Original Article

Factorial Design Methodology for Development of Pediatric Nasal Spray: Study on Xylometazoline Nasal Solution Used For Treatment of Nasal Congestion

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ABSTRACT

Objective: The objective of the present work is to develop the meter dose pediatric formulation of xylometazoline hydrochloride for enhanced effectiveness for nasal decongestion. **Experimental Work:** The 3^2 factorial experimental design was employed for optimization of sodium cholate (X_1) and Polyethyleneglycol 400 concentration (X_2) in the formulation. The optimized formulation contains 1.18 % w/v sodium cholate and 13 % v/v Polyethyleneglycol 400. The formulation was further evaluated for its drug content, pH, viscosity, spray content uniformity, *In vitro*-diffusion studies, pump delivery, spray pattern, sterility and stability study. **Results:** The percentage diffusion and viscosity was observed 91.33 ± 1.15 % and 19.33 ± 0.57 centipoise respectively. The meter dose nasal formulation shows the superior performance during *in-vitro* drug release compared with current marketed formulations, which provides alternative option for treatment of nasal decongestion.

Key words: Nasal congestion, Factorial design, Meter dose formulation, Xylometazoline hydrochloride, Sodium cholate.

INTRODUCTION

The pharmaceutical arena is constantly in search for novel drug delivery systems that can overwhelmed the existing issues. The nasal drug delivery is unique route for inhibiting drug degradation from first pass metabolism.[1,2] About 2% of the drug that is conveyed through nasal route, since relatively large surface is available for this route which is vascularized along with the leaky epithelium.[3,4,5]

Allergic Rhinits or Sinusitis has one of the main indication as nasal congestion, which is related to enlargement of blood vessels and contracting of nasal passages.The nasal mucosa is destined tightly and depict its less permeability. It is postulated that the nasal mucosal integrity is refurbished because of creation of protective barrier preventing contact with external irritants. This barrier mingles with the mucosal layer thus upholding protective coating upto extended time period, thus ensuring insulation towards substances involved in local infection.[6]

Imidazole class derivatives like xylometazoline act on alpha-adrenergic receptors of nasal mucosal arterioles leading to decrease of blood flow. Thus reducing the swelling of nasal turbinate's, relating to amplification of nasal lumen.[7,8,9]

Nasal congestion was the most frequent primary presenting complaint that is associated with rhinitis. It has been proposed after assessments that approximately 31 million patients across United States are affected every year which have been progressively recognized that larger populations are to be affected (considering mixed rhinitis).[8,10,11]

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The present work was performed out with a vision to develop pediatric nasal spray of Xylometazoline hydrochloride by utilization of sodium cholate (as bile salt) and PEG400 to provide effective and enhanced drug delivery of xylometazoline hydrochloride. The meter dose spray formulation helps to prevent the uneven delivery of formulation, inconstant bioavailability and nasal irritation. The formulation pediatric nasal spray was characterized for drug content, spray content uniformity, pump delivery and spray pattern.

MATERIALS AND METHOD

Materials

Xylometazoline HCl was acquired from Anish Chemicals, Gujarat, India. Sodium cholate was procured from National Chemicals Pvt. Ltd, Vadodara, India. PEG 400 was obtained as gift sample from Sigma Aldrich, USA. Methyl Paraben was obtained from S.D. Fine Chem. Ltd, Mumbai, India and Sodium carboxymethyl cellulose was obtained from Amar cellulose industries, Gujarat, India. The container closure system was obtained from Aptar Pharma, Mumbai, India.

Methods

Preparation of Xylometazoline HCl nasal solution

The drug substance contained nasal solution was developed by sequential mixing of various excipients as shown in Figure 1.

Experimental Design

The independent variables were optimized using 3^2 factorial experimental design. The preliminary data from experiment advise that the excipients sodium cholate (X_1) and PEG 400 (polyethyleneglycol 400) (X_2) have foremost impact on the other formulation parameters, henceforth selected as independent variables. The optimization was accomplished by utilization of 3 level 2 factors factorial (3^2) experimental design. (Stat- Ease Design Expert®, v 9).

The levels of independent variables were designated as depicted in Table 1 and all the batches were formulated conferring to experimental design. (As per Table 2) The batches were then assessed for different parameters.

Equation 1 below demonstrations the polynomial equation for 3^2 experimental design.

$$Yi = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 12X1X2 \quad \text{(Eq.1)}$$

Where Y = dependent variable

β_0 = Intercept (arithmetic mean of all the batches) runs,

β_1 = estimated coefficient for the factor X_1.

