Study of Drug Exposure in Systemic Circulation of Primatene® Mist (0.25mg) by Oral Inhalation, versus Epinephrine Injection (0.30mg) by IM and ProAir® (0.18mg) by Oral Inhalation in Healthy Individuals

(A Randomized, Safety Evaluator-blind, Three-Treatment, Crossover, Fasting Study)

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SYNOPSIS

General Information

(1) Protocol No.	API-E004-CL-I
(2) Title	Study of Drug Exposure in Systemic Circulation of Primatene® Mist (0.25mg) by Oral Inhalation, versus Epinephrine Injection (0.30mg) by IM and ProAir® (0.18mg) by Oral Inhalation, in Healthy Individuals
(3) Subtitle	A Randomized, Safety Evaluator-blind, Three-treatment, Crossover, Fasting Study
(4) Study Drug	Primatene® Mist (**E004**), Epinephrine HFA MDI Inhalation Aerosol 0.125mg/inhalation, NDA 205920
(5) Comparator Treatments	Epinephrine Injection (Generic of EpiPen®), Auto-Injector, 0.3 mg by [redacted] ProAir® HFA MDI Inhalation Aerosol by [redacted] (0.090mg/inhalation)
(6) Delivery Path	Oral Inhalation (E004, ProAir) Intramuscular Injection (Epinephrine Injection)
(7) Manufacturer	[redacted]
(8) Sponsor	Amphastar Pharmaceuticals, Inc.
(9) Objectives	To assess the drug exposure profile in systemic circulation of Primatene® Mist by inhalation, versus Epinephrine by IM and ProAir® HFA by inhalation in healthy adults
(10) Phase	IV

I. Study Design

This is a randomized, safety evaluator-blind, three-treatment, crossover, fasting study in healthy adults (18 - 50 years of age). The design includes a Screening Visit, three Dosing Visits and a follow-up phone evaluation. All study subjects must be properly consented, and screened against the inclusion and exclusion criteria prior to randomization.

1) Screening Visit (3 – 7 days before Visit-1):
Prospective subjects will sign informed consent and be assigned a screening ID, ***EIS-mm***, where *EI* represents "E004-CL-I Study", ***S*** stands for screening, and ***mm*** is the screening sequential number. Subjects will be screened per inclusion/exclusion criteria. The

following screening activities will be conducted: (i) physical examination (PE), (ii) measurements of vital signs, (iii) ECG, (iv) urinalysis (UA), (v) urine drug screen, (vi) alcohol screen (urine or breathalyzer), (vii) urine or serum pregnancy test (all female subjects), and (viii) fasting (≥ 8 hours) blood tests [comprehensive metabolic panel, complete blood count (CBC) with differentials, thyroid function tests, HIV, hepatitis B and C tests].

The subject's recent medication history will include a review for any use of medicines as listed in the prohibited drug list, **Appendix III**. Qualified subjects will be scheduled for Dosing Visit.

Per FDA guidance for Metered Dose Inhaler (MDI), each subject will be trained on the correct self-administration of MDI using placebo, a MDI training unit that contains no active drug, to assure a relatively consistent inspiratory flow rate and inspiratory duration [1]. A video on how to use the MDI will be provided at each training session at Screening and dosing visits. Subjects will be retrained on the use of the MDI at each dosing visit. Vitalograph AIM™ (Aerosol Inhalation Monitor) will be used to monitor the duration of the inhalation during the training at Screening and the dosing visit. **In order to be enrolled into the study, subject must demonstrate proficiency in the use of MDI after training**.

2) Dosing Visits (separated by 2 to 7 day between the three visits):
Subject qualification will be confirmed at the beginning of the Dosing Visit. After satisfying the enrollment criteria, each subject will be assigned a unique study subject ID as ***EIPK-nn***, where *nn* is the sequential number based on the order of enrollment.

At each dosing visit, subjects will either self-administer the MDI inhaler provided (Primatene Mist for Treatment-A, or ProAir HFA for Treatment-C), or receive an IM injection (0.30mg Epinephrine injection for Treatment-B)

Fasting (≥ 10 hours) is required prior to study drug dosing. Meals will be served after the 4 hour post-dose PK sampling point and after completion of End-of-Study (EOS) blood draw.

Record concomitant drugs or ongoing medications since the Screening Visit, and ensure that all prohibited drugs washout have been met.

One (1) baseline and twenty-five (25) serial post-dose PK blood samples will be collected for PK analysis.

At each Dosing Visit, vital signs will be measured at baseline, 5±2 min, 15±5 min, 60±5 min, 120±15 min and 24 ±2 hrs after dosing. ECG will be measured at baseline, 20±5 min, 120±15 min and 24 ±2 hrs after dosing.

Serum potassium and glucose levels will be measured at baseline, 10±2 min, 60±5 min and 24 ± 2 hrs post-dose.

An EOS evaluation will be performed, which includes reviews and collection of vital signs, ECG, clinical lab tests (≥8 hrs fasting metabolic panel, CBC with differentials, and UA), and PE.

3) A Follow-up Evaluation:
Subjects who experienced any serious adverse event (SAE) or other adverse events (AE) that requires follow-up will remain under medical supervision until the AE is resolved or stabilized. A follow-up phone call will be made to all subjects within 7 days after the last dosing visit, or at subject discontinuation from the study.

II. Study Subjects

A sufficient number of candidates will be screened and enrolled to target approximately 24 evaluable subjects.

All subjects will be enrolled based on the following inclusion/exclusion criteria.

Inclusion Criteria:

(1) Agree to participate; understand and sign informed consent;
(2) Male and female adults, aged 18 to 50 years, inclusive at Screening;
(3) Generally healthy and medically stable, with no clinically significant abnormalities based on physical examination and laboratory tests as determined by the Investigators;
(4) Have good venous access;
(5) Have normal resting blood pressure and normal heart rate (HR) without history of syncope; a subject with out of range blood pressure may be enrolled in the study at the discretion of the Principal Investigator;
(6) Have a body mass index (BMI) of 18.0 – 30.0 kg/m^2;
(7) Female candidates must be >1 year post-menopausal or practicing a clinically acceptable form of birth control and confirmed by negative urine or serum pregnancy test at Screening;
(8) Negative HIV-Ab, HBs-Ag and HCV-Ab;
(9) Negative alcohol test (urine or breathalyzer);
(10) Negative drug screening results;

(11) Currently non-smoker; have not used any tobacco products for at least three (3) months prior to Screening; and
(12) Demonstrate proficiency in the use of MDI and a consistent inhalation time >2.0 seconds after training, for at least three (3) times, with a maximum of 5 attempts.

Exclusion Criteria:

1) Concurrent clinically significant cardiovascular, hematological, renal, neurologic, hepatic, endocrine, psychiatric, or malignant diseases.
2) Known intolerance or hypersensitivity to any component of the study drugs (i.e., Epinephrine, Albuterol Sulfate or any sympathomimetic drugs, HFA-134a, thymol, ethanol, ascorbic acid, nitric acid, and hydrochloric acid).
3) Upper or lower respiratory tract infection, or other systemic infection within 6 weeks prior to Screening;
4) Clinically significant abnormalities in the screening/baseline ECG; prolonged corrected QT interval (QTcF) on ECG: men >450ms, women: >470ms; single or multiple premature ventricular contractions (PVC);
5) Abnormal thyroid function test (if TSH is out of range, refer to T3/T4 for thyroid function assessment);
6) Subject has been on other investigational drug/device studies within 30 days of Screening Visit or planned participation in another investigational drug trial at any time during this trial;
7) Women who are pregnant or lactating or planning a pregnancy during the study period;
8) Subject has donated or lost > 500 mL of blood within 3 months of Screening;
9) Evidence of alcohol or drug abuse or dependency within 6 months prior to screening; or
10) Use of any of the prohibited drugs listed in Appendix III without appropriate washout.

III. Study Drugs and Dosing Regimens

Qualified subjects will participate in three (3) dosing visits separated by a period of 2 – 7 days. Each subject will receive one study treatment at each dosing visit per the randomized sequences, as described in ***Table A***.

MDIs will be dispensed by the designated site professional, and the correctness of the initial dose indicator mark shall be verified prior to dispensing. The reading on the dose indicator of each MDI unit will be recorded prior to and after dosing in the study source documents. IM injection will be dispensed using aseptic techniques by the designated site personnel.

Due to the difference in dosing position for IM and oral inhalation, and to preserve blinding from evaluators, subjects will be escorted to a separated dosing area for study drug administration.

Table A. Study Arms and Treatments

Items	Treatment-A	Treatment-B	Treatment-C
Study Drug Name	Primatene Mist, E004	Epinephrine Injection Auto-Injector (Generic of EpiPen®)	ProAir
Manufacturer	[redacted]		
API	Epinephrine	Epinephrine	Albuterol Sulfate
Dosage Form	Microcrystalline Suspension HFA MDI	Sterile Solution	Microcrystalline Suspension HFA MDI
Strength	0.125 mg/inh	0.3mg/0.3mL	0.09 mg/inh
Dose Regimen	2 inhalations, 0.25mg	IM Injection of 0.30 mg Epinephrine in 0.30 mL	2 inhalations, 0.180 mg
Route of Administration	Oral Inhalation	IM Injection	Oral Inhalation

Subjects will be retrained on the use of the MDI at each Dosing Visit. Vitalograph AIM™ (Aerosol Inhalation Monitor) will be used to monitor and measure the duration of the inhalation during the training at Screening and prior to dosing.

Each MDI must be primed at the study site prior to the first use by releasing 4 test sprays (E004 for Treatment-A), or 3 test sprays (ProAir for Treatment-C) into the air away from the face, shake the inhaler well before each spray. Priming will be performed in an area that is well ventilated (preferably a fume hood, or an open space), and physically separated from the study evaluation area.

Subjects will self-administer two (2) inhalations (within 30 seconds interval between each inhalation) of E004 for Treatment-A, or two (2) inhalation of ProAir for Treatment-C. The study drug administration process will also be recorded, and the video will be used to investigate apparent irregularities. Time of the start of each inhalation will be recorded. The used MDI inhaler will be collected at the end of each Dosing Visit.

IM injection (0.30mg of epinephrine solution in 0.30 mL) for Treatment-B will be given on the thigh. Subjects will be lying down in a supine position. The 0.30 mL dose will be given perpendicularly as a single deep IM injection into anterolateral aspect of the thigh (vastus lateralis site). Epinephrine injection (Treatment B) will be administered as follows [2]:

1) Doser or designated site personnel will first pull off the blue safety release to unlock the auto injector.
2) Subject will hold the leg firmly in place.

3) Doser will place the orange tip against the middle of the outer thigh (upper leg) at a right angle (perpendicular) to the thigh.
4) Swing and push the injector firmly until it 'clicks.' The click signals that the injection has started.
5) Hold firmly in place for 3 seconds.
6) Remove the injector from the thigh and massage (apply gentle pressure) with dry gauze to the injection area for 10 seconds. The orange tip automatically extends out after use.
7) The injection site will be observed for any medication leakage for approximately 30 seconds after the dosing is completed. If there is observed leakage, PK samples will not be collected, subject will be early terminated (ET).

The study medication administration should be conducted between 07:00 and 11:00 AM on the Dosing Visit.

IV. PK Blood Sampling

1) Sampling Schedules and Time Window

A total of twenty-six (26) PK blood samples will be collected from each Dosing Visit. The blood sampling schedule and allowed time window are listed in **Table B**.

