Chapter 7
Formulation of nasal spray

7 Formulation of nasal spray

7.1 Introduction

Currently, nose to brain delivery systems are widely explored as these can bypass blood brain barrier facilitating entry of therapeutic agents into brain. This route has been employed for local as well as systemic delivery of active agents and provides advantages including cost-effectiveness, user friendly, possibility of self-administration and improved patient compliance. Various formulations including nasal spray, nasal powder, nasal drops, nasal inserts and nasal gel can be administered intranasally and employed for nose to brain delivery. Amongst all the available formulations, nasal spray provides non-invasive administration approach along with fast onset of therapeutic activity augmenting its importance over other formulations. Additionally, market share of nasal spray in 2016 was around 23 billion US$ which is expected to increase to about 35 billion US $ by 2023 [1].

Nasal spray can be delivered using actuators and pumps with precise dosing within 25-200 µl per spray. Dosing accuracy in nasal spray can be achieved by controlling droplet size distribution, spray pattern and plume geometry. This factors in-turn depend on various formulation characteristics including formulation thixotropy, viscosity and surface tension [2]. Additionally, design of nasal spray delivery device also has important role in regulating dosage accuracy and thus various parameters of delivery device like actuation force, pump design, orifice size etc need to be controlled and optimized [3].

Nasal spray can be delivered using spray pumps like – (i) Metered dose spray pumps which replace generated liquid spray with air and the dose administration from such device is accurate despite of variation in position and spraying angle and thus it is favorable in pediatric and bed-ridden patients. Antioxidants and preservatives are required to be added in formulation as administered dose is replaced with air. But, currently evolution in design of such delivery device has led to filtration of air before it enters the container and thus facilitating formulation of preservative free nasal spray. (ii) Nasal pressurized metered dose inhalers which employs propellants for delivery of therapeutics and widely used for local effect.

The excipients employed in formulation of nasal spray and their role are summarized in Table 7. 1 along with some marketed nasal spray formulations in Table 7. 2 [4].

Table 7. 1: Excipients used in nasal spray

Role	Excipients	IIG limit for nasal route (%w/w)
Tonicity agent	Anhydrous dextrose	0.5
	Sodium chloride	1.9
pH adjusting agents	Hydrochloric acid	Not reported
	Sodium hydroxide	0.004
	Sulfuric acid	0.4
Preservatives	Benzyl alcohol	0.0366
	Benzalkonium chloride	0.119
	Chlorobutanol	0.5
	Methyl paraben	0.7
	Propyl paraben	0.3
Buffering agent	Anhydrous trisodium citrate	0.0006
Surfactant	PEG 400	20
	PEG 3500	1.5
	Polyoxyl 400 stearate	15
	Polysorbate 20	2.5
	Polysorbate 80	10
Suspending agent	Cellulose microcrystalline	2
	CMC-Na	0.15
Antioxidants	Butylated hydroxy anisole	0.0002
	Butylated hydroxy toluene	0.01
Cation chelating agent	Disodium EDTA	0.5
Co-solvent	Alcohol	2
	PEG-400	20
	Propylene glycol	20
Humectant	Glycerine	0.233
Penetration enhancer	Oleic acid	0.132

Table 7. 2: Marketed nasal spray formulations

Class	Active drug	Brand name	Manufacturer
Antihistamine	Azelastine	Astelin Nasal Spray	Meda Pharmaceuticals
		Astepro	Meda Pharmaceuticals
	Olopatadine	Patanase	Alcon laboratories
Nasal Antihistamine and Nasal Steroid	Azelastine and Fluticasone Propionate	Dymista	Meda Pharmaceuticals
Nasal Steroids	Beclomethasone Diproprionate	Q-Nasl	Teva Respiratory
	Budesonide	Rhinocort	AstraZeneca
	Ciclesonide	Omnaris Nasal Spray	Sunovion Pharmaceuticals Inc.
		Zetonna	Sunovion Pharmaceuticals Inc.
	Flunisolide	Flunisolide 0.025% Solution	Apotex
	Fluticasone Furoate	Veramyst nasal spray	GlaxoSmithKline
		Flonase Sensimist	GlaxoSmithKline
	Mometasone Furoate Monohydrate	Nasonex Nasal Spray	MSD
	Triamcinolone Acetonide	Nasacort AQ	Chattem, Inc.
Decongestant	Oxymetazoline	Afrin	Merck
Anticholinergic	Ipratropium Bromide	Atrovent Nasal Spray	Roxane Laboratories, Inc.
Protein/ Peptide	Salmon calcitonin	Karil 200 I.E.	Novartis Pharma

	Desmopressin	Minirin Nasenspray	Ferring Arzneimitted
	Buserelin	Profact nasal	Aventis Pharma
	Nafarelin	Synarela	Pharmacia
	Oxytocin	Syntocinon	Novartis Pharma
	Protirelin	Antepan nasal	Aventis Pharma
		Relefact TRH nasal	Aventis Pharma
	Insulin	Nasulin	CPEX Pharmaceuticals
Triptans	Zolmitriptan	AscoTop Nasal	Astra Zeneca
	Sumatriptan	Imigran nasal	Glaxo SmithKline
Hormone replacement	Estradiol	Aerodiol	Servier

7.2 Procedure

For preparation of nasal spray, Glycerine and propylene glycol were taken and stirred well. Simultaneously, citric acid and trisodium citrate, dissolved in water were added to above mixture. The formulations (biodegradable bPEI-Chi polyplex or lipopolyplex) were incubated with Tween-80 for 15 min, which were later added to above mixture. Finally, benzalkonium chloride was added as preservative and volume was made up with nuclease free water. The optimized formula for the preparation of nasal spray is represented in Table 7. 3.

Table 7. 3: Optimized formula for nasal spray

Ingredients	**Quantity (%w/v)**
Glycerine	0.5%
Propylene glycol	2.5%
Citric acid	0.1407%
Trisodium citrate	0.3728%
Tween-80	1%
Benzalkonium chloride	0.1%

7.3 Characterization

7.3.1 pH

pH of prepared nasal spray was determined using Labindia pH meter. Briefly, fixed sample volume was taken in beaker and glass electrode was dipped in it. This was allowed to stabilize for some time and then pH was recorded.

7.3.2 Osmolality

Osmolality was determined using Advanced® 3250 Single-Sample Osmometer (Advanced Instruments, Inc., Norwood, MA, USA). The instrument was operated according to manufacturer's specifications. Briefly, empty sample tube was placed inside sample well and test was started. After some preliminary testing 0.25 ml test sample was added in the sample tube and the result displayed was recorded.

7.3.3 Viscosity

The viscosity of each nasal spray formulation was measured using a Brookfield DV-II+ Pro viscometer (Brookfield Engineering Laboratories, Middleboro, MA, USA). The test formulation was loaded in sample holder with a constant volume and rested for 30 min with the solvent cap on before the measurement. An appropriate spindle (number #63) was immersed in the test liquid and rotated at different speed (10, 20, 50, 100 rpm). The reading was taken after five full spindle rotations. Three replicate measurements per formulation were carried out at a temperature of 25.5 ± 0.2 °C.

7.3.4 Pump delivery volume

To determine pump delivery volume of CPS Technology Platform spray pump (Aptar Pharma, Illinois, USA), 5 spray pumps were filled with 10 gm nasal spray. These were then primed, followed by 5 test actuations and weighed prior to and after each test actuation using analytical balance (Shimadzu, Japan). Pump delivery was determined using formula

$$Pump\ delivery\ = \frac{(W_1 - W_2)}{D}$$

Where,

W_1 = Initial weight

W_2 = Weight after actuation

D = Density of liquid formulation (1.032 g/ml)

7.3.5 Priming

10 gm of nasal spray was filled in the CPS Technology Platform spray pump (Aptar Pharma, Illinois, USA) and priming was determined by manually actuating the pump. Number of sprays required to achieve delivery of desired dose/volume of nasal spray was considered priming. Additionally, manual actuation was continued and the number of sprays that could be delivered uniformly were counted before priming the spray pump again.

7.3.6 Repriming

In order to test whether resting time has any effect on unprimed nasal sprays, CPS Technology Platform spray pumps filled with approximately 10 gm nasal spray were primed before initiation of test. Subsequently, the spray pumps were kept under resting time for 0, 6, 12, 24, 48 and 72 h. After resting time, the pumps were manually actuated and % pump delivery volume for 5 actuations from each pump, without priming, was determined using analytical balance (Shimadzu, Japan). This study was used to determine length of time for which nasal spray can deliver uniform dose without repriming and number of actuations required for repriming.

7.3.7 Spray pattern

For spray pattern determination, FD&C Blue No. 2 dye was dissolved in the formulated nasal spray in concentration such that it does not affect nasal spray properties. This was filled in nasal spray bottles and the pumps were actuated manually. The resultant spray was captured on a white cardboard sheet located at 3 or 6 cm above the tip of spray nozzle. The longest chord (LL) and shortest chord (LS) were measured across the spray pattern and ratio of LL to LS was also calculated. This ratio, defined as the ovality ratio, characterizes the general shape of each pattern. Each sample was tested in triplicate.

7.3.8 Plume geometry

Plume geometry was measured using a SprayVIEW™ NSP system and analyzed by SprayVIEW™ software version 3.6.1 (Proveris Scientific Corporation, USA). All units were actuated with an automated NSx Actuation Station. The SprayVIEW™ NSP combines laser sheet illumination and high-speed digital imaging and is designed specifically to characterize pharmaceutical nasal spray pumps. The plume geometry was characterized by the following metrics: spray angle (the angle of emitted plume measured from vertex of the spray cone and spray nozzle) and plume

width (the width of plume at a given distance from the spray nozzle) per FDA Guidance for Industry. The software allows user to select a single frame from fully developed plume phase by observing intensity profile throughout the life of the spray. The user then manipulates cursors on this image using the mouse to determine plume angle and plume width at a user selected actuation distance. Plume width was calculated at 3 and 6 cm from nozzle orifice.

