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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Introduction

* Ipratropium bromide is a synthetic anticholinergic drug which acts as a non-selective antagonist at the muscarinic receptor. It is a short-acting bronchodilator
* It is indicated for the regular treatment of reversible bronchospasm associated with chronic obstructive pulmonary disease (COPD) and chronic asthma
* The recommended dose in adults (including the elderly) is usually 1 or 2 puffs three or four times daily, although some patients may need up to 4 puffs at a time to obtain maximum benefit during early treatment. The recommended dose in children of 6-12 years is usually 1 or 2 puffs three times daily and in children under 6 years is usually 1 puff three times daily
* The inhaler can be used with the Aerochamber Plus™ spacer device. This may be useful for patients, e.g. children, who find it difficult to synchronize breathing in and inhaler actuation

Study Products

* Innovator- ATROVENT CFC-free (containing ipratropium bromide 20 µg per actuation) marketed by BOEHRINGER INGELHEIM LIMITED, UK)
* Generic- ipratropium bromide 20 µg per actuation

Rationale

* The sponsor of these studies was interested in obtaining the marketing authorization for Ipratropium Bromide HFA pMDI 20 µg/actuation in European Union
* Three bioequivalence studies were submitted to demonstrate therapeuticequivalence between the test and the reference formulation of IpratropiumBromide HFA pMDI 20 µg/actuation

Objective

The aim of these three studies was to evaluate the rate and extent of absorption of test formulation of Ipratropium Bromide HFA pMDI 20 µg/actuationagainst the reference 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

Methods

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

Study Protocols

- The protocols were approved by an independent ethics committee prior to study initiation
- The studies were conducted in accordance with the ethical principles of the Declaration of Helsinki and its amendments and the International Conference on Harmonization for Good Clinical Practice Guideline
- All the three studies were conducted at Sitec Labs. Pvt. Ltd., Navi Mumbai, India

Study volunteers and eligibility criteria

- Volunteers were screened within 21 days prior to dosing
- 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/m2 and ⩽ 25.00 kg/m2, 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
- Before inclusion into the study, the volunteers were judged to be healthy by physician based on previous medical history, physical examination, vital signs examination, ECG, chest X-ray, pulmonary function test, pulse oximetry, and clinical laboratory test results- CBC, LFT, RFT, FBS, serological tests for hepatitis B and C and HIV antibodies, Urine routine

	N		Age (yrs)	Weight (kgs)	Height (m)	BMI (Kg/m²)
Ipratropium 80 mcg (with charcoal)	90	Mean	28	64.9	1.68	23.0
		SD	6	6.8	0.05	1.9
Ipratropium 80 mcg (without charcoal)	24	Mean	28.04	63.69	1.68	22.38
		SD	6.09	7.79	0.06	1.89
Ipratropium 40 mcg (with spacer)	64	Mean	28	65.4	1.68	23.0
		SD	5	6.7	0.05	1.8

Table 1

Training On the Inhalation Technique

- 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

Study procedures

On check in day-

- written informed consent was obtained from all willing volunteers
- Volunteers who fulfilled all the criteria for inclusion were admitted to the study center at least 12hrs prior to each dosing
- In the study-1, a total of 90 were recruited
- In the study-2, a total of 24 were recruited
- In the study-3, a total of 64 were recruited

Drug administration

- After priming, investigational product was inhaled; there was a gap of one minute with an allowed deviation of ± 5 seconds between each puff inhaled by the volunteer
- In study-1, 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 gm) of activated charcoal suspension was given 2 minutes prior to the 1st puff, immediately after dosing and also at 1.00, 2.00 and 4.00 hours post-dose
- In study-2, 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, 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 Aerochamber Plus valved holding chamber as per the randomized sequence in the standing position on two separate treatment days

Study restrictions

- Volunteers remained seated at least for the first 2 hrs after dosing
- Water was not permitted from 1 hr before dosing until 1 hr following dosing, but it was allowed at all other times
- Post dosing, food was withheld for at least 4 hrs. Standardized lunch, snack and dinner were served at 4, 9, and 13 hrs after dosing
- They were asked not to take any drugs at least two weeks before the first dosing, to avoid taking food or drinks that contain xanthine: chocolate, tea, coffee and cola drinks, not to drink alcoholic products 48 hours before dose administration and during the study

Blood sampling

- In study-1 and 2, Blood samples (1 x 5 mL) were collected at -0.00 hours (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 hours post dose
- In study-3, blood samples (1 x 6 mL) were collected at -0.00 hours (pre-dose) and blood samples (1 x 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 hours 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
- The blood sample tubes were centrifuged to separate plasma and plasma samples were stored at −70°C or below until sample analysis

Bioanalysis

- Ipratropium plasma concentrations were determined using validated LC-MS/MS method
- 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

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.

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). 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}. 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 even
- 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.
- 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

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

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. 2 volunteers were dropouts. 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 volunteers was considered for pharmacokinetic and statistical analysis who had completed at least two treatment periods
- 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 were dropouts. 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
- The blood samples were collected up to 24 hrs post dose. Mean plasma concentration profiles of ipratropium bromide under linear over the 24-hour pharmacokinetic study are presented in Figure 1 for with charcoal study-1, without charcoal study-2, and with spacer study-3)

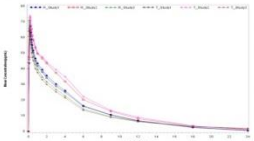

Figure 1

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

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	—	77.75±25.88
AUC_{0-t} (hr.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
$AUC_{0-\infty}$ (hr.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_e (1/hr)	0.143 ±0.034	0.139 ± 0.033	0.144±0.044	0.143± 0.038	0.119±0.025	0.118±0.024
$T_{1/2}$ (hr)	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 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

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} (hr.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} (hr.pg/mL)	329.99	318.97	103.46	.. ..	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} (hr.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

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 BoehringerIngelheim Limited, UK with and without charcoal blockade; and with spacer device according to the European Medicines Agency Guidelines on the Investigation of Bioequivalence

References:

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003504.pdf