FLUIDDA

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Project No: FLUI-2012-94	**Study code: E-RES/12/12-Q13**

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Study Title:
A double blind, double dummy, randomized, two way cross-over study to compare the effects of Seretide® Evohaler (supplied by Allen & Hanburys, UK) and a generic salmeterol/fluticasone HFA pMDI (manufactured by Cipla Ltd, India) on functional respiratory imaging parameters in asthmatic patients.

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Date first patient enrolled: 2 April 2013

Last patient last visit: 19 July 2013

INVESTIGATOR'S SIGNATURE PAGE FOR CLINICAL TRIAL REPORT

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Principal Investigator

Date: 12 / 03 / 2014
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Prof. Dr. W. De Backer

Principal Investigator

Antwerp University Hospital

1 Study Synopsis

STUDY TITLE	A double blind, double dummy, randomized, two way cross-over study to compare the effects of Seretide® Evohaler (supplied by Allen & Hanburys, UK) and a generic salmeterol/fluticasone HFA pMDI (manufactured by Cipla Ltd, India) on functional respiratory imaging parameters in asthmatic patients.
INVESTIGATIONAL DRUG, DOSE AND MODE OF ADMINISTRATION	**Test product:** Salmeterol xinafoate/Fluticasone propionate combination hydrofluroalkane (HFA) pressurised metered dose inhaler (pMDI) (Cipla Ltd., India) Strength: 25/250 mcg per actuation Dose: single dose of 2 puffs; a total dose of 50/500 mcg
	Reference product: Salmeterol xinafoate/Fluticasone propionate combination HFA pMDI (Seretide Evohaler, Allen & Hanburys, UK) Strength: 25/250 mcg per actuation Dose: single dose of 2 puffs; a total dose of 50/500 mcg
	Placebo: To ensure blinding, each patient received two inhalers (one active and one placebo) on each dosing day.
	The investigational medicinal product (IMP) was inhaled orally. Spacers were not allowed for the inhalation of the IMP.
PHASE:	Phase III

INDICATION	Chronic Asthma
OBJECTIVES:	**Primary** The primary objective of this study was to evaluate the effect of both the study drugs under investigation on Functional Respiratory Imaging (FRI) parameters and to evaluate the particle deposition in the lungs using Computational Fluid Dynamics (CFD). **Secondary** The secondary objectives were the assessment of the effect of both the study drugs on lung function (spirometry and body plethysmography), on exercise capacity (6 Minutes Walking Test = 6MWT) and on dyspnea (Borg Category (C) Ratio (R) 10 Scale and Visual Analog Scale (VAS) dyspnea). Furthermore the safety of the 2 products under investigation were evaluated through monitoring of adverse events (AEs) throughout the study.
STUDY DESIGN	This study was conducted as a randomized, double blind, double dummy two period crossover study in stable asthma patients. A total of 16 stable asthma patients treated in accordance with the Global Initiative for Asthma (GINA) guidelines[1], were included. On the screening visit day, patients were explained the full details of the study procedures and it was asked if they voluntarily wished to participate in the study, they were then asked to sign the informed consent form (ICF). Patients were given a chance to ask questions and clarify their doubts regarding the study if any either at the screening visit or anytime during the study. Study procedures started only after the patient signed the ICF. Study procedures such as demographic data, medical/surgical history, prior medication data (in last 3 months), physical examination, safety laboratory investigations, spirometry, body plethysmography, 6MWT were performed. Patients were assessed for inclusion exclusion criteria based on the results of above tests. Patients were also assessed for the correctness

	of their inhaler technique. In case the patient was not able to perform the inhalation technique satisfactorily, a retraining was provided with the placebo inhalers. Only patients who met all the inclusion criteria and none of the exclusion criteria were enrolled into the study and they entered in to the run-in period of minimum 7 days or a maximum of 11 days. Patients were asked to report to the clinic for the dosing days. On the first dosing day (visit 2) patients asthma stability was first assessed during the predose measurements and based on review of the monitoring on the pharmacologic and non-pharmacologic treatment. Stable asthma patients were then randomized into the study and were allocated a treatment sequence. According to the sequence patients either received a single dose of 2 puffs of either the test product or the reference product in the presence of the investigator or authorized site personnel. Additionally patients also received a matching placebo of the alternate treatment as a dummy inhaler to achieve double blinding. Randomization codes were assigned strictly sequentially as patients became eligible for randomization. There was a washout period of at least 3 days (not more than 7 days) between visit 2 and visit 3. Patients were given the similar instructions as during run-in period. There was also a follow up period of 4 - 7 days after the visit 3 (2nd dosing day). Safety assessments such as safety laboratory investigations and spirometry were performed at this visit. If there were no follow up actions for a patient at this visit, it was considered as a completion of study participation / end of study for that particular patient. AEs were monitored and recorded throughout the study.
SAMPLE SIZE	A total of 16 stable asthma patients were enrolled and randomized in a two-way crossover design.
STUDY POPULATION: INCLUSION CRITERIA	A patient was eligible for inclusion in this study only if all of the following criteria apply. 1. Male or female patient ≥ 18 years old

	2. Written informed consent obtained 3. Patient with a documented diagnosis of asthma according to the GINA guidelines[1] 4. Patient with a co-operative attitude and ability to be trained to correctly use the pMDI 5. Female patient of childbearing potential who confirmed that a contraception method was used at least 14 days before visit 1 and who agreed to continue to use a contraception method during the study 6. Patient must be stable and treated in accordance with the GINA guidelines 7. Patient must be a non-smoker or ex-smoker who have stopped smoking at least 1 year prior to visit 1 and has a smoking history of < 10 pack years 8. Patient must be able to understand and complete the protocol requirements, instructions, questionnaires and protocol-stated restrictions.
EXCLUSION CRITERIA	A patient was not eligible for inclusion in this study if any of the following criteria apply: 1. Pregnant or lactating female 2. Unstable patient who developed an exacerbation during the last 8 weeks 3. Patient with upper or lower airways infection 4. Patient unable to perform pulmonary function testing 5. Patient with an uncontrolled disease or any condition that might, in the judgment of the investigator, place the patient at undue risk or potentially compromise the results or interpretation of the study 6. Patient with cancer or any other chronic disease with poor prognosis and /or affecting patient status 7. Patient with allergy, sensitivity or intolerance to study drugs and/or study drug formulation ingredients

	8. Patient unlikely to comply with the protocol or unable to understand the nature, scope and possible consequences of the study 9. Patient who received oral corticosteroids within the last 4 weeks prior to visit 1 10. Patient who received any investigational new drug within the last 4 weeks prior to visit 1 or twice the duration of the biological effect of any drug (whichever is longer). 11. Patient with a history of alcohol or substance abuse that in the opinion of the investigator may be of clinical significance 12. Patient who had undergone major surgery in the last 12 weeks before the visit 1 or had planned to undergo a major surgery before the end of the trial. 13. Patient with diagnosis of chronic obstructive pulmonary disease
STABILITY CHECK	Following stability criteria were also checked on each dosing day prior to start of dosing and if the patient fulfilled any one of them the patient could be withdrawn from the study based on the investigator's decision. • Any change in the asthma therapy apart from the use of rescue medication including use of any other asthma medication • Visit 3 only: Absolute Forced Expiratory Volume in one second (FEV_1) measured on visit 3 predose is > 10% (variation should not be more than 10% on either side) of the value recorded at visit 2 predose
PRIMARY ENDPOINT	1. Total airway volume and total airway resistance 2. The number of deposited particles per pre-defined airway section
SECONDARY	Spirometry parameters - Lung function:

ENDPOINTS	• FEV_1, • Forced Vital Capacity (FVC), • Peak Expiratory Flow (PEF), • Maximum Expiratory Flow at 25% of FVC (MEF50), • Maximum Expiratory Flow at 50% of FVC (MEF25), • Inspiratory Vital Capacity (IVC), • Tiffeneau Index (FEV_1/FVC ratio) Body plethysmography: • Functional Residual Capacity (FRC), • Total Lung Capacity (TLC) • Airway resistance: Airway Resistance (Raw), Specific airway resistance (sRaw) 6MWT: • Exercise capacity: distance walked in 6 minutes (m) • Borg CR10 Scale: measure of the present dyspnea • VAS: measure of the difference in dyspnea before and after treatment
SAFETY ENDPOINTS	Incidence of all AEs
DURATION OF THE STUDY	There was a run-in period of 7-11 days prior to randomization or the first dosing day. The study comprised 2 separate dosing days of one day duration each. Two dosing days were separated by a minimum of 3 days (maximum 7 days), followed by a follow up period of 4-7 days. Hence an entire study duration for each patient could vary from a minimum of 16 days to a maximum of 27 days.
STATISTICAL	All variables were tested on normality using Shapiro-Wilk W

METHODS	test. Correlations between lung function tests and FRI parameters were examined using Spearman correlation coefficient or the Pearson correlation coefficient, as well as changes in deposition and effect. For all analysis, $p<0.05$ is defined as statistically significant. To be on the conservative side, no correction for multiple testing was performed. Comparisons of airway characteristics before and after medication were assessed using Wilcoxon matched-pairs test. Comparisons between the different medications were performed with the Mann Whitney U test. Post-hoc effect size calculations were performed for future research.

2 Table of contents

3 List of abbreviations

3D	Three-Dimensional
6MWT	6 Minutes Walking Test
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartase aminotransferase
Borg CR10 Scale	Borg Category Ratio Scale
BUN	Urea Nitrogen
CFD	Computational Fluid Dynamic
COPD	Chronic obstructive pulmonary disease
CRF	Case Report Form
CT	Computed Tomography
EC	Ethics Committee
ECG	Electrocardiography
FEV1	Forced Expiratory Volume in one second
FP	Fluticasone Propionate
FRC	Functional Residual Capacity
FRI	Functional Respiratory Imaging
FVC	Forced Vital capacity
GCP	Good Clinical Practice
GINA	Global Initiative for Asthma
GMP	Good Manufacturing Practise
GOLD	The Global Initiative for Chronic Obstructive Lung Disease
HFA	Hydrofluroalkane
HRCT	High Resolution Computed Tomography
ICF	Informed Consent Form
ICS	Inhaled Corticosteroid
IEC	Institutional ethics committee
IMP	Investigational Medicinal Product
iRaw	Airway resistance

iSaw	Surface area
iVaw	Airway Volume
IVC	Inspiratory Vital Capacity
LABA	Long-acting β2-agonists
LES	Large Eddy Simulation
MEF25	Maximum Expiratory Flow at 25% of FVC
MEF50	Maximum Expiratory Flow at 50% of FVC
MMAD	Mass median aerodynamic diameter
PEF	Peak Expiratory Flow
pMDI	Pressurised Metered Dose Inhaler
RANS	Reynolds averaged Navier-Stokes
Raw	Airway resistance
Re	Reynolds number
SAE	Serious Adverse Event
sRaw	Specific airway resistance
TLC	Total Lung Capacity
QC	Quality Control
VAS	Visual Analog Scale

4 Ethics

4.1 Ethical Conduct of the Study

Prior to the initiation of the study, the protocol, the investigator's brochure, the curriculum vitae of the principal investigator, the patient information leaflet and the informed consent (ICF) were submitted to the local institutional ethics committee (IEC) for review and approval.

