

*Contains Nonbinding Recommendations*  
*Draft – Not for Implementation*  
**Draft Guidance on Fluticasone Propionate**  
**May 2023**

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<b>Active Ingredient:</b>	Fluticasone propionate
<b>Dosage Form; Route:</b>	Spray, metered; Nasal
<b>Strength:</b>	0.05 mg/spray
<b>Recommended Studies:</b>	Two options: (1) eight in vitro bioequivalence studies, or (2) six in vitro bioequivalence studies, one in vivo bioequivalence study with pharmacokinetic endpoints, and one comparative clinical endpoint bioequivalence study

FDA recommends the following in vitro or in vitro and in vivo studies to establish bioequivalence of test (T) and reference (R) nasal spray products containing fluticasone propionate. The recommendations provided here supersede information provided in the most recent version of the FDA draft guidance for industry on *Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action*.<sup>a</sup>

### **I. Option 1: In vitro bioequivalence studies**

To be eligible for this recommended option to demonstrate bioequivalence, the T and R product formulations should be qualitatively (Q1)<sup>1</sup> and quantitatively (Q2)<sup>2</sup> the same, and the nasal spray device (e.g., pump and actuator design) of the T product is appropriate for an abbreviated new drug application (ANDA).

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<sup>1</sup> Q1 (qualitative sameness) means that the T product uses the same inactive ingredient(s) as the R product.

<sup>2</sup> Q2 (quantitative sameness) means that concentrations of the inactive ingredient(s) used in the T product are within ± 5% of those used in the R product.

FDA recommends that prospective applicants conduct the following in vitro bioequivalence studies on samples from each of three or more batches of the T product and three or more batches of the R product, with no fewer than 10 units from each batch. FDA recommends that three primary stability batches be also used to demonstrate in vitro bioequivalence. The three batches of the T product should be manufactured from, at minimum, three different batches of the drug substance, three different batches of critical excipients, and three different batches of the device components (e.g., pump and actuator) proposed for the final device configuration of the commercial product. The T product should consist of the final device constituent part and final drug constituent formulation intended to be marketed.

For comparative in vitro studies, T and R products should be studied under the same instrumental conditions. Actuation should be conducted in a manner that removes potential operator bias, either by employing automatic actuation or by employing blinded procedures when manual actuation is used, where feasible. The analyst performing the post-actuation evaluations of the collected data should be blinded to the identity of the samples. Method validation should be performed using R product, and the lot number(s) used for the validation should be provided.

The following in vitro bioequivalence tests are recommended:

1. Type of study: Single actuation content (SAC)  
Design: The SAC test should be performed at the beginning (B) and end (E) lifestages of the product.<sup>3</sup> An appropriate apparatus may be used to determine the SAC using a validated assay. The number of actuations per determination should be one.

**Equivalence based on:** The SAC comparison of the T and R products is based on the population bioequivalence (PBE). Refer to the most recent version of the FDA product-specific guidance on *Budesonide Inhalation Suspension* (NDA 020929)<sup>b</sup> for additional information regarding PBE analysis procedures.

2. Type of study: Droplet size distribution by Laser Diffraction  
Design: Droplet size distribution should be determined using laser diffraction or an appropriately validated alternate methodology. Droplet size distribution should be measured for fully developed phase only at B and E lifestages. It is recommended that the studies be performed within a range of 2 to 7 cm from the actuator orifice, with the two distances separated by 3 cm or more.

Additional comments: Single spray droplet size distribution and span should be reported based on volume (mass). Mean  $D_{10}$ ,  $D_{50}$ ,  $D_{90}$  values for a given unit can be computed from the mean of up to three consecutive sprays from that unit at each lifestage. Span can be computed as  $((D_{90} - D_{10})/D_{50})$ . To assess precision, the data of each spray should also be reported.