Table B. PK Blood Sampling Schedule (~5 mL per sample)

Seq. #	Scheduling	Time window	Sample No.,"XX"	Seq. #	Scheduling	Time window	Sample No.,"XX"
1	Baseline	within 30 min pre-dosing	01	14	40 min	±5 min	14
2	1 min	± 0.5 min	02	15	50 min		15
3	2 min		03	16	60 min		16
4	3 min		04	17	70 min		17
5	5 min	±1 min	05	18	80 min		18
6	7 min		06	19	90 min		19
7	9 min		07	20	120 min	±10 min	20
8	12 min	±1 min	08	21	4 hrs		21
9	15 min		09	22	6 hrs	±15 min	22
10	18 min		10	23	8 hrs		23
11	21 min		11	24	12 hrs	±20 min	24
12	25 min	±2 min	12	25	18 hrs		25
13	30 min		13	26	24 hrs		26

One (1) pre-dose baseline sample will be collected within 30 minutes before dosing. Time 0 is defined as the beginning of the last inhalation of the MDI, or at the completion of IM administration.

2) Sampling Procedure

A catheter will be inserted into one of the antecubital region veins for blood sampling. Alternatively, if there is a clot in the catheter, collection from a vein of the hand or forearm for easier access or via venipuncture is also acceptable. After each blood sample collection, a catheter flush will be performed to avoid catheter clot formation and prevent interference between samples.

At each sampling point, the first 1 mL of blood will be discarded for waste (utilizing a disposable tube), and blood samples (~5 mL) will be collected in ice-chilled potassium-EDTA sample tubes, mixed well immediately following collection, kept on ice or refrigerated and centrifuged within 20 minutes of collection. All blood samples will be centrifuged at 4°C, 2,000-3,000 *g* for 20 minutes for plasma isolation. Isolated plasma will be distributed into two (2) aliquots, approximately 1.5 mL in the first aliquot and the remainder in the second aliquot. The plasma sample vials will be frozen immediately on dry ice and then stored (within 60 minutes following plasma separation) in a freezer at -20°C or lower until analysis.

3) PK Sample ID

The sample ID for PK blood samples (before centrifugation) is defined as:

"**EIPK-nn-V-XX**"

where **EIPK-nn** is the subject ID, *V* is the dosing visit code, V=1 to 3, and ***XX*** is the two-digit time-point sequential number of the sample: 01, 02, 03, … 26 as listed in **Table B**.

The sample ID for PK plasma samples (after centrifugation) is defined as "**EIPK-nn-V-XX-k**" and ***k*** is the aliquot number (A or B). The PK plasma sample ID labels, with the size of 1/2"×1¾" (Avery label 5667), should be printed in blue font as demonstrated below:

EIPK-nn-V-XX-k

4) Sample Transportation and Analysis

The chain of custody, temperature control, and related documents for sample transportation must

be recorded. During transportation of PK samples from the site to Amphastar, the temperature should be below -20ºC. PK samples will be analyzed with an established LC/MS/MS method for detection of Epinephrine and Albuterol.

V. Study Evaluations

1) Primary Endpoints
   - $AUC_{0\text{-}6h}$, defined as area under the curve (AUC) concentration curve over time, from time 0 to 6 hours post-dose for epinephrine;
   - $AUC_{0\text{-}24h}$, defined AUC of plasma Albuterol concentration curve over time 0 to 24 hours post-dose;
   - $AUC_{0\text{-}\infty}$, defined as AUC in the plot of plasma Albuterol, or Epinephrine versus time from time 0 to infinity; and
   - C_{max}, defined as the maximum plasma concentration of Albuterol, or Epinephrine.

2) Secondary Endpoints
   - t_{max} of Albuterol, or Epinephrine, defined as the time, at which the C_{max} is observed; and
   - $T_{1/2}$, terminal elimination half-life of each analyte.

3) Additional Evaluations
   - Relative Bioavailability of Treatment-A (E004 inhalation) over Treatment-B (IM epinephrine injection);
   - Epinephrine concentrations versus time; and
   - The mean and standard deviation of study endpoints will be estimated. The variances within and between subjects will be estimated.

4) Statistical Analysis:

All endpoint will be listed and compared between treatments.

VI. Safety Evaluations

1) Monitor changes in vital signs and ECG will be analyzed between Screening, designated post-dose time points and EOS;
2) Heart rate, and systolic and diastolic blood pressure will be measured at baseline, 5±2 min, 15±5 min, 60±5 min, 120±15 min and 24 ±2 hours after dosing;
3) Monitor serum potassium and glucose at study baseline, designated post-dose time points and EOS;
4) Physical examinations at Screening, and at the end of the last Dosing Visit, or study termination;

5) The results of laboratory tests, including CBC (with differential), metabolic panel and UA, will be assessed and compared between data at Screening and that at EOS;
6) All subjects will be queried for ADEs. All ADEs must be recorded and assessed.

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1. INTRODUCTION AND RATIONALE

1.1. Introduction of the Drug Product

Epinephrine, also known as adrenaline, is an adrenal hormone and sympathetic neurotransmitter with both α- and β-adrenergic activities. Systemically administered epinephrine can result in a whole array of adrenergic responses, resembling those induced by activation of the sympathetic nervous system and the adrenal glands. Epinephrine administered by oral inhalation is associated with a rapid and effective delivery to the respiratory tract, making it ideal for rescue of acute asthma symptoms. It has been well established that orally inhaled epinephrine is associated with a minimal systemic exposure [3], due partly to its mode of pulmonary delivery and partly to its post-absorption degradation by catechol-O-methyltransferase (COMT) and monoamine oxidases (MAO) in the gastrointestinal tract and liver [4]. In addition, its local α-adrenergic vasoconstrictive actions in the lungs have been postulated to further reduce its systemic exposure after inhalation.

Primatene® MIST (E004) is a epinephrine inhalation aerosol, supplied in metered dose inhaler (MDI), which allows 0.125 mg per spray of epinephrine. Primatene® MIST is the only FDA-approved over-the-counter asthma inhaler that is indicated for temporary relief of mild symptoms of intermittent asthma.

Epinephrine Injection by [redacted] (therapeutically equivalent to EpiPen®) is a patient-actuated, auto-injection system that delivers a single dose of 0.30 mg epinephrine injection. Each auto-injector contains 1 mL epinephrine solution. Approximately 0.7 mL remains in the auto-injector after activation, but is not available for future use, and should be discarded. This auto-injector is used for immediate administration in patients who are determined to be at increased risk for anaphylaxis, including individuals with a history of anaphylactic reactions.

PROAIR HFA Inhalation Aerosol is made of albuterol sulfate, a racemic salt, of albuterol. PROAIR is supplied as a pressurized aluminum canister, with a plastic actuator, a dose indicator and dust cap. Each canister contains 8.5 g of the formulation and provides 200 actuations. Each actuation delivers 120 mcg of albuterol sulfate from the canister valve and 108 mcg of albuterol sulfate from the actuator mouthpiece (equivalent to 90 mcg of albuterol base). Albuterol HFA is prescribed to treat bronchospasm, and also commonly used as rescue inhalers (short-term bronchodilators) to alleviate asthma attacks.

1.2. Mechanism of Action

Although systemically administered epinephrine binds to all α- and β-adrenergic receptors non-selectively, the inhaled epinephrine is delivered to the pulmonary airways and binds to the β2-adrenergic receptors which are the predominant type in the bronchial smooth muscles [3]. This leads to the activation of adenylcyclase, and to an increase in the intracellular concentration of cyclic-3',5'-adenosine monophosphate (cAMP). This increase of cAMP in turn leads to the activation of protein kinase A, which then inhibits the phosphorylation of myosin and lowers intracellular ionic calcium concentrations, and results in relaxation of smooth muscles of all airways. Increased cAMP concentrations are also associated with the inhibition of release of inflammatory mediators from mast cells in the airway. These events lead to dilation of the tracheal bronchial lumens with substantial increase in air passage. Epinephrine acts as a functional antagonist to relax the airway smooth muscles irrespective of the spasmogen involved, thus protecting against most if not all bronchoconstrictor challenges.

Similarly, Albuterol relaxes the smooth muscle of all airways, from the trachea to the terminal bronchioles.

1.3. Indications and Dose

Primatene Mist, Epinephrine inhalation aerosol is indicated for temporary relief of mild symptoms of intermittent asthma (wheezing, tightness of chest, and shortness of breath) in patients age 12 years and older The recommended dose is 1-2 actuations every 3 hours.

Epinephrine injection is indicated in the emergency treatment of allergic reactions (Type I) including anaphylaxis.

PROAIR HFA Inhalation Aerosol is a beta2-adrenergic agonist indicated for treatment or prevention of bronchospasm in patients 4 years of age and older with reversible obstructive airway disease, as well as for prevention of exercise-induced bronchospasm in patients 4 years of age and older.

1.4. Pharmacokinetics (PK) and Pharmacodynamics (PD) Profile

Pharmacokinetics:

The absorption of inhaled epinephrine is rapid. In a multiple-dose systemic absorption of inhaled epinephrine study which enrolled six normal healthy volunteers, a rapid rise of plasma epinephrine levels with short duration were observed after administration of 15 puffs

followed by 30 puffs, with a >5 minute interval, at 160 mcg per puff. One minute after the initial 15 inhalations (2.4 mg) of epinephrine, the mean base-line epinephrine levels rose from 0.23 (±0.02) to a peak of 1.50(±0.61) nmol/L (i.e., 275 pg/ml). One minute after the subsequent 30 inhalations (a dose of 4.8 mg epinephrine), the epinephrine level rose to a peak of 4.22 (1.93) nmol/L (i.e., 772 pg/ml). The plasma epinephrine fell to baseline at 20 min after 30 inhalations of epinephrine [2].

Overall systemic sympathomimetic effects are minimized due to a very low systemic exposure (~5% bioavailability) after oral inhalation [3].

The systemic levels of albuterol are low after inhalation of recommended doses. The terminal plasma half-life of albuterol delivered by PROAIR HFA was approximately 6 hours.

Information from published literature suggests that the primary enzyme responsible for the metabolism of albuterol in humans is SULTIA3 (sulfotransferase). The primary route of elimination of albuterol is through renal excretion (80% to 100%) of either the parent compound or the primary metabolite. Less than 20% of the drug is detected in the feces.

Pharmacodynamics:

In a double-blind, randomized, two-period crossover study with mild-to-moderate asthma patients, the FEV_1 measurements in subjects receiving inhalation of epinephrine were compared with those receiving placebo inhalations. Clinically significant improvement in FEV_1, defined as an increase of ≥15% above baseline, has been observed. The results also showed that at 15 seconds after one inhalation, 11 of the 24 subjects receiving epinephrine, but only 1 of the 23 subjects receiving placebo, exhibited significant improvement in FEV_1 ($P < 0.004$). Mean volume (and %) increments (± 1 SEM) in FEV_1 at 15 sec after 1 inhalation were 390±60 ml (16.8±2.6%) after epinephrine, and -120±7 ml (-6.1±2.9%) after placebo ($P < 0.0001$). Mean peak volume (and %) increments in FEV_1 were 800±80 ml (34.5±3.3%) after epinephrine, and 180±60 ml (6.9±2.5%) after placebo ($P<0.0001$). The same study also demonstrated that the mean time (±SD) to peak increase in FEV1 (T_{max}) was 7.5±7.8 min. With 2 inhalations administered 1 min apart, average T_{max} was less than 10 min [5].

