Protocol No: v20170305

Title of Study:
Evaluation of the sensitivity of pharmacokinetics to differences in the aerodynamic particle size distribution of three different formulations of fluticasone propionate dry powder inhalers

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1. Synopsis

Objectives

The aim of the study is to determine if pharmacokinetics (**PK**) is sensitive to differences in the aerodynamic particle size distribution of three different formulations of fluticasone propionate (**FP**) dry powder inhalers (**DPI**).

Methodology (Design)

This is a single center, double-blind, single-dose (500 mcg), randomized, four period, four sequence cross over PK study to evaluate the local (lung) bioequivalence **(BE)** of 100 mcg fluticasone propionate formulation 1(**FPF I**), 100 mcg fluticasone propionate formulation 2(**FPF II**) and 100 mcg fluticasone propionate formulation 3 (**FPF III**). FPF III will be replicated in the study to provide an assessment of bioequivalence when FPF III is tested against itself.

During the screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. The informed consent will be reviewed with the subject by a member of the study team and the subject will be encouraged to ask questions to ensure the subject has a good understanding of the study. If the subject is eligible and agrees to participate, the subject will be asked to sign the informed consent form prior to any study specific procedures. After their consent, demographic data, medical history and concomitant medications will be recorded. A physical examination will be performed after the vital sign measurements are obtained. A pregnancy test for female subjects must be negative (test repeated if the subject continues in the study for more than 30 days). Spirometry testing and inhalation training will be performed to ensure the suitability of subjects. Laboratory tests such as Complete Blood Count with differential, urinalysis and basic metabolic panel will be performed. Screening tests will be performed within 28 days of study visit 1 and no later than 2 days before study visit 1. All screening results will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm the eligibility of the subjects.

A minimum period of 5 days should lapse between the subsequent study visits. At each study visit, review of the eligibility criteria, documentation of any changes in medical history including concomitant medications, vital signs, inhalation training, insertion of the indwelling catheter into the forearm region of the subject for blood collection, administration of the formulation by inhalation, blood sampling as per protocol will be performed. The total duration of the study is estimated to be approximately 10 weeks.

Number of Subjects

The number of subjects required was calculated to be 24**,** which takes into account a 10% dropout rate to ensure 22 subjects complete the study. Subjects will include healthy males and females aged 18 and 50 years who meet the inclusion and exclusion criteria.

Study Medication, Dose and Mode of Administration

On each study visit the subject will receive 5 inhalations from one of the following 3 devices:

Study Drug	Device*	Formulation
1	Plastiape Monodose DPI	Fluticasone Propionate Formulation 1 (FPF I)
2	Plastiape Monodose DPI	Fluticasone Propionate Formulation 2 (FPF II)
3	Plastiape Monodose DPI	Fluticasone Propionate Formulation 3 (FPF III)

* Dry Powder Inhaler.

Inclusion Criteria

1. Healthy male or female subjects aged 18 to 50 years (inclusive).

2. Females will be eligible only if they are currently non-lactating and demonstrate a negative urine pregnancy test. Female subjects must be willing to use highly effective methods of contraception throughout the study. A highly effective method of birth control is defined as one which results in a low failure rate (i.e. less than 1% per year) when used consistently and correctly e.g. no sexual intercourse, an intrauterine device (IUD), using contraceptive foam **AND** a condom (double-barrier).

3. Body weight ranging from 50 to 100 kg, corresponding to a BMI of 18-29 kg/m^2.

4. Non-smoker for at least 12 months prior to study screening and a maximum smoking history of less than ten-pack years (i.e. the equivalent of one-pack per day for ten years).

5. Healthy and free of significant abnormal findings as determined by medical history, physical examination, vital signs, laboratory tests (including serum cortisol at screening), complete blood count (CBC) with differential, urinalysis and basic metabolic panel.

6. Ability to read, comprehend and sign the consent form.

7. Ability and willingness to comply with all study procedures, discontinue and/or withhold medications as specified in the protocol, and attend scheduled study visits.

8. No history of respiratory disease.

9. Normal baseline spirometry as predicted for age, sex and height, including forced expiratory volume in 1 second / forced vital capacity (FEV1/FVC) $\geq$ 0.8.

