Available online on 15.10.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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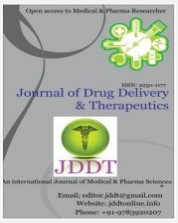

Open Access Research Article

PEDIATRIC NASAL SPRAY SOLUTION: FACTORIAL DESIGN DEVELOPMENT AND EVALUATION

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ABSTRACT

The work was aimed to develope the meter dose formulation of pediatric xylometazoline nasal spray formulation. The 3^2 factorial design was utilized for development of the pediatric formulation. The independent factors were sodium cholate (X_1) and Polyethyleneglycol 400 concentration (X_2). The optimized formulation composition consisted of 0.105 g sodium cholate and 1.35 ml polyethyleneglycol 400. The formulation was evaluated for solution parameters like drug content, pH, viscosity, % diffusion, sterility and spray evaluation parameters like spray content uniformity, pump delivery, spray pattern, and weight loss. The results for spray content uniformity were in the range of 95-102 %, while pH in the range of 6.5 ± 0.3. The ovality of spray was 1.118, while perimeter and area of spray pattern were found to be 57.42 mm and 258.8 mm^2 respectively for optimized formulation. The least value for repriming was 97.6 % while the extreme value was 102.2 % indicating one actuation was satisfactory for repriming. The results for droplet size test were obtained in the range of 51.63 to 58.90 µm. The formulation along with its container closure was evaluated for its stability up to 12 months at long term conditions. The formulated batch PFE showed better performance for in-vitro drug release with marketed product thus providing another option for treatment of nasal congestion.

Keywords: Nasal decongestion, Xylometazoline hydrochloride, Sodium cholate, Metered dose delivery, Drug diffusion.

Article Info: Received 02 Sep, 2018; Review Completed 12 Oct 2018; Accepted 12 Oct 2018; Available online 15 Oct 2018

Cite this article as:

Bhuva F, Patel LD, Pediatric nasal spray solution: factorial design development and evaluation, Journal of Drug Delivery and Therapeutics. 2018; 8(5-s):355-365 **DOI:** http://dx.doi.org/10.22270/jddt.v8i5-s.2067

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List of Abbreviations: PEG400– polyethylene glycol 400; US– United States; Sodium CMC– Sodium carboxy methyl cellulose; USP– United States Pharmacopoeia; RH– Relative Humidity; Cp– centipoises

INTRODUCTION

The pharmaceutical arena is constantly in search for novel drug delivery systems that can overwhelm the existing issues. The suitability of administration and enhanced patient amenableness are essential in the strategy of nasal drug delivery system. [1,2] About 2% of the drugs that are conveyed through nasal route due to the reason for availability of large surface area. In comparison to alternative routes of drug delivery, nasal route is found more prominent. [3,4, 5.]

There is wide range of differences observed between adults and children in various aspects of pharmacotherapy which are mandatory for achieving accurate and safe dosage administration. The drug product formulated for pediatric use must take into consideration factors such as age targeted, physiological conditions and type of requirement of treatment. Some advancement in development of pediatric formulations is observed due to new regulations, as well as collaborative research work. Allergic rhinits or sinusitis has one indication as nasal congestion in pediatric patients. [6]

Drug substance like xylometazoline, which is of imidazole class, acts on alpha-adrenergic receptors of nasal mucosal arterioles leading to decrease of blood flow. It reduces the swelling of nasal turbinate's, relating to amplification of nasal lumen. [7,8,9] Various excipients were used in novel drug delivery system with aim of taking specific roles in drug delivery system such as carriers, bioavailability enhancers, masking of taste,

stabilizer, solubilizer etc. In recent days, various natural as well as synthetic polymers are widely used in nasal drug delivery. The present work described the formulation development and evaluation of nasal solution dosage form for pediatric use of xylometazoline as a model drug. The meter dose spray formulation helps to deliver known amount of formulation.

MATERIALS AND METHODS

Materials

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

Methods

Preparation of Xylometazoline pediatric nasal solution: The pediatric nasal solution was prepared by sequential mixing of various excipients as shown in Figure 1.