**Equivalence based on:** PBE analysis of  $D_{50}$  and span at two selected distances.

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<sup>3</sup> Based on the labeled number of actuations, the terms B lifestage, M lifestage, and E lifestage represent the first actuation(s) following the labeled number of priming actuations, the actuation(s) corresponding to 50 percent of the labeled number of actuations, and the actuation(s) corresponding to the labeled number of actuations, respectively.

3. Type of study: Drug in small particles/droplets  
Design: Determination of drug in small particles/droplets is recommended to be performed at the B lifestage of the product using the U.S. Pharmacopeia (USP) <601> Apparatus 1 (flow rate of 28.3 L/min), Apparatus 6 (flow rate of 15 L/min), or another appropriate method using a validated, highly sensitive assay. Drug in small particles/droplets should be determined using fewest numbers of actuations (generally not exceeding 10 actuations) justified by the sensitivity of the assay, to be more reflective of individual doses.

Additional comments: Drug deposition should be reported in mass units. Mass balance should be based on drug deposition on each of the valve stem, actuator, adapters, induction port, any other accessories, the top stage, and all lower stages to the filter. Mass balance accountability should be reported based on the sum of all deposition sites. The total mass of drug collected on all stages and accessories is recommended to be between 85% and 115% of the amount labeled on a per actuation basis.

**Equivalence based on:** PBE modified to be one-sided for mean comparison of drug mass in the small particles/ droplets less than 9.0  $\mu\text{m}$ . See the Appendix for the step-wise procedure for PBE modified to be one-sided for mean comparison analysis.

4. Type of study: Spray pattern  
Design: The spray pattern test should be performed at the B lifestage of the product and at two different distances from the actuator orifice. The selected distances should be at least 3 cm apart and based on the range of 3 to 7 cm from the R actuator mouthpiece. Impaction (thin-layer chromatography plate impaction), non-impaction (laser light sheet and high-speed digital camera), or other suitable method may be used to determine the spray pattern.

Additional comments: Spray pattern should be measured quantitatively in terms of ovality ratio and area within the perimeter (to include a high proportion, e.g., 95%, of the total pattern) of the true shape for the automated analysis, or ovality ratio and  $D_{\text{max}}$  for the manual analysis. Ovality ratio is defined as the ratio of  $D_{\text{max}}$  to  $D_{\text{min}}$ , which are the longest and shortest diameters, respectively, that pass through the center of mass or the center of gravity, as appropriate. The number of sprays per spray pattern should preferably be one.

**Equivalence based on:** At two selected distances, (i) qualitative comparison of spray shape, and (ii) PBE analysis of ovality ratio and area for automated analysis, or ovality ratio and  $D_{\text{max}}$  for manual analysis.

5. Type of study: Plume geometry  
Design: The plume geometry test should be performed at B lifestage of the product. The time sequence sound-triggered flash photography method, laser light sheet technology, and high-speed digital camera, or other suitable method may be used to determine the plume geometry at the appropriate post-actuation delay time.

Additional comments: Plume geometry measurements should be reported at a single delay time while the fully developed plume is still in contact with the actuator tip. Plume geometry should be measured quantitatively in terms of plume angle and width of one side view. The plume angle is based on the conical region of the plume extending from a vertex that occurs at or near the actuator tip. The plume width is measured at a distance equal to the greater of the two distances selected for characterization of the spray pattern.

**Equivalence based on:** Ratio of the geometric mean of the three batches of T to that of the three batches of R (based on log-transformed data) for both plume angle and width, which should fall within 90-111% of plume angle and plume width.

6. Type of study: Priming and repriming

Design: Priming and repriming tests should be based on the emitted dose (ex-actuator) of a single actuation immediately following the specified number of priming or repriming actuations specified in the R product labeling. The repriming test should be performed following storage for the specified period of non-use after initial use and/or other conditions (e.g., dropping), if the R product labeling provides such repriming information.

Additional comments: For BE evaluation, the priming and repriming tests should be based on products stored in the valve-upright position, with the exception of a nasal spray for which the R labeling recommends storage in the valve-down position. The priming data can be based on the SAC data at the B lifestage. Repriming would be similarly established based on a single actuation following the specified number of repriming actuations in the R product labeling.

