



A Rugged and Sensitive Method for the Determination of Albuterol in Human Plasma by LC/MS/MS

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Overview

OBJECTIVE

To develop a rugged assay with the lowest LLOQ available for the determination of albuterol in human plasma by LC/MS/MS.

METHOD

Samples were extracted by a weak cation-exchange solid-phase extraction (SPE) procedure and analyzed by liquid chromatography/tandem mass spectrometry. A Sciex API 5000™ was used to detect positive ions using electrospray ionization.

RESULTS

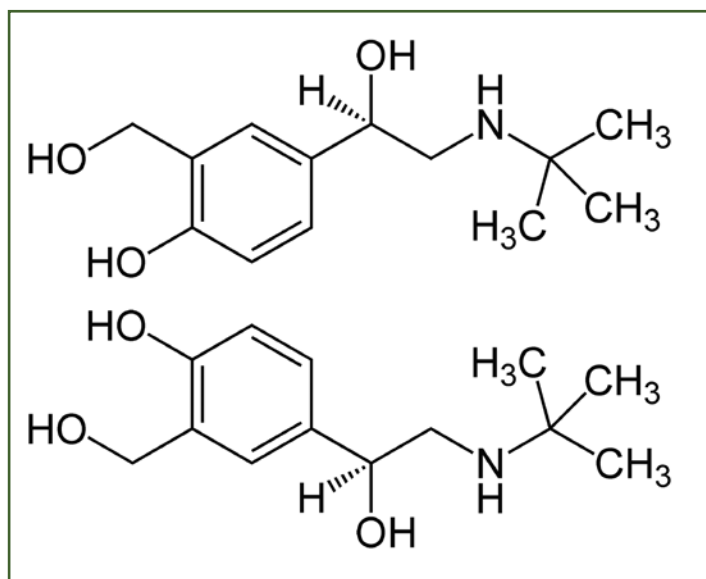
Analytical challenges such as imprecision, contamination, instability and impact from hemolysis were overcome during method development. The assay was validated and several thousand samples were successfully analyzed with the method.



Introduction

Albuterol (Figure 1) is a highly polar compound prescribed for the relief of bronchospasms resulting from conditions such as asthma and chronic obstructive pulmonary disease (COPD). Albuterol is typically administered by inhalation in low doses either on its own or in combination with other drugs. Increased cases of COPD have led to interest in developing improved therapeutical treatments. Due to its low delivered dose, analytical methods for the determination of albuterol in human plasma require a low limit of quantitation (LLOQ). We recently developed, validated, and analyzed clinical samples for the determination of albuterol using an ion-exchange SPE extraction with LC/MS/MS with an LLOQ of 1.00 pg/mL. To the best of our knowledge, this is currently the lowest published LLOQ for the determination of albuterol in human plasma.

FIGURE 1: Structure of Albuterol





Method

- 0.5 mL Human plasma (K2EDTA) aliquot volume
- Add the internal standard, albuterol-D3
- Add water
- Extract using Phenomenex Strata™ X-CW SPE plate
 - wash with water and then methanol with neutral pH
 - elute with 5% ammonium hydroxide in water: methanol
- Evaporate and reconstitute with aqueous solvent
- Inject onto a Waters Xbridge™ C18 column (2.1 x 50 mm, 5 μm)
- Use slight gradient, 3 minute cycle time
 - Flow rate is 0.5 mL/min
- Use Sciex API 5000™ to detect positive ions in ESI mode

Results

During method development, extensive pre-emptive stability testing was performed. Different extraction techniques were screened (Figure 2) and the method was optimized to maximize recovery (Figures 3a/3b and Table 1).

Challenges that were encountered and overcome in method development:

- Imprecision at the LLOQ in different lots of matrix: This was overcome by requiring a fresh bottle of ammonium hydroxide with each extraction.
- Contamination during the extraction: This was overcome by using the Tomtec to mix and transfer samples and using the centrifuge (instead of a positive pressure manifold) to elute from SPE.
- Instability of albuterol in whole-blood at room temperature: This was overcome by collecting samples on ice (Table 2).
- Hemolysis at higher levels ($\geq 5\%$) affects quantitation (Table 3): This was addressed by documenting hemolysis levels in samples and determining impact.

