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Bioequivalence of Ipratropium Bromide HFA pMDI 20 µg/actuation in Healthy Volunteers with and without Charcoal Blockade; and with Spacer Device

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Abstract

Ipratropium Bromide is a short-acting (lasting for 6-8 h) anticholinergic bronchodilator used in the management of chronic obstructive pulmonary disease (COPD). The aim of these three studies was to determine the bioequivalence of test and reference formulations of Ipratropium Bromide HFA pMDI 20 µg/actuation with and without charcoal blockade; and with spacer device. Study-1 was single dose, randomized, 4-period, 2-sequence, laboratory-blinded, crossover, replicate design conducted in 90 healthy volunteers under fasting conditions with concurrent oral charcoal blockade with a washout period of 7-14 days. Study-2 was single dose, randomized, 2-period, 2-sequence, laboratory-blinded, crossover design conducted in 24 healthy volunteers under fasting conditions without concurrent oral charcoal blockade with a washout period of 6 days. Study-3 was single dose, randomized, 2-period, 2-sequence, laboratory-blinded, crossover design conducted in 64 healthy volunteers under fasting conditions with Aero Chamber Plus valved holding chamber with a washout period of 7-10 days. Blood samples were collected up to 24 h post-dose for pharmacokinetic profiling. Safety evaluations included monitoring adverse events and vital signs as well as performing clinical laboratory tests. Plasma concentrations of Ipratropium were determined with a validated LC-MS/MS method. The 90% CI of Ipratropium were 91.30-99.91, and 90.42-97.77 for C_{max}, and AUC_{0-t} respectively for study-1. The 90% CI of Ipratropium were 87.33-121.30, and 88.94-120.34 for C_{max}, and AUC_{0-t} respectively for study-2. The 90% CI of Ipratropium were 87.21-99.83, and 91.66-97.94 for C_{max}, and AUC_{0-t} respectively for study-3. Since the 90% CI for C_{max} and AUC_{0-t} were within the 80-125% interval, it was concluded that test and reference formulation of Ipratropium Bromide HFA pMDI 20 µg per actuation are bioequivalent in their rate and extent of absorption with and without charcoal blockade; and with spacer device.

Keywords: Ipratropium bromide; Metered dose inhaler, Inhalational; Charcoal blockade; Spacer device; Bioequivalence; Pharmacokinetics

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; COPD: Chronic Obstructive Pulmonary Disease; CFC: Chlorofluorocarbon; C_{max}: Maximum Plasma Concentration; CI: Confidence Interval; CV: Coefficient of Variation; °C: Degree Centigrade; cm: Centimeter; ECGs: Electrocardiograms; gms: Grams; ≥: Greater than or Equal to; GCP: Good Clinical Practice; HFA: Hydrofluoroalkane; h(s): Hour(s); K_e: Elimination Rate Constant; kg(s): Kilogram(s); LC-MS/MS: Liquid Chromatography-Mass Spectroscopy/Mass Spectroscopy; ≤ Less than or Equal to; 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; 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; yr.(s): Year(s)

Introduction

Chronic obstructive pulmonary disease (COPD) is a lung disease that includes the conditions chronic bronchitis and emphysema. COPD is mainly caused by smoking or inhaling dust, which leads to blockage or narrowing of the airways. The symptoms include breathlessness and a chronic cough. Ipratropium bromide is a short-acting anticholinergic, with effects lasting six to eight hours. It is a non-selective muscarinic antagonist and therefore blocks M2 receptors as well as M1 and M3 receptors in airway smooth muscle and prevents the increases in intracellular concentrations of cyclic guanosine monophosphate (cyclic GMP). M2 receptors at cholinergic nerve endings inhibit the release of acetylcholine and therefore act as inhibitory feedback receptors. Inhibition of these receptors with ipratropium bromide results in increased acetylcholine release in the airways, which may overcome the blockade of other muscarinic receptors in the muscle [1,2].

The aim of these three studies was to evaluate the rate and extent of absorption of generic formulation of Ipratropium Bromide HFA pMDI 20 µg/actuation against that of innovator formulation (ATROVENT® CFC-free (containing ipratropium bromide 20 µg per actuation) marketed by BOEHRINGER INGELHEIM LIMITED, UK), with concurrent oral charcoal blockade, without concurrent oral charcoal blockade and with AeroChamber Plus valved holding chamber under fasting conditions in order to assess bioequivalence.

A single dose of Ipratropium Bromide HFA pMDI 80 µg (20 µg/actuation X 4 puffs) was evaluated in study-1 with concurrent oral charcoal blockade.

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A single dose of Ipratropium Bromide HFA pMDI 80 µg (20 µg/actuation X 4 puffs) was evaluated in study-2 without concurrent oral charcoal blockade.

A single dose of Ipratropium Bromide HFA pMDI 40 µg (20 µg/actuation X 2 puffs) was evaluated in study-3 with AeroChamber Plus valved holding chamber.