The study was approved by the ethics committee (EC) of the Antwerp University Hospital on 4 March 2013.

The study was conducted in full conformity with the current revision and clarifications of the Declaration of Helsinki.

4.2 Patient Information and Consent

Prior to entry in the study, the investigator or a person designated by the investigator explained the study to the patient and the implications of participation. Patients were

informed that the participation was voluntary and that they could withdraw from the study at any time. They were informed that choosing not to participate or to withdraw from the study had no impact on the care they would receive for the treatment of his/her disease. Finally, they were told that their records could be accessed by the EC, regulatory authorities and authorized representatives of the sponsor without violating the confidentiality of the patient, to the extent permitted by the applicable law and/or regulations. By signing the ICF, the patient authorized such access.

The patient was given sufficient time to read the ICF and to ask additional questions. After this explanation and before entry in the study, consent was appropriately recorded by means of the patient's personally dated signature. After having obtained the consent, a copy of the signed and dated ICF was given to the patient.

5 Introduction

5.1 Pathology

Asthma is a condition that affects the airways. When a person with asthma comes into contact with an asthma trigger, their airways become irritated and the muscles around the walls of the airways tighten so that the airways become narrower and the lining of the airways become inflamed and starts to swell. Sometimes, sticky mucus or phlegm builds up, which can further narrow the airways. These reactions cause the airways to become narrower and irritated - making it difficult to breathe and leading to coughing, wheezing, shortness of breath and tightness in the chest. People with asthma are generally advised to take inhaled steroids to treat the underlying inflammation, but if asthma is still not controlled, current clinical guidelines recommend the introduction of additional medication to help.

5.2 Medication

A common strategy in these situations is to use a Long-acting β2-agonists (LABA): formoterol or salmeterol. A LABA is an inhaled drug which opens the airways (bronchodilator). A bronchodilator relieves the symptoms of asthma and other chest conditions.

Seretide® metered dose inhaler (MDI) contains two medicines, fluticasone propionate (FP) and salmeterol xinafoate. FP belongs to a group of medicines known as corticosteroids, frequently called 'steroids'. Corticosteroids have an anti inflammatory action. They reduce the swelling and irritation in the walls of airways and so help to breathe more easily. Corticosteroids are used to treat asthma and chronic obstructive pulmonary disease (COPD). Salmeterol xinafoate is a bronchodilator.

Cipla Ltd. India has developed a generic inhaler product containing a combination of salmeterol xinafoate and FP hydrofluroalkane (HFA) pressurized metered dose inhaler (pMDI) (here after referred to as a test product). This product is pharmaceutically

equivalent to the innovator product Seretide Evohaler, supplied by Allen & Hanburys, UK (here after referred to as reference product.)

5.3 Functional respiratory imaging

In the functional respiratory images (FRI) workflow, patient-specific anatomical images of computed tomography (CT) scans are combined with functional information that are calculated using computational fluid dynamics (CFD). In this way, it is possible to assess how inhalation medication behaves in the airways and lungs of a specific patient[2]. To be able to use this validated technique[2,3], patient-specific three-dimensional models of the airway and lungs needed to be extracted from medical images and suitable boundary conditions were defined based on patient specific internal airflow distribution. This technology led to obtaining airway resistance comparisons between the test product and reference product. Additionally, other parameters that were studied include airway bronchodilation comparison in terms of the airway volume and airway surface areas.

6 Study objectives

In this study the possible effects of the Seretide® reference product and the test product were evaluated in asthma patients after the administration of a single dose in a crossover manner.

The primary objective of this study was to evaluate the effect of both the study drugs under investigation on FRI parameters.

The secondary objectives were the assessment of the effect of both the study drugs on lung function (spirometry and body plethysmography), on exercise capacity (6 Minutes Walking Test = 6MWT) and on dyspnea (Borg Category (C) Ratio (R) 10 Scale and Visual Analog Scale (VAS) dyspnea). Furthermore the safety of the 2 products under investigation were evaluated through monitoring of adverse events (AEs) throughout the study.

7 Competent authorities

The study was approved by the competent authorities on 22 February 2013. The study was authorized in accordance with article 12 of the Law of 7 May 2004 concerning experiments on the human person.

8 Investigational plan

8.1 Overall Study Design and Plan-Description

This study was conducted as a randomized, double blind, double dummy two period crossover study in stable asthma patients. A total of 16 stable asthma patients treated in accordance with the Global Initiative for Asthma (GINA) guidelines[1], were included.

On the screening visit day, patients were explained the full details of the study procedures and it was asked if they voluntarily wished to participate in the study, they were asked to sign the informed consent form (ICF). Patients were given a chance to ask questions and clarify their doubts regarding the study if any either at the screening visit or anytime during the study. Study procedures started only after the patient signed the ICF.

Study procedures such as demographic data, medical/surgical history, prior medication data (in last 3 months), physical examination, safety laboratory investigations, spirometry, body plethysmography, 6MWT were performed. Patients were assessed for inclusion exclusion criteria based on the results of above tests. Patients were also assessed for the correctness of their inhaler technique. In case the patient was not able to perform the inhalation technique satisfactorily, a retraining was provided with the placebo inhalers. Only patients who met all the inclusion criteria and none of the exclusion criteria were enrolled into the study and they entered in to the run-in period of minimum 7 days or a maximum of 11 days. Patients were asked to report to the clinic for the dosing days.

On the first dosing day (visit 2) patients asthma stability was first assessed during the predose measurements and based on review of the monitoring on the pharmacologic and non-pharmacologic treatment. Stable asthma patients were then randomized into the study and were allocated a treatment sequence. According to the sequence patients either received a single dose of 2 puffs of either the test product or the reference product in the presence of the investigator or authorized site personnel. Additionally patients also received a matching placebo of the alternate treatment as a dummy inhaler to achieve double blinding.

Randomization codes were assigned strictly sequentially as patients became eligible for randomization.

There was a washout period of at least 3 days (not more than 7 days) between visit 2 and visit 3. Patients were given the similar instructions as during run-in period. There was also a follow up period of 4 - 7 days after the visit 3 (2nd dosing day). Safety assessments such as safety laboratory investigations and spirometry were performed at this visit. If there were no follow up actions for a patient at this visit, it was considered as a completion of study participation / end of study for that particular patient. AEs were monitored and recorded throughout the study. This is a randomized, double-blind, double dummy, two way cross-over study. An overview can be found in Figure 1.

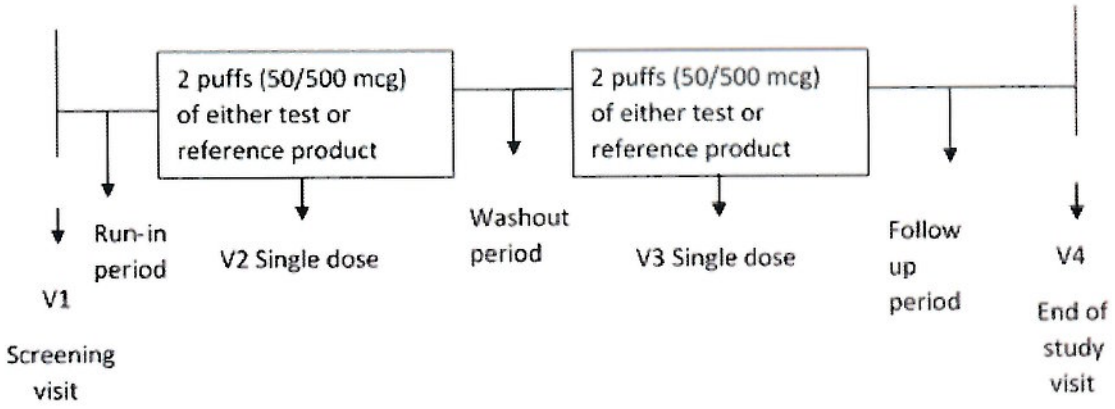


Figure 1: Study design

8.2 Selection of study population

8.2.1 Inclusion criteria

A patient was eligible for inclusion in this study only if all of the following criteria apply.

1. Male or female patient ≥ 18 years
2. Written informed consent obtained
3. Patient with a documented diagnosis of asthma according to the GINA guidelines[1]
4. Patient with a co-operative attitude and ability to be trained to correctly use the pMDI
5. Female patient of childbearing potential who confirmed that a contraception method was used at least 14 days before visit 1 and who agreed to continue to use a contraception method during the study
6. Patient must be stable and treated in accordance with the GINA guidelines
7. Patient must be a non-smoker or ex-smoker who have stopped smoking at least 1 year prior to visit 1 and has a smoking history of < 10 pack years
8. Patient must be able to understand and complete the protocol requirements, instructions, questionnaires and protocol-stated restrictions.

8.2.2 Exclusion criteria

A patient was not eligible for inclusion in this study if any of the following criteria apply:

1. Pregnant or lactating female
2. Unstable patient who developed an exacerbation during the last 8 weeks

3. Patient with upper or lower airways infection
4. Patient unable to perform pulmonary function testing
5. Patient with an uncontrolled disease or any condition that might, in the judgment of the investigator, place the patient at undue risk or potentially compromise the results or interpretation of the study
6. Patient with cancer or any other chronic disease with poor prognosis and /or affecting patient status
7. Patient with allergy, sensitivity or intolerance to study drugs and/or study drug formulation ingredients
8. Patient unlikely to comply with the protocol or unable to understand the nature, scope and possible consequences of the study
9. Patient who received oral corticosteroids within the last 4 weeks prior to visit 1
10. Patient who received any investigational new drug within the last 4 weeks prior to visit 1 or twice the duration of the biological effect of any drug (whichever is longer).
11. Patient with a history of alcohol or substance abuse that in the opinion of the investigator may be of clinical significance
12. Patient who has undergone major surgery in the last 12 weeks before the visit 1 or has planned to undergo a major surgery before the end of the trial.
13. Patient with diagnosis of chronic obstructive pulmonary disease

8.3 Treatment

8.3.1 Treatments administered

	Test (T)	Reference (R)
Name of product	Salmeterol xinafoate /Fluticasone propionate HFA pMDI 25/250 µg per actuation manufactured by Cipla Limited, India.	Seretide Evohaler pMDI (containing salmeterol xinafoate/fluticasone propionate 25/250 µg per actuation) supplied by Allen and Hanburys Ltd., UK.

