Research Article

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Bioequivalence of Two Formulations of Salmeterol Xinafoate/Fluticasone Propionate HFA pMDI in Healthy Volunteers

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Abstract

In the treatment of asthma, salmeterol xinafoate and fluticasone propionate are known to be effective and well accepted. These studies determined the bioequivalence between the test and reference formulations of salmeterol xinafoate/fluticasone propionate HFA pMDI, in healthy volunteers. Four pharmacokinetic studies were performed with the two higher strengths (25/250 mcg per actuation) and the two lower strengths (25/125 mcg per actuation) of the test and reference formulation. In all studies, the evaluation was based on a single dose, randomized, crossover design with a minimum washout period of 14 days. Out of the four studies, two studies also evaluated pulmonary deposition by blocking gastrointestinal absorption using charcoal administration for each strength. Examinations for safety included monitoring of adverse events and vital signs along with clinical laboratory assessments. A validated LC-MS/MS technique was used to determine the plasma concentrations of salmeterol xinafoate and fluticasone propionate. For the studies without charcoal blockade for salmeterol, the 90% CI for C_{max} and AUC_{0-t} for 25/250 mcg was 83.44-100.29 and 104.08-120.08 respectively, while for 25/125 mcg it was 88.33-106.08 and 100.49-114.88 respectively. Similarly, in the studies with charcoal blockade for salmeterol, the 90% CI for C_{max} and AUC_{0-t} for 25/250 mcg was 94.10-113.20 and 96.44-116.69 respectively, while for 25/125 mcg it was 100.70-115.72 and 104.99-122.70 respectively. For fluticasone, the 90% CI for C_{max} and AUC_{0-t} for 25/250 mcg was 91.08-105.07 and 99.86-115.61 respectively and for 25/125 mcg, it was 87.04-105.03 and 85.38-103.42 respectively. Since the 90% CI for C_{max} and AUC_{0-t} for both salmeterol and fluticasone were within the 80-125% interval in all the studies, it was concluded that test and reference formulations of salmeterol xinafoate/fluticasone propionate HFA pMDI are bioequivalent in their rate and extent of absorption with and without charcoal blockade for both the strengths.

Abbreviations: AE: Adverse event; AUC_{0-t}: Area under the plasma concentration versus time curve from time 0 to time t; AUC_{0-inf}: Area under the plasma concentration versus time curve from time 0 extrapolated to infinity; CFC: Chlorofluorocarbon; C_{max}: Maximum plasma concentration; CI: Confidence interval; CV: coefficient of variation; °C: Degree centigrade; cm: Centimeter; EMA: European Medicine Agency; ECGs: Electrocardiograms; gms: Grams; ≥: Greater than or equal to; GCP: Good clinical practice; GINA: Global Initiative for Asthma; GI: Gastro-intestinal; HFA: Hydrofluoroalkane; hr(s): Hour(s); ICS: Inhaled corticosteroids; K_e: Elimination rate constant; kg(s): Kilogram(s); LC-MS/MS: Liquid Chromatography-Mass Spectroscopy/ Mass Spectroscopy; ≤: less than or equal to; LABA: Long-acting β_2-agonist; LOQ: Lower limit of quantification; L/min: Liters/minute; Min(s): Minute(s); mm: Millimeter; m: Meter; mL: Milli liter; mM: millimol; μl: Microliter; μg: Micro gram; ng/mL: Nano gram/Milliliter; OIPs: Orally inhaled products; pMDI: pressurized metered dose inhaler; %: Percent; PD: pharmacodynamics; PK: Pharmacokinetic; rpm: Rotations per minute; SAE: Serious adverse event; SAS: Statistical analysis software; T_{max}: Time to reach C_{max}; $t_{1/2}$: Elimination half-life; UK: United Kingdom; yr.(s): Year(s).

Introduction

Asthma is an inflammatory airway disease and it causes serious health complications to patients and a massive economic burden on societies [1].

For patients with persistent asthma, inhaled corticosteroids (ICS) have been the first-line treatment regardless of disease severity. Considering the guidelines, patients with asthma who are not sufficiently well controlled with ICS alone (plus a fast-acting bronchodilator used whenever required) should have added a long-acting β_2-agonist (LABA). As reported in the GINA guidelines, the administration of a combination inhaler containing both ICS and LABA in patients with asthma ensures that the LABA is not administered alone [1,2].

Seretide Evohaler (UK) (Salmeterol/fluticasone propionate metered dose pressurised inhalation suspension-reference combination inhaler) is a fixed-dose combination containing a LABA+ICS. Salmeterol is a selective LABA, which causes bronchodilation and inhibition of the release of hypersensitivity mediators from mast cells. The corticosteroid fluticasone propionate inhibits eosinophil activation and the subsequent release of inflammatory mediators [2].

This combination therapy serves as an evident scientific rationale as LABA and ICS may optimize each other's favorable actions in the airways [3].

Cipla Ltd has developed a salmeterol/fluticasone combination delivered by a metered dose inhaler (test combination inhaler).

The product has been developed by following the European Medicine Agency (EMA) guideline on the requirements for clinical documentation for orally inhaled products (OIPs) [4]. According to the guideline, a second entry orally inhaled combination product has to demonstrate therapeutic equivalence with the reference combination product for both active substances of the test combination product. In case therapeutic equivalence cannot be proven based on *in vitro* data, pharmacokinetic (PK) and/or clinical studies are required. We report here the results of four PK studies that evaluated equivalence in pulmonary deposition (lung dose after blocking of the gastro-intestinal (GI) uptake with charcoal) and systemic exposure (without charcoal blockade) after inhalation of a single dose of two formulations of salmeterol/fluticasone.

Materials and Methods

Study drugs

Seretide Evohaler (25 mcg salmeterol/125 mcg fluticasone per inhalation and 25 mcg salmeterol/250 mcg fluticasone per inhalation) were the reference products (hereafter referred to as Seretide Evohaler 25/125 and Seretide Evohaler 25/250). For charcoal block charcoal powder (Carbomix 50 gm, Beacon Pharmaceuticals, UK) was utilized [5].

In all the studies, a single dose of the test formulation of salmeterol xinafoate/fluticasone propionate HFA pMDI 50/500 (25/250 mcg per actuation × 2 puffs) or salmeterol xinafoate/fluticasone propionate HFA pMDI 50/250 (25/125 mcg per actuation × 2 puffs) manufactured by CIPLA LIMITED, INDIA was compared with similar doses of the reference formulation of Seretide™ Evohaler™ supplied by ALLEN AND HANBURYS LTD., UK. The doses assessed with each strength were the recommended doses as mentioned in the summary of product characteristics.

Volunteers

For these studies, healthy male volunteers aged 18-45 years with a body mass index ≥ 18.5 kg/m^2 and ≤ 25.00 kg/m^2, a forced expiratory volume in one second (FEV$_1$) ≥ 80% of predicted normal, and good general health were selected. The volunteers were declared to be healthy based on prior medical history, physical examination, ECG, chest X-ray, pulmonary function test (spirometry), pulse oximetry, and clinical laboratory test results.