**Equivalence based on:** PBE analysis of the emitted dose of a single actuation immediately following the specified number of priming or repriming actuations specified in the R product labeling.

7. Type of study: Drug particle size distribution

Design: The emitted drug particle size distribution (PSD) should be determined using an optimized and validated analytical method (e.g., morphologically-directed Raman spectroscopy). The emitted drug PSD should be measured at the B lifestage of the product.

Additional comments: Samples for drug PSD measurement should be prepared to ensure that the drug is in its suspended state post-actuation. The sample preparation method and selected particle sizing methodology should be adequately optimized and validated to demonstrate the adequacy of the selected method in accurately and reliably identifying and measuring the size of the drug particles without any interference from the excipient particles that are also suspended in the formulation. Drug PSD and span should be reported. An orthogonal method may be required if the selected methodology is not sensitive to measure particles beyond a certain size range.

**Equivalence based on:** PBE analysis of  $D_{50}$  and span.

8. Type of study: Dissolution

Design: Dissolution tests are recommended to be performed at the B lifestage of the product. An appropriate apparatus (e.g., USP <711> Apparatus 2, USP <724> Apparatus 5, or Transwell system) may be used to determine dissolution measurements using a sufficiently developed and validated method to support its sensitivity in detecting differences in performance between the T and R products.

Additional comments: Information should be provided to explain the selection of dissolution method parameters such as equipment, product dose amount, media, media volume, stirring/agitation rate, sampling times, etc. The submitted study method information should detail each parameter value and its sensitivity and reproducibility. Dissolution tests should be performed on samples with sufficiently similar drug mass for T and R products. Dissolution measurements should be reported in mass units and as percent drug dissolved.

**Equivalence based on:** Comparative analysis of dissolution profiles should be established using an appropriate statistical method (e.g., model independent approach using similarity factor (f<sub>2</sub>)). For more information on calculation of f<sub>2</sub> factor, refer to the most recent version of the FDA guidance for industry on *Dissolution Testing of Immediate Release Solid Oral Dosage Forms*.<sup>a</sup>

**In vitro bioequivalence data submission recommendations:**

For data summary tables and SAS data tables for the in-vitro data recommended for nasal spray products, see the templates in the FDA's ANDA Forms and Submissions Requirements website (<https://www.fda.gov/drugs/abbreviated-new-drug-application-anda/abbreviated-new-drug-application-anda-forms-and-submission-requirements>), should be used to ensure completeness and consistency of the data. The dissolution data summary table template can be found within Model Bioequivalence Data Summary Tables.

In addition to submission of all raw data, the following supporting documentation for droplet size distribution by Laser Diffraction, spray pattern, plume geometry, drug PSD, and dissolution studies should be provided:

- Documentation includes instrument output reports and photographic or graphic material as applicable. Documents should be clearly labeled to indicate the product (e.g., T or R), batch number, and testing conditions (e.g., distance, lifestage, delay time), as appropriate.
- For droplet size distribution by Laser Diffraction, profiles of droplet size and obscuration or percent transmission over the complete life of the single sprays should be submitted.
- Supporting documentation for droplet size distribution by Laser Diffraction, spray pattern, and plume geometry studies should include representative copies, preferably electronic, of >20% of the total observations.
- For spray pattern and plume geometry studies quantitated by automatic image analysis, representative electronic images rather than paper copies of >20% of the total observations should be submitted, as electronic files are definitive.
- Drug PSD, comprehensive method optimization and validation data should be submitted for the sample preparation method (e.g., number of actuations, sample volume, particle

settling time) and selected particle sizing methodology. If, for example, MDRS is the selected particle sizing method, prospective applicants should submit optimization and validation data, at minimum, for particle imaging settings (e.g., scan area, magnification settings, illumination settings, threshold value, trash settings, number of particles for analysis), morphology filter selection (e.g., aspect ratio, circularity, convexity, elongation, intensity mean, solidity, etc., as applicable), and Raman spectroscopy settings (e.g., exposure time, spectral correlation range, Raman spectral correlation score, as applicable). In addition, comprehensive optimization and validation data for any orthogonal analytical methods used for particle size determination should be submitted.

