Official Title:	A Randomized, Single-dose, Open-label, Two-way Crossover Pivotal Study to Assess the Bioequivalence between Albuterol Sulfate inhalation aerosol 108mcg per actuation (MDI, eq. to albuterol 90mcg /puff) and Proair HFA (albuterol sulfate) Inhalation Aerosol 90 mcg per actuation (MDI, eq. to albuterol 90mcg /puff) in Healthy Volunteers under Fasting Conditions
NCT Number:	NCT04803734
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Statistical Analysis Plan

TITLE OF STUDY:	**A Randomized, Single-dose, Open-label, Two-way Crossover Pivotal Study to Assess the Bioequivalence between Albuterol Sulfate inhalation aerosol 108mcg per actuation (MDI, eq. to albuterol 90mcg/puff) and Proair HFA(albuterol sulfate) Inhalation Aerosol 90 mcg per actuation (MDI, eq. to albuterol 90mcg/puff) in Healthy Volunteers under Fasting Conditions**
PROTOCOL NO.:	**TW20-4502**
PROTOCOL VERSION :	**1.0**
PROTOCOL VERSION DATE:	**Jan-08-2021**
PPC STUDY NO.:	**TW20-4502**
SPONSOR:	**Intech Biopharm Ltd.** **3F., No.36, Ln. 358, Ruiguang Rd., Neihu Dist.,** **Taipei City 114, Taiwan** **Phone: +886-2-7721-8877** **Fax: +886-2-7721-8800**
CRO:	**Protech Pharmaservices Corporation** **11F., No.3, Park St., Nangang District,** **Taipei City 11503, Taiwan** **Phone: +886-2-5558-0000** **Fax: +886-2-5558-0055**
STATUS:	**Final**
DATE/VERSION:	**May-13-2021 v1.0**
AUTHOR:	[redacted] **Protech Pharmaservices Corporation**

1. SIGNATURE PAGE

Prepared by:

[redacted]

May - 13 - 2021
Date

Biostatistician, Biostatistics & Data Management Dept.

Protech Pharmaservices Corp.

Approved by:
By signing this Statistical Analysis Plan, I tender it for review.

[redacted]

May - 13 - 2021
Date

Head of Biostatistician, Biostatistics & Data Management Dept.
Protech Pharmaservices Corp.

Approved by:
On behalf of Intech Biopharm Ltd., I have reviewed this Statistical Analysis Plan, and I accept and approve of the document provisions as stated herein.

[redacted]

Intech Biopharm Ltd.

14.05.2021

Date

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2. STUDY DESIGN

Using a single-dose, randomized, open-label, single-centre, two-way crossover study design, is examined after administration to 60 healthy volunteers under fasting conditions on this study. The subjects are assigned to one of the treatment sequences as follows:

Sequence	Period 1	Washout	Period 2
1	T	≥ 14 days	R
2	R		T

The following tables show the information about study treatments:

Test Product (T)

Drug name:	Albuterol Sulfate inhalation aerosol 108mcg per actuation (equivalent to 90mcg of Albuterol)
Active ingredient:	Albuterol sulfate
Strength:	eq. to albuterol 90mcg/puff
Dosage form:	MDI
Manufactured by:	Intech Biopharm Ltd.
Mode of administration:	Inhalation

Reference Product (R)

Drug name:	Proair HFA(albuterol sulfate) Inhalation Aerosol 90 mcg per actuation
Active ingredient:	Albuterol sulfate
Strength:	eq. to albuterol 90mcg/puff
Dosage form:	MDI
Manufactured by:	IVAX Pharmaceuticals Ireland Waterford, Ireland
Mode of administration:	Inhalation

The scheduled sampling times for drug's concentration analysis are pre-dose and at 2, 5, 10, 15, 20, 30, 45 minutes, and 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 4, 6, 8, 12, 16 and 24 hours after dosing, a total of 22 sampling points are drawn for each study period.

3. STUDY OBJECTIVES

3.1 Primary Objective

This study is designed to assess the bioequivalence of Albuterol sulfate of the Test product vs. the Reference product under fasting conditions. Bioequivalence will be assumed if the 90% confidence interval of the AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} ratio are within the 80-125% interval for ln-transformed values.

3.2 Secondary Objective

The other PK-variables will be assessed for descriptive purposes. As for safety parameters, AE and vital signs (BP, PR and body temperature) will be recorded.

4. SPONSOR

Intech Biopharm Ltd.

3F., No.36, Ln. 358, Ruiguang Rd., Neihu Dist., Taipei City 114, Taiwan

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5. STATISTICAL ANALYSIS FACILITY

Protech Pharmaservices Corporation

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6. STUDY PERSONNEL

Biostatistician: [redacted], Biostatistics & Data Management Department

7. SUBJECT POPULATIONS

The data from the following subjects will be included in the final pharmacokinetic and statistical analysis:

1. Subjects who complete all study periods.

2. Subjects who have missed samples that have been determined prior to the start of bioanalytical analysis as not significantly impacting the overall outcome of the study.