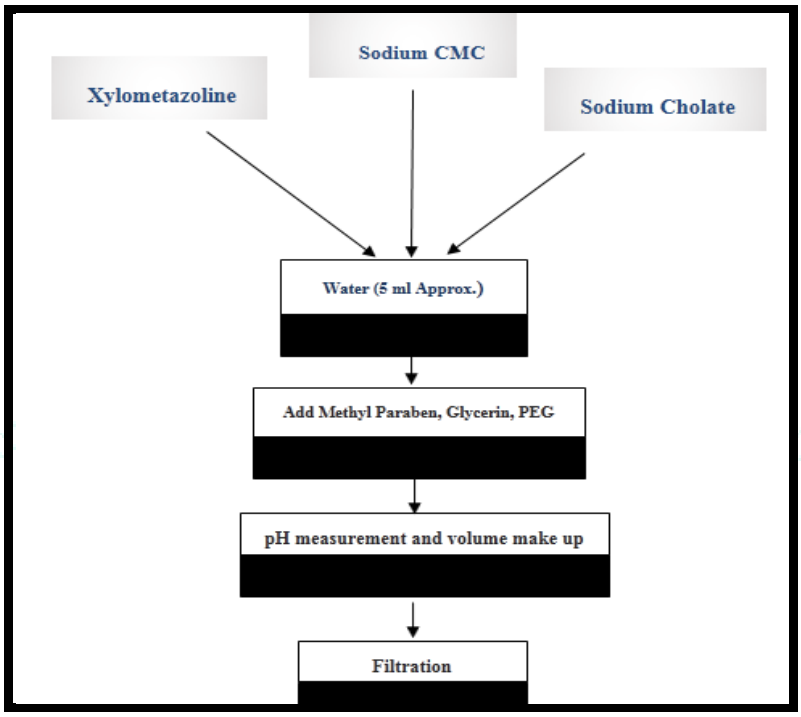

Figure 1: Method of preparation of nasal solution

Experimental Design

A 3 level 2 factors factorial design (3^2) was adopted using concentration of sodium cholate (X_1) and PEG 400 (X_2) as independent variables for formulation development of pediatric xylometazoline nasal solution. (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 (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

β0 = Intercept (arithmetic mean of all the batches) runs,
β1 = estimated coefficient for the factor X1.
B2-Estimated coefficient for the factor X2
B12= Estimated coefficient of the interaction between X1 and X2

Table 1: Independent variables and their levels for Xylometazoline Solution for Pediatric Use

Level	Independent Variables	
	X1= Concentration of Sodium Cholate (g)	X2 = Concentration of PEG 400 (ml)
−1(Low Level)	0.02	0.50
0 (Medium Level)	0.07	1.00
+1 (High level)	0.12	1.50

Table 2: Composition of 3^2 factorial design of Pediatric use nasal formulations of xylometazoline*

Batch	X1, Sodium Cholate Concentration (g)	X2, PEG 400 Concentration (ml)	X1	X2
PFA1	0.02	0.5	-1	-1
PFA2	0.07	0.5	0	-1
PFA3	0.12	0.5	1	-1
PFA4	0.02	1.0	-1	0
PFA5	0.07	1.0	0	0
PFA6	0.12	1.0	1	0
PFA7	0.02	1.5	-1	1
PFA8	0.07	1.5	0	1
PFA9	0.12	1.5	1	1

* Each factorial design batch contained 10 mg of xylometazoline, 0.01 g of Na CMC, 0.15 ml of Glycerin, 0.0033g Methyl Paraben, q.s. of NaCl, and volume was made with purified water to 10 ml

Evaluation parameters of formulation:

Clarity:

The formulated solutions were evaluated as in-house test for clarity. The color and clarity were visually examined against black and white surface in inspection booth. [10]

Drug Content:

The drug content of the nasal formulation was measured using standard curve equation obtained by calibration curve using UV spectrophotometer at 268 nm. The sample of the formulation was diluted using distilled water and absorbance was measured at 268 nm using water as blank. The mean of three results was used as drug content of the sample. [11]

pH:

The pH was measured in triplicate for pH using digital pH meter. The model pH range of nasal formulations was in range of 6.5 ± 0.3. [12,13,14]

Viscosity:

Viscosity has influence on the dwelling time of formulation, which is associated to rate of drug absorption through nasal mucosa. The viscosity was measured through Brookfield Viscometer (Spindle no S18 at 100 rpm) (Brookfield Viscometer, Model no DV-II PRO, Dolphine instrument, Mumbai). [10]

Spray Content Uniformity:

