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In-Vitro Equivalence Design for Nasal Spray Product Containing Corticosteroids Active Ingredient

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Abstract **Original Research Article**

The in-vitro bioequivalence study of nasal sprays is useful to understand the efficiency of test product in comparison to reference product so that the equivalency in the in -vivo studies can be assessed. Analytical in vitro release tests were performed to statistically evaluate the performance of the nasal spray suspension formulation containing the corticosteroid active ingredient and compare its performance with the reference drug product. When performing in vitro testing, took into account the EMA Guide on Pharmaceutical Quality of Respiratory and Nasal Products (EMEA/CHMP/QWP/49313/2005 Corr) and the Food and Drug Administration (FDA, Draft Guideline). According to the results of all in vitro bioequivalence studies, it was concluded that Nasal Spray Product containing corticosteroids active ingredient, which contains the corticosteroid active ingredient developed and produced by Abdi İbrahim R&D center, is therapeutically equivalent to the reference product.

Keywords: Corticosteroids active ingredient; Nasal spray; In vitro; Performance tests; Statistical tests; Bioequivalence.

INTRODUCTION

A dosage form is a pharmaceutical preparation consisting of drug substance(s) and/or excipient(s) to facilitate dosing, administration, and delivery of the content of the drug product or placebo to the patient. The design, materials, manufacturing, and testing of all dosage forms target drug product quality (The United States Pharmacopeial Convention, 2014).

Nasal spray drug products contain therapeutically active ingredients (drug substances) dissolved or suspended in solutions or mixtures of excipients (e.g., preservatives, viscosity modifiers, emulsifiers, buffering agents) in nonpressurized dispensers that deliver a spray containing a metered dose of the active ingredient. Nasal sprays are applied to the nasal cavity for local and/or systemic effects. Although similar in many features to other drug products, some aspects of nasal sprays may be unique (e.g., formulation, container closure system, manufacturing, stability, controls of critical steps, intermediates, and drug product). These aspects should be considered carefully during the development program because changes can affect the ability of the product to deliver reproducible doses to patients throughout the products shelf life (U.S. Food and Drug Administration [FDA], 2002). Despite the simple appearance and easy access of the human nose, the development of nasally administered therapies requires excellent and accurate development encompassing drug characterization, formulation development and aerosol evaluation to ensure optimal drug delivery. While such an approach ensures that the drug is effective, it also enables it to reach the desired anatomical region (Dondeti, Zia, & Needham, 1996), (Guo & Doub, 2006).

Intranasal corticosteroids are considered a very safe and appropriate treatment for allergic rhinitis. There are several intranasal corticosteroids available today. All of them are used in the treatment of seasonal allergic rhinitis and long-term allergic rhinitis. (Kaliner, 2011). It treats nasal congestion, itching, runny nose and sneezing, which generally occur as allergic symptoms; Studies show that these symptoms are almost completely prevented. (Naclerio & Solomon, 1997). The reason for using intranasal corticosteroids in the treatment of allergic rhinitis is that sufficient drug concentrations can be achieved at the receptor sites in the nasal mucosa. In this way, symptoms can be controlled and the risk of

systemic adverse effects is reduced (Tripathy & Patterson, 2001), (Spector, 1999) (Spector, 1999). Differences between intranasal corticosteroids are limited to potency, dosing regimens, patient preference, administration, and device (Howarth, 2000).

An in vitro release rate reflects the combined effect of several physical and chemical parameters, including the solubility of the active ingredient, particle size, and rheological properties of the dosage form. The in vitro release rate is a useful test to evaluate product identity between products before and after exchange (U.S. Food and Drug Administration [FDA], 1997). The in-vitro bioequivalence study of nasal sprays is useful to understand the efficiency of test product in comparison to innovator so that the equivalency in the in -vivo studies can be assessed. The in -vitro bio equivalency characterization of nasal sprays helps us to correlate the efficiency of test product with respect to the reference product, so that the efficacy and safety can be achieved in to be equal Reference listed drug (Cheekti, Kumar, Dhage, Gappa, & Sahoo, 2021).

Analytical in vitro tests should be performed to demonstrate similarity between the test product and the reference product. When establishing equivalence based solely on in vitro data, the in vivo correlation of in vitro parameters should be considered. To this end, the set of quality attributes tested should cover all relevant parameters, acceptance criteria and the method of evaluation of results should be justified, and finally the results should be appropriate. For in vitro parameters, MAH has considered the EMA Guidance on Pharmaceutical Quality of Respiratory and Nasal Products (EMEA/CHMP/QWP/49313/2005 Corr.) and the Food and Drug Administration (FDA, Draft Guidance) (Medicines Evaluation Board, n.d.).

Tests performed according to the EMA Guidance on Pharmaceutical Quality of Respiratory and Nasal Products (EMEA/CHMP/QWP/49313/2005 Corr.), which is taken as a reference for the application of in vitro tests: Particle Size Distribution (PSD), Shaking Requirement, Performance After Temperature Cycling is in the form of robustness, viscosity, osmolality, pH, density. The tests performed according to the Food and Drug Administration (FDA, Draft Guidance), another source we refer to, unlike EMA Guidance: Single Actuation Content through Container Life (SCU), Droplet size distribution by laser diffraction (DSD), Spray pattern, Plume geometry, Profiling of Sprays Near Container Exhaustion (Tail Off Characteristics), Effect of Dosing Orientation (European Medicines Agency [EMA], 2006), (U.S. Food and Drug Administration [FDA], 2019).

In this study, the performance similarity of Test product developed and produced by Abdi İbrahim R&D Center and Reference product was evaluated by considering the in vitro study parameters of the EMA Guide (EMEA/CHMP/QWP/49313/2005 Corr.) and the Food and Drug Administration (FDA, Draft Guidance).

MATERIALS AND METHODS

The standards, reagents and equipment used within the scope of in vitro analyzes of nasal spray, which contains the corticosteroid active ingredient developed by Abdi İbrahim R&D Center, are as follows.

MATERIALS

a) Working standard
b) Anhydrous Potassium dihydrogen phosphate (Merck, catalog number:104873)
c) Acetonitrile (Merck, catalog number:1.00030.4000)

EQUIPMENTS

a) Waters High Performance Liquid Chromatography equipped with an ultraviolet/visible detector Waters 2489; a reversed phase column: Waters BEH C18 50 mm x 2.1mm x 1.7 µm; a data recorder and processor system: Empower Software 3
b) Brookfield DV II+Pro
c) Malvern Mastersizer (3000E, 2000)
d) Spraytech-Open Spray
e) Renishaw inVia Confocal Raman Microscope

Composition of the formulations

Nasal spray product containing corticosteroid active ingredient was developed by Abdi İbrahim İlaç R&D Center. The Reference Listed Drug (RLD) was obtained from Germany. The composition of the formulation contains active ingredient, tonicity agent, suspending agent, surfactant, antimicrobial preservative, chelating agent, solvent.

METHODS

Single Actuation Content through Container Life (SCU):

Validated UPLC uniformity of delivered dose and uniformity of dosage units methods will be used for the assay of the finished product, the content of active ingredient in bottle and assay per dose.

Droplet Size Distribution by Laser Diffraction (DSD)

The droplet size distribution is measured by using the method of Nasal Spray in Spraytech. The studies will be performed within a range of 2 to 7 cm from the actuator orifice, with the two distances separated by 3 cm or more.

Particle Size Distribution

Drug Particle Size Distribution in suspension formulations has the potential to influence the rate and extent of drug availability to nasal sites of action and to systemic circulation. If drug Particle Size Distribution in the Test and Reference products can be accurately measured using an analytical method such as Drug in Small Droplets (using Copley Next Generation Impactor

(NGI)) and morphology directed Raman spectroscopy or any other advanced methodology, may submit comparative particle size distribution data as part of their drug characterization within their application.

Drug in Small Droplets

Drug in Small Droplets studies on the nasal spray product using Copley Next Generation Impactor (NGI) instrument are performed to determine the relative amount of small droplets (< 9 µm, below the top stage) in the test and reference products. Since the amount of drug deposited below the top stage is of primary interest, the drug deposition should be categorized in two groups. Group 1 should include all drug deposited below the top stage which is < 9 µm in size. Group 2 should include the total mass of drug collected on all stages and accessories. A validated UPLC Drug in Small Droplet method will be used for the assay of the finished product, the content of active ingredient in bottle and assay per dose.

PSD by Raman Spectroscopy

Raman spectroscopy is used to distinguish suspended drug particles from other particles and suspended excipients. With the Raman microscope, only the size of the particles is analyzed by the spectrum of the drug substance.

Spray Pattern

Spray pattern studies characterize the spray either during the spray prior to impaction or following impaction on an appropriate target such as a thin-layer chromatography (TLC) plate. Spray patterns for certain nasal spray products may be spoked or otherwise irregular in shape. Spray patterns can be characterized and quantitated by either manual or automated image analysis. Both analyses will allow shape and size to be determined.

Plume Geometry

Plume geometry describes a side view of the aerosol cloud parallel to the axis of the plume, and we recommend it be based on high-speed photography, a laser light sheet and high speed digital camera, or other suitable methods. The image would be snapshot, not time averaged. Quantitation can be by manual analysis or automated image analysis.