Dosage Form	Pressurised MDI	Pressurised MDI
Content and Strength	Each puff releases 25 µg of salmeterol xinafoate and 250 µg of fluticasone propionate.	Each puff releases 25 µg of salmeterol xinafoate and 250 µg of fluticasone propionate.
Dose	Single dose of 50/500 µg (25/250 µg per actuation X 2 puffs)	Single dose of 50/500 µg (25/250 µg per actuation X 2 puffs)
Storage condition	Do not store above 25° C. Do not freeze.	Do not store above 25° C. Do not freeze.
Batch number and expiry	G22237 April 2014	F0033 & F3856 April 2013 & January 2015
Information about Placebos		
	Test Placebo	**Reference Placebo**
Name of product	Placebo inhaler containing HFA propellant resembling the test active inhaler	Placebo inhaler containing HFA propellant resembling the reference active inhaler
Manufacturer	Cipla Ltd.	Cipla Ltd.
Dosage Form	Pressurised MDI	Pressurised MDI
Dose	Single dose of 2 puffs	Single dose of 2 puffs
Storage condition	Do not store above 25° C. Do not freeze.	Do not store above 25° C. Do not freeze.
Batch number & expiry	GPL2203	GPL2203

	February 2017	February 2017

Test active, test placebo and the reference placebo canisters (only) were manufactured and supplied by Cipla Ltd. as per Good Manufacturing Practice (GMP). The reference active drugs and reference placebo actuators were manufactured by GlaxoSmithKline, supplied by Allen & Hanburys, UK and obtained from the commercial lot for the purpose of the study.

8.3.2 Method of Assigning Subjects to Treatment Sequence

Patients were assigned a sequential screening number at the first study visit (Screening, Visit 1). On the first dosing day (Visit 2) after the confirmation of the investigator that patient fulfilled all the required criteria, the patient was assigned a unique randomisation number. Supplies were pre-packed and numbered serially so that randomisation is achieved by assigning the patient to the next available number in the sequence. Once a randomisation number was assigned to a patient, it was not re-used.

8.3.3 Selection and Timing of Dose for Each Patient

This was a single dose study and 2 puffs of study medication were self-administered by the patient at the clinic approximately at the same time on both the dosing days.

8.3.4 Blinding

The trial was conducted under double-blind, double dummy conditions. The study treatment included two treatments containing the active ingredients (test and reference inhaler) i.e. salmeterol xinafoate and FP in combination 25/250 mcg per actuations and respective matching placebo inhalers containing only the propellant.

In order to blind the active treatments, a double dummy technique was applied in the study. Therefore on each dosing day every patient received study medication from two inhalers out of which one contained the active drug (i.e. either the test or the reference product) while the other inhaler contained placebo matching the alternate treatment/product i.e. test active inhaler was combined with the reference placebo inhaler and vice versa. The patient inhaled two puffs from both the inhalers.

With such a design neither the investigator nor the patient was aware of the identity of the active drug.

On each dosing day the patient received a study kit containing the study drugs/IMP to be used on that particular dosing day. Each study kit contained two inhalers labelled as "A" and "B" indicating the order in which they should be used. One of these inhalers was an active inhaler and one was a placebo inhaler. However since the placebo inhaler was being used as a dummy inhaler the identity of these inhalers was not revealed. The label "A" was always associated with a reference active or a reference placebo. The label "B" was always associated with a test active or a test placebo.

Each patient randomly received one of the two treatment sequence associated with that randomisation number. The treatment sequence was predefined in a randomisation scheme (computer generated). The randomisation scheme for the study was prepared by an independent statistician.

The investigator was issued with individual patient emergency code break sealed envelopes for use in emergency situations only. During the study none of the codes were opened. The individual patient emergency code break sealed envelopes were also present with Sponsors medical and safety expert. At the end of the study all of the sealed patient emergency code break envelopes were resend to the independent statistician.

In the protocol, it was defined that the patients would be randomized (1:1) in two treatment sequences as shown in the following table.

	Treatment sequence 1 (8 patients)	**Treatment sequence 2 (8 patients)**
Visit 2	Reference active single dose 50/500µg (= 2 puffs) and Test placebo single dose (= 2 puffs)	Test active single dose 50/500µg (= 2 puffs) and Reference placebo single dose (= 2 puffs)
Visit 3	Test active single dose 50/500µg (= 2 puffs) and Reference placebo single dose (= 2 puffs)	Reference active single dose 50/500µg (= 2 puffs) and Test placebo single dose (= 2 puffs)

Instead of equal number of patients in each group, there were 7 patients who received treatment sequence 1 while 9 patients received treatment sequence 2. However since this was a crossover study where every patient was exposed to each treatment, it did not have any impact/influence on the accuracy or reliability of the study.

8.3.5 Prior and concomitant therapy

All medications taken after signing the ICF and continued at the start of the study, as well as for treatment of adverse events (AEs), are recorded in the case report form (CRF). Reported information includes a description of the type of pharmacologic and non-pharmacologic therapy, generic name of the medicine, treatment period, dosing regimen, route of administration and its indication, start date and stop date.

Following medication were not permitted:

- Investigational drugs were not allowed for at least 4 weeks, or twice the duration of the biological effect of any drug (whichever is longer) prior to visit 1 and throughout the study period
- Oral corticosteroids were not allowed for at least 4 weeks prior to visit 1 and throughout the study period
- systemic steroids
- Beta blockers
- Anticholinergic agents such as ipratropium, tiotropium
- Any herbal medicine
- Mast cell stabilisers
- Leukotriene receptor antagonists
- Theophylline

8.3.6 Treatment compliance

Patients self-administered the single dose (2 puffs) of IMP at the clinic under the supervision of the investigator or the authorised site personnel. The supervised administration of the IMP ensured direct compliance with the IMP.

8.4 Efficacy and Safety Variables

Primary endpoints in this study were the changes in airway volume (iVaw), airway resistance (iRaw) and particle deposition per pre-defined airway section. As for this evaluation FRI with CFD was used a High Resolution Computed Tomography (HRCT) scan was taken before and after the administration of a single dose of both the test and the reference product. The scan was taken at two different lung volumes, i.e. Total Lung Capacity (TLC) and Functional Residual Capacity (FRC), during breath hold. During visit 2 predose an additional scan of the upper airway was taken.

Secondary in this study the effect of salmeterol and fluticasone combination therapy on lung function (spirometry and body plethysmography), on exercise capacity (6MWT) and on dyspnea (Borg Category Ratio Scale (Borg CR10 Scale) and Visual Analog Scale (VAS) dyspnea) was assessed.

Outcome variables that were obtained with respective tests are listed below:

- Spirometry - Lung function:
    - Forced Expiratory Volume in one second (FEV1),
    - Forced Vital Capacity (FVC),

- Peak Expiratory Flow (PEF),
- Maximum Expiratory Flow at 25% of FVC (MEF50),
- Maximum Expiratory Flow at 50% of FVC (MEF25),
- Inspiratory Vital Capacity (IVC),
- Tiffeneau Index (FEV_1/FVC ratio)

- Body plethysmography
  - FRC,
  - TLC,
  - Airway resistance: Raw, Specific airway resistance (sRaw)
- 6MWT
  - Exercise capacity: distance walked in 6 minutes (m)
- Borg CR10 Scale: measure of the present dyspnea
- VAS: measure of the difference in dyspnea

To ensure the patients safety a complete physical examination was performed before inclusion, other safety tests that were performed were:

- Electrocardiography (ECG) (visit 1)
- Blood and urine collection (visit 1 and visit 4)

The following parameters were determined:

- Haematology:
  - Haematocrit, haemoglobin, erythrocytes (red blood cells), leucocytes (white blood cells), differential blood count (neutrophils, lymphocytes, monocytes, eosinophils and basophils), thrombocytes (platelets).
- Biochemistry:
  - Electrolytes: sodium, potassium, calcium, chloride and inorganic phosphorus.
  - Enzymes: Aspartase aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase, Gamma glutamyl transpeptidase, lactate dehydrogenase and creatine kinase.

- Substrates: total cholesterol, triglycerides, creatinine, total bilirubin, total protein, albumin, uric acid and urea nitrogen (BUN)

- Urine Analysis:
  - Measurement of protein, pH, glucose, blood and bilirubin
- Vital signs (visit 1, visit 2, visit 3 and visit 4): blood pressure, pulse rate
- Urine pregnancy test with woman of childbearing potential (visit 1)

Furthermore the safety of the 2 study drugs under investigation was evaluated through monitoring of AEs throughout the study.

8.5 Data Quality Assurance

Study monitors contacted the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and sponsor requirements in accordance with applicable regulations including GCP.

Fluidda monitored the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of patients are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, Good Clinical Practice (GCP), and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agreed to allow the monitor direct access to all relevant documents.

The investigator ensured that appropriate Quality Control (QC) steps were included into the different clinical processes to guarantee adequate protection of the study patients and quality of the data.

To ensure compliance with GCP and all applicable regulatory requirements, Fluidda had the rights conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

8.6 Statistical Methods Planned in the Protocol and Determination of Sample Size

In a previous study where 10 mild to moderately severe asthma patients were actively treated with carmoterol, FRI was able to determine a significant increase in imaging based total (13.31±6.11 [%]) and distal airway volume (54.90±22.51 [%]) between the baseline and post-treatment scan. A one mean t-test sample size calculation on this data showed that 5 patients were needed to obtain significance for these outcome parameters (power goal 90%, alpha 0.05)[5].

In another trial, where 24 asthma patients (steroid-naïve, n=7; partially-controlled, n=6; well-controlled, n=11) were switched from a fine to an extrafine beclomethasone / formoterol compound, a one mean t-test sample size calculation (power goal 90%, alpha 0.05) on the chronic effects in well controlled patients showed that 25 patients were needed to obtain significance for the imaging based airway volume[6].

The sensitivity of FRI was also calculated for another patient population. In a double blind cross over study where 10 COPD GOLD III patients (GOLD, The Global Initiative for Chronic Obstructive Lung Disease) received a budesonide / formoterol combination, FRI was able to quantify the effects of the active compound. A one mean t-test sample size calculation (power goal 90%, alpha 0.05) revealed that in order to have a well-powered study with change in imaging based airway volume as primary outcome parameter, a total of 16 patients would be required[4].

Based on this data and the powerful design of this trial, a sample size of 16 patients was chosen.

8.7 Changes in the Conduct of the Study or Planned Analyses

The initial protocol of this study was approved on 4 Mar 2013. A non-substantial amendment was subsequently made to this protocol with the following most important changes:

The questionnaires (Borg CR10 dyspnea scale and VAS dyspnea) will be performed at two different time points. At visit 2 and visit 3 the Borg CR10 Scale will be performed twice predose and twice postdose (i.e. before and after the 6MWT). The VAS will be completed twice at visit 2 postdose and twice at visit 3 postdose. The VAS will be filled out once at the start of the post dose measurements and once after the post dose 6MWT.

Due to the repetition of the questionnaire the sequence order described in section 5 and 7.2 is slightly adapted.

The stability check written in the synopsis as well as in section 6.4 is corrected as there was written: `absolute FEV1 is > 10% of the value recorded at the study start on the screening visit day`. This should be: `Visit 3 only: Absolute FEV1 measured on visit 3 predose is > 10% of the value recorded at visit 2 predose`

We cannot compare the predose values with the values measured on the screening visit day as the patients have to follow some wash out requirements before dosing days. Only the FEV_1 measured on visit 3 predose can be compared with those measured on visit 2 predose.