B_2-Estimated coefficient for the factoe X_2

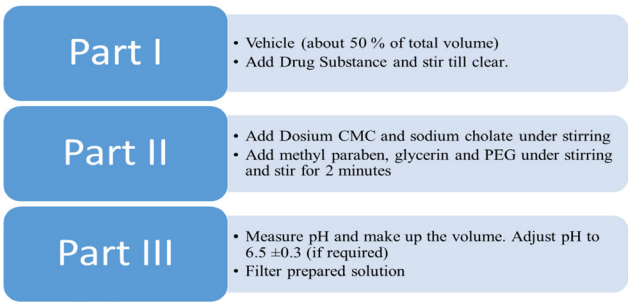

Figure 1: Method of preparation of nasal solution

B_{12}= Estimated coefficient of the interaction between X_1 and X_2

Evaluation parameters of formulation

Appearance and Clarity

The developed nasal solution were assessed as in-house test for appearance (color) as well as their clarity. The color and clarity were visually examined against black and white surface in inspection booth.[12]

Drug Content

The drug content estimation of prepared pediatric nasal solution was accomplished as per the method reported in the USP product monograph.[13] The amount of xylometazoline hydrochloride in mg per mL of the nasal solution was calculated by the formula stated in equation 2:

$$\left(\frac{0.05C}{V}\right)\left(\frac{Au}{As}\right) \quad \text{(Eq.2)}$$

Where, C is the concentration, in µg per mL, in the Standard preparation,

V is the volume, in mL of Nasal Solution,

A_U is the absorbance of the solution for the Assay preparation

A_S is the absorbance of the solution from the Standard preparation

pH

The pH of the pediatric nasal formulation play important role to withstand normal physiological ciliary movement. Lysozyme present in secretions of nasal mucosa, is mainly responsible for destroying certain types of pathogenic micro-organisms at acidic pH. This Lysozyme is neutralized or disabled at basic pH leading to susceptible to microbial infection. All prepared formulations were measured in triplicate for pH using by digital pH meter and average value was considered. The ideal pH range of nasal formulations was in range of 6.5 ± 0.3.[14,15,16]

Viscosity

Viscosity has noteworthy influence on the residence time of formulation, which is directly related to rate of drug absorption through nasal mucosa. Higher the viscosity of the solution, it will shows the higher rate of absorption due to increased residence time. The viscosity of the prepared solution was measured through Brookfield Viscometer (Spindle no S18 at 100 rpm) (Brookfield Viscometer, Model no LVDL-2T, Dolphine instrument, Mumbai).[11]

Spray Content Uniformity

The spray content uniformity as important parameter for nasal spray, was studied to investigate the spray discharged from the nosepiece for the drug substance content. The examination was carried out for multiple spray form single container and in different container for the same. This test will determine an overall performance evaluation of the formulated batch, which will assess the pump selection. The test was accomplished with units primed following the instructions of the labeling. The amount of drug substance delivered from the nose-piece can be stated as the percent of label claim. The acceptance criteria was nominated as the amount of drug substance not outside of 80-120 percent of label claim for more than 1 of 10 containers, none of the determinations is outside of 75–125 percent of the label claim, and the mean is not outside of 85-115 percent of label claim.[17,18]

Pump Delivery

The pump delivery and spray content uniformity are inter connected parameters demonstration substantial impact on the product performance. The formulation was tested for pump-to-pump reproducibility and the metering ability of the pump. The formulation was filled into the container closure, which was further actuated for 10 times in a pre-weighed bottle. The weight of the bottle was reweighed after 10 actuations and the difference was calculated.[12]

Spray Pattern

The charactarization of spray is the medium through which the performance of the pump and the nozzle of container closure system need to be assessed. Those factors which have noteworthy impact on the pattern of spray comprises of nozzle size and shape, pump, and the formulation. In the evaluation of the spray pattern, the spray distance between the nose-piece and the collection surface, orientation of the nose-piece, and visualization procedure are specified. Spray Pattern of prepared pediatric nasal spray formulation was measured by the SprayVIEW system (Proveris Scientific Cooperation, USA) furnished with the SprayVIEW automated pump actuation system. The parameters of spray pattern assessment are height at 30 mm, evacuation time 15000 millisecond, inclination as 63.4 ° and summation mode as automatic.[17,18]

Net Content and Weight Loss

The regulatory guildeline published by US FDA for nasal products displays that the net content and weight loss are important parameters that determines the product performance and stability. Inverted and Horizontal positions (i.e. both the orientations plays significant role in weight loss) were kept for the drug product storage to assess this characteristic. The net content of formulation in the container closure system was determined as per USP chapter <755> and it is essentially to be in accordance with the predefined specification.[13,17,19]

Priming and Repriming

The first priming essentially to demonstrate the minimum amount of drug released from the product, while repriming shows the ability of product to delivery same amount of drug content after storage of product. The length of storage for conducting the study is defined as 5, 10 and 30 days. The number of actuations are determined that are required for priming until the subsequent doses meet the specification limits (80-120 % label claim). The number of actuations are determined that are required for re-priming up to the subsequent doses meets the specification limits.[13, 18]