Epinephrine injection is mostly indicated for emergency treatment for anaphylactic reactions. When given subcutaneously or intramuscularly, epinephrine has a rapid onset and short duration of action [2]. Through its action on alpha-adrenergic receptors, epinephrine lessens the vasodilation and increased vascular permeability that occurs during anaphylaxis, which can lead to loss of intravascular fluid volume and hypotension. Through its action on beta-adrenergic receptors, epinephrine causes bronchial smooth muscle relaxation and helps alleviate bronchospasm, wheezing and dyspnea that may occur during anaphylaxis. Epinephrine injectionalso alleviates pruritus, urticaria, and angioedema and may relieve

gastrointestinal and genitourinary symptoms associated with anaphylaxis because of its relaxer effects on the smooth muscle of the stomach, intestine, uterus and urinary bladder.

1.5. Contraindications, Warnings, Precautions, and Adverse Reactions

1.5.1. Contraindications

Epinephrine Inhalation Aerosol is contraindicated in patients who are taking a prescription drug that contains phenylephrine, pseudoephedrine, ephedrine, or caffeine (for allergy, cough-cold, or pain), monoamine oxidase inhibitor (MAOI) (in certain drugs for depression, psychiatric or emotional conditions, or Parkinson's disease), or for 2 weeks after stopping the MAOI drug.

Albuterol Sulfate HFA is contraindicated in patients with a history of hypersensitivity to albuterol and any other Albuterol Sulfate HFA components.

1.5.2. Warnings and Precautions

Primatene Mist should be taken with precaution under certain conditions:

- ever been hospitalized for asthma
- heart disease
- high blood pressure
- diabetes
- trouble urinating due to an enlarged prostate gland
- thyroid disease
- seizures
- narrow angle glaucoma

Epinephrine injection should be used with precaution:

- more than two sequential doses of epinephrine should only be administered under direct medical supervision.
- Epinephrine injection should **only** be injected into the anterolateral aspect of the thigh
- rare cases of serious infections at the injection site may occur
- may trigger allergic reactions associated with sulfite in certain susceptible individuals
- patients with heart disease, hyperthyroidism, diabetes, Parkinson's disease (may notice a temporary worsening of symptoms), elderly individuals, and pregnant women should be carefully instructed in regard to the circumstances under which epinephrine should be used.

PROAIR should be taken with precaution and discontinue use under the following circumstances:

- paradoxical bronchospasm may occur
- deterioration of asthma
- not to substitute for use of anti-inflammatory agents
- cardiovascular effects may occur
- exceeding recommended dose may be fatal
- immediate hypersensitivity reactions may occur
- hypokalemia and changes in blood glucose may occur.

1.5.3. Adverse Reactions

The clinically observed adverse events are mainly related to its systemic adrenergic actions exerted by the small fraction of epinephrine that enters into the systemic blood circulation and interacts with the α- and β-adrenoceptors on the endothelia cells. The common and expected adverse event include: Increased blood pressure and heart rate (which could lead to more serious problems such as heart attack and/or stroke), nervousness, sleeplessness, rapid heartbeat, tremor, seizure, etc. Rarely, bronchial irritation and edema may occur. In some patients, severe prolonged asthma attacks may be precipitated. Rebound bronchospasm may occur when the effects of epinephrine end.

Adverse reactions to epinephrine include anxiety, apprehensiveness, restlessness, tremor, weakness, dizziness, sweating, palpitations, pallor, nausea and vomiting, headache, and/or respiratory difficulties.

Most common adverse reactions associated with the use of PROAIR are headache, tachycardia, pain, dizziness, pharyngitis, and rhinitis.

Use of Albuterol Sulfate HFA may be associated with the following:

- paradoxical bronchospasm;
- cardiovascular effects;
- immediate hypersensitivity reactions; and
- hypokalemia
- Hyperglycemia.

1.6. Drugs with Strong Impact on PK or PD

Drugs with impact on PK: Short-acting sympathomimetic aerosol bronchodilators and adrenergic drugs.

Drug with Impact on PD and safety: (i) Non-selective α-blockers; (ii) Beta-blockers; (iii) Diuretics, or non-potassium sparing diuretics may potentiate hypokalemia or ECG changes; (iv) Digoxin; (v) Monoamine oxidase (MAO) inhibitors; (vi) Sympathomimetics; (vii) Thyroid hormones; and (viii) tricyclic antidepressants.

A list of drugs, which have strong impact on PK or PD profile for epinephrine and Albuterol, is provided in Appendix III.

2. OBJECTIVES

2.1. Objective

To assess the drug exposure profile in systemic circulation of Primatene® Mist by inhalation, versus Epinephrine by IM, and ProAir® HFA by inhalation in healthy adults

2.2. Primary Endpoints

- $AUC_{0\text{-}6h}$, defined as area under the curve (AUC) concentration curve over time, from time 0 to 6 hours post-dose for Epinephrine;
- $AUC_{0\text{-}24h}$, defined AUC of plasma Albuterol concentration curve over time 0 to 24 hours post-dose;
- $AUC_{0\text{-}\infty}$, defined as AUC in the plot of plasma Albuterol, or Epinephrine versus time from time 0 to infinity; and
- C_{max}, defined as the maximum plasma concentration of Albuterol, or Epinephrine.

2.3. Secondary Endpoint

- t_{max} of Albuterol, or Epinephrine, defined as the time, at which the C_{max} is observed; and
- $T_{1/2}$, terminal elimination half-life of each analyte.

3. INVESTIGATORS AND STUDY SITES

3.1. Principal Investigator(s)

Principal Investigators (PI) responsible for the conduct of this study will be board certified physicians who are familiar with the study medications and trained to handle any untoward events related to medications.

The PI is responsible for the global aspects of the research study. For safety monitoring, a study physician or healthcare professional, who is certified for Advanced Cardiac Life Support, will be available at the study site at the time of study drug administration and during the entire time of PK blood sampling period. The Institutional Review Board (IRB) acknowledges one PI per study site. Qualifications for the PI and Co-Investigator(s) are listed on the Form FDA 1572.

3.2. Investigational Site(s)

The detailed information about the study site can be found on Form FDA 1572, including: Name, address, and statement of qualifications of each Principal Investigator; name of each sub-Investigator working under the supervision of the Investigator; name and address of the research facilities to be used; name and address of each reviewing IRB per US 21 CFR 312.23 section 6(iii)(b).

3.3. Laboratory

Samples of blood for evaluation of hematology and chemistry (metabolic panels) will be analyzed by the clinical laboratory with certification from a recognized accreditation agency (e.g., College of American Pathology or Clinical Laboratory Improvement Amendments certification). Refer to Appendix IV for a list of parameters analyzed, and the laboratory manual for details regarding specimen sample collection, processing, and shipment procedures. PK samples will be processed and analyzed by R&D Department, Amphastar Pharmaceuticals, Inc.

4. MATERIALS AND METHODS

4.1. Study Design

This is a randomized, safety evaluator-blind, three-treatment, crossover, fasting study in healthy adults (18 - 50 years of age).

4.2. Study Subjects

4.2.1. Sample Size

A total of at least 24 evaluable healthy adult volunteers will take part in this crossover study.

4.2.2. Subject Enrollment

A sufficient number of candidates will be screened to ensure 24 evaluable subjects will be enrolled into the study based upon the satisfaction of the inclusion and exclusion criteria. Informed consent will be obtained prior to performing any screening procedures.

4.2.3. Inclusion Criteria:

Candidates will be qualified only if they meet **all** of the following criteria:

(1) Agree to participate; understand and sign informed consent;
(2) Male and female adults, aged 18 to 50 years, inclusive at Screening;
(3) Generally healthy and medically stable, with no clinically significant abnormalities based on physical examination and laboratory tests as determined by the Investigators;
(4) Have good venous access;
(5) Have normal resting blood pressure and normal heart rate (HR) without history of syncope; a subject with out of range blood pressure may be enrolled in the study at the discretion of the Principal Investigator;
(6) Have a body mass index (BMI) of 18.0 – 30.0 kg/m^2;
(7) Female candidates must be >1 year post-menopausal or practicing a clinically acceptable form of birth control and confirmed by negative urine or serum pregnancy test at Screening;
(8) Negative HIV-Ab, HBs-Ag and HCV-Ab;
(9) Negative alcohol test (urine or breathalyzer);
(10) Negative drug screening results;
(11) Currently non-smoker; have not used any tobacco products for at least three (3) months prior to Screening; and

(12) Demonstrate proficiency in the use of MDI and a consistent inhalation time >2.0 seconds after training, for at least three (3) times, with a maximum of 5 attempts.

4.2.4. Exclusion Criteria

Candidates will be excluded from the study for **any** of the following reasons:

(1) Concurrent clinically significant cardiovascular, hematological, renal, neurologic, hepatic, endocrine, psychiatric, or malignant diseases.
(2) Known intolerance or hypersensitivity to any component of the study drugs (i.e., Epinephrine, Albuterol Sulfate or any sympathomimetic drugs, HFA-134a, thymol, ethanol, ascorbic acid, nitric acid, and hydrochloric acid).
(3) Upper or lower respiratory tract infection, or other systemic infection within 6 weeks prior to Screening;
(4) Clinically significant abnormalities in the screening/baseline ECG; prolonged corrected QT interval (QTcF) on ECG: men >450ms, women: >470ms; single or multiple premature ventricular contractions (PVC);
(5) Abnormal thyroid function test (if TSH is out of range, refer to T3/T4 for thyroid function assessment);
(6) Subject has been on other investigational drug/device studies within 30 days of Screening Visit or planned participation in another investigational drug trial at any time during this trial;
(7) Women who are pregnant or lactating or planning a pregnancy during the study period;
(8) Subject has donated or lost > 500 mL of blood within 3 months of Screening;
(9) Evidence of alcohol or drug abuse or dependency within 6 months prior to screening; or
(10) Use of any of the prohibited drugs listed in Appendix III without appropriate washout.

4.3. Randomization Procedures

4.3.1. Subject Identification Assignment

Subject will sign informed consent and be assigned a screening ID, as

EIS-mm,

where ***EI*** represents "E004-CL-I", ***S*** stands for screening, and ***mm*** is the screening sequential number. The subject will be evaluated per inclusion/exclusion criteria. The

following procedures will be performed at Screening: (i) physical examination (PE), (ii) measurements of vital signs, (iii) ECG, (iv) urinalysis (UA), (v) urine drug screen, (vi) alcohol screen (urine or breathalyzer), (vii) urine or serum pregnancy test (female subjects), and (viii) fasting (≥ 8 hours) blood tests [comprehensive metabolic panel, complete blood count (CBC) with differentials, thyroid function tests, HIV, hepatitis B and C tests].

After satisfying the enrollment criteria, subjects will be assigned a unique subject ID as

EIPK-nn,

where ***nn*** is the sequential number in order of enrollment.

The same Subject ID will be used throughout the Dosing Visits. The Subject ID numbers will be recorded on the study source documents, the case report forms, and other appropriate study documents. In addition, all subjects will be identified by their first, (middle), and last initials.

4.3.2. Subject Randomization

The Investigator must ensure that all inclusion/exclusion criteria are met, and will endorse the subject enrollment at the beginning of Dosing Visit 1.

A set of computer generated randomization codes will be supplied by Amphastar IT department in accordance with the Standard Operating Procedure (SOP). A 3 × 3 Latin Square method will be used for randomization. The randomization code will dictate the sequence of the three (3) crossover treatments for individual subject.

4.4. Dispensing, Returning, and Storage of Material and Drug Supplies

All study drug units are prepared and packaged as individual units by the Amphastar manufacturing team under GMP conditions and QA oversight. Study drugs will be packaged and delivered to the investigational site. All study units are labeled with proper subject ID#, and Visit#, but are devoid of any treatment-identifying information.