Materials and Methods

Volunteers

A total of 90, 24 and 64 Indian adult male non-smoker volunteers, between 18 and 45 years of age (inclusive), having body mass index ≥ 18.5 kg/m^2 and ≤ 25.00 kg/m^2, in general good health were enrolled in study-1, 2 and 3 respectively. The demographics of 90, 24 and 64 recruited volunteers of study-1, 2 and 3 respectively are summarized in Table 1.

Screening of the volunteers was done within 21 days prior to recruitment. Before inclusion into the study, the volunteers were judged to be healthy by a GCP certified physician based on previous medical history, physical examination, ECG, chest X-ray, pulmonary function test (spirometry), pulse oximetry, and clinical laboratory test results.

Informed consent and ethical approval

The protocol was approved by an independent ethics committee prior to study initiation. The volunteers were informed about the study, verbally and in writing, and written consent was obtained from all the volunteers before participation. The volunteers were free to withdraw from the study at any time without providing a reason. These studies were conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with all International Conference on Harmonization Good Clinical Practice Guidelines and national regulatory guidelines [3-6]. These studies were conducted from October to December, 2014 (Ipratropium Bromide HFA pMDI 80 µg with charcoal study), December, 2011 (Ipratropium Bromide HFA pMDI 80 µg without charcoal study), and August to September, 2015 (Ipratropium Bromide HFA pMDI 80 µg with spacer study).

Study design

Study-1 was single dose, randomized, 4-period, 2-sequence, laboratory-blinded, crossover, replicate design conducted in 90 healthy volunteers each under fasting conditions with concurrent oral charcoal blockade with a washout period of 7-14 days.

Study-2 was single dose, randomized, 2-period, 2-sequence, laboratory-blinded, crossover design conducted in 24 healthy volunteers each under fasting conditions without concurrent oral charcoal blockade with a washout period of 6 days.

Study-3 was single dose, randomized, 2-period, 2-sequence, laboratory-blinded, crossover design conducted in 64 healthy volunteers each under fasting conditions with Aero Chamber Plus valved holding chamber with a washout period of 7-10 days.

In study-1 and 2, a single dose of test formulation of Ipratropium Bromide HFA pMDI 80 µg (20 µg per actuation X 4 puffs) was compared with reference formulation of ATROVENT® CFC-free 80 µg (20 µg per actuation X 4 puffs) marketed by BOEHRINGER INGELHEIM LIMITED, UK. In study-3, a single dose of test formulation of Ipratropium Bromide HFA pMDI 40 µg (20 µg per actuation X 2 puffs) was compared with reference formulation of ATROVENT® CFC-free 40 µg (20 µg per actuation X 2 puffs) marketed by BOEHRINGER INGELHEIM LIMITED, UK.

Preparation of charcoal slurry

Measured 400 mL water was added to Carbomix bottle (containing 50 g of activated charcoal) and shaken thoroughly. Prepared suspension was poured in an empty bottle with lid and measuring scale. Measured 50 mL water added to Carbomix bottle to rinse residual charcoal. Again measured 50 mL water added to Carbomix bottle to rinse residual charcoal. After rinsing, residual slurry added to bottle with lid and measuring scale. This total 500 mL activated charcoal suspension (containing 50 g of activated charcoal) was stored below 25°C and was used for the study within 24 h.

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). Volunteers were excluded if they took prescription medications or over-the-counter products including herbal products within the 14 days prior to the study drug dosing and also during the study. Exclusion criteria included a history of drugs of abuse, heavy alcohol consumption, active smoking, and inability to use metered dose inhaler satisfactorily.

On check in day, at least 12 h 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. In the study-1, a total of 90 volunteers who fulfilled all the criteria for inclusion were admitted to the study center in the evening before dosing. Study-1 was conducted in three batches (Batch A, Batch B and Batch C). Batch A consisted of 42 volunteers (subject No. 01 to 42), batch B consisted of 18 volunteers (subject No. 43 to 60), and batch C consisted of 30 volunteers (subject No. 61 to 90). In the study-2, a total of 24 volunteers each who fulfilled all the criteria for inclusion were admitted to the study center in the evening before dosing. In the study-3, a total of 64 volunteers who fulfilled all the criteria for inclusion were admitted to the study center in

	Ipratropium 80 mcg (with charcoal)				Ipratropium 80 mcg (without charcoal)				Ipratropium 40 mcg (with spacer)			
	Age (yrs)	Weight (kgs)	Height (m)	BMI (Kg/m^2)	Age (yrs)	Weight (kgs)	Height (m)	BMI (Kg/m^2)	Age (yrs)	Weight (kgs)	Height (m)	BMI (Kg/m^2)
Number of observations	90	90	90	90	24	24	24	24	64	64	64	64
Mean	28	64.9	1.68	23.0	28.04	63.69	1.68	22.38	28	65.4	1.68	23.0
Standard Deviation	6	6.8	0.05	1.9	6.09	7.79	0.06	1.89	5	6.7	0.05	1.8
Median	27	65.8	1.67	23.8	27	62.8	1.68	22.55	27	65.3	1.68	23.7
Minimum	19	51.0	1.49	18.6	21	51.4	1.60	18.8	20	51.8	1.58	18.9
Maximum	43	81.2	1.81	24.9	41	83.1	1.83	24.8	41	86.3	1.87	24.8

