Contains Nonbinding Recommendations

Draft Guidance on Fluticasone Propionate

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA, or the Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the Office of Generic Drugs.

Active Ingredient: Fluticasone propionate

Dosage Form; Route: Metered, spray; Nasal

Strength: 0.05 mg/spray

Recommended Studies: In vitro and in vivo studies

FDA recommends the following in vitro and in vivo studies to establish bioequivalence (BE) of the test (T) and reference (R) nasal sprays containing fluticasone propionate.

In Vitro Studies

FDA recommends that applicants conduct the following in vitro BE 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 BE. The batches should be prepared from three different batches of the drug substance, different batches of critical excipients, and different batches of the same device components (e.g., pump and actuator).

For comparative in vitro studies, T and R 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) for the R bottles used for the validation should be provided.

The following in vitro studies are recommended to establish in vitro BE of the T and R nasal sprays containing fluticasone propionate:

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.[1] 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 product-specific guidance for *Budesonide Inhalation Suspension* 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 should 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.

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 µm. See the Appendix for the step-wise procedure for PBE modified to be one-sided for mean comparison analysis.

[1] 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.

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_{max} for the manual analysis. Ovality ratio is defined as the ratio of D_{max} to D_{min}. D_{max} and D_{min} 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_{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.

In vitro BE data submission recommendations

- For data summary tables and SAS data tables for the in-vitro data recommended for nasal spray products, the templates in the FDA website *"Abbreviated New Drug Application (ANDA) Forms and Submissions Requirements"* should be used, to ensure completeness and consistency of the data.

- In addition to submission of all raw data, the following supporting documentation for Droplet Size Distribution by Laser Diffraction, Spray Pattern, and Plume Geometry 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 should include representative copies, preferably electronic, of >20 percent of the total observations.

  - For Spray Pattern and Plume Geometry 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. For automated image analysis of Spray Pattern and Plume Geometry, in addition to the electronic images, we recommend paper copies of a few screen images be submitted as reference samples.

Pharmacokinetic (PK) BE Study

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, general population
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) ≤ 1 pg/mL is suggested).

Analyte to measure (in appropriate biological fluid): 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 baseline-corrected AUC and C_{max} should fall within the limits of 80.00-125.00%.

Comparative Clinical Endpoint BE Study

The following BE study with a clinical endpoint is recommended.

The recommendations provided here supersede information provided in the draft guidance for industry *Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action* (April 2003). These recommendations are specific to this product and may not be appropriate for comparative clinical endpoint BE studies of any other product, including any other dosage form or strength of fluticasone propionate.

Type of study: BE Study with Clinical Endpoint
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: 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 BE study

1. The Office of Generic Drugs (OGD) recommends conducting a BE study with clinical endpoint in the treatment of seasonal allergic rhinitis 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 test product, RLD, 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 sponsor 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 seasonal allergic rhinitis (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 sponsor 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. 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 sponsor should provide a list of medications and treatments, with justification/rationale provided for duration of the washout period prior to screening].
    l. Planned travel outside the study area from the time of enrollment to completion of the study.
    m. 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 7 scores from the placebo run-in period. The final 7 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. The OGD recommends that each of the test and reference batches used in the clinical endpoint BE study be at least one of the three batches used for the in vitro and in vivo PK BE studies.

14. We recommend 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 product-specific guidance on Adapalene; Benzoyl Peroxide Topical Gel 0.3%; 2.5% for a recommended approach to statistical analysis and study design for bioequivalence studies with clinical endpoints.[2]

16. Study data should be submitted in a standardized format. Please refer to the study data standards published at www.fda.gov.[3]

Alternate approach to the comparative clinical endpoint BE study

A comparative clinical endpoint BE study is recommended for T fluticasone propionate nasal spray product because of an inability to adequately characterize drug particle size distribution (PSD) in aerosols and sprays using commonly used analytical methods. Drug PSD 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 PSD in the T and R products can be accurately measured using a validated analytical method such as morphology-directed Raman spectroscopy or any other advanced methodology, sponsors may submit comparative particle size distribution data as part of their drug characterization within their ANDA application. In such case, comprehensive method validation data should be submitted to demonstrate the adequacy of the selected method in identifying and measuring the size of the drug particles without any interference from the excipient particles that are also suspended in the formulation. An orthogonal method may be required if the selected methodology is not sensitive to measure particles beyond a certain size range. Equivalence between T and R drug PSD should be based on PBE analysis on D_{50} and span.

