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DESIGN UTILIZING BILE SALT SURFACTANTAND *IN VITRO* EVALUATION: IMIDAZOLE CLASS DRUG NASAL SPRAY FOR TREATMENT OF NASAL CONGESTION

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Nasal drug delivery, Nasal decongestion, Meter dose formulation, Xylometazoline hydrochloride, Sodium cholate

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ABSTRACT: The objective of the present work is to develop the meter dose nasal formulation of xylometazoline hydrochloride with a view to improve the effectiveness as nasal decongestion. The 3^2 factorial experimental design was employed for optimization of sodium cholate (X_1) and PEG 400 concentration (X_2) in the formulation. All the possible experimental trials were carried out according to design layout. The experimental model was further validated by six check point batches. The optimized formulation contains 1.5% w/v sodium cholate and 20% v/v PEG 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. The percentage diffusion and viscosity were observed 89.33 ± 0.57% and 25.33 ± 0.57 centipoise respectively. For spray pattern of optimized formulation, the ovality obtained was 1.217, while perimeter and area were found to be 104.56 mm and 791.5 mm^2 respectively. The meter dose nasal formulation shows the superior performance during *in-vitro* drug release compared with currently marketed formulations, which provides alternative option for treatment of nasal congestion.

INTRODUCTION: The pharmaceutical sectors are currently engaged with the novel drug delivery systems to overcome the current issues regarding nasal decongestion treatment. Nasal therapy also knowas 'Nasya karma' in ayurvedic medicine has been renowned form of dealing in the Ayurvedic system of Indian medicine [1, 2]. The nasal drug delivery system is one of the options for preventing drug degradation from first pass metabolism and relative quick absorbance compared to oral route [3]. About 2% of the drug is delivered by nasal route due to relatively large surface for this route which is vascularized along with the leaky epithelium [4, 5].

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Sinusitis is one of the major influences, which boost complications during the upper respiratory tract infections. The acute rhinosinusitis for the condition as the symptoms involve the nasal cavity and the sinuses [6]. Nasal decongestants are widely used in concomitant remedy for nasal congestion that is related to sinusitis, rhinitis or other allergies. Nasal symptoms such as congestion, itching, sneezing and rhinorrhoea are conditions mostly associated with allergic rhinitis, sinusitis and the common cold [7].

The pathophysiology of nasal congestion can be designated as an intelligence of declined nasal airflow. In another sense it can be as of facial fullness which involves a number of essential mechanisms, including problems of nasal passage structure, nasal sensory, perception variation, mucosal inflammation, increased venous engorgement, amplified nasal secretions, and tissue edema [8].

The conventional cause for nasal congestion is allergic rhinitis and it has been witnessed that worldwide, there is a countless increase in the prevalence of allergic rhinitis, which has been oscillating from as short as 10% to as high as 40%. The predict occurrence of nasal decongestion with allergic rhinitis in paediatrics and young populations in Unites States lies between 9% to 16% along with emerging symptoms before 20 years of age [9].

The bile salts which significantly improves reverse micelles in the membrane and forms aqueous channels, acts on proteins or lipids of membrane, generating fluidization of membrane, thus improving the permeation rate across the membrane. It is also testified that at higher concentrations of bile salts, membrane lipids may be extracted, which create micelles and thus enhancing transcellular transport [10]. Moghimipour *et al.*, described the role of bile salts for improve transport of hydrophilic drugs *via* paracellular route by amalgamation into the cell membrane. Sodium deoxycholate is extreme ciliotoxic that causes ciliary arrest happening within 1 min at a concentration of 5 mM.

Thexylometazoline solution is currently available in the market in the form of nasal drops, which is leading to uneven delivery of dose resulting in variable bioavailability of drug and nasal irritation.

The present work was carried out with a view to develop the nasal spray of Xylometazoline hydrochloride by using sodium cholate (bile salt) and PEG 400 to provide effective and improved drug delivery of xylometazoline hydrochloride. The meter dose spray formulation also prevents the uneven delivery, variable bioavailability and nasal irritation. The developed spray was characterized by drug content, spray content uniformity, pump delivery, spray pattern and other related parameters.

MATERIALS AND METHOD:

Materials: Xylometazoline HCl was obtained from Anish Chemicals Pvt. Ltd., Gujarat. Sodium cholate was purchased from National Chemicals Pvt. Ltd., Vadodara. PEG 400 was obtained gift samplefrom Sigma Aldrich, USA. Sodium carboxymethyl cellulose was obtained from Amar cellulose industries, Gujarat, Methyl Paraben was obtained from S.D. Fine Chem. Ltd., Mumbai.

Methods:

Preparation of Xylometazoline HCl Nasal Solution: The drug loaded nasal solution was developed by sequential mixing of various excipients as shown in **Fig. 1**.