7.3.9 Droplet size distribution

Droplet size distribution was determined by laser diffraction technique employing HELOS BR instrument with SPRAYER module and force actuator (Sympatech GmbH, Clausthal-Zellerfeld, Germany). Various instrumental parameters were varied and effect of such parameters in droplet size distribution of nasal spray was studied. The spraying angle was either 30°, 60° or 90° while the actuation force was kept 35 or 45 N. Actuation distance was set as 3 or 6 cm and hold time was kept either 1, 2 or 3 sec. Time resolved measurement was performed and data were analyzed by Fraunhofer theory.

7.3.10 Weight loss

This test was performed to assess sealing characteristics of nasal spray pumps. The nasal spray pumps were filled with approximately 10 gm formulated nasal spray and the pumps were kept in inverted, upright and horizontal position and weight of pump along with spray was determined using analytical weight balance at fixed time intervals for 3 months.

7.3.11 Tail-off characteristics

Approximately, 500 mg of nasal spray was filled in the CPS Technology Platform spray pump (Aptar Pharma, Illinois, USA) and tail-off characteristic including droplet size distribution, spray pattern and shot weight were determined employing procedure as described in previous sections.

7.3.12 Microbial limit

Microbial limit is expressed in terms of TAMC (total aerobic microbial count) and TYMC (total yeast mould count). For determination, ready prepared media plates of TSA and SDA were employed respectively for TAMC and TYMC. 1 ml suitably diluted nasal spray sample was pipetted out on both TSA and SDA plates and was spread uniformly on media plates using glass spreader. This was allowed to settle for 30 min and later the plates were incubated in lid down position. TSA plates were

incubated at 30-35 °C for 3-5 days while SDA plates were incubated at 20-25 °C for 5-7 days. After incubation, microbial colonies were counted and data were recorded. The criteria for passing the test according to USP is depicted in Table 7. 4 [5, 6].

Table 7. 4: Criteria for passing the microbial limit test for nasal spray

Microbial count	Criteria
TAMC	NMT 10^2 CFU/ml
TYMC	NMT 10^1 CFU/ml
Absence of specified microorganism (1 ml)	*Staphylococcus aureus* *Pseudomonas aeruginosa*

7.3.13 Preservative efficacy study

Preservative efficacy study was carried out using 5 strains - *Aspergillus niger* ATCC No. 16404, *Candida albicans* ATCC No. 10231, *Escherichia coli* ATCC No. 8739, *Pseudomonas aeruginosa* ATCC No. 9027, *Staphylococcus aureus* ATCC No. 6538. The nasal spray was inoculated with each organism such that final concentration of test preparation was 1 x 10^5 to 1 x 10^6 cfu/ml. The volume of inoculum used was between 0.5% to 1% of total volume of the product. 1 ml of the inoculated sample was pipetted out on both TSA and SDA plates for bacteria and fungi respectively and was spread uniformly on media plates using glass spreader. This was allowed to settle for 30 min and later the plates were incubated in lid down position. TSA plates were incubated at 30-35 °C for 3-5 days while SDA plates were incubated at 20-25 °C for 5-7 days. After incubation, microbial colonies were counted and the data were recorded. This procedure was performed at regular time intervals (0 day, 7 days, 14 days and 28 days) and log reduction in concentration of cfu/ml of each microorganism was calculated. The criteria for passing the test is depicted in Table 7. 5.

Table 7. 5: Criteria of tested microorganism for passing the preservative efficacy test

Organism	Criteria
Bacteria	NLT 1.0 log reduction from intial at 7 days NLT 3.0 log reduction from intial at 14 days No increase from 14 days at 28 days.
Yeast and molds	No increase from initial at 7, 14 and 28 days

7.4 Result and discussion

7.4.1 Physicochemical properties

The pH of nasal cavity has been found to have great impact on absorption of drugs and other therapeutics through nasal cavity [7]. According to a study performed by Washington et al. average nasal pH is 6.3 which may increase in presence of mildly acidic solutions or buffer with higher buffering capacity [8]. This study thus signified pH regulation and buffer concentration in nasal spray formulations. This formed the basis for FDA's recommendation for optimal pH range viz. 4.5-6.5 [9]. The pH of optimized formulation for nasal spray was 5.12 ± 0.01.

FDA recommends osmolality of nasal spray containing tonicity agents should be checked and controlled. Ideally, osmolality should be in range of 300-700 mOsmol/kg as hypotonic nasal sprays have shown improvement in nasal mucosal permeability [10, 11]. The osmolality of final optimized formulation was 535 ± 5 mOsmol/kg.

The guidance document released by FDA for nasal spray suggest to check and control viscosity of nasal spray on release as well as stability [10]. The results for viscosity of optimized formulation of nasal spray at different rotations is depicted in Table 7. 6.

Table 7. 6: Viscosity of nasal spray formulation

Spindle Rotation (RPM)	**Viscosity (cP)**
10	40.5 ± 1.5
20	11.25 ± 0.75
50	3.9 ± 0.3
100	1.35 ± 0.15

The results of viscosity for the optimized formulation suggest that formulated nasal spray showed shear thinning properties and thus viscosity decreased proportionally as shear increased. A previous study reported viscosities for four marketed formulations including Nasacort, Beconase, Nasonex and Flixonase, which showed similar trend of shear thinning property [12]. Additionally, shear thinning property proves beneficial in maintaining properties like plume geometry, droplet size distribution and spray pattern, on actuation of highly viscous nasal spray. Furthermore, viscosity of nasal spray is very important in prevention of dripping and increasing residence time and thereby prolonging therapeutic action [13].

7.4.2 Pump delivery volume

The results of pump delivery volume of 5 different spray pumps, each pump actuated for 5 actuations, are demonstrated in Table 7. 7. The average pump delivery volume of nasal spray was 73.524 ± 0.995 µl.

Table 7. 7: Pump delivery volume for nasal spray pump

Spray number	**Delivery volume (µl)**				
	Pump 1	**Pump 2**	**Pump 3**	**Pump 4**	**Pump 5**
1	73.2	75.5	72.9	72.9	73.9
2	72.1	73.9	74.8	74	73.1
3	73.4	73.7	74.1	72.9	73.2
4	74	74.9	74.6	70.8	74.5
5	72.5	76.4	73.4	70.5	72.9
Average	73.04	74.88	73.96	72.22	73.52

7.4.3 Priming

The result of priming of nasal spray pump is depicted in Table 7. 8.

Table 7. 8: % Pump delivery for nasal spray pump

Spray number	**Shot weight (mg)**	**Pump delivery (µl)**	**% pump delivery**
1	47.0	45.5	62.0
2	49.2	47.7	64.9
3	55.7	54.0	73.4
4	59.9	58.0	79.0
5	62.8	60.9	82.8
5 actuations are required for priming			
1	74.3	72.0	98.0
2	76.1	73.7	100.3
3	75.6	73.3	99.7
4	76.4	74.0	100.7
5	73.3	71.0	96.6
6	72.8	70.5	96.0
7	76.7	74.3	101.1
8	73.1	70.8	96.4
9	74	71.7	97.6

10	75.7	73.4	99.8
11	73.7	71.4	97.2
12	72.9	70.6	96.1
13	76.3	73.9	100.6
14	75.6	73.3	99.7
15	75.8	73.4	99.9
16	76	73.6	100.2
17	74	71.7	97.6
18	75.7	73.4	99.8
19	75.4	73.1	99.4
20	75.9	73.5	100.1
21	74.5	72.2	98.2
22	75.4	73.1	99.4
23	75.6	73.3	99.7
24	76	73.6	100.2
25	74.6	72.3	98.3
26	76.2	73.8	100.5
27	75.6	73.3	99.7
28	74.9	72.6	98.7
29	76.4	74.0	100.7
30	75.7	73.4	99.8
31	75.9	73.5	100.1
32	76	73.6	100.2
33	76.3	73.9	100.6
34	76.3	73.9	100.6
35	76.8	74.4	101.2
36	75.7	73.4	99.8
37	74.5	72.2	98.2
38	76.3	73.9	100.6
39	76.1	73.7	100.3
40	76.2	73.8	100.5
41	75.6	73.3	99.7

42	75.5	73.2	99.5
43	76.8	74.4	101.2
44	76.1	73.7	100.3
45	75.9	73.5	100.1
46	75.9	73.5	100.1
47	75.2	72.9	99.1
48	75.6	73.3	99.7
49	76.3	73.9	100.6
50	75.4	73.1	99.4
51	75.7	73.4	99.8
52	74.6	72.3	98.3
53	77.1	74.7	101.6
54	75.9	73.5	100.1
55	75.1	72.8	99.0
56	75.6	73.3	99.7
57	75.9	73.5	100.1
58	76.5	74.1	100.9
59	75.6	73.3	99.7
60	76.1	73.7	100.3
61	76.2	73.8	100.5
62	76.2	73.8	100.5
63	76.3	73.9	100.6
64	76.7	74.3	101.1
65	75.9	73.5	100.1
66	76	73.6	100.2
67	75.9	73.5	100.1
68	73.3	71.0	96.6
69	74.4	72.1	98.1
70	76	73.6	100.2
71	75.2	72.9	99.1
72	75.7	73.4	99.8
73	76.2	73.8	100.5

74	73.9	71.6	97.4
75	76	73.6	100.2
76	76.1	73.7	100.3
77	75.8	73.4	99.9
78	76.2	73.8	100.5
79	76.4	74.0	100.7
80	75.5	73.2	99.5
81	76.3	73.9	100.6
82	76.2	73.8	100.5
83	75.9	73.5	100.1
84	76.2	73.8	100.5
85	76.2	73.8	100.5
86	76.1	73.7	100.3
87	76.4	74.0	100.7
88	76	73.6	100.2
89	76.5	74.1	100.9
90	75.4	73.1	99.4
91	75.8	73.4	99.9
92	76.5	74.1	100.9
93	76.3	73.9	100.6
94	75.8	73.4	99.9
95	76	73.6	100.2
96	75.8	73.4	99.9
97	75.3	73.0	99.3
98	75.3	73.0	99.3
99	74.7	72.4	98.5
100	75.8	73.4	99.9
101	76.4	74.0	100.7
102	75.2	72.9	99.1
103	75.3	73.0	99.3
104	73	70.7	96.2
105	74.1	71.8	97.7

106	74.8	72.5	98.6
107	74.7	72.4	98.5
108	74.4	72.1	98.1
109	72.9	70.6	96.1
110	74.7	72.4	98.5
Total number of sprays allowed: 110 sprays			
111	64	62.0	84.4
112	62.9	60.9	82.9
113	55.4	53.7	73.0
114	54.2	52.5	71.5
115	50.6	49.0	66.7

The data depicted above is graphically represented in Figure 7. 1. According to FDA guidance document, the % pump delivery of individual spray should be within 15% of target delivery. Thus, in order to achieve uniformity in pump delivery, this nasal spray pump should be primed with 5 actuations. Additionally, nasal spray pump delivers accurately up to 110 sprays and after that the % pump delivery exceeds the ± 15% limit. Furthermore, the average % pump delivery of 110 actuations was 99.58 ± 1.25% which was well within 10% limit recommended by FDA for average pump delivery.