In-vitro Diffusion study

The *In-vitro* diffusion study was performed using the Franz diffusion cell. The recently detached sheep nasal mucosa, was collected from a local slaughter house. The superior nasal membrane was identified and separated from the nasal cavity. The nasal mucosa was sensibly removed and submerged in phosphate buffer (along with aeration) for conserving the tissue. Sheep nasal mucosa was mounted on diffusion chamber with the mucosal side facing the donor phase. The doner medium comprised of formulation, while the receptor medium comprised of phosphate buffer. The temperature of the medium was maintained at 37 °C ± 1 °C. The receptor medium was refilled with the equivalent volume of the fresh solution as the samples withdrawn. The samples were then analyzed spectrophotometrically at a wavelength of 265 nm against blank. The obtained results are compared with the current marketed formulation Otrivin® Pediatric (Novartis).[20,21,22]

Droplet Size Distribution

The *in-vivo* deposition of the formulation in cavity is affected by droplet size distribution of the formulation spray. The major factor that influences the droplet size is the delivery device and the formulation. The USFDA guideline for nasal aqueous spray formulation states that there must be appropriate control for droplet size distribution of the delivered plume. The droplet size distribution can be controlled in terms of D_{10}, D_{50}, and D_{90} bu utilization of laser diffraction.[23,24]

Sterility

Sterility is one of the most mandetory requirement for nasal preparation. The tests for sterility are expected for discovering the presence of possible forms of microorganisms in the formulation. The reason behind the test is that at favorable temperature and nutrition conditions the microorganisms will grow, and this can be identified by turbidity in clear medium. The method used to perform sterility of the product to explore the presence of aerobic, anaerobic bacteria and fungi was as per USP general chapter sterilitiy tests <71>. [25]

Stability study

The stability studies were executed at 25 ± 2°C/ 60 ± 5% RH for stability stations 3, 6, 9 and 12 months for long term studies, while at 40 ± 2°C/ 75 ± 5% RH for accelerated studies at stations 3 and 6 months.[26]

RESULTS AND DISCUSSION

Priliminary experiments and composition of nasal formulation

For nasal solution formulations, viscosity enrichers could be essential for prevention of formulation drainage. Conversely, an extreme enhancement in viscosity of the nasal formulation may not be valuable because it leads to inconvenience accompanying with delivering such an high viscosity formulations with comfort and uniformity. A nasal solution formulation appears very pretty since this formulation is fluid or liquid as nasal administration and can straightforwardly be inculcated. Nasal solutions with appropriate viscosity will prevent drainage and thus increase the maintenance time of the dosage form at the mucus membranes. The long dwelling time of the solution formulation surges the geometric probability for satisfactory drug permeation.[20]

Eskandar Moghimipour *et al.* investigated bile salts as absorption enhancers for nasal delivery.[13] The bile salt absorption enhancement mechanism comprises of membrane protein or lipid extraction, fluidization of membrane, reverse micelle production within membrane thus creating aqueous channels. Higher concentrations of bile salts, leads to membrane lipids extraction forming micelles, enhancing transcellular transport. Sodium Cholate is an excellent bile salt, which one of the most studied member of bile salt family. Polyethylene glycol (PEG) is a polymer of prime for drug delivery since USFDA has approved this polymer owing to its properties and well-known safety profile.[27] With several preliminary trials the concentration range for sodium cholate was finalized as 0.2 % - 1.2 %, while that of PEG400 as 5.0 % -15.0 %.

Experimental design

Nasal Solution for pediatric dose was successfully framed by applying 3^2 factorial design. As per the design layout, all the nine possible experimental trials were performed and further evaluation was conceded using polynomial equation of design. Additional four centre point trails baches thave been as per design of experiment. The invitro assessment for appearance, pH, viscosity, assay and % diffusion at 10 minutes was performed for formulated batches. However, by fluctuating the level of indeen pendent variables sodium cholate (X_1) and PEG 400 (X_2), the remarkable difference was observed in % diffusion at 10 minutes and viscosity, henceforward it was considered as dependent parameters.

The effects of independent variables (X_1 and X_2) on percentage diffusion at 10 minutes is depicted by equation 3:

Diffusion at 10 minutes (Y1)= 62.16 + 75.05 * X1 + 1344.82 * (X1)2 (Eq.3)

The value of sodium cholate changing from -1 to +1 shows the diffusion higher value obtained as 93 % at higher concentration of sodium cholate (X_1) with higher value of PEG 400 (X_2), while at lower value of X_1 with the same value of X_2 was found 64 %. It is observed from the different batches results that there is a linear relationship between percentage diffusion and sodium cholate (X1). The fabrication of countour and surface response graphs was performed by fluctuating level of independent variables for -1 to +1 for dependent parameters.