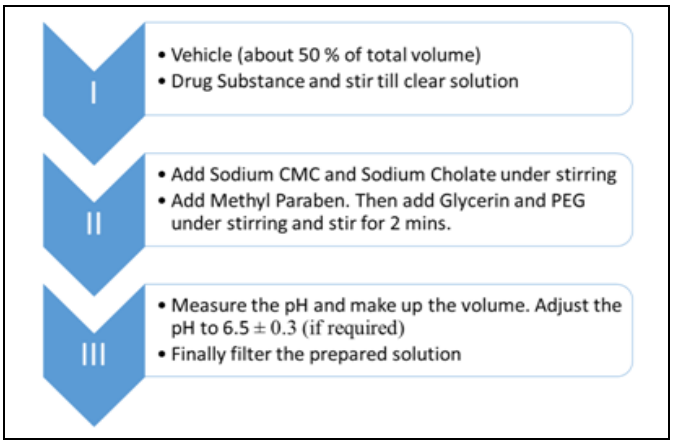

FIG. 1: METHOD OF PREPARATION OF NASAL SOLUTION

Experimental Design: The independent variables values were optimized using experimental design. The preliminary experimental data suggest the sodium cholate (X_1) and PEG 400 (X_2) have major impact on the other formulation parameters, hence selected as independent variables. The optimization was performed utilizing 3 level 2 factors factorial (3^2) experimental design. (Stat- Ease Design Expert®, v 9). Equation 1 shows the polynomial equation for 3^2 experimental design.

$$Yi = \beta_0 + \beta_1 X_1 + \beta_2 X2 = \beta_{12} X_1 X_2 \text{.....Eq. (1)}$$

Where Y = dependent variable

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

β_1 = Estimated coefficient for the factor X_1.

β_2 = Estimated coefficient for the factor X_2

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

TABLE 1: INDEPENDENT VARIABLES AND THEIR LEVELS

Levels	Independent Variables	
	X_1 = Conc. of Sodium Cholate	X_2 = Conc. of PEG400
1(Low Level)	0.05 gm	1 ml
0 (Medium Level)	1.0 gm	1.5 ml
+1 (High level)	1.5 gm	2 ml

The levels of independent variables were selected as displayed in **Table 1** and all the experimental design batches were formulated according to **Table 2**. The batches were evaluated for different parameters.

TABLE 2: COMPOSITION OF FORMULATION

Ingredients	FA1	FA2	FA3	FA4	FA5	FA6	FA7	FA8	FA9	FA10	FA11	FA12	FA13
Xylometazoline HCl (mg)	10	10	10	10	10	10	10	10	10	10	10	10	10
Sodium Cholate (gm)	0.1	0.05	0.15	0.1	0.05	0.15	0.05	0.1	0.15	0.1	0.1	0.1	0.1
PEG400(ml)	1.5	1.5	1.5	1	1	2	2	2	1	1.5	1.5	1.5	1.5
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

Evaluation Parameters of Formulation:

Appearance and Clarity: The developed nasal solutions were evaluated for its appearance color and clarity. The color and clarity were visually inspected against black and white surface in inspection booth [11].

Drug Content: The drug content of nasal solution was performed as per the method reported in the USP product monograph [12]. The quantity of xylometazoline hydrochloride in mg per mL of the nasal solution taken was calculated by the formula as per equation 2:

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

Where, C is the concentration, in µg per mL, in the standard preparation; V is the volume, in mL of nasal solution taken; A_U is the absorbance of the solutions for the Assay preparation; A_S is the absorbance of the solutions from the Standard preparation.

pH: The pH of the nasal formulation play important role in preventing irritation in the nasal mucosa, and provides to sustain normal physiological ciliary movement. All prepared formulations were measured in triplicate for pH using by digital pH meter and average value was considered (Equiptronic pH meter, Model no: EQ-610, Mumbai). The ideal pH range of nasal formulations was in range of 6.5 ± 0.3 [13, 14, 15].

Viscosity: Viscosity has significant impact on the residence time of formulation, which is directly related to rate of drug absorption. Higher the viscosity of the solution shows the higher rate of absorption. The viscosity of the prepared solution was measured through Brookfield Viscometer (Spindle no S18 at 100 rpm) (Brook field Viscometer, Model no LVDL-2T, Dolphine instrument, Mumbai) [11].

Spray Content Uniformity: The spray content uniformity was analyzed to confirm the uniformity of drug substance delivery in each spray. The analysis was carried out for multiple spray form single container and in different container for the same. This test will provide an overall performance evaluation, which will assess the formulation, as well as the pump selection. The test was performed with units primed following the instructions of the labelling. The amount of active pharmaceutical ingredient or drug substance supplied from the nose-piece can be stated as a percent of label claim. The test was performed for uniformity of content per spray, discharged from the nose-piece (appropriate numbers of containers from a batch). The acceptance criteria was selected as the amount of active ingredient not outside of 80 - 120% of label claim for more than 1 of 10 containers, none of the determinations is outside of 75 - 125% of the label claim, and the mean is not outside of 85 – 115% of label claim [16, 18].