The spray content uniformity as critical parameter for nasal spray, was studied to explore the spray discharged containing the amount of active ingredient from the nosepiece. The examination was carried out for multiple spray form single container and in different container for the same. The acceptance criteria was defined as the amount of active ingredient 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. [15,16]

Pump Delivery:

The pump delivery is crucial parameter interrelated with spray content uniformity for product performance. The pediatric nasal spray formulation was assessed for pump-to-pump reproducibility and the pump metering capability. The formulation was actuated for 10 times in a pre-weighed bottle. The bottle was reweighed after 10 actuations and the difference was calculated. [10]

Spray Pattern:

Spray Pattern of prepared nasal spray formulation was measured by the SprayVIEW system (Proveris Scientific Corperation, 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 65.4^o and summation mode as automatic. [15,16]

Weight Loss:

The weight loss is parameters that determines the product performance and stability. Inverted and Horizontal positions shows noteworthy role in weight loss, hence both orientations were kept for the drug product storage. The weight loss 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. [11,15,17]

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. [11, 16]

In-vitro Diffusion:

This study was performed using the Franz diffusion cell, where recently isolated sheep nasal mucosa obtained from a local slaughter house was utilized for attachment on diffusion chamber. The doner medium comprised of formulation, while the receptor medium comprised of phosphate buffer, while the temperature of the medium was maintained at 37 ^{o}C ± 1 ^{o}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). [18,19,20,25]

Droplet Size Distribution:

The deposition of the nasal formulation in cavity is impacted by droplet size distribution of the spray. The delivery device and the formulation are the two major factors that influence the droplet size. There must be appropriate control for droplet size distribution of the delivered plume. The droplet size distribution can be measured in terms of D10, D50, and D90 by utilization of laser diffraction. [21,22]

Sterility:

Sterility is an important requirement for nasal formulation. The method used was as per USP chapter for sterility tests <71>. [23]

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. [24]

RESULTS AND DISCUSSION

Preliminary trials and composition of nasal formulation

The preliminary formulation trials were designed for the selection of surfactant concentration, and co-solvent amount. The preliminary parameters like clarity, pH, drug content, % diffusion at 10 minutes and viscosity were optimized by varying one parameter at a time, while keeping the others constant, so that the effect of varied parameter could be evaluated. The concentration range 0.02 g to 0.12 g of sodium cholate as surfactant was selected, while the concentration range of 0.50 ml to 1.50 ml of polyethylene glycol 400 was selected for further studies.

Experimental design

Pediatric nasal solution was formulated by utilizing 3^2 factorial design. These batches were assessed using polynomial equation of design and two check point batches were confirmatory for authentication of design of experiment. The assessment tests parameters consisted for appearance, pH, viscosity, drug content and % diffusion at 10 minutes (Table 3). The % diffusion at 10 minutes and viscosity displayed differences based on the values of independent variables, henceforward they were defined as dependent parameters.

Table 3: Results of responses of 3^2 factorial design of pediatric xylometazoline nasal solution

Parameter: → Batch * ↓	% Diffusion at 10 minutes [Y_1]	Viscosity (cp)[Y_2]
PFA1	65.33 ± 1.52	13.33 ± 0.57
PFA2	74.33 ± 1.52	14.33 ± 1.15
PFA3	90.33 ± 0.57	15.33 ± 0.57
PFA4	64.33 ± 1.15	17.00 ± 1.00
PFA5	75.33 ± 0.57	18.33 ± 0.57
PFA6	93.33 ± 0.57	18.33 ± 0.57
PFA7	66.66 ± 0.57	21.33 ± 0.57
PFA8	76.33 ± 1.52	19.33 ± 0.57
PFA9	91.66 ± 1.52	20.00 ± 1.73

The polynomial equation was obtained by regression analysis of the results of % diffusion at 10 minutes for 3^2 factorial design batches by equation 3:

$$\%\ Diff.\ at\ 10\ mins\ (Y1) = 75.4814 + 13.1666 * X1 + 0.7777 * X2 + 0.00 * X1 * X2 + 3.2777\ (X1)2 - 0.2222(X2)2 \ldots (\text{Eq.3})$$

\

Among the independent variables, X_1 had prominent positive effect (β_1= 13.1666) on diffusion at 10 minutes. The X_2 variable had very less effect (β_2= 0.7777), while the interaction between X_1 and X_2 showed no impact on diffusion at 10 minutes (β_{12}= 0.00). Sodium cholate exhibited prominent impact on % diffusion at ten minutes. The contour and surface response plot for % diffusion at 10 minutes were displayed in Figure 2. It could be inferred from the contour and response surface plot that excipient sodium cholate has correlation to % diffusion at 10 minutes.