Table 1: The demographics of all recruited volunteers in Ipratropium 80 mcg with charcoal, Ipratropium 80 mcg without charcoal and Ipratropium 40 mcg with spacer studies are summarized.

the evening before dosing. Study-3 was conducted in two batches (Batch A and Batch B). Batch A consisted of 32 volunteers (subject No. 01 to 32), and batch B consisted of 32 volunteers (subject No. 33 to 64). On the check-in day, volunteers' belongings were thoroughly checked for any restricted items. Volunteers wore uniform provided by Sitec for the housing period. Then, they were assigned to each treatment sequence as per the randomization scheme. Test and reference formulations of Ipratropium Bromide HFA pMDI 20 µg per actuation were stored in a pharmacy under controlled conditions of temperature (20 ± 2°C) and 40 to 50% relative humidity and were monitored continuously.

Drug administration

The investigational product was primed within 10 minutes prior to dosing by releasing 2 test sprays into the cardboard box, away from the volunteer's blood sample tubes or supplies and away from the analytic laboratory. After priming, investigational product was inhaled; there was a gap of one minute with an allowed deviation of ± 5 s between each puff inhaled by the volunteer and the dosing supervisor was notifying the volunteer when it is time to begin the next inhalation. The volunteers were supervised for the inhalation technique during the dosing activity.

In study-1, all the volunteers received doses of Ipratropium Bromide HFA pMDI 80 µg (20 µg per actuation X 4 puffs) of test or the reference formulation on the dosing day. While volunteer was in standing position, a single dose of 4 puffs (each puff releases 20 µg of Ipratropium Bromide) of the test or the reference product were inhaled by the volunteer as per the randomized sequence in the standing position on four separate treatment days. 50 mL (approximately 5 g) of activated charcoal suspension was given 2 min prior to the 1st puff, immediately after dosing and also at 1.00, 2.00 and 4.00 h post-dose as per the method described by Bennett et al. [7]. Volunteers were instructed to rinse their mouth with the activated charcoal suspension before swallowing, to make sure the buccal mucosa is coated with charcoal (before dosing only).

In study-2, all the volunteers received doses of Ipratropium Bromide HFA pMDI 80 µg (20 µg per actuation X 4 puffs) of test or the reference formulation on the dosing day. While volunteer was in standing position, a single dose of 4 puffs (each puff releases 20 µg of Ipratropium Bromide) of the test or the reference product were inhaled by the volunteer as per the randomized sequence in the standing position on two separate treatment days.

In study-3, all the volunteers received doses of Ipratropium Bromide HFA pMDI 40 µg (20 µg per actuation X 2 puffs) of test or the reference formulation on the dosing day. While volunteer was in standing position, a single dose of 2 puffs (each puff releases 20 µg of Ipratropium Bromide) of the test or the reference product were inhaled by the volunteer with the aid of the Aero Chamber Plus valved holding chamber as per the randomized sequence in the standing position on two separate treatment days.

The treatments were self-administered by the volunteers after an overnight fast of at least 10 h in each period under the supervision of the trained and qualified pharmacist, quality assurance personnel, quality control personnel and the sponsor's monitor. The time at which all puffs were administered was captured in the respective case record forms. Time of first puff was considered as time zero for all post-dose activities.

Volunteers were trained on the inhalation technique with the help of an in-check dial, aerosol inhalation monitor and a placebo (inactive) inhaler at least for 5 days continuously prior to dosing. Volunteers were instructed to inhale at a flow rate of 30-60 L/min (this was checked using the in-check dial). The volunteers were carefully instructed by the trainer on the inhalation technique as described in the manufacturer's leaflet. The inhalation technique was checked on check-in day of all the periods before check-in and prior to dosing. The inhalation technique performance was documented for each volunteer. The key points emphasized while training for correct inhalation technique were: complete exhalation before beginning of inhalation; Ensuring a firm seal with the lips around the device mouth piece; For metered dose inhaler, the most important thing is co-ordination of actuation and inhalation; After complete exhalation, volunteers were asked to breathe in slowly and deeply at least for 5 s; The device should be actuated while the inhalation is going on; After this the volunteers should be asked to hold his breath at least for 10 s and then breath out normally through the nose.

Study volunteers were confined to the study facility from at least 12 h prior to dosing until at least 24 h after dosing. Each dosing period was separated by 7-14 days for with charcoal study; 6 days for without charcoal study and 7-10 days for with spacer study. Volunteers remained seated at least for the first 2 h after dosing. During housing, post-dose meals were identical for both periods of the study. Lunch, snack and dinner were served at 4.0, 9.0 and 13.0 h, respectively, after dosing. Water was not permitted from 1 h before dosing until 1 h following dosing, but it was allowed at all other times.

Safety evaluations included monitoring adverse events and vital signs as well as performing clinical laboratory tests. All adverse events that occurred during the study were documented. Volunteers were questioned about any symptoms or unexpected occurrences during the study. All adverse events, regardless of severity or relationship to the study drug, were recorded in the case report forms. The principal investigator or clinical-investigator was present at the site throughout the study.