Pump Delivery: The pump delivery parameter shows substantial impact on the product performance. The products were tested for pump-to-pump reproducibility and the metering ability of the pump. The formulation was filled into the container which is to be actuated for 10 times in a pre-weighed weighing bottle, the weight of the bottle was reweighed. The difference was calculated [11].

Spray Pattern: The performance of the pump and the nozzle of container closure system need to be evaluated in context of characterizations of spray. The factors which have significant impact on the pattern of spray include 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, the orientation of the nose - piece, and visualization procedure are specified. Spray Pattern of prepared nasal spray formulation was measured by the SprayVIEW system (Proveris Scientific Cooperation, USA) which is furnished with the SprayVIEW automated pump actuation system.

The parameters that were kept for the determination of spray pattern are spray pattern height of 30 mm, evacuation time 15000 millisecond, inclination as 14.3° and summation mode was kept automatic [16, 18].

Net Content and Weight Loss: The regulatory guideline (USFDA) for nasal products shows the net content and weight loss are critical parameters which depicts the performance and stability of product [12]. The drug product was stored in inverted and horizontal positions (*i.e.* both the orientations play a key role in any weight loss) to assess this characteristic. The net content of the formulation in the container was determined. The net content of each of containers needs to be in accordance with the predefined specification [17, 21].

Priming and Repriming: The first priming of the product must show the minimum amount of drug released from the product according to label claim as per USFDA guideline.

Repriming shows the ability of product to delivery same amount of drug content after storage of product. The length of storage for conducting study is defined as 5, 10 and 30 days. The number of actuations required for priming until the subsequent doses meet the specification limits (80 - 120% label claim) for delivered dose are determined. The number of actuations required for re-priming up to the subsequent doses meets the specification limits for delivered dose are also to be determined [12, 18].

***In-vitro* Diffusion Study:** The *in vitro* diffusion study was performed using the Franz diffusion cell as per the method reported in the literature [19]. The recently expunged sheep nasal mucosa, was collected from a local slaughter house (except septum part). The superior nasal membrane was recognized, separated from the nasal cavity by removal of adhered tissue. The nasal mucosa was sensibly removed, followed by immediate submerged in phosphate buffer for 15 minutes (along with aeration) for conserving viability of the tissue. The donor medium consisted of prepared nasal formulation, while the receptor medium consisted of phosphate buffer. The temperature of the medium was maintained at 37 °C ± 1 °C. The samples were analyzed spectrophotometrically at a wavelength of 265 nm against the blank. The receptor medium was refilled with the equivalent volume of the fresh solution as the samples withdrawn. The obtained results are compared with the current marketed formulation Otrivin® Adult (Novartis) [20, 21].

Sterility: Sterility is one of the most vivacious necessities for nasal preparation. The tests for sterility are anticipated for discovering the presence of possible forms of microorganisms in nasal preparations. The principle behind the tests is that at favourable temperature and nutrition conditions, the microorganisms will grow, which can be identified by turbidity in medium. The sterility of the product was performed as per USP general chapter sterility tests <71> to explore the presence of aerobic, anaerobic bacteria and fungi in the nasal solution [22].

Stability Study: The stability studies were performed at 25 ± 2 °C / 60 ± 5% RH for 3, 6, 9 and 12 months for long term studies and accelerated studies were performed at 40 ± 2 °C / 75 ± 5% RH for 3, 6 months [23].

RESULTS AND DISCUSSION:

Design of Experiment: Nasal Solution was optimized by applying 3^2 factorial design. All the possible experimental trials were carried out according to design layout and further evaluation was performed using 3^2 factorial design polynomial equation. The different batches were evaluated for clarity, pH, assay, viscosity and % diffusion at 10 minutes and data for different experimental batches were shown in **Table 3**. While changing the level of independent variables [sodium cholate (X_1) and PEG 400 (X_2)], the significant difference was observed in % diffusion at 10 minutes and viscosity of formulation, hence it was deliberated as dependent parameters.