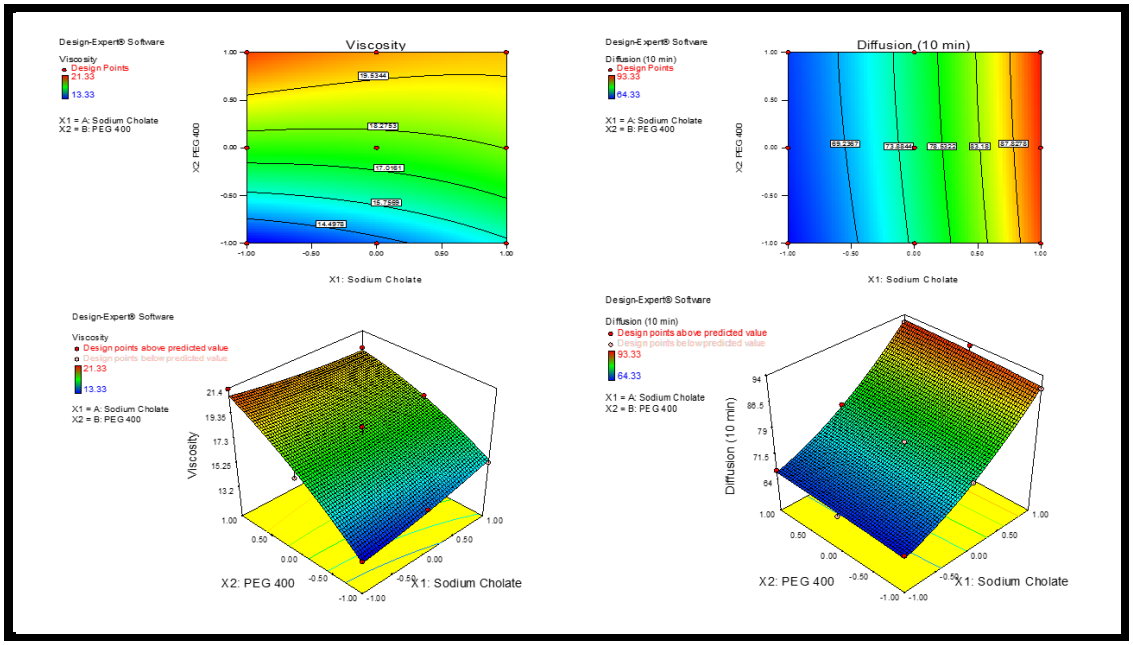

Figure 2: Contour and Response Surface Plots for % Diffusion and viscosity

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. 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 sodium cholate have substantial impact on diffusion rate on formulation.

Table 4: ANOVA for % Diffusion at 10 minutes

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Coefficient
Model	1065.38	5	213.07	115.57	0.0012 (significant)	
Intercept[(β_0)]						75.4814
Sodium cholate [(β_1)]	1040.17	1	1040.17	564.00	0.0001	13.1666
PEG 400[(β_2)]	3.62	1	3.62	1.96	0.2551	0.7777
[(β_{12})]	0.000	1	0.000	0.000	1.0000	0.0000
[$(\beta_1)^2$]	21.47	1	21.47	11.64	0.0420	3.2777
[$(\beta_2)^2$]	0.100	1	0.100	0.054	0.8318	– 0.2222
Residual	5.53	3	1.843			
Cor Total	1070.91	8				

The polynomial equation 4 was obtained by regression analysis of the results of viscosity for 3^2 factorial design batches as below:

$$Viscosity\ (Y2) = 17.7407 + 0.3333 * X1 + 2.9444 * X2 - 0.8333 * X1 * X2 + 0.2222\ (X1)2 - 0.6111(X2)2 \quad \ldots\ldots(Eq.4)$$

The quadratic model shows the enhanced fitting related to other models. The influences of independent factors were as evaluated as displayed in Table 5. The p value of the model was found (P = 0.0168) < 0.05 indicating that the model was significant. The independent variable X_2, had prominent positive effect (β_2= 2.9444) on viscosity, while the X_1 variable had very minor impact (β_1= 0.3333) viscosity. The changing of factor (PEG 400) X_2 from -1 to +1 showed the viscosity change from 13 cp to 21 cp.