- For dissolution, a comprehensive method development report should be submitted in the ANDA to show how the dissolution method was optimized, and to support a demonstration that the method parameters selected for the dissolution method are appropriate. The equipment, methodologies, and study conditions used in the dissolution study should be appropriately validated. The dissolution method should be able to demonstrate discriminatory ability (e.g., ability to detect meaningful differences in formulation or manufacturing process, such as a difference in drug particle size) in measuring the dissolution kinetics of the product.

## **II. Option 2: In vitro bioequivalence studies, one in vivo bioequivalence study with pharmacokinetic endpoints and one comparative clinical endpoint bioequivalence study**

If the T product formulation is not Q1 and Q2 the same as the R product formulation and the nasal spray device (e.g., pump and actuator design) of the T product is appropriate for an ANDA, then in vitro bioequivalence studies #1 through #6 (as described in Option 1), and the following in vivo studies are recommended to establish bioequivalence between the T and R products:

### **In vivo bioequivalence study with pharmacokinetic endpoints:**

1. Type of study: Fasting  
Design: Single-dose, two-way crossover  
Strength: 0.05 mg/spray  
Dose: 0.2 mg, administered as two sprays in each nostril  
Subjects: Healthy males and non-pregnant, non-lactating females  
Additional comments: 1) Follow the reference listed drug (RLD) labeling for the method of drug administration; 2) The analytical method should have sufficient sensitivity to adequately quantify the concentration of fluticasone propionate in plasma (assay method with Limit of Quantification (LOQ)  $\leq 1$  pg/mL is suggested).

**Analyte to measure:** Fluticasone propionate in plasma

**Equivalence based on:** AUC and  $C_{max}$  for fluticasone propionate. The 90% confidence intervals for the geometric mean T/R ratios of AUC and  $C_{max}$  should fall within the limits of 80.00% - 125.00%.

### **Comparative clinical endpoint bioequivalence study:**

These recommendations are specific to this product and may not be appropriate for comparative clinical endpoint bioequivalence studies of any other product, including any other dosage form or strength of fluticasone propionate.

1. Type of study: Comparative clinical endpoint bioequivalence study  
Design: Randomized, double-blind, three-arm, placebo-controlled, parallel group  
Strength: 0.05 mg/spray  
Dose: 0.2 mg once-daily, administered as two 0.05 mg sprays in each nostril  
Subjects: Adult males and non-pregnant, non-lactating females with seasonal allergic rhinitis  
Additional comments: Specific recommendations are provided below.

### **Additional comments regarding the comparative clinical endpoint bioequivalence study:**

1. FDA recommends conducting a single comparative clinical endpoint bioequivalence study in the treatment of seasonal allergic rhinitis (SAR) consisting of 2 periods: a 7-day, single-blinded, placebo run-in period (Study Days -7 to -1) to establish a baseline and to identify placebo responders, followed by a 14-day treatment period (Study Days 1 to 14). Prime each product as per the RLD labeling prior to initial dosing. During the placebo run-in period, all subjects are to receive the placebo vehicle administered as two sprays in each nostril once daily for 7 days. All subjects who qualify after the placebo run-in period are to be randomized to receive the T product, R product, or placebo (vehicle) control during the treatment period, administered as two sprays in each nostril once daily for 14 days. The primary endpoint is the difference in the mean change in reflective total nasal symptom scores from baseline through the treatment period.
2. A multi-center study is recommended to avoid potential investigator bias.
3. A double dummy design is not recommended for study blinding due to a concern that the doubled fluid volume may result in washing the drug from its nasal deposition sites, potentially resulting in an altered safety and efficacy profile.
4. Inclusion criteria (the prospective applicant may add additional criteria):
  - a. Males and non-pregnant, non-lactating females, 18 years of age and older. For female subjects of childbearing potential: agreement to practice an approved method of birth control
  - b. History of SAR
  - c. A positive test for relevant specific allergens (e.g., allergen skin test).
  - d. Demonstration of significant symptoms during screening and randomization visits, measured by a reflective total nasal symptom score (rTNSS) of, for example, at least 6 at the time of enrollment (see items 7 and 8)