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	98.8 ± 0.43	24.3 ± 0.57	81.0 ± 2.0
FA2	Clear Solution	6.6 ± 0.05	100.62 ± 0.75	22.3 ± 1.15	72.3 ± 1.52
FA3	Clear Solution	6.56 ± 0.05	99.14 ± 0.95	23.3 ± 0.57	82.67 ± 1.52
FA4	Clear Solution	6.5 ± 0.0	98.73 ± 0.95	20.3 ± 0.57	80.33 ± 0.57
FA5	Clear Solution	6.8 ± 0.0	101.21 ± 1.16	19.0 ± 1.00	75.33 ± 0.57
FA6	Clear Solution	6.5 ± 0.0	102.42 ± 1.62	26.0 ± 0.00	90.67 ± 0.57
FA7	Clear Solution	6.7 ± 0.0	97.95 ± 1.64	25.33 ± 0.57	70.33 ± 1.15
FA8	Clear Solution	6.53 ± 0.05	96.77 ± 0.34	25.67 ± 0.57	78.33 ± 1.52
FA9	Clear Solution	6.6 ± 0.0	97.8 ± 0.62	20.33 ± 1.52	81.00 ± 1.73
FA10	Clear Solution	6.53 ± 0.0	99.2 ± 0.41	24.3 ± 0.57	81.66 ± 0.57
FA11	Clear Solution	6.6 ± 0.0	98.9 ± 0.54	25.0 ± 0.00	81.33 ± 1.15
FA12	Clear Solution	6.5 ± 0.0	99.75 ± 0.39	23.3 ± 0.57	80.33 ± 1.52
FA13	Clear Solution	6.53 ± 0.05	100.26 ± 0.69	24.3 ± 0.57	81.00 ± 1.00
FAE (Exhibit/Optimized Batch)	Clear Solution	6.5 ± 0.0	89.7 ± 0.38	25.33 ± 0.57	89.33 ± 0.57

The polynomial equation 3 shows the effects of independent variables X1 and X2 on % diffusion for 10 minutes.

Diffusion at 10 min (Y1) = 87.91 – 98.33 × X1 – 14 × X2 + 150 × X1 × X2 .....Eq.(3)

The value of sodium cholate changing from -1 to +1 show the diffusion higher value obtained as 91% 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 70%. The different batches results depict that the % diffusion (Y_1) has a linear relationship with sodium cholate (X_1).

The contour and surface response plot for dependent parameters were constructed by changing the level of independent variables from -1 to +1 as displayed in **Fig. 2**.

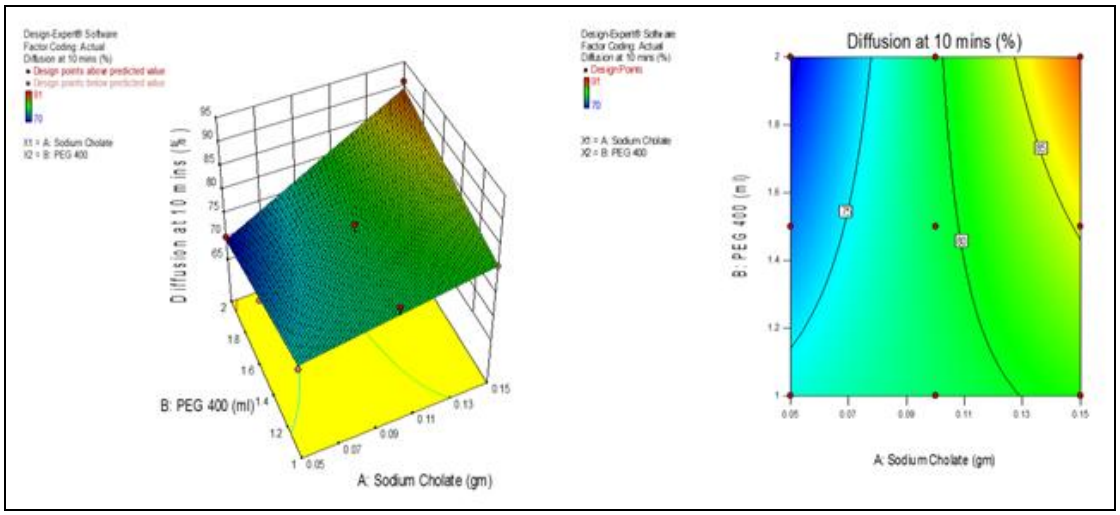

FIG. 2: 3D RESPONSE SURFACE AND COUNTER PLOT FOR % DIFFUSION AT 10 MINUTES

The **Table 4** shows the model summary statistic for diffusion at 10 minutes (Y_1). The data suggest the 2FI model shows relatively better fitting compared to other statistical models. The **Table 5** shows the impact of independent variables on the percentage diffusion at 10 minutes.

The data shows p-value < 0.05 indicating the factor has significant impact on dependent parameter. Hence the both independent variables (Sodium cholate and PEG 400) have significant impact on diffusion rate on formulation.