The contour and surface plot for viscosity were constructed as represented in Figure 2. It can be interpreted that concentration of PEG 400 and viscosity of the formulation were interrelated.

Table 5: ANOVA for response Viscosity

	Sum of		Mean	F	p-value	Coefficient
Source	Squares	df	Square	Value	Prob > F	
Model	56.30	5	11.26	19.68	0.0168 (significant)	
Intercept[(β_0)]						17.7407
Sodium cholate [(β_1)]	0.67	1	0.67	1.17	0.3593	0.3333
PEG 400[(β_2)]	52.04	1	52.04	91.12	0.0024	2.9444
[(β_{12})]	2.77	1	2.77	4.85	0.1148	– 0.8333
[$(\beta_1)^2$]	0.10	1	0.10	0.17	0.7056	0.2222
[$(\beta_2)^2$]	0.75	1	0.75	1.31	0.3360	– 0.6111
Residual	1.71	3	0.57			
Cor Total	58.02	8				

The Table 6 displayed the percentage error observed between the experimental value and the predicted value of various experimental batches. The % bias value of diffusion at 10 minutes was found in the range of -1.72% to 1.67 % and the % bias value for viscosity was found in the range of - 5.64% to 6.52 %. The percentage bias/error for both the variables were found satisfactory, which endorsed the model selected was successful to predict the response of the experimental design. The summary of the results of regression analysis of batches of xylometazoline nasal solution for full and reduced model was provided in Table 7.

Table 6: % bias for experimental batches for xylometazoline nasal solution for pediatric use

Batch No.	Composition of Sodium Cholate	Composition of PEG	Calculated Value 0f Diff.	Exp Value of Diff	% Bias for Diff.	Calculated Value of Visc.	Exp Value of Vis.	% Bias for Visc.
PFA1	-1	-1	64.66	65.33	1.02	14.20	13.33	6.52
PFA2	0	-1	74.55	74.33	-0.29	14.53	14.33	-1.39
PFA3	1	-1	90.99	90.33	-0.73	14.87	15.33	3.00
PFA4	-1	0	65.44	64.33	-1.72	17.14	17.00	-0.82
PFA5	0	0	75.33	75.33	0.00	17.48	18.33	4.63
PFA6	1	0	91.77	93.33	1.67	17.81	18.33	2.83
PFA7	-1	1	66.22	66.66	0.66	20.09	21.33	5.81
PFA8	0	1	76.11	76.33	0.28	20.42	19.33	-5.64
PFA9	1	1	92.55	91.66	-0.97	20.75	20.00	-3.75

Table 7: Summary of the results of regression analysis of batches of pediatric xylometazoline nasal solution for factorial design

Response	**Model**	**β_0**	**β_1**	**B_2**	**β_{11}**	**β_{22}**	**β_{12}**	**R^2**
% Diff. at 10 mins.	Full	75.4814	13.1666	0.7777	0.000	3.2777	-0.2222	0.9948
	Reduced	75.3333	13.1666	0.7777	–	3.2777	–	0.9947
Viscosity	Full	17.7407	0.3333	2.9444	-0.8333	0.2222	-0.6111	0.9704
	Reduced	17.4814	0.3333	2.9444	–	–	–	0.9079

Optimization of experimental design

The design of experimental model was validated by two check point batches. The verification for % diffusion and viscosity of checkpoint batches was confirmed by utilizing mathematics equation (3,4).

The Table 8 depicts the results for check point batches for % diffusion at 10 minutes and viscosity. The Figure 2 displays contour and surface response plots. The optimization of formulation was performed by utilizing the Design® Expert software by using desirability approach (Figure 3).