10. Healthy and without any pre-existing medical conditions.

Exclusion Criteria

1. Any history and/or conditions that might interfere with drug absorption, distribution, metabolism or excretion of FP, e.g., pre-existing lung and liver disease.
2. Known or suspected sensitivity to Flonase (Fluticasone Propionate), Veramyst (Fluticasone Furoate), or related compounds in that class.
3. Hypersensitivity to milk proteins or lactose (inactive ingredients in the formulation).
4. Having a history and/or currently having the medical condition in the opinion of medically accountable investigator and hence taking any medication for the following (including but not limited to):
   - **4.1** Significant cardiac, dermatologic, gastrointestinal, hepatic, renal, hematological, neurological and psychiatric disease (determined by physical exam, CBC with differential, urinalysis, basic metabolic panel and medical history).
   - **4.2** Presence of glaucoma, cataracts, ocular herpes simplex or carcinoma (other than basal cell).
   - **4.3** Presence of tuberculosis and other respiratory diseases (including but not limited to intermittent or persistent asthma, emphysema and chronic bronchitis); or respiratory infection, common cold, sinusitis or ear infections.
5. Current use of hormone replacement therapy (HRT), hormonal contraceptives and/or corticosteroid treatment within the last 2 months.
6. Smoker during the last 1 year prior to study screening (self-report).
7. Evidence of a positive pregnancy urine test for female volunteers or females who are pregnant or breast-feeding or are likely to become pregnant during the trial. Women of child-bearing potential may be included in the study if, in the opinion of the investigator, they are taking adequate contraceptive precautions as described above.
8. Exposure to any investigational drug within 30 days of enrolment.
9. Subjects who are unable to demonstrate proper inhalation of the test products.
10. Subjects who have a history of anemia.
11. Exposure to any medication that alters CYP3A4 activity within last 2 weeks (e.g.: azole antifungals, rifampin).
12. Nausea, vomiting or diarrhoea within 7 days of dosing.
13. Subjects who have donated 1 pint (450 mL) of blood or more within the previous 8 weeks prior to study administration.
14. Any history or current drug or alcohol abuse, which would interfere with the subject's completion of the study and with adherence to the protocol.
15. A subject will not be eligible for this study if he/she is an immediate family member of the participating investigator, sub-investigator, study coordinator, or employee of the participating investigator.
16. The subject is the student of the Principal Investigator (PI).
17. Lack of willingness to have personal study related data collected, archived and transmitted according to the protocol.

Pharmacokinetic Evaluation

Blood samples will be drawn by inserting an indwelling catheter into the subject's median antecubital vein in the forearm region. Blood samples will be taken within 30 minutes prior to the dosing of the product (pre-dose sample) and at approximately 5, 10, 15, 20, 30, 45, 60 minutes, 1.5, 2, 3, 4, 6, 8, 10, 12, 14 and 24 hours post dosing (approximately means within ±2 min of the scheduled time during the first hour, within ±5 min of the scheduled time for the 1.5 to 14 h samples and within ±1 hour for the 24 h sample). The actual sampling time will be recorded for all samples. At each time point 0.24 ounces (7.5 mL) of blood sample will be collected, of which 0.033 ounces (1 mL) will be discarded in waste tubes and the remaining 0.21 ounces (6.5 mL) will be collected in a EDTA-pretreated blood collection tube (vacutainer) for plasma preparation and storage. A HPLC-MS-MS method capable of quantifying FP in human plasma at levels down to 1.5 picogram /mL (or better) will be used. Bioanalysis will be performed at Worldwide Clinical Trials (Austin, TX, USA).

Safety Evaluation

Safety parameters will include vital signs (temperature, heart rate, respiratory rate and blood pressure), physical examinations, and routine clinical laboratory tests.

Statistical Methods

Non-Compartmental Analysis will be performed in WinNonlin using an inbuilt extra-vascular PK model. Standard PK metrics such as $AUC_{(0\text{-}30min)}$, $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}inf)}$, C_{max}, t_{max}, terminal slope/half-life, CL/F, Vdss/F, $AUMC_{(0\text{-}t)}$, $AUMC_{(0\text{-}inf)}$, and MRT will be calculated for each individual plasma-concentration-time profile. ANOVA model will be used to construct univariate 90% confidence intervals for $AUC_{(0\text{-}t)}$ and C_{max} ratios and applied to conventional 0.8 – 1.25 average BE limits. If needed, alternative BE evaluation methodology and or BE criteria may be investigated. Data will also be assessed by ANOVA methods (SAS 9.2 or another equally suitable software package) to test for statistical differences ($p<0.05$).