Volunteers were excluded from the PK studies if they had known history of hypersensitivity to salmeterol xinafoate or fluticasone propionate or any component of the product, or related class of drug; had history of chronic bronchitis, emphysema, asthma or any other lung disease of clinical significance; had recent upper or lower respiratory tract infection; had consumed drugs that induce/inhibit the hepatic microsomal enzymes two months prior to dosing; and had ingested any herbal product, prescribed or non-prescribed drug four weeks prior to dosing and throughout the study.

Informed and ethical consent

The Independent Ethics Committee (IEC) reviewed the protocol and informed consent forms (ICFs) and approved them before the initiation of the studies. The IEC was Dakshata, an Independent Ethics Committee which approved all the studies. Volunteers were informed in the language they understand about the purpose, nature, procedure, duration, anticipated risks and discomfort of the study. They were given sufficient time to read and understand the ICF and a written informed consent was obtained from each one of them prior to study

participation. All studies were conducted at Sitec Labs, Mumbai India as per the Declaration of Helsinki, Good Clinical Practice guidelines and national regulatory guidelines [6-9].

Study design

This report summarizes the four PK studies comparing the two strengths of the test combination inhaler with similar strengths of the reference combination inhaler. For each strength, lung deposition of salmeterol was assessed by blocking gut absorption using charcoal blockade. The charcoal regimen used to block the GI absorption of salmeterol was as follows: immediately before study drug administration (2 minutes prior to the first puff), the mouth was thoroughly rinsed with 50 mL (approximately 5 gm) of charcoal suspension before swallowing. This was repeated at 2 minutes after the first puff, followed by 100 mL (approximately 10 gm) of charcoal suspension at 1.00, 2.00 and 3.00 hours post-dose. The method of charcoal administration has been validated in a study conducted by Bennett et al. [10].

Fluticasone propionate has negligible oral bioavailability (<1%) due to a combination of incomplete absorption from the gastro-intestinal tract and extensive first-pass metabolism, therefore systemic exposure arises only from pulmonary absorption i.e. lungs [11-13]. The amount of drug that reaches the blood via absorption from the lungs is same as the total bioavailability of the drug. Therefore, use of oral charcoal blockade to block the gastrointestinal absorption of inhaled fluticasone propionate for comparison of the pulmonary bioavailability of the test product and the reference product was not required.

The systemic exposure of the active moieties was assessed in the PK studies without charcoal blockade. All studies were single dose, randomized, crossover studies. The studies consisted of a screening period, two or four treatment days separated by at least 14-day wash-out periods, and an end-of-study visit occurring at least 14 days after the last study treatment administration.

Studies with 25/250 mcg strength: The pivotal Study-1 (four-way, replicate design) evaluated lung deposition of salmeterol after a single dose 50/500 mcg (administered as 2 puffs of 25/250 mcg/puff) in 42 healthy volunteers. This study utilized a replicate design with a large sample size to take into account the potential for a high coefficient of variation that can be observed for the PK parameters for SM in charcoal blockade studies. Further, since the use of charcoal is unlikely to have a significant effect on the magnitude of bioavailability of fluticasone due to its negligible oral bioavailability, fluticasone was not evaluated in this study.

In the pivotal study-2 (two-way design), 74 healthy male volunteers were studied after a single dose of 50/500 mcg (administered as 2 puffs of 25/250 mcg/puff) on each of the treatment days (without charcoal blockade). This study evaluated the systemic exposure of both salmeterol and fluticasone and also the lung deposition of fluticasone.

Studies with 25/125 mcg strength: The pivotal Study-3 was a randomized, single dose, four-way crossover pharmacokinetic study in 80 volunteers to compare the test product with the reference product with and without charcoal administration. A single dose of 50/250 mcg (administered as 2 puffs of 25/125 mcg/puff) was administered. As the oral bioavailability of fluticasone propionate is very low (<1%), fluticasone propionate was only evaluated in the treatment arms without charcoal administration while salmeterol was evaluated both with and without charcoal administration.

The pivotal Study-4 (four-way, replicate design) evaluated lung

deposition of salmeterol after a single dose 50/250 mcg (administered as 2 puffs of 25/125 mcg/puff) using charcoal blockade in 72 healthy volunteers. This study was conducted as the earlier pivotal study-3 showed high variability in the treatment arms using charcoal blockade.

In all studies the volunteers were trained in the correct use of the inhalers at the screening visit and before each study drug administration. They were trained on the inhalation technique with the help of an in-check dial, aerosol inhalation monitors and a placebo (inactive) inhaler. The volunteers were carefully instructed by the trainer on the inhalation technique as described in the manufacturer's leaflet.

Study drug administration

The test and the reference products were primed within 10 minutes prior to dosing by releasing 2 test sprays, away from the volunteer's blood sample tubes or supplies. After priming, the test or the reference product was inhaled; there was a gap of at least 30 seconds between each puff inhaled by the volunteer. The treatments were self-administered by the volunteers after an overnight fast of at least 10 hours in each period under the supervision of the trained and qualified pharmacist, quality assurance personnel, quality control personnel and the sponsor's monitor. Study volunteers were confined to the study facility from at least 12 hr prior to dosing until at least 24 hr after dosing. During housing, post-dose meals were identical for all the periods of the study. Lunch, snacks and dinner were served at 4.0, 9.0 and 13.0 hours, respectively, after dosing. Water was not permitted from 1 hour before dosing until 1 hour following dosing, but it was allowed thereafter.

The test and reference formulations of salmeterol xinafoate/fluticasone propionate HFA pMDI were stored in a pharmacy under controlled conditions of temperature ($22 \pm 3°C$) and at 50 to 60% relative humidity and the conditions were monitored continuously.

Blood sampling

The samples of blood for salmeterol xinafoate/salmeterol xinafoate and fluticasone propionate test, were collected via an indwelling catheter (intra-venous) with respect to start time of first puff in vacutainers containing (dipotassium ethylene diamine tetraacetic acid) K_2EDTA anticoagulant.

The content and the blood sample in the vacuum collection tubes are mixed well by inverting them gently, after the collection of the blood. Tubes containing blood samples were immediately placed in an iced water bath at approximate temperature below 12°C till they were centrifuged. The blood sample tubes were centrifuged to separate plasma as soon as possible at 3000 rpm for 10 minutes in a centrifuge set at a temperature of 8°C. The plasma samples were divided in two portions (main and reserve). Then plasma samples were stored at -70°C or below until sample analysis.

Blood samples for the determination of fluticasone and salmeterol concentrations in plasma were drawn before the administration of the study treatments and up to 36 h after drug administration (except in pivotal study-1 where the sampling was up to 18 h post dose).