Table 1: Independent variables and their levels

Levels	Independent Variables	
	X1= Concentration of Sodium Cholate	X2 = Concentration of PEG400
-1(Low Level)	0.02 gm	0.5 ml
0 (Medium Level)	0.07 gm	1.0 ml
+1 (High level)	0.12gm	1.5 ml

The data of model summary statistic for diffusion at 10 minutes (Y_1) clearly suggest the quadratic model shows better suitability as compared to other statistic models. The Table 4 displays the independent variables impact on the percentage diffusion at 10 minutes. It can be concluded that the quadratic effect was found significant for sodium cholate (factor A) which is shown by its respective p value from Table 4. The data obtained reveals that the p-value < 0.05 stating that the factor has significant impact on dependent parameter. Hence conclusion can be derived that the both independent variables (Sodium cholate and PEG 400) have substantial impact on diffusion rate on formulation.

The effects of independent variables (X_1 and X_2) on viscosity is depicted by equation 4 as below:

Viscosity (Y2) = 10.01 + 13.33 * X1 + 6.67 * X2 (Eq.4)

It is also observed that the concentrations of sodium cholate (X_1) and PEG 400 (X_2) considerably affect the viscosity. The linear model shows the better fitting compared to other models as it is clearly depicted by different coefficient of linearity value. The influences of independent factors were as evaluated displayed in table 5. There was significant impact on viscosity observed for the both factors. The varying of the factor (X_1) form -1 to +1 demonstrates that there is minor impact on the viscosity of the formulation, while varying factor (X_2) from -1 to +1 demonstrates that the viscosity changes from 13 cp to 22 cp. It was observed that there was linear relationship between viscosity and PEG400 concentration. The creation was performed for contour and surface plot for viscosity.

The Table 6 indicates the percentage bias/error observed between the experimental value and the predicted value of various experimental batches. The percentage bias/error value for factor (X_1) diffusion at 10 minutes was found in the range of -1.81% to 3.14%, while the percentage bias/error value for factor (X_2) viscosity was found in the range of -4.62% to 4.40%. The percentage bias/error for both the variables were found below 5 %, which expressively shows that the model selected successfully fits to predict the response of the experimental design zone.

Optimization of experimental design

The design of experimental model was supplementarily authenticated by four check point batches. The evaluation for diffusion and viscosity of checkpoint batches was executed by using mathematics equation (3,4).

The Table 7 depicts the check point batches results for percentage diffusion at 10 minutes and viscosity. The optimization of formulation was conceded using the design expert software by creation of overlay plot as shown in the figure 2.

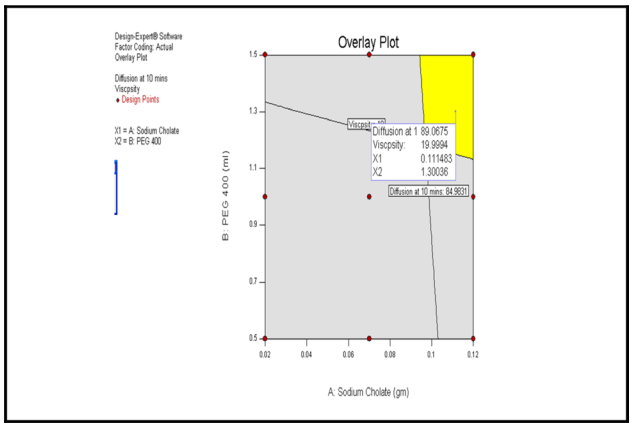

Figure 2: Overlay plot of optimized formulation

The optimized formulation contained sodium cholate 0.12 g (1.2 % w/v) and PEG 400 1.5 mL (15 % v/v) displayed in Table 8. This optimized formulation (batch number : PFE) displays the observed value for % diffusion at 10 min and viscosity are 91.33 % and 19.33 cps respectively, while the percentage bias/error with respect to the observed and the predicted responses are as per Table 7. The comparision of *in-vitro* diffusion results between marketed product and optimized formulated batch is revealed in Figure 4, indicating that the optimized formulation demonstrating higher diffusion at 10 minutes. (Refer Supplimentary Table 2) .

Other Evaluation parameters

pH of formulation

The importance of pH arises as higher or lower pH of formulation encourages irritation at the application site, thus making it necessity to be controlled for effective delivery. The formulation pH was found 6.5 ± 0.1 as depticted in Table 3 showing better compatibility at the delivery site.

Spray Content Uniformity

The formulations must be assessed in its relationships of emitted dose content uniformity. The performance evaluation of system by assessing the formulation, valve and the actuator is observed by Regulatory bodies like FDA as the control of content uniformity.