The assay was validated with a linear quantitative range from 1.00 to 1000 pg/mL. Inter-day accuracy (% bias) and precision (% CV) for the LLOQ were -5.1% and 8.4%, respectively (Table 4). Intra-day accuracy (% bias) and precision (% CV) ranges for three runs for the LLOQ were -10.6% to 5.0% and 3.7% to 11.6%, respectively (Table 5). Recoveries across the entire concentration range were 78.6 to 84.3% (Table 6). Four freeze/thaw cycles were established. Stability in matrix was established at -20C and -70C for 66 days. There were no matrix effects or chromatographic interferences observed within different lots of plasma after ion exchange SPE.



Results (continued)

FIGURE 2: Method Development: SPE Screening

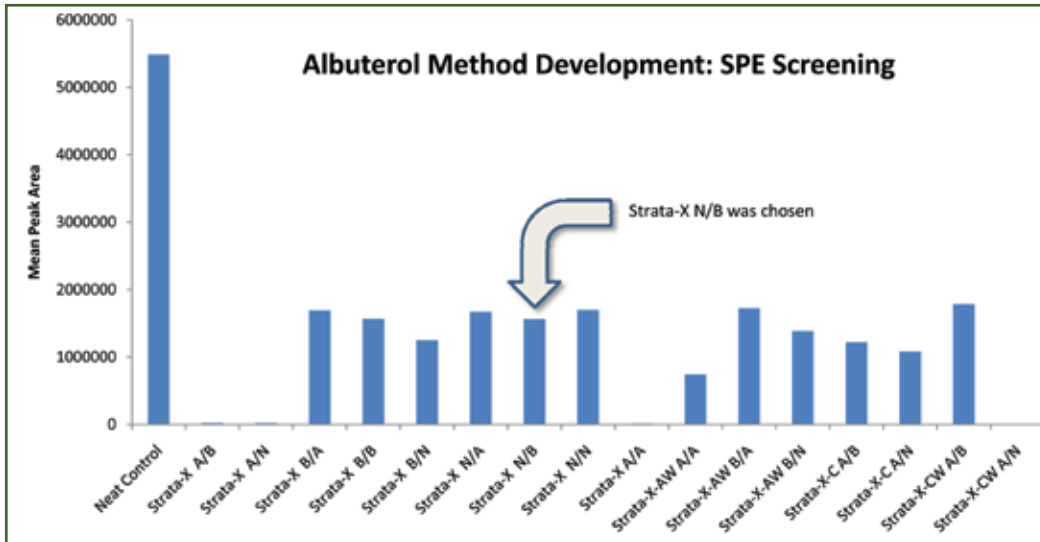
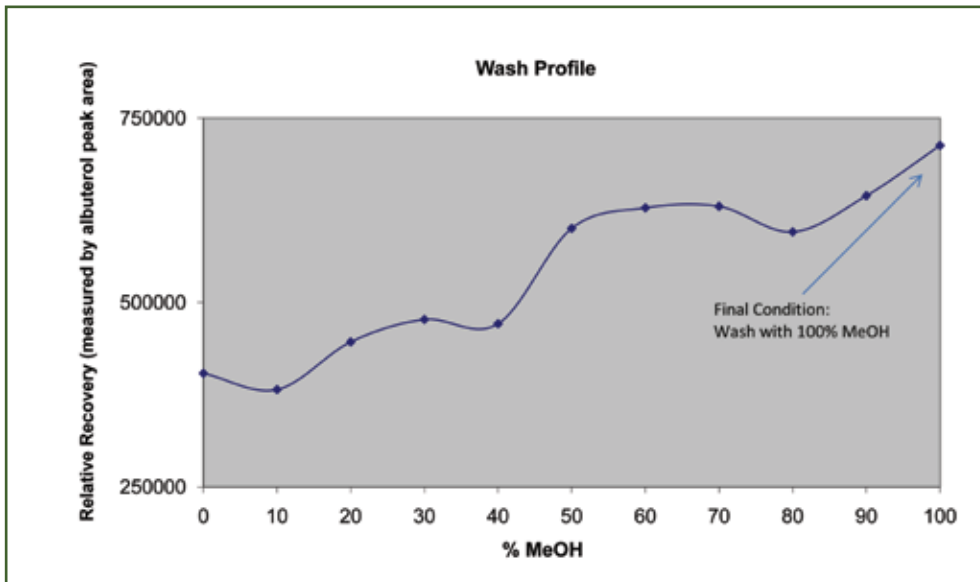