Blood sampling

In study-1 and 2, Blood samples (1 × 5 mL) were collected at -0.00 h (pre-dose) and at 0.08, 0.17, 0.25, 0.33, 0.42, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 9.00, 12.00, 18.00 and 24.00 h post dose. In study-3, blood samples (1 × 6 mL) were collected at -0.00 h (pre-dose) and blood samples (1 × 5 mL) were collected at 0.017, 0.05, 0.08, 0.17, 0.25, 0.33, 0.42, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 9.00, 12.00, 18.00 and 24.00 h post dose. Blood samples for ipratropium analysis were collected via an indwelling catheter (intra-venous) with respect to start time of first puff in vacutainers containing sodium heparin anticoagulant. After blood collection, vacuum collection tubes were inverted gently several times to ensure the mixing of tube content and blood sample. 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 min 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.

Randomization and blinding

The volunteers were randomized into the test and reference group by using SAS software. The study was an open-label, where the investigators knew the type of the formulations administered at each

study phase. However, the randomization list was not available to the bio-analytical team of Sitec until the analysis was completed.

Analytical methods

Bio-analysis of ipratropium (with charcoal study-1, and without charcoal study-2) was performed using high-performance liquid chromatography with mass spectrometry detection (LC-MS/MS) based method. The bio-analytical method for estimation of Ipratropium from human plasma was developed and validated as per the international guidelines [8,9]. Ipratropium was extracted from human plasma using Solid Phase extraction technique. Solid phase extraction was performed using Stratta X-CW 1 cc cartridges. Extracted samples were injected into the liquid chromatograph coupled with tandem MS/MS detector; LC: Agilent 1200; MS/MS: API 4000. Separation between matrix and analyte/internal standard was achieved by reverse phase chromatography using Thermo Hypersil BDS C8 column.

Bio-analysis of ipratropium (with spacer study-3) was performed using LC-MS/MS based method. Ipratropium was extracted from human plasma using Solid Phase extraction technique. Solid phase extraction was performed using Waters WCX 1 cc cartridges. Extracted samples were injected into the liquid chromatograph coupled with tandem MS/MS detector; LC: Shimadzu UFLC XR; MS/MS: API 5500. Separation between matrix and analyte/internal standard was achieved by reverse phase chromatography using ACE 3C18 PFP column.

Quantitation was performed using Internal Standard method. Ipratropium-d3-iodide was used as the Internal Standard. Ipratropium and IS were monitored by LC-MS/MS in the MRM with positive polarity mode using the mass transitions 332.30/166.20, 332.30/124.20 amu and 335.40/169.00, 335.40/127.00 amu respectively. A weighted linear regression using weighting 1/concentration^2 was prepared to determine concentration of Ipratropium in human plasma.

The lowest limit of quantitation of method was 3.0 pg/ml for the method used for analysis with and without charcoal blockage studies and 1.0 pg/ml for the spacer study. The calibration standards ranged from 3.0 pg/ml to 180 pg/ml for the analysis of ipratropium (with charcoal study-1, and without charcoal study-2). The calibration standards ranged from 1.0 pg/ml to 115 pg/ml for the analysis ipratropium (with spacer study-3). The calibration curve was linear and regression coefficient (r value) was greater than 0.995. Matrix effect was evaluated by performing post-extraction addition and post-column infusion experiment and results of both experiments were within acceptance criteria. The extraction recovery was greater than 80%. Analyte was found stable in plasma at room temperature for 6 h and three freeze-thaw cycles did not alter the concentration significantly. Post-preparative stability was evaluated for 52 h at room temperature and refrigerator temperature and was found stable. Long-term stability at -70°C was evaluated for 190 days and the analyte was found stable.

Pharmacokinetic analysis

The following PK parameters were calculated using validated PK software (WinNonlin version 6.3 for study-1; WinNonlin version 5.3 for study-2; WinNonlin version 6.4 for study-3), namely, maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under the plasma concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) and the total area under the plasma concentration-time curve ($AUC_{0-\infty}$). These parameters were derived from the plasma concentration-time data. C_{max} and T_{max} were obtained directly from the plasma values, while AUC_{0-t} was calculated using the trapezoidal formula and $AUC_{0-\infty}$ was obtained by dividing the last measurable plasma drug concentration with the elimination rate constant (Ke). $AUC_{0-\infty}$ was obtained by summing both values of AUC_{0-t} and $AUC_{0-\infty}$. The elimination rate constant, Ke, was derived from the terminal slope of the individual, logarithmic (ln) transformed, plasma concentration values (at least three concentration values were used) and the application of linear regression. The half-life of ipratropium ($t_{1/2}$) was calculated with the following equation: $t_{1/2} = \ln 2 / Ke$.