Nasal sprays are consist of active pharmaceutical ingredient dissolved in solutions or group of excipients in non-pressurized dispenser which transport/delivers a spray enclosing a metered dose of the active ingredient. The Table 9 data shows the spray content (Drug substance content) of the same container and among different containers. The results obtained are in the range of

Table 2: Composition of experimental batches													
Ingredients	**FA1**	**FA2**	**FA3**	**FA4**	**FA5**	**FA6**	**FA7**	**FA8**	**FA9**	**FA10**	**FA11**	**FA12**	**FA13**
Xylometazoline HCl(mg)	5	5	5	5	5	5	5	5	5	5	5	5	5
Sodium Cholate(gm)	0.02	0.12	0.02	0.12	0.07	0.12	0.07	0.2	0.07	0.07	0.07	0.07	0.07
PEG400(ml)	0.5	1	1.5	1.5	0.5	0.5	1.5	1	1	1	1	1	1
Sodium CMC (gm)	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
Glycerin (ml)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methyl Paraben(gm)	0.0033	0.0033	0.0033	0.0033	0.0033	0.0033	0.0033	0.0033	0.0033	0.0033	0.0033	0.0033	0.0033
NaCl	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs
Purified Water (ml)	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10	qs to 10

Table 3: Results of Clarity, pH, Assay, Viscosity and Diffusion as per factorial design layout					
Parameter: → Formulation ↓	**Clarity**	**pH**	**Assay (%)**	**Viscosity (cp)**	**% Diffusion at 10 minutes**
FA1	Clear Solution	6.5 ± 0.0	96.26 ± 0.40	13.33 ± 0.57	65.66 ± 1.52
FA2	Clear Solution	6.6 ± 0.0	102.91 ± 0.64	18.33 ± 0.57	93.33 ± 0.57
FA3	Clear Solution	6.6 ± 0.0	97.52 ± 0.74	21.33 ± 0.57	66.67 ± 0.57
FA4	Clear Solution	6.6 ± 0.0	104.25 ± 0.72	20.00 ± 1.73	91.67 ± 1.15
FA5	Clear Solution	6.6 ± 0.0	99.12 ± 0.75	14.33 ± 1.15	74.33 ± 1.52
FA6	Clear Solution	6.53 ± 0.05	104.09 ± 0.34	15.33 ± 0.57	90.33 ± 0.57
FA7	Clear Solution	6.6 ± 0.0	93.83 ± 0.85	19.33 ± 0.57	76.67 ± 1.52
FA8	Clear Solution	6.6 ± 0.0	101.97 ± 1.52	17.00 ± 1.00	64.33 ± 1.15
FA9	Clear Solution	6.6 ± 0.0	99.75 ± 1.89	18.33 ± 0.57	75.33 ± 0.57
FA10	Clear Solution	6.53 ± 0.05	99.41 ± 0.37	18.33 ± 0.57	75.33 ± 0.57
FA11	Clear Solution	6.6 ± 0.0	98.79 ± 0.44	18.00 ± 0.00	75.33 ± 0.57
FA12	Clear Solution	6.5 ± 0.0	99.17 ± 0.29	18.33 ± 0.57	75.33 ± 0.57
FA13	Clear Solution	6.53 ± 0.05	99.94 ± 0.61	18.33 ± 0.57	74.67 ± 1.52
PFE (Exhibit/Optimized Batch)	Clear Solution	6.5 ± 0.0	100.11 ± 0.40	19.33 ± 0.57	91.33 ± 1.15

Table 4 : ANOVA for Response Diffusion at 10 minutes						
ANOVA for Response Surface 2FI Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	**Coefficient**
Model	1088.272	5	217.6545	223.9047	< 0.0001 (significant)	
Intercept						62.1678
A-Sodium cholate	315.4212	1	315.4212	324.4789	< 0.0001	75.0574
B-PEG 400	2.096928	1	2.096928	2.157144	0.1854	
AB	0	1	0	0	1.0000	
A^2	31.21921	1	31.21921	32.11571	0.0008	1344.827
B^2	0.362069	1	0.362069	0.372466	0.5609	
Residual	6.804598	7	0.972085			
Pure Error	0.8	4	0.2			
Cor Total	1095.077	12				

Table 5: ANOVA for Response Viscosity						
ANOVA for Response Surface Linear Model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	**Coefficient**
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	69.3333	2	34.6666	60.3571	< 0.0001 (significant)	
						10.0153
A-Sodium cholate	2.6666	1	2.6666	4.6428	< 0.0001	13.3333
B-PEG 400	66.6667	1	66.6666	116.0714	0.0566	6.6667
Residual	5.7435	10	0.5743			
Pure Error	0	4	0			
Cor Total	75.0769	12				

95-102 %, which passes the test according to FDA guidelines for nasal products.

Pump Delivery

The suitable performance from pump is essentially required for precise drug delivery to targeted area. The assurance is provided by this test that the pump conveys the predefined dose consistently. The container closure system which was acquired from Aptar Pharma, is qualified with tip seal technology having capacity for 360 degree possible applications. The preliminary weight of filled nasal spray was 15 g. The final weight of spray system was found the 15 g after 10 actuations, with the actuation volume for each spray as 100 µl. The data recommend that the system was performing accurate pump delivery of the spray.