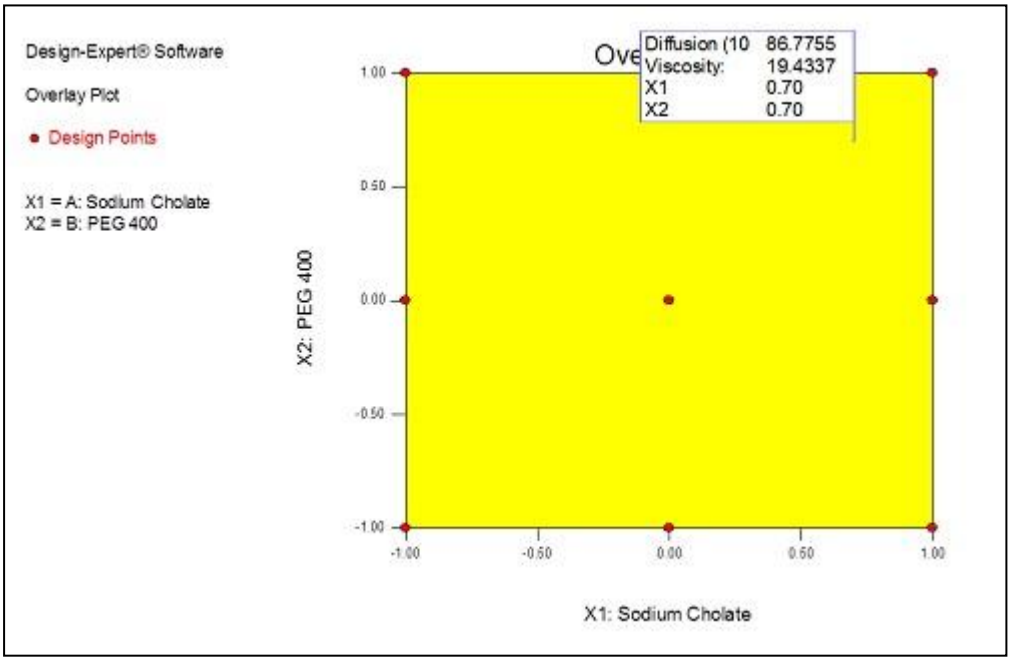

Figure 3: Overlay plot of optimized formulation

Table 8: Check point batches results for dependent variables with % bias

Sr. No.	Conc. of sodium cholate*	Conc. of PEG 400*	Diffusion at 10 min (%)			Viscosity (cp)		
			Actual	Predicted	% Bias	Actual	Predicted	% Bias
1	-0.08	0.0	73.3	74.44	-1.55	18.0	17.71	1.61
2	0.2	-1	76.3	77.24	-1.23	14.0	14.42	-3.00

* Coded values are indicated

The optimized formulation contained sodium cholate 0.105 g and PEG 400 1.35 mL as displayed in Table 9. This formulation PFE displayed values for % diffusion at 10 min and viscosity is 87.33 % and 19.33 cps respectively. The comparision of in-vitro diffusion results between otrivin® pediatric and batch PFE is revealed in Figure 4, indicating that the optimized formulation demonstrating superior % diffusion at 10 minutes. (Refer supplementary Table 2).

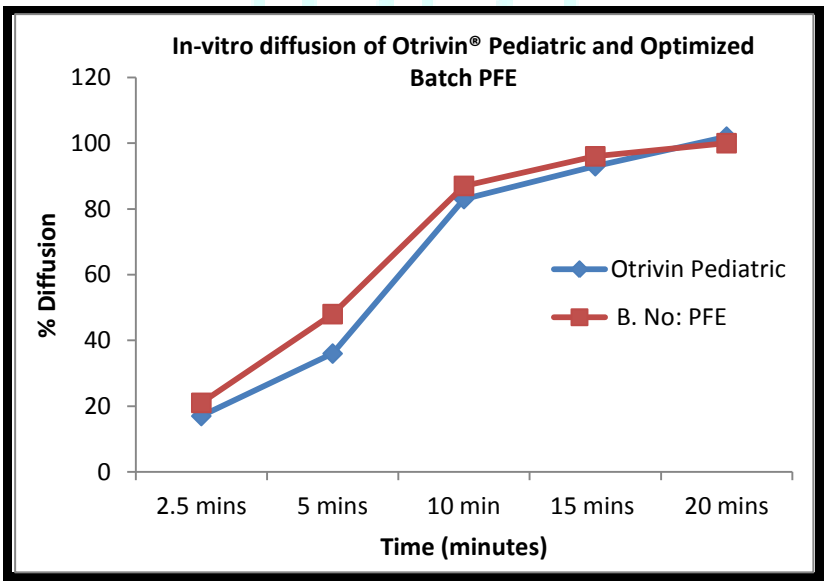

Figure 4: *In-vitro* diffusion comparison of marketed formulation and optimized batch

Table 9: Optimized Batch Composition (PFE) and results of market and optimized batch preparation