In section 9.9.3 was written that patients using already combination therapy such as Seretide®, Symbicort®, Inuvair® should be switched to Flixotide®. This is changed into: Patient using already combination therapy (such as Seretide®, Symbicort®, Inuvair®) will be switched to Inhaled Corticosteroid (ICS) (such as Flixotide®, Pulmicort®, Qvar®) on the evening before the dosing days (visit 2, visit 3). As this is Flixotide® for Seretide® but for Symbicort®, Inuvair® this is Pulmicort® respectively Qvar®.

In section 9.3 it was written that the temperature of the medication should be controlled and documented once a day (only on week days), this is changed into twice a day (only on week days)

The amendment dated 20/03/2013 was sent as a non-substantial amendment to the ethics committee to request the notification by the ethics committee. The written confirmation of the notification was received on 8/04/2013.

9 Imaging Protocol and Methodology

9.1 Input data and three-dimensional model generation

9.1.1 Imaging

The CT scans were taken with a multi-slice scanner with 64 receptors (GE VCT Lightspeed). These scans were performed with low dose radiation using the multi-slice CT scan. Scanning was performed at FRC and TLC. The lung volumes were controlled using adapted spirometry during the CT procedure. The radiation dose was reduced by reduction of the tube current and voltage depending on the patients' weight (1mAs/kg). Previous studies have shown that a three to six-fold reduction in the radiation dose can be obtained without a loss in image quality[7,8] due to the natural contrast between air and the surrounding airway tissue. The CT data for the asthmatic subjects was utilized for constructing computer models which will be analyzed numerically using CFD for airway resistance, and for bronchodilation comparison in terms of airway volume and surface area for the test and reference products.

9.1.2 Model development

The CT images were used to perform functional imaging for accurate three-dimensional (3D) reconstructions of the airway geometries using a semi-automatic algorithm of the airways up to the point where no distinction could be made between the intra-luminal and alveolar air (7^{th}-10^{th} generation). The segmentation and three-dimensional reconstruction was performed. The segmented airway model was then smoothed with a volume compensation algorithm in order to eliminate the staircasing effect (error introduced during model smoothing). A fully developed 3D model is illustrated in Figure 2. The parts represented the mouth, upper airway region, the central region and distal region. Additionally the peripheral airways were studied by comparing the lobar expansion between FRC and TLC scans as show in Figure 3 below.

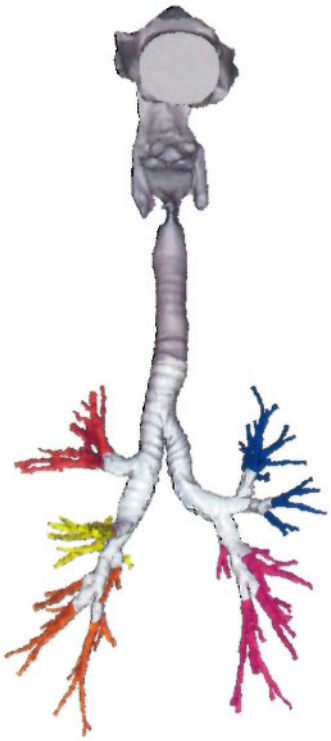


Figure 2: A complete airway model shown with Mouth (dark grey), Upper airway (light grey), Central airways (white) Distal airways (colored). Peripheral airways are not detectable in a conventional thoracic CT scan.

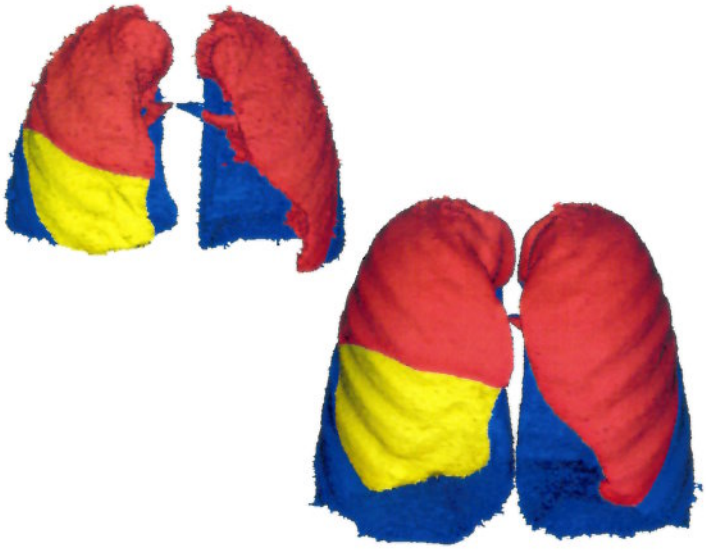


Figure 3: Peripheral airway volumes are considered using volumetric differences between FRC and TLC scans.

9.1.3 Simulation Methodology

CFD can be defined as the science of solving mathematical flow equations to obtain flow properties throughout the entire domain of a computer model. In the past this technique has been, and still is, extensively used and validated in the field of aerospace engineering and turbomachinery. Typically these 'virtual prototypes' are used to optimize the design prior to production.

Since the governing mathematical flow equations (Navier-Stokes) are too complex to be solved analytically it is necessary to use the numerical approach. To this end the computer model is required to be converted into a computational grid or mesh. Meshing of the computer models means that the geometry is divided into finite number of small cells called control volumes. The governing flow equations are subsequently solved for these discrete control volumes. Initial and boundary conditions known from the problem definition are needed to close the system of equations to make them solvable. These CFD techniques can also be applied to evaluate the flow through respiratory airways to provide information on deposition of aerosols in 3D lung models.

The Reynolds averaged Navier-Stokes equations (RANS) for the conservation of mass and momentum are

$$\frac{\partial \overline{u}_i}{\partial x_i} = 0$$

$$\frac{\partial \overline{u}_i}{\partial t} + \overline{u}_j \frac{\partial \overline{u}_i}{\partial x_j} = -\frac{1}{\rho}\frac{\partial p}{\partial x_i} + \frac{\partial}{\partial x_j}\left[\left(\nu + \nu_T\right)\left(\frac{\partial \overline{u}_i}{\partial x_j} + \frac{\partial \overline{u}_j}{\partial x_i}\right)\right]$$

Where $\bar{u}_i$ is the time-averaged velocity in three coordinate directions, i.e., i = 1, 2, and 3, p is the time-averaged pressure, ρ is the fluid density, v is the kinematic viscosity and v_T is the turbulent viscosity. The Reynolds number (Re) is defined as the ratio of the viscous forces to the inertial forces experienced by the fluid. The Re is the most significant dimensionless number affecting flow behavior, and is given by

$$\mathrm{Re} = \frac{\rho u d}{\mu}$$

Where μ is the dynamic viscosity and d is the pipe diameter. The Large Eddy Simulation technique (LES) is used to model turbulence. Unlike the RANS wherein the fluctuating part of the flow is ensemble averaged or the Direct Numerical Simulation wherein the

fluctuating part of the flow is fully resolved, the LES resolves only the larger eddies. In the description of RANS above, $\bar{u}_i$ is defined as time-averaged velocity, and turbulence is modeled using the term v_T as an effective viscosity based on the model used. In LES, turbulence is fully resolved for the larger eddies, which carry most of the energy in a flow. For smaller eddies, which are responsible for dissipating the energy carried by the larger eddies, turbulence is modeled using a sub-grid scale similar to the RANS approach. The description of turbulence using LES can be defined using the following equations.

$$\frac{\partial u_i}{\partial t} + u_j \frac{\partial u_i}{\partial x_j} = \frac{g_i}{T_0}\theta - \frac{1}{\rho}\frac{\partial p}{\partial x_i} + \upsilon \frac{\partial^2 u_i}{\partial x_j{}^2}$$

For the discrete phase (particle) transport, the governing transport equation is written as

$$\frac{dv_i}{dt} = \alpha \frac{Du_i}{Dt} + \frac{f}{\tau_p}\left(u_i - v_i\right) + g_i(1-\alpha) + f_{i,\text{lubrication}} + f_{i,lift}$$

$$\frac{dx_i}{dt} = v_i(t)$$

where v_i and u_i are the components of the particle and local fluid velocity, respectively, and g_i denotes gravity. The drag factor is given by 'f', the response time for particles is denoted by $\tau_p = \rho_p d_p^2/18\mu$, and the density ratio $\alpha = \rho/\rho_p \approx 10^{-3}$.

9.2 Bronchodilation Comparison Methodology

Bronchodilation was compared between the test product and the reference product for all 16 patients included in this study. The comparison was based on changes in airway volumes (iVaw) and surface area (iSaw) between baseline and post treatment for the test product and the reference product over all patients studied.

Furthermore, the airway models were imported into a meshing program in order to create a high quality computational grid. This grid was then exported to a validated flow solver and airway resistance (iRaw) was calculated. For the determination of iRaw of the lower airway, steady calculations were performed. The same mass flow rate was employed at the inlet for all models studied. Outlet conditions were derived from the internal mass flow distributions for the individual lobes, based on the volume changes between the FRC and TLC scans.

These measurements were performed on the central and distal airway region as illustrated in Figure 4.

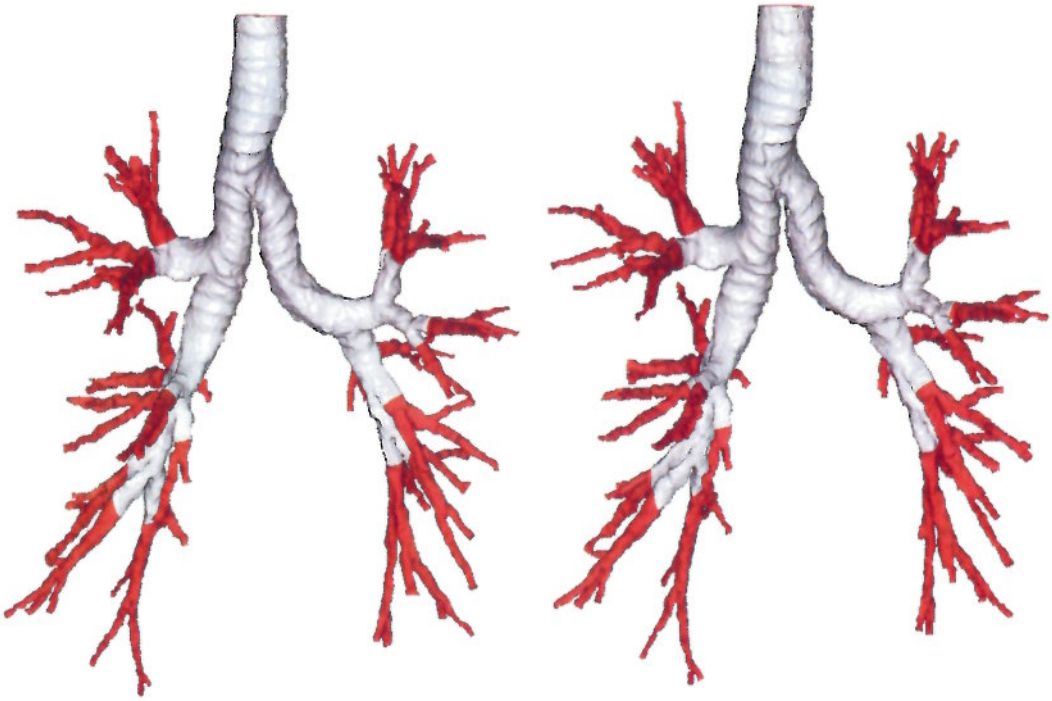


Figure 4: The central airway region is shown in grey color and the distal airway region is shown in red

9.3 Dosimetry

The dosimetry analysis included a numerical evaluation of particle deposition results for these models using unsteady (time dependent) conditions to simulate actual inhalation. The inhalation profiles for this study were measured during treatment maneuver (inhaler usage) for all patients for the test product and reference product, separately.