The sampling time points (hours: minutes) were at -1.00 h (predose) and after the administration of the study drugs at: 0.05, 0.08, 0.17, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 14.00, 16.00, 18.00, 24.00 and 36:00 hr post dose. In pivotal study-1 the sampling time points post dose excluded 0.75, 1.25, 1.50, 1.75, 2.50, 3.00 and 24 h. Since, this was the first study to be conducted with charcoal blockade assessing only salmeterol, hence the intermediate time points relevant to fluticasone (0.75, 12.5, 1.50,

1.75, 2.50 and 3 h) were not included. Further, since lower plasma concentrations were expected due to charcoal blockade, sampling for salmeterol was done only till 18 hours.

Fluticasone and salmeterol concentrations in plasma were determined by separate, validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods at Sitec Labs, Mumbai, India.

The volunteers were required to refrain from consuming any food and beverages containing xanthine or alcohol (48 h before dosing and for 24 h after each dose), grapefruit (7 days before dosing and throughout the study), or vitamins (throughout the confinement period). On check in day, at least 12 hr prior to each dosing, all volunteers were screened for drugs of abuse (cocaine, cannabinoids, benzodiazepines, Opioids, Amphetamines, and barbiturates) by urine test, and for alcohol consumption by breath alcohol test.

Pharmacokinetic analysis

As primary markers of efficacy and safety, the following variables were calculated from concentration-time curves for fluticasone and salmeterol after study drug administration: the maximum observed concentration of concentration-time curve (C_{max}) and the area under the concentration-time curve from time zero to the last sample with quantifiable drug concentration (AUC_t) calculated with the linear trapezoidal rule. The secondary PK parameters were the area under the concentration-time curve from time zero to infinity (AUC_∞) determined by adding AUC_t to the extrapolated area that was determined dividing the last quantifiable concentration by K_e (K_e = the terminal elimination rate constant from log-linear portion of a concentration-time curve), the time to reach the maximum concentration (t_{max}), and the terminal elimination half-life ($t_{1/2}$) calculated with the equation $\ln_2/K_e$. The same PK variables as above were calculated in all the 4 studies. The PK parameters were calculated by a non-compartmental method using the WinNonlin version 3.6 computer program. The actual time of sampling was used in the calculations. The zero time was the start of the first inhalation of the active study treatment.

Clinical safety was assessed by monitoring adverse events, physical examination and vital signs as well as performing clinical laboratory tests.

Statistical analysis

The determination of sample size for individual studies was based on previous studies with the developmental formulations of fluticasone/salmeterol product. The reported variability of salmeterol was the highest for the primary parameters and was therefore used in the sample size calculations. It was assumed that the expected ratio of means would be 0.95-1.1 in order to demonstrate that the 90% CI for bioequivalence in a crossover study design will be within 80-125% with 90% power at a 5% level of significance. Per protocol (PP) data set was used when comparing the PK results.

The PP data set excluded all the subjects who discontinued, had a major protocol deviation, or insufficient number of PK samples for the calculation of reliable PK parameters.

The primary PK variables C_{max} and AUC_t, were analyzed using analysis of variance (ANOVA). The responses were modeled using logarithmic transformations. By taking exponential back-transformations, the results were returned to the original scale, yielding the ratio of geometric means and their 90% confidence intervals (CIs). These CIs were evaluated against the conventional BE region from 0.80 to 1.25. The secondary PK variables were AUC_∞, AUC_∞ was analyzed

in the same way as C_{max} and AUC_t. Wilcoxon Signed Rank Sum Test for paired samples was used for analysis of T_{max}. All statistical analyses were performed with SAS* for Windows release 9.3 (SAS Institute Inc., Cary, NC, USA).

Randomization and Blinding techniques

The volunteers were randomized to the test and reference group by using SAS software. All four studies were open-label, where the investigators knew the type of the formulations administered in each study period. However, the randomization list was not available to the bio-analytical team at Sitec Labs until the analysis was completed.

Analytical methods

The concentrations of Fluticasone and Salmeterol in the plasma samples of the subjects were determined using separate validated LC-MS/MS based bio-analytical methods in accordance with the principles of Good Laboratory Practice (GLP). The bio-analytical methods were developed and validated at Sitec Labs (P) Ltd. as per the international guidelines [14,15]. Fluticasone and Salmeterol were extracted from human plasma using Solid Phase Extraction (SPE) procedure and injected into the liquid chromatograph coupled with tandem MS/MS detector. To avoid bias, the analyst did not have access to the randomization code. Samples for any given subject for all time points were assayed under similar chromatographic conditions that were validated for the analysis of salmeterol/fluticasone in human plasma. For both the analytical methods, the validation parameters were system suitability, carry over test, specificity and selectivity, matrix effect (post-extraction addition and post-column infusion), sensitivity, linearity (calibration curve), precision and accuracy, ruggedness, haemolysis effect, recovery, stability under different conditions, plasma dilution integrity and re-injection reproducibility.

All the analytical methods were validated according to the latest regulatory guidelines (CDER 2013 and EMA 2012).

Fluticasone assay: Fluticasone propionate was extracted from plasma samples (500 µl) using SPE method involving C18 cartridges. Plasma samples were spiked with internal standard and subsequently 500 µl of 30% methanol was added to each sample. These samples were loaded on cartridges and washed with a mixture of acetonitrile and water followed by elution with acetonitrile. The eluted solvent was then mixed with 2 mM Ammonium trifluoroacetate buffer and samples were analysed on LC-MS/MS. The LC-MS/MS comprised of Shimadzu UFLC (LC) and Sciex 5500 (MS/MS) triple quadrapole mass spectrometer. A monolithic RP18 column was used with a mobile phase comprising of acetonitrile and buffer. The flow rate of mobile phase was 1.0 mL/min and the chromatographic run time was 2.0 min. The mass spectrometer was operated in positive ionization mode and Fluticasone and internal standard (Fluticasone D3) were monitored using MRM transition 501.20/293.20 and 504.20/313.20 respectively. The lower

limit of quantitation was 2.0 pg/mL and calibration standards ranged from 2.0 to 250 pg/mL.

Four precision and accuracy sets (P & A set) were analysed during method validation and each P & A set consisted of seven QCs each at five different concentration levels (LLOQ QC, Low QC, Mid QC - A, Mid QC-B & High QC). The within batch precision (%CV) ranged from 1.38 to 19.18% and the within batch accuracy (%Nominal) ranged from 90.00% to 110.00%. The between batch precision (%CV) ranged from 2.39% to 14.55% and the between batch accuracy (%Nominal) ranged from 96.72% to 105.00%. The between batch precision and accuracy during the study are presented in Table 1.

For 25/250 mcg, the performance of analytical method was monitored during the study using quality control samples at concentrations 6.0, 30, 100 and 200 pg/mL.

For the 25/125 mcg, the first ten subjects were analysed with calibration range of 2.0 to 250 pg/mL and based on the concentration data obtained the calibration range was modified to 2.0 to 100 pg/mL for the remaining part of the study. The performance of analytical method was monitored during the study using quality control samples at concentrations 6.0, 30, 100 and 200 pg/mL for calibration ranged 2.0 to 250 pg/mL and 6.0, 20, 55 and 85 pg/mL for the calibration range of 2.0 to 100 pg/mL. The performance of the Fluticasone assay method during pivotal studies is summarized in Table 1.