5. Exclusion criteria (the prospective applicant may add additional criteria):
  - a. Pregnant or lactating or planning to become pregnant during the study period
  - b. Asthma, with the exception of mild intermittent asthma
  - c. Active or quiescent tuberculous infections of the respiratory tract; untreated local or systemic fungal, bacterial, viral, or parasitic infections
  - d. Presence of glaucoma, cataracts, ocular herpes simplex, conjunctivitis, or other eye infection
  - e. Presence of any nasal mucosal erosion, nasal septal ulcers, or septum perforation on focused nasal examination at screening or randomization
  - f. Recent nasal sinus surgery or nasal trauma
  - g. Other nasal disease(s) likely to affect deposition of intranasal medication, such as acute or chronic sinusitis, rhinitis medicamentosa, nasal polyps, or nasal septal abnormalities
  - h. Presence or history of any clinically significant condition that, in the opinion of the investigator, would compromise the safety of the subject or the conduct of the study
  - i. Respiratory tract infection requiring antibiotic within 4 weeks prior to screening.
  - j. Use of any investigational drug within 30 days prior to screening
  - k. Initiation of immunotherapy or its dose escalation for 1 month prior to screening and during the study (it is acceptable if subjects are on a stable regimen for at least 30 days prior to screening and they should maintain the same dose during the study)
  - l. Use of any prohibited medications and treatments (e.g., systemic or intranasal decongestants, anti-allergy therapy as antihistamines, leukotriene antagonists, corticosteroid therapy, and potent cytochrome P450 3A4 inhibitors as ketoconazole) prior to screening [the prospective applicant should provide a list of medications and treatments, with justification/rationale provided for duration of the washout period prior to screening]
  - m. Planned travel outside the study area from the time of enrollment to completion of the study
  - n. Known hypersensitivity to fluticasone propionate, or to similar drug, or to any of the study medications or inactive ingredients
6. The protocol should include a list of the prescription and over-the-counter drug products, procedures, and activities that are prohibited during the study, such as systemic or intranasal decongestants, anti-allergy therapy as antihistamines, leukotriene antagonists, corticosteroid therapy (parenteral, intranasal, oral, inhaled, or potent topical), anti-IgE antibodies (e.g., omalizumab), immunosuppressive therapy, and potent cytochrome P450 3A4 inhibitors as ketoconazole.
7. Subjects should self-score their symptoms twice daily (AM and PM, 12 hours apart at the same times daily) throughout the 7-day placebo run-in period and the 14-day randomized treatment period. Scoring should be made immediately prior to each dose (and 12 hours after the AM dose for once-daily dosing), to reflect the previous 12 hours (*reflective* scores) and how the subject is feeling at the time of evaluation, i.e., at the end of dosing



interval (*instantaneous* scores). Each of the following symptoms should be scored using the following scale:

- a. Symptoms: Runny nose, sneezing, nasal itching, and congestion
- b. Scoring Scale: The following is an example of an acceptable scale. Each score should be objectively defined.