Plume geometry would be performed at:

- Beginning lifestage only
- One side view only
- A single delay times

Priming and repriming

This study carried out to determine the number of sprays should be evaluated as waste when using the product stored in different positions such as horizontal and vertical. At the same time, when the bottle is re-sprayed at the end of its unused period, a re-spraying study is carried out to determine the number of sprays that should be considered as waste.

Profiling of Sprays Near Container Exhaustion (Tail Off Characteristics)

For nasal spray drug products, a study should be conducted to determine the profiles of SCU each individual spray after the point at which the labeled number of sprays have been dispensed until no more sprays are possible.

Shaking Requirement

The possibility of incorrect dosing which may occur with foaming or insufficient shaking, will be tested by conducting the dose uniformity tests and appropriate shaking time will be determined.

Performance After Temperature Cycling

For nasal spray suspension drug products, a stress temperature cyclic study should be performed to evaluate the effects of high and low temperature variations that may be encountered during shipping and handling on the quality and performance of the drug product.

This study is conducted to evaluate the effect of the temperature cycling on Nasal Spray Reference product and the Nasal spray test product delivered dose homogeneity.

Robustness

The product performance should be investigated under conditions to simulate use by patients. This includes activating the delivery device at the frequency indicated in the instructions for use. Device robustness should be studied for nasal spray drug products and the performance characteristics of the device should be studied after different handling situations (e.g., dropping, shaking, vibrating).

Effect of Dosing Orientation

For nasal spray drug products, studies should be undertaken to determine the comparative performance of the devices in terms of SCU and droplet size distribution at various dosing orientations.

Density

The study will be performed with 10 bottles selected from each of 3 batches of the reference product and 10 bottles from each of 3 batches of the test product nasal spray.

Viscosity (S62, 90 rpm, Turque: 50%±10%, Room Temperature)

The study will be performed with 50 bottles (5 bottles are used for each analysis sample and a total of 10 viscosity analysis samples are obtained) selected from each of 3 batches of the reference product nasal spray and 50 bottles (5 bottles are used for each analysis sample and a total of 10 viscosity analysis samples are obtained) selected from each of 3 batches of the test product nasal spray.

Osmolality

The study will be performed with 10 bottles selected from each of 3 batches of the Reference product 10 bottles from each of 3 batches of the test product, Nasal Spray, Suspension.

pH

The study will be performed with 10 bottles selected from each of 3 batches of the Reference product and 10 bottles from each of 3 batches of the test product, Nasal Spray, Suspension.

EXPERIMENTAL

Single Actuation Content through Container Life (SCU):

The pump primes by using 6 actuations (Reference product label recommends "wasting" of the first six actuations for priming). Testing performs at the beginning of life stage, actuation # 7 (referred as #1), middle of life stage, actuation # 66 (referred as #60), and at the end of bottle life actuation #126 (referred as #120). Each single actuation collects into a 5 mL volumetric flask and assay is performed by UPLC.

Droplet Size Distribution by Laser Diffraction (DSD)

The pump primes by using 6 actuations (Reference product label recommends "wasting" of the first six actuations for priming). Testing performs at the beginning of life stage, actuation # 7 (referred as #1) and at the end of bottle life actuation #126 (referred as #120). Samples test in the study is two different distance which are 30 mm and 60 mm and 10 bottles selected from each of 3 batches of the Reference product and 10 bottles from each of 3 batches of the test product, Nasal Spray, Suspension. Spray bottle should be shaken vigorously before measurement. The device is primed at least five spray actuations.

Method for Vertical Distance from Laser is 30 mm

Device	**Spraytech-Open Spray**
Hardware	NSS System
Vertical Distance from Laser	30 mm
Angle (º)	0º
Number Of Events	1
Particle Name	Water
Particle Refractive Index	1.33
Particle Density (g/mL)	1.00
Dispersant Name	Air
Dispersant Refractive Index	1.00
Profile Type	Velocity based
Actuation Velocity (mm/s)	100
Hold Time (ms)	500
Return Velocity (mm/s)	100
Interaction Delay (ms)	1000

Method for Vertical Distance from Laser is 60 mm

Device	**Spraytech-Open Spray**
Hardware	NSS System
Vertical Distance from Laser	60 mm
Angle (º)	0º
Number Of Events	1
Particle Name	Water
Particle Refractive Index	1.33
Particle Density (g/mL)	1.00
Dispersant Name	Air
Dispersant Refractive Index	1.00
Profile Type	Velocity based
Actuation Velocity (mm/s)	100
Hold Time (ms)	500
Return Velocity (mm/s)	100
Interaction Delay (ms)	1000

Single spray droplet size distribution and span are report based on volume (mass). Span can be computed as ((D90 - D10)/D50).

Particle Size Distribution

Drug Particle Size Distribution in suspension formulations has the potential to influence the rate and extent of drug availability to nasal sites of action and to systemic circulation. If drug Particle Size Distribution in

the Test and Reference products can be accurately measured using an analytical method such as Drug in Small Droplets (using Copley Next Generation Impactor (NGI)) and morphology directed Raman spectroscopy or any other advanced methodology, have been submitted comparative particle size distribution data as part of their drug characterization within their application.

In order to identify and measure the size of drug particles without any interference from excipient particles suspended in the formulation, Particle Size Distribution has been performed by Raman spectroscopy as a comprehensive method to demonstrate the adequacy of the chosen method.

Drug in Small Droplets

The pump primes by using 6 actuations (Reference product label recommends "wasting" of the first six actuations for priming). Testing is performed at the beginning of life stage, and at the end of bottle life actuation. The sample placed in the nosepiece adapter is sprayed once into the Glass Expansion Chamber. The process is repeated 10 times in the same way for 1 sample preparation for each part shown in the table below, the sample collected at the stages is taken by washing with the solvent in the specified volumetric flasks. Each single head and stage sample collect into a 50 mL volumetric flask and assay is performed by UPLC.

Table 1: Sample preparation for Drug in Small Droplets Test

Part	Sample	Volume of Volumetric Flask (mL)
Head	Glass Expansion Chamber + Induction port + Nosepiece adapter	50
Stage	Stage 1 + Stage 2 + Stage 3 + Stage 4 + Stage 5 + Stage 6 + Stage 7 + Stage 8	50

PSD by Raman spectroscopy

Renishaw's inViaTM Qontor Raman microscope, equipped with 532 nm laser excitation and Stream HR Raman mapping, use to analyse samples of nasal spray containing an API of corticosteroid active ingredient. Renishaw's Particle statistics app is then used to analyse the shape and particle size distribution of the APIs.

Analysis is performed on a single dried droplet from each batch. Samples tested in the study will be 10 bottles from each of 3 lots of the Reference product and 10 bottles from each of 3 lots of the test product.

Spray Pattern

Analysis will be carried out using the manual method. Samples are tested in the study from two different distances which are 30 mm and 60 mm and 10 bottles selected from each of 3 batches of the Reference product, and 10 bottles from each of 3 batches of the test product, Nasal Spray, Suspension. The lot numbers are given in Section C.1 and C.2.

The pump primes by using 6 actuations (Reference product label recommends "wasting" of the first six actuations for priming). Testing is performed at the beginning of life stage, actuation # 7 (referred as #1).

In order to get accurate and repeatable puffs every time, analyzes will be made using the spray head of the Spraytech-Open Spray device. The distance to be measured is measured from the puff head and vertically sprayed on the filter paper kept at the desired distance with the help of clips.

Spray bottle should be shaken vigorously before measurement. After each actuation, the spray pattern of the regarding actuation is determined. D_{MAX} (mm), D_{MIN} (mm), Average, Spray Pattern (D_{MAX}/D_{MIN}) and Angle values are saved.

Plume Geometry

The pump primes by using 6 actuations (Reference product label recommends "wasting" of the first six actuations for priming). Testing performs at the beginning of life stage, actuation # 7 (referred as #1).

Samples are tested in the study from a distance which is 30 mm and 10 bottles selected from each of 3 batches of the Reference product and 10 bottles from each of 3 batches of the test product, Nasal Spray, Suspension. Spray bottle should be shaken vigorously before measurement. The device is primed at least five spray actuations.

Analysis Method

Device	Spray VIEW® Measurement System
Vertical Distance from Laser	30 mm
Actuation	automatically
Return velocity	100 mm/s
Return acceleration	5700 mm/s²
Initial delay	0 ms
Hold time	100 ms
Final delay	0 ms

Priming and Repriming

20 unused spray bottles are taken. 10 spray bottles are tested in vertical position and 10 spray bottles are tested in horizontal position. Each spray bottle is sprayed 6 times and the assay of active ingredient in the sample obtained by each spray is determined. After the test is completed, the bottles are removed to the cabinets in two positions which are vertical and horizontal at 25 °C 60% RH condition. The first spraying and re-spraying test is repeated on the 15th day and 30th day.

Each single actuation is collected into a 5 mL volumetric flask and are assayed by UPLC. A validated UPLC uniformity of delivered dose and uniformity of dosage units' method will be used for the assay of the finished product, the content of active ingredient in bottle and assay per dose.

Profiling of Sprays Near Container Exhaustion (Tail Off Characteristics)

Samples tested in the study will be 3 bottles selected from each 3 batches of the Reference product 3 bottles from each 3 batches of the test product, Nasal Spray, Suspension.

A validated UPLC uniformity of delivered dose and uniformity of dosage units' method will be used for the assay of the finished product, the content of active ingredient in bottle and assay per dose. Each single actuation is collected into a 5 mL volumetric flask and are assay by UPLC.