During study sample analysis, quality control samples were distributed throughout each batch. Samples for any given subject for all time points were assayed under similar chromatographic conditions that were validated for the analysis of Fluticasone in human plasma.

Salmeterol assay: Salmeterol was extracted from plasma samples using SPE method involving mixed mode anion exchange cartridges. Plasma samples were spiked with internal standard and subsequently 500 µl of 100 mM Ammonium acetate was added to each sample. These samples were loaded on cartridges and washed with a mixture of methanol and water followed by elution with 20% acetonitrile. The eluted samples were analysed on LC-MS/MS. The LC-MS/MS comprised of Shimadzu UFLC (LC) and Sciex 5500 (MS/MS) triple quadrapole mass spectrometer.

The analytical column used in the bioanalytical method for the 25/250 mcg study (Study-1) was ACE 3 C18 (100 mm, 3 mm) column. The mobile phase comprised of mixture of Acetonitrile, 5 mM ammonium trifluroacetate buffer and isopropyl alcohol. The flow rate of mobile phase was 0.5 mL/min and the chromatographic run time was 3.2 min. The mass spectrometer was operated in positive ionization mode and Salmeterol and internal standard (Salmeterol D3) were monitored using MRM transition 416.30/232.10 and 419.30/235.20 respectively.

The lower limit of quantitation was 2.0 pg/mL and calibration standards ranged from 2.0 to 2000 pg/mL. Four precision and accuracy

	Between batch precision (% CV)	Between batch accuracy (%Nominal)	Calibration standard range	Incurred sample reanalysis: (%)
25/250 mcg (Dose 50/500 mcg)				
During Pivotal study 2	3.10 to 8.05	99.67 to 100.79	2.0 to 250 pg/mL	91.96
25/125 mcg (Dose 50/250 mcg)				
During study- 3	3.84 to 6.55	93.08 to 94.06	2.0 to 250 pg/mL (for first 10 subjects)	84.58
During study- 3	5.47 to 8.20	98.36 to 101.86	2.0 – 100 pg/mL (subject number 11 - 80)*	
* A partial validation was performed for the modified calibration range prior to continuing analysis from subject number 11				

Table 1: Precision and Accuracy of batches during the analytical run of Fluticasone.

sets (P & A set) were analysed during method validation and each P & A set consisted of seven QCs each at five different concentration levels (LLOQ QC, Low QC, Mid QC - A, Mid QC-B & High QC). The within batch precision (%CV) ranged from 0.85 to 7.87% and the within batch accuracy (%Nominal) ranged from 96.44% to 105.00%. The between batch precision (%CV) ranged from 1.86% to 6.12% and the between batch accuracy (%Nominal) ranged from 98.31% to 100.00%. The between batch precision and accuracy during the study are presented in Table 2.

The performance of analytical method was monitored during the with charcoal study was monitored using quality control samples at concentrations 6.0, 60, 500 and 1500 pg/mL for calibration ranged 2.0 to 2000 pg/mL and 6.0, 50, 100 and 400 pg/mL for the calibration range 2.0 to 500 pg/mL.

The analytical method was subsequently modified to improve the chromatography and reduce the run time. The analytical column used in the bioanalytical method for the 25/250 mcg study (without charcoal pre-treatment- Study-2) was Chromolith RP18 (100 mm, 3 mm) column. The mobile phase comprised of mixture of Acetonitrile, 5 mM ammonium trifluroacetate buffer and isopropyl alcohol. The flow rate of mobile phase was 0.8 mL/min and the chromatographic run time was 2.0 min. The mass spectrometer was operated in positive ionization mode and Salmeterol and internal standard (Salmeterol D3) were monitored using MRM transition 416.30/232.10 and 419.30/235.20 respectively. The performance of analytical method was monitored during the study using quality control samples at concentrations 6.0, 50, 100 and 400 pg/mL. During study sample analysis, quality control samples were distributed throughout each batch.

In the study-3, the performance of analytical method was monitored during the study using quality control samples at concentrations 6.0, 50, 100 and 400 pg/mL. The analytical method was modified to change the concentration of quality control samples in order to validate the unknown concentration in better manner for the charcoal pre-treatment study. There was no change in the extraction, chromatographic and mass spectrometric method.

In the study-4, the performance of analytical method was monitored during the study using quality control samples at concentrations 6.0, 25, 50 and 400 pg/mL. The performance of the Salmeterol assay method during the studies is summarized in Table 2.

Results

Volunteer disposition and baseline demographic characteristics

In study-1, a total of 42 volunteers were recruited, but only 39 volunteers completed the study. 3 volunteers were drop-outs in period-4 for personal reasons. Data of remaining 39 volunteers was considered for pharmacokinetic and statistical analysis.

In study-2, a total of 74 volunteers were recruited, but only 66 volunteers completed the study. 8 volunteers were drop-outs in period-2 for personal reasons. Leakage of drug was observed during dosing for 10 volunteers. One volunteer was excluded from fluticasone analysis due to pre-dose concentration >5% of C_{max} value reported for period-1. Therefore, data of remaining 56 volunteers was considered for salmeterol xinafoate; and 55 volunteers was considered for fluticasone propionate for pharmacokinetic and statistical analysis except for volunteers who were dropped out or discontinued from the study before dosing of period-2.

In study-3, a total of 80 volunteers were recruited, but only 72 volunteers completed the study. 7 volunteers were drop-outs for personal reasons. 1 volunteer was discontinued due to AE. Leakage of drug was observed during dosing for 9 volunteers. Data of remaining 63 volunteers was considered for pharmacokinetic and statistical analysis of salmeterol xinafoate and fluticasone propionate without charcoal.

In study-4, a total of 72 volunteers were recruited, but only 69 volunteers completed the study. 2 volunteers were drop-outs for personal reasons. 1 volunteer was discontinued due to AE. Leakage of drug was observed during dosing for 1 volunteer. 1 volunteer was dropped from the statistical analysis due to poorly characterized concentration profile (having less than four (<4) consecutive non-zero concentration data points over the entire sampling duration for all periods). Data of remaining 68 volunteers was considered for pharmacokinetic and statistical analysis of salmeterol xinafoate with charcoal. The demographic data of all recruited volunteers for all the 4 studies are presented in Table 3.