9.4 Particle size distribution data

A polydisperse particle size distribution was studied with a mass median aerodynamic diameter (MMAD) of 3.83 for fluticasone and 3.73 for salmeterol for the test product and the reference product as reported by Cipla Ltd., India. The particle distribution was simulated using computer models in patient-specific respiratory airway models as illustrated in Model Development Section (Figure 2). The patient-specific airway models were coupled with the specific inhaler after un-randomization was available to perform deposition simulations. The simulations were performed for both, test products and reference product independently.

9.5 Inhalation profiles

The inhalation profiles were developed using data from patient-specific respiration belt data. The respiration belt data was calibrated using a pneumotach device. The resulting data included inhalation flow and time. A parabolic breathing profile was fitted to the inhalation flow and time data resulting in the profile that was used for performing the

deposition simulations. The average inhalation profiles for both products are illustrated in Figure 5.

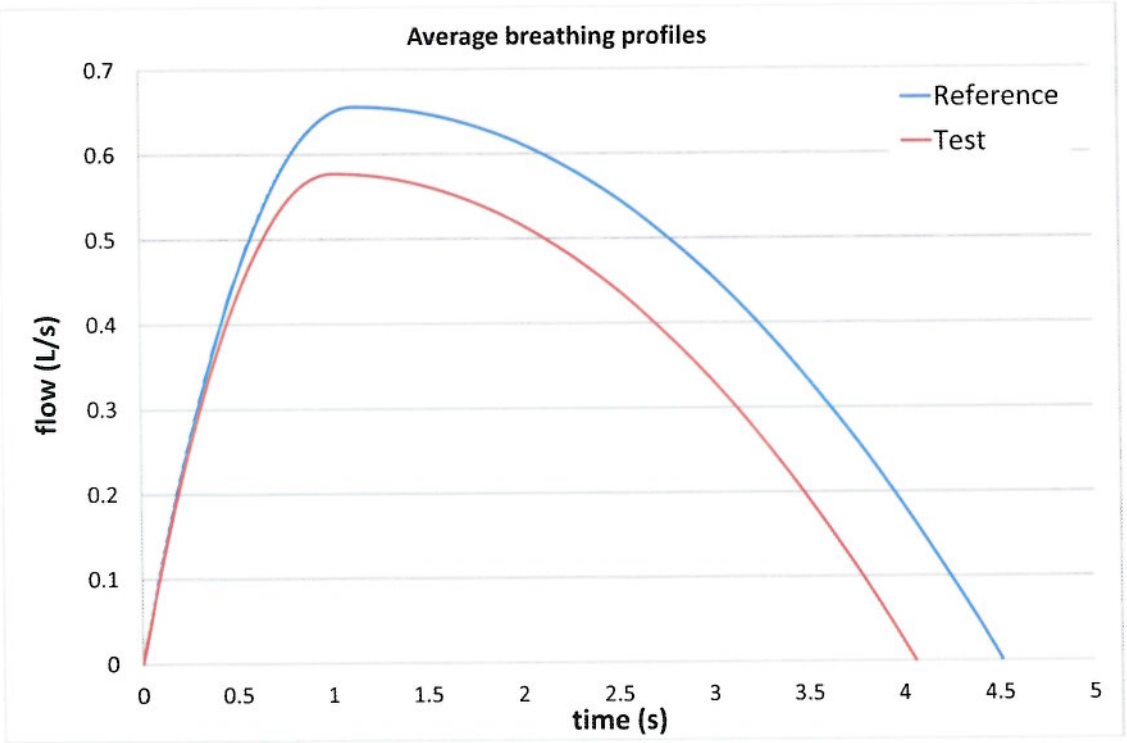


Figure 5: Inhalation profile

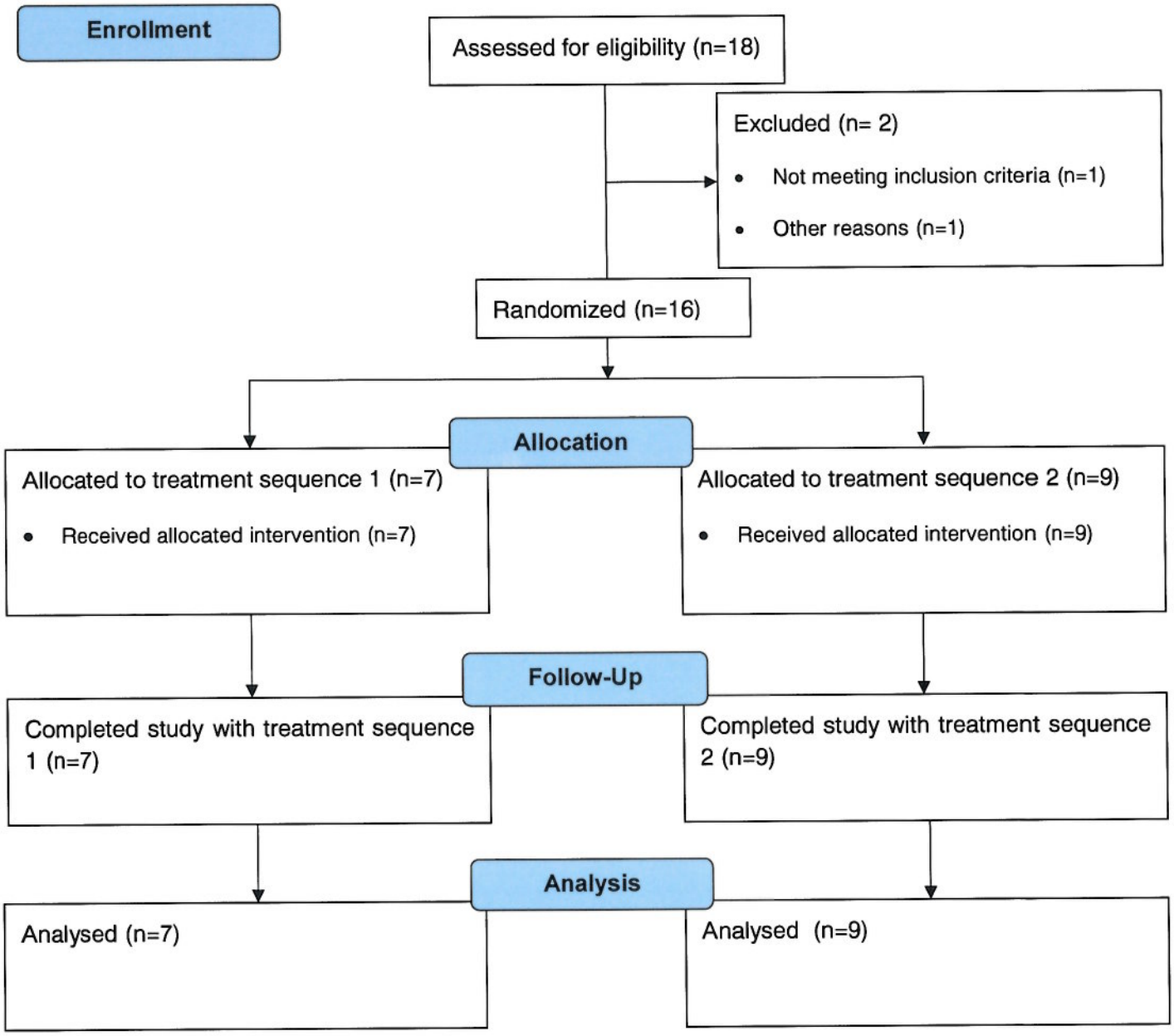
9.6 Statistical Methodology

The clinical results for the analyses are analyzed statistically for the test product between the baseline and post intervention. Similarly, analysis is performed for the reference product. These initial tests provide evidence for the effect of treatment for each product separately. The statistical test used was the non-parametric Wilcoxon matched pair test since some of the clinical data was not normally distributed.

The differences between the test product and reference product were then checked by analyzing the changes pre vs. post for both the products. Therefore, treatment effect for test product (post_test_product – pre_test_product) was statistically analyzed with treatment effect for reference product (post_reference_product – pre_reference_product) for all clinical and imaging parameters involved.

10 Study Patients

10.1 Disposition of Patients


Enrollment
Assessed for eligibility (n=18)
Excluded (n= 2)
Not meeting inclusion criteria (n=1)
Other reasons (n=1)
Randomized (n=16)
Allocation
Allocated to treatment sequence 1 (n=7)
Received allocated intervention (n=7)
Allocated to treatment sequence 2 (n=9)
Received allocated intervention (n=9)
Follow-Up
Completed study with treatment sequence 1 (n=7)
Completed study with treatment sequence 2 (n=9)
Analysis
Analysed (n=7)
Analysed (n=9)

10.2 Protocol Deviations

General protocol deviations:

- Instead of equal number of patients in each group, there were 7 patients who received treatment sequence 1 while 9 patients received treatment sequence 2.
- The temperature of the IMP was not documented during public holidays.

Subject specific protocol deviations:

- The oxygen level was not measured during 6MWT for 4 patients
- The oxygen level was not registered every minute during 6MWT. This deviation was noted for visits of 12 patients
- The post dose assessments were not started at the correct time according to protocol with 3 patients
- The study visit was not performed within timeframes stated in the protocol with 2 patients
- 1 patient received 3 puffs of study medication label A instead of 2
- For 2 patients the physical exam was partially performed
- 1 Patient has a smoking history of 22,5 pack years
- For 1 patient not all the study assessments were performed, questionnaires were missing
- The priming was not performed according to protocol for 3 patients. During the visits there was primed more than 2 times
- During the administration of 1 patient 3 puffs were used as the patient did not inhale the 2nd puff

A detailed list of protocol deviations with subject numbers are provided in section 16.

11 Efficacy Evaluation

11.1 Demographic and Other Baseline Characteristics

The baseline characteristics of the patients, measured at visit 1, are shown in Table 1.

