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Development and Validation of a Sensitive LC-MS/MS Method for the Determination of Albuterol in Human Plasma

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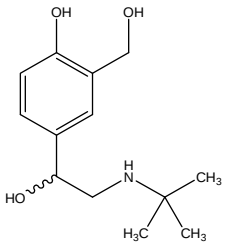
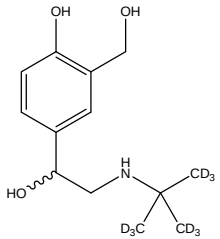
INTRODUCTION

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.



Albuterol
$C_{13}H_{21}NO_3$



Albuterol-d_9
$C_{13}H_{12}D_9NO_3$

EXPERIMENTAL

The sample extraction of albuterol from human plasma employed the polymeric cation exchange solid-phase extraction using Statra 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-d_9, was m/z 249.2→148.1. The total analysis time was 3 min.

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 H_2O

Wash: 800 µL H_2O and then 800 µL MeOH

Elution: 300 µL MeOH: H_2O : NH_4OH / 50:50:5 (v:v:v)

Reconsititution: 200 µL ACN: H_2O / 95:5 (V:V)

LC-MS/MS ANALYSIS

Mass Spectrometry: Sciex, API 4000. TIS +

HPLC: Shimadzu Nexera HPLC

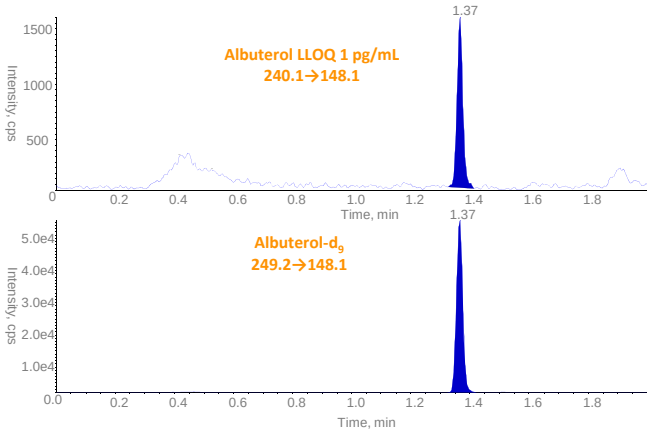
Mobile Phase: A: 5mM Ammonium Formate in Water pH=2.5
B: Acetonitrile:Water / 95:5 (V:V)

Program: Gradient. Starting at 95%B and ramping to 70%B.

Flow rate: 0.5 mL/min

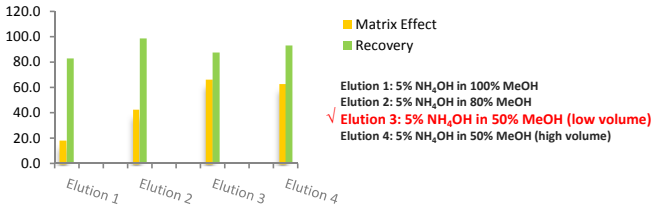
HPLC column: Atlantis HILIC, 2.1 x 50 mm, 3 µm, Waters

REPRESENTATIVE CHROMATOGRAMS



RESULTS

- **Matrix Effect and Recovery**



Strata X-CW 96-Well Plate (Weak Cation Exchange SPE)

- **Inter-day Accuracy and Precision from Validation**

	Albuterol Concnetration (pg/mL)				
	LLOQ	LQC	MQC1	MQC2	HQC
	1.00	3.00	50.0	450	800
Overall Mean	0.938	3.04	52.6	441	781
Inter-run CV%	11.4	5.5	2.7	2.6	2
Inter-run RE%	-6.2	1.3	5.2	-2.0	-2.4
n	18	18	18	18	18

- **Additional Stability Established in Human Plasma**

  - 19 hours in plasma at ambient temperature
  - 2 hours in whole blood at ambient temperature
  - 5 freeze/thaw cycles at -20 & -70 °C
  - 115 days frozen at -20 & -70 °C
  - 0.5% hemolyzed human plasma

CONCULSIONS

- **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**

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