Spray Pattern

The performance of the pump can be evaluated with the help of spray pattern, which can be affected by various factors like the size and shape of the nozzle of pump, the design of the pump, and the formulation characteristics. The ovality was attained was 1.118, while perimeter and area were found to be 57.42 mm and 258.8 mm^2 respectively. Image actuation graph, along with intensity graph for the formulation are as presented in figure 3. The image actuation graph illustrates the force in kg and position in mm relating to spray pattern,. while the intensity graph illustrates the time in ms (millisecond) related to spray pattern. The figure confirms that the spray pump successfully delivered medication without any difficulty.

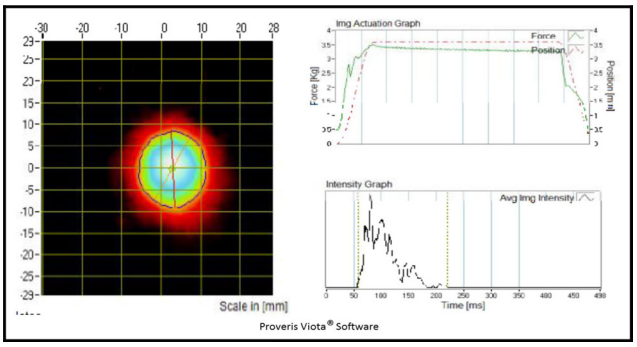

Figure 3: Spray pattern, Image Actuation Graph and Intensity Graph of formulation

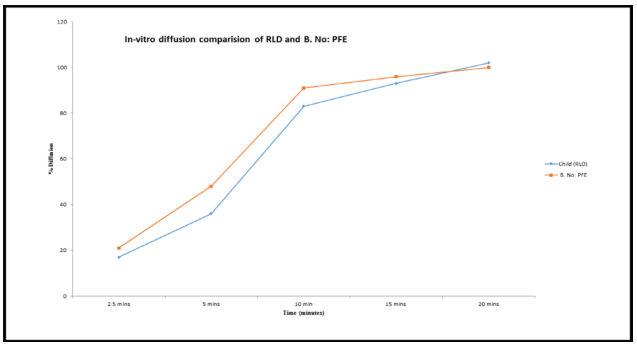

Figure 4: *In-vitro* diffusion comparison of marketed formulation and optimized batch

Table 6: Percentage Bias for experimental batches

Composition of Sodium Cholate	Composition of PEG400	Formulation	Calculated Value of Equation of viscosity	Exp Value of Viscosity	% Bias for Viscosity	Calculated Value of Equation of Diffusion	Exp Value of Diffusion	% Bias for Diffusion
0.02	0.5	FA1	13.53612	13	4.12	66.04829	65.0	1.61
0.12	1.0	FA2	18.33593	18	2.58	92.25872	93.0	-0.79
0.02	1.5	FA3	21.57293	21	4.40	68.19847	67.0	1.78
0.12	1.5	FA4	21.39826	22	-4.62	90.78253	92.0	-1.32
0.07	0.5	FA5	14.09836	14	0.75	76.32391	74.0	3.14
0.12	0.5	FA6	14.63972	15	-2.77	91.89379	90.0	2.10
0.12	1.5	FA7	19.39863	19	3.06	75.92832	77.0	-1.39
0.02	1.0	FA8	17.42961	17	3.30	62.84018	64.0	-1.81
0.07	1.0	FA9	18.19732	18	1.51	76.49572	75.0	1.99
0.07	1.0	FA10	18.14639	18	1.12	74.82321	75.0	-0.23
0.07	1.0	FA11	18.06472	18	0.49	75.71845	75.0	0.95
0.07	1.0	FA12	18.06928	18	0.53	74.56862	74.0	0.76
0.07	1.0	FA13	18.26249	18	2.01	75.45628	75.0	0.60

Table 7 : Check point batches results for dependent variables with % bias and comparison of predicted and observed responses of optimized formulation

Sr. No.	Conc. of sodium cholate	Conc. of PEG 400 Actual	Diffusion at 10 min (%)		Viscosity (cp)			
			Predicted	% Bias	Actual	Predicted	% Bias	
1	0.03	1.0	68	69.39	2.04	18.0	18.73	4.05
2	0.08	0.5	76	74.79	- 1.59	14.0	14.56	4.00
3	0.10	1.5	84	86.39	2.84	21.0	21.81	3.85
4	0.12	0.8	92	92.78	0.84	18.0	17.24	-1.44
Comparison of predicted and observed responses of optimized formulation (PFE)								
Response			Predicted		Observed		% Bias	
Viscosity (cps)			19.99		19.33		3.30	
% diffusion at 10 mins			89.06		91.33		2.54	