FIGURE 3A: Method Development: SPE Wash Profile





Results (continued)

FIGURE 3B: Method Development: SPE Elution Profile

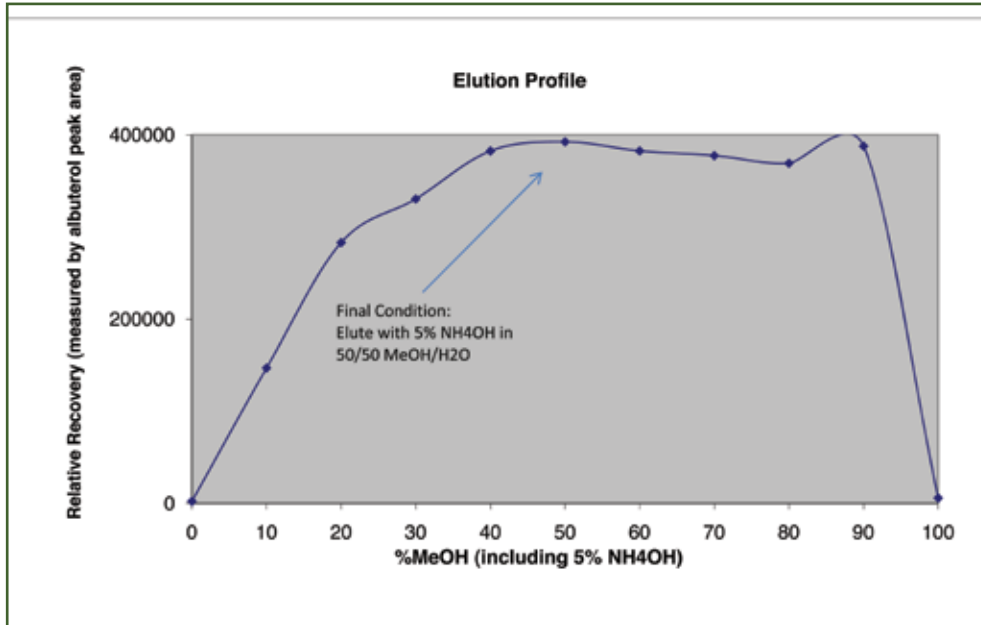


TABLE 1. Method Development: SPE Elution Optimization
(Yellow indicates condition that was chosen for method).

Elution Volume (mL)	Mean Peak Area	%CV
0.2	947806	21%
0.3	1194041	26%
0.5	875070	54%

%NH4OH in Elution Solvent	Mean Peak Area	%CV
0.2	20240	90%
0.5	226327	46%
1	1194041	26%
5	2969785	3%



Results (continued)

TABLE 2A. First attempt to establish stability of Albuterol (500 pg/mL) in Whole Blood

Results expressed as Instrument Response (analyte/internal standard area) for QCs at n=6

	0 Hour (Control)	1 Hour (Room Temp)	2 Hours (Room Temp)	1 Hour (1 to 8 °C)	2 Hours (1 to 8 °C)
Mean	1.516	1.068	0.980	1.268	1.264
S.D.	0.038	0.015	0.019	0.026	0.018
%CV	2.5	1.4	1.9	2.1	1.4
%Difference	N/A	-29.59	-35.40	-16.35	-16.65

TABLE 2B. Second attempt to establish stability of Albuterol (500 pg/mL) in Whole Blood

Results expressed as Instrument Response (analyte/internal standard area) for QCs at n=6

	0 Hour (Control)	0.5 Hour (wet ice)	0.75 Hour (wet ice)	1 Hour (wet ice)
Mean	0.959	0.970	0.959	0.961
S.D.	0.040	0.014	0.015	0.016
%CV	4.2	1.5	1.5	1.6
%Difference	N/A	1.08	0.02	0.23

TABLE 3A. Evaluation of Hemolysis for Albuterol 3.00 pg/mL (First Experiment)

Results expressed as Instrument Response (analyte/internal standard area) for QCs at n=6

	0.0% Hemolysis	0.5% Hemolysis	5.0% Hemolysis
Mean	0.00653	0.00622	0.00923
S.D.	0.000266	0.000367	0.000743
%CV	4.1	5.9	8.1
%Difference	N/A	-4.67	41.47



Results (continued)

TABLE 3B. Evaluation of Hemolysis for Albuterol at 3.00 pg/mL (Second Experiment)