Study drugs will be properly labeled and delivered to the dosing area for immediate use. All study drug units must be returned to the site or designee when the visit is completed, and its use documented.

At each Dosing Visit, one (1) study treatment (Treatment-A, B, or C) will be assigned to subjects per the randomization code (**Table 1**).

Table 1. Treatments and Dose

Items	Treatment-A	Treatment-B	Treatment-C
Study Drug Name	Primatene Mist, E004	Epinephrine Injection Auto-Injector (Generic of EpiPen®)	ProAir
Manufacturer	[redacted]		
API	Epinephrine	Epinephrine	Albuterol Sulfate
Dosage Form	Microcrystalline Suspension HFA MDI	Sterile Solution	Microcrystalline Suspension HFA MDI
Strength	0.125 mg/inh	0.3mg/0.3mL	0.09 mg/inh
Dose Regimen	2 inhalations, 0.25mg	IM Injection of 0.30 mg Epinephrine in 0.30 mL	2 inhalations, 0.180 mg
Route of Administration	Oral Inhalation	IM Injection	Oral Inhalation

Study drugs should be stored at USP defined, controlled room temperature, with excursions permitted from 15°-25°C (59°-77°F). The study drugs will be protected from environmental extremes and stored in a locked cabinet or room to which only designated persons have access. The inhalers will be stored with the mouthpiece down.

4.5. Study Drugs Administration and Training

4.5.1. Training for Correct Use of MDI Inhaler

During the Screening and at the beginning of every Dosing Visit, each subject will be trained on the correct use of the MDI inhalation aerosols in a standard fashion to assure a relatively consistent inspiratory flow rate and duration. A video on how to use the MDI will be provided at each training session. The training will use placebo, a MDI simulation unit that contains no active drug. Subjects have to demonstrate a consistent inhalation time >2.0 seconds (at least 3 times, with a maximum of 5 attempts) after training. Vitalograph AIM™ (Aerosol Inhalation Monitor) will be used to monitor and measure the duration of the inhalation during the training at Screening and prior to dosing.

4.5.2. Preparation and Training of MDIs at the Dosing Visit

Each MDI must be primed at the study site prior to the first use by releasing 4 test sprays (E004 for Treatment-A), or 3 test sprays (ProAir for Treatment-C) into the air away from the face, **shaking well before each spray**. Priming will be performed in an area that is well ventilated (preferably a fume hood, or an open space), and physically separated from the study evaluation area. Refer to the training video for details on the priming procedure.

(1) **Correct sitting position**: In order to get a deep proper breath during dosing, the subject must sit on a stool, not a chair, with their posture straight in a relaxed calm state for at least 1 min prior to dosing. Do not slouch or lean forward or backward.
(2) **Finger placement for dosing**: To ensure proper drug delivery, subject is required to hold the canister with the thumb at the bottom and place the index finger of the same hand on the top of the canister to release the drug. For each actuation, subject **must fully depress the top of the metal canister**, and **hold it for at least one second before releasing**.
(3) **Critical Timing**: Subject begins a **slow and deep inhalation before pressing the inhaler**. A slight delay in the sequence can result in deposition of inhaled drugs on the back of the throat and tongue.
(4) **Deep inhalation as long as possible**: After pressing the inhaler, subject should continue to inhale slowly and deeply for as long as possible (at least 3-5 seconds, refer to the training video). Keep tongue away from the opening of the mouthpiece.
(5) After deep inhalation, **hold the breath for** approximately **10 seconds** (5- 10 seconds) [6] to allow deposition of drug particles into the lower level of the lung.

4.5.3. Detailed MDI Dosing Procedure

(1) The designated study site personnel will verify if the canister is fully inserted into the actuator.
(2) The designated study site personnel will remove the cap from the mouthpiece of the actuator, and check the mouthpiece for foreign objects prior to use;
(3) Subjects will **first shake and spray into the air one time to mix the medicine.**
(4) Subjects will exhale deeply through the mouth and empty the lungs as completely as possible by exhaling fully (exhalation must be deep and slow, similar to the deep inhalation step);
(5) Immediately after the full exhalation, subjects will hold the inhaler with the mouthpiece down, then places the mouthpiece fully into their mouth, with lips sealed around the mouthpiece;
(6) While inhaling, subjects will fully depress the top of the canister with an index finger. Subjects should keep inhaling **deeply and slowly for as long as possible (at least 3-5 seconds)** through the mouth;
(7) After breathing in fully, remove the inhaler from the mouth, subjects keep the mouth closed, hold the breath for approximately10 seconds (5- 10 seconds) [6];
(8) Subjects will exhale slowly through the nose (keep lips nearly closed); and
(9) Repeat the above (1) to (7) steps for the second inhalation. The time interval between each actuation will be within 30 seconds.

4.5.4. IM Injection Procedure

IM injection (0.30mg of epinephrine solution in 0.30 mL) for Treatment-B will be given on the thigh. Subjects will be lying down in a supine position. The 0.30 mL dose will be given perpendicularly as a single deep IM injection into anterolateral aspect of the thigh (vastus lateralis site). Epinephrine auto-injector (Treatment B) will be administered as follows [2]:

(1) Doser or designated site personnel will quickly twist off the yellow cap off the auto-injector.
(2) Subject will hold the leg firmly in place.
(3) Doser or designated site personnel will first pull off the blue safety release to unlock the auto injector.
(4) Doser will place the orange tip against the middle of the outer thigh (upper leg) at a right angle (perpendicular) to the thigh.
(5) Swing and push the injector firmly until it 'clicks.' The click signals that the injection has started.
(6) Hold firmly in place for 3 seconds. Time 0 begins once the holding is completed.
(7) Remove the injector from the thigh and massage (apply gentle pressure) with dry gauze to the injection area for 10 seconds. The orange tip automatically extends out after use.
(8) The injection site will be observed for any medication leakage for approximately 30 seconds after the dosing is completed. If there is observed leakage, PK samples will not be collected, subject will be early terminated (ET).

4.6. Blinding Methodology and Procedure

The preparation area will be physically separated from the dosing area to ensure the evaluator remains blinded. All treatments will be prepared by qualified personnel at the study site prior to dosing at each study visit according to the order of treatment arms assigned to subjects per the randomization code. Subjects will be escorted to a separated dosing area for study drug administration to preserve blinding from evaluators.
Study drugs will be dispensed by CRC or pharmacist per randomization.

Safety evaluator(s) will remain blinded during the course of the study and will have the sole responsibility of evaluating any Adverse events.

4.7. Unblinding

Should any safety evaluator become "unblinded" to any subject's treatment assignment during the study, the Investigator shall notify Amphastar Clinical Research Department within 24 hours. The Justification for Code Breaking Form will be signed and dated by the

Investigator, and the Amphastar Clinical Research Associate (CRA). The form will be placed back into the envelope, which is not to be resealed, and kept in study site regulatory files, separated from the other envelopes. Any subject whose envelope seal has been broken will be subject to QA audit and may be excluded from the BE data analyses. Subject should also be reminded to maintain the identity/configuration of the study drug provided to them and must not disclose this information to the blinded study team or other subjects in order to maintain the blinding of the evaluators.

5. STUDY PROCEDURE

5.1. Global Plan

The design includes (i) a Screening Visit (3-7 days prior to Visit-1), (ii) three (3) Dosing Visits separated by a 2-7 day washout period, and (iii) a post-study follow-up evaluation 1-7 days after Dosing Visit-3 or study termination.

5.2. Screening Procedure and Scheduling

5.2.1. Schedule and Purpose of Screening Visit

The Screening Visit takes place 3-7days prior to Dosing Visit-1. During Screening, subject qualification will be determined based on the Inclusion/Exclusion Criteria (Sections 4.2.3 and 4.2.4), the physical examination, and laboratory test results. These data will be documented on the subject source documents.

5.2.2. Informed Consent Form

The Informed Consent document will be used to explain the purpose, risks, and benefits of study participation to the subject in simple terms at the Screening Visit. The Investigator must obtain a signed informed consent form from each subject before proceeding to any screening and study activities. A copy of the signed Informed Consent Form should be given to the subject. The Informed Consent Form includes compliance with the privacy provisions of HIPAA.

5.2.3. Medical History, PE, and General Safety (Screening Activities)

The Screening Visit entails the following screening activities:

(1) Interview for demographic information and medical history;
(2) Body Mass Index (BMI) needs to meet the requirements in Section 4.2.3. BMI can be calculated and checked by the formula and chart in Appendix II, or by utilizing the NIH website;
(3) Review medication history for any prohibited drugs (Appendix III). Ensure any appropriate washout has been observed;
(4) A full physical examination and measurements of vital signs will be performed to ensure that the subject is generally healthy;
(5) The subject has to rest in a secluded area for at least 10 minutes before measurements of vital signs. The blood pressure and heart rate measurement can be repeated twice, each separated by a 15 minute interval;

(6) 12-lead ECG will be conducted (routine and QT/QTc) to ensure absence of overt cardiac illnesses;
(7) Collect urine samples for urinalysis (routine and microscopic), alcohol/drug screens (urine or breathalyzer test allowed for alcohol) for all subjects, and pregnancy test for all female subjects (serum pregnancy test allowed);
(8) Collect blood samples for CBC, thyroid function, HIV-Ab, HBs-Ag, and HCV-Ab tests;
(9) Collect fasting (≥ 8 hours) blood samples for serum comprehensive metabolic panel (per Appendix IV); and
(10) At the Screening Visit, subjects should be trained on the use of the MDI inhalation aerosols in a standard fashion to assure a relatively consistent inspiratory flow rate and inspiratory duration as described in Section 4.5. Eligible subjects must demonstrate the correct use of the MDI unit and a consistent inhalation time >2.0 seconds after training, for at least three (3) times, with a maximum of 5 attempts. AIM™ (Aerosol Inhalation Monitor) will be used to monitor and measure the duration of the inhalation during the training at Screening and prior to dosing.

Out of range lab results may be repeated once at the discretion of the Investigator. Subject enrollment will be confirmed at the beginning of Dosing Visit-1 (Section 5.3.1). The final decision of enrollment will be determined by the Investigator.

5.2.4. Instruction to Subjects at the End of Screening Visit

(1) Dates for Dosing Visit-1 Visit-2, and Visit-3 should be separated by 2 to 7 days.
(2) Subject must be at least 10-hours fasting for each Dosing Visit.
(3) Subjects must refrain from alcohol consumption and caffeine-containing (including decaffeinated) beverages or foods (coffee, tea, applicable soft drinks including cola and analogs, or chocolate) for 24 hours prior to each Dosing Visit.
(4) At each Dosing Visit, subjects are required to check in at the site the night before. Dinner will be provided at study site.

5.3. Dosing Visits

5.3.1. General Profile for Dosing Visits

The same procedures will be performed at each Dosing Visit, except that the subject will be given different study treatments based on the randomization code.

At Dosing Visit-1 the following activities will be conducted:

(1) Enrollment is verified at the beginning of Dosing Visit-1, by reviewing all inclusion/exclusion criteria and other requirements.

