 was used as a dissolution medium. The paddles were stirred at 50 RPM. Suspension samples equivalent to 200mg/5ml of Azithromycin was transferred to the vessel. A 5 ml samples were withdrawn at 30, 45 minutes time interval and dissolved amount was determined by HPLC (waters; 2928) at 210nm.

Sedimentation characteristics

To study sedimentation in suspensions, the sedimentation volume was determined as a function of time. The sedimentation volume, F, is the ratio of the final equilibrium volume of the sediment Vu, to the total volume V_o before settling, as expressed $F = V_u/V_o$.[14]

In this study 30.0 ml suspension was transferred in a 50 ml measuring cylinder. The sedimentation volume F was determined after 24 hr.

Rheology study

Rheology study helps in determining the settling behavior of the suspension. Brookfield viscometer can be used for evaluating viscosity of suspensions. In this study Brookfield digital viscometer (Model DV-II+PRO) equipped with spindle No. 2 at 60 RPM was used to measure the viscosities.[6]

Reconstitution study

The reconstitution study was carried out after the preparation of trial batches. The amount of water (ml) required for the reconstitution was measured. This study was carried out according to the final weight of dry powder and final volume of dry powder suspension (ml) for that what amount of purified water is required, the amount of water is measured.

pH

It is another important parameter for the suspension stability. The pH determination study was carried out by using digital pH meter(Mettler Toledo).The pH meter was calibrated and the electrode was dipped into the suspension sample and pH was measured at room temperature.[15]

Deliverable volume

Contents of the container were emptied as completely as possible and the volume of the contents was determined. The volume should not be less than amount stated on the label.[16]

Resuspendability

If a pharmaceutical suspension produces sediment on storage, it is essential that it should be readily dispersible on shaking so that uniformity of dose is assured. Resuspendability of suspension was checked by inversion of cylinder normally to 180^o and number of inversions required to redisperse the sediment layer into pourable suspension was noted.[17]

Stability study

Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. In this study the formulations are charged at 30^0C/75%RH, 40^0C/75% RH continuously for six months. After completion of each time interval of 1, 2, 3, 6 months formulation are analyzed for assay, dissolution and physical parameters.[18]

RESULTS AND DISCUSSION

The formulation development of Azithromycin reconstitutable oral suspension 200 mg/5 ml was carried out with different concentrations of XG and HPC by using QbD approach which includes identification and risk assessment of QbD parameter such as drug substance attributes, formulation variables and manufacturing process variables. Justification for the risk assessment of drug substance attributes, formulation variables and manufacturing process variables are shown in Table 7, 8, 9.

Table No.7: Justification for the risk assessment of drug substance attributes.

Drug substance attributes	Drug Product CQAs	Justification
Appearance	Organoleptic properties	API color, odor, texture and taste have major impact on final product appearance hence risk is high.
Particle size	Dissolution	API is Class II drug; micronization may be required for better dissolution, hence risk is high.
	Blend uniformity	Particle size affects the uniform distribution of API, hence risk is high.
	Powder flow	Flow of the powder and fill weight may vary, risk is high.
	Sedimentation	A larger particle settles faster, risk is medium.
	Redispersibility	Fine particles form smooth and easy to redisperse suspension, risk is medium.
Crystallinity	Stability	Different temperature dependent polymorphic conversion leads to instability, risk is high
Polymorphism	Stability	Dihydrate form is stable during stability, risk is high.
Impurities	Stability	API is highly prone to oxidative degradation leading to potency loss hence risk is high.

Table No. 8: Justification for the risk assessment of formulation variables.

Formulation variables	Drug Product CQAs	Justification
Sucrose	Organoleptic properties	Drying of the sugar to remove water in crystal lattice is highly important as it causes powder to form agglomerates leads to changing physical description of powder, risk is high.
HPC	Sedimentation and Viscosity	Concentration must be optimized. Risk of phase separation sedimentation is high.
XG	Sedimentation and Viscosity	Concentration must be optimized. Risk of phase separation and sedimentation is high.

Table No. 9: Justification for risk assessment of manufacturing process variables.

Process Steps	Drug Products CQAs	Justification
Sugar drying	Moisture absorbance	If sugar is not properly dry, it causes lumps formation hence risk is medium.
	Appearance	Lumps formation takes place, which affect the appearance of final formulation, hence risk is medium.
Dry-mixing	Assay	Mixing process variables may impact the distribution of API in the blend which could impact blend uniformity and ultimately content uniformity hence risk is high.
	Blend Uniformity	Assay can get affected by mixing. The risk is high.
Granulation	Assay	During granulation processing can potentially impact the PSD of the granules, thus impact on flow .Thus risk is Medium.
	Dissolution	During granulation processing can potentially impact the PSD of the granules, thus impact on flow .The risk is Medium.
Sifting (Milling)	Assay	If milling generates excessive fines, both bulk density and flow of the blend may be impacted. This may impact content uniformity hence risk is medium.
Blending and filling	Assay	Improper blending of granules affects assay and content uniformity of final product.
	Dose uniformity	Improper filling of a container leads to dose variation.