TABLE 4: MODEL SUMMARY STATISTICS

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	2.895691	0.742804	0.691365	0.359971	208.6603
2FI	1.751198	0.915341	0.887121	0.795826	66.56413 (Suggested)
Quadratic	1.610245	0.944327	0.904561	0.52981	153.2898
Cubic	1.365057	0.971422	0.931413	-2.31094	1079.424

TABLE 5: 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	298.4167	3	99.47222	32.43629	< 0.0001 (significant)	
Intercept						87.91795
A-Sodium cholate	240.6667	1	240.6667	78.47753	< 0.0001	-98.3333
B-PEG 400	1.5	1	1.5	0.489126	0.04020	-14
AB	56.25	1	56.25	18.34222	0.0020	150
Residual	27.60026	9	3.066695			
Lack of Fit	27.57226	5	5.514451	787.7788	< 0.0001 (significant)	
Pure Error	0.028	4	0.007			
Cor Total	326.0169	12				

The polynomial equation 4 shows the effects of independent variables X1 and X2 on viscosity of formulation.

Viscosity (Y2) = 13.56+10×X1+5.66×X2... Eq.(4)

The viscosity is also significantly affected by conc. of sodium cholate (X_1) and PEG 400 (X_2). The **Table 6** displays the model summary of statistical analysis for viscosity. The different coefficient of linearity value expresses that the linear model shows the better fitting compared to other models.

TABLE 6: MODEL SUMMARY STATISTICS FOR VISCOSITY

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.841488	0.875219	0.850263	0.795998	11.57667 (Suggested)
2FI	0.887007	0.875219	0.833626	0.652461	19.72206
Quadratic	0.514359	0.967365	0.944054	0.75855	13.7017
Cubic	0.551112	0.973239	0.935774	-2.09348	175.5479

The impacts of independent factors were evaluated as per **Table 7**. The both factors were found to have significant impact on viscosity. The changing factor X_1 from -1 to +1 shows the least impact on the viscosity of the formulation, while varying PEG 400 (X_2) from -1 to +1 show the viscosity change 20 cp to 25 cp. The different batches results show the viscosity has a linear relationship with PEG 400 concentration. The contour and surface plot for viscosity were constructed as represented in **Fig. 3**.

TABLE 7: ANOVA FOR RESPONSE VISCOSITY

ANOVA for Response Surface Linear 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	49.66667	2	24.83333	35.07025	< 0.0001 (significant)	
Intercept						13.56923
A-Sodium cholate	1.5	1	1.5	2.118337	0.1762	10
B-PEG 400	48.16667	1	48.16667	68.02216	< 0.0001	5.666667
Residual	7.081026	10	0.708103			
Lack of Fit	7.073026	6	1.178838	589.4188	< 0.0001 (significant)	
Pure Error	0.008	4	0.002			
Cor Total	56.74769	12				

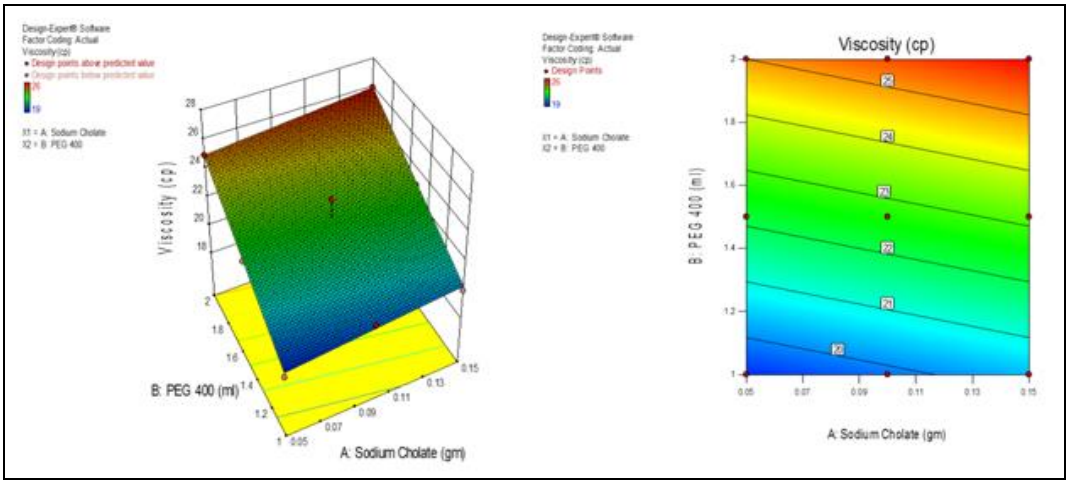

FIG. 3: 3D RESPONSE SURFACE AND COUNTER PLOT FOR VISCOSITY

The **Table 8** shows the percentage bias between the experimental and predicted value of different experimental batches.