Ingredients		**Composition**
Xylometazoline HCl		5 mg
Sodium Cholate		0.105 g
PEG400		1.35 ml
Sodium CMC		0.01 g
Glycerin		0.15 ml
Methyl Paraben		0.0033 g
NaCl		qs
Purified Water		qs to 10 ml
Parameters	**Market Formulation (B. No: 31090V)**	**Optimized Batch (B. No: PFE)**
Assay (% w/w)	99.59 ± 0.83	100.41 ± 0.89
Clarity	Clear Solution	Clear Solution
pH of solution	6.40 ± 0.0	6.50 ± 0.00
Drug Diffusion	83.0 ± 1.00	87.33 ± 1.15
Viscosity	4.33 ± 0.57	19.33 ± 0.57

Other Evaluation parameters

pH :

The formulation pH for factorial design batches was found in the range of 6.50 ± 0.3 showing better compatibility at the delivery site.

Spray Content Uniformity:

The formulations must be assessed in its relationships of emitted dose content uniformity. The Table 10 data indicated the drug substance content sprayed within same container and among the different containers. The results are in the range of 95-102 %.

Table 10: Drug substance content among different containers

Sr. No	**Batch No**	**PC1***	**PC2***	**PC3***
1	PFE	102 %	97 %	99 %
Drug substance content among same containers				
Sr. No	**Batch No**	**S1#**	**S2#**	**S3#**
1	PFE	100 %	99 %	95 %

* indicates different containers labelled PC1, PC2 and PC3 used for Spray content test.

# indicates different sprays (S1, S2 and S3) from container PC5.

Pump Delivery:

The relevant performance from pump is primarily essential for precise delivery of drug to targeted part. This test guarantees specific dose is constantly delivered by the pump. The initial weight of filled nasal spray was 15 g. The final weight of spray system was found the 14.5 g after 10 actuations, with the actuation volume for each spray as 100 µl. The results showed that the pump delivery of xylometazoline nasal spray formulation for pediatric use was satisfactory.

Spray Pattern:

The pumping performance can be estimated with the help of spray pattern test. This test could be impacted by factors such as the size and shape of the nozzle of pump, the design of the pump, the formulation characteristics etc. The ovality of spray was 1.118, while perimeter and area of spray pattern 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 5. The figure 4 endorses that the pump delivered the required medication appropriately without any concerns.

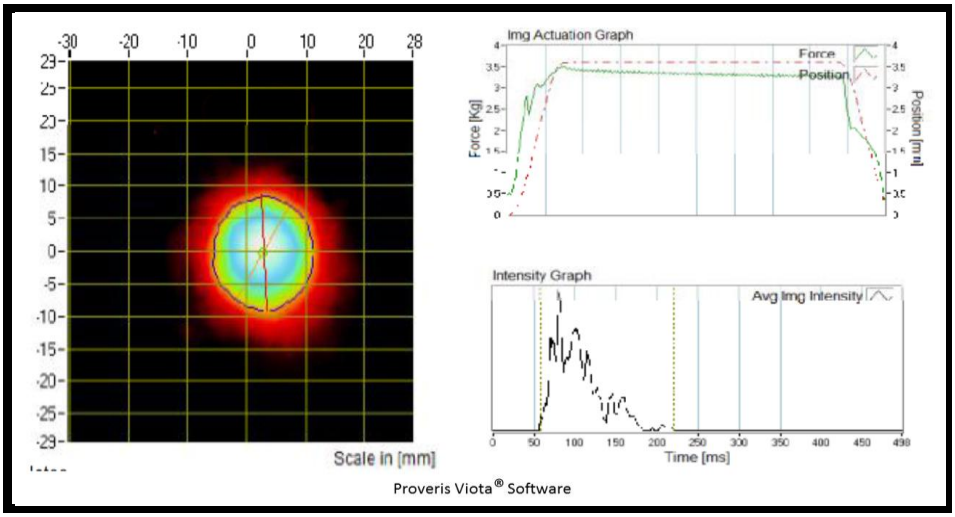

Figure 5: Spray pattern, Image Actuation Graph and Intensity Graph of formulation

Weight Loss:

The formulation was stored in both i.e. inverted and horizontal position. Weight loss study was performed for stability time points of 3 and 6 months. As shown in Table 11, the results indicated that there was no any change for weight loss study demonstrating the reliability of packaging components.