**Table 1: Sample Scoring Scale**

Score	Description
0	absent (no symptom evident)
1	mild (symptom clearly present, but minimal awareness; easily tolerated)
2	moderate (definite awareness of symptom that is bothersome but tolerable)
3	severe (symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping)

8. Total nasal symptom score (TNSS) is the sum of each individual symptom rating for runny nose, sneezing, nasal itching, and congestion.
9. Baseline mean rTNSS is the mean of the final seven scores from the placebo run-in period. The final seven scores from the placebo run-in period consist of the AM and PM scores on Days -3, -2, and -1 and the AM score (prior to drug dosing) on Day 1 of the 14-day randomized treatment period.
10. Placebo responders should be excluded from the study to increase the ability to show a significant difference between active and placebo treatments, and to increase sensitivity to detect potential differences between active products.
11. Treatment mean rTNSS is the average of 27 scores from the randomized treatment period. The 27 scores consist of the PM score on Day 1 and the AM and PM scores on Days 2 to 14.
12. The recommended primary endpoint is the change from the baseline mean rTNSS to the treatment mean rTNSS, expressed in absolute units rather than percent change from baseline.
13. FDA recommends that each of the test and reference batches used in the comparative clinical endpoint bioequivalence study be at least one of the three batches used for the in vitro bioequivalence studies and the in vivo bioequivalence study with pharmacokinetic endpoints.
14. FDA recommends using a statistical model for the endpoint data that takes into account baseline values. If the study was conducted at multiple clinical centers, the center should also be considered in the data analysis.

15. Refer to the most recent version of the FDA product-specific guidance on *Adapalene; Benzoyl Peroxide Topical Gel* (NDA 207917)<sup>b</sup> for a recommended approach to statistical analysis and study design for bioequivalence studies with clinical endpoints.

16. Refer to the study data standards resources, <https://www.fda.gov/industry/fda-resources-data-standards/study-data-standards-resources>.

**Additional information:**

Device:

The RLD is presented in a bottle with a nasal pump and actuator. The pump with actuator is the device constituent part.

FDA recommends that prospective applicants examine the size and shape, the external critical design attributes, and the external operating principles of the reference product when designing the test device including:

- Active, metered, multi-dose format of the reference product
- Number of doses of the reference product

User interface assessment:

An ANDA for this product should include complete comparative analyses so FDA can determine whether any differences in design for the user interface of the proposed generic product, as compared to the RLD, are acceptable and whether the product can be expected to have the same clinical effect and safety profile as the RLD when administered to patients under the conditions specified in the labeling. For additional information, refer to the most recent version of the FDA guidance for industry on *Comparative Analyses and Related Comparative Use Human Factors Studies for a Drug-Device Combination Product Submitted in an ANDA*.<sup>a</sup>

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<sup>a</sup> For the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

<sup>b</sup> For the most recent version of the product-specific guidance, check the FDA product-specific guidance web page at <https://www.accessdata.fda.gov/scripts/cder/psg/index.cfm>.

## Appendix

Method for Statistical Analysis Using Population Bioequivalence (PBE) Modified to be One-Sided with Respect to the Mean Comparison for Drug in Small Particles/Droplets by Cascade Impactor In Vitro Bioequivalence Test for Fluticasone Propionate Nasal Spray

### Step 1. Establish Modified One-Sided PBE Criterion:

Modified One-Sided PBE BE criterion:

$$\frac{(\mu_T - \mu_R)^2 + (\sigma_T^2 - \sigma_R^2)}{\sigma_R^2} = \theta_p; \text{ If } \mu_T \geq \mu_R$$

$$\frac{(\sigma_T^2 - \sigma_R^2)}{\sigma_R^2} = \theta_p; \text{ If } \mu_T < \mu_R$$

where,

$\mu_T - \mu_R$ : Mean difference of T (log scale) and R (log scale) products

$\sigma_T^2, \sigma_R^2$ : Total variance of T and R products

$\sigma_{TO}$ : Regulatory constant ( $\sigma_{TO} = 0.1$ )

$\theta_p$ : Regulatory constant ( $\theta_p = 2.0891$ )

### Step 2: When $\mu_T \geq \mu_R$ , use Traditional PBE Analysis

When  $\mu_T \geq \mu_R$ , proceed 95% upper bound calculation, as described in the most recent version of the FDA product-specific guidance on *Budesonide Inhalation Suspension*<sup>a</sup>.