In order to save time and consumption and increase analytical efficiency, all puffs after the end of bottle life actuation #126 (referred as #120) puff will be analyzed as Based on the information we obtained from the Number of Actuations per Container test, the Profiling of Sprays Near Container Exhaustion analysis will be applied for the test product and the reference product as described in the Table 2 and Table 3 below.

Table 2: Profiling of Sprays Near Container Exhaustion analysis for test product

Number of puffs	**Explanation**
From 120th puff to 170th puff	Every 10th puff will be analyzed.
From 170th to 180th puff	Every 5th puff will be analyzed.
From 180th puff to Exhaustion puff	All puffs will be analyzed.

Table 3: Profiling of Sprays Near Container Exhaustion analysis for reference product

Number of puff	**Explanation**
From 120th puff to 140th puff	Every 10th puff will be analyzed.
From 140th to 150th puff	Every 5th puff will be analyzed.
From 150th puff to Exhaustion puff	All puffs will be analyzed.

Shaking Requirement

10 different bottles of Nasal Spray, Suspension are shaken slowly (with agitation rate once per second) for 3 seconds, 5 seconds and 10 seconds, respectively. At the end of each shaking period, the dose analysis will be made within the scope of single-dose weight and dose uniformity for the dose sprayed from each bottle.

The study will be performed 10 bottles selected from each of 3 batches of the Reference product and 10 bottles from each of 3 batches of the test product, Nasal Spray, Suspension. Samples will be shaken slowly (with agitation rate once per second) for 3 seconds, 5 seconds and 10 seconds, respectively. Each single actuation is collected into a 5 mL volumetric flask and are assay by UPLC.

A validated UPLC uniformity of delivered dose and uniformity of dosage units' method will be use for the assay of the finished product, the content of active ingredient in bottle and assay per dose.

Performance After Temperature Cycling

The bottles are analyzed to determine initial values which are packaging material observation for any visual defects, mean weight change of bottles (Weight Loss), particle size distribution, droplet size distribution, delivered dose uniformity, density, active ingredient assay and related substances.

Nasal spray bottles will be stored in two position which are vertical and horizontal and cycled between recommended storage conditions Temperature cycling conditions are 25 °C 60% RH and 40 °C 75% RH. The nasal spray bottles are stored with 6 recurrent at each temperature condition. Each temperature cycle time was applied for 24 hours. The temperature cycle procedure is started at 25 °C 60% RH, and this cycle is continued for 12 days by changing every 24 hours between 40 °C 75% RH and 25 °C 60% RH. Intermediate condition analysis will be done on the 6th day and final analyzes are made at the end of the 12th day. Packaging material observation for any visual defects, mean weight change of bottles (Weight Loss), particle size distribution, droplet size distribution, delivered dose

uniformity, density, active ingredient assay and related substances analyzes are performed on the samples stored in two different positions on the 6th and 12th days.

Robustness

The bottles are analyzed to determine initial values which are appearance, particle size distribution, droplet size distribution and Single Actuation Content through Container Life (SCU).

Nasal spray bottles will be transported in two distance which are Istanbul and Izmir (nearly 500 km) several time. Appearance, particle size distribution, droplet size distribution and Single Actuation Content through Container Life (SCU) analyzes will be made for the test and reference product at the 3rd arrival (approximately 3000 km) and 6th arrival (approximately 6000 km) to the starting point (Istanbul).

Effect of Dosing Orientation

Samples tested in the study will be 10 bottles selected from each of 3 batches of the Reference product and 10 bottles from each of 3 batches of the test product, Nasal Spray, Suspension. For the SCU analysis, the sample will be puffed at a spray angle of 45º.

Droplet size distribution method is worked according to the method below.

Metot for Vertical Angle from Laser is 45º

Device	**Spray tech-Open Spray**
Hardware	NSS System
Vertical Distance from Laser	45 mm
Angle (º)	0º
Number Of Events	1
Particle Name	Water
Particle Refractive Index	1.33
Particle Density (g/mL)	1.00
Dispersant Name	Air
Dispersant Refractive İndex	1.00
Profile Type	Velocity based
Actuation Velocity (mm/s)	100
Hold Time (ms)	500
Return Velocity (mm/s)	100
Interaction Delay (ms)	1000

DENSITY

Density of sample at 20 °C is measured according to Ph.Eur. 2.2.5. Sample bottle should be shaken vigorously before measurement.

Viscosity (S62, 90 rpm, Torque: 50%±10%, Room Temperature)

Device	**Brookfield DV II+Pro**
Spindle	S62
Rotation Speed	90 rpm
Sample Volume	50 mL
Sample Temperature	Room temperature
Torque	50 % ± 10 %

Final product viscosity measurement should be performed at the earliest 24 hours after production.

50 mL of analysis sample is transferred directly to the viscosity measuring tube without shaking. By adjusting the device parameters, the sample is mixed at 90 rpm, using the S62 spindle until the torque value is 50% ± 10%. When the target value is reached, the viscosity value is measured according to Ph. Eur.2.2.10 and the result is recorded.

Osmolality

One bottle is shaken well and opened. The osmolality value of the sample is measured according to Ph.Eur.2.2.35.

pH

10 mL sample is taken into a suitable container. The pH value of the sample is measured with a calibrated pH meter according to Ph.Eur.2.2.5.

RESULTS AND DISCUSSIONS

A summary of the design used in the PBE (Population Bioequivalence) and ABE (Average Bioequivalence) statistical calculations performed for all tests is as shown in Table 4.

Table 4: A summary of the design used in the PBE (Population Bioequivalence) and ABE (Average Bioequivalence) statistical calculations.

	REFERENCE	TEST
Number of Batches	3	3
Number of Container per Batches	10	10

Single Actuation Content Through Container Life

The results are as shown in Table 5.

Table 5: Active ingredient % results of the beginning, middle and end spray for the test and reference product.

	Test Product			Reference Product		
	1st batch	2nd batch	3rd batch	1st batch	2nd batch	3rd batch
	Active ingredient %					
Beginning Spray (10 bottles)						
AVG	98.7	100.9	99.5	97.4	94.5	94.1
MIN	92.8	94.2	93.9	95.7	90.4	92.3
MAX	104.2	109.6	103.8	100.9	98.3	95.9
Middle Spray (10 bottles)						
AVG	102.9	99.0	99.2	98.9	95.6	94.0
MIN	96.6	94.2	95.9	96.3	93.8	91.6
MAX	107.4	108.8	105.7	112.6	98.8	96.6
End Spray (10 bottles)						
AVG	102.6	98.5	98.8	98.3	94.4	93.3
MIN	96.2	95.4	95.5	95.3	89.4	89.1
MAX	107.1	104.6	101.4	103.8	99.6	95.1

Evaluation Result

- All results found meet limits of 85 to 115 percent.
- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of the statistical calculation of ABE and PBE are as shown in Table 6.

Table 6: Summary results of statistical evaluation for ABE and PBE

ABE no LS (Least Squares) means			
Overall mean	97.49325013		
CV (Coefficient of variation) %	2.82242431	**CV ref %**	2.993343941
ABE	**Ratio=theta**	**LL (Lower limit)**	**UL (Upper Limit)**
	1.046666827	1.036374331	1.05706154
Limits	**Non scaled**	0.9	1.1111
	Scaled ABE	0.9	1.1111
Conclusion based on non scaled	BIOEQUIVALENT		
PBE Results			
σR	0.029926737	CONSTANT SCALED	
Ratio	1.046666827		
Hη	-0.017116579	As <0: BIOEQUIVALENT	
Fieller on non transformed data			
CV% within	3	CV ref %	4
	Ratio	LL	UL
	1.0469	1.0379	1.0559
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed			
ABE	**Dif**	**LL**	**UL**
	4.466666667	3.625464816	5.307868517
Limits	Non scaled	-9.533666667	9.533666667
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference			
ABE	**Dif**	**LL**	**UL**
Dif (Difference) in % ref	1.0469	1.0380	1.0557
	BIOEQUIVALENT		

According to Guidance for Industry Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action, reference product and test product have been proven to be similar for Single Actuation Content through Container Life test.

Droplet Size Distribution by Laser Diffraction (DSD)

The results obtained are as shown in Table 7 (During analysis, 10 bottles were used for each batch).

Table 7: Droplet size distribution by laser diffraction (DSD) results at 30 mm and 60 mm distance of the beginning and end spray for the test (batch 1, batch 2 and batch 3) and reference (batch 1, batch 2 and batch 3) product

	Test Results							
	30 mm from the actuator orifice far							
	Beginning Spray				**End Spray**			
	D10 µm	**D50 µm**	**D90 µm**	**SPAN**	**D10 µm**	**D50 µm**	**D90 µm**	**SPAN**
AVG	23.14	45.19	91.69	1.51	22.94	44.87	89.61	1.49
MIN	20.09	41.82	78.97	1.28	18.19	41.57	78.35	1.27
MAX	24.96	50.37	125.66	2.10	26.05	47.87	107.16	1.98
	60 mm from the actuator orifice far							
AVG	24.31	44.02	81.81	1.30	24.29	45.88	90.37	1.44
MIN	20.53	38.47	73.75	1.13	20.72	40.63	74.96	1.12
MAX	26.94	48.96	92.14	1.58	27.69	55.37	114.93	1.89
	Reference Results							
	30 mm from the actuator orifice far							
AVG	22.89	45.45	95.81	1.61	23.25	46.24	95.28	1.56
MIN	21.57	42.02	85.52	1.43	22.31	43.67	86.92	1.42
MAX	24.26	48.67	113.83	2.04	24.38	49.11	109.91	1.78
	60 mm from the actuator orifice far							
AVG	25.51	46.17	84.71	1.28	25.68	46.30	84.38	1.27
MIN	24.06	42.13	70.64	1.08	23.78	43.10	75.20	1.12
MAX	27.22	49.52	100.29	1.65	27.69	48.85	95.13	1.43

Evaluation Result

- All results obtained were found suitable within the scope of in vitro studies.
- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of the statistical calculation of ABE and PBE are as shown in Table 8 and Table 9.