(2) Upon verification of subject enrollment, a Subject ID will be assigned and the randomization procedure will be completed (see Section 4.3.1 and 4.3.2)

5.3.2. Pre-dose Activities

At each Dosing Visit, the following review and verification will be performed prior to study drug administration:

(1) A urine or serum pregnancy test must be performed on all female subjects. If it is positive, the subject will be terminated from the study;
(2) Any concomitant medication since the last visit will be recorded, including name of medication, route of administration, dosage, indication, and start and end dates where available. If any medications listed in Appendix III were used after the last visit without the proper washout period, the subject will be terminated;
(3) Perform alcohol and drug test; if any is positive, subject will be terminated;
(4) Subjects will be trained again on the use of the MDI inhalation aerosols prior to each treatment session, as described in Section 4.5.1;
(5) Placement of ECG leads;
(6) Subjects need to rest for at least 10 minutes prior to the baseline vital signs evaluations. Vital signs should be taken from the opposite arm of the IV catheter arm if possible; Vital signs measurement may be repeated twice to PI discretion.
(7) An indwelling IV catheter shall be placed into one of the antecubital region veins for blood drawing, or separate venipunctures may be used; and
(8) One (1) baseline blood samples will be collected within 30 minutes before dosing.

5.3.3. Instructions to the Subjects before Dosing at Dosing Visit

(1) No food will be allowed for 10 hours prior to dosing and until 4 hour post-dose PK samples have been collected.
(2) Meals will be provided at normal times after the 4 hour post-dose blood draw.
(3) Water intake is not allowed 1 hour before and 1 hour after drug administration.
(4) All physical activities during the Dosing Visits should be limited with the exception of bathroom visits until the completion of the 120-minute post-dose PK sampling at each Dosing Visit.
(5) The subject will remain in a semi-recumbent or seated position for 120 minutes after drug dosing. After the 120-minute PK sampling, the subject has no movement restrictions, but should refrain from any excessive physical activity.
(6) During each Dosing Visit, subjects should refrain from alcohol consumption and caffeine-containing (including decaffeinated) beverages or foods (coffee, tea, applicable soft drinks including cola and analogs, or chocolate).

5.3.4. Study Medication Administration

At each dosing visit, subjects will either self-administer 2actuations from the MDI inhaler provided, or receive an IM injection (0.30mg Epinephrine injection for Treatment-B).

IM Injection into the vastus lateralis site:

IM injection (0.30mg of epinephrine solution in 0.30 mL) for Treatment-B will be given on the thigh. Subjects will be lying down in a supine position. The 0.30 mL dose will be given perpendicularly as a single deep IM injection into anterolateral aspect of the thigh (vastus lateralis site). Refer to Section 4.5.4 for detailed IM injection procedure.

MDI Self-Administration:

The designated unblinded dispenser at the study site will prime each unit prior to the first use (Section 4.5). Each subject will also be retrained on the use of the MDI inhalation aerosols as described in Section 4.5.1 to Section 4.5.3.

Study drugs will be dispensed by the designated site professional, and the reading on the dose indicator of the MDI unit will be recorded prior to dosing.

Subject will **shake the inhaler well before each spray**. Subjects will self-administer 2 inhalations from the inhaler unit (within 30 seconds interval between each inhalation). The study drug administration process will be video-recorded and used for an investigation of apparent dosing irregularity. Refer to Section 4.5.3 for critical instructions for correct dosing techniques.

The study medication administration should be conducted between 07:00 and 11:00 AM on the Dosing Visit. Time of the start of each inhalation will be recorded.

Once dosing is completed, CRC or other site professionals will record the readings of each dose-indicator in the study source documents. The used MDI inhaler will be collected at the end of the Dosing Visit.

5.3.5. PK Blood Sampling

At each Dosing Visit, PK blood samples will be collected and plasma will be isolated for analyzing the concentrations of Albuterol, and epinephrine. A total of 26 blood samples will be drawn from each subject at each Dosing Visit as indicated in **Table 2**.

(1) A catheter will be inserted into one of the antecubital region veins for blood sampling. Alternatively, collection from a vein of the hand or forearm for easier access or via venipuncture is also acceptable;
(2) One (1) pre-dose samples (baseline) will be collected within 30 minutes before dosing;
(3) Time 0 is defined as the beginning of the last inhalation of the MDI, or after completion of IM injection, Section 4.5.3.

Table 2. PK Blood Sampling Schedule

Seq. #	Scheduling	Time window	Sample No.,"XX"
1	Baseline	within 30 min pre-dosing	01
2	1 min	± 0.5 min	02
3	2 min		03
4	3 min		04
5	5 min	±1 min	05
6	7 min		06
7	9 min		07
8	12 min	±1 min	08
9	15 min		09
10	18 min		10
11	21 min		11
12	25 min	±2 min	12
13	30 min		13
14	40 min	±5 min	14
15	50 min		15
16	60 min		16
17	70 min		17
18	80 min		18
19	90 min		19
20	120 min	±10 min	20
21	4 hrs		21
22	6 hrs	±15 min	22
23	8 hrs		23
24	12 hrs	±20 min	24
25	18 hrs		25
26	24 hrs		26

(4) A catheter flush will be performed between each blood sample collection to avoid catheter clot formation and prevent interference between samples. At each sampling point, the first 1.0 mL portion of the blood (or saline flush) will be collected into a disposable tube and discarded;
(5) At each PK sampling point, blood samples (~5 mL), will be collected in ice-chilled potassium-EDTA sample tubes, mixed well (gently invert the tube 8-10 times) immediately following collection, kept on ice or refrigerated and centrifuged within 20 minutes of collection.
(6) If a tube breaks, a new sample can be collected if it is within the allowed time window (**Table 2**).
(7) Plasma isolation (i.e., centrifugation of the blood samples) will start within 20 minutes of blood collection. Blood samples will be centrifuged at 4°C, 2,000-3,000 *g* for 20 minutes for plasma isolation.
(8) Isolated plasma will be distributed into two (2) 2 mL cryo vials, approximately 1.5

mL in the first vial and the remainder in the second vial. The plasma sample vials will be frozen immediately on dry ice (within 60 minutes following plasma separation) and then stored (within 60 minutes following plasma separation) in a freezer at -20°C or lower until analysis.

5.3.6. PK Blood Sample ID and Labeling

The sample ID for PK blood samples (before centrifugation) is defined as

"**EIPK-nn-V-XX**"

Where, **EIPK-nn** is the subject ID; **V** is the Dosing Visit code, V=1, 2 or 3; and **XX** is the two-digit time-point sequential number of the sample: 01, 02, 03... 26 as listed in **Table 2**.

The sample ID for PK plasma samples (after centrifugation) is defined as "**EIPK-nn-V-XX-k**" and **k** is the aliquot number (A or B). The PK plasma sample ID labels, with the size of 1/2"×1¾" (Avery label 5667), should be printed in blue font as demonstrated below:

EIPK-nn-V-XX-k

5.3.7. Safety Evaluations/Monitoring

The following safety measurements will be performed during each Dosing Visit:

(1) Vital signs (HR, SBP/DBP): at baseline, 5±2 min, 15±5 min, 60±5 min, 120±15 min and 24 ±2 hrs post-dose.
(2) 12-lead ECG (routine QT and QTc analysis): at baseline, 20±5 min, 120±15 min and 24 ±2 hours post-dose.
(3) Serum potassium and glucose levels will be measured at baseline, 10±2 min, 60±5 min, and 24 ± 2 hrs post-dose.
(4) All subjects will be queried for adverse events (AEs) and AEs must be recorded with all related information.

5.4. Activity at End of Study (EOS)

The following EOS evaluations will be conducted at the end of study as follows:

(1) The Investigator(s) will ensure that all study drugs and study records are accountable.
(2) At Visit-3, blood and urine samples will be taken for fasting comprehensive metabolic panel (Refer to Appendix IV). Lunch will be provided **after** the EOS

blood draw. The meals and fasting schedule is indicated in **Table 3**.

Table 3. Meals and fasting schedule

	Night before Dosing	Day 1	Day 2 (Visit-3 Only)
Morning	NA	Dosing	Breakfast will be provided after EOS blood draw
Mid-Day	NA	Lunch (≥ 4 hr post-dose)	NA
Evening	Dinner, then fast overnight (≥ 10 hr)	Dinner, then fast overnight(≥8hr)*	NA

* At Visit-3, breakfast will be provided after EOS blood draws

(3) Conduct EOS physical examination between 16 and 26 hours post-dose. EOS Vital signs, ECG, CBC, fasting comprehensive metabolic panel and serum potassium/glucose will be measured at the end of PK sample collection (24 ± 2 hrs post-dose) at Visit-3.
(4) Adverse events, whether reported by the subjects or observed by study staff, will need to be documented, and reported or **followed up** where applicable.
(5) Review all safety and compliance data.
(6) For Early Termination (ET), an ET evaluation will be conducted with the same safety assessments as listed (items 1-5 above) when appropriate and feasible.

5.5. Follow-up Activities

All treated subjects will be followed up by a phone call scheduled 1-7 days after the completion of Dosing Visit-3 or study termination. Subjects will be asked if they have experienced any adverse event after the last Dosing Visit.

5.6. PK Sample Transfer and Laboratory Analysis

The plasma samples will be transferred at temperature below -20˚C from the site to the Amphastar R&D Laboratory. The chain of custody, temperature control and related documents for sample transfer must be recorded and documented.

All plasma samples should be stored at -80ºC or lower in the laboratory. Epinephrine and Albuterol concentration in the PK plasma samples will be analyzed by the R&D Department of Amphastar with validated methods under GMP.

5.7. Summary and Schema of Study Activities

5.7.1. Data collection

Data collection for this study is summarized as follows:

- PK samples for evaluation of plasma epinephrine or Albuterol concentrations;
- Safety data as vital signs, ECG, urinalysis, serum potassium, glucose and clinical laboratory testing, etc.; and
- Adverse events reported.

5.7.2. Schema of Study Activities

A schematic outline of the study activities is provided in **Table 4** below.