Statistical analysis

A statistical analysis was performed using the SAS® GLM procedure (SAS® system for windows® release 9.3 for study-1; SAS® system for windows® release 9.2 for study-2; SAS® system for windows® release 9.4 for study-3). Concentration values below the LOQ of the assay for ipratropium (3.0 pg/mL for study-1 and 2; 1.0 pg/mL for study-3) were set to zero. Analysis of variance (ANOVA) was used to analyze C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and ke because it can distinguish the effects due to participants, periods, and treatment. Wilcoxon Signed Rank Sum Test for paired samples was used for analysis of T_{max}. Bioequivalence was assessed based on the ratio of the C_{max}, and AUC_{0-t} values of test-versus-reference formulation. The 90% confidence intervals were calculated using the two one-sided test procedure where α=5% level of significance. The 90% confidence interval of the ratio of C_{max}, and AUC_{0-t} should fall between 80.00-125.00% (transformed values).

Results

Safety

In study-1, a total of 90 volunteers were recruited. There were 26 adverse events of mild and moderate severity. Overall, 26/90 (28.89%) volunteers experienced an adverse event.

In study-2, a total of 24 volunteers were recruited. There were no adverse events reported during the study. In study-3, a total of 64 volunteers were recruited. There were 9 adverse events of mild and moderate severity. Overall, 9/64 (14.06%) volunteers experienced an adverse event.

No deaths occurred during conduct of all the three studies. One serious adverse event (hospitalization) occurred during conduct of study-1. This subject experienced Seizure (convulsion) of moderate severity after 11 days of administration of the reference investigational product during period-1. It was considered "not related" to investigational product. Subject was discontinued from the study and he was hospitalized for observation and treatment. He was resolved after completing the treatment for about 1 month.

No serious adverse events (SAE) occurred during conduct of study-2 and 3. Adverse events of study-1 and 3 are summarized in Table 2.

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.

Pharmacokinetics and statistics

In study-1, a total of 90 volunteers were recruited, but only 82 volunteers completed the study. 3 volunteers were discontinued from the study in period-1 due to AE/SAE. 2 volunteers did not complete period-2, 3 and 4; and 3 volunteers did not complete period-3 and 4 for personal reasons. The plasma samples of all 90 volunteers were analyzed for ipratropium bromide. Leakage of drug was observed during dosing for 4 volunteers. Therefore, data of 4 volunteers was not considered for final pharmacokinetic and statistical analysis. Data of remaining 81

Adverse Event (Preferred Term)	Frequency (Percentage)	Relationship	Number of Adverse Events	
			Test product (T)	Reference product (R)
Ipratropium 80 mcg (with charcoal)				
Cough	5.56%	Not Related	4	1
Headache	4.44%	Related	1	3
Injury	3.33%	Not Related	1	2
Abdominal Pain	2.22%	Not Related	2	0
Nasopharyngitis	2.22%	Not Related	1	1
Pain	2.22%	Not Related	0	2
Vomiting	1.11%	Not Related	1	0
Oropharyngeal pain	1.11%	Not Related	1	0
Musculoskeletal chest pain	1.11%	Not Related	1	0
Wrist fracture	1.11%	Not Related	0	1
Convulsion	1.11%	Not Related	0	1
Syncope	1.11%	Not Related	0	1
Renal colic	1.11%	Not Related	1	0
Pyrexia	1.11%	Not Related	0	1
Ipratropium 40 mcg (with spacer)				
Pyrexia	3.13%	Not Related	1	1
Electrocardiogram PR shortened	3.13%	Not Related	2	0
Diarrhea	1.56%	Not Related	1	0
Oropharyngeal pain	1.56%	Not Related	0	1
Cough	1.56%	Not Related	1	0
Pain in extremity	1.56%	Not Related	0	1
Mucocutaneous rash	1.56%	Not Related	0	1

Table 2: Adverse events of Ipratropium 80 mcg with charcoal and Ipratropium 40 mcg with spacer studies are summarized.

volunteers was considered for pharmacokinetic and statistical analysis who had completed at least two treatment periods (one with the test and one with the reference) without any major protocol violation like leakage of drug during dosing.

In study-2, a total of 24 volunteers were recruited, and all 24 volunteers completed the study. The plasma samples of all 24 volunteers were analyzed for ipratropium bromide. No leakage of drug was observed during dosing for all 24 volunteers. Therefore, data of all 24 volunteers was considered for final pharmacokinetic and statistical analysis.

In study-3, a total of 64 volunteers were recruited, but only 58 volunteers completed the study. 1 volunteer was discontinued from the study in period-1 due to AE. 5 volunteers did not complete period-2 for personal reasons. The plasma samples of all 64 volunteers were analyzed for ipratropium bromide. No leakage of drug was observed during dosing for all 64 volunteers. Data of remaining 58 volunteers was considered for pharmacokinetic and statistical analysis except for volunteers who were dropped out or discontinued from the study before dosing of period-2.

Data of only those volunteers was considered for the final pharmacokinetic analyses and the conclusion of bioequivalence who satisfactorily completed the study or volunteers who completed at least two treatment periods (one with the test and one with the reference) without any major protocol violation (defined as any protocol violation which may affect the primary PK endpoint parameters) and for whom the primary PK parameters (C_{max} and AUC_{0-t}) were calculable for both the treatments (test and reference); irrespective of whether there are equal or unequal number of volunteers for a particular sequence.

