INTRODUCTION
Albuterol is a beta-adrenergic receptor agonist used for the treatment of bronchial asthma and chronic obstructive pulmonary disease. As one of the most common routes of administration, the inhalation therapy of albuterol results in very low concentration in plasma, which requires low limit of quantitation for pharmacokinetics studies. The purpose of this study is to develop a rapid and sensitive assay based on LC-MS/MS to quantify albuterol in human plasma as low as 1 pg/mL with limited sample volume.

SAMPLE PREPARATION
300 μL of human plasma was mixed with internal standard and 300 μL water, and then extracted the mixture using Phenomenex X-CW SPE plate.
- Equilibration: 800 µL MeOH and then 800 µL H₂O
- Wash: 800 µL H₂O and then 800 µL MeOH
- Reconstitution: 200 µL ACN: H₂O / 95:5 (V:V)

LC-MS/MS ANALYSIS
Mass Spectrometry: Sciex, API 4000. TIS +
HPLC: Shimadzu Nexera HPLC
- Mobile Phase: A: 5mM Ammonium Formate in Water pH=2.5
- B: Acetonitrile:Water / 95:5 (V:V)
- Program: Gradient. Starting at 95%B and ramping to 70%B.
- Flow rate: 0.5 mL/min
- HPLC column: Atlantis HILIC, 2.1 x 50 mm, 3 µm, Waters

EXPERIMENTAL
The sample extraction of albuterol from human plasma employed the polymeric cation exchange solid-phase extraction using Strata X-CW 96-well plate. The LC-MS/MS analysis was performed using a HILIC silica column and the mobile phase was 5 mM ammonium formate in water (pH=2.5) and acetonitrile:water/ (95:5:v:v). The MS detection in multiple-reaction-monitoring (MRM) mode for albuterol was m/z 240.1→148.1 and for the internal standard, albuterol-d₉, was m/z 249.2→148.1. The total analysis time was 3 min.

RESULTS
- Matrix Effect and Recovery
- Inter-day Accuracy and Precision from Validation
- Additional Stability Established in Human Plasma

CONCLUSIONS
- The LLOQ level of 1 pg/mL was achieved on API 4000 MS by implementing HILIC chromatography for this polar analyte
- The SPE extraction was optimized to achieve high recovery and minimize the matrix suppression
- The validated method shows adequate selectivity, sensitivity, specificity, accuracy, and reproducibility for analysis of albuterol in human plasma