Table 4. Summary of Activities in All Visits and EOS

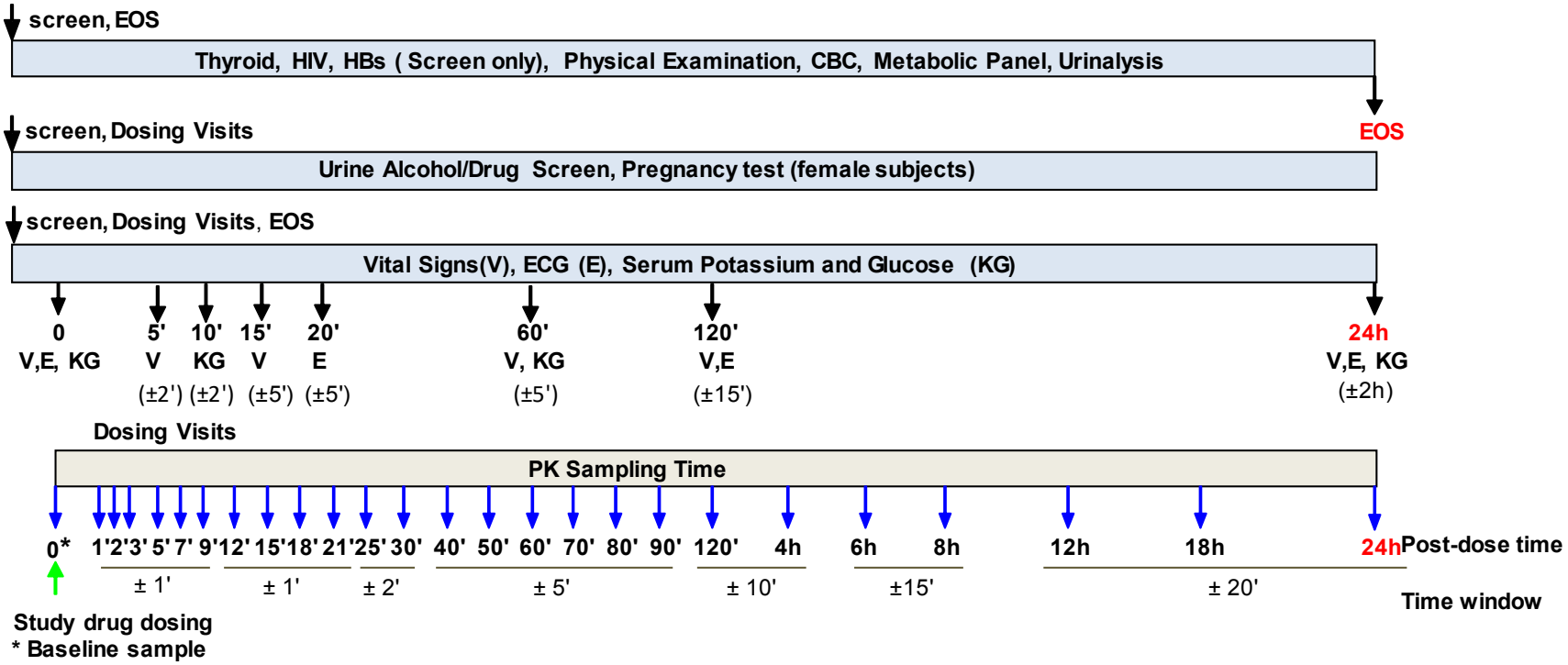
Study Activities & Evaluations	Screen Visit	Dosing Visit-1	Dosing Visit-2 and 3	EOS[1]
Informed Consent / HIPAA	X			
Medical history / demographics	X			
Physical Exam (PE)	X			X
Lab: Urinalysis (UA)	X			X
Lab: CBC/Comp. metab. Panel	X			X
Lab: Thyroid Function	X			
Lab: HIV-Ab, HB-sAg, HCV-Ab	X			
Inclusion/Exclusion	X	X		
Confirmation of enrollment		X		
Assign subject ID		X		
Randomization		X		
Training/Dosing of MDI	X	X	X	
Concomitant Medicines	Record throughout study period			
Urine or serum pregnancy test	X	X	X	
Urine drug screen	X	X	X	
Alcohol screen (urine or breathalyzer)	X	X	X	
Food / beverage restrictions		X	X	
Meals served		X	X	
PK Baseline samples		X (1)	X (1)	
PK samples		X (25)	X (25)	
Vital signs	X	X (6)	X (6)	X[2]
12-lead ECG	X	X (4)	X (4)	X[2]
Serum potassium and glucose	X	X (4)	X (4)	X[2]
Adverse event reporting	Record throughout study period			

1. The fasting comprehensive metabolic panel (≥8 hours) for EOS will be taken at the Study Visit-3 or study termination
2. Vital signs, ECG and serum potassium and glucose at 24 ± 2h at Visit-3 can be used for EOS

5.8. Chart for Study Activities

Study activities are summarized in **Figure 1**.

Figure 1. Summary of Study Activities



6. STATISTICAL ANALYSIS

6.1. Populations

6.1.1. Per-Protocol Population (PPP)

The "Per Protocol" population is defined as all subjects who have received all study medications during the study and are evaluable for all treatments. The primary analyses will be performed based on "Per Protocol" population (PPP).

An evaluable subject for primary analyses of this study must meet all of the following ten (10) items for both treatments:

(1) Correct dose and administration;
(2) Nineteen (19) or more of 25 post-dose PK data points (>75%) are available;
(3) There are no more than four (4) consecutive missing PK data points;
(4) The baseline PK data point is available;
(5) For Treatment-A, at least four (4) of the five (5) PK data points at 1, 2, 3, 5, and 7 minutes post-dose are available;
(6) For Treatment-B, at least four (4) of the five (5) PK data points at 9, 12, 15, 18, and 21 minutes post-dose are available;
(7) For Treatment-C, at least four (4) of the five (5) PK data points at 25, 30, 40, 50, and 60 minutes post-dose are available;
(8) For Treatment-A, at least three (3) of the PK data points at 25, 30, 40, and 50 minutes post-dose are available.
(9) For Treatment-B, at least three (3) of the PK data points at 80, 90, 120 minutes and 4 hours post-dose are available; and
(10) For Treatment-C, at least three (3) of the PK data points at 6, 8, 12 and 18 hours post-dose are available.

6.1.2. Treated Population (TP)

The "treated" population is defined as all subjects who have been randomized and treated with any dose of the study drugs.

The safety evaluation will be performed base on treated population (TP).

6.1.3. Intent-to-treat Population (ITT)

The "Intent-to-Treat" population is defined as all subjects who have been randomized.

6.2. PK Results Evaluations

6.2.1 Calculation of Primary Endpoints

- C_{max} will be obtained directly by observing the plot of PK curve;
- $AUC_{0\text{-}6h}$ and $AUC_{0\text{-}24h}$ will be calculated with trapezoid method;
- $AUC_{0\text{-}\infty}$ will be calculated with extrapolation method.

6.2.2 Calculation of Secondary Endpoints

- $\mathbf{t_{max}}$ is an observed values, at which $\mathbf{C_{max}}$ is achieved.

6.2.3 Missing Data Handling

Missing data includes missing samples and missing data points for various cases, such as:

(1) Missed scheduled points for sampling;
(2) Tube breakage;
(3) Inadequate amount of sample for analysis;
(4) Unexpected reasons (i.e., medical condition such that the subject is unable to complete the study).

A table of all missing samples will be provided with explanations.

6.2.4 Relative Bioavailability

The relative bioavailability of Treatment-A over Treatment-B (IM) can be defined as follows:

$$\text{RBA} = \frac{\text{AUC}^{\text{A}}_{0-\infty}}{\text{AUC}^{\text{B}}_{0-\infty}}$$

6.3. Safety Assessment

(1) Changes in vital signs and ECG will be analyzed between Screening, designated post-dose time points and EOS;
(2) Changes in serum potassium and glucose at study baseline, designated post-dose time points and EOS;
(3) Physical examinations at Screening, and at the end of the last Dosing Visit, or study termination;
(4) The results of laboratory tests, including CBC (with differential), metabolic panel and UA, will be assessed and compared between data at Screening and that at EOS;

(5) AEs will be observed and documented as described in Sections 5.3.7 **and** 7.2.1. AEs of each study treatment will be assessed per seriousness, severity, expected/unexpected, etc. The occurrence of AEs as absolute case number and relative ratio will be compared between each treatment.

6.4. Interim Analyses

No interim analyses are planned for this study.

7. STUDY MANAGEMENT

7.1. Discontinued Subjects or Withdrawal

Subjects can withdraw or be withdrawn from the study at any time. In addition, it may be necessary to discontinue subjects from the study due to medical safety considerations, noncompliance or administrative concerns.

If for any reason a subject is withdrawn or discontinued before completing the study, the reasons for withdrawal or discontinuation must be entered on the End-of-Study and/or Subject Withdrawal or Discontinuation source document page.

7.2. Adverse Events

The Investigator will be required to provide the sponsor with any information concerning any findings that suggest any adverse event pertinent to the investigation.

The study may be stopped at any time due to safety concerns. Subject safety will be constantly monitored throughout the study. However, if and when a significant safety concern arises, such as occurrence of serious and unexpected adverse drug events that are considered associated with the study drug(s) per regulatory guidelines and SOP, the study may be discontinued and safety will be reviewed.

Any such safety analysis will be presented and discussed in the final study report.

7.2.1. Types of Adverse Events

The term "adverse event" could include any of the following events that arise, or increase in severity and/or frequency, during the course of the study:

a) Any signs or symptoms whether thought to be related or unrelated to the condition under study.
b) Any clinically significant laboratory abnormality.
c) Any physical abnormality detected.

These data will be recorded on the appropriate case report forms, including "Adverse Event Form", regardless of whether they are thought to be associated with the study or the drug under investigation. Per FDA definition, "associated with the use of the drug" means that "there is a reasonable possibility that the experience may have been caused by the drug".

An adverse event is considered associated with the use of the drug if the attribution is possible, probable, or very likely according to definitions specified in the AE reporting SOP and the CRF for adverse event.

Signs or symptoms will be graded by the Investigator as mild, moderate or severe according to the following definitions:

Severity	Definition
Mild	Causing no limitation of usual activities
Moderate	Causing some limitation of usual activities
Severe	Causing inability to carry out usual activities

When a subject experiences any significant adverse event or laboratory abnormality, the event must be followed based on good clinical practice until it becomes resolved or stable.

7.2.2. Definition of Serious and Unexpected Adverse Events

According to the FDA guidelines, a serious adverse event will refer to any adverse drug experience occurring at any dose results in any of the following outcomes:

a) Cancer [6].
b) Death.
c) A life-threatening adverse drug experience.
d) Hospitalization or prolongation of existing hospitalization.
e) A persistent or significant disability/incapacity.
f) A congenital anomaly/birth defect.
g) Other important medical events, while not resulting in death, being life-threatening, or requiring hospitalization, may still be considered as serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An unexpected event is any adverse experience, the specificity or severity of which is not consistent with the approved labeling. “Unexpected”, thus refers to an adverse drug experience that has not been previously observed rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

Expected adverse events of Primatene Mist include: headache, nausea, vomiting, nervousness, dizziness, shaking (tremor), trouble sleeping, stomach upset, sweating, dry mouth, bad taste, cough, sore throat, difficult or painful urination, fast heartbeat, weakness, high blood pressure, loss of appetite, loss of skin color, or dry throat. Severe adverse reactions are very rare.

7.2.3. Obligations of AE Reporting to Sites, FDA and IRB

Sponsor Reporting to Investigator: Sponsor shall promptly notify the Investigators of any material discoveries made during monitoring of the sites which could affect the safety of participants or influence the conduct of the study.

Investigator Reporting to Sponsor: Any serious events, whether or not unexpected or considered to be associated with the use of the drug, must be communicated by the Investigator to Amphastar Pharmaceuticals, Inc. immediately upon discovery of the event. The Clinical Research Associate (CRA) will then advise the Investigator regarding the nature of any further information or documentation that is required.

Sponsor/Investigator Reporting to FDA: It is required to report to FDA any serious adverse event observed during the conduct of the study, regardless of whether the event is considered drug related.

Each notification shall be made as soon as possible and in no event later than 15 calendar days after the sponsor's initial receipt of the information. Each notification shall be submitted via the FDA Safety Reporting Portal (SRP). The FDA must be notified via telephone of the Sponsor's initial notification of any fatal or life-threatening experiences associated with the use of the drug as soon as possible but in no event later than seven (7) calendar days.

Sponsor/Investigator Reporting to IRB: All serious adverse events or an unanticipated problem must be reported to the appropriate IRB/Ethics Committee promptly.

1. Serious Adverse Event (SAE) Reporting Policy:
An adverse event is considered **serious** if it results in any of the outcomes listed in Section 7.2.2. Study sites are required to report any SAE to the IRB, regardless of whether the event is considered drug related. The specific reporting policy defined by the IRB will be followed for the reporting of SAE to IRB within the required time period.

Note: IRB does not require the event to be unexpected. The IRB feels that this category is too subjective, and can change over time, to be a criterion for deciding whether the event is reportable or not.

2. Unanticipated Problem/Event Report Form:
In order for the event to be reportable to the IRB, it must meet all of the following criteria:

- The event is unexpected (in terms of nature, severity, or frequency) given the research procedures described in the study Protocol and/or Informed Consent

Form.

