T2304

Method Development of an LC-MS/MS Assay for Therapeutic Dosages of Albuterol by Metered Dose Inhaler in Human Plasma

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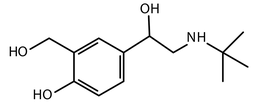
PURPOSE

- To develop a highly sensitive LC-MS/MS method for measurement of albuterol (Figure1) in human plasma from patients using a therapeutic dose delivered by a metered dose inhaler (MDI).
- The LLOQ of 10 pg/mL was lower than previously seen with albuterol LC-MS/MS assays in order to measure 5 half-lives of elimination following a single therapeutic, rather than supratherapeutic, dose in the majority of subjects.

Figure 1: Albuterol structure



METHODS (DEVELOPMENT)

- Several challenges were faced in the method development of the assay, primarily achieving adequate recovery while minimizing matrix effects.
- Many extraction solvents were investigated, including MTBE, ethyl acetate, and n-butyl chloride.
- A variety of solid-phase sorbent types were tested (HLB, WCX, SCX, MCX, and Plexa PCX), and none produced acceptable results (Table 2).
- With few extraction options remaining, a phenylboronic acid (PBA) sorbent was attempted even though albuterol is a less than ideal candidate for the retention mechanism. PBA sorbent has a strong affinity for cis-diol containing compounds such as catechols, proteins, nucleic acids, and carbohydrates. Although albuterol is not the typical molecule used with a (PBA) plate, it had the highest recovery and least matrix effect after method optimization of load buffer, washes, and elution.

RESULTS (DEVELOPMENT)

- Liquid-liquid recovery in all attempted conditions was poor (Table 1).
- HLB, WCX, SCX, and Plexa PCX SPE materials gave recoveries of 6% to 41%. MCX resulted in near 100% recovery with a matrix suppression of 53% which could not be eliminated with wash solutions (Table 2).
- Average recovery with the PBA plate was 74% with minimal matrix enhancement (5%).

Table 1. Liquid-Liquid Solvent Recovery

Solvent	Modifier	Recovery (%)
ethyl acetate	1% NH4OH in water	10
methyl tert-butyl ether	5% NH4OH in water	3
methyl tert-butyl ether	ultrapure water	0.6
methyl tert-butyl ether	50 mM CH3COONH4, pH 3.0	0
n-butyl chloride	5% NH4OH in water	0
n-butyl chloride	ultrapure water	0
n-butyl chloride	50 mM CH3COONH4, pH 3.0	0

Table 2. Recovery and Matrix Effect for Solid Phase Materials*

Sorbent	Recovery (%)	Matrix Effect (%)
Oasis MCX (30 um, 10 mg)	30	11
Varian Bond Elut SCX	41	-12
BondElut Plexa	6	-56
Oasis MCX (30 um, 30 mg)	106	-53
Oasis HLB (30 um, 30 mg)	6	(not evaluated)
Oasis WCX (30 ug, 30 mg)	14	27.3
BondElut PBA (low QC/high QC)	72/76	4/5

* data presented is highest recovery amongst several different load buffers and wash conditions tested for each sorbent

METHODS (VALIDATION)

- Albuterol human plasma samples (0.250 mL) with 0.0500 mL of d_4-albuterol as internal standard, were diluted with an ammonium acetate buffer (pH 9.0), and loaded onto a conditioned Varian Bond Elut PBA plate.
- After washes of buffer, methanol, acetonitrile, and 4% formic acid in acetonitrile, samples were eluted with acidic methanol and evaporated to dryness followed by reconstitution in solvent for injection.
- Albuterol was chromatographically separated from other matrix components on a Thermo Scientific, BioBasic SCX, 50 x 3.0 mm, 5 µm column with an isocratic mobile phase of acetonitrile and an ammonium formate buffer.
- An AB SCIEX API 4000, using an ESI interface, detected positive ions in the MRM mode.

RESULTS (VALIDATION)

- Average recovery with the PBA plate was 70.3%.
- Matrix factor (Table 3) and post-column infusion assessments (Figure 2) demonstrated an absence of significant matrix effect.
- Assay selectivity was demonstrated by quantitation of six separate plasma lots fortified at the LLOQ and high quality control concentrations. No significant matrix effect was observed (Table 4).
- Signal to noise at the LLOQ was 16.
- Representative chromatograms of extracted albuterol and d_4-albuterol (IS) from validation are presented in figures 3-6.