The blood samples were collected up to 18 hrs post dose for study-1. Mean plasma concentration profile of salmeterol xinafoate with charcoal over the 18-hour pharmacokinetic study is presented in Figure 1 (with charcoal FPSM 25/250 mcg study-1). The blood samples were collected up to 36 hrs post dose for study-2. Mean plasma concentration profile of salmeterol xinafoate without charcoal is presented in Figure 2 and for fluticasone propionate without charcoal is presented in Figure 3 over the 36-hour pharmacokinetic study (without charcoal FPSM 25/250 mcg study-2). The blood samples were collected up to 36 hrs post dose for study-3. Mean plasma concentration profile of salmeterol xinafoate without charcoal is presented in Figure 4 and fluticasone propionate without charcoal are presented in Figure 5 over the 36-hour pharmacokinetic study (with and without charcoal FPSM 25/125 mcg study-3). The blood samples were collected up to 36 hrs post dose for study-4. Mean plasma concentration profile of salmeterol

	Between batch precision (% CV)	Between batch accuracy (%Nominal)	Calibration standard range	Incurred sample reanalysis: (%)
25/250 mcg (Dose 50/500 mcg)				
During study- 1 (with charcoal)	1.43 to 4.08%	96.72 to 100.09%	2.0 – 2000 pg/mL (for 1st 9 subjects analysed)	93.75%
During study- 1 (with charcoal)	1.79 to 6.29%	99.60 to 100.89%	2.0 – 500 pg/mL (remaining 33 subjects)	
During study- 2 (without charcoal)	2.61 to 10.45%	96.72 to 101.36%	2.0 – 500 pg/mL	87.14%
	Between batch precision (% CV)	Between batch accuracy (%Nominal)	Calibration standard range	Incurred sample reanalysis: (%)
25/125 mcg (Dose 50/250 mcg)				
During study- 3 without charcoal	3.37 to 8.13%	95.08 to 100.82%	2.0 to 500 pg/mL.	87.50%
During study- 4 with charcoal	2.30 to 8.97%	95.96 to 99.52%	2.0 to 500 pg/mL.	95.36%

Table 2: Precision and Accuracy of batches during study of Salmeterol.

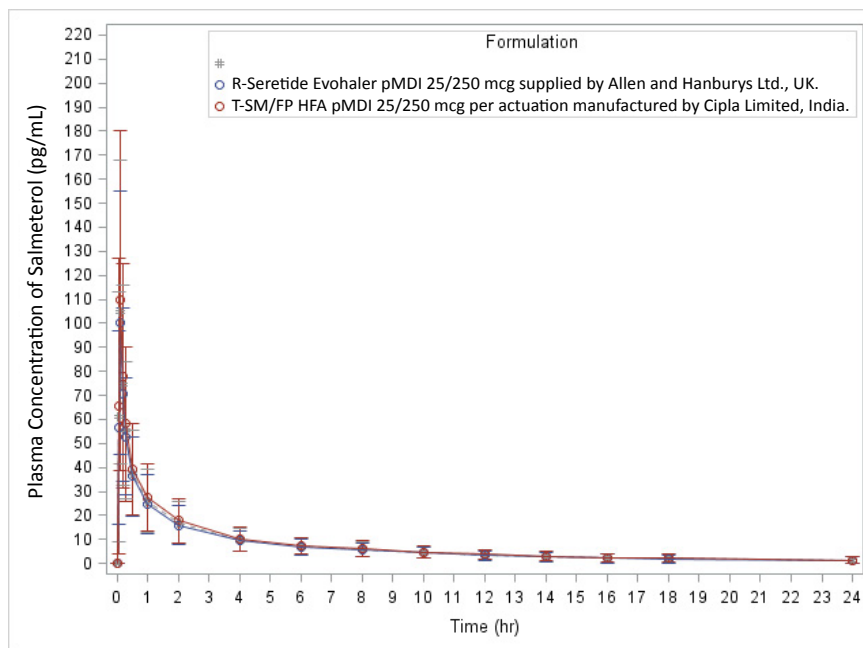|	**Study-1**	**Study-2**	**Study-3**	**Study-4**
Number of volunteers	42	74	80	72
Mean Age ± SD (yrs)	28 ± 6	28 ± 6	27 ± 6	28 ± 5
Mean Weight ± SD (kg)	64.0 ± 7.6	61.8 ± 6.2	64.9 ± 6.9	63.8 ± 6.4
Mean Height ± SD (m)	1.69 ± 0.06	1.67 ± 0.05	1.69 ± 0.06	1.68 ± 0.04
Mean BMI ± SD (Kg/m^2)	22.3 ± 2.3	22.1 ± 2.1	22.7 ± 1.9	22.7 ± 2.0

Table 3: Demographic data.

Figure 1: Mean graph (linear) for plasma concentration vs. time profile of Salmeterol xinafoate after inhalational dose of Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 50/500 mcg in Study-1 (Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 25/250 mcg per actuation with charcoal).
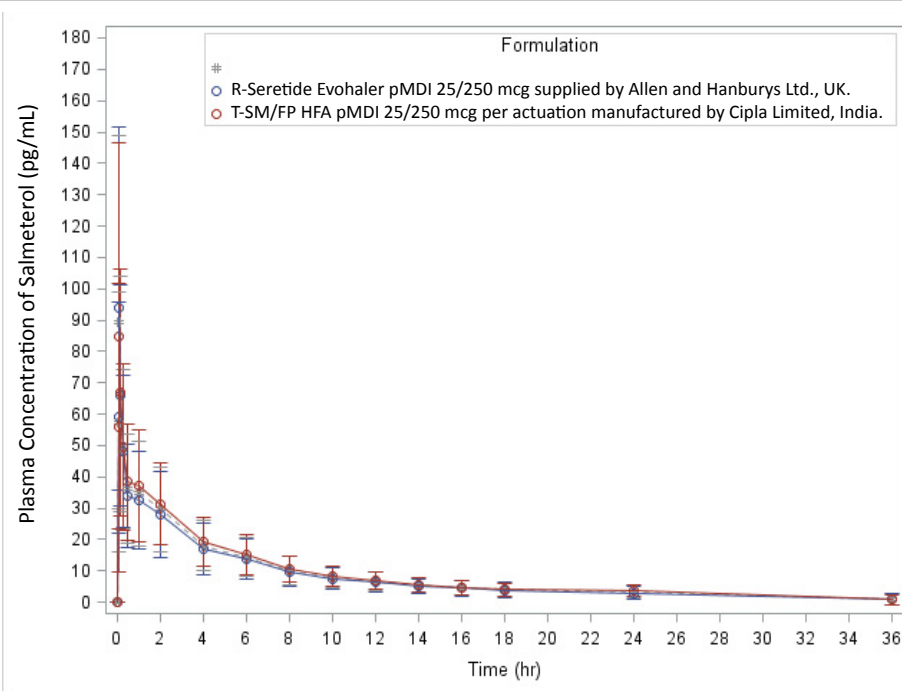

Figure 2: Mean graph (linear) for plasma concentration vs. time profile of Salmeterol xinafoate after inhalational dose of Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 50/500 mcg in Study-2 (Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 25/250 mcg per actuation without charcoal).