Table 1: Baseline characteristics

9F/7M	Mean	SD	Min	Max
Age [y]	58.88	8.70	44	73
Height [cm]	169.69	9.46	158	192
Weight [kg]	81.16	16.25	55	102.5
BP Sys [mmHg]	121.44	7.70	107	130
BP Dia [mmHg]	79.94	9.65	60	92
HR [bpm]	70.25	12.73	56	102
FVC [L]	4.23	1.25	2.46	6.74
FVC [%p]	120.53	13.20	90.4	141.9
FEV1 [L]	2.97	0.91	1.77	5.27
FEV1 [%p]	104.50	18.87	65.7	133.9
FEV1/FVC [%]	70.95	9.61	41.7	80.6
PEF [L/s]	8.21	2.71	5.59	15.75
MEF50 [L/s]	2.70	1.37	0.9	6.24
FEF75 [L/s]	0.73	0.38	0.23	1.69
FEF25 [L/s]	5.54	2.33	1.95	11.45
RV [L]	2.50	0.75	1.51	4.14
RV [%p]	116.19	23.77	90	170
TLC [L]	6.84	1.88	4.48	10.65
TLC [%p]	114.44	13.79	91	142
FRC [L]	3.45	0.95	2.06	5.36
FRC [%p]	109.88	18.39	77	149
Raw [kPas/L]	0.328	0.130	0.119	0.640
sRaw [kPas]	1.236	0.533	0.560	2.109
6MWT [m]	605.06	75.15	473	735
6MWT [%p]	92.75	11.96	75	118

11.2 Measurements of Treatment Compliance

At visit 1, visit 2 predose and visit 3 predose a training with the placebo for both study drugs was performed. In this way, the patient was taught a correct inhalation technique. All patients were able to use the training device correctly.

11.3 Efficacy Results

11.3.1 Analysis of imaging efficacy

The results for the imaging tests are illustrated in Table 2 and Table 3. In Table 2, baseline stability and differences between test product treatment effect and reference product treatment effect are shown. The changes post treatment for test product and changes post treatment for reference product are given in Table 3. The results indicate significant changes post treatment for all the imaging tests. Significant changes were observed for the test product as well as the reference product post treatment. The difference in treatment effects between the test product and reference product are not statistically significant for the imaging tests.

The images with the results of all individual patients are given in a separate document.

Table 2: Imaging test results for a) differences at baseline, b) differences between 'Test' product treatment effect and 'Reference' product treatment effect

IMAGING TESTS		Pre Test		Pre Reference		p-value	Change Test		Change Reference		p-value
		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
iVaw [cm^3]	Total	52.59	24.10	51.93	23.59	0.14060	3.72	3.36	3.23	1.82	0.39360
	Central	40.72	18.92	40.36	18.26	0.28910	1.39	1.61	1.03	1.17	0.26630
	Distal	11.87	6.04	11.58	6.23	0.18730	2.34	2.12	2.20	1.37	0.69820
iSaw [cm^2]	Total	295.50	89.34	290.24	93.13	0.17060	22.33	17.37	20.25	12.02	0.66030
	Central	129.95	34.29	128.03	32.93	0.28910	3.23	2.68	2.92	3.12	0.33880
	Distal	165.55	60.75	162.22	65.29	0.17060	19.10	15.73	17.32	12.00	0.58720
iRaw [kPas/L]	Total	0.039	0.023	0.040	0.024	0.45340	-0.012	0.013	-0.014	0.013	0.45340
	Central	0.013	0.010	0.014	0.009	0.36550	-0.002	0.003	-0.002	0.003	0.89710
	Distal	0.026	0.017	0.026	0.017	0.77610	-0.010	0.011	-0.012	0.011	0.48510

Table 3: Imaging test results for a) changes post treatment for 'Test' product, b) changes post treatment for 'Reference' product

IMAGING TESTS		Pre Test		Post Test		p-value	Pre Reference		Post Reference		p-value
		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
iVaw [cm^3]	Total	51.93	23.59	55.66	25.85	0.00048	52.59	24.10	55.82	25.16	0.00058
	Central	40.36	18.26	41.74	19.26	0.00176	40.72	18.92	41.75	19.26	0.01048
	Distal	11.58	6.23	13.91	7.61	0.00048	11.87	6.04	14.07	6.67	0.00048
iSaw [cm^2]	Total	290.24	93.13	312.57	103.03	0.00048	295.50	89.34	315.75	94.06	0.00048
	Central	128.03	32.93	131.26	33.92	0.00058	129.95	34.29	132.87	34.53	0.00085
	Distal	162.22	65.29	181.32	74.42	0.00048	165.55	60.75	182.87	64.96	0.00048
iRaw [kPas/L]	Total	0.040	0.024	0.028	0.016	0.00147	0.039	0.023	0.025	0.013	0.00048
	Central	0.014	0.009	0.012	0.007	0.01215	0.013	0.010	0.011	0.007	0.00209
	Distal	0.026	0.017	0.017	0.011	0.00123	0.026	0.017	0.014	0.007	0.00048

11.3.2 Analysis of deposition efficacy

No differences in both inhalation time (Figure 6) and volume (Figure 7) were observed between the test and the reference product.

Deposition results show that the test product and the reference product were not statistically different for deep lung deposition (distal + peripheral airways) for fluticasone and salmeterol. The boxplots and p-values for the statistical analyses are illustrated in Figure 8 and Figure 9, respectively.