Data from subjects who were dismissed or withdrew due to AE(s) will not be included in the pharmacokinetic and statistical analysis.

8. STATISTICAL METHODOLOGY

8.1 Objective

In this bioequivalence study, the concentration value of Albuterol sulfate for the Test product [Albuterol Sulfate inhalation aerosol 108mcg per actuation (equivalent to 90mcg of Albuterol)] and the Reference product [Proair HFA(albuterol sulfate) Inhalation Aerosol 90 mcg per actuation] will be used to calculate the pharmacokinetic parameters, to perform the statistical analysis, to compare the bioavailability between Test product and Reference product, and will be used to assess the bioequivalence between Test product and Reference product.

8.2 General Considerations

8.2.1 Continuous Variables

The raw data will be summarized with the number of available observations, mean, standard deviation, minimum and maximum on visit basis.

8.2.2 Categorical Variables

The raw data will be summarized with the number of available observations, frequency of each class on visit basis.

8.3 Pharmacokinetic Analysis

The following pharmacokinetic parameters will be estimated (where possible) for Albuterol sulfate included in the pharmacokinetic and statistical analysis for the subjects in the final data set:

C_{max}:	The maximal observed plasma concentration.
T_{max}:	Time when the maximal plasma concentration is observed.
AUC_{0-t}:	Area under the concentration-time curve from time zero until the last measurable concentration or last sampling time t, whichever occurs first. AUC_{0-t} is estimated using the trapezoidal method.
$AUC_{0-\infty}$:	Area under the concentration-time curve from time zero to infinity, calculated as $AUC_{0-t} + C_{last}/\lambda$, where C_{last} is the last measurable concentration.
$k_{el}(\lambda)$:	Terminal elimination rate constant, estimated by linear regression analysis of the terminal portion of the ln-concentration vs. time plot.
$T_{1/2}$:	Terminal elimination half-life, estimated as $\ln(2)/\lambda$.
MRT:	Mean residence time

If a subject's pre-dose concentration is less than or equal to 5% of the C_{max} value for that subject in the given period, then the subject's data without any adjustments can be included in all pharmacokinetic measurements and calculations. If the pre-dose value is greater than 5% of the C_{max}, data from that subject will be dropped from the pharmacokinetic and statistical analysis.

During pharmacokinetic and statistical analyses, drug concentrations below the lower limit of quantitation (<LLOQ) of an assay will be considered as zero except when they occur between two measurable concentrations where they will be considered as missing during pharmacokinetic calculations and estimations.

Missed samples and non-reportable concentrations (e.g. quantity not sufficient) from the analytical laboratory will be treated as they had not been scheduled for collection in the pharmacokinetic analysis.

The $AUC_{0-\infty}$, $k_{el}(\lambda)$, $T_{1/2}$ and MRT parameters will not be estimated for plasma concentration-time profiles where the terminal linear phase is not clearly defined, only the AUC_{0-t}, C_{max} and T_{max} will be presented and included in the statistical analysis.

8.4 Statistical Analysis for Pharmacokinetic Parameters

Pharmacokinetic and statistical analysis will be performed by PPC. The actual sampling times will be used in the pharmacokinetic calculations.

Descriptive statistics of all pharmacokinetic parameters (min, max, median, mean, standard deviation and coefficient of variability) will be provided for Albuterol sulfate for the Test and Reference products. Parameter differences will be tabulated without coefficient of variability and geometric mean.

ANOVA including sequence, subjects nested within sequence, period and treatment will be performed on the ln-transformed data for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}, and on the raw data for k_{el} and $T_{1/2}$. T_{max} will be analyzed using an additional non-parametric test (Wilcoxon test).

The 90% CI of the Test/Reference ratios of geometric means for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} will be calculated based on the LSMEANS and ESTIMATE of the ANOVA. Schuirmann's two one-sided test procedures will be evaluated.

For Albuterol sulfate, the 90% CI of the Test/Reference ratios of geometric means for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} will be calculated based on the LSMEANS and ESTIMATE of the ANOVA. Schuirmann's two one-sided test procedures will be evaluated.

If study is carried out in two or more groups of subjects, the model should reflect the fact that the periods for the first group are different from the periods for the second group.

The model of ANOVA including Group, Sequence, Treatment, Subject (nested within Group × Sequence), Period (nested within Group), Group-by-Treatment Interaction will be performed on the ln-transformed data for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}.

Subject (nested within Group×Sequence) is a random effect, and all other effects are fixed effects.