The design of experiments (DoE) technique was used to provide an efficient means to optimize the process variables. DoE is an approach for effectively and efficiently exploring the effect relationship between numerous process variables and the output. A sequence of experiments were performed that would yield the most information about the factors and their interactions in as few experiments as possible. A CCD technique was employed to investigate the variables like viscosity and phase separation volume using the Design Expert Software (version 10.05 state-ease). Actual design with factors and responses are shown in Table 10.[19]

Table No. 10: Actual design with factors and responses.

std	run	Factor 1:HPC(mg)	Factor 2:XG (mg)	Response 1:viscosity (cps)	Response 2: phase separation volume (ml)
12	1	4	0.3	1.23	2
6	2	6.82843	0.3	1.39	13
8	3	4	0.582843	3.49	10
9	4	4	0.3	1.22	2
7	5	4	0.0171573	0.74	14
11	6	4	0.3	1.26	3
1	7	2	0.1	0.49	17
13	8	4	0.3	1.24	2
3	9	2	0.5	1.96	10
2	10	6	0.1	0.98	18
10	11	4	0.3	1.25	3
4	12	6	0.5	3.25	1
5	13	1.17157	0.3	0.76	15

The responses given by the software are expressed in terms of the Quadratic equations which are given below.

Final Equation in Terms of Coded Factors for viscosity

Viscosity = +1.24 +0.33*A+0.95*B+0.20*AB-0.064*A^2+0.46*B^2

The Model F-value of 121.55 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

The R^2 (0.9886) was high indicating the adequate fitting of the quadratic model. The "Pred R-Squared" of 0.9196 is in reasonable agreement with the "Adj R-Squared" of 0.9805; i.e. the difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here the ratio of 34.123 indicates an adequate signal. The polynomial equations can also be used to draw conclusions considering the magnitude of co-efficient and the mathematical sign it carries; i.e. positive or negative.

Final equation in terms of coded factors for phase separation volume

Phase separation volume = +2.40-1.35*A-3.71*B-2.50*AB+5.43*A^2+4.42*B^2

The Model F-value of 12.39 implies the model is significant. There is only a 0.23% chance that an F-value this large could occur due to noise.

The R^2 (0.8985) was high indicating the adequate fitting of the quadratic model. The "Pred R-Squared" of 0.2911 is not as close to the "Adj R-Squared" of 0.8259 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with model. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio is 8.010 indicates an adequate signal.

Response surface plot

The quadratic model obtained from the regression analysis used to build a 3D graphs in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots. The response surface plot were generated using Design Expert 10.05 software presented in Figure 1 and Figure 2 to observe the effects of independent variables on the response studied such as viscosity and phase separation volume respectively. Graphical presentation of the data helped to show the relationship between the response and the independent variables.

The positive coefficient of variable X1 i.e. XG in case of response viscosity indicates that as the XG concentration was increased the viscosity was also increased, where the increase in concentration of HPC does not have significant effect on the increase in viscosity and thus the effect of concentration of XG is more than the HPC on viscosity. The relationship between the variables was further elucidated by using the response surface plot Figure 1.

As the concentration of HPC increases from 2-4mg, the decrease in phase separation volume was observed. Similarly, as the concentration of HPC increases from 4-6mg, the increase in phase separation volume was observed. However, the concentration of XG increases from 0.1 to 0.5 mg, there was slight increase in phase separation volume Figure 2.