Schedules[+]

Activity	Screening Visit	Study Visit*	
Visit Window (Days)	2-28 Days prior study visit	Day 1	Day 2
Subject Information	X		
Informed Consent	X		
Review Inclusion/Exclusion Criteria	X		
Demographic Data	X		
Medical History	X	X	
Concomitant Medications	X	X	X
Measure BMI	X		
Vital Signs	X	X	
Physical Examination	X		
Lung Function Measurements (FEV1)	X		
Pregnancy Test**	X	X	
Laboratory Assessments	X		
ECG	X		
Adverse Events	X	X	X
Proper Inhalation Technique	X	X	
Inhalation of Study Formulation		X	
Blood Sampling		X	X

* At least 5 days (maximum 3 weeks) of washout between each study visit.

+ A more detailed chart is given in Appendix A.

** To be repeated if subject's participation exceeds 30 days.

2. Glossary

List of Abbreviations and Definitions of Terms

ANOVA	Analysis of Variance
APSD	Aerodynamic Particle Size Distribution
$AUC_{(0\text{-}t)}$	Area under the concentration of analyte *vs.* time curve (AUC) from time zero to the last quantifiable concentration
$AUC_{(0\text{-}inf)}$	AUC from time zero extrapolated to infinity
$AUMC_{(0\text{-}t)}$	Area under the moment curve from time zero to the last observation
$AUMC_{(0\text{-}inf)}$	Area under the moment curve when the concentration time curve is extrapolated to infinity
BE	Bioequivalence
BMI	Body Mass Index
β-hCG	Serum beta-human chorionic gonadotropin
C_{max}	The peak or maximum concentration
CL/F	Ration of the total body clearance and the fraction of dose absorbed (F)
DPI	Dry Powder Inhaler
ECG	Electrocardiogram
ED	Emitted Dose
FEV1	Forced Expiratory Volume in 1 second
FDA	Food and Drug Administration
FP	Fluticasone Propionate
FPF I	100 mcg Fluticasone Propionate Formulation 1
FPF II	100 mcg Fluticasone Propionate Formulation 2
FPF III	100 mcg Fluticasone Propionate Formulation 3
FPF III*	100 mcg Fluticasone Propionate Formulation 3 replicate
GLP	Good Laboratory Practices
GSD	Geometric Standard Deviation
ISM	Impactor-Sized Mass
IUD	Intrauterine Device
Lamda Z	First order rate constant associated with the terminal (log-linear) elimination phase
LC-MS/MS	Liquid Chromatography – Mass Spectroscopy/Mass Spectroscopy
MMAD	Mass Median Aerodynamic Diameter
MRT	Mean Residence Time, is the amount of time a particle remains in a system , calculated as AUMC/AUC
NCA	Non Compartmental Analysis
t_{max}	The time of peak concentration
VZ/F	Volume of distribution based on the terminal phase corrected with F

***** The asterisk is used to distinguish the replicate from the original formulation.

3. Study Design

The study comprises of 5 visits: 1 screening visit and 4 study visits.

On each study visit day, subjects will get 5 inhalations from one of the following formulations:

Study Drug	Device	Formulation
1	Plastiape Monodose DPI	Fluticasone Propionate Formulation 1 (FPF I)
2	Plastiape Monodose DPI	Fluticasone Propionate Formulation 2 (FPF II)
3	Plastiape Monodose DPI	Fluticasone Propionate Formulation 3 (FPF III)

The following sequences will be used in the study:

Sequence	Study Visit 1	Study Visit 2	Study Visit 3	Study Visit 4
1	FPF I	FPF II	FPF III	FPF III*
2	FPF II	FPF III*	FPF I	FPF III
3	FPF III	FPF I	FPF III*	FPF II
4	FPF III*	FPF III	FPF II	FPF I

Note that in this design every study drug appears once in each period. Also every study drug is immediately followed only once by each of the other study drugs.

4. Risk/Benefit Considerations

Benefits: Participation in a human pharmacokinetic study like the present one cannot be of benefit to healthy volunteers. Nevertheless, the information form the physical examination, vital signs, ECG and the breathing tests will be shared with the personal physician if the subject chooses.