TABLE 8: PERCENTAGE BIAS FOR EXPERIMENTAL BATCHES

Composition of Sodium Cholate	Composition of PEG 400	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
0.10	2.0	FA8	25.9026	25	3.60	80.0846	78.0	3.64
0.05	1.0	FA5	19.7359	19	3.84	76.5012	75.0	2.0
0.10	1.5	FA10	23.0692	24	3.91	79.5846	81.0	1.83
0.15	1.0	FA9	20.7359	20	3.65	81.6679	81.0	-0.81
0.10	1.5	FA1	23.0692	23.9	3.51	79.5846	80.9	1.63
0.15	1.5	FA3	23.5692	23	-2.43	85.9179	83.0	3.49
0.15	2.0	FA6	26.4026	26	-1.53	90.1679	91.0	0.92
0.05	2.0	FA7	25.4026	25	-1.60	70.0012	70.0	-0.001
0.10	1.5	FA12	23.0692	24	3.91	79.5846	80.8	1.50
0.10	1.0	FA4	20.2359	20	-1.15	79.0846	80.0	1.15
0.10	1.5	FA13	23.0692	24	3.91	79.5846	80.9	1.63
0.10	1.5	FA11	23.0692	24	3.91	79.5846	81.0	1.75
0.05	1.5	FA2	22.5692	22	-2.54	73.2512	72.0	-1.73

The % bias value of diffusion at 10 minutes (X_1) was found in the range of -1.73% to 3.64% and the % bias value for viscosity (X_2) was found in the range of -2.54% to 3.91%. The % bias for both the variables were found below 4 %, which significantly shows the selected model successfully fit to predict the response the experimental design area.

Optimization of Experimental Design: The experimental model was further validated by six check point batches. The checkpoint batches were further evaluated for diffusion and viscosity by using mathematics equation (3, 4). The **Table 9** shows the check point batches results for % diffusion at 10 minutes and viscosity results.

TABLE 9: CHECK POINT BATCHES RESULTS FOR DEPENDENT VARIABLES WITH % BIAS

S. no.	Conc. of sodium cholate	Conc. of PEG 400	Diffusion at 10 min (%)			Viscosity (cp)		
			Actual	Predicted	% Bias	Actual	Predicted	% Bias
1	0.07	1.1	77.36	77.17	0.36	21.12	20.49	2.98
2	0.12	1.3	82.36	81.31	1.14	21.76	22.11	-1.61
3	0.06	1.7	75.02	73.51	2.2	23.00	23.78	-3.39
4	0.12	1.5	83.78	82.11	1.99	22.8	23.25	-1.97

The optimization of formulation was carried out using the design expert software by construction of overlay plot as shown in **Fig. 4**. The optimized formulation (**Table 10**) contained sodium cholate 0.15 g and PEG 400 2.00 mL, which shows the observed value for % diffusion at 10 minutes and viscosity are 89.33% and 25.33 cps respectively. The percentage biases with respect to observed and predicted responses for optimized formulation are as displayed in **Table 11**.

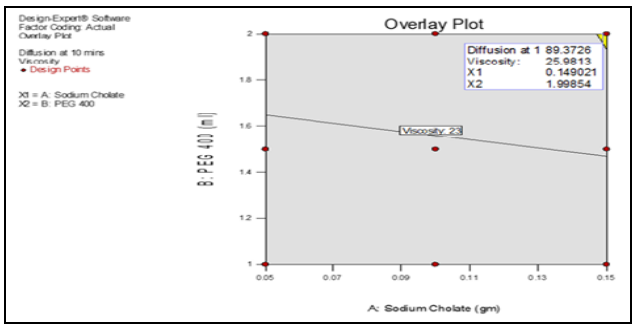

FIG. 4: OVERLAY PLOT OF OPTIMIZED FORMULATION

TABLE 10: OPTIMIZED BATCH COMPOSITION (FAE)

Ingredients	Composition
Xylometazoline HCl	100 mg
Sodium Cholate	1.50 gm
PEG 400	20 ml
Sodium CMC	0.1 gm
Glycerin	2.5 ml
Methyl Paraben	0.033 gm
NaCl	qs
Purified Water	qs to 100 ml

TABLE 11: COMPARISON OF PREDICTED AND OBSERVED RESPONSES OF OPTIMIZED FORMULATION

Response	Predicted	Observed	% Bias
Viscosity (cps)	25.98	25.33	2.50
% diffusion at 10 min	89.37	89.33	0.04

Other Evaluation Parameters:

pH of Formulation: The higher or lower pH of formulation induces irritation at the application site, hence the pH of the formulation is necessity to be controlled for effective delivery. The formulation pH was found 6.5 ± 0.3 as depicted in **Table 3**, which shows better compatibility at the delivery site.