Table 8: Summary results of statistical evaluation for ABE and PBE (D50 results of beginning and end spray at 30 mm and 60 mm distance)

D50 Results						
ABE no LS means						
	30 mm			**60 mm**		
Overall mean	45.40208652			45.48689426		
CV %	3.747460608	**CV ref %**	2.993343941	5.372928202	**CV ref %**	4.097002078
ABE	**Ratio=theta**	**LL**	**UL**	**Ratio=theta**	**LL**	**UL**
	0.982162134	0.9711	0.9934	0.965402353	0.9498	0.9812
Limits	**Non scaled**	0.9	1.11	**Non scaled**	0.9	1.11
	Scaled ABE	0.9	1.11	**Scaled ABE**	0.9	1.11
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
PBE Results						
σR	0.029926737	CONSTANT SCALED		0.040952844	CONSTANT SCALED	
Ratio	1.046666827			0.965402353		
Hη	-0.017116579	As <0: BIOEQUIVALENT		-0.014288449	As <0: BIOEQUIVALENT	
Fieller on non transformed data						
CV% within	3	CV ref %	5	5	CV ref %	6
	Ratio	LL	UL	Ratio	LL	UL
	0.9823	0.9712	0.9935	0.9666	0.9512	0.9823
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**

	-0.812166667	-1.327370313	-0.296963021	-1.547	-2.280583472	-0.813416528
Limits	Non scaled	-4.584133333	4.584133333	Non scaled	-4.633133333	4.633133333
Classical CI based on non transformed as percentage of the reference						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
Dif in % ref	0.9823	0.9710	0.9935	0.9666	0.9508	0.9824
	BIOEQUIVALENT			BIOEQUIVALENT		

Table 9: Summary results of statistical evaluation for ABE and PBE (span results of beginning and end spray at 30 mm and 60 mm distance)

Span Results						
ABE no LS means						
	30 mm			**60 mm**		
Overall mean	1.532525467			1.313273598		
CV %	10.0635551	**CV ref %**	7.133102086	9.487161066	**CV ref %**	7.894485478
ABE	**Ratio=theta**	**LL**	**UL**	**Ratio=theta**	**LL**	**UL**
	0.94449967	0.9162	0.9736	1.064193729	1.0341	1.0951
Limits	Non scaled	0.9	1.11	**Non scaled**	0.9	1.11
	Scaled ABE	0.9	1.11	**Scaled ABE**	0.9	1.11
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
PBE Results						
σR	0.029926737	CONSTANT SCALED		0.078822266	CONSTANT SCALED	
Ratio	1.046666827			1.064193729		
Hη	-0.017116579	As <0: BIOEQUIVALENT		-0.004547645	As <0: BIOEQUIVALENT	
Fieller on non transformed data						
CV% within	9	CV ref %	10	8	CV ref %	12
	Ratio	LL	UL	Ratio	LL	UL
	0.9494	0.9193	0.9804	1.0671	1.0360	1.0991
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
	-0.08000000	-0.12946277	-0.03053723	0.085666667	0.04674194	0.124591394
Limits	Non scaled	-0.15810000	0.15810000	Non scaled	-0.1277	0.1277
Classical CI based on non transformed as percentage of the reference						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
Dif in % ref	0.9494	0.9181	0.9807	1.0671	1.0366	1.0976
	BIOEQUIVALENT			BIOEQUIVALENT		

As a result, according to Guidance for Industry Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action, reference product and test product have been proven to be similar for Droplet Size Distribution.

Drug in Small Droplets

The results obtained are as shown in Table 10. (During analysis, 10 bottles were used for each batch.)

Table 10: Drug in Small Droplets test results of the beginning and end spray for the test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product

	Drug in Small Droplets		
Batches	**% Drug in Small Droplet head part**	**% Drug in Small Droplet NGI Part (sum of all stages)**	**Total Mass (%)**
AVG	**95.6**	**< LOQ**	**95.6**
SD	**3.56**		**3.56**
RSD %	**3.72**		**3.72**

LOQ: Limit of Quantitation

Evaluation Result

- All the total mass of drug collected on all stages and accessories results meet 85% and 115% of the amount labeled on a per actuation basis and the results of the samples taken from all Stage part meet less than 5% (9 µm ≤5%) limits.
- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of

the statistical calculation of ABE and PBE have been as shown in Table 11.

Table 11: Summary results of statistical evaluation for ABE and PBE (Total Mass (%) result)

ABE no LS means			
Overall mean	95.55761379		
CV %	2.94714878	**CV ref %**	3.23038008
ABE	**Ratio=theta**	**LL**	**UL**
	1.047643601	1.0344	1.0611
Limits	**Non scaled**	0.9000	1.1100
	Scaled ABE	0.9000	1.1100
Conclusion based on non scaled	BIOEQUIVALENT		
PBE Results			
σR	0.032295378	CONSTANT SCALED	
Ratio	1.047643601		
Hη	-0.017619586	As <0: BIOEQUIVALENT	
Fieller on non transformed data			
CV% within	2	CV ref %	2
	Ratio	LL	UL
	1.0475	1.0343	1.0608
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed			
ABE	**Dif**	**LL**	**UL**
	4.433333333	3.226746915	5.639919752
Limits	Non scaled	-9.340666667	9.340666667
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference			
ABE	**Dif**	**LL**	**UL**
Dif in % ref	1.0475	1.0345	1.0604
	BIOEQUIVALENT		

As stated in method validation studies performed for Drug in Small Droplets, Limit of Quantitation (LOQ) value was found to be 0.5%. As seen in Table 10, all the results obtained by adding all the stages of the NGI device were found to be less than or equal to the LOQ limit. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with satisfactory accuracy and precision. For this reason, statistical evaluation was not made for the samples collected from the stage parts of the NGI device.

PSD by Raman Spectroscopy

The results obtained are as shown in Table 12 (During analysis, 10 bottles were used for each batch).

Table 12: Particle Size Distribution by Raman Microscope for actuation and without actuation for the test (batch 1, batch 2 and batch 3) and reference (batch 1, batch 2 and batch 3) product

Test Results								
	Actuation				**Without Actuation**			
	D90	**D50**	**D10**	**SPAN**	**D90**	**D50**	**D10**	**SPAN**
AVG	5.48	2.02	1.17	2.14	22.94	44.87	89.61	2.13
MIN	4.36	1.63	0.78	1.72	18.19	41.57	78.35	1.52
MAX	6.25	2.39	1.36	2.61	26.05	47.87	107.16	2.49
Reference Results								
AVG	5.67	2.07	1.29	2.12	5.72	2.07	1.25	2.17
MIN	4.24	1.81	1.01	1.57	3.15	1.5	0.90	1.43
MAX	6.28	2.26	1.36	2.41	6.49	2.43	1.36	2.90

*AVG: For 3 batches

Evaluation Result

- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of the statistical calculation of ABE and PBE are as shown in Table 13 and Table 14.

Table 13: Summary results of statistical evaluation for ABE and PBE (actuation and without actuation of D50 results)

D50 Results						
ABE no LS means						
	Actuation			**Without Actuation**		
Overall mean	2.041721172			2.08110494		
CV %	7.459088065	**CV ref %**	4.601458375	8.914333024	**CV ref %**	10.93542888
ABE	**Ratio=theta**	**LL**	**UL**	**Ratio=theta**	**LL**	**UL**
	0.973089969	0.9423	1.0049	1.02367113	0.9851	1.0637
Limits	**Non scaled**	0.9	1.11	**Non scaled**	0.9	1.11
	Scaled ABE	0.9	1.11	**Scaled ABE**	0.9	1.11
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
PBE Results						
σR	0.045990254	CONSTANT SCALED		0.109029465	CONSTANT SCALED	
Ratio	0.973089969			1.02367113		
Hη	-0.006875377	As <0: BIOEQUIVALENT		-0.01982711	As <0: BIOEQUIVALENT	
Fieller on non transformed data						
CV% within	5	CV ref %	6	8	CV ref %	10
	Ratio	LL	UL	Ratio	LL	UL
	0.9763	0.9458	1.0078	1.0200	0.9832	1.0582
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
	-0.049077024	-0.114005069	0.015851022	0.041353809	-0.035383779	0.118091397
Limits	Non scaled	-0.207186258	0.207186258	Non scaled	-0.206827961	0.206827961
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
Dif in % ref	0.9763	0.9450	1.0077	1.0200	0.9829	1.0571
	BIOEQUIVALENT			BIOEQUIVALENT		

Table 14: Summary results of statistical evaluation for ABE and PBE (actuation and without actuation of span results)