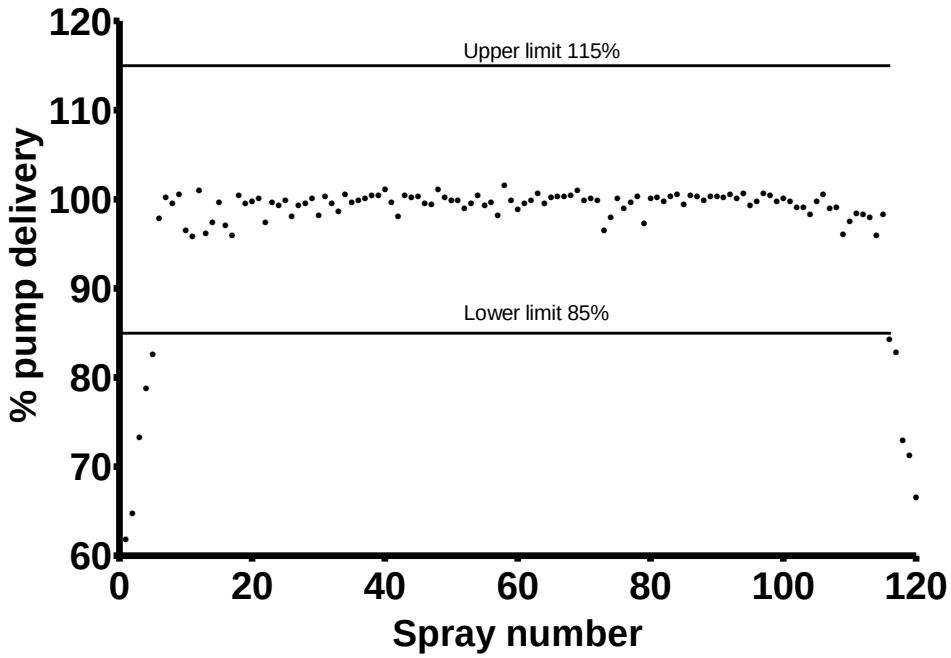

Figure 7. 1: Graphical representation of % pump delivery of nasal spray pump

7.4.4 Repriming

The data depicting effect of resting time on pump delivery and thereby having decisive role on repriming of nasal spray pumps, is demonstrated in Table 7. 9 and Figure 7. 2. The data concluded that % pump delivery of nasal pumps was within FDA's recommended limit i.e. 85-115% but after 24 h the % pump delivery falls beyond the allowed range. Thus, such pump can be used without priming if kept unused for not more than 24 h. Additionally, it was found that 3 actuations were required for repriming of the nasal spray pump.

Table 7. 9: % Pump delivery volume for nasal spray pump to determine effect of resting time

Resting time (h)	**% Pump delivery**					
	Pump 1	**Pump 2**	**Pump 3**	**Pump 4**	**Pump 5**	**Mean ± SD**
Initial	98.09	97.69	100.46	100.72	98.22	99.03 ± 1.44
6	98.61	100.72	98.74	99.01	97.43	98.90 ± 1.18
12	99.14	99.27	97.29	99.40	99.80	98.98 ± 0.98
24	94.92	95.05	97.03	99.54	98.35	96.98 ± 2.02
48	77.91	70.00	78.31	76.73	80.02	76.60 ± 3.87
72	68.95	69.87	64.47	72.11	60.38	67.16 ± 4.70

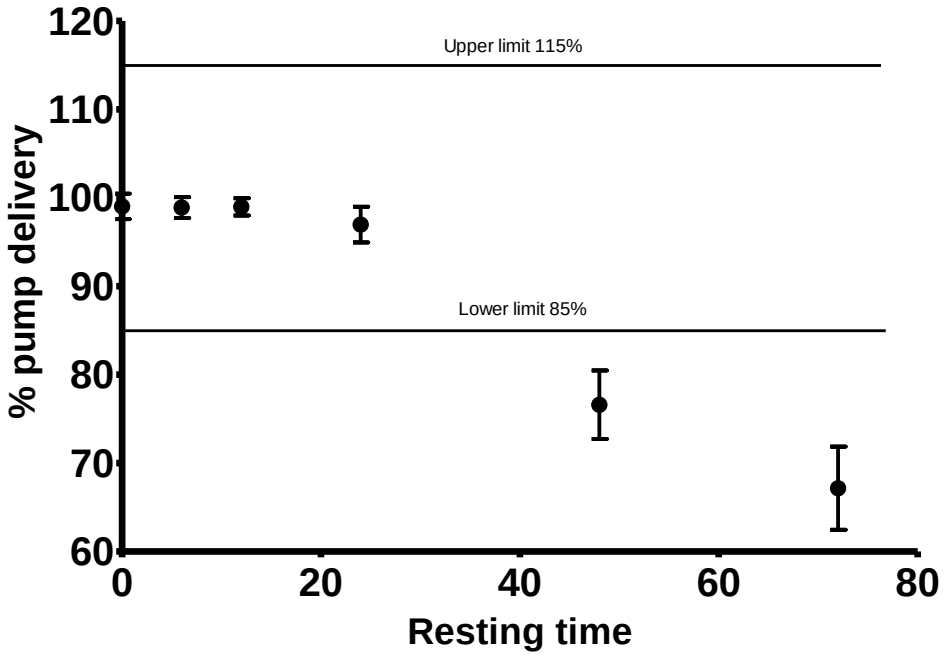

Figure 7. 2: Graphical representation of effect of resting time on % pump delivery from nasal spray pump

7.4.5 Spray pattern

The results of spray pattern of nasal spray, determined by impaction method, are shown in Figure 7. 3 and Figure 7. 4.

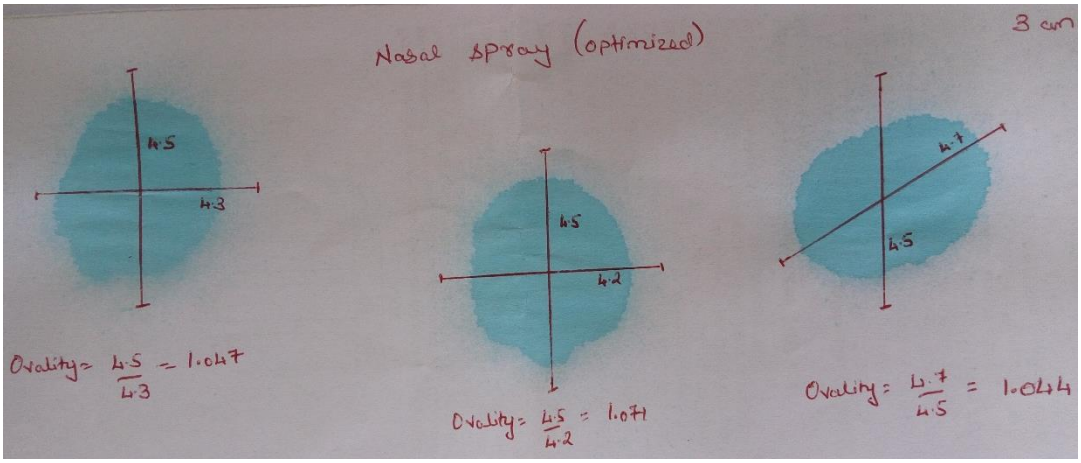

Figure 7. 3: Spray pattern for nasal spray at 3 cm

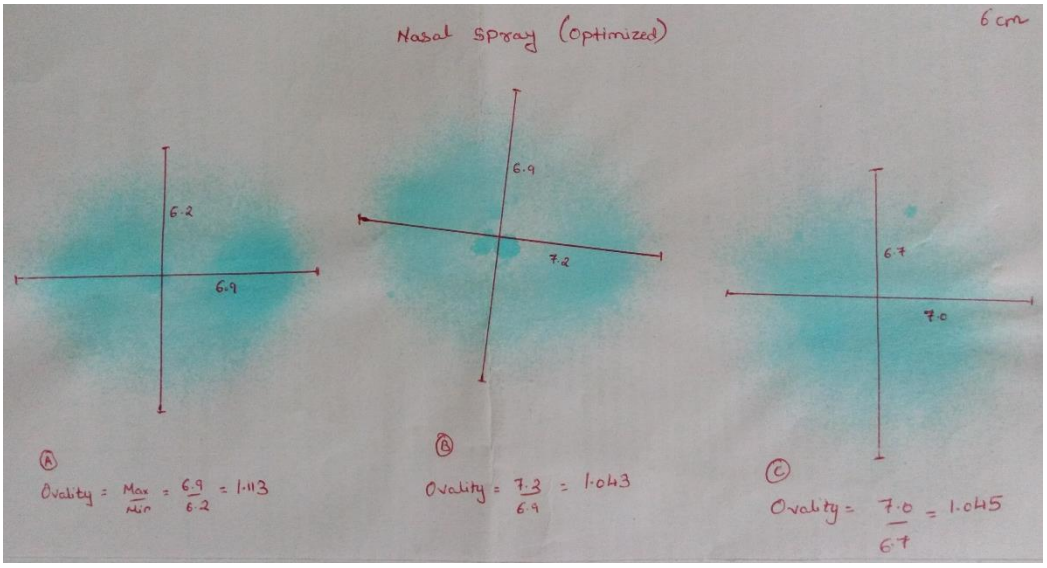

Figure 7. 4: Spray pattern for nasal spray at 6 cm

The spray pattern as recommended by FDA was measured at 2 distances from nozzle tip, 3 cm apart. The results of nasal spray showed that shape of the spray pattern was ellipsoidal in shape with no axis longer than 4.7 cm and 7.2 cm at distance of 3 cm and 6 cm, respectively. The data for ovality ratio are depicted in Table 7. 10.