Spray Content Uniformity: These formulations need to be assessed in terms of emitted dose content uniformity. The control of content uniformity is observed as the global performance evaluation of a system by assessing the formulation, manufacturing process, valve and the actuator. Nasal spray formulations are comprised of therapeutically active ingredient dissolved in solutions or mixtures of excipients in non-pressurized vending machine (dispenser) which transport a spray enclosing a metered dose of the active ingredient. The **Table 12** data shows the spray content (Drug content) of the same container and among different containers. The results show the spray content among same container and different containers are in the range of 95 - 102 %, which passes the test results criteria according to FDA guidelines for nasal products.

TABLE 12: DRUG SUBSTANCE CONTENT AMONG SAME CONTAINERS

S. no.	Batch no.	C1	C2	C3
1	FAE	96 %	94 %	101 %
Drug substance content among same containers				
S. no.	**Batch no.**	**S1**	**S2**	**S3**
1	FAE	98%	97 %	102 %

Pump Delivery: The appropriate performance from pump must be required for accurate nasal drug delivery. The container closure system procured from Aptar Pharma is qualified with tip seal technology, with 360 possible applications. The initial weight of filled nasal spray was 15 g, while after 10 actuations, the final weight of spray system was found the 14.5 g. The actuation volume for each spray was 100 µl. The data suggest the system was showing the accurate pump delivery of xylometazoline nasal spray.

Spray Pattern: Spray pattern is important for evaluating the performance of the pump. Various factors can affect the spray pattern, which includes the size and shape of the nozzle, the design of the pump, and the characteristics of the formulation. The ovality obtained was 1.217, while perimeter and area were found to be 104.56 mm and 791.5 mm^2 respectively. Image actuation graph and intensity graph for the formulation are as shown in **Fig. 5**, where the image actuation graph depicts the force in kg and position in mm relating to spray pattern. While the intensity graph depicts the time in millisecond to relate to spray pattern. The image shows the spray pump successfully delivered medication without any problem.

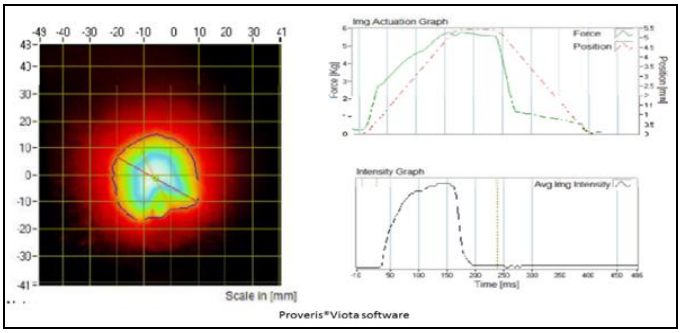

FIG. 5: SPRAY PATTERN, IMAGE ACTUATION GRAPH AND INTENSITY GRAPH OF FORMULATION

Net Content and Weight Loss: The formulation stability was evaluated according to ICH guideline for conducting stability of drug products, where the formulation was stored in inverted and horizontal position evaluating for weight loss and net content

test. Net content in the product container closure was checked initially and weight loss study was performed for 3 and 6 months. As shown in **Table 13**, the results obtained revealed that no any significant change was observed with respect to horizontal and inverted position for weight loss study at 3 months and 6 months. Thus indicating the integrity of container closure system.

TABLE 13: HORIZONTAL POSITION FOR NET CONTENT AND WEIGHT LOSS EVALUATION

S. no.	Batch no.	Container	Initial wt.	Wt. after 3 months	Wt. after 6 months
1	FAE	1	15 gm	15 gm	15 gm
2		2	15 gm	15 gm	15 gm
Horizontal position for net content and weight loss evaluation					
S. no.	**Batch no.**	**Container**	**Initial wt.**	**Wt. after 3 months**	**Wt. after 6 months**
1	FAE	1	15 gm	15 gm	15 gm
2		2	15 gm	15 gm	15 gm

Priming and Repriming Study: Priming and Repriming study was performed to support the number of actuations to be suggested which need to be fired to discarded prior to the end user using the product for the first time and subsequent use afterwards. A study, better be accompanied to support the length of time that the prepared formulation might be stored deprived of use after initial priming, but before re-priming, as the number of repriming actuations essential.

The priming study was performed on container closure and the results obtained were 97.6% and 98.5% respectively for first actuation. Priming study results reveal that first actuation itself delivered greater than 95% of drug content thus indicating that only one actuation requirement as priming. The minimum results found for priming study was 97.6%.

Repriming study was implemented on formulation at interval of 5 days, 10 days and 30 days and the results obtained as shown in table14. The minimum result for repriming within 5, 10 and 30 days was found to be 95.4% while the maximum result obtained was 100.2%. It was observed that only one actuation was sufficient for priming as well as repriming to meeting the drug product specification criteria.