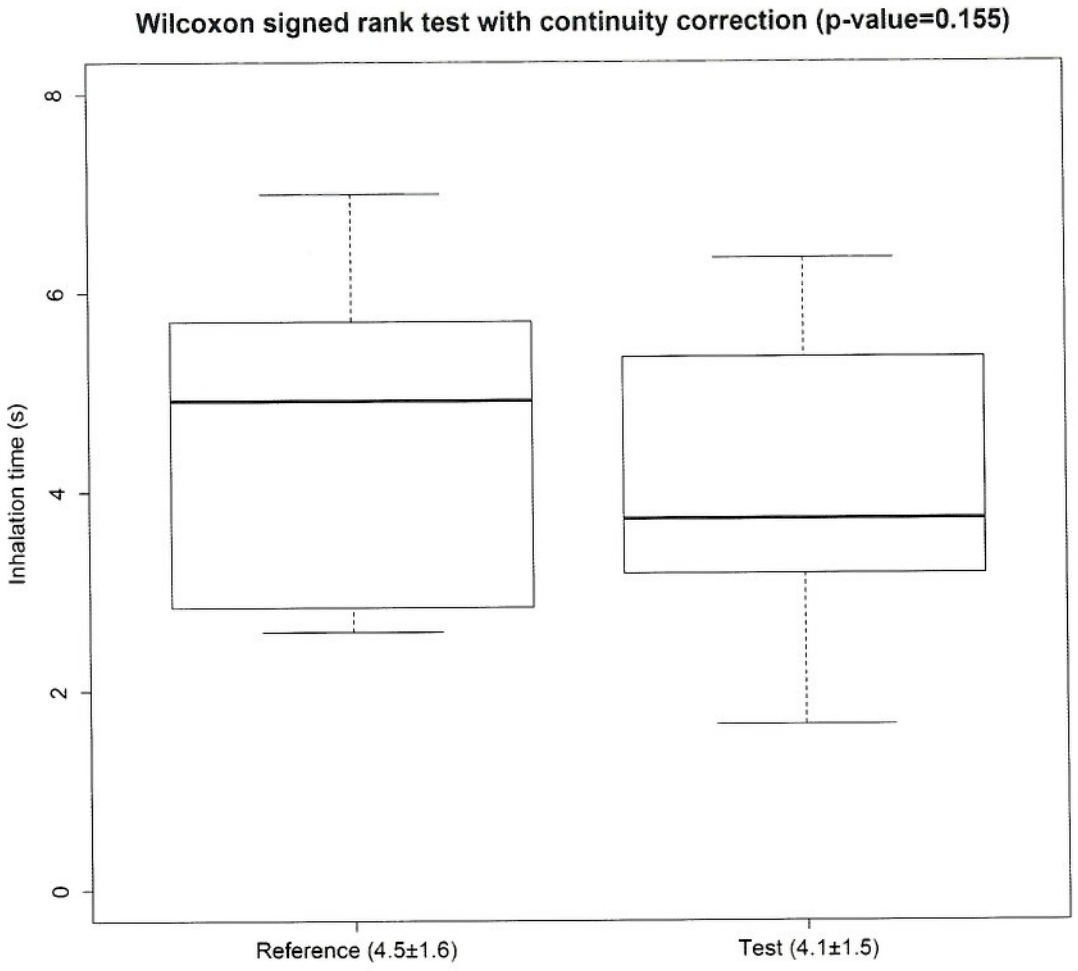


Figure 6: Inhalation time for test and reference products

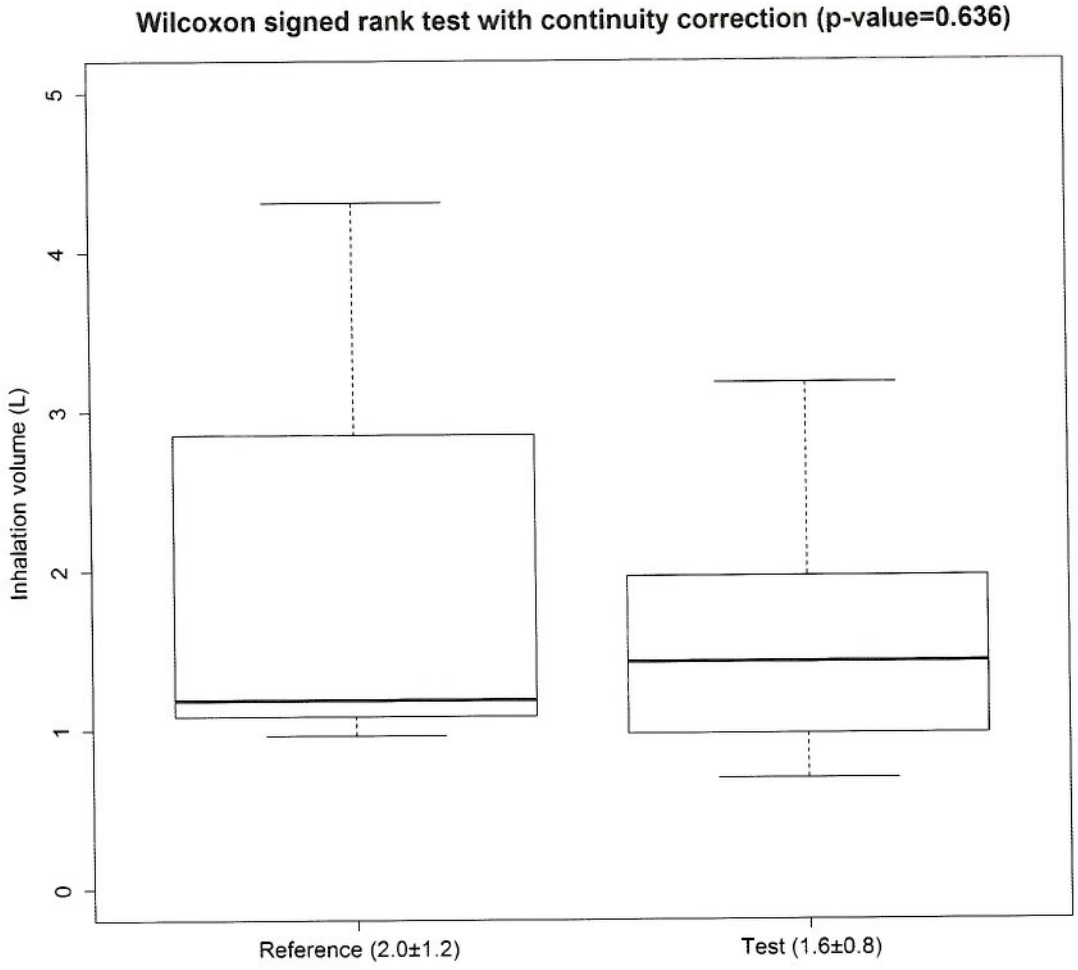


Figure 7: Inhalation volume for test and reference product

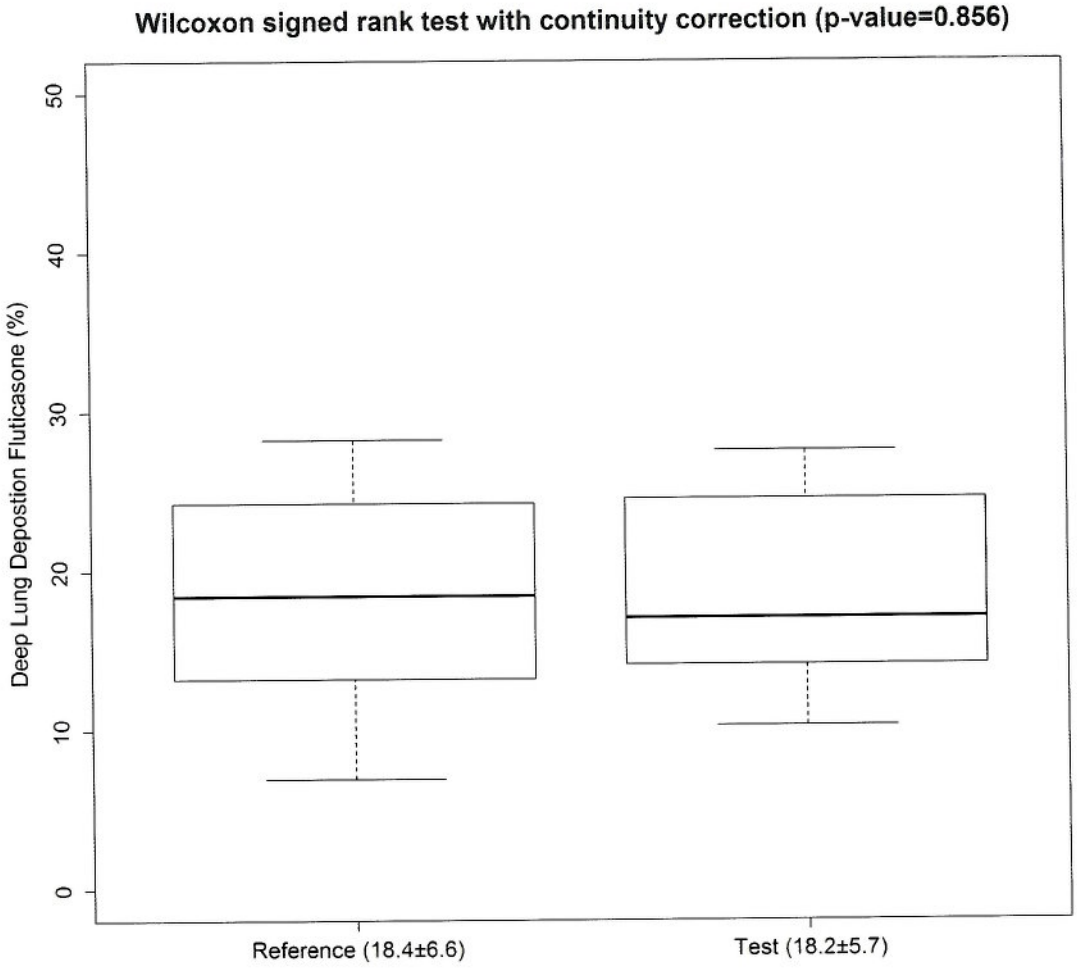


Figure 8: Lung deposition estimates of Fluticasone for test and reference products

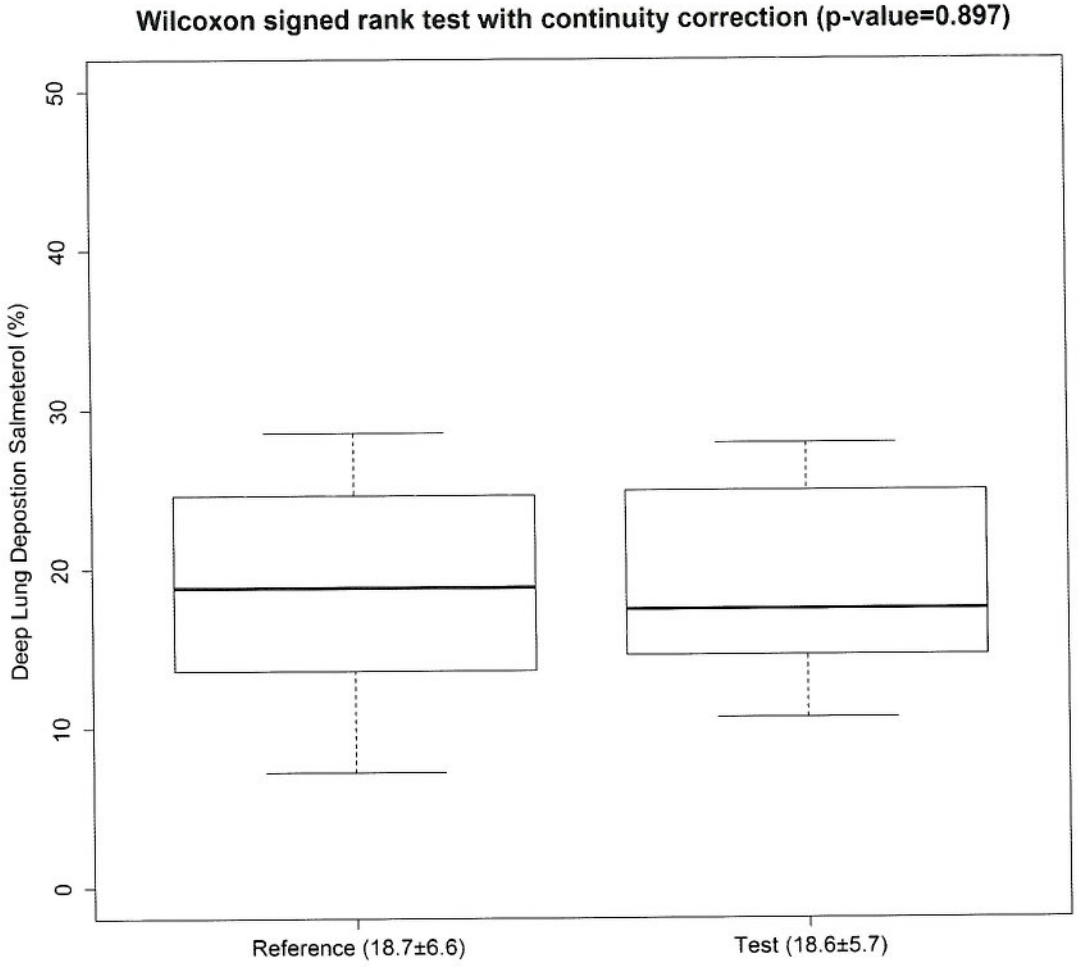


Figure 9: Lung deposition estimates of Salmeterol for test and reference products

11.3.3 Analysis of clinical efficacy

The results for the clinical tests are illustrated in Table 4 and Table 5. In Table 4, baseline stability and differences between test product treatment effect and reference product treatment effect are shown. The changes post treatment for test product and changes post treatment for reference product are given in Table 5. Significant changes were observed for the test product as well as the reference product post treatment except with the FVC and 6MWT. The difference in treatment effects between the test product and reference product were not statistically significant for the clinical tests.

Table 4: Clinical test results for a) differences at baseline, b) differences between 'Test' product treatment effect and 'Reference' product treatment effect.

CLINICAL TESTS	Pre Test		Pre Reference		p-value	Change Test		Change Reference		p-value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
FVC [L]	4.21	1.40	4.20	1.27	0.90950	0.02	0.18	0.10	0.26	0.16390
FVC [%p]	119.49	16.88	120.03	15.48	0.93820	0.28	5.13	2.38	6.53	0.14050
FEV1 [L]	2.92	1.03	2.91	0.96	1.00000	0.13	0.13	0.18	0.13	0.23290
FEV1 [%p]	102.01	21.74	102.32	21.10	0.79590	4.46	4.01	5.99	4.04	0.29340
FEV1/FVC [%]	69.69	9.62	69.71	10.09	0.97730	3.05	2.43	3.10	1.77	1.00000
PEF [L/s]	7.94	2.93	7.90	2.76	0.73680	0.26	0.39	0.32	0.51	0.58720
MEF50 [L/s]	2.62	1.24	2.55	1.27	0.55090	0.41	0.24	0.35	0.33	0.28910
FEF75 [L/s]	0.69	0.34	0.68	0.30	0.60900	0.15	0.16	0.19	0.21	0.24430
FEF25 [L/s]	5.26	2.15	5.22	2.10	0.55200	0.72	0.47	0.74	0.87	0.85640
RV [L]	2.59	0.86	2.58	0.84	0.95880	-0.14	0.20	-0.11	0.27	0.50140
RV [%p]	120.06	28.61	120.25	28.94	1.00000	-6.38	8.52	-4.44	11.77	0.46850
TLC [L]	6.86	1.93	6.83	1.85	0.45330	-0.10	0.13	-0.01	0.17	0.13940
TLC [%p]	114.75	14.19	114.19	13.74	0.54790	-1.50	2.19	-0.19	3.04	0.23210
FRC [L]	3.57	1.09	3.61	1.13	0.53200	-0.21	0.21	-0.20	0.22	0.81590
FRC [%p]	113.69	23.10	114.94	24.79	0.38150	-6.69	6.26	-6.00	6.30	0.73600
R [kPas/l]	0.39	0.19	0.36	0.15	0.23410	-0.07	0.08	-0.14	0.11	0.06636
sR [kPas]	1.54	1.01	1.43	0.89	0.35190	-0.35	0.40	-0.59	0.59	0.08323
6MWT [m]	626.73	57.29	636.67	61.09	0.18410	-4.73	17.02	4.47	11.13	0.05693
6MWT [%p]	96.40	10.13	98.00	11.67	0.16830	-0.80	2.46	0.53	1.92	0.15010
SaO2 [%]	96.64	1.95	97.21	1.25	0.14630	-0.36	1.28	0.21	1.81	0.35070
SaO2_low [%]	93.50	5.26	94.43	3.69	0.23460	-0.64	1.78	-0.21	1.81	0.32370
Borg_Dyspnea [0-10] pre 6MWT	0.80	0.94	0.63	0.97	0.73490	-0.34	0.78	-0.37	0.50	0.67400
Borg_Dyspnea [0-10] post 6MWT	2.40	2.20	2.34	2.28	0.96870	-0.81	1.26	-0.51	1.45	0.34930

Table 5: Clinical test results for a) changes post treatment for 'Test' product, b) changes post treatment for 'Reference' product