Span Results						
ABE no LS means						
	Actuation			**Without Actuation**		
Overall mean	2.119522351			2.133021801		
CV %	10.03287624	**CV ref %**	9.980164265	11.58001901	**CV ref %**	13.11469568
ABE	**Ratio=theta**	**LL**	**UL**	**Ratio=theta**	**LL**	**UL**
	1.011493511	0.9687	1.0561	0.983784735	0.9360	1.0340
Limits	**Non scaled**	0.9	1.11	**Non scaled**	0.9	1.11
	Scaled ABE	0.9	1.11	**Scaled ABE**	0.9	1.11
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
PBE Results						
σR	0.099554459	CONSTANT SCALED		0.130588234	CONSTANT SCALED	
Ratio	1.011493511			0.983784735		
Hη	-0.012821863	As <0: BIOEQUIVALENT		-0.02449695	As <0: BIOEQUIVALENT	
Fieller on non transformed data						
CV% within	5	CV ref %	6	11	CV ref %	13
	Ratio	LL	UL	Ratio	LL	UL
	1.0118	0.9703	1.0550	0.9803	0.9339	1.0289
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
	0.024877242	-0.064097218	0.113851703	-0.042749651	-0.146568638	0.061069335
Limits	Non scaled	-0.207186258	0.207186258	Non scaled	-0.216792156	0.216792156
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
Dif in % ref	1.0118	0.9697	1.0538	0.9803	0.9324	1.0282
	BIOEQUIVALENT			BIOEQUIVALENT		

As a result, according to Guidance for Industry Bioavailability and Bioequivalence Studies for Nasal Aerosols

and Nasal Sprays for Local Action, reference product and test product have been proven to be similar for Particle Size Distribution.

Spray Pattern

The results obtained are as shown in Table 15 (During analysis, 10 bottles were used for each batch).

Table 15: Spray Pattern results at 30 mm and 60 mm distance for the test product (batch 1, batch 2 and batch 3) and reference product (batch 1, batch 2 and batch 3)

Test Product							**Reference Product**					
30 mm							**30 mm**					
1st Batch							**1st Batch**					
	Dmax (mm)	**Dmin (mm)**	**Average (mm)**	**Spray Pattern**	**Angle**			**Dmax (mm)**	**Dmin (mm)**	**Average (mm)**	**Spray Pattern**	**Angle**
AVG	33.20	27.00	30.10	1.24	53.25		**AVG**	31.70	26.80	29.25	1.19	51.94
2nd Batch							**2nd Batch**					
AVG	32.00	26.90	29.45	1.19	52.23		**AVG**	30.40	25.80	28.10	1.18	50.16
3rd Batch							**3rd Batch**					
AVG	30.10	25.80	27.95	1.17	49.90		**AVG**	30.20	26.10	28.15	1.17	50.20
60 mm							**60 mm**					
1st Batch							**1st Batch**					
AVG	46.70	37.70	42.20	1.24	70.22		**AVG**	45.10	39.10	42.10	1.16	70.07
2nd Batch							**2nd Batch**					
AVG	44.40	34.30	39.35	1.30	66.44		**AVG**	44.70	37.70	41.20	1.19	68.91
3rd Batch							**3rd Batch**					
AVG	44.20	37.90	41.05	1.17	68.72		**AVG**	42.10	34.70	38.40	1.22	65.15

Evaluation Result

- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of the statistical calculation of ABE and PBE are as shown in Table 16 and Table 17.

Table 16: ABE and PBE Results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product at 30 mm and 60 mm- Spray Pattern (Ovality ratio)

Ovality Ratio						
ABE no LS means						
	30 mm			**60 mm**		
Overall mean	1.185849361			1.208679544		
CV %	6.523326379	**CV ref %**	6.432711701	6.906320462	**CV ref %**	6.041671109
ABE	**Ratio=theta**	**LL**	**UL**	**Ratio=theta**	**LL**	**UL**
	1.017405818	0.9892	1.0464	1.03885035	1.0069	1.0718
Limits	Non scaled	0.9	1.11	**Non scaled**	0.9	1.11
	Scaled ABE	0.9	1.11	**Scaled ABE**	0.9	1.11
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
PBE Results						
σR	0.06426072	CONSTANT SCALED		0.060361687	CONSTANT SCALED	
Ratio	1.017405818			1.03885035		
Hη	-0.016813377	As <0: BIOEQUIVALENT		-0.01065064	As <0: BIOEQUIVALENT	
Fieller on non transformed data						
CV% within	7	CV ref %	7	7	CV ref %	6
	Ratio	LL	UL	Ratio	LL	UL
	1.0175	0.9892	1.0467	1.0404	1.0078	1.0741
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
	0.020666667	-0.012852014	0.054185348	0.048	0.009480428	0.086519572
Limits	Non scaled	-0.1178	0.1178	Non scaled	-0.1188	0.1188
Classical CI based on non transformed as percentage of the reference						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
Dif in % ref	1.0175	0.9891	1.0460	1.0404	1.0080	1.0728
	BIOEQUIVALENT			BIOEQUIVALENT		

Table 17: ABE & PBE Results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product at 30 mm and 60 mm distance – Dmax

Dmax

ABE no LS means						
	30 mm			**60 mm**		
Overall mean	31.16554614			44.43355926		
CV %	2.94714878	**CV ref %**	3.23038008	6.749673677	**CV ref %**	6.663328483
ABE	**Ratio=theta**	**LL**	**UL**	**Ratio=theta**	**LL**	**UL**
	1.030842997	0.9977	1.0505	1.025664281	0.9962	1.0559
Limits	Non scaled	0.9	1.11	**Non scaled**	0.9	1.11
	Scaled ABE	0.9	1.11	**Scaled ABE**	0.9	1.11
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
PBE Results						
σR	0.068801849	CONSTANT SCALED		0.066559499	CONSTANT SCALED	
Ratio	1.030842997			1.025664281		
Hη	-0.010174209	As <0: BIOEQUIVALENT		-0.015991759	As <0: BIOEQUIVALENT	
Fieller on non transformed data						
CV% within	8	CV ref %	7	6	CV ref %	6
	Ratio	LL	UL	Ratio	LL	UL
	1.0475	1.0343	1.0608	1.0258	0.9970	1.0554
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
	1	-0.026277956	2.026277956	1.133333333	-0.133445075	2.400111742
Limits	Non scaled	-9.340666667	9.340666667	Non scaled	-4.396666667	4.396666667
Classical CI based on non transformed as percentage of the reference						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
Dif in % ref	1.0325	0.9991	1.0659	1.0258	0.9970	1.0546
	BIOEQUIVALENT			BIOEQUIVALENT		

As a result, according to Guidance for Industry Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action, reference product and test product have been proven to be similar for Spray Pattern.

Plume Geometry

The results obtained are as shown in Table 18 (During analysis, 10 bottles were used for each batch).

Table 18: Plume Geometry results at 30 mm distance for the test product (batch 1, batch 2 and batch 3) and reference product (batch 1, batch 2 and batch 3)

30 mm				
Test Product				
1st batch				
Batches	**Plum Angle**	**Plume Angel_Log**	**Plume Width**	**Plume Width Log**
AVG	43.00	1.63	23.99	1.37
2nd batch				
AVG	42.19	1.62	23.46	1.37
3rd batch				
AVG	42.3	1.60	23.6	1.35
Reference Product				
1st batch				
AVG	44.6	1.65	24.6	1.39
2nd batch				
AVG	43.5	1.64	24.0	1.38
3rd batch				
AVG	43.1	1.63	23.8	1.37

Evaluation Result

- Ratio of the geometric mean of the three batches of test to that of the three batches of reference (based on log-transformed data) for both plume angle and width, meet limits. The results are given in Table 19.

Table 19: Results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding Plume Geometry-30 mm

	30 mm (Plume Angle Log)	**30 mm (Plume Width Log)**
Reference product Geo. Average (batch 1, batch 2, batch 3)	1.63831442	1.379340654
Test product Geo. Average (batch 1, batch 2, batch 3)	1.61379833	1.357497966
Similarity	**98.5**	**98.4**

As a result, reference product and test product have been proven to be similar for plume geometry.

Priming and Repriming

The results obtained are as shown in Table 20 (During analysis, 10 bottles held in horizontal and vertical positions were used for each batch).

Table 20: Results for test (batch 1, batch 2 batch 3) and reference (batch 1, batch 2 batch 3) product regarding Priming and Repriming Tests

		Initial (Active ingredient, %)						**15th day**	**30th day**
Batches	**Stage**	**1st Puff**	**2nd Puff**	**3rd Puff**	**4th Puff**	**5th Puff**	**6th Puff**	**7th Puff**	**8th Puff**
Test Product									
Batch 1									
AVG	**Vertical**	N.D	16.3	49.2	80.4	99.3	98.0	103.5	100.9
	Horizontal	N.D	16.3	49.7	81.2	100.2	98.5	105.0	104.5
Batch 2									
AVG	**Vertical**	N.D	17.3	60.0	80.4	90.7	97.2	101.8	99.9
	Horizontal	N.D	35.0	65.8	84.6	95.6	98.1	102.0	99.9
Batch 3									
AVG	**Vertical**	N.D	28.5	67.8	85.6	97.7	99.7	103.2	102.2
	Horizontal	N.D	19.9	59.8	80.3	94.6	97.3	100.6	100.1
Reference Product									
Batch 1									
AVG	**Vertical**	N.D	N.D	41.1	86.1	92.7	95.0	96.8	96.4
	Horizontal	N.D	N.D	33.6	85.9	91.4	93.4	96.2	94.9
Batch 2									
AVG	**Vertical**	N.D	N.D	36.9	83.5	88.6	92.1	98.9	95.9
	Horizontal	N.D	N.D	29.4	81.3	88.6	92.8	96.8	95.4
Batch 3									
AVG	**Vertical**	N.D	N.D	35.2	78.4	88.3	89.2	94.1	93.1
	Horizontal	N.D	N.D	45.3	78.4	87.5	89.6	94.8	92.0

N.D: Not Detected

Evaluation Result

- The obtained results for 6th puff on initial, 7th puff on15th day and 8th puff on 30th day meet the requirements.
- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of the statistical calculation of ABE and PBE are as shown in Table 21 and Table 22.