Risks: The subjects are exposed to risks associated with the pharmacological properties of the investigational product and the study procedures. One single-dose of 500 mcg of fluticasone propionate rarely has side effects. Bad reactions can happen, but are very rare. Some of the bad reactions we know about happen in people using the drug for a long time and with larger doses (e.g., 500 mcg of fluticasone propionate twice daily for 28 weeks, as per labeling information of the FDA-approved **ADVAIR DISKUS**). These reactions are pharyngitis (sore throat), nasal congestion, allergic rhinitis (runny nose), oral candidiasis (yeast infections in the mouth), upper respiratory infection, nausea, vomiting and headache.

Risk/Benefit Ratio: There is no direct benefit to the healthy volunteer in participating of the study. The subjects' contribution to the study is of major importance to agencies like the U.S. Food and

Drug Administration (FDA) for helping them better evaluate the generic alternatives and thus make available cheaper and effective formulations for asthmatic subjects.

Women of Childbearing Age: This drug will not be given to anyone who is pregnant. All women must take a pregnancy test before getting any study drug on this study. All woman of child bearing potential enrolled on this study must use effective birth control during the study. These include: no sexual intercourse, an IUD, using contraceptive foam **AND** a condom (double-barrier). You must notify the doctor if you become pregnant during the course of the study.

Intravenous (IV) Catheter Insertion: During the insertion of the IV catheter, soreness or bruising at the insertion site can occur but is unlikely. Infection at the IV site is possible but unlikely. Dizziness and lightheadedness can occur during insertion of IV catheter or during the blood draws.

4.1 Adverse Events (AEs)

An adverse reaction is defined as a response to a drug that is unintended and which occurs at doses normally used in man for prophylaxis, diagnosis or therapy of a disease. Since it is a single-dose study adverse reactions are rare but the following adverse reactions can occur with the use of fluticasone propionate – pharyngitis (sore throat), nasal congestion, allergic rhinitis (runny nose), oral candidiasis (yeast infections in the mouth) and upper respiratory infection.

The drug is contraindicated when there is severe hypersensitivity to milk proteins (exclusion criteria) and as a primary treatment of status asthmatics. The latter is not an issue since healthy subjects are enrolled in the study.

A very serious allergic reaction to this drug is rare. However if any symptoms of a serious allergic reaction, including: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing are observed, medical help will be immediately provided.

The physicians associated with this study are: Mutasim Abu-Hasan, M.D., and Brandon Seay, M.D., and they will review all AEs monthly and SAEs as it occurs, for its severity and causality assessment, using Common Terminology Criteria for Adverse Events Version 5 (CTCAE V5).

4.2 Serious Adverse Events (SAEs)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the principal investigator or sponsor (FDA), it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

All serious and unexpected adverse events will be reported to UF IRB-01within 5 working days using the *"Adverse Event Reporting Form"* (available at http://irb.ufl.edu/irb01/forms/htm)

All serious but expected adverse events will be reported UF IRB-01 at Continuing Review on the *"Cumulative Adverse Event Summary Table"* (available at: http://irb.ufl.edu/irb01/forms.htm)

4.3 Adverse Event Reporting to RIHSC

The Principal Investigator must report to the FDA Sponsor and the RIHSC (FDA IRB) any unanticipated problems involving risk to human subjects or others 45 CFR 46.103(b)(5)(i). Anticipated adverse events may be reported to the RIHSC in the annual report for continuing review. However, certain adverse events must be reported to the RIHSC within 10 working days after identifiying the event. The RIHSC defines these adverse events as including, but not limited to, the following:

• An adverse event that is not expected, i.e., not listed in the informed consent document;

• An expected adverse event that occurs at a greater frequency or duration than expected;

• Any adverse event that would require modification of the protocol and/or informed consent document.

When reporting any adverse event to the RIHSC, the Principal Investigator should address the significance of the adverse event including, whether it was related to the study and whether there is a need to communicate information to the subjects. The Principal Investigator should discuss whether a change in description of risk is warranted in the protocol and consent form is needed, and whether a change in the study design is warranted.

Selection of Study Population

Asthma subjects typically experience a higher degree of central deposition of the inhaled drug than healthy subjects. Because of the skewed central/peripheral ratio towards a more central deposition, asthmatics are a less sensitive study population; therefore healthy volunteers will be used as they will be more sensitive to detect potential differences in the deposition profile between the three formulations. Healthy volunteers also represent a homogeneous study population for investigations of bioequivalence, owing to a lesser scope for variability as compared to subjects with asthma. Higher variability leads to lesser sensitivity in detecting differences.

[Please see synopsis (above) for details on the inclusion and exclusion criteria.]