CLINICAL TESTS	Pre Test		Post Test		p-value	Pre Reference		Post Reference		p-value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
FVC [L]	4.20	1.27	4.21	1.28	0.82020	4.21	1.40	4.31	1.46	0.15550
FVC [%p]	120.03	15.48	120.30	14.83	0.77610	119.49	16.88	121.87	15.77	0.28910
FEV1 [L]	2.91	0.96	3.04	0.96	0.00058	2.92	1.03	3.09	1.03	0.00058
FEV1 [%p]	102.32	21.10	106.78	19.98	0.00058	102.01	21.74	108.01	20.16	0.00058
FEV1/FVC [%]	69.71	10.09	72.76	10.25	0.00123	69.69	9.62	72.79	10.41	0.00048
PEF [L/s]	7.90	2.76	8.15	2.68	0.01212	7.94	2.93	8.26	2.75	0.02137
MEF50 [L/s]	2.55	1.27	2.96	1.32	0.00085	2.62	1.24	2.97	1.31	0.00103
FEF75 [L/s]	0.68	0.30	0.82	0.42	0.00102	0.69	0.34	0.88	0.48	0.00287
FEF25 [L/s]	5.22	2.10	5.94	2.03	0.00058	5.26	2.15	6.01	2.42	0.00348
RV [L]	2.58	0.84	2.44	0.74	0.02613	2.59	0.86	2.48	0.70	0.17870
RV [%p]	120.25	28.94	113.88	26.25	0.01832	120.06	28.61	115.63	23.04	0.21440
TLC [L]	6.83	1.85	6.73	1.82	0.01507	6.86	1.93	6.85	1.94	1.00000
TLC [%p]	114.19	13.74	112.69	13.63	0.02273	114.75	14.19	114.56	14.41	0.81300
FRC [L]	3.61	1.13	3.40	1.03	0.00270	3.57	1.09	3.38	0.96	0.00209
FRC [%p]	114.94	24.79	108.25	22.85	0.00234	113.69	23.10	107.69	20.29	0.00264
R [kPas/l]	0.36	0.15	0.28	0.12	0.00270	0.39	0.19	0.25	0.09	0.00048
sR [kPas]	1.43	0.89	1.08	0.66	0.00103	1.54	1.01	0.94	0.46	0.00048
6MWT [m]	636.67	61.09	631.93	62.96	0.21080	626.73	57.29	631.20	62.45	0.20850
6MWT [%p]	98.00	11.67	97.20	12.26	0.23480	96.40	10.13	96.93	10.15	0.46400
SaO2 [%]	97.21	1.25	96.86	1.41	0.32750	96.64	1.95	96.86	1.41	1.00000
SaO2_low [%]	94.43	3.69	93.79	4.64	0.21220	93.50	5.26	93.29	5.38	0.71980
Borg_Dyspnea [0-10] pre 6MWT	0.63	0.97	0.28	0.34	0.12500	0.80	0.94	0.41	0.47	0.02225
Borg_Dyspnea [0-10] post 6MWT	2.34	2.28	1.53	1.63	0.03333	2.40	2.20	1.89	2.07	0.38710

12 Safety Evaluation

12.1 Adverse events

During the study there were reported 18 AE`s of which 1 AE was serious. A detailed overview of the AE`s are listed in appendix 1. All the reported AE`s were not related with the study treatment except for AE 1, heart palpations, of patient 2012-94-01 which was possibly related and AE 1, headache of patient 2012-94-002 which was unlikely related with the study treatment. The reason of the serious adverse event (SAE) for patient 2012-94-04 was a hospitalization or prolonged hospitalization due to a pneumonia in the left lung. Further details are given in section 15.

12.2 Clinical Laboratory Evaluation

Laboratory safety reports showed that none of the abnormal laboratory values had clinical relevance.

13 Discussion and Overall Conclusions

The objective of this study was to compare the effects of two different metered dose inhalers containing fluticasone propionate and salmeterol xinafoate. A comparison between the Seretide Evohaler (reference product) and a generic inhaler product from Cipla Ltd. India (test product) was performed in stable asthma patients after the administration of a single dose in a crossover manner. A total of 16 stable asthma patients treated in accordance with the Global Initiative for Asthma guidelines were included and stability checks were performed on each dosing day prior to start of dosing.

The baseline values for both treatment groups showed no significant differences, indicating that there was baseline stability. For both test and reference product, large positive acute effects were observed in both clinical and imaging parameters, meaning that both products lead to a beneficial effect on lung function. FEV1 was found to be the most sensitive clinical marker with a Cohen's d of 1.11 for the test product and a Cohen's d of 1.48 for the reference product. On the imaging side, distal airway volume showed the largest effect size with a Cohen's d of 1.10 for the test product and a Cohen's d of 1.61 for the reference product.

No significant differences were found between the effects of the test product and the effects of the reference product. This finding is consistent for all the parameters: the clinical tests, the patient reported outcomes, the imaging data and the lung deposition estimates. It has to be noted that the p-values are on the conservative side as no correction for multiple testing was performed.

Only two mild adverse events were possibly related to the study medication, but the outcome of the adverse events is that the patients are recovered. One adverse event was not closed during the follow up visit. The study team tried to contact the patient during the monitoring visit, but was unable to contact him. This means that the outcome of this adverse event is unknown. However, the adverse event was not related to the study medication. Furthermore, laboratory safety reports showed that none of the abnormal lab values had clinical relevance.

14 References

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15 Appendix 1

All safety data was clearly reported. A summary can be retrieved underneath.

Subject trial number	Name AE	Start date	Stop date	Relatedness to study treatment	SAE?	Intensity ?
2012-94-01	Heart palpations	2/04/2013	2/04/2013	Possibly	No	Mild
2012-94-02	Headache	3/04/2013	3/04/2013	Unlikely	No	Mild
2012-94-04	Abnormal lab findings	26/04/213	11/07/2013	Not related	No	Moderate
2012-94-04	Pneumonia left lung	15/04/2013	7/05/2013	Not related	Yes	Moderate
2012-94-04	Abnormality CT scan	15/04/2013	7/05/2013	Not related	No	Moderate
2012-94-08	Nose bleed	29/05/2013	3/06/2013	Not related	No	Mild
2012-94-09	Gastro-oesophageal reflux	17/06/2013	25/06/2013	Not related	No	Moderate
2012-94-12	Fracture foot (right): TMS	21/06/2013	5/08/2013	Not related	No	Severe
2012-94-12	Sinusitis	29/06/2013	5/07/2013	Not related	No	Mild
2012-94-12	Abnormal lab findings	12/07/2013	19/07/2013	Not related	No	Mild
2012-94-13	Depression	21/06/213	1/07/2013	Not related	No	Mild

2012-94-13	Abnormal lab findings	5/07/2013	23/07/2013	Not related	No	Mild
2012-94-14	Allergy for latex	16/06/2013	18/06/2013	Not related	No	Mild
2012-94-14	Core biopsy left axilla	27/06/2013	27/06/2013	Not related	No	Mild
2012-94-14	Fungal infection vagina	16/06/2013	22/06/2013	Not related	No	Mild
2012-94-15	Fatigue	7/07/2013	9/07/2013	Not related	No	Mild
2012-94-15	Headache	8/07/2013	8/07/2013	Not related	No	Moderate
2012-94-16	Infected toe	4/07/2013	ongoing	Not related	No	Mild

The reason of the SAE for patient 2012-94-04 was a hospitalisation or prolonged hospitalization due to a pneumonia in the left lung.

16 Appendix 2

Subject specific protocol deviations:

CRF ID Number	Subject trial Number (if applicable)	Subject Initials	Visit Designator	Description of Deviations	Code
94-001	2012-94-01	MLO	visit 1, visit 2	Oxygen level not measured during 6MWT	6
94-001	2012-94-01	MLO	visit 3	Oxygen level not registered every minute during 6MWT predose	6
94-001	2012-94-01	MLO	visit 2	Postdose assessments started 1min too early	6
94-003	2012-94-02	FHE	visit 1, visit 2	Oxygen level not measured during 6MWT	6
94-003	2012-94-02	FHE	visit 3	Oxygen level not registered every minute during 6MWT postdose	6
94-004	2012-94-03	MGO	visit 1	Oxygen level not measured during 6MWT	6
94-004	2012-94-03	MGO	visit 2	Postdose assessments started 2min too early	6
94-004	2012-94-03	MGO	visit 3	Oxygen level not registered every minute during 6MWT	6

				postdose	
94-005	2012-94-04	TVL	visit 3	3 puffs of study medication label A instead of 2	2
94-005	2012-94-04	TVL	visit 2, visit 3	Oxygen level not registered every minute during 6MWT postdose	6
94-006	2012-94-05	DBE	visit 1	physical exam partially performed	6
94-006	2012-94-05	DBE	visit 2	Priming medication label A: 3 puffs instead of 2 as nothing was released during the first puff	2
94-007	2012-94-06	PAR	visit 1	physical exam partially performed	6
94-007	2012-94-06	PAR	Visit 3	Oxygen level not registered every minute during 6MWT predose	6
94-009	2012-94-08	VKO	visit 2, visit 3	Oxygen level not registered every minute during 6MWT postdose	6
94-009	2012-94-08	VKO	visit 3	During 1st attempt administration no medication released so priming was repeated (2 puffs)	2
94-012	2012-94-10	ARO	visit 2	Oxygen level not registered every minute during 6MWT predose	6

94-013	2012-94-11	FAR	visit 1	22,5 pack years	1
94-013	2012-94-11	FAR	visit 2	Oxygen level not registered every minute during 6MWT postdose	6
94-014	2012-94-12	CBA	visit 2, visit 3	Predose: 6MWT not performed, BORG CR10 Dyspnea 2 not performed Postdose: 6MWT not performed, BORG CR10 Dyspnea 2, VAS Dyspnoe 2 not performed	6
94-014	2012-94-12	CBA	visit 2	Priming medication label B: 3 puffs instead of 2 as nothing was released during one of puffs	2
94-014	2012-94-12	CBA	visit 3	visit not performed within timeframes stated in the protocol	10
94-015	2012-94-13	IGO	visit 3	Postdose assessments started 2min too early	6
94-015	2012-94-13	IGO	visit 2	3 puffs of study medication label B instead of 2, second puff was not inhaled by patient	2
94-016	2012-94-14	HDV	visit 2	Oxygen level not registered every minute during 6MWT pre- and postdose	6
94-016	2012-94-14	HDV	visit 3	Oxygen level not registered every	6

				minute during 6MWT predose	
94-017	2012-94-15	JSM	visit 2	Oxygen level not registered every minute during 6MWT postdose	6
94-017	2012-94-15	JSM	visit 3	visit not performed within timeframes stated in the protocol	10
94-018	2012-94-16	JBA	visit 3	Postdose assessments started 29min too early	6
94-018	2012-94-16	JBA	visit 1	Oxygen level not registered every minute during 6MWT	6

Protocol Deviation Code List

1. Inclusion/Exclusion
2. Investigational Product
3. Concomitant Medications
4. Lab
5. Visit Schedule
6. Procedures/Tests
7. Randomization
8. Safety Reporting
9. Protocol Specific Discontinuation Criteria
10. Other