Figure 3: Mean graph (linear) for plasma concentration vs. time profile of Fluticasone propionate after inhalational dose of Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 50/500 mcg in Study-2 (Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 25/250 mcg per actuation without charcoal).
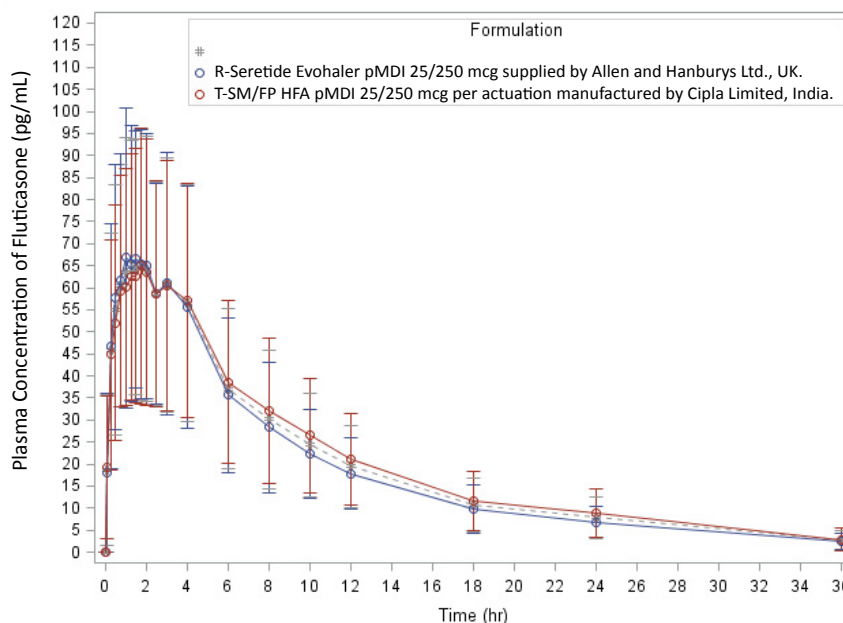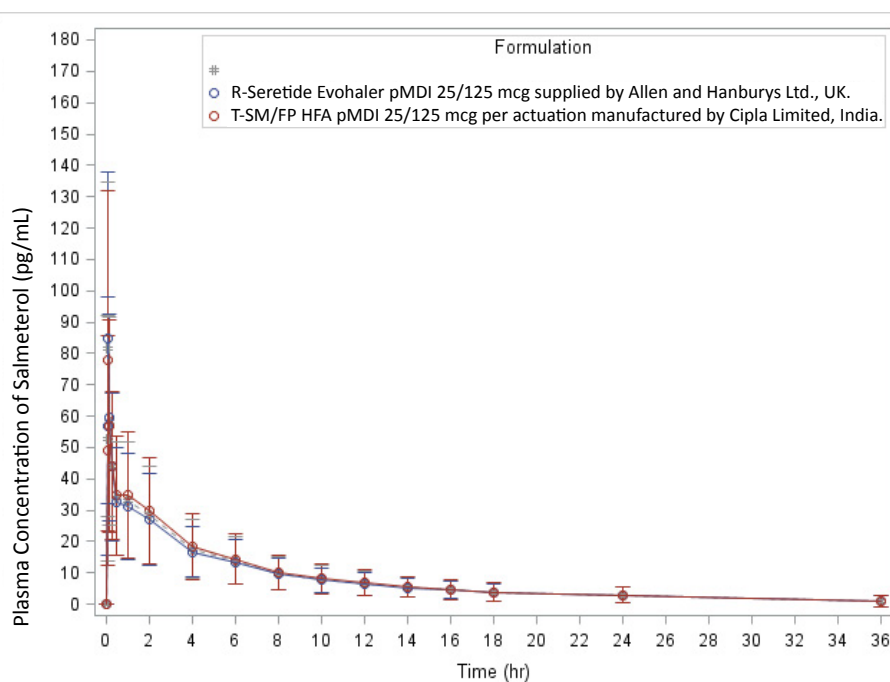

Figure 4: Mean graph (linear) for plasma concentration vs. time profile of Salmeterol xinafoate after inhalational dose of Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 50/250 mcg in Study-3 (Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 25/125 mcg per actuation with and without charcoal).

xinafoate with charcoal over the 36-hour pharmacokinetic study is presented in Figure 6 (with charcoal FPSM 25/125 mcg study-4). These figures suggest comparable mean plasma concentration-time curves for reference-test formulation corresponding to each study.

The mean ratios of $AUC_{0-t}/AUC_{0-\infty}$ for all volunteers were found to be more than 80%, indicating that blood samples collected adequately characterized the pharmacokinetic profile of the drug. The statistical results of the pharmacokinetic parameters of salmeterol xinafoate and
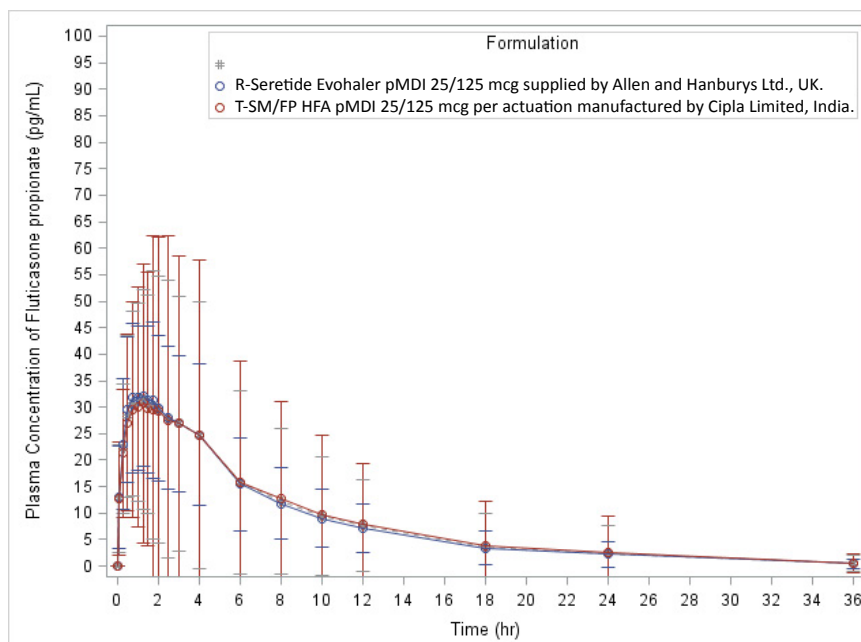
Figure 5: Mean graph (linear) for plasma concentration vs. time profile of Fluticasone propionate (without charcoal) after inhalational dose of Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 50/250 mcg in Study-3 (Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 25/125 mcg per actuation with and without charcoal).
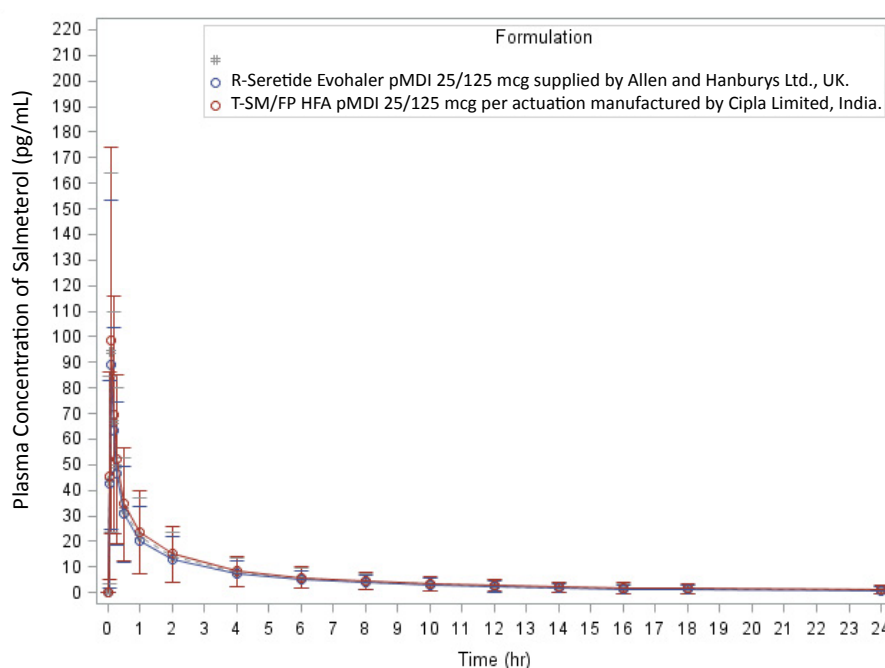