### Step 3. When $\mu_T < \mu_R$ , follow Step 3A to Step 3E.

#### Step 3A: Estimate the Linearized Criteria:

$$\hat{\eta}_1 = \frac{MSB_T}{m} + \frac{(m-1)MSW_T}{m} - (1 + \theta_p) \frac{MSB_R}{m} - (1 + \theta_p) \frac{(m-1)MSW_R}{m} \quad \text{for } \sigma_R > \sigma_{TO}$$

$$\hat{\eta}_2 = \frac{MSB_T}{m} + \frac{(m-1)MSW_T}{m} - \frac{MSB_R}{m} - \frac{(m-1)MSW_R}{m} - \theta_p \sigma_{TO}^2 \quad \text{for } \sigma_R \leq \sigma_{TO}$$

where,

m: number of life stages

MSW<sub>T</sub>: within-bottle variability for test product

$MSW_R$  : within-bottle variability for reference product

$(MSB_T - MSW_T)/m$  : between-bottle variability for test product

$(MSB_R - MSW_R)/m$  : between-bottle variability for reference product

### Step 3B: Calculate MSB and MSW

Calculation for  $MSW_T$ ,  $MSW_R$ ,  $MSB_T$  and  $MSB_R$  can be conducted as follows.

$$MSB_k = \frac{m \cdot \sum_{j=1}^{\ell_k} \sum_{i=1}^{n_k} (\bar{X}_{ijk} - \bar{X}_{..k})^2}{n_k \cdot \ell_k - 1} \quad \text{k refers to either test or reference product}$$

$$MSW_k = \frac{\sum_{j=1}^{\ell_k} \sum_{i=1}^{n_k} \sum_{s=1}^m (X_{ijks} - \bar{X}_{ijk})^2}{n_k \cdot \ell_k \cdot (m - 1)}$$

$$\bar{X}_{ijk} = \frac{\sum_{s=1}^m X_{ijks}}{m}; \quad \bar{X}_{..k} = \frac{\sum_{i=1}^{\ell_k} \sum_{j=1}^{n_k} \bar{X}_{ijk}}{n_k \cdot \ell_k}$$

$n_T, n_R$  : Number of canisters or bottles per batch, for T and R products

$\ell_T, \ell_R$  : Number of batches of T and R products

$X_{ijks}$  is the  $i^{\text{th}}$  bottle in batch #  $j$  at life stage  $s$  for test or reference product;

$\bar{X}_{ijk}$  is the average  $m$  life stages for  $i^{\text{th}}$  bottle in batch #  $j$ ;

$\bar{X}_{..k}$  is the population mean for the reference or test products.

### Step 3C. Calculate $\sigma_R$

$\sigma_R$  can be conducted as follows:

$$\sigma_R = \sqrt{\frac{MSB_R}{m} + \frac{(m-1)MSW_R}{m}}$$

- a. If  $\sigma_R > \sigma_{TO}$  (regulatory constant, 0.1), using the reference-scaled procedure to determine BE for the measured parameter(s)

- b. If  $\sigma_R \leq \sigma_{T0}$  (regulatory constant, 0.1), using the constant-scaled procedure to determine BE for the measured parameter(s)

**Step 3D. Calculate Linearized Point Estimate and 95% Upper Confidence Bound:**

**1). Reference-scaled Criterion ( $\hat{\eta}_1$ ): Use  $\alpha=0.05$  for a 95% upper confidence bound:**

**Equation for Linearized Point Estimate:**

$$E_q = E1 + E2 + E3s + E4s$$

**95% upper confidence bound ( $H\eta_1$ ):**

$$H\eta_1 = (E1 + E2 + E3s + E4s) + (U1 + U2 + U3s + U4s)^{1/2}$$

Following are the equations to compute each component:

$E_q$ = Point Estimate	$H_q$ = Confidence Bound	$U_q=(H_q- E_q)^2$
$E1 = \frac{MSB_T}{m}$	$H1 = \frac{(\ell_T \cdot n_T - 1) \cdot E1}{\chi_{\ell_T \cdot n_T - 1, \alpha}^2}$	$U1$
$E2 = \frac{(m-1) \cdot MSW_T}{m}$	$H2 = \frac{\ell_T \cdot n_T \cdot (m-1) \cdot E2}{\chi_{\ell_T \cdot n_T \cdot (m-1), \alpha}^2}$	$U2$
$E3s = -(1 + \theta_p) \frac{MSB_R}{m}$	$H3s = \frac{(\ell_R \cdot n_R - 1) \cdot E3s}{\chi_{\ell_R \cdot n_R - 1, 1 - \alpha}^2}$	$U3s$
$E4s = -(1 + \theta_p) \frac{(m-1)MSW_R}{m}$	$H4s = \frac{\ell_R \cdot n_R \cdot (m-1) \cdot E4s}{\chi_{\ell_R \cdot n_R \cdot (m-1), 1 - \alpha}^2}$	$U4s$

Where  $\chi_{\ell_T \cdot n_T - 1, \alpha}^2$  is from the cumulative distribution function of the chi-square distribution with  $\ell_T \cdot n_T - 1$  degrees of freedom, i.e.  $\Pr(\chi_{\ell_T \cdot n_T - 1}^2 \leq \chi_{\ell_T \cdot n_T - 1, \alpha}^2) = \alpha$

For data collected on one life stage ( $m=1$ ), ignore E2 and E4s and their corresponding H and U terms in the calculation. For data collected on more than one stage ( $m \geq 2$ ), use the equations listed above.

**2). Constant-scaled Criterion ( $\hat{\eta}_2$ ): Use  $\alpha=0.05$  for a 95% upper confidence bound:**

**Equation for Linearized Point Estimate:**

$$E_q = E1 + E2 + E3c + E4c - \theta_p \sigma_{T0}^2$$

**95% upper confidence bound (H<sub>η2</sub>):**

$$H_{\eta_2} = (E1 + E2 + E3c + E4c - \theta_p \sigma_{T0}^2) + (U1 + U2 + U3c + U4c)^{1/2}$$

Following are the equations to compute each component:

$E_q$ = Point Estimate	$H_q$ = Confidence Bound	$U_q=(H_q- E_q)^2$
$E1 = \frac{MSB_T}{m}$	$H1 = \frac{(\ell_T \cdot n_T - 1) \cdot E1}{\chi_{\ell_T \cdot n_T - 1, \alpha}^2}$	$U1$
$E2 = \frac{(m-1) \cdot MSW_T}{m}$	$H2 = \frac{\ell_T \cdot n_T \cdot (m-1) \cdot E2}{\chi_{\ell_T \cdot n_T \cdot (m-1), \alpha}^2}$	$U2$
$E3c = -\frac{MSB_R}{m}$	$H3c = \frac{(\ell_R \cdot n_R - 1) \cdot E3c}{\chi_{\ell_R \cdot n_R - 1, 1-\alpha}^2}$	$U3c$
$E4c = -\frac{(m-1)MSW_R}{m}$	$H4c = \frac{\ell_R \cdot n_R \cdot (m-1) \cdot E4c}{\chi_{\ell_R \cdot n_R \cdot (m-1), 1-\alpha}^2}$	$U4c$

For data collected on one life stage (m=1), ignore E2 and E4c and their corresponding H and U terms in the calculation. For data collected on more than one stage (m≥2), use the equations listed above.

The method of obtaining the upper confidence bound is based on two FDA guidance documents: 1) Statistical information from the June 1999 draft guidance and statistical information for in vitro bioequivalence posted on August 18, 1999, accompanying the Guidance for Industry *Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action* (April 2003)<sup>b</sup>; and 2) Guidance for Industry *Statistical Approaches to Establishing Bioequivalence* (January 2001)<sup>b</sup>. The concept is adapted from the method for the two-sequence, four-period using T-distribution.

**Step 3E. For the test product to be bioequivalent to the reference product, the following conditions must be satisfied.** The 95% upper confidence bound for linearized criteria H<sub>η</sub> must be ≤ 0