- The event is related or possibly related to participation in the research study.
- The event suggests that the research places subjects or others at a greater risk of harm than was previously realized or anticipated.

It is required to report unanticipated problem(s) to the IRB promptly after the Investigator becomes aware of them. The specific reporting policy defined by the IRB will be followed for the reporting of unanticipated problem to IRB within the required time period.

Sponsors are specifically required to "keep each participating Investigator informed of new observations discovered by or reported to the sponsor on the drug, particularly with respect to adverse effects and safe use".

7.2.4. Follow-up of Adverse Events

The Sponsor's CRA will periodically request follow-up information of the AE from the PI. The CRA will ensure that all fatal or serious adverse events are reported to the IRB, per Amphastar SOP. All serious adverse events must be followed up for at least 30 days or until resolved or having become stable.

The sponsor shall promptly investigate all safety information received by it from the clinical studies. Follow up information to a safety report shall be submitted as soon as the relevant information becomes available.

7.3. Clinical Laboratory Test and Normal Laboratory Ranges

Each standard clinical laboratory test, i.e., CBC, serum comprehensive metabolic panel and urinalysis, will be performed by a single laboratory for all subjects enrolled in the study. The laboratory that will perform these tests required by the protocol must transmit normal ranges of the clinical laboratory tests to Amphastar Pharmaceuticals, Inc. Urinary pregnancy and 9 panel drug tests may be performed using a clinically accepted test kit.

8. STUDY MONITORING, DOCUMENTATION AND RECORD KEEPING

8.1. Data Quality Assurance and Monitoring

The trial will be monitored according to the current Amphastar SOP.

The site monitor will conduct monitoring of the investigational activities for the purpose of ensuring subject safety and study compliance to applicable regulatory guidelines. The Investigator will permit Amphastar authorized monitors to access the subject source documents, eCRFs, clinical supplies dispensing and storage area and study documentation as frequently as necessary and agrees to assist the site monitors with their activities. The study site will make the eCRF available, provide missing or correct data and PI will sign the eCRFs. Personal or subject identifying information will be treated as confidential and will NOT be publicly accessible.

The trial information may be reviewed by regulatory authorities or independent QA auditors. The study site may be inspected during or after completing the study. The Investigators agree to allow inspectors from regulatory agencies to have access to all trial records, including subject source documents. By participating in this study, the Investigator

8.2. Electronic Case Report Forms (eCRFs)

Electronic Data Capture (EDC) system will be used for recording of electronic CRF (eCRF) for the conduct of the study by Amphastar, eCRF will be used for the purpose of capturing data for all consented participants in the study. The 24 hour clock will be used for all time entries.

Subject source documents are the study subject records maintained at the study site. In some cases, the source documents may be the hospital's or the physician's clinical records. Therefore, the information collected on the eCRFs must match those records. Amphastar personnel will review all eCRFs to ensure the data is verifiable and accurate. At all times, the PI has final responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the eCRFs. The eCRFs will be completed as soon as possible after the data become available.

8.3. Clinical Trial Drug Accountability Data

The Study Site Research Pharmacist or designated dispenser acknowledges that the drug supplies are investigational and, as such, must be handled in accordance with the protocol and labeling. The Research Pharmacist or designated personnel will maintain adequate

records showing the receipt, dispensing, return, or other disposition of the investigational study material, including the date, quantity, lot or code number, and identification of subjects (number, initials) who received or returned study drug.

The Research Pharmacist or designated dispenser and Amphastar's unblinded site monitor will review study supply records, including dispensing and accountability records, at monitoring visits.

It is the responsibility of the Investigator and research pharmacist/designated dispenser to ensure that, when the study is completed or discontinued, unused study supplies will be returned or disposed of, as directed by Amphastar's site monitors or CRA. Unused and returned medication must be available for verification by Amphastar's site monitors or CRA.

8.4. Trial Documents

The trial documents, including the protocol signature page signed by the Investigators, Investigators' curriculum vitae, IRB approval notice and IRB member list, approved Informed Consent Form, laboratory verification and institutional accreditation, will be submitted to Amphastar Pharmaceuticals, Inc.

8.5. Reporting and Publication

The clinical trial report will be prepared according to the Amphastar SOP for Clinical Trial Reporting.

8.6. Archive

The Investigator will retain a copy of all study documents, including reports to the IRB/Ethics Committee and to the sponsor, in accordance with the FDA and local regulations. The FDA regulations and/or ICH guidelines state that the Investigator must maintain study documents for a set period of time. The Investigator will arrange for the retention of the Subject Identification for at least 15 years after the Final Study Report has been signed. All other documents should be kept for the maximum period of time permitted by the institution.

9. ETHICS

9.1. Institutional Review Board (IRB) or Ethics Committee Approval

This study will be conducted in conformance with all regulatory guidelines, particularly the FDA guidance on MDI and DPI drug products [1]. This trial can be conducted only after full approval of the final protocol (and its addendum) has been obtained from the involved Institutional Review Board (IRB) or Ethics Committee. The approval must be received by Amphastar before the initiation of the study.

9.2. ICH GCP Guidelines and Declaration of Helsinki

This trial will be undertaken in accordance with the current ICH Good Clinical Practice (GCP) guidelines and adheres to the core principles of the Declaration of Helsinki of the World Medical Association to assure that the rights, safety and well-being of the study subjects are protected.

9.3. Informed Consent

The Investigator must explain to subjects the purpose, methods, potential benefits and risks of the trial. Subjects will be informed that they are free not to participate in the trial and they may withdraw from the study at any time. Written informed consent from each subject enrolled in the study, in accordance with the Code of Federal Regulations Title 21, Part 50, Subpart B will be recorded by means of dated signatures of the subjects.

Informed Consent will include authorization to use data from the trial for all purposes relating to approval of the drug under investigation.

9.4. HIPAA Compliance

The Investigator is responsible for compliance with the privacy provisions of the Health Insurance Portability and Accountability Act including obtaining informed consent for use of data for marketing purposes.

10. REFERENCES

[1] FDA Draft Guidance for Industry Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products - Quality Considerations, 04/2018.

[2] Instructions for use from Epinephrine Injection prescribing information, Revised 9/2018.

[3] Warren JB, Doble N, etc: Systemic absorption of inhaled epinephrine. Clin Pharmacol Ther, 40:673-678, 1986.

[4] Westfall TC, Westfall DP: Adrenergic agonists and antagonists, in Brunton LL, Lazo JS, Parker KL (eds): Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th Ed. P 237-296., 2006.

[5] Dauphinee B, Tashkin DP, et al: Placebo-controlled evaluation of the speed of onset of epinephrine metered-dose aerosol (Primatene® Mist) in mild to moderate asthmatics. Am J Respir Crit Care Med, 149:A204, 1994.

[6] American Thoracic Society, "Using Your Metered Dose," *Am J Respir Crit Care Med,* vol. 190, pp. 5-6, 2014.

[7] CDISC SDTM Implementation Guide (Version 3.2). 11/26/2013.

[8] Prescribing Information for ProAir HFA Inhalation Aerosol ([redacted]), revised 06/2016.

[9] FDA Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies, December 2012.

[10] Guidance for Industry: Handling and Retention of BA and BE Testing Samples and Retention
http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126836.pdf.

[11] FDA Draft Guidance on Albuterol Sulfate, Recommended Apr 2013; Revised Jun 2013; Dec 2016.

Appendix I. Approval by an IRB/Ethics Committee

In performing this study, both the Investigator and Sponsor endorse, as a minimum, the standards for conduct of clinical research activities as set forth in the FDA regulations and/or adhere to the core principles of the Declaration of Helsinki and local country laws and regulations.

The Investigator will submit the protocol, any amendments, and informed consent for IRB/Ethics Committee approval prior to initiation of the study. This will be appropriately documented. The IRB/Ethics Committee must give their approval in writing. The names and qualifications of the members of the review committee will be recorded and submitted to the sponsor with the written approval for the conduct of the study. The members of the IRB/Ethics Committee approving the protocol must be independent of the Sponsor and the Investigator. The written approval will consist of written documentation from the IRB/Ethics Committee containing the approval information.

Until written approval by the IRB/Ethics Committee has been received by the Investigator, no subject may undergo any procedures solely for the purpose of determining eligibility for this study.

Protocol amendments must also be reviewed and approved by the IRB/Ethics Committee. Written approval from the IRB/Ethics Committee, or a designee, must be received by the sponsor, Amphastar Pharmaceuticals, Inc. before implementation. This written approval will consist of documentation from the IRB/Ethics Committee containing the approval information.

Protocol Signature Page

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the ICH Good Clinical Practice guideline and adhere to the core principles of the Declaration of Helsinki, or the applicable local laws and regulations of the study site for which I am responsible, whichever provides the greater protection of the individual. I will abide by the publication rules set forth in my agreement with Amphastar Pharmaceuticals, Inc. I will accept the monitor's inspection and overseeing of the study. I will promptly submit the protocol to the applicable Institutional Review Board/Ethical Committee for approval.

_____ _____

Signature of Investigator Date

_____

Investigator Name (Print)

_____

Title

_____

Name of Facility

_____

Street Address

_____

City, State, ZIP Code

_____ _____

Signature of Representative of
Amphastar Pharmaceuticals, Inc. Date

Appendix II. Body Mass Index (BMI) Table

BMI Formula:

BMI=Weight [in kilograms]/ (Height [in meters]) ^{2} *Or*

BMI=Weight [in pounds] X703.7/ (height [in inches]) ^{2}

BMI CHART

HEIGHT feet/ inches	cm																											
6'4"	192.5	12	13	13	14	15	16	17	18	18	19	20	21	22	22	23	24	24	26	26	27	28	29	29	30	31	32	33	34
6'3"	190	12	13	14	15	16	16	17	18	19	20	20	21	22	23	24	24	25	26	27	28	29	29	30	31	32	33	34	34
6'2"	187.5	13	13	14	15	16	17	18	18	19	20	21	22	23	24	24	25	26	27	28	29	29	30	31	32	33	34	34	36
6'1"	185	13	14	15	15	16	17	18	19	20	21	22	22	23	24	25	26	27	28	29	29	30	31	32	33	34	34	36	37
6'0"	182.5	13	14	15	16	17	18	19	20	20	21	22	23	24	24	26	27	28	29	29	30	31	32	33	34	34	36	37	38
5'11"	180	14	15	15	16	17	18	19	20	21	22	23	24	24	26	27	27	28	29	30	31	32	33	34	34	36	37	38	39
5'10"	177.5	14	15	16	17	18	19	20	21	22	23	23	24	25	26	27	28	29	30	31	32	33	34	34	36	37	38	39	40
5'9"	175	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	34	36	37	38	39	40	41
5'8"	172.5	15	16	17	18	19	20	21	22	23	24	24	26	27	28	29	29	31	32	33	34	34	36	37	38	39	40	41	42
5'7"	170	15	16	17	18	19	20	21	22	24	24	26	27	28	29	29	31	32	33	34	34	36	37	38	39	40	41	42	43
5'6"	167.5	16	17	18	19	20	21	22	23	24	25	26	27	29	29	31	32	33	34	34	36	37	38	39	40	41	42	43	45
5'5"	165	16	17	18	19	21	22	23	24	24	26	27	28	29	30	32	33	34	34	36	37	38	39	40	42	43	44	45	46
5'4"	162.5	17	18	19	20	21	22	23	24	26	27	28	29	30	31	33	34	34	36	37	38	39	41	42	43	44	45	46	47
5'3"	160	17	18	20	21	22	23	24	25	27	28	29	30	31	32	34	34	36	37	38	39	41	42	43	44	45	46	48	49
5'2"	157.5	18	19	20	21	23	24	24	26	27	29	29	31	32	33	34	36	37	38	40	41	42	43	44	46	47	48	49	50
5'1"	155	18	20	21	22	23	24	26	27	28	29	31	32	33	34	36	37	38	40	41	42	43	45	46	47	48	50	51	52
5'0"	152.5	19	20	21	23	24	25	27	28	29	31	32	33	34	36	37	38	40	41	42	43	45	46	47	49	50	51	52	54
4'11"	150	20	21	22	24	24	26	28	29	30	32	33	34	36	37	38	40	41	42	44	45	46	48	49	50	52	53	54	56
4'10"	147.5	20	22	23	24	26	27	28	29	31	33	34	35	37	38	40	41	42	44	45	46	48	49	51	52	53	55	56	57
4'9"	145	21	22	24	25	27	28	29	31	32	34	35	37	38	39	41	42	44	45	47	48	49	51	52	54	55	57	58	59
4'8"	142.5	22	23	24	26	28	29	31	32	33	34	36	38	39	41	42	44	45	47	48	50	51	53	54	56	57	59	60	62
WEIGHT (kg)		44	47	50	53	56	59	62	65	68	71	74	77	80	83	86	89	92	95	98	101	104	107	110	113	116	119	122	125
WEIGHT (lbs)		97	103	110	117	123	130	136	143	150	156	163	169	176	183	189	196	202	209	216	222	229	235	242	249	255	262	268	275