The blood samples were collected up to 24 h post dose. Mean plasma concentration profiles of ipratropium bromide under linear over the 24 h pharmacokinetic study are presented in Figure 1 (for with charcoal study-1, without charcoal study-2 and with spacer study-3). This figure suggest comparable mean plasma concentration-time curves for each pair of reference-test formulation corresponding to each study. A washout period of 7-14 days (for with charcoal study-1); 6 days (for without charcoal study-2); and 7-10 days (for with spacer study-3) prior to dosing was sufficient since none of the volunteers had pre dose concentration levels of ipratropium bromide greater than 5 percent of the C_{max} in Period 2. 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.

In addition, 81 volunteers provided 100% power to detect a difference of at least 20% in C_{max}, and AUC_{0-t} between the two treatments for with charcoal study-1; and 24 volunteers provided >70% power to detect a difference of at least 20% in C_{max}, and AUC_{0-t} between the two treatments for without charcoal study-2; and 58 volunteers provided >99% power to detect a difference of at least 20% in C_{max}, and AUC_{0-t} between the two treatments for with spacer study-3.

The statistical results of the primary pharmacokinetic parameters of ipratropium bromide (with charcoal study-1; without charcoal study-2; and with spacer study-3) are presented in Table 3. The geometric mean ratios, 90% CI, power and intra subject coefficient of variation of test and references for Ln transformed pharmacokinetic parameters C_{max}, and AUC_{0-t} for ipratropium bromide (with charcoal study-1; without charcoal study-2; and with spacer study-3) are presented in Table 4.

Discussion

In these studies, we investigated the bioequivalence of test and reference formulations of Ipratropium Bromide HFA pMDI 20 µg/actuation with and without charcoal blockade; and with spacer device under fasting conditions. Demonstrating bioequivalence of inhaled

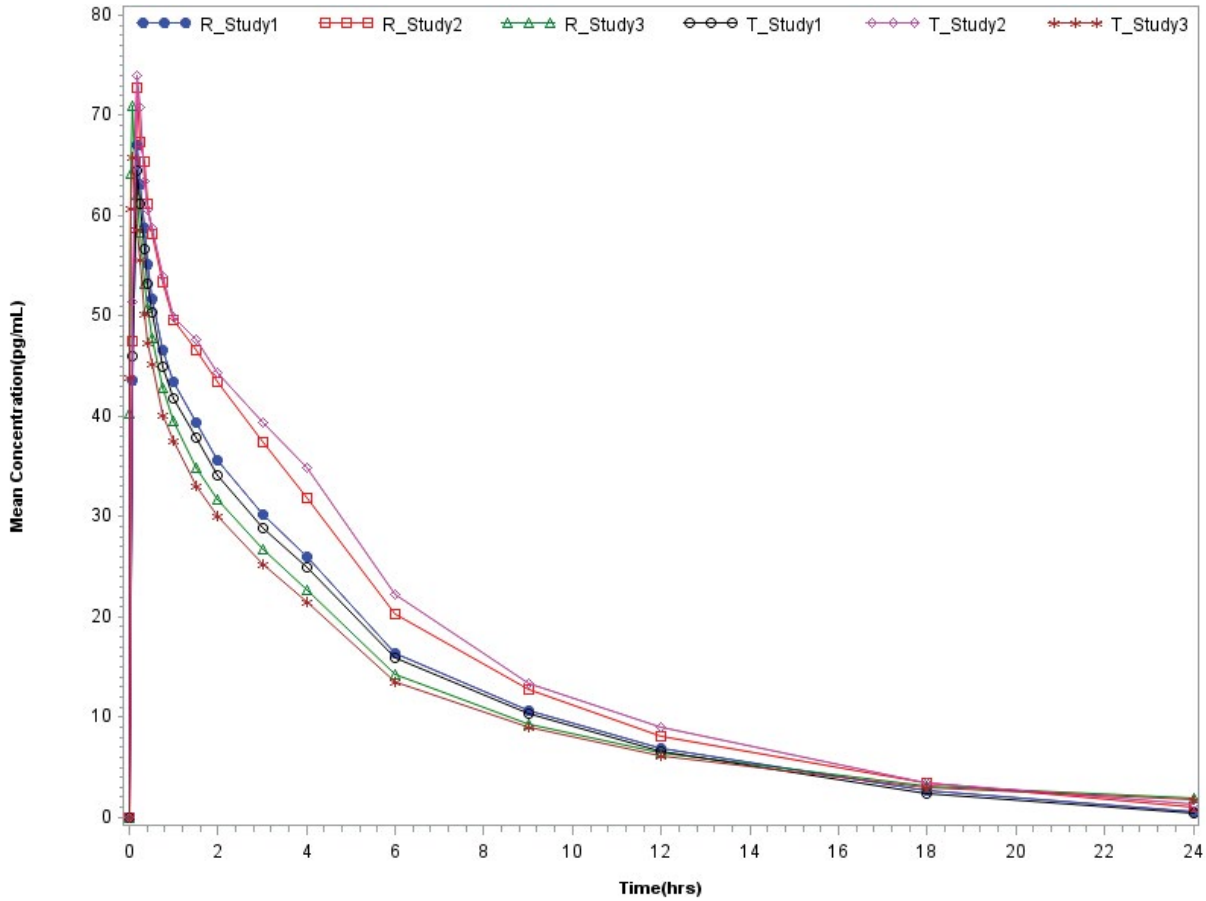

Figure 1: Mean graph (linear) for plasma concentration vs. time profile of Ipratropium after inhalational dose of Ipratropium 80 mcg study-1 (with charcoal), Ipratropium 80 mcg study-2 (without charcoal), and Ipratropium 40 mcg study-3 (with spacer).