If the Group-by-Treatment interaction test or Group test is not statistically significant ($p \geq 0.05$), the bioequivalence will be demonstrated by pooled group of all subjects.

If the Group-by-Treatment interaction test or Group test is statistically significant ($p<0.05$), the bioequivalence will be demonstrated by pooled group of all subjects and separated group.

Additional statistical and alternate tests will be performed if necessary.

8.5 Bioequivalence Criteria

Bioequivalence will be demonstrated under fasting conditions if the 90% CI for the Test/Reference ratios of geometric means for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} are completely contained within the FDA defined acceptance range of 80.00%-125.00% for Albuterol sulfate.

8.6 Safety parameters

The safety data for each subject will be included in subject listings, separate from the statistical analysis report.

8.6.1 Adverse event

Subjects will be instructed to inform clinical personnel of any untoward medical symptoms and/or events that may arise during the course of the study. Prior to subsequent study periods, subjects will be questioned concerning symptoms that may have occurred after the previous administration of the study drug(s).

This study will collect pretreatment event(s) and adverse event after subjects provide written consent in the ICF. Only AE occur after first dosing will be analyzed for safety assessment.

8.6.2 Vital signs

Vital signs (blood pressure [BP], pulse rate [PR] and body temperature) will be obtained at pre-dose and at 0.5, 16 and 24 hours after dosing in each study period. Additional vital signs measurements will be taken if deemed necessary by the Principal Investigator/Sub-Investigator.

9. RECORD AND ARCHIVE

After completing this study, the statistical analysis plan, statistical analysis report, and the storage media (if necessary) should be archived in PPC for 2 years following the date on which the study is completed, then returned according to the sponsor's demand.

10. CHANGES TO PLANNED ANALYSES FROM CLINICAL PROTOCOL

There is no change section.

11. STATISTICAL ANALYSIS PLAN AMENDMENT

Changes to the statistical analysis plan must be authorized by the biostatistician, and the sponsor. Any sentences, reasons and content of the modification will be attached to the statistical analysis plan as an amendment and signed by the biostatistician. The head of division and the sponsor should keep a copy version of amendment.

12. PROGRAMMING CONSIDERATIONS

Statistical Software

The calculation of pharmacokinetic parameters will be obtained from software WinNonlin version 6.4 or higher. All tables, listing and analyses of pharmacokinetic parameters will be produced using the SAS® version 9.2 or higher.

Format of Table and Figure

The following is the table and figure list for the format of output, they will be adjusted when perform real analytical figures in these table.

Statistical Table and Figure Name
Table 14.2.1.1. The concentration vs. time value for Test product
Table 14.2.1.2. The concentration vs. time value for Reference product
Table 14.2.1.3. The actual sampling time for test product
Table 14.2.1.4. The actual sampling time for reference product
Table 14.2.2.1. The individual pharmacokinetic parameters for Test product
Table 14.2.2.2. The individual pharmacokinetic parameters for Reference product
Table 14.2.3.1. The summary of AUC_{0-t} and ln-transformed AUC_{0-t} - Test vs Reference
Table 14.2.3.2. The summary of $AUC_{0-\infty}$ and ln-transformed $AUC_{0-\infty}$ - Test vs Reference
Table 14.2.3.3. The summary of C_{max} and ln-transformed C_{max} - Test vs Reference
Table 14.2.4. Statistical analysis for pharmacokinetic parameters
Figure 14.2.1.1. The individual concentration-time profile on linear scale
Figure 14.2.1.2. The mean concentration-time profile on linear scale
Figure 14.2.2.1. The individual concentration-time profile on semi-logarithmic scale
Figure 14.2.2.2. The mean concentration-time profile on semi-logarithmic scale

Format of listing

The following is the subject listing for the format of output, they will be adjusted if required. The output of subject listing will be separate from the statistical analysis report.

Subject Listing Name
Listing 16.1. Subject disposition
Listing 16.2. Study termination
Listing 16.3. Date of consent and demography
Listing 16.4. Vital signs
Listing 16.5. Medical history
Listing 16.6. Physical examination
Listing 16.7. Drugs of Abuse

Listing 16.8. Breath Alcohol Test
Listing 16.9. 12-lead ECG
Listing 16.10. Study drug administration and blood sampling
Listing 16.11. Urine pregnancy test
Listing 16.12. Adverse event
Listing 16.13. Inclusion criteria
Listing 16.14. Exclusion criteria.
Listing 16.15. Concomitant medication

13. REFERENCE(S)

1. Guidance for Industry. Statistical Approaches to Establishing Bioequivalence. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), January 2001.
2. Chow, S.C., and Liu, J.P. (2009) Design and Analysis of Bioavailability and Bioequivalence Studies, Third Edition. Marcel Dekker, Inc. New York.