Additional Information

Number of Reserve Samples:

Please refer to 21 CFR 320.38, 320.63 and the Guidance for Industry, "Handling and Retention of BA and BE Testing Samples", regarding retention of study drug samples and 21 CFR 320.36 for requirements for maintenance of records of bioequivalence testing. In addition, the

[2] Product-Specific Guidances for Generic Drug Development available at: https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm075207.htm

[3] Study Data Standards for Submission to CDER and CBER available at: https://www.fda.gov/ForIndustry/DataStandards/StudyDataStandards/ucm587508.htm

investigators should follow the procedures of 21 CFR 58 and ICH E6, "Good Clinical Practice: Consolidated Guideline", for retention of study records and data in order to conduct their studies in compliance with Good Laboratory Practices (GLP) and Good Clinical Practices (GCP). Retention samples should be randomly selected from the drug supplies received for each shipment prior to dispensing to subjects. Retention samples should not be returned to the Applicant at any time.

Formulation:

FDA recommends that the T formulation be qualitatively (Q1)[4] and quantitatively (Q2)[5] the same as the R formulation.

Device:

Sponsors should refer to the FDA guidance for industry entitled, *Comparative Analyses and Related Comparative Use Human Factors Studies for a Drug-Device Combination Product Submitted in an ANDA*, which, when finalized, will provide the Agency's current thinking on the identification and assessment of any differences in the design of the user interface for a proposed generic drug-device combination product when compared to its RLD.

FDA recommends that applicants consider the following characteristics of the R product in designing the T product:

- External operating principles and external critical design attributes of the R product
- Size and shape of the R product
- Number of doses in the R product

In addition, in vitro studies should be conducted to support the functionality, accuracy, and robustness of the proposed T product.[6]

[4] Q1 (qualitative sameness) means that the T formulation uses the same inactive ingredient(s) as the R formulation.

[5] Q2 (quantitative sameness) means that concentrations of the inactive ingredient(s) used in the T formulation are within ± 5% of those used in the R formulation.

[6] Refer to the FDA *Guidance for Industry Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products – Chemistry, Manufacturing, and Controls Documentation* for relevant principles regarding studies to support nasal spray devices.

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:

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

$$\frac{\left(\sigma_T{}^2-\sigma_R{}^2\right)}{\sigma_R{}^2}=\theta p \text{ ; If } \mu_T < \mu_R$$

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

σ_{TO} : Regulatory constant (σ_{TO}= 0.1)

$\theta_{p:}$ Regulatory constant (θ_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 FDA product-specific guidance on *Budesonide Inhalation Suspension.*

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

Step 3A: Estimate the Linearized Criteria:

$$\hat{\eta}_1=\frac{MSB_T}{m}+\frac{(m-1)MSW_T}{m}-(1+\theta_p)\frac{MSB_R}{m}-(1+\theta_p)\frac{(m-1)MSW_R}{m} \quad \textbf{for } \sigma_R > \sigma_{T0}$$

$$\hat{\eta}_2=\frac{MSB_T}{m}+\frac{(m-1)MSW_T}{m}-\frac{MSB_R}{m}-\frac{(m-1)MSW_R}{m}-\theta_p\sigma_{T0}{}^2 \quad \textbf{for } \sigma_R \leq \sigma_{T0}$$

Where,

m: number of life stages

MSW_T : 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.