Table 7. 10: Data depicting ovality ratio of nasal spray at 3 cm and 6 cm

Distance (cm)	Longest chord (LL)	Shortest chord (LS)	Ovality ratio (LL/LS)	Mean ± SD
3 cm	4.5	4.3	1.047	1.054 ± 0.015
	4.5	4.2	1.071	
	4.7	4.5	1.044	
6 cm	6.9	6.2	1.113	1.067 ± 0.039
	7.2	6.9	1.043	
	7.0	6.7	1.045	

According to previous studies, ovality ratio near 1 depicts ideal and uniform spray pattern [7] and data of optimized nasal spray for ovality ratio comply with it at both distances. Although, increase in distance form nozzle tip resulted in proportional increase in spray area, longest as well as shortest chord length, there was negligible change in ovality ratio. This proved that measurement distance from nozzle does not have significant effect on spray pattern [14].

7.4.6 Plume geometry

The results of plume geometry of nasal spray, determined by SprayVIEW™ NSP system, are shown in Figure 7. 5.

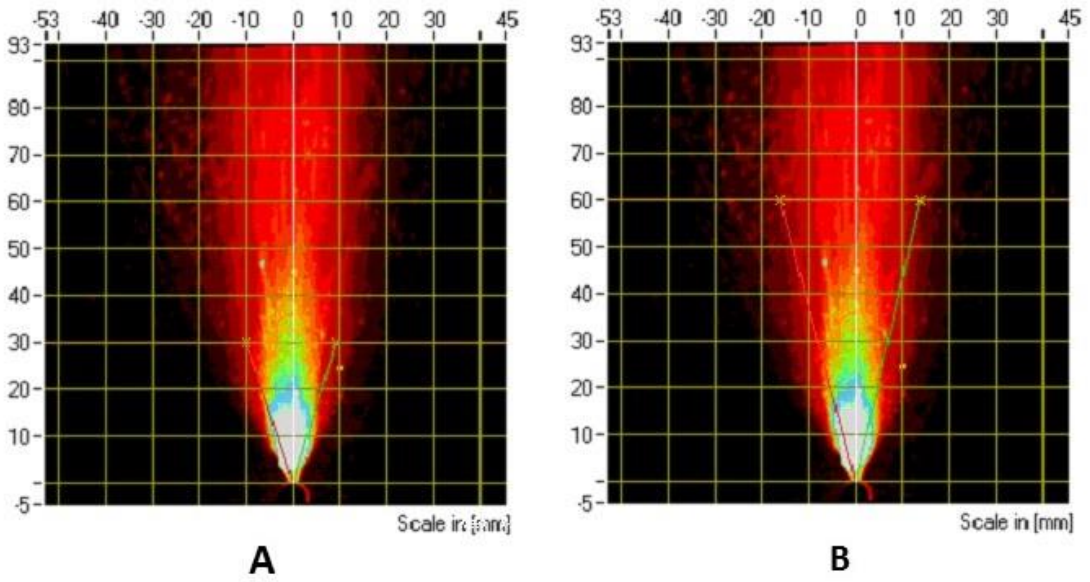

Figure 7. 5: Plume geometry of nasal spray at (A) 3 cm (B) 6 cm

When the results were analyzed by SprayVIEW™ software version 3.6.1 the data showed that plume angle and plume width were 35.4 ± 2.5° and 19.2 ± 1.4 mm,

respectively at 3 cm. Similarly, plume angle and plume width at 6 cm were 28.1 ± 0.4° and 30.0 ± 0.5 mm, respectively. This optimized nasal spray may improve nose to brain delivery of therapeutics. This was supported by various in-vitro and in-vivo studies, which were carried out to determine influence of plume angle on nasal spray deposition characteristics [15, 16]. Such studies concluded that nasal spray formulations with plume angle less than 35° showed more than 90% deposition in posterior nasal cavity which potentiated nose to brain delivery through olfactory neuroepithelium. Additionally, much of the nasal spray therapeutics were lost in anterior region on augmentation of spray width.

7.4.7 Droplet size distribution

The droplet size distribution of nasal spray was determined using HELOS BR Sympatech instrument. Various actuation parameters including spray angle, actuation force and actuation distance were optimized to select ideal actuation parameters to obtain desired droplet size distribution of nasal spray formulation. As per FDA's recommendation, D_{50} should be between 30-70 µm while D_{90} should be less than 200 µm. Also, % droplets having size less than 10 µm should be minimum as these are absorbed systemically via lungs [7].

7.4.7.1 Effect of spraying angle on droplet size distribution

The results of droplet size distribution at spray angle varied at 30°, 60° or 90° are demonstrated in Figure 7. 6, Figure 7. 7 and Figure 7. 8, respectively. The results infer that there was no significant influence of spraying angle on droplet size distribution. The individual parameters i.e. D_{10}, D_{50}, D_{90}, span value and % droplets having size less than 10 µm are enlisted in Table 7. 11