Figure 6: Mean graph (linear) for plasma concentration vs. time profile of Salmeterol xinafoate after inhalational dose of Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 50/250 mcg in Study-4 (Salmeterol xinafoate/Fluticasone propionate (SM/FP) HFA pMDI 25/125 mcg per actuation with charcoal).

fluticasone propionate (for all studies) are presented in Tables 4 and 5 respectively. The geometric mean ratios, 90% CI, and intra subject coefficient of variation of test and reference for Ln transformed pharmacokinetic parameters C_{max}, and AUC_{0-t} for salmeterol xinafoate and fluticasone propionate for all studies are presented in Tables 6 and 7 respectively. In the 2 pivotal PK studies with each strength,

the absorption of fluticasone was equivalent to the reference product (Seretide Evohaler, UK) after administration. For salmeterol, in all the 4 pivotal PK studies (with and without charcoal blockade), almost all 8 of the 10 primary parameters fulfilled the pre-specified bioequivalence (BE) criteria (except for salmeterol in the third pivotal PK study due to very high variability in the charcoal arms).

Pharmacokinetic Parameters	Salmeterol xinafoate (study-1)		Salmeterol xinafoate (study-2)		Salmeterol xinafoate (study-3)		Salmeterol xinafoate (study-4)	
	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)
N	78	78	56	56	63	63	68	68
C_{max} (pg /ml)	104.11 ± 54.80	114.67 ± 74.86	90.97 ± 60.06	96.19 ± 57.08	85.20 ± 55.20	89.55 ± 53.91	105.91 ± 75.47	94.26 ± 63.49
$AUC_{0-0.50}$ (hr.pg/ml)	30.28 ± 17.04	27.71 ± 12.49	25.69 ± 14.17	25.54 ± 13.03	24.46 ± 13.29	24.57 ± 13.01	27.55 ± 16.63	24.57 ± 14.42
AUC_{0-t} (hr.pg /ml)	149.64 ± 68.33	163.85 ± 80.82	290.36 ± 122.04	263.73±129.68	296.37 ± 168.72	275.17 ± 147.62	153.03 ± 109.37	128.21 ± 89.51
$AUC_{0-\infty}$ (hr.pg /ml)	180.74 ± 85.37	195.46 ± 94.40	345.76 ± 151.31	310.86 ± 147.21	342.29 ± 192.47	325.89 ± 177.27	190.65 ± 137.90	162.21 ± 113.73
*T_{max} (hr)	0.08 (0.05-0.50)	0.08 (0.05-0.50)	0.08 (0.05 −2.00)	0.08 (0.05 −2.00)	0.08 (0.08-2.00)	0.08 (0.05-2.00)	0.08 (0.08 −0.52)	0.08 (0.07 −0.50)
K_{el} (1/hr)	0.115 ± 0.049	0.125 ± 0.122	0.085± 0.041	0.084 ± 0.040	0.078 ± 0.032	0.078 ± 0.037	0.195 ± 0.503	0.180 ± 0.317
$T_{1/2}$ (hr)	7.23 ± 3.47	7.03 ± 3.13	11.36 ± 8.46	10.82 ± 6.43	10.57 ± 4.65	11.34 ± 5.60	10.72 ± 15.11	9.85 ± 10.40

*Median (range)

Table 4: The statistical results of primary pharmacokinetic parameters of Salmeterol xinafoate for Study-1 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/250 mcg per actuation with charcoal); Study-2 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/250 mcg per actuation without charcoal); Study-3 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/125 mcg per actuation with and without charcoal) and Study-4 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/125 mcg per actuation with charcoal) are presented.

Pharmacokinetic Parameters	Fluticasone propionate (Study-2)		Fluticasone propionate (Study-3)	
	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)
N	55	55	63	63
C_{max} (pg /ml)	74.09 ± 32.27	76.50 ± 34.50	39.75 ± 37.15	38.79 ± 16.29
AUC_{0-t} (hr.pg /ml)	713.02 ± 337.41	652.39 ± 301.32	295.37 ± 435.55	275.32 ± 159.19
$AUC_{0-\infty}$ (hr.pg /ml)	771.55 ± 359.04	703.05 ± 320.30	330.04 ± 454.81	302.03 ± 164.25
*T_{max} (hr)	1.50 (0.25 −4.02)	1.25 (0.25 − 4.00)	1.00 (0.25-4.00)	1.00 (0.25-4.00)
K_{el} (1/hr)	0.083 ± 0.023	0.085 ± 0.023	0.120 ± 0.057	0.119 ± 0.045
$T_{1/2}$ (hr)	8.97 ± 2.59	8.82 ± 2.65	7.03 ± 3.46	6.60 ± 2.31

*Median (range)

Table 5: The statistical results of primary pharmacokinetic parameters of Fluticasone propionate for Study-2 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/250 mcg per actuation without charcoal), and Study-3 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/125 mcg per actuation with and without charcoal) are presented.

Pharmacokinetic Parameters	Geometric Mean		*(%)T/R	90% Confidence Interval	Intra subject CV%
	Test	Ref			
N (Study -1)	78	78	-	-	-
C_{max} (pg/mL)	93.73	90.82	103.21	94.10-113.20	34.20**
AUC_{0-t} (hr.pg/mL)	138.76	130.80	106.08	96.44-116.69	38.99**
N (Study-2)	56	56	-	-	
C_{max} (pg/mL)	75.24	82.25	91.47	83.44-100.29	29.60
AUC_{0-t} (hr.pg/mL)	265.59	237.57	111.79	104.08-120.08	22.82
N (Study -3)	63	63	-	-	
C_{max} (pg/mL)	72.03	74.26	96.80	88.33-106.08	31.45
AUC_{0-t} (hr.pg/mL)	255.39	237.06	107.44	100.49-114.88	22.73
N (Study -4)	68	68	-	-	-
C_{max} (pg/mL)	84.19	77.99	107.95	100.70-115.72	28.09**
AUC_{0-t} (hr.pg/mL)	109.39	96.38	113.50	104.99-122.70	32.78**

*(%) T/R is ratio of Test Geometric Mean/Ref Geometric Mean
**intra-subject variability for reference product

Table 6: The Geometric mean ratios, 90% CIs, power and intra subject coefficient of variation of test and reference for Ln transformed pharmacokinetic parameters C_{max}, and AUC_{0-t} for Salmeterol xinafoate of Study-1 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/250 mcg per actuation with charcoal); Study-2 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/250 mcg per actuation without charcoal); Salmeterol xinafoate of Study-3 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/125 mcg per actuation without charcoal) and Study-4 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/125 mcg per actuation with charcoal) are presented.