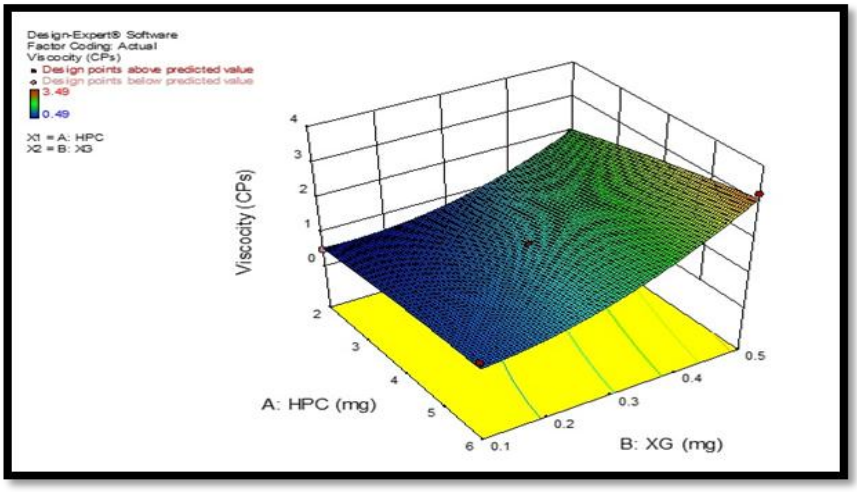

Figure 1: Response surface plot showing, influence of XG & HPC on viscosity of suspension.

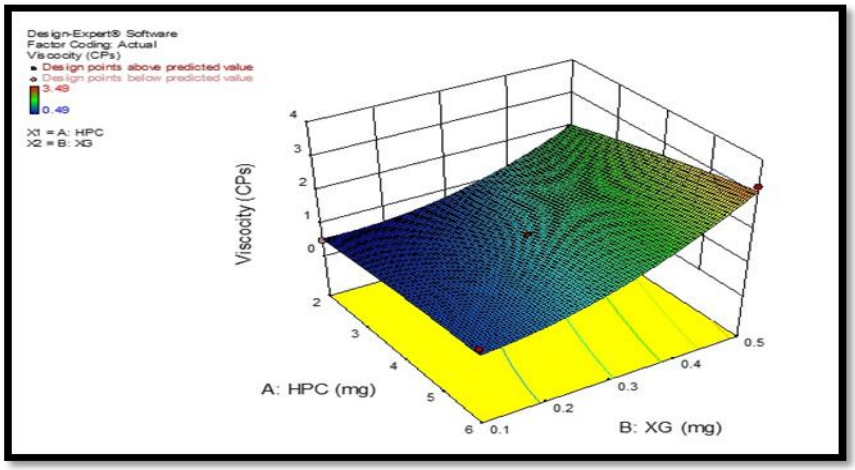

Figure 2: Response surface plot showing, influence of XG & HPC on phase separation volume of suspension.

Compatibility studies confirmed that there were no chemical interactions between the drug and excipients Table 11.

Table No.11: Compatibility study.

Drug+ Excipients	Proportion (w/w)	Observation		Conclusion
		Initial	At the end of 4th week	
Drug	1	White	No change	Compatible
Drug+ lactose supertab11SD	1:0.25	White	No change	Compatible
Drug + castor sugar	1:1	White	No change	Compatible
Drug+ HPC	1:1	off White	No change	Compatible
Drug + Xanthan gum	1:1	off White	No change	Compatible
Drug + sodium phosphate	1:1	White	Compatible	Compatible
Drug + Menthol	1:0.25	White	No change	Compatible
Drug + flavour peppermint trusil	1:0.25	White	No change	Compatible
Drug +Aerosil	1:0.25	White	No change	Compatible
Drug + Sucralose	1:0.25	off White	No change	Compatible

The pH of all formulations was found in the range of 8.51-8.67. All formulations fulfilled requirement of deliverable volume. Results of resuspendability showed that F1 to F7, F12, and F13 were readily redispersed without any signs of caking. F8, F9, F10 and F11 required more number of inversions to redisperse the sedimented layer into pourable suspension. The water content of all formulations was found in the range of 0.75-0.45% Table 12. The granule flow of all formulations was found to be excellent. The water (ml) required for the reconstitution was found in the range of 17-19ml. The F1, F4, F8, and F12 showed minimum phase separation volume. The assay was found in the range of 99-105% for all formulations and it was present in the specified limit (i.e.±10%). The dissolution of all formulations was found in the range of 87-97% Table 13.

Table No. 12: Evaluation of formulated oral reconstituted suspension of Azithromycin.

Trials	pH	Time for Sedimentation (hr)	No. of strokes for redispersible	Deliverable volume (ml)	Water content (%)
1	8.51	3	3	30±1	0.83
2	8.63	2.30	3	27±1	0.79
3	8.64	3	3	26±2	0.84
4	8.48	2.45	3	29±1	0.80
5	8.62	3	3	28±1.5	0.75
6	8.52	3	3	27±1	0.79
7	8.73	2	3	30±1.5	0.83
8	8.47	3	4	28±1.5	0.81
9	8.61	3	5	28±1	0.78
10	8.64	2	5	27±1.5	0.82
11	8.51	3	5	27±2	0.82
12	8.63	2.30	3	27±1	0.79
13	8.67	2.30	3	29±1.5	0.85

Table No. 13: Evaluation of formulated oral reconstituted suspension of Azithromycin.