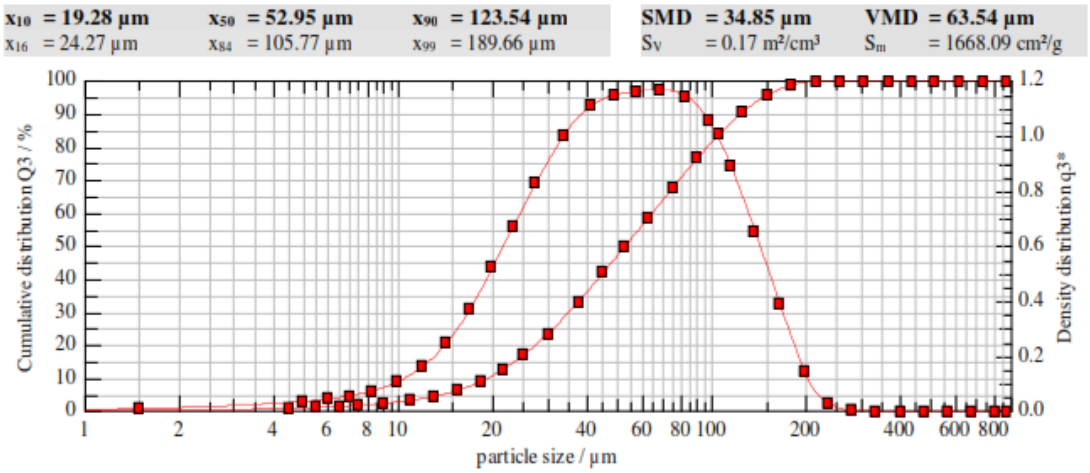

Figure 7. 6: Droplet size distribution of nasal spray at spraying angle 30°

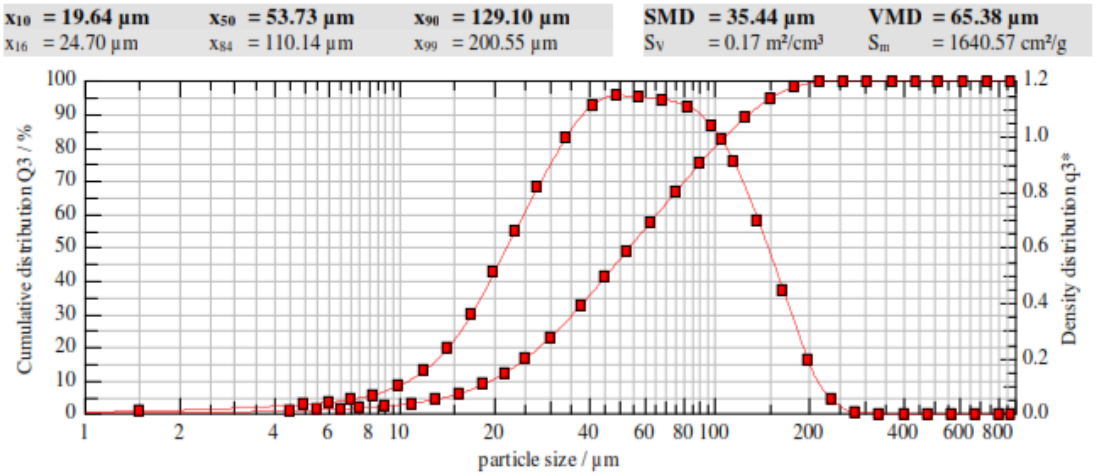

Figure 7. 7: Droplet size distribution of nasal spray at spraying angle 60°

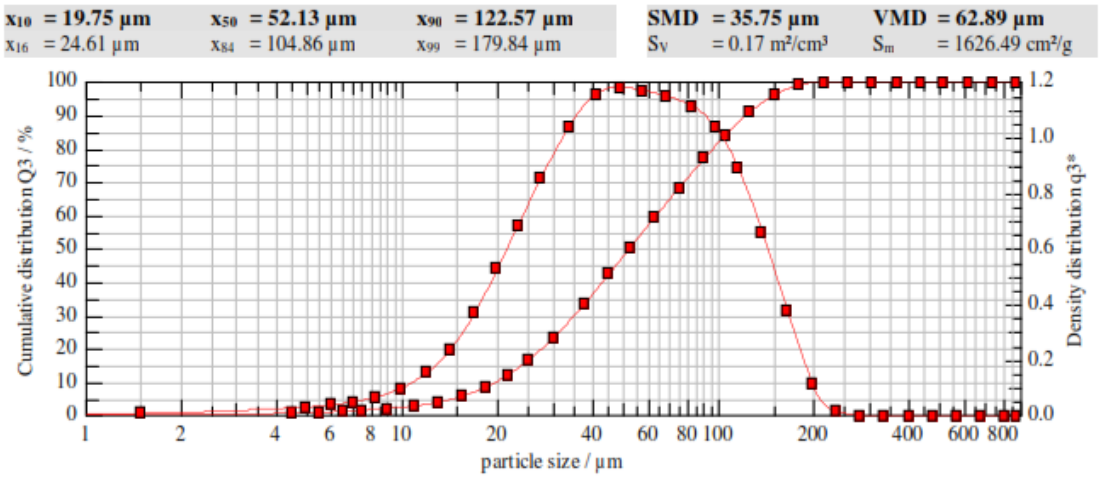

Figure 7. 8: Droplet size distribution of nasal spray at spraying angle 90°

Table 7. 11: Influence of spraying angle on various parameters of droplet size distribution

Parameters	**Spraying angle**		
	30°	**60°**	**90°**
D_{10} (µm)	19.28	19.64	19.75
D_{50} (µm)	52.95	53.73	52.13
D_{90} (µm)	123.54	129.10	122.57
Span	1.97	2.03	1.97
% droplets less than 10 µm	2.27	2.18	2.80

Although, spraying angle did not have any significant influence on droplet size distribution practically, it was considered very important parameter for optimizing nasal spray and its efficient delivery [17]. Additionally, spraying angle of 30° and 45° are recommended for nasal spray formulations and spraying angle more than 60° would cause deposition of droplets in nasal valve region [18]. Furthermore, spraying angle along with plume geometry decides region of nasal spray deposition. Thus, lesser spraying angle with narrower plume would deposit the droplets in posterior region while for higher spraying angle more than 60° would require wider plume [17]. Lower spraying angle < 30° would deposit droplets on anterior region.

7.4.7.2 Effect of actuation force on droplet size distribution

The effect of actuation force on droplet size distribution was studied by varying actuation force at 35 and 45 N and the results obtained are demonstrated in Figure 7. 9 and Figure 7. 10, respectively and the parameters are summarized in Table 7. 12.

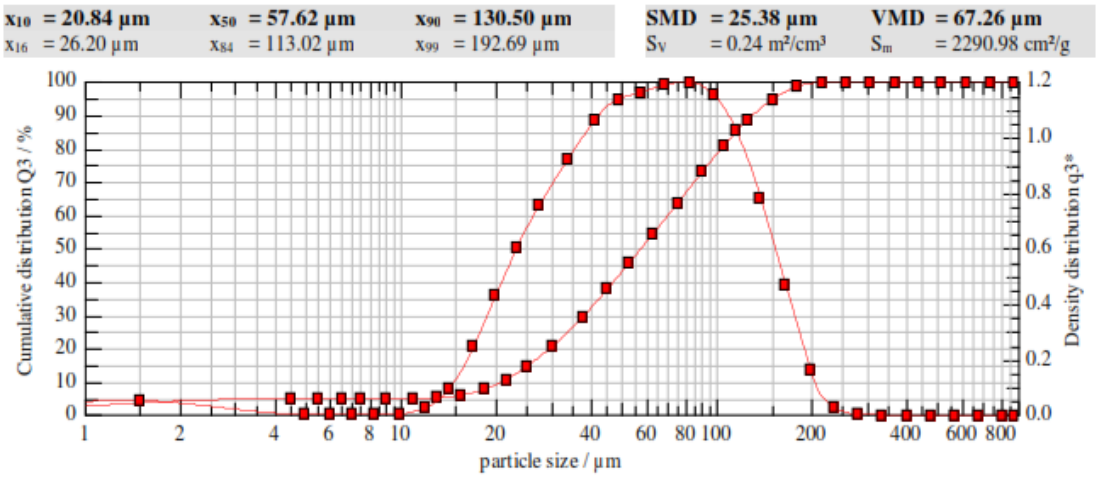

Figure 7. 9: Droplet size distribution of nasal spray at actuation force 35 N

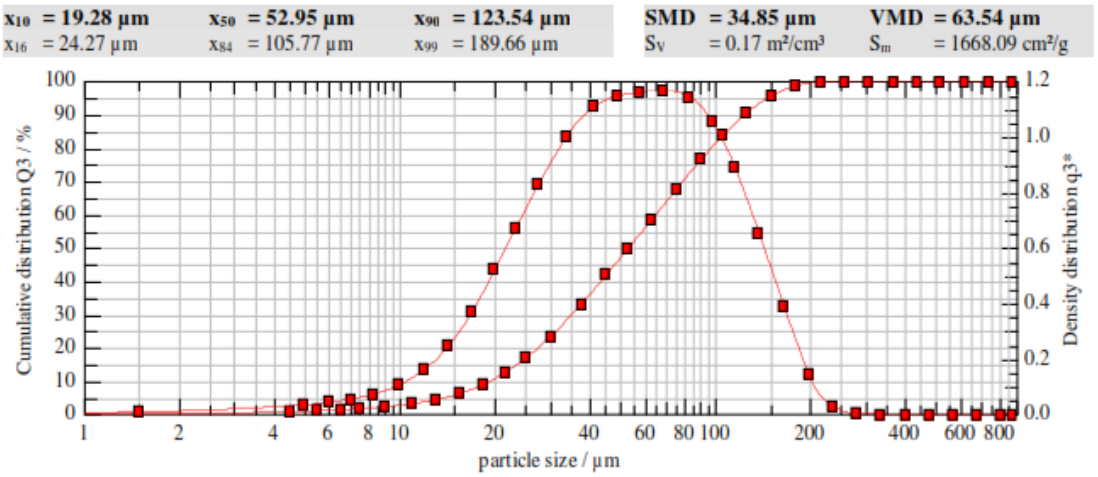

Figure 7. 10: Droplet size distribution of nasal spray at actuation force 45 N

Table 7. 12: Influence of actuation force on various parameters of droplet size distribution

Parameters	Actuation force	
	35 N	45 N
D_{10} (µm)	20.84	19.28
D_{50} (µm)	57.62	52.95
D_{90} (µm)	130.5	123.54
Span	1.90	1.97
% droplets less than 10 µm	4.96	2.27

From the results it was evident that there was significant reduction in the droplet size with increase in actuation force. This may be attributed to increased atomization which may produce smaller droplets and stronger plume [14].

7.4.7.3 Effect of actuation distance on droplet size distribution

The results of droplet size distribution at actuation distance varied at 3 and 6 cm are demonstrated in Figure 7. 11 and Figure 7. 12, respectively and the parameters described in Table 7. 13.

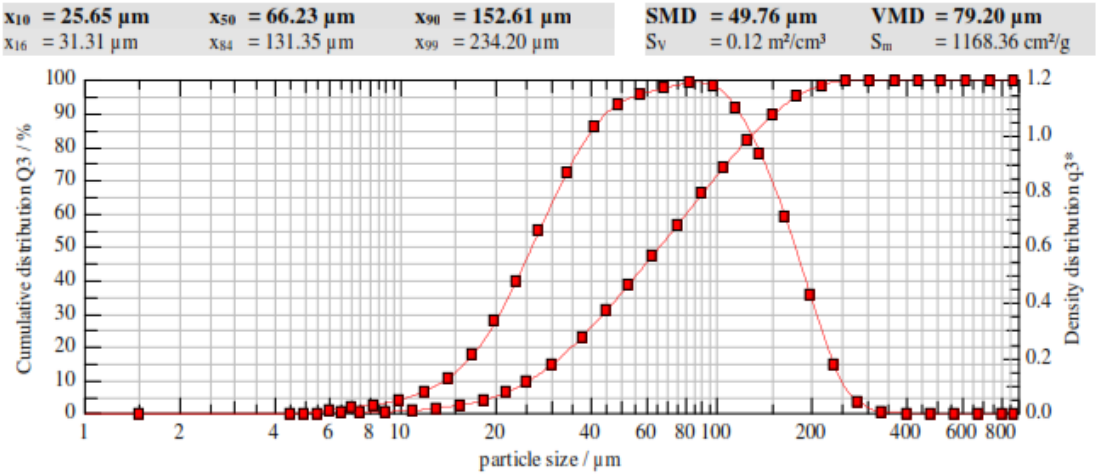

Figure 7. 11: Droplet size distribution of nasal spray at actuation distance 3 cm