5. Study Procedures and Scheduling

The study is comprised of 5 visits in total – a screening visit and 4 study visits. At least 5 days (washout period, maximum 3 weeks) should lapse between the study visits. A phone call within 7 days after the last study visit will be performed for follow-up end of the study.

5.1 Screening Visit

During the screening visit (please see Appendix A for location), the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. The informed consent will be reviewed with the subject by a member of the study team and the subject will be encouraged to ask questions to ensure the subject has a good understanding of the study. If the subject is eligible

and agrees to participate, the subject will be asked to sign the informed consent form prior to any study specific procedures including randomization. After the subject signs the informed consent, the subject will be interviewed and demographic data, medical history and concomitant medications will be collected and recorded. A physical examination will be performed after the vital sign measurements are obtained. A pregnancy test for female subjects will be obtained. Spirometry testing and inhalation training will be performed by a qualified study clinician/investigator to ensure the suitability of subjects. Laboratory tests including a CBC with differential, urinalysis and basic metabolic panel will be collected via venipuncture and processed in the lab. Screening tests will be performed within 28 days of study visit 1 and no later than 2 days before study visit 1. All screening results will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm the eligibility of the subjects. Subject ID will be assigned sequentially during the screening visit.

Inhalation Training: Inhalation training will be performed by a qualified study team member at the screening visit and at each study visit. The training will be accomplished by instructions and subsequent inhalation via Vitalograph AIM™ inhalation trainer. The instructions on how to use the device correctly are similar to the Foradil Aerolizer DPI product information label. (http://www.rxlist.com/foradil-drug/medication-guide.htm)

5.2 Study Visits 1, 2, 3 and 4

Eligible subjects will be asked to return for study visit 1 (see Appendix A for location). A minimum period of 5 days should lapse between the subsequent study visits. Each study visit is scheduled for 28 hours over two days. The study will be conducted at the UF CRC (Clinical Research Center). It is an outpatient study and the subject will be asked to come back the following day for the 24 hour blood sample. The subject will be asked to stay in an outpatient room during the study visit. The same activities are carried out at the other study visits.

At each study visit, changes in medical history including concomitant medications will be documented. Vital signs will be obtained. Inhalation training will be provided to the subjects as mentioned in the section above. An IV catheter will be inserted in a vein located in the forearm region of the subject. The IV catheter is used to avoid multiple pricks while collecting blood samples. The IV catheter is not used for the administration of the drug. The subject inhales 5 times from a given inhaler during each study visit. Each inhaler will be used only once and by only one subject to ensure the subject's safety from infectious agents. The schedule below summarizes the procedures performed at the screening visit and a single study visit.

Activity	Screening Visit	Study Visit	
Visit Window (Days)	2-28 Days prior study visit	Day 1	Day 2
Subject Information	X		
Informed Consent	X		
Review Inclusion/Exclusion Criteria	X		
Demographic Data	X		
Medical History	X	X	
Concomitant Medications	X	X	X
Measure BMI	X		
Vital Signs	X	X	
Physical Examination	X		
Lung Function Measurements (FEV1)	X		
Pregnancy Test	X	X	
Laboratory Assessments	X		
ECG	X		
Adverse Events	X	X	X
Proper Inhalation Technique	X	X	
Inhalation of Study Formulation		X	
Blood Sampling for PK		X	X

5.3 Washout Period

The washout period between study drug administrations will be at least 5 days (maximum 3 weeks).

6. Blood Sample Collection

Blood samples will be drawn by inserting an indwelling catheter into the subject's median cubital vein in the forearm region. Blood samples will be taken within 30 minutes prior to the dosing of the product (pre-dose sample) and at approximately 5, 10, 15, 20, 30, 45, 60 minutes, 1.5, 2, 3, 4, 6, 8, 10, 12, 14 and 24 hours post dosing (approximately means within ±2 min of the scheduled time during the first hour, within ±5 min of the scheduled time for the 1.5 to 14 h samples and within ±1 hour for the 24 h sample). The actual sampling time will be recorded for all samples. At each time point 0.24 ounces (7.5 mL) of blood sample will be collected of which the first 0.033 ounces (1 mL) will be discarded and the remaining 0.21 ounces (6.5 mL) will be stored in a blood collection tube for plasma preparation and storage. The volume of blood to be collected at the screening visit is 0.25 ounces (7.5 mL), and that at each study visit (from 15 min pre-dose to 24 hours post-dose) is 4.56 ounces (135 mL). The total volume of blood to be collected in the study is 18.51 ounces (547.5 mL).