TABLE 14: REPRIMING STUDY FOR 5 DAYS

Cont. no.	Duration	Repriming	No. of Actuations	Assay Results
1	5 d	Yes	1	95.4 %
2	5 d	Yes	1	99.7 %
Repriming study for 10 days				
1	10 d	Yes	1	100.2%
2	10 d	Yes	1	96.8 %
Repriming study for 30 days				
1	30 d	Yes	1	98.5 %
2	30 d	Yes	1	99.4%

***In vitro* Diffusion:** The *in vitro* diffusion of developed formulation was compared with current marketed (Refer supplementary **Table 2**) formulation Otrivin® Adult (Novartis). The *in-vitro* diffusion comparison data (**Fig. 6**) shows that the percentage drug release was found superior for developed nasal spray formulation in comparison with the marketed formulation. The results obtained at 10 minare 89.00% and 81.00% for optimized and marketed formulation respectively. The developed formulation shows the higher amount of drug released for same duration compared to marketed formulation. Hence, it was concluded that developed formulation is more superior compared to current marketed formulation.

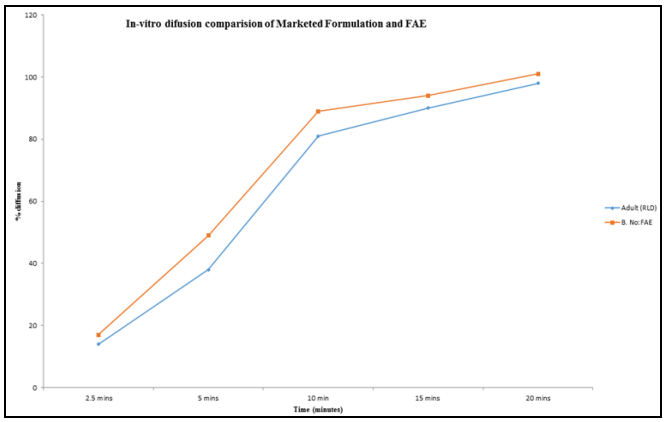

FIG. 6: *IN VITRO* DIFFUSION COMPARISON OF MARKETED FORMULATION AND OPTIMIZED BATCH

Sterility: The sterility of product was performed according to USP sterility tests. The sterility test results show no any type of microbial growth was observed, which endorses that the formulation is sterile.

Stability Studies: The stability of formulation was carried out at 25 ± 2 °C / 60 ± 5% RH (long term conditions) and accelerated condition studies were

performed at 40 ± 2 °C/ 75 ± 5% RH. The results obtained as displayed in **Table 15**. All the results related long and accelerated stability studies were found well within predefined specification and no significant change was observed in context of initial results. During and at the completion of the stability study (accelerated and long term), the formulation disclosed drug content comparable to original results. Formulation also demonstrated the sensible appearance, viscosity, pH and *in vitro* diffusion at the completion of the stability study. The stability study results the formulation remained unique without any significant change until 12 months.

TABLE 15: 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	6.50 ± 0.0	6.40 ± 0.0	6.5 ± 0.0	6.53 ± 0.05	6.50 ± 0.0
Viscosity (cp)	25.33 ± 0.57	24.33 ± 0.57	24.33 ± 0.57	25.33 ± 0.57	25.00 ± 1.00
Assay (%)	98.7 ± 0.38	99.37± 0.75	99.40 ± 0.76	99.07 ± 1.23	98.60 ± 0.83
% diffusion	89.33 ± 0.57	91.66 ± 1.52	90.66 ± 1.52	90.33 ± 1.52	88.66 ± 0.57
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	6.50 ± 0.0	6.53 ± 0.05	6.50 ± 0.0		
Viscosity (cp)	25.33 ± 0.57	25.66 ± 0.57	24.33 ± 0.57		
Assay (%)	98.7 ± 0.38	100.44 ± 0.46	99.16 ± 0.35		
% diffusion	89.33 ± 0.57	90.66 ± 0.57	90.33 ± 1.15		
Net content*	10 ml	--	10 ml		
Sterility*	confirms	--	confirms		

* indicates test performed at initial and end of shelf life

CONCLUSION: The nasal solution of xylometazoline spray was successfully developed using surfactant sodium cholate and PEG 400. The pre-formulation study was supported for active pharmaceutical ingredient xylometazoline. The drug-excipient compatibility study exposed there is no interaction between xylometazoline and excipients used in the formulation. Optimization was completed using 3^2 full factorial design, where sodium cholate (X1) and PEG400 (X2) were taken as independent factors. The formulation was evaluated for pH, viscosity of solution, assay, diffusion, plume geometry, priming and repriming, weight loss and sterility. The different parameters results show there is no any significant change in formulation. The developed formulation was evaluated for its stability up to 12 months long term conditions and 6 months accelerated conditions. The meter dose nasal formulation shows the superior performance during *in vitro* drug release compared with current marketed formulation thus providing alternative choice for treatment of nasal decongestion.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interests.

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