Table 8: Optimized Batch Composition (PFE) and results of market and optimized batch preparation		
Ingredients		**Composition**
Xylometazoline HCl		50 mg
Sodium Cholate		1.20 gm
PEG400		15 ml
Sodium CMC		0.1 gm
Glycerin		2.5 ml
Methyl Paraben		0.033 g
NaCl		qs
Purified Water		qs to 100 ml
Parameters	**Market Preparation (B. No: 30484M)**	**Optimized Batch (B. No: PFE)**
Assay (% w/w)	99.59 %	100.11 %
Clarity	Clear Solution	Clear Solution
pH of solution	6.4	6.5
Drug Diffusion	83 % (10 minutes)	91 % (10 minutes)
Viscosity	4 cp	19 cp

Table 9: Drug substance content among different containers and same containers				
Sr. No	**Batch No**	**C1**	**C2**	**C3**
1	PFE	102 %	97 %	99 %
Drug substance content among same containers				
Sr. No	**Batch No**	**S1**	**S2**	**S3**
1	PFE	100 %	99 %	95 %

Table 10: Inverted and Horizontal position for net content and weight loss evaluation					
Sr. No	**Batch No**	**Container**	**Initial Wt**	**Wt after 3 months**	**Wt after 6 months**
1	PFE	1	15 gms	15 gms	15 gms
2		2	15 gms	15 gms	15 gms
Horizontal position for net content and weight loss evaluation					
Sr. No	**Batch No**	**Container**	**Initial Wt**	**Wt after 3 months**	**Wt after 6 months**
1	PFE	1	15 gms	15 gms	15 gms
2		2	15 gms	15 gms	15 gms

Net Content and Weight Loss

The FDA guideline for nasal sprays clearly state that the weight loss and net content study must be performed considering the worst case scenario for orientation of container closure. Thus the formulation was stored in both i.e. inverted and horizontal position. Net content in the product container closure was checked initially, while weight loss study was performed for stability time points of 3 and 6 months. As shown in Table 10, the results exposed that there is no any noteworthy change with respect to horizontal and inverted position for weight loss study indicating the integrity of container closure system.

Priming and Repriming study

Priming Study

These studies were executed to support the number of actuations to be suggested which essentially to be fired to discarded prior to the end user using the product for the first time and subsequent use afterwards. The priming study was performed on formulation nasal spray and the results obtained were 101.2 % and 98.1 % respectively for first actuation. The study results indicate that the first actuation itself delivered greater than 95 % of

Table 11: Repriming study for 5, 10 and 30 days

Cont. No.	Duration	Repriming	No of Actuations	Assay Results
1	5 d	Yes	1	99.9 %
2	5 d	Yes	1	98.4 %
Repriming study for 10 days				
1	10 d	Yes	1	102.2%
2	10 d	Yes	1	98.1 %
Repriming study for 30 days				
1	30 d	Yes	1	97.6 %
2	30 d	Yes	1	100.3%

Table 12: Results for Droplet size (D_{50}) distribution

Container Number	D_{50} Value (µm) Droplet size distribution
1	58.90 ± 3.28
2	51.63 ± 4.72
3	52.42 ± 2.97
4	56.59 ± 3.19
5	57.77 ± 3.63

Table 13: Long Term stability study results at 25 ± 2 °C/ 60 ± 5% RH

Specifications	Initial (0 m)	At 3 months	At 6 months	At 9 months	At 12 months
Appearance	Clear solution	Clear solution	Clear solution	Clear solution	Clear solution
pH	6.5 ± 0.00	6.4 ± 0.53	6.5 ± 0.00	6.5 ± 0.00	6.5 ± 0.00
Viscosity (cp)	19.33 ± 0.57	18.33 ± 0.57	18.66 ± 0.57	19. 33 ± 0.57	19. 33 ± 0.57
Assay (%)	100.11 ± 0.40	98.85 ± 0.29	99.57 ± 0.86	97.47 ± 0.61	99.25 ± 0.74
% diffusion	91.33 ± 1.15	91.66 ± 0.57	90.33 ± 1.52	88.33 ± 1.52	90.00 ± 1.00
Net content*	10 ml	--	--	--	10 ml
Sterility*	confirms	--	--	--	confirms
Accelerated stability study results for batch no: FAE at 40 ± 2 °C/ 75 ± 5% RH					
Specifications	Initial (0 m)	At 3 months	At 6 months		
Appearance	Clear solution	Clear solution	Clear solution		
pH	6.5 ± 0.00	6.5 ± 0.00	6.5 ± 0.00		
Viscosity (cp)	19.33 ± 0.57	20.66 ± 1.15	19.33 ± 0.57		
Assay (%)	100.11 ± 0.40	99.55 ± 1.21	99.17 ± 0.72		
% diffusion	91.33 ± 1.15	91.00 ± 1.00	89.66 ± 1.52		
Net content*	10 ml	--	10 ml		
Sterility*	confirms	--	confirms		

*These tests were performed initially and at end point

drug content. Thus representing that only one actuation requirement as priming.