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

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

$$\overline{\boldsymbol{X}}_{\boldsymbol{ijk\cdot}} = \frac{\sum_{\boldsymbol{s}=1}^{\boldsymbol{m}} \boldsymbol{X}_{\boldsymbol{ijks}}}{\boldsymbol{m}}; \quad \overline{\boldsymbol{X}}_{\boldsymbol{\cdot\cdot k\cdot}} = \frac{\sum_{\boldsymbol{i}=1}^{\ell_k} \sum_{\boldsymbol{j}=1}^{\boldsymbol{n_k}} \overline{\boldsymbol{X}}_{\boldsymbol{ijk\cdot}}}{\boldsymbol{n_k} \cdot \ell_{\boldsymbol{k}}}$$

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

ℓ_T, ℓ_R : Number of batches of T and R products

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

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

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

Step 3C. Calculate σ_R

σ_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 α=0.05 for a 95% upper confidence bound:

Equation for Linearized Point Estimate:

Eq = E1+ E2+ E3s+ E4s

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

$H\eta_1$ = (E1+ E2+ E3s+ E4s) + (U1+ U2+U3s+U4s)^{½}

Following are the equations to compute each component:

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

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

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≥2), use the equations listed above.

2). Constant-scaled Criterion ($\hat{\eta}_2$): Use α=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 ($H\eta_2$):

$H\eta_2 = (E1 + E2 + E3c + E4c - \theta_p\sigma_{T0}^2) + (U1 + U2 + U3c + U4c)^{½}$

Following are the equations to compute each component:

E_q= Point Estimate	H_q= Confidence Bound	$U_q=(H_q - E_q)^2$
$\boldsymbol{E}1 = \frac{\boldsymbol{MSB_T}}{\boldsymbol{m}}$	$\boldsymbol{H}1 = \frac{(\ell_{\boldsymbol{T}} \cdot \boldsymbol{n_T} - 1) \cdot \boldsymbol{E}1}{\boldsymbol{\chi}^2_{\ell_{\boldsymbol{T}} \cdot \boldsymbol{n_T} - 1, \boldsymbol{\alpha}}}$	$U1$
$\boldsymbol{E}2 = \frac{(\boldsymbol{m} - 1) \cdot \boldsymbol{MSW_T}}{\boldsymbol{m}}$	$\boldsymbol{H}2 = \frac{\ell_{\boldsymbol{T}} \cdot \boldsymbol{n_T} \cdot (\boldsymbol{m} - 1) \cdot \boldsymbol{E}2}{\boldsymbol{\chi}^2_{\ell_{\boldsymbol{T}} \cdot \boldsymbol{n_T} \cdot (\boldsymbol{m} - 1), \boldsymbol{\alpha}}}$	$U2$
$E3c = -\frac{MSB_R}{m}$	$\boldsymbol{H}3\boldsymbol{c} = \frac{(\ell_{\boldsymbol{R}} \cdot \boldsymbol{n_R} - 1) \cdot \boldsymbol{E}3\boldsymbol{c}}{\boldsymbol{\chi}^2_{\ell_{\boldsymbol{R}} \cdot \boldsymbol{n_R} - 1, 1 - \boldsymbol{\alpha}}}$	$\boldsymbol{U}3\boldsymbol{c}$
$\boldsymbol{E}4\boldsymbol{c} = -\frac{(\boldsymbol{m} - 1)\boldsymbol{MSW_R}}{\boldsymbol{m}}$	$H4c = \frac{\ell_R \cdot n_R \cdot (m-1) \cdot E4rc}{\chi^2_{\ell_R \cdot n_R \cdot (m-1), 1-\alpha}}$	$\boldsymbol{U}4\boldsymbol{c}$

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, companying the Guidance for Industry *Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action* (April 2003); and 2) Guidance for Industry *Statistical Approaches to Establishing Bioequivalence* (January 2001). 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 Hη must be ≤ 0