Table 3. Multiple Lot Matrix Effect Factor

		Low QC		ISTD				REC Samples	
Batch	Lot#	REX Peak Area	MF	REX Peak Area	MF	MF Normalized	Low QC	ISTD
22	1	7368	0.941	3674	0.871	1.08	8552	4810
	2	8007	1.02	4395	1.04	0.982	7405	3932
	3	8199	1.05	4273	1.01	1.03	7711	4259
	4	8392	1.07	4349	1.03	1.04	7972	4303
	5	7915	1.01	4335	1.03	0.984	8221	4071
	6	8273	1.06	4447	1.05	1.00	7099	3944
Mean						1.02	7826	4220
% CV						3.8		
n						6		

		High QC		ISTD			REC Samples	
Batch	Lot#	REX Peak Area	MF	REX Peak Area	MF	MF Normalized	High QC	ISTD
22	1	408412	1.04	4622	1.08	0.958	394696	3985
	2	358646	0.911	3872	0.907	1.00	384822	4382
	3	384924	0.977	4209	0.986	0.992	398989	4695
	4	407307	1.03	4526	1.06	0.976	360668	3965
	5	*	*	*	*	*	471407	4869
	6	408263	1.04	4552	1.07	0.972	352700	3725
Mean						0.980	393880	4270
% CV						1.8		
n						5		

* = unacceptable chromatography

Table 4. Matrix Effect for Albuterol in Human Plasma (EDTA)

		LLOQ		High	
Batch	Lot#	10.0 pg/mL	% Dev.	1500 ng/mL	% Dev.
27	1	9.11	-8.9	1430	-4.7
	2	11.7	+17.0	1400	-6.7
	3	9.17	-8.3	1520	+1.3
	4	8.86	-11.4	1500	+0.0
	5	9.17	-8.3	1510	+0.7
	6	9.14	-8.6	1500	+0.0
	7	9.35	-6.5	1540	+2.7
	8	9.00	-10.0	1510	+0.7
	9	10.2	+2.0	1450	-3.3
	10	9.58	-4.2	1450	-3.3
Mean		9.53		1480	
% CV		8.9		3.1	
% Theoretical		95.3		98.7	
n		10		10	

Figure 2. Post-Column Matrix Infusion

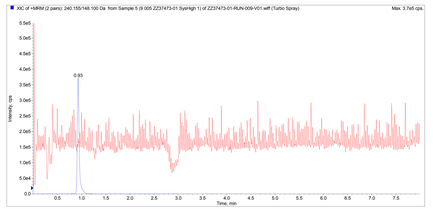


Figure 3. Representative Chromatograms of Albuterol and d_4-Albuterol (IS) from an Extracted Blank Human Plasma (EDTA) Sample

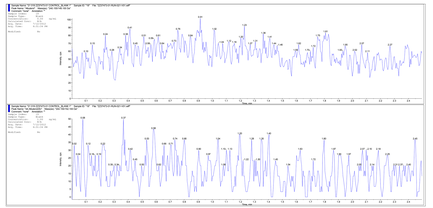


Figure 4. Representative Chromatograms of Albuterol and d_4-Albuterol (IS) from an Extracted Human Plasma (EDTA) Sample Fortified with d_4-Albuterol (IS) Only

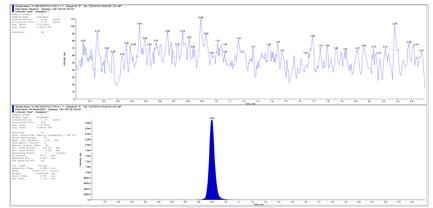


Figure 5. Representative Chromatograms of Albuterol and d_4-Albuterol (IS) from an Extracted Human Plasma (EDTA) LLOQ Sample

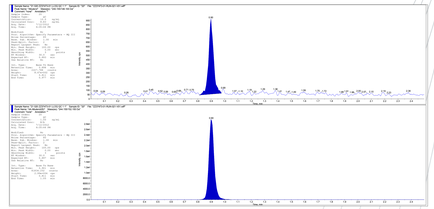
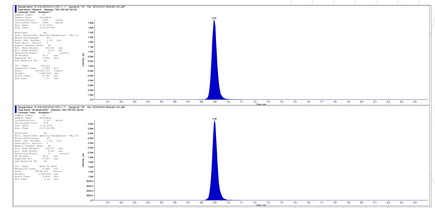


Figure 6. Representative Chromatograms of Albuterol and d_4-Albuterol (IS) from an Extracted Human Plasma (EDTA) ULOQ Sample



CONCLUSIONS

- A bioanalytical assay for the quantitation of albuterol in human plasma was developed with an LLOQ of 10 pg/mL.
- The method was validated and has been used to analyze 760 clinical samples with acceptable Incurred Sample Reproducibility.

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