Pharmacokinetic Parameters	Ipratropium 80 mcg with charcoal		Ipratropium 80 mcg without charcoal		Ipratropium 40 mcg with spacer	
	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	81	81	24	24	58	58
C_{max} (ng/mL)	69.96 ± 28.05	72.66 ± 29.97	80.08 + 39.24	77.54 + 33.07	72.78 ± 25.89	77.75 ± 25.88
AUC_{0-t} (h.ng /mL)	271.73 ± 98.27	283.83 ± 94.54	363.39 + 169.02	344.14 + 121.98	260.59 ± 45.27	274.92 ± 48.37
$AUC_{0-\infty}$ (h.ng /mL)	302.12 ± 98.64	314.56 ± 96.18	398.73 + 173.14	375.77 + 121.08	277.69 ± 48.21	292.53 ± 51.69
***T_{max} (hr)**	0.17 (0.08-1.50)	0.17 (0.08-2.00)	0.17 (0.08-3.00)	0.17 (0.17-2.00)	0.08 (0.02-0.42)	0.08 (0.02-0.50)
K_{el} (1/h)	0.143 ± 0.034	0.139 ± 0.033	0.144 + 0.044	0.143 + 0.038	0.119 ± 0.025	0.118 ± 0.024
$T_{1/2}$ (h)	5.11 ± 1.22	5.27 ± 1.31	5.27 + 1.62	5.17 + 1.28	6.08 ± 1.16	6.11 ± 1.13

*Median (range)

Table 3: The statistical results of primary pharmacokinetic parameters of Ipratropium 80 mcg with charcoal, Ipratropium 80 mcg without charcoal and Ipratropium 40 mcg with spacer studies are presented.

drugs is challenging. There are certain issues in conducting PK studies for proving bioequivalence of inhaled drugs, e.g. Dose selection: sometimes drug doses are too low to be detected by the standard bio-analytical methods. This either requires increasing the drug or developing more sensitive methods of drug assay. Volunteer selection: healthy and non-smokers are to be selected for the PK study as smokers are more liable to have respiratory morbidities which may affect the comparative pharmacokinetics. Volunteer training: this is one of the most important factors for assuring proper performance of pulmonary function testing and, more importantly, for correct and consistent inhalation technique.

The study was designed and conducted in accordance to the European Medicines Agency Guideline on the Investigation of Bioequivalence. European medical agency requires two studies: one with and one without charcoal blockade. Equivalence in terms of efficacy is typically recommended to be established via a PK systemic exposure equivalence study where charcoal is administered to block gastro-intestinal absorption so that only the exposure of the active pharmaceutical ingredient absorbed via the lung is compared. Equivalence in terms of safety is done via a PK systemic exposure equivalence study but where charcoal is not administered, so that the total systemic exposure of the generic versus the reference product are compared, not just what is absorbed via the lung [10].

Pharmacokinetic Parameters	Geometric Mean		*(%)T/R	90% Confidence Interval	Power (%)	Intra subject CV%
	Test	Ref				
Ipratropium 80 mcg with charcoal						
N	81	81	-	-	-	-
C_{max} (pg/mL)	63.76	66.76	95.51	91.30-99.91	100.00	25.00**
AUC_{0-t} (h.pg/mL)	251.12	267.08	94.03	90.42-97.77	100.00	19.95**
Ipratropium 80 mcg without charcoal						
N	24	24	-	-		
C_{max} (pg/mL)	71.10	69.07	102.93	87.33-121.30	72.76	34.07
AUC_{0-t} (h.pg/mL)	329.99	318.97	103.46	88.94–120.34	78.86	31.23
Ipratropium 40 mcg with spacer						
N	58	58	-	-	-	-
C_{max} (ng/mL)	68.44	73.35	93.31	87.21-99.83	99.98	21.99
AUC_{0-t} (h.ng/mL)	255.81	270.00	94.75	91.66-97.94	100.00	10.68

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

Table 4: 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 Ipratropium 80 mcg with charcoal, Ipratropium 80 mcg without charcoal and Ipratropium 40 mcg with spacer studies are presented.

Following inhalation, 10 to 30% of a dose is generally deposited in the lungs, depending on the formulation, device and inhalation technique. The major part of the dose is swallowed and passes through the gastro-intestinal tract [11]. Activated charcoal has been shown to effectively block the swallowed portion of inhaled drugs [7,12-14]. The aim of study-1 was to use oral charcoal blockade to block the gastrointestinal absorption of inhaled Ipratropium and thus allow a comparison of the pulmonary bioavailability of the test product and the reference product to be made (as an indicator of efficacy).