Appendix III. Prohibited Drug List

	Drug Category	Major Indications	Type of Impact	Description of Impact	Route	Brand Name	Generic Name	Washout Period*
1	Anti-depressant (Tricyclic)	Depression	Safety	May ↑the cardiovascular side effects	PO	Anafranil	Clomipramine	4 weeks
2						Asendin	Amoxapine	
3						Norpramin	Desipramine	
4						Pamelor	Nortriptyline	
5						Sinequan	Doxepin	
6						Surmontil	Trimipramine	
7						Tofranil	Imipramine	
8					PO/IV	Elavil	Amitriptyline	
9	Anti-depressant (Polycyclic)				PO	Desyrel	Trazodone	
10						Ludiomil	Maprotiline	
11						Serzone	Nefazodone	
12	Antihistamine	Allergy	Safety	Potentiate side effect of Primatene and Albuterol	PO	Benadryl	Diphenhydramine	6 hours
13						Chlortrimeton	Chlorpheneramine	
14	Alpha1-blockers	Hypertension/ Prostate enlargement	PK	↑PK level by competing for α-receptors	PO	Cardura	Doxazosin	3 days
15						Dibenzyline	Phenoxybenzamin	5 days
16						Flomax	Tamsulosin	3 days
17						Hytrin	Terazosin	3 days
18						Minipress	Prazosin	3 days
19						Rapaflo	Silodosin	3 days
20						Uroxatral	Alfluzosin	3 days
21	β1/β2-Blockers (Selective & Non-selective)	Hypertension/ angina	Safety PD	↓Effect of Primatene and Albuterol, also affect safety in QTc prolongation	PO/IV	Betapace	Sotolal	4 weeks
22					PO	Blocadren	Timolol	
23						Bystolic	Nebivolol	
24						Coreg	Carvediol	
25					PO/IV	Inderal	Propranolol	
26					PO	Lopressor	Metoprolol	
27					PO	Tenormin	Atenolol	
28					PO/IV	Trandate	Labetalol	
29					PO	Zebeta	Bisprolol	
30	Cardiac Glycoside	Congestive heart failure	Safety	May induce cardiac arrhythmia with Primatene; Albuterol may ↓digoxin level	PO	Lanoxin	Digoxin	10 days
31	Diuretics	Edema/ Hypertension	Safety	ECG changes/ Hypokalemia/ hyperkalemia	PO	Aldactone	Spironolactone	1 day
32						Bumex	Bumetanide	
33						Diuril	Chlorothiazide	
34						Demadex	Torsemide	
35						HydroDiuril	Hydrochlorothiazid	3 days
36						Hygroton	Chlorthalidone	1 day
37						Lasix	Furosemide	
38						Lozol	Indapamide	3 days
39						Zaroxolyn	Metolazone	1 day

*This drug list is provided based on the drug interaction listed in package insert of Epi-Pen. Some drugs that have been discontinued per FDA website will not be listed here. Asthma medications are also not listed because this study is for healthy volunteers only.

Appendix III. Prohibited Drug List (Continued)

	Drug Category	Major Indications	Type of Impact	Description of Impact	Route	Brand Name	Generic Name	Washout Period*
40	Monoamine Oxidase-A Inhibitor	Major depression	PK/ Safety	↑PK and cardiac side effects of Primatene and Albuterol	PO	Marplan	Isocaarboxazid	14 days
41						Nardil	Phenelzine	
42						Parnate	Tranylcypromine	
43	Monoamine Oxidase-B Inhibitor	Parkinsonism				Eldepryl	Selegiline	
44						Zelapar	Selegiline	
45						Xadago	Safinamide	
46	Oral Decongestant /Oral or Parenteral Sympathomimetics	Attention Deficit Hyperactivity Disorder (ADHD)	PK/ Safety	↑PK level and ↑cardiac side effects of Primatene and Albuterol	PO	Adderall	Dextroamphetamine	2 days
47						Adderall XR	mixed salts	5 days
48						Dexedrine	Dextroamphetamine	4 days
49						Ritalin	Methylphenidate	2 days
50						Ritalin LA	Methylphenidate	5 days
51						Vyvanse	Lisdexamfetamine	1 day
52		Anaphylaxis			IV/IM/ SC	Adrenalin	Epinephrine	1 day
53		Nasal Congestion			Nasal	Afrin	Oxymetazoline	2 days
54						Neo-synephrin	Phenlephrine	1 day
55		Allergy/nasal congestion		↑ PK level by competing α-receptor and ↑cardiac side effects of Primatene and Albuterol	PO	Advil Cold & Sinus	Ibuprofen/ Pseudoephedrine	1 day
56						Allegra D	Fexofenadine/ Pseudoephedrine	
57						Claritin D	Loratadine/ Pseudoephedrine	
58		Allergy/Nasal & Chest Congestion				Mucinex D	Guaifenesin/ Pseudoephedrine	
59		Nasal Congestion				Sudafed/PE	Phenylephrine	
60						Sudafed/SA	Pseudoephedrine	
61		Allergy/Nasal congestion				Zyrtec D	Cetirizine/ Pseudoephedrine	
62	Phenothiazines	Nausea & Vomiting	Safety	May potentiate side effect of Primatene and Albuterol	PO	Compazine	Prochlorperazine	7 days
63		Schizophrenia				Etrafon*	Perphenazine/ amitriptyline	
64						Mellaril*	Thioridazine	
65						Serentil*	Mesoridazine	
						Stelazine*	Trifluoperazine	
66					PO/IV	Thorazine*	Chlorpromazine	
67	Thyroid hormones	Hypothyroidism	Safety	↑ cardiac effects of Primatene and Albuterol	PO	Synthroid	Levothyroxine	6 weeks

*This drug list is provided based on the drug interaction listed in package insert of Epi-Pen. Some drugs that have been discontinued per FDA website will not be listed here. Asthma medications are also not listed because this study is for healthy volunteers only.

Appendix IV. Blood Metabolic/CBC and Urinalysis Tests

Based on the ICH guidelines (i) Guideline for Good Clinical Practice E6 (R1), version 4, dated 10 June 1996; and (ii) Safety Pharmacology Studies for Human pharmaceuticals S7A, version 4, dated 8 November 2000. For safety assessment, the following items will be conducted at screening.

#	Test Categories	Description	#	Test Items	*Reference Normal Range	Footnote
1	Comprehensive Metabolic Panel	≥ 8 hr fasting	1	Total protein	6.2 to 8.3 g/dL	Albumin/globulin
			2	Sodium	133 to 145	
			3	Potassium	3.4 to 5.4 mEq/L	
			4	Calcium	8.4 to 10.6 mg/dL	
			5	Chloride	96 - 110 mmol/L	
			6	Glucose	60 to 99 mg/dL	
			7	CO2	22 to 32 mEq/L	
			8	BUN	6 to 21 mg/dL	Blood urea nitrogen
			9	Creatinine	0.4 to 1.54 mg/dL	
			10	ALP	35 to 104 U/L	Alkaline Phosphatase
			11	ALT	0 to 40 U/L	Alanine Transaminase
			12	AST	10 to 35 U/L	Aspartate Transaminase
			13	Bilirubin	0.0 to 1.4 mg/dL	
2	Urine analysis, routine and microscopic	fasting not required	14	Color	yellow	
			15	Character (Clarity)	clear	
			16	Specific gravity	1.003-1.029	
			17	PH	4.5 - 8.0	
			18	Protein	negative	
			19	Glucose	negative	
			20	Nitrite	negative	
			21	Ketone	negative	
			22	Leukocyte esterase	negative	
			23	Blood	negative	
			24	Bilirubin	negative	
			25	Crystals	negative	
			26	Bacteria/Yeast	negative	
			27	RBC/WBC/Epithelial cells	negative	
			28	Casts	negative	
3	CBC with differentials	*ibid*	29	RBC	3.9-5.2 $\times 10^6$/µl	Red blood cells
			30	MCV	80 to 100 fL	Mean Corpuscular Volume
			31	MCH	26 to 34 pg/cell	Mean Corpuscular Hemoglobin
			32	MCHC	32 to 37 g/dL	Mean Corpuscular Hemoglobin Concentration
			33	Hemoglobin	11.0-15.5 g/dL	
			34	Hematocrit	34.0-45.0 %	
			35	Platelet	150-450 $\times 10^3$/µl	
			36	WBC	4.3-11.0 $\times 10^3$/µl	White Blood Cell

* Normal value ranges may vary slightly among different laboratories, the value listed here are only for reference, the actual values will be determined by study site.

Appendix IV. Blood Metabolic/CBC and Urinalysis Tests (Continued)

#	Test Categories	Description	#	Test Items	*Reference Normal Range	Footnote
4	Urinary or serum pregnancy test**	*ibid*	37	Pregnancy	negative	
5	Urinary alcohol/nine panel drug screen tests	*ibid*	38	amphetamines	negative	
			39	cannabinoids	negative	
			40	methadone	negative	
			41	barbiturates	negative	
			42	PCP	negative	
			43	opiates	negative	
			44	benzodiazepines	negative	
			45	cocaine/metabolites	negative	
			46	methamphetamines	negative	
			47	alcohol	negative	
6	Tests for Infectious diseases	fasting not required	48	HIV Ab	negative	Human Immunodeficiency Virus Antibody
			49	HBs Ag	negative	Hepatitis B Surface Antigen
			50	HCV Ab	negative	Hepatitis C Virus Antibody
7	Thyroid Function	fasting not required	51	Free T3	200-500 pg/dL	Triiodothyronine
			52	Total T4	4.5-11.2 µg/dL	Thyroxine
			53	TSH	0.4 - 4.0 mIU/L	Thyroid Stimulating Hormone

* Normal value ranges may vary among different laboratories, the value listed here are only for reference, the actual values will be determined by study site.
** all female subjects

* Normal value ranges may vary slightly among different laboratories, the value listed here are only for reference, the actual values will be determined by study site.
** All female subjects