Repriming study

Repriming study was employed on formulation nasal spray at intervals of 5 days, 10 days and 30 days and the results obtained as shown in Table 11. The minimum result for repriming within 5, 10 and 30 days was found to be 97.6 % while the maximum result obtained was 102.2 %. It was observed that only one actuation was sufficient for repriming to meeting the drug product specification criteria.

Droplet Size Distribution

In this study for droplet size distribution determination, laser diffraction method was utilized. The instrument used was automatatic nasal actuator (Malvern Instruments, UK) assembled onto spraytech.Droplet size distribution was carried out for five containers of the batch., where each container was actuated in triplicate at 3 cm and 6 cm distance from orifice of actuator. Single scan was used for performing droplet size distribution at fully developed spray stable phase. The results of median droplet size (D_{50}) mean from 6 cm actuation distance are shown in Table 12.

Sterility

The sterility of product was performed according to USP sterility tests. The sterility test results demonstrates that no any type of microbial growth was observed. This depicts that the formulation is sterile.

Stability Studies

The stability study for the prepared formulation was performed at 25 ± 2°C/ 60 ± 5% RH as long term conditions and while at 40 ± 2°C/ 75 ± 5% RH as accelerated conditions. The results obtained as presented in Table 13. All the results related to stability studies were

found well within the acceptance criteria without any significant change comparing initial results. During and at the finish point of the stability study (both conditions), the formulation revealed the drug content equivalent to original results. Formulation also confirmed the apposite appearance, viscosity, *in vitro* diffusion and pH at the finish point of the stability study. The stability study result the formulation endured without any significant change unto 12 months.

CONCLUSION

The current study proved how factorial design approach can be utilized towards development and optimization of solution type of nasal formulation sprays. The pre-formulation study was supported for active pharmaceutical ingredient xylometazoline. The drug-excipient compatibility study indicated no interaction occurance between xylometazoline and excipients. Optimization was concluded using 3^2 full factorial design, where sodium cholate (X1) and PEG400 (X2) were reserved as independent factors. The formulation was evaluated for various *in-vitro* evaluation parameters such as pH, assay, viscosity, diffusion, primimg and repriming, weight loss, sterility. The results obtained for these parameters demonstrates results in the required range of specifications for formulation. The formulation along with its container closure was evaluated for its stability up to 12 months at long term conditions. The pediatric nasal spray formulation displays superior performance for *in-vitro* drug release compared with current marketed drops formulation thus providing substitute choice for treatment of nasal congestion.

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CONFLICT OF INTEREST

The authors indicates that there is no conflict of interests.

ABBREVIATION USED

PEG400: polyethylene glycol 400; **US:** United States; **Sodium CMC:** Sodium carboxy methyl cellulose; **USP:** United States Pharmacopoeia; **RH:** Relative Humidity; **Cp:** Centipoise.

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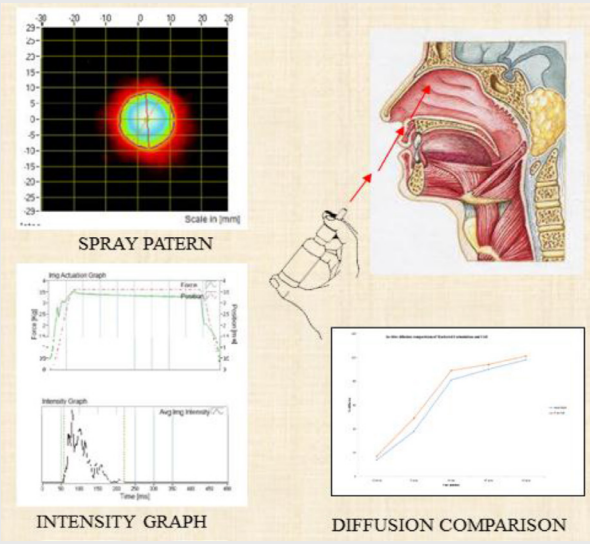
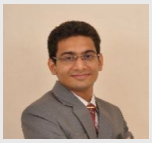
PICTORIAL ABSTRACT



SUMMARY

- The current study proved how factorial design approach can be utilized towards development and optimization of solution type of nasal formulation sprays.
- The formulation was evaluated for various *in-vitro* evaluation parameters such as pH, assay, viscosity, diffusion, primimg and repriming, weight loss, sterility.
- The pediatric nasal spray formulation displays superior performance for *in-vitro* drug release compared with current marketed drops formulation

About Authors



Falgun Bhuva: PhD research scholar with research interest in formulation development in pharmaceutical science.

Dr. L. D. Patel: Academic researcher, with research interest in pharmaceutical science and in the development of novel drug delivery formulation.

Krutagn Patel: Academic researcher, with research interest in pharmaceutical sciences and novel drug delivery system.

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