The systemic exposure that occurs following inhaled dosing is assumed to come from absorption via the lungs, and the swallowed portion of the dose. The aim of study-2 was to allow a comparison of total systemic bioavailability of the test product and the reference product to be made (as an indicator of safety, to ensure that test and reference do not differ in systemic side effects). A single dose of 80 µg of Ipratropium Bromide HFA pMDI (20 µg per actuation X 4 puffs) was selected in the study-1 and 2 as this is the maximum recommended dose in adults at one time [11].

As Atrovent® inhaler can be used with the AeroChamber Plus™ spacer device. The spacer device helps to eliminate the need for coordination between the actuation and inhalation and ensures the proper drug delivery. Overall the use of spacer results in to decreased swallowed dose and increased lung dose [11]. As per EMA guidelines, if the reference product is recommended to be administered with or without a spacer, two PK studies would be required, one with and the other without the spacer device [15]. Therefore, the aim of study-3 was to allow a comparison of total systemic bioavailability of the test product and the reference product with spacer device to be made. The use of spacer device results in to reduce oropharyngeal deposition and increase lung deposition [16]. Therefore, a single lower dose of 40 µg of Ipratropium Bromide HFA pMDI (20 µg per actuation X 2 puffs) was selected in the study-3 which was sufficient to produce detectable Ipratropium concentrations in the blood in order to permit the comparison of complete pharmacokinetic profiles of the analyte.

All the three studies demonstrate generic and innovator formulations of Ipratropium Bromide HFA pMDI 20 µg/actuation displayed similar rate and extent of bioavailability of Ipratropium Bromide. The median T_{max} for both test and reference was found to be 0.17 h. for both with charcoal study-1, and without charcoal study-2. The median T_{max} for both test and reference was found to be 0.08 hr. for with spacer study-3. The T_{max} is comparable. The C_{max} was found to be consistent both for test and reference in all the 3 studies, indicating the attainment of similar body peak levels. The mean data are also comparable. For the AUC parameter, the results were found to be similar and there was not much difference in inter-subject variability. The $T_{1/2}$ values are also comparable and in the elimination phase there is no variation.

The statistical analysis was carried out for both untransformed and log transformed data. The data showed statistical equivalence for the important pharmacokinetic parameters, i.e., C_{max}, and AUC_{0-t}. A power of 100% was achieved for the pharmacokinetic parameters for with charcoal study-1. A power of >70% was achieved for the pharmacokinetic parameters for without charcoal study-2. A power of >99% was achieved for the pharmacokinetic parameters for with spacer study-3. However this power refers to the manufacturer's risk of erroneously concluding bioequivalence when the two formulations were indeed bioequivalent. The consumer's risk of erroneously accepting bioequivalence remained unchanged at 5% level (type I error).

Considering that all 90% CI of the ratios of the pharmacokinetic parameters (C_{max} and AUC_{0-t}) were found to be within the predetermined ranges of bioequivalence and that the two one-sided t tests found all of the probability values to be <0.05, the results of both studies satisfied the accepted regulatory requirements to assume bioequivalence.

The intra-subject CV was found to be 25.00 % for C_{max} and 19.95 % for AUC_{0-t} for log-transformed data for with charcoal study-1.

The intra-subject CV was found to be 34.07 % for C_{max} and 31.23 % for AUC_{0-t} for log-transformed data for without charcoal study-2.

The intra-subject CV was found to be 21.99 % for C_{max} and 10.68 % for AUC_{0-t} for log-transformed data for with spacer study-3.

The sample size of 90 volunteers, 24 volunteers, and 64 volunteers selected for with charcoal study-1, without charcoal study-2 and with spacer study-3 respectively was considered to be sufficient to provide adequate power to meet bioequivalence criteria. All the volunteers were dosed between 08:00 to 10:06 in both the periods in (with charcoal) study-1. All the volunteers were dosed between 08:00 to 09:35 in both the periods in (without charcoal) study-2. All the volunteers were dosed between 08:00 to 09:34 in both the periods in (with spacer) study-3.

During the clinical study there were no significant protocol/ standard operating procedure (SOP) deviations and adverse events

were mild to moderate in nature. The volunteers tolerated the study medications well. The biological samples were successfully analyzed by LCMS/MS. The quality control data are found to be consistent and precise.

Conclusion

The 90% CI of Ipratropium Bromide for C_{max} and AUC_{0-t} were within 80.00-125.00% for all the three studies, suggesting the generic formulation of Ipratropium Bromide HFA pMDI 20 µg/ actuation was bioequivalent with the innovator formulation of ATROVENT® CFC-free 40 µg (20 µg per actuation X 2 puffs) marketed by BOEHRINGER INGELHEIM LIMITED, UK with and without charcoal blockade; and with spacer device according to the European Medicines Agency Guidelines on the Investigation of Bioequivalence.

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Disclosure

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Conflict of Interest

The authors have indicated that they have no other conflicts of interest regarding the content of the article.

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