Table 21: ABE and PBE results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding 6th puff on initial for priming and 7th puff on 15th day for repriming (in vertical and horizontal position)

ABE no LS means						
	6th puff on inital			**7th puff on 15th day**		
Overall mean	94.94899632			99.35547169		
CV %	3.970353532	**CV ref %**	3.193735733	3.484041716	**CV ref %**	2.300581492
ABE	**Ratio=theta**	**LL**	**UL**	**Ratio=theta**	**LL**	**UL**
	1.066079064	1.0533	1.0790	1.06594345	1.0548	1.0772
Limits	**Non scaled**	0.9000	1.1100	**Non scaled**	0.9000	1.1100
	Scaled ABE	0.9000	1.1100	**Scaled ABE**	0.9000	1.1100
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
PBE Results						
σR	0.031929218	CONSTANT SCALED		0.023002772	CONSTANT SCALED	

Ratio	1.066079064			1.049141326		
Hη	-0.013387139	As <0: BIOEQUIVALENT		-0.013590539	As <0: BIOEQUIVALENT	
Fieller on non transformed data						
CV% within	4	CV ref %	4	3	CV ref %	3
	Ratio	LL	UL	Ratio	LL	UL
	1.0667	1.0538	1.0796	1.0667	1.0552	1.0783
Conclusion based on non scaled	BIOEQUIVALENT			BIOEQUIVALENT		
Classical CI based on non transformed						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
	6.133333333	4.985725009	7.280941657	6.416666667	5.344168968	7.489164365
Limits	Non scaled	-9.2005	9.2005	Non scaled	-9.625833333	9.625833333
Classical CI based on non transformed as percentage of the reference						
ABE	**Dif**	**LL**	**UL**	**Dif**	**LL**	**UL**
Dif in % ref	1.0667	1.0542	1.0791	1.0667	1.0555	1.0778
	BIOEQUIVALENT			BIOEQUIVALENT		

Table 22: ABE and PBE results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding 8th puff on 30th day for repriming (in vertical and horizontal position)

ABE no LS means			
Overall mean	97.81603262		
CV %	3.459420847	**CV ref %**	2.463196772
ABE	**Ratio=theta**	**LL**	**UL**
	1.069524935	1.0584	1.0808
Limits	**Non scaled**	0.9000	1.1100
	Scaled ABE	0.9000	1.1100
Conclusion based on non scaled	BIOEQUIVALENT		
PBE Results			
σR	0.024628233	CONSTANT SCALED	
Ratio	1.069524935		
Hη	-0.013335056	As <0: BIOEQUIVALENT	
Fieller on non transformed data			
CV% within	3	CV ref %	3
	Ratio	LL	UL
	1.0701	1.0587	1.0817
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed			
ABE	**Dif**	**LL**	**UL**
	6.636666667	5.588644655	7.684688679
Limits	Non scaled	-9.461166667	9.461166667
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference			
ABE	**Dif**	**LL**	**UL**
Dif in % ref	1.0701	1.0591	1.0812
	BIOEQUIVALENT		

As a result, according to Guidance for Industry Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action, reference product and test product have been proven to be similar for priming and repriming test. Reference product and test product can be used after the 6th puff. It has been proven that it is not affected by the waiting conditions in horizontal and vertical positions for 30 days and can be used directly after the waiting period.

Profiling of Sprays Near Container Exhaustion (Tail Off Characteristics)

The results obtained are as shown in Table 23 (During analysis, 3 containers were used for each batch).

Table 23: Results of test product (batch 1, batch 2 and batch 3) and reference product (batch 1, batch 2 and batch 3) for Profiling of Sprays Near Container Exhaustion (Tail Off Characteristics)

Test Product				**Reference Product**			
Tail Off							
	1st batch (3 container)	**2nd batch (3 container)**	**3rd batch (3 container)**		**1st batch (3 container)**	**2nd batch (3 container)**	**3rd batch (3 container)**
AVG-130th stage	103.0	94.3	99.0	**AVG-130th**	94.8	93.8	100.6
AVG-140th stage	103.8	92.9	92.4	**AVG-140th**	94.6	88.4	97.1
AVG-150th stage	97.3	94.6	94.5	**AVG-145th**	93.8	90.7	98.2
AVG-160th	97.9	96.2	95.0	**AVG-150th**	92.2	89.8	95.3
AVG-170th	99.4	99.1	99.0	**AVG-151th**	91.3	90.0	94.9
AVG-175th	96.3	95.8	97.0	**AVG-152th**	86.0	91.9	95.8
AVG-180th	94.9	98.1	97.8	**AVG-153th**	91.5	90.5	95.9
AVG-181th	93.8	93.3	91.5	**AVG-154th**	91.3	90.4	96.7
AVG-182th	95.3	85.3	94.7	**AVG-155th**	92.9	90.1	95.4
AVG-183th	93.9	85.8	84.6	**AVG-156th**	86.0	91.9	91.7
AVG-184th	84.4	74.9	73.8	**AVG-157th**	81.0	86.0	62.2
AVG-185th	66.4	49.2	52.4	**AVG-158th**	63.6	39.9	58.1
AVG-186th	60.3	48.0	53.5	**AVG-159th**	60.0	37.9	54.4
AVG-187th	N.D	N.D	N.D	**AVG-160th**	N.D	N.D	N.D

N.D: Not Detected

Evaluation Result

The results of samples performed have been calculated by taking 3 bottles selected from each 3 batches of the reference product and 3 bottles from each 3 batches of the test product.

The last puff results that meet the 85 to 115 percent of label claim are as shown in Table 24.

Table 24: Sum of Profiling of Sprays Near Container Exhaustion (Tail Off Characteristics) test results

Batches	**Number of doses**
Test Product	
1st batch	182
2nd batch	181
3rd batch	182
Reference Product	
1st batch	156
2nd batch	156
3rd batch	156

Shaking Requirement

The results obtained are as shown in Table 25 (During analysis, 10 bottles were used for each batch).

Table 25: Results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding shaking requirement

Batches	**Average**	**Shaking Time (sec)**	**Shaking Requirement & In Vitro Dose Proportionality (%)**
Test Product			
Batch 1	**AVG**	**3**	94.2
		5	97.2
		10	97.6
Batch 2	**AVG**	**3**	97.9
		5	95.0
		10	97.6
Batch 3	**AVG**	**3**	95.6
		5	97.4
		10	96.0

Reference Product			
Batch 1	**AVG**	**3**	94.8
		5	95.7
		10	96.8
Batch 2	**AVG**	**3**	93.6
		5	92.7
		10	94.2
Batch 3	**AVG**	**3**	95.5
		5	95.9
		10	95.9
AVG			95.8
SD			4.14
RSD %			4.32
MIN			85.9
MAX			109.7

Evaluation Result

In the end of the Shaking Requirement test which 10 different bottles of test product were shaken vigorously (with agitation rate once per second) for 3 seconds, 5 seconds and 10 seconds, respectively, all results meet requirements.

Performance After Temperature Cycling

Results are given between in Table 26- Table 32. (During analysis, 10 bottles held in horizontal and vertical positions were used for each batch.)

Table 26: Results of Performance After Temperature Cycling -Initial

	Initial					
	Appearance (Limit: White colored, homogenous suspension)	**Density at 20 °C**	**PSD**	**DSD**	**Assay of Active ingredient (Limit: 95.0% - 105.0%)**	**Assay of Preservative active ingredient (Limit: 90.0% - 110.0%)**
Test Product						
Batch 1	Conforms	1.03 g/mL	8 µm	5% < 10 µm: 0.9 D50: 46 µ	100.9	103.0
Batch 2	Conforms	1.02 g/mL	8 µm	5% < 10 µm: 0.4 D50: 45 µ	100.8	103.4
Batch 3	Conforms	1.02 g/mL	7 µm	5% < 10 µm: 0.8 D50: 46 µ	99.0	103.8
Reference Product						
Batch 1	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 0.6 D50: 47 µ	92.3	90.5
Batch 2	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 1.0 D50: 45 µ	94.2	93.2
Batch 3	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 0.2 D50: 45 µ	93.0	91.8

Table 27: Results of Performance After Temperature Cycling -Initial

	Initial					
	Test Product			**Reference Product**		
	Batch 1	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Test Product Related Substances						
Impurity D	N.D	N.D	N.D	N.D	N.D	N.D
Maximum Unknown Impurity	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Total Impurity	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

N.D: Not Detected
LOQ: Limit of Quantitation

Table 28: Results of Performance After Temperature Cycling - 6th Day (H:Horizontal and V:Vertical)