Trials	Granules Flow	Water required for reconstitution(ml)	Phase separation volume (ml)	Viscosity (CPs)	Assay (%)	Dissolution (%)	Blend uniformity (%)
1	Excellent	19 ±0.57	2	1.23 ±0.02	101 ±1.5	94 ±1.5	103.9 ±1.5
2	Excellent	18 ±0.53	13	1.39 ±0.01	99.3 ±1.0	89 ±2.0	93.54 ±1.0
3	Excellent	19±0.45	10	3.49 ±0.04	105 ±1.0	87 ±1.0	101.1 ±1.4
4	Excellent	19±0.54	2	1.30 ±0.02	101 ±1.5	88 ±1.5	98.32 ±1.0
5	Excellent	19±0.57	14	0.74 ±0.01	105 ±1.0	97 ±2.0	98.59 ±1.0
6	Excellent	19±0.54	3	1.26 ±0.01	99.7 ±1.0	97 ±1.0	97.65 ±2.0
7	Excellent	17±0.57	17	0.49 ±0.02	98.2 ±2.0	81 ±1.5	91.23 ±1.0
8	Excellent	18±0.55	2	1.22 ±0.01	99.4 ±1.0	97 ±2.0	90.15 ±1.0
9	Excellent	17±0.54	10	0.96 ±0.01	100 ±1.5	97 ±1.0	95.67 ±2.1
10	Excellent	18±0.52	18	0.98 ±0.02	98.4 ±2.0	90 ±1.5	98.75 ±1.5
11	Excellent	18±0.54	3	1.25 ±0.03	101 ±1.5	87 ±1.0	93.56 ±1.0
12	Excellent	18±0.57	1	1.39 ±0.02	99.5 ±1.0	92 ±1.5	98.1 ±2.0
13	Excellent	17±0.55	15	0.76 ±0.01	98.2 ±2.0	90 ±1.5	95.67 ±1.4

The optimized batches given by Design of Experiment software according to the criteria having HPC, XG and viscosity were selected in range, and phase separation volume selected as a minimum. Table14. Stability studies were accomplished for a period of 6 months at 30^0C/75 RH and 40^0C/75RH Table 15.

Table No. 14: Optimize batches given by the DoE software.

Number	HPC(mg)	XG(mg)	Viscosity	Phase separation volume(ml)	Desirability	
1	4.473	0.397	1.910	1.339	0.980	Selected
2	4.469	0.395	1.896	1.340	0.980	

Table No. 15: Stability data stored at 30^0C/75RH and 40^0C/75RH.

Time in months (30^0C/75RH)					
Parameter	**Initial**	**1**	**2**	**3**	**6**
Colour	**White coloured free flowing**	**Complies**	**Complies**	**Complies**	**Complies**
pH	8.36	8.31	8.30	8.27	8.25
Viscosity (CPs)	1.23	1.23	1.23	1.21	1.19
Assay (%)	104	98.4	95.4	87.6	87.2
Dissolution (%)	89	96	86.5	85.2	85.0
40^0C/75RH					
Colour	**White coloured free flowing**	**Moisture absorption and lumps formation**	**Complies**	**Complies**	**Complies**
pH	8.36	8.31	8.27	8.29	8.25
Viscosity(CPs)	1.23	1.23	1.23	1.20	1.20
Assay (%)	104	99.3	96.9	86.4	86.1
Dissolution (%)	89	96	85.1	83.7	82.3

Analysis of marketed product

The marketed product ZITHROMAX 200 mg/5ml manufactured by Pfizer, Singapore was evaluated for the parameters like viscosity, pH, phase separation volume, dissolution and assay. The optimized formulation was compared with the marketed product Table16.

Table No. 16: Comparison of marketed product with optimized formulation.

Evaluation parameter	Marketed product	Optimized formulation
Assay (%)	103.5	101
Dissolution (%)	103	94
Viscosity (CPs)	2.45	1.25
Phase separation volume (ml)	No phase separation	2
pH	7.89	8.51

CONCLUSION

A CCD was performed to study the effect of formulation variables on viscosity and phase separation volume applying a QbD optimization technique. XG is the major factor affecting the viscosity and phase separation volume. The statistical approach for formulation optimization is a useful tool, particularly in simultaneously evaluating several variables. The observed responses were in close agreement with the optimized formulations, demonstrating the feasibility of the optimization procedure in developing stable suspension. The developed formulation was found comparable with the marketed formulation (Zithromax suspension, Pfizer) and suitable for large scale manufacturing.

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