6.1 Labeling

The blood collection tubes and the cryovials containing whole blood and plasma samples respectively will be labeled with subject number, date and time of sampling.

6.2 Bioanalytical Method

A HPLC-MS-MS method capable of quantifying FP in human plasma at levels down to 1.5 picogram /mL (or better) will be used. Bioanalysis will be performed at Worldwide Clinical Trials (Austin, TX).

7. Statistical Methods and Analysis of Data

7.1 Sample Size Calculation

Using in-house and literature PK data for fluticasone propionate, the variability in the PK parameters C_{max} and t_{max} were estimated to be between 20% to 40% (%CV).[24,25,26] If the %CV is 30% and the products differ by only 5%, a sample size of 40 (no dropouts) was initially thought to be appropriated to have 80% power to show that the products are equivalent.[27]

$$N = 2\left(t_{\alpha,2N-2} + t_{\beta,2N-2}\right)^2 * [CV/(V-\delta)]\text{^}2$$

N = total number of subjects required to be in the study

t = the appropriate value from the t distribution

α = the significant level

1-β = the power, usually 80%

CV = the coefficient of variation

V = the bioequivalence limit (ln 1.25 = 0.223)

δ = the difference between the products (for 5% difference, delta equals (ln(1.05) = 0.0488)

However, due to budgetary constraints, the sample size was re-assessed and it was determined that the minimum number of subjects necessary to complete the study is approximately **22** subjects (considering 10% dropout rate, 24 subjects will be recruited). This sample size number does not affect the study design, which is kept as a four-way crossover (i.e., no study drug was removed from the study), and is sufficient to be utilized to achieve 80% power to show that the products are equivalent, as shown in the table below.

Case	Test / Reference Ratio (point estimator)	Intra-Individual Variability (ANOVA-CV)	Power	Sample Size (without dropouts)	Dropout Rate	Final Sample Size
1	0.95	30%	80%	40	~10%	**44** (previous sample size)
2	**0.95**	**21%**	**80%**	**22**	**~10%**	**24 subjects**
3	**1.00**	**24%**	**80%**	**22**	**~10%**	**24 subjects**
4	**1.05**	**22%**	**80%**	**22**	**~10%**	**24 subjects**

Safety Population: All subjects randomized who received at least one dose of study drugs.

Study Population to be Analyzed: Analyses and summaries of pharmacokinetic data will be based on subjects who are administered all doses, complete at least 75% of blood sampling and follow the protocol procedures.

Data Analysis: Once the bioanalytical data is available, the pharmacokinetic data analysis will be conducted under blinded conditions. Afterwards, formulation-based analysis will be conducted under unblinded conditions.

7.2 Non-Compartmental Analysis (NCA)

Hypothesis of the proposed research is that the three test formulations (differing in the c/p ratio) will differ in AUC and Cmax estimates. Three multiple comparisons will be performed i.e. Reference against the reference, reference against test 1 and reference against test 2. A multiplicity adjustment (e.g. bonferroni type) will be made for the 3 multiple comparisons. No additional adjustment will be made for the use of multiple metrics. NCA will be performed in WinNonlin using an inbuilt extra-vascular PK model. Standard PK metrics such as $AUC_{(0\text{-}30min)}$, $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}inf)}$, C_{max}, t_{max}, terminal slope/half-life, CL/F, Vdss/F, $AUMC_{(0\text{-}t)}$, $AUMC_{(0\text{-}inf)}$, and MRT will be calculated for each individual plasma-concentration-time profile. ANOVA model will be used to construct univariate 90% confidence intervals for $AUC_{(0\text{-}t)}$ and C_{max} ratios and applied to conventional 0.8 – 1.25 average BE limits. If needed, alternative BE evaluation methodology and or BE criteria may be investigated. Data will also be assessed by ANOVA methods (SAS 9.2 or another equally suitable software package) to test for statistical differences ($p<0.05$).

7.3 Compartmental Analysis

In addition to NCA analysis, compartmental data analysis methodology will be applied to the observed in vivo pharmacokinetic (PK) data. Compartmental PK analysis using NONMEM (or another equally suitable software package) will incorporate information on physiological processes such as pulmonary absorption, mucociliary clearance rates and dissolution rates (bulk and after cascade impactor separation), and cascade impactor information.