Table 11: Inverted 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 g	15 g	15 g
2		2	15 g	15 g	15 g
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 g	15 g	15 g
2		2	15 g	15 g	15 g

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. This study referenced that only one actuation requirement as priming since the results obtained were higher than 95 %.

Repriming study:

Repriming study results at 5 days, 10 days and 30 days are as shown in Table 12. The least value for repriming within 5, 10 and 30 days was obtained to be 97.6 % while the extreme value acquired was 102.2 %. It was concluded that one actuation was satisfactory for repriming.

Table 12: Repriming study for 5 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%

Droplet Size Distribution:

The droplet size study was performed based on laser diffraction method. The instrument used was automatatic nasal actuator (Malvern Instruments,UK) further assembled onto spraytech. The droplet size distribution was carried out for five containers labelled C1 to C5, where each container was actuated at 3 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 (D50) mean from 6 cm actuation distance are shown in Table 13. The results were obtained in the range of 51.63 to 58.90 µm.

Table 13: Results for Droplet size (D_{50}) distribution

Container Number	D_{50} Value (µm) Droplet size distribution
PC1	58.90 ± 3.28
PC2	51.63 ± 4.72
PC3	52.42 ± 2.97
PC4	56.59 ± 3.19
PC5	57.77 ± 3.63

Sterility:

The sterility test of optimized formulation batch PFE was carried out as per USP criteria using container PC8. The sterility test showed no microbial growth, which indicated that the formulation was sterile.

Stability Studies:

The stability of optimized pediatric nasal formulation (Batch PFE) was carried at 25 ± 2 ºC / 60 ± 5% RH for 12 months and accelerated condition study was performed at 40 ± 2°C/ 75 ± 5% RH for 6 months. The results were displayed in Table 14. No significant change was observed in context of initial results. Batch number PFE also demonstrated the satisfactory appearance, pH, *in vitro* diffusion, viscosity and sterility at the completion of the stability study.

Table 14: Results of stability study 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 ± 0.05	6.5 ± 0.00	6.5 ± 0.00	6.4 ± 0.05
Viscosity (cp)	19.33 ± 0.57	18.33± 0.57	18.66± 0.57	19.33± 0.57	19.33± 0.57
Assay (%)	100.41±0.89	98.81± 0.62	99.60± 1.29	99.34± 0.72	98.86± 0.93
% diffusion	87.33 ± 1.15	89.33± 1.52	88.33± 0.57	88.33± 1.52	90.00± 1.00
Net content*	10 ml	--	--	--	10 ml
Sterility*	confirms	--	--	--	confirms

Results of stability study 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 ± 0.57	19.33 ± 0.57
Assay (%)	100.41±0.89	99.20 ± 1.05	99.18 ± 0.42
% diffusion	87.33 ± 1.15	89.00 ± 1.73	89.66 ± 1.52
Net content*	10 ml	--	10 ml
Sterility*	confirms	--	confirms

*These tests were performed initially and at end point

CONCLUSION

The pediatric nasal spray formulation was developed by utilizing factorial design approach. The drug-excipient compatibility study endorsed no interaction between xylometazoline and the excipients. The 3^2 full factorial design was utilized, where sodium cholate (X_1) and PEG 400 (X_2) were allocated as independent factors. The parameters for solution formulation such as drug content, pH, viscosity, % diffusion, sterility and spray evaluation like spray content uniformity, pump delivery, spray pattern, and weight loss were evaluated. The results for spray content uniformity were in the range of 95-102 %, while pH in the range of 6.5 ± 0.3. The ovality of spray was 1.118, while perimeter and area of spray pattern were found to be 57.42 mm and 258.8 mm^2 respectively for optimized formulation. The least value for repriming was 97.6 % while the extreme value was 102.2 % indicating one actuation was satisfactory for repriming. The results for droplet size test were obtained in the range of 51.63 to 58.90 µm. The formulation along with its container closure was evaluated for its stability up to 12 months at long term conditions. The formulated batch PFE showed better performance for in-vitro drug release with marketed

product thus providing another option for treatment of nasal congestion.

Acknowledgements: We are extremely thankful to Anish chemicals, Gujarat for providing drug substance xylometazoline hydrochloride. We are also extremely thankful to Aptar pharma for providing container closure system.

Conflict of interests: The Authors indicate that there is no conflict of interest.

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