	6th Day						
	Appearance (Limit: White colored,	**Density at 20 °C**	**PSD (D50)**	**DSD (D50)**	**Weight loss**	**Assay of active ingredient (Limit: 95.0% - 105.0%)**	**Assay of preservative active ingredient**

	homogenous suspension)						(Limit: 90.0% - 110.0%)
Test Product							
Batch 1 / H	Conforms	1.03 g/mL	7 µm	5% < 10 µm: 0.7 D50: 44 µ	-0.002	100.7	103.9
Batch 1 / V	Conforms	1.02 g/mL	7 µm	5% < 10 µm: 0.3 D50: 43 µ	-0.004	101.0	104.5
Batch 2 / H	Conforms	1.02 g/mL	7 µm	5% < 10 µm: 0.6 D50: 44 µ	-0.002	100.7	103.9
Batch 2 / V	Conforms	1.02 g/mL	7 µm	5% < 10 µm: 0.0 D50: 44 µ	-0.0002	99.8	104.4
Batch 3 / H	Conforms	1.02 g/mL	7 µm	5% < 10 µm: 0.2 D50: 43 µ	-0.002	100.8	104.4
Batch 3 / V	Conforms	1.02 g/mL	7 µm	5% < 10 µm: 0.1 D50: 43 µ	-0.0005	100.8	104.4
Reference Product							
Batch 1 / H	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 0.5 D50: 45 µ	-0.06	94.2	91.6
Batch 1 / V	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 1.2 D50: 44 µ	-0.03	93.8	91.2
Batch 2 / H	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 0.0 D50: 43 µ	-0.06	94.5	91.9
Batch 2 / V	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 0.6 D50: 44 µ	-0.03	93.7	91.9
Batch 3 / H	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 0.0 D50: 44 µ	-0.06	94.4	91.9
Batch 3 / V	Conforms	1.02 g/mL	6 µm	5% < 10 µm: 0.4 D50: 43 µ	-0.03	93.5	90.9

Table 29: Results of Performance After Temperature Cycling - 6th Day

	6th Day											
	Test Product						**Reference Product**					
	Horizontal			**Vertical**			**Horizontal**			**Vertical**		
	Batch 1	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Test Product Related Substances												
Impurity D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Max. Unknown impurity	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Total Impurity	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

N.D: Not Detected
LOQ: Limit of Quantitation

Table 30: Results of Performance After Temperature Cycling - 12th Day

	12th Day											
	Test Product						**Reference Product**					
	Horizontal			**Vertical**			**Horizontal**			**Vertical**		
	Batch 1	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Test Product Related Substances												
Impurity D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Maximum Unknown Impurity	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Total Impurity	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

N.D: Not Detected
LOQ: Limit of Quantitation

Table 31: Results of Performance After Temperature Cycling - 12th Day

	12th Day						
	Appearance (Limit: White colored, homogenous suspension)	**Density at 20 °C**	**PSD (D50)**	**DSD (D50)**	**Weight loss**	**Assay of active ingredient (Limit: 95.0% - 105.0%)**	**Assay of preservative active ingredient (Limit: 90.0% - 110.0%)**
Test Product							
Batch 1 / H	Conforms	1.02 g/mL	8 µm	5%< 10µ : 0.5 D50: 44 µ	-0.006	102.4	104.0
Batch 1 / V	Conforms	1.03 g/mL	8 µm	5%< 10µ: 0.8 D50: 44 µ	-0.005	102.1	104.2
Batch 2 / H	Conforms	1.02 g/mL	8 µm	5%< 10µ: 0.0 D50: 44 µ	-0.004	101.6	104.2
Batch 2 / V	Conforms	1.02 g/mL	8 µm	5%< 10µ: 0.8 D50: 45 µ	-0.004	100.8	104.2
Batch 3 / H	Conforms	1.03 g/mL	8 µm	5%< 10µ: 0.6 D50: 45 µ	-0.003	100.9	103.8
Batch 3 / V	Conforms	1.02 g/mL	7 µm	5%< 10µ: 0.0 D50: 45 µ	-0.005	101.9	103.8
Reference Product							
Batch 1 / H	Conforms	1.02 g/mL	7 µm	5%< 10µ: 1.1 D50: 44 µ	-0.05	94.4	91.3
Batch 1 / V	Conforms	1.02 g/mL	6 µm	5%< 10µ: 1.2 D50: 44 µ	-0.03	94.4	91.1
Batch 2 / H	Conforms	1.02 g/mL	6 µm	5%< 10µ: 0.9 D50: 44 µ	-0.04	94.6	91.2
Batch 2 / V	Conforms	1.02 g/mL	6 µm	5%< 10µ: 0.9 D50: 44 µ	-0.03	94.1	91.7
Batch 3 / H	Conforms	1.03 g/mL	6 µm	5%< 10µ: 0.9 D50: 44 µ	-0.04	94.4	91.1
Batch 3 / V	Conforms	1.02 g/mL	6 µm	5%< 10µ: 0.7 D50: 44 µ	-0.03	94.2	91.3

Table 32: Results of Performance After Temperature Cycling Mean Delivered Dose& Uniformity of Delivered Dose - Initial, 6th Day, 12th Day

	Mean Delivered Dose (Active ingredient, %)			**Uniformity of Delivered Dose (Active ingredient, %)**		
	Initial	**6th Day**	**12th Day**	**Initial**	**6th Day**	**12th Day**
AVG	99.9	97.5	96.6	99.0	97.5	96.6
SD	5.3	4.3	4.3	4.9	4.3	5.2
RSD %	5.3	4.4	4.5	5.0	4.4	5.3
MIN	92.1	88.6	88.3	91.9	88.8	88.1
MAX	112.0	109.5	109.8	111.2	109.5	109.9

Evaluation Result

Initial results are expected to comply with release specifications and 6th and 12th day results with shelf-life specifications. All results have been compared with initial values and the results have been seen to be appropriate for both release and shelf-life specifications.

Robustness

Results are given in Table 33 and Table 34 (During the analysis, 10 bottles were used for the initial stage and 10 bottles for the final stage).

Table 33: Results of Robustness

SCU (Active ingredient %)				
Batches	**Average**	**Stage**	**3000 km**	**6000 km**
Test Product				

Batch 1	**AVG**	**Beginning**	99.1	100.9
		End	96.8	100.8
Batch 2	**AVG**	**Beginning**	99.8	102.6
		End	99.4	98.6
Batch 3	**AVG**	**Beginning**	100.7	101.5
		End	98.7	100.4
Reference Product				
Batch 1	**AVG**	**Beginning**	95.1	99.0
		End	94.0	99.3
Batch 2	**AVG**	**Beginning**	95.4	96.9
		End	95.2	97.3
Batch 3	**AVG**	**Beginning**	90.0	92.7
		End	92.5	94.1
AVG			**95.7**	**97.8**
SD			**4.6**	**4.0**
RSD %			**4.9**	**4.1**
MIN			**87.3**	**90.9**
MAX			**108.0**	**112.0**

Table 34: Results of Robustness (3000 km and 6000 km)

3000 km			
	Appearance (Limit: White colored, homogenous suspension)	**PSD (Limit: D50: 3 µm - 10 µm)**	**DSD (Maximum 5%<10 µm D50: 30 µm - 70 µm)**
Test Product			
Batch 1	Conforms	8µ	5%<10 µ: 0.3 D50: 43 µ
Batch 2	Conforms	8µ	5%<10 µ: 0.0 D50: 42 µ
Batch 3	Conforms	8µ	5%<10 µ: 0.2 D50: 43 µ
Reference Product			
Batch 1	Conforms	6µ	5%<10 µ: 0.4 D50: 44 µ
Batch 2	Conforms	6µ	5%<10 µ: 0.8 D50: 43 µ
Batch 3	Conforms	6µ	5%<10 µ: 1.3 D50: 44 µ
6000 km			
	Appearance (Limit: White colored, homogenous suspension)	**PSD (Limit: D50: 3 µm - 10 µm)**	**DSD (Maximum 5%<10 µm D50: 30 µm - 70 µm)**
Test Product			
Batch 1	Conforms	8µ	5%<10 µ: 0.3 D50: 42 µ
Batch 2	Conforms	8µ	5%<10 µ: 0.1 D50: 43 µ
Batch 3	Conforms	8µ	5%<10 µ: 0.9 D50: 42 µ
Reference Product			
Batch 1	Conforms	6µ	5%<10 µ: 0.9 D50: 43 µ
Batch 2	Conforms	6µ	5%<10 µ: 1.1 D50: 43 µ
Batch 3	Conforms	6µ	5%<10 µ: 1.3 D50: 43 µ

Evaluation Result

All results are compared with initial values. Initial results are expected to comply with release specifications and 3000 km and 6000 km results with shelf-life specifications. All results were found to be appropriate in line with expectations.

Effect of Dosing Orientation

Results are given in Table 35 and Table 36 (During the analysis, 10 bottles were used for the initial stage and 10 bottles for the final stage).