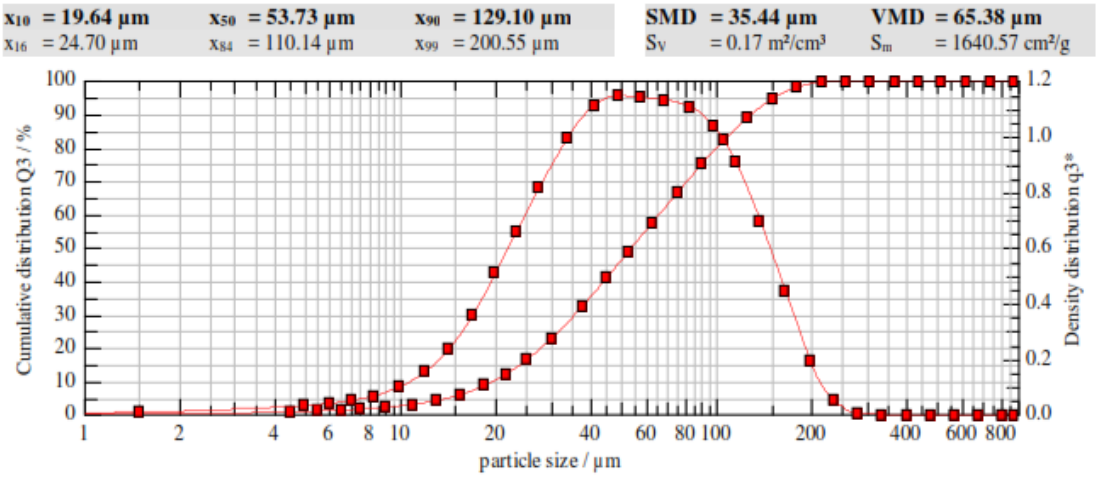

Figure 7. 12: Droplet size distribution of nasal spray at actuation distance 6 cm

Table 7. 13: Influence of actuation distance on various parameters of droplet size distribution

Parameters	Stroke length	
	3 cm	6 cm
D_{10} (µm)	25.65	19.64
D_{50} (µm)	66.23	53.73
D_{90} (µm)	152.61	129.10
Span	1.92	2.04
% droplets less than 10 µm	2.18	0.44

The results infer that there was significant reduction in droplet size distribution with increase in stroke length. Such alteration in droplet size distribution may be attributed to plume geometry, less representation of droplets in laser beam and varied settling velocities. On increasing the stroke length, there was proportional reduction in % droplets missing the laser beam. Thus, even though there is reduction in droplet size, it does not actually represent the droplet size distribution. Consequently, actuation distance selection is considered important actuation parameter in optimizing droplet size distribution.

7.4.7.4 Effect of hold time on droplet size distribution

The results of droplet size distribution at hold time varied at 1, 2 and 3 s are demonstrated in Figure 7. 13, Figure 7. 14 and Figure 7. 15, respectively and the parameters are described in Table 7. 14.

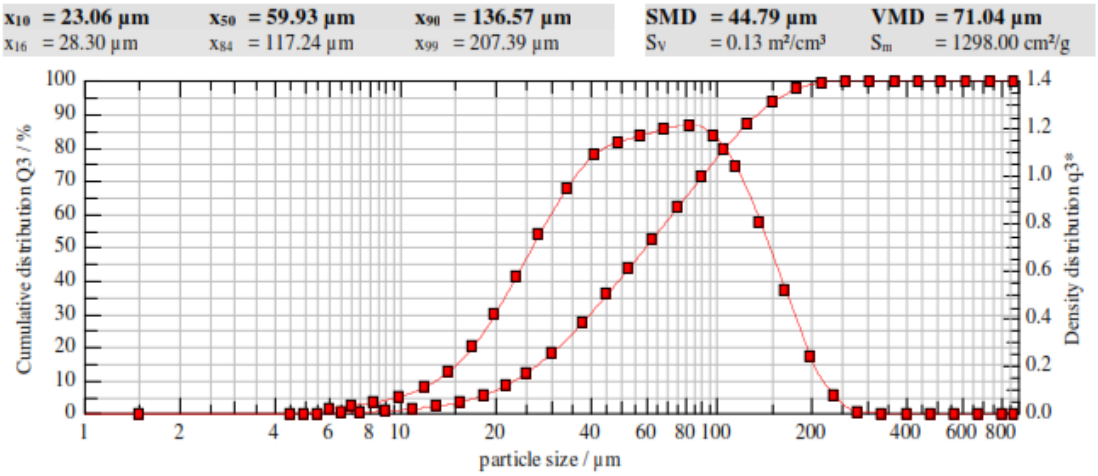

Figure 7. 13: Droplet size distribution of nasal spray at hold time 1 s

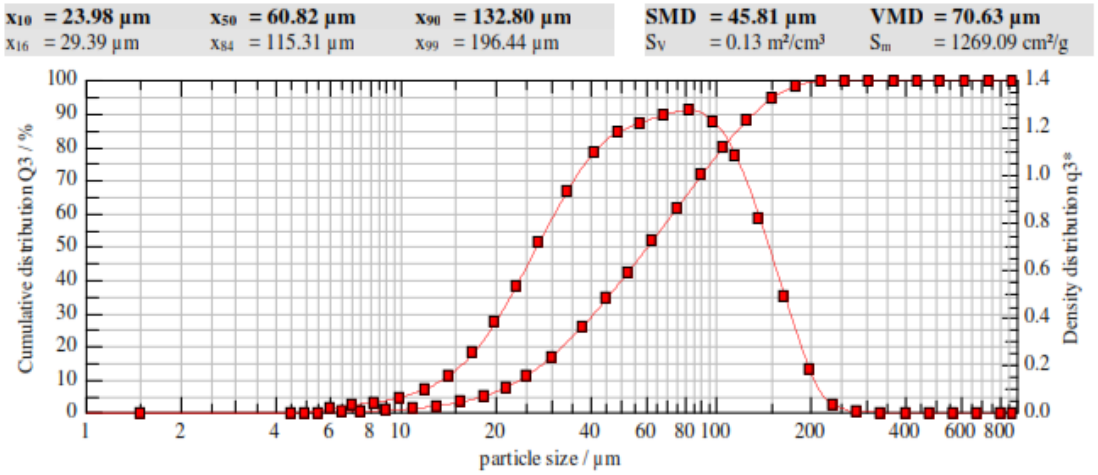

Figure 7. 14: Droplet size distribution of nasal spray at hold time 2 s

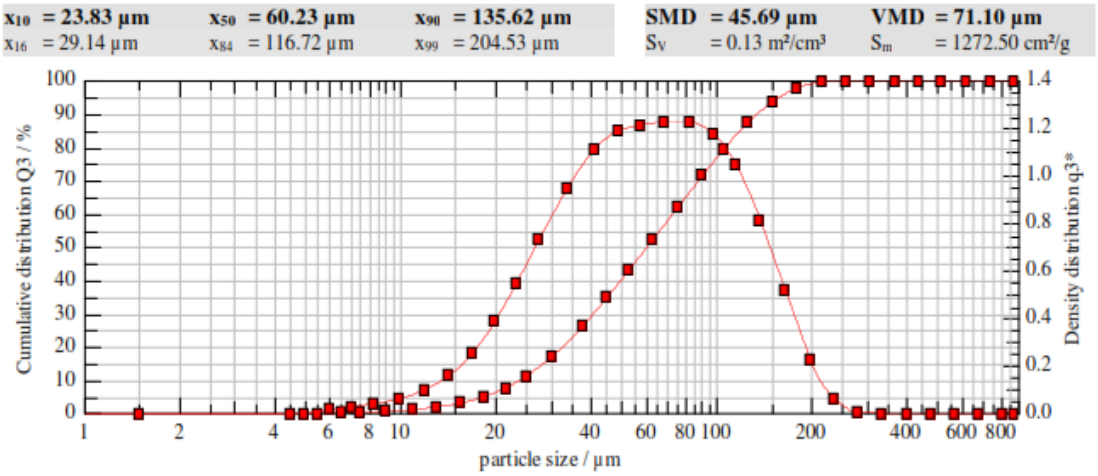

Figure 7. 15: Droplet size distribution of nasal spray at hold time 3 s

Table 7. 14: Influence of hold time on various parameters of droplet size distribution

Parameters	**Hold time**		
	1 s	**2 s**	**3 s**
D_{10} (µm)	23.06	23.98	23.83
D_{50} (µm)	59.93	60.82	60.23
D_{90} (µm)	136.57	132.80	135.62
Span	1.89	1.79	1.86
% droplets less than 10 µm	0.68	0.64	0.61

The results infer that there was no significant change in droplet size distribution with change in hold time. The time had no influence on formation of spray plume as complete spray was developed in minimum time selected. Additionally, hold time as such did not have significant effect as once actuated, it will result in formation of complete spray plume.

After preliminary optimization, the final optimized parameters for preferable droplet size distribution is depicted in Table 7. 15.

Table 7. 15: Optimized actuation parameters for preferable droplet size distribution

Parameters	Spraying angle	Actuation force	Actuation distance	Hold time
Optimized value	30°	45 N	3 cm	2 s

The droplet size distribution of formulated nasal spray at optimized actuation parameters is shown in Figure 7. 16 and the size distribution parameters are depicted in Table 7. 16.

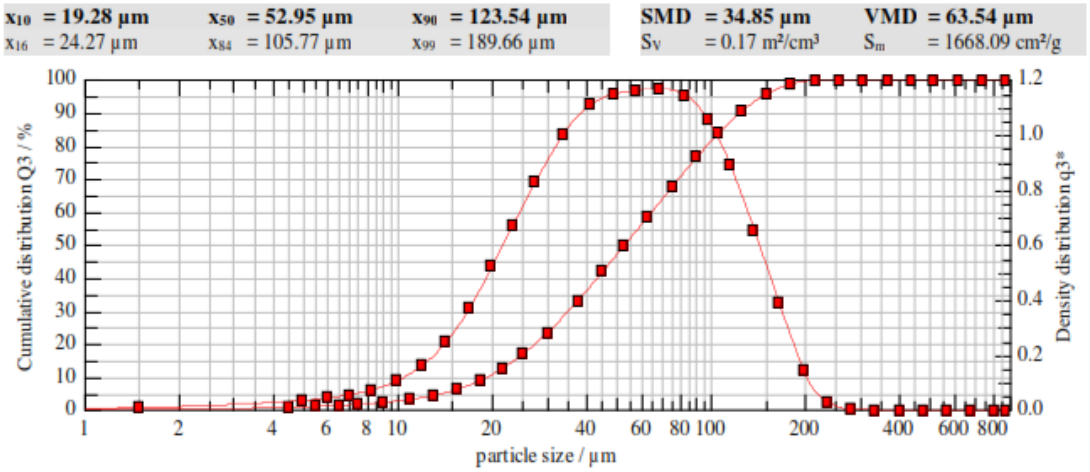

Figure 7. 16: Optimized droplet size distribution of nasal spray

Table 7. 16: Optimized droplet size distribution parameters

Parameters	Results
D_{10} (µm)	19.28
D_{50} (µm)	52.95
D_{90} (µm)	123.54
Span	1.97
% droplets less than 10 µm	2.27

7.4.8 Weight loss

The results of weight loss are depicted in Table 7. 17 and represented graphically in Figure 7. 17.