Results expressed as Instrument Response (analyte/internal standard area) for QCs at n=6

	0.0% Hemolysis	1.0% Hemolysis	2.0% Hemolysis
Mean	0.00637	0.00607	0.00616
S.D.	0.000582	0.000171	0.000413
%CV	9.1	2.8	6.7
%Difference	N/A	-4.72	-3.26

TABLE 4. Accuracy and Precision for Albuterol QCs (from ANOVA)

Nominal Conc.	LLOQ QC 1.00 pg/mL	Low QC 3.00 pg/mL	Medium QC 400 pg/mL	High QC 800 pg/mL
Mean Observed Conc.	0.949	2.99	401	781
%Bias	-5.1	-0.3	0.3	-2.4
Between Run Precision (%CV)	8.4	3.8	3.0	2.2
Within Run Precision (%CV)	9.6	3.0	1.4	0.7
Total Variation (%CV)	12.8	4.8	3.3	2.3
n	17	18	18	18
Number of Runs	3	3	3	3



Results (continued)

TABLE 5. Intra-Assay Accuracy and Precision for Albuterol QCs				
Nominal Conc.	LLOQ QC 1.00 pg/mL	Low QC 3.00 pg/mL	Medium QC 400 pg/mL	High QC 800 pg/mL
08-Oct-2010	*1.21	3.18	410	760
	1.08	3.24	408	772
	0.968	3.07	400	774
	1.10	3.04	400	773
	1.02	3.05	408	770
	0.901	3.08	403	775
Mean	1.05	3.11	405	771
SD	0.108	0.0810	4.40	5.50
%CV	10.3	2.6	1.1	0.7
%Theoretical	105.0	103.7	101.3	96.4
%Bias	5.0	3.7	1.3	-3.6
n	6	6	6	6
12-Oct-2010	0.972	2.82	391	766
	0.853	3.06	392	777
	0.904	2.91	386	770
	*0.777	2.72	380	768
	0.806	2.88	388	770
	1.05	2.86	389	778
Mean	0.894	2.88	388	772
SD	0.104	0.112	4.32	4.89
%CV	11.6	3.9	1.1	0.6
%Theoretical	89.4	96.0	97.0	96.5
%Bias	-10.6	-4.0	-3.0	-3.5
n	6	6	6	6
16-Oct-2010	0.923	3.03	419	800
	0.929	2.92	421	798
	0.854	3.05	411	800
	‡1.76	2.98	413	800
	0.913	2.99	402	796
	0.874	2.87	405	812
Mean	0.899	2.97	412	801
SD	0.0329	0.0677	7.49	5.62
%CV	3.7	2.3	1.8	0.7
%Theoretical	89.9	99.0	103.0	100.1
%Bias	-10.1	-1.0	3.0	0.1
n	5	6	6	6

* > ±20% deviation from theoretical
‡ Sample deactivated as an outlier



Results (continued)

TABLE 6. Extraction Recovery for Albuterol and Albuterol-d3		
Results expressed as Instrument Response (analyte/internal standard area) for QCs at n=6		
	Low AB/IB	Low AA/IB
Mean	0.006078	0.007728
S.D.	0.000316	0.000728
%CV	5.2	9.4
Low Analyte Recovery: 78.6%		
	Medium AB/IB	Medium AA/IB
Mean	0.785403	0.960491
S.D.	0.007683	0.022480
%CV	1.0	2.3
Medium Analyte Recovery: 81.8%		
	High AB/IB	High AA/IB
Mean	1.610649	1.909573
S.D.	0.016023	0.045631
%CV	1.0	2.4
High Analyte Recovery: 84.3%		
	Medium AB/IB	Medium AB/IA
Mean	0.822390	0.606282
S.D.	0.028210	0.044261
%CV	3.4	7.3
IS Recovery: 73.7%		

AB/IB = Analyte and internal standard added before extraction.

AA/IB = Analyte added after extraction. Internal standard added before extraction.

AB/IA = Analyte added before extraction. Internal standard added after extraction.

Analyte recovery = 100 x Instrument response of (AB/IB) / (AA/IB)



Conclusion

The assay was successfully used to analyze several thousand study samples. Subsequent incurred sample reanalysis (ISR) testing yielded a 100% pass rate with a mean absolute bias of 2.2%, demonstrating the assay's precision and ruggedness, even at these low levels of quantitation.