Table 35: Results of Effect of Dosing Orientation

45°			
Batches	**Average**	**Stage**	**SCU (%)**
Test Product			
Batch1	**AVG**	Beginning	98.7
		End	101.9
Batch 2	**AVG**	Beginning	100.2
		End	99.6
Batch 3	**AVG**	Beginning	97.3
		End	100.4
Reference Product			
Batch1	**AVG**	Beginning	93.6
		End	94.2
Batch 2	**AVG**	Beginning	93.8
		End	92.6
Batch 3	**AVG**	Beginning	93.2
		End	93.4
AVG			**96.6**
SD			**5.3**
RSD %			**5.5**
MIN			**87.6**
MAX			**110.5**

Table 36: Results of Effect of Dosing Orientation

45°	
DSD (Maximum 5%<10 µm D50: (30 µm - 70 µm)	
Test Product	
Batch 1	D50: 44 µm 5%< 10µ: 1.1
Batch 2	D50: 43 µm 5 %< 10µ: 0.02
Batch 3	D50: 44 µm 5%< 10µ: 0.0
Reference Product	
Batch 1	D50: 43 µm 5%< 10µ: 0.2
Batch 2	D50: 43 µm 5%< 10µ: 0.7
Batch 3	D50: 44 µm 5%< 10µ: 0.2

Evaluation Result

All results were compared with the initial values and found to be appropriate.

Density

Results are as shown in Table 37 (During analysis, 10 bottles were used for each batch).

Table 37: Results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding Density

	Density, g/mL					
	Test Product			**Reference Product**		
Batches	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
AVG	1.0241	1.02361	1.02320	1.02370	1.02137	1.02370

Evaluation Result

- All results found to be appropriate.
- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of

the statistical calculation of ABE and PBE are as shown in Table 38.

Table 38: ABE and PBE results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding density

ABE no LS means			
Overall mean	1.023272876		
CV %	0	**CV ref %**	0
ABE	**Ratio=theta**	**LL**	**UL**
	1.00068469	1.000297418	1.001072112
Limits	**Non scaled**	0.9000	1.1100
	Scaled ABE	0.9000	1.1100
Conclusion based on non scaled	BIOEQUIVALENT		
PBE Results			
σR	0.001092848	CONSTANT SCALED	
Ratio	1.00068469		
Hη	-0.020890502	As <0: BIOEQUIVALENT	
Fieller on non transformed data			
CV% within	3	CV ref %	3
	Ratio	LL	UL
	1.0000	0.9995	1.0005
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed			
ABE	**Dif**	**LL**	**UL**
	-0.366666667	-4.822938356	4.822938356
Limits	Non scaled	-1022.923333	1022.923333
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference			
ABE	**Dif**	**LL**	**UL**
Dif in % ref	1.0000	0.9995	1.0005
	BIOEQUIVALENT		

As a result, according to Guidance for Industry Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action, reference product and test product have been proven to be similar for density.

Viscosity (S62, 90 rpm, Torque: 50%±10%, at Room Temperature)

Results are given in Table 39 (During analysis, 10 bottles were used for each batch)

Table 39: Results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding viscosity

	Viscosity, cP (S62, 90 rpm, Torque: 50%±10%, at Room Temperature)					
	Test Product			**Reference Product**		
Batches	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
AVG	166	166	167	167	167	167

Evaluation Result

- All results found to be appropriate.
- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of the statistical calculation of ABE and PBE are as shown in Table 40.

Table 40: ABE and PBE results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding viscosity

ABE no LS means			
Overall mean	166.6772751		
CV %	0.860272734	**CV ref %**	0.829660388
ABE	**Ratio=theta**	**LL**	**UL**
	0.997797656	0.9941	.1.0015
Limits	**Non scaled**	0.9000	1.1100

	Scaled ABE	0.9000	1.1100
Conclusion based on non scaled	BIOEQUIVALENT		
PBE Results			
σR	0.008296461	CONSTANT SCALED	
Ratio	0.997797656		
Hη	-0.020813043	As <0: BIOEQUIVALENT	
Fieller on non transformed data			
CV% within	1	CV ref %	1
	Ratio	LL	UL
	0.9978	0.9941	1.0015
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed			
ABE	**Dif**	**LL**	**UL**
	-0.366666667	-0.984787114	0.25145378
Limits	Non scaled	-9.625833333	9.625833333
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference			
ABE	**Dif**	**LL**	**UL**
Dif in % ref	0.9978	0.9941	1.0015
	BIOEQUIVALENT		

As a result, according to Guidance for Industry Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action, reference product and test product have been proven to be similar for viscosity.

Osmolality

Results are given in Table 41 (During analysis, 10 bottles were used for each batch).

Table 41: Results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding Osmolality

	Osmolality, mOsm/kg					
	Test Product			**Reference Product**		
Batches	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
AVG	343	342	345	321	318	318

Evaluation Result

- All results found to be appropriate.
- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of the statistical calculation of ABE and PBE are as shown in Table 42.

Table 42: ABE and PBE results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding Osmolality

ABE no LS means			
Overall mean	330.7368486		
CV %	1.17757007	**CV ref %**	1.334531768
ABE	**Ratio=theta**	**LL**	**UL**
	1.076473629	1.0710	1.0820
Limits	**Non scaled**	0.9000	1.1100
	Scaled ABE	0.9000	1.1100
Conclusion based on non scaled	BIOEQUIVALENT		
PBE Results			
σR	0.013344724	CONSTANT SCALED	
Ratio	1.076473629		
Hη	-0.01476008	As <0: BIOEQUIVALENT	
Fieller on non transformed data			
CV% within	1	CV ref %	1
	Ratio	LL	UL
	1.0764	1.0710	1.0819
Conclusion based on non scaled	BIOEQUIVALENT		

Classical CI based on non transformed			
ABE	**Dif**	**LL**	**UL**
	24.36666667	22.69995625	26.03337708
Limits	Non scaled	-9.625833333	9.625833333
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference			
ABE	**Dif**	**LL**	**UL**
Dif in % ref	1.0764	1.0712	1.0817
	BIOEQUIVALENT		

As a result, according to Guidance for Industry Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action Reference product and test product have been proven to be similar for Osmolality.

pH

Results are given in Table 43 (During analysis, 10 bottles were used for each batch).

Table 43: Results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding pH

	pH					
	Test Product			**Reference Product**		
Batches	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
AVG	6.23	6.19	6.21	6.23	6.33	6.28

Evaluation Result

- All results found to be appropriate.
- ABE and PBE statistical evaluations were made in the Excel using SAS language. The results of the statistical calculation of ABE and PBE are as shown in Table 44.

Table 44: ABE and PBE results for test (batch 1, batch 2, batch 3) and reference (batch 1, batch 2, batch 3) product regarding pH

ABE no LS means			
Overall mean	6.245654987		
CV %	0.530208245	**CV ref %**	0.705482806
ABE	**Ratio=theta**	**LL**	**UL**
	0.989139616	0.9869	0.9914
Limits	**Non scaled**	0.9000	1.1100
	Scaled ABE	0.9000	1.1100
Conclusion based on non scaled	BIOEQUIVALENT		
PBE Results			
σR	0.00705474	CONSTANT SCALED	
Ratio	0.989139616		
Hη	-0.020757495	As <0: BIOEQUIVALENT	
Fieller on non transformed data			
CV% within	0	CV ref %	0
	Ratio	LL	UL
	0.9891	0.9868	0.9914
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed			
ABE	**Dif**	**LL**	**UL**
	-0.068333333	-0.082694322	-0.053972345
Limits	Non scaled	-0.628	0.628
Conclusion based on non scaled	BIOEQUIVALENT		
Classical CI based on non transformed as percentage of the reference			
ABE	**Dif**	**LL**	**UL**
Dif in % ref	0.9891	0.9868	0.9914
	BIOEQUIVALENT		

CONCLUSIONS

The in-vitro bioequivalence study of nasal sprays is useful to understand the efficiency of test

product in comparison to innovator so that the equivalency in the in-vivo studies can be assessed.

Bioequivalence testing is an essential step during the development of generic drugs. Regulatory agencies have drafted recommendations and guidelines to frame this step but without finding any consensus. Different methodologies are applied depending on the geographical region. For instance, in the EU, EMA recommends using average bioequivalence test (ABE), while in the USA, FDA recommends using population bioequivalence (PBE) test.

The methodology of PBE is based on the calculation described in the FDA Budesonide Individual Bioequivalence Guideline (U.S. Food and Drug Administration [FDA], 2015). Basically, the calculation takes into account the variability of the reference formulation to scale acceptance limits. A Hn value limit below 0 indicates similarity.

The methodology of the ABE calculation is based on the EMA guideline, it is based on an ANOVA with product type (test or reference) as factor and a 90% CI is calculated based on residual variance. Limits are fixed 0.9000-1.1111.

Tests are performed according to the EMA Guidance on Pharmaceutical Quality of Respiratory and Nasal Products (EMEA/CHMP/QWP/49313/2005 Corr), which is taken as a reference for the application of in vitro tests: Particle Size Distribution (PSD), Shaking Requirement, Performance After Temperature Cycling is in the form of robustness, viscosity, osmolality, pH, density. The tests performed according to the Food and Drug Administration (FDA, Draft Guidance), another source we refer to, unlike EMA Guidance: Single Actuation Content through Container Life (SCU), Droplet size distribution by laser diffraction (DSD), Spray pattern, Plume geometry, Profiling of Sprays Near Container Exhaustion (Tail Off Characteristics), Effect of Dosing Orientation.

In line with all in vitro bioequivalence studies carried out and presented in this article, taking into account EMA Guidance and FDA Guidance, and the statistical approaches carried out to support these studies; It was concluded that the Nasal Spray Product, which contains the corticosteroid active ingredient developed by Abdi İbrahim R&D Pharmaceuticals Center, is therapeutically equivalent to the Reference Product.

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