Table 7. 17: Influence of time on weight of nasal spray pump

Time points	Weight of nasal spray pump at different orientations		
	Inverted	Upright	Horizontal
Initial	19805.5 ± 3.30	19812.0 ± 3.44	19811.4 ± 5.55
1 month	19808.2 ± 4.69	19813.1 ± 7.84	19806.0 ± 5.73
2 months	19809.6 ± 5.09	19803.4 ± 3.48	19813.9 ± 5.46
3 months	19810.4 ± 6.35	19808.3 ± 8.97	19811.7 ± 9.44

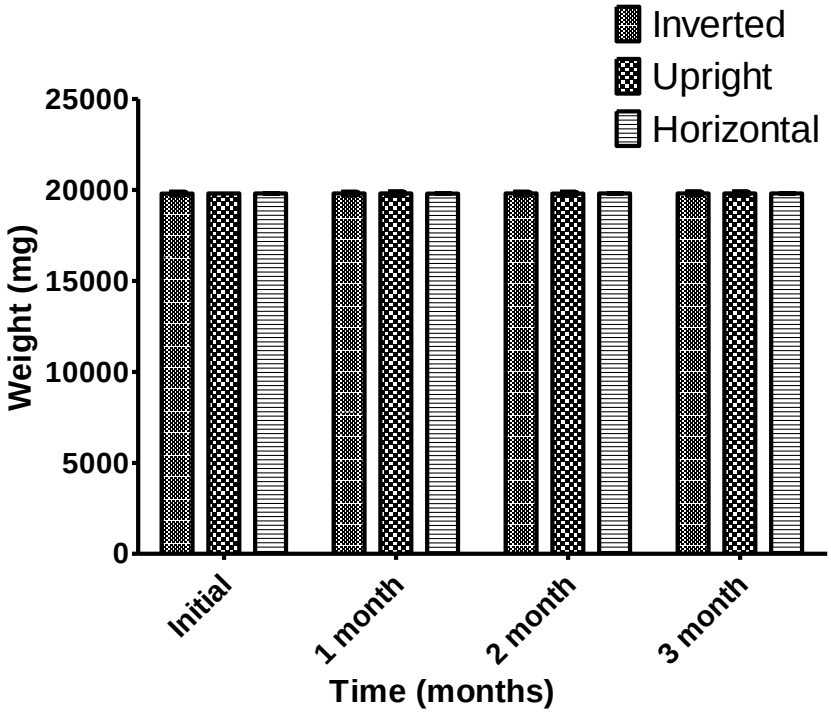

Figure 7. 17: Influence of time on weight of nasal spray pump

The results conclude that there was no significant change in weight of nasal spray filled pump even after 3 months at various orientations i.e. inverted, upright and horizontal. Thus, it was concluded that there was no dripping or leakage from the nasal spray pump and thus qualifies sealing characteristics of nasal spray pumps.

7.4.9 Tail-off characteristics

Tail-off characteristics was used to determine performance of nasal spray formulation at near completion stage of the nasal spray in the pump. For this, various parameters including droplet size distribution, spray pattern and shot weight were determined. The result of droplet size distribution is depicted in Figure 7. 18 and the parameters are summarized in Table 7. 18.

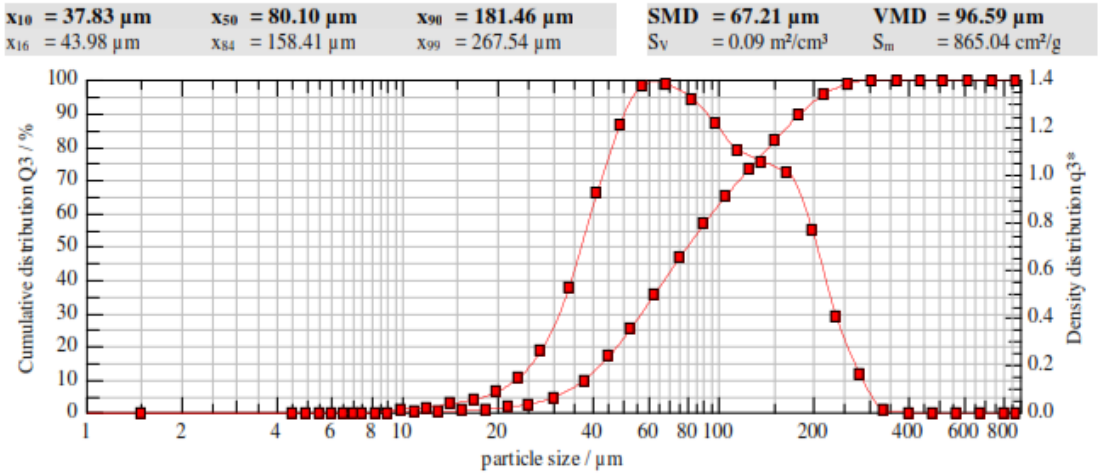

Figure 7. 18: Droplet size distribution of nasal spray as tail-off characteristics

Table 7. 18: Droplet size distribution parameters as tail-off characteristics

Parameters	Results
D_{10} (µm)	37.83
D_{50} (µm)	80.10
D_{90} (µm)	181.46
Span	1.79
% droplets less than 10 µm	0.12

From the results it was found that the D_{50} value of nasal spray was 80.10 µm which exceeds the ideal limit for D_{50} value. Additionally, the D_{90} values were higher as compared to optimized value and thus it was concluded that the resultant droplet size distribution at near completion stage was bit higher as compared to optimized values.

The result of spray pattern is depicted in Figure 7. 19 and the ovality ratio is summarized in Table 7. 19.

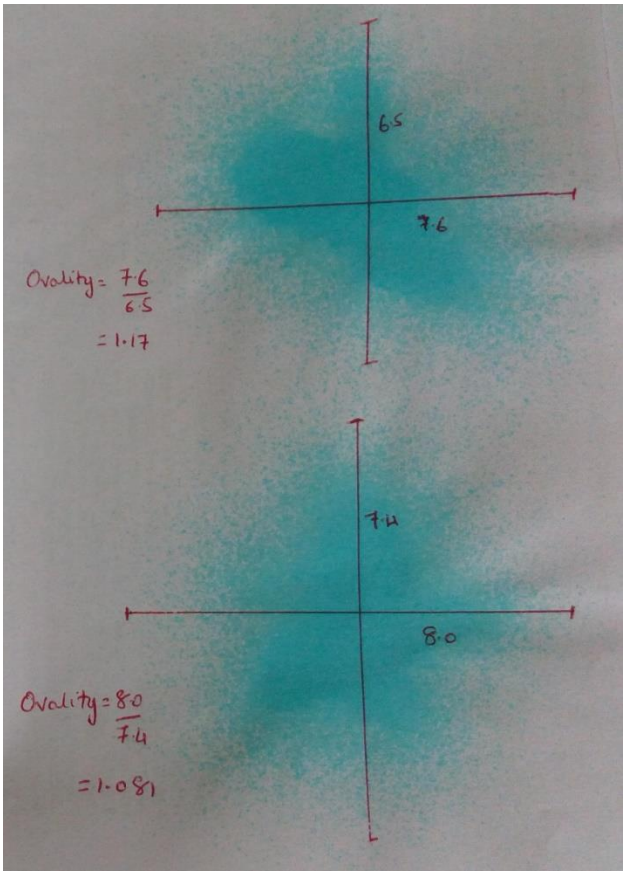

Figure 7. 19: Spray pattern for nasal spray as tail-off characteristic

Table 7. 19: Data depicting ovality ratio of nasal spray at 3 cm

Distance (cm)	**Longest chord (LL)**	**Shortest chord (LS)**	**Ovality ratio (LL/LS)**	**Mean ± SD**
3 cm	7.6	6.5	1.069	1.075 ± 0.008
	8.0	7.4	1.081	

From the results it was concluded that the spray pattern of the formulation at near completion stage was star shaped instead of ellipsoidal shape. Additionally, localized hotspots were observed supporting increased droplet size distribution of nasal spray. Furthermore, shot weight of nasal spray formulation was about 53.4 ± 2.5 mg with average pump delivery volume of 51.7 ± 2.42 µl averaging around 70.4 ± 3.3% of total deliverable volume. Thus, the results confirmed augmentation in droplet size distribution with altered spray pattern along with reduction in shot weight influencing nasal spray characteristics.

7.4.10 Microbial limit test

The results of TAMC and TYMC are depicted in Figure 7. 20 and Figure 7. 21, respectively.