In the pivotal study-3, for salmeterol with charcoal administration, both C_{max} and AUC_{0-t} for salmeterol with charcoal were marginally outside the accepted bioequivalence range of 80-125% (90% CI for C_{max} was 77.23 - 94.94; 90% CI for AUC_{0-t} was 71.25 - 96.11). However, it is important to note that the % CV was high (>36% for C_{max} and >55% for AUC_{0-t}) for both the test and reference products in the presence of oral charcoal blockade. As a result, there was inadequate power for the salmeterol PK bioequivalence assessments in the presence of oral

Pharmacokinetic Parameters	Geometric Mean		*(%)T/R	90% Confidence Interval	Intra subject CV%
	Test	Ref			
N (Study -2)	55	55	-	-	
C_{max} (pg/mL)	67.83	69.34	97.83	91.08-105.07	22.60
AUC_{0-t} (hr.pg/mL)	631.67	587.89	107.45	99.86-115.61	21.84
N (Study -3)	63	63	-	-	
C_{max} (pg/mL)	33.60	35.07	95.61	87.04-105.03	32.31
AUC_{0-t} (hr.pg/mL)	217.69	231.10	93.97	85.38-103.42	32.99

*(%) T/R is ratio of Test Geometric Mean / Ref Geometric Mean
**intra-subject variability for reference product

Table 7: The Geometric mean ratios, 90% CIs, power and intra subject coefficient of variation of test and reference for Ln transformed pharmacokinetic parameters C_{max}, and AUC_{0-t} for Fluticasone propionate of Study-2 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/250 mcg per actuation without charcoal) and Study-3 (Salmeterol xinafoate/Fluticasone propionate HFA pMDI 25/125 mcg per actuation with and without charcoal) are presented.

Study No (Number of subjects)	Strength	Number of volunteers reporting AEs (Number of AEs)	Number of Adverse Events	
			Test product (T)	Reference product (R)
Study 1 (N=42)	25/250	11 (19)	12	7
Study 2 (N=74)	25/250	11 (14)	8	6
Study 3 (N=80)	25/125	22 (28)	13	15
Study 4 (N=72)	25/125	15 (16)	9	7
Total		59 (77)	42	35

Table 8: Incidence of adverse events pooled from all the 4 studies.

charcoal blockade, particularly for AUC_{0-t}. Hence, definitive conclusions regarding salmeterol PK bioequivalence between the test and reference product in the presence of oral charcoal blockade could not be made on the basis of this study. Hence, assessment of bioequivalence for salmeterol with charcoal blockade was done in the fourth pivotal PK study with a much larger sample size.

The secondary parameters T_{max} and $T_{1/2}$ were comparable between the test and the reference products in all studies. The median fluticasone t_{max} varied from 1.00 to 1.50 h and median salmeterol T_{max} was 0.08 h in different studies irrespective of the product. Mean $T_{1/2}$ for fluticasone varied from 7.03 to 8.97 h and mean $T_{1/2}$ for salmeterol from 7.03 to 11.36 h irrespective of the product. The obtained $AUC\infty$ values were in line with the corresponding AUC_{0-t} values.

There were no safety issues in any of the studies and no serious AEs were reported. AE profiles were similar after both inhalers (Table 8). Most of the adverse events were mild to moderate intensity.

No deaths/ serious adverse event occurred during conduct of all the four studies. No clinically relevant changes were observed during vital signs examination, ECGs, and post-study clinical laboratory data. All volunteers were medically fit in post-study safety assessment.

Discussion

All 4 PK studies were conducted to compare the pulmonary deposition and total systemic exposure of the 2 strengths of fluticasone/salmeterol MDI (Cipla Ltd) with the reference product. All studies were single dose, randomized crossover studies.

In accordance with the OIP guidance, PK studies in healthy volunteers were performed which together with the required *in vitro* investigations formed the basis of test product fluticasone/salmeterol HFA MDI marketing authorizations in Europe. The findings of studies in healthy volunteers can be bridged to patients as the MDI device do not have flow rate dependency characteristics.

Generally, there is good agreement between PK and *in vitro* data

which aids in product development and helps ensure that the relative clinical efficacy and safety of the given drug formulation will be comparable to that of the reference product [16,17].

PK studies can differentiate between systemic absorption (surrogate of safety) and lung absorption (surrogate of efficacy) using validated methodologies such charcoal blockade. For a PK study with charcoal blockade, or for a product with very low oral bioavailability, AUC is considered to be a direct reflection of the dose that reaches the lungs and subsequently passes from the lung epithelium into the systemic circulation. This is reflected in PK profiles as evident from studies with large versus small particle formulations of an inhaled drug [17]. Therefore a PK study can effectively evaluate relative lung distribution for an OIP through the standard AUC and C_{max} parameters.

The established relationship between dose and lung/systemic exposure is generally dose proportional for both LABAs and ICSs. By contrast, the clinical efficacy dose response for both ICSs and LABAs are very flat and successive doses which result in marked increases in AUC and C_{max} are not generally associated with any appreciable differences in efficacy. Therefore efficacy measures are very insensitive for detecting difference between corresponding doses of a test and reference product.

It has been shown that the same doses of a test and reference product which have comparable systemic exposure profiles also have comparable efficacy profiles [18]. However even when significant difference in the PK profiles are present when the same doses of a test and reference product are administered, no appreciable clinical differences are observed [19,20]. Hence, PK studies, unlike clinical efficacy studies are more sensitive to identify differences between formulations and assess equivalence.

In all the PK studies, the systemic exposure and pulmonary deposition of fluticasone/salmeterol MDI (Cipla Ltd, India) was equivalent to the reference product (Allen and Hanburys, UK).

Conclusions

The 90% CI (T/R) for salmeterol xinafoate and fluticasone propionate

for both C_{max} and AUC_{0-t} was within 80.00-125.00% with and without charcoal blockade, suggesting that both the formulations of salmeterol xinafoate/fluticasone propionate HFA pMDI are bioequivalent in their rate and extent of absorption for both the strengths. Since the test product has been shown to have equivalent pulmonary absorption and systemic exposure as that of the reference product, it is expected to have equivalent efficacy and safety as well.

Acknowledgement

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