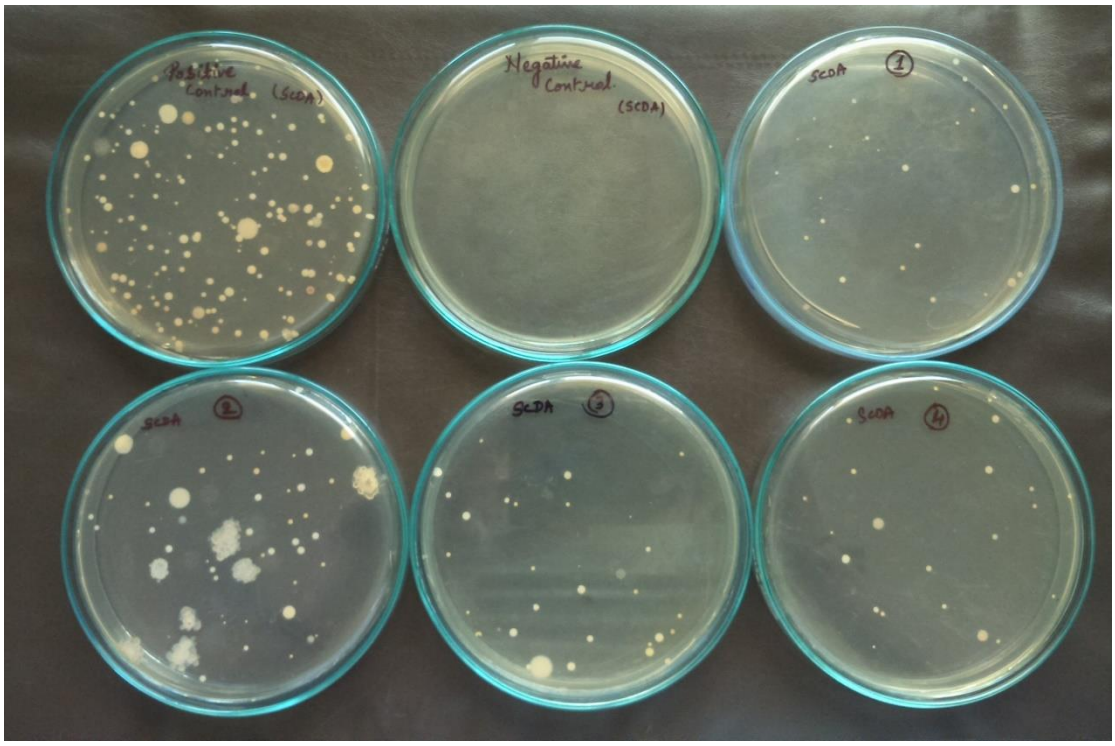

Figure 7. 20: TAMC determination by spread plate technique on TSA plates

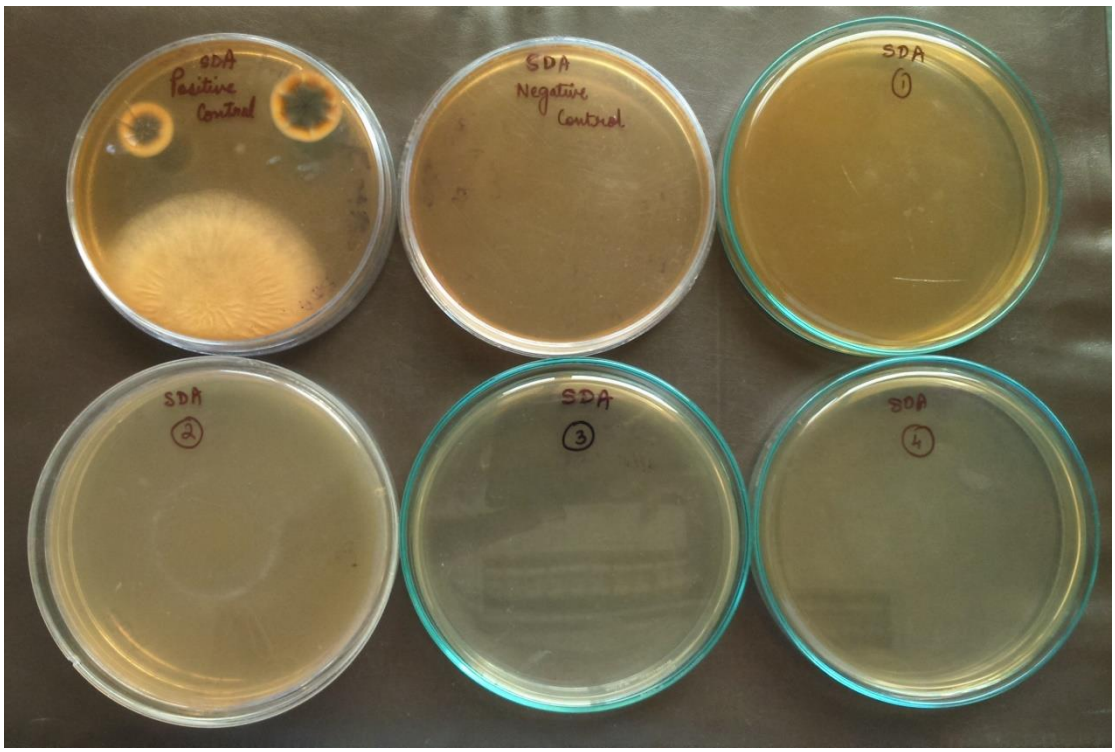

Figure 7. 21: TYMC determination by spread plate technique on SDA plates

The results demonstrated approximately 31 CFU on TSA plates when 1 ml formulation was spread on plates while CFU on SDA plates were not found indicating absence of yeast and mold. Both TAMC and TYMC count complied USP defined limits along with absence of *S. aureus* and *P. aeruginosa* fulfilling criteria for passing microbial limit test for nasal spray.

7.4.11 Preservative efficacy study

The results of preservative efficacy test, as performed by USP, in terms of colony forming units/ml and log reduction are depicted in Table 7. 20 and Table 7. 21, respectively

Table 7. 20: Results of preservative efficacy study depicting colony forming units/ml of different microorganisms at sampled time points

Microorganism	**Colony forming units / ml**			
	0 days	**7 days**	**14 days**	**28 days**
E. coli	3.9×10^5	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$
S. aureus	2.1×10^5	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$
P. aeruginosa	2.2×10^5	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$
A. niger	3.3×10^5	3.2×10^4	7.0×10^1	$<1.0 \times 10^1$
C. albicans	4.8×10^5	8×10^3	1.0×10^2	1.0×10^1

Table 7. 21: Results of preservative efficacy study depicting log reduction of different microorganisms at sampled time points when compared with 0 days

Microorganism	**Log reduction**		
	7 days	**14 days**	**28 days**
E. coli	4.59	4.59	4.59
S. aureus	4.32	4.32	4.32
P. aeruginosa	4.34	4.34	4.34
A. niger	1.01	3.67	4.52
C. albicans	1.78	3.68	4.68

From the results it was concluded that the log reduction profiles of both bacteria and fungi including yeast and molds were complying the limit specified by USP at all the time points i.e. 7 days, 14 days and 28 days. Thus, the nasal spray formulation was found to have desired preservative action fulfilling the criteria for passing preservative efficacy test.

7.5 References

1. Market, O.D., *Global Industry Analysis, Size, Share, Growth, Trends and Forecast, 2013-2019, Transparency Market Research, 2014.* There is no corresponding record for this reference, 2015.
2. Kublik, H. and M. Vidgren, *Nasal delivery systems and their effect on deposition and absorption.* Advanced drug delivery reviews, 1998. **29**(1): p. 157-177.
3. Djupesland, P.G., *Nasal drug delivery devices: characteristics and performance in a clinical perspective—a review.* Drug delivery and translational research, 2013. **3**(1): p. 42-62.
4. Santosh, T., *Formulation and Product Development of Nasal Spray: An Overview.* Scholars Journal of Applied Medical Sciences, 2016. **4**(8D): p. 2976-2985.
5. Vu, N., J.R. Lou, and T.C. Kupiec, *Quality control analytical methods: microbial limit tests for nonsterile pharmaceuticals, Part 1.* Int J Pharm Compd, 2014. **18**(3): p. 213-21.
6. Vu, N., J. Lou, and T. Kupiec, *Quality Control: microbial limit tests for nonsterile pharmaceuticals, part 2.* International journal of pharmaceutical compounding, 2013. **18**(4): p. 305-310.
7. Kulkarni, V. and C. Shaw, *Formulation and characterization of nasal sprays. An examination of nasal spray formulation parameters and excipients and their influence on key in vitro tests*, 2012, Inhalation.
8. Washington, N., et al., *Determination of baseline human nasal pH and the effect of intranasally administered buffers.* Int J Pharm, 2000. **198**(2): p. 139-46.
9. Aurora, J., *Development of nasal delivery systems: a review.* Drug Deliv Technol, 2002. **2**(7): p. 1-8.
10. Food, U. and D. Administration, *Guidance for industry: Nasal spray and inhalation solution, suspension, and spray drug products—Chemistry, manufacturing, and controls documentation.* Fed. Regist, 2002: p. 1-49.
11. Farina, D.J., *Regulatory aspects of nasal and pulmonary spray drug products.* Handbook of Non-Invasive Drug Delivery Systems, 2010: p. 247-290.
12. Eccleston, G.M., et al., *Rheological behavior of nasal sprays in shear and extension.* Drug Dev Ind Pharm, 2000. **26**(9): p. 975-83.

13. Pu, Y., et al., *A comparison of the deposition patterns of different nasal spray formulations using a nasal cast.* Aerosol Science and Technology, 2014. **48**(9): p. 930-938.
14. Guo, C. and W.H. Doub, *The influence of actuation parameters on in vitro testing of nasal spray products.* Journal of pharmaceutical sciences, 2006. **95**(9): p. 2029-2040.
15. Newman, S., F. Moren, and S. Clarke, *Deposition pattern of nasal sprays in man.* Rhinology, 1988. **26**(2): p. 111-120.
16. Cheng, Y., et al., *Characterization of nasal spray pumps and deposition pattern in a replica of the human nasal airway.* Journal of Aerosol Medicine, 2001. **14**(2): p. 267-280.
17. Foo, M.Y., et al., *The influence of spray properties on intranasal deposition.* Journal of Aerosol Medicine, 2007. **20**(4): p. 495-508.
18. Benninger, M.S., et al., *Techniques of intranasal steroid use.* Otolaryngology-Head and Neck Surgery, 2004. **130**(1): p. 5-24.