Determination of Albuterol Sulfate using LC-MS/MS and Evaluation of Lung Microphysiological Systems Used for Inhaled Drugs

Isra Tariq, Md Shadiqur Rashid Roni, Shekh Rahman, Robert Geiger, Vikram Patel, Kevin Ford, Rodney Rouse, Omnia A. Ismaiel

Abstract

A sensitive LC-MS/MS was developed and validated for the determination of Albuterol (salbutamol) Sulfate (SS) over a concentration range of 10-2000 nM in serum-free media (PneumaCult™-ALI Medium and Endothelial Cell Growth Basal Medium) used for lung MPS. Excellent peak shape was obtained for both analyte and internal standard using a reversed phase C18 column, 0.05:2:98 formic acid/methanol/water, v/v/v as mobile phase A, methanol with 0.1% formic acid, v/v as mobile phase B, and a positive ESI MRM mode. Sample dilution with organic solvent was applied for sample processing followed by online sample clean up. SS was stable for 3 freeze/thaw cycles and for 8 hours at RT in low-binding tubes, extracted samples were stable for 29 hours at 2-8 ◦C. No light sensitivity, non-specific binding, or degradation were observed after incubation at 37 ◦C for 180 minutes in the study media. The proposed method was applied to evaluate the permeability and epithelial solubility of SS using two different lung MPS. Preliminary results showed low intracellular concentrations for human lung epithelial and endothelial cells using different MPS. The results were consistent with drug properties and clinical information.

Introduction

Albuterol (salbutamol) Sulfate (SS) is an inhaled beta2-adrenergic agonist used to treat or prevent bronchospasms in patients with reversible obstructive airway disease [1]. Several Analytical methods have been developed for the determination of SS in biological samples, using solid phase extraction (SPE) and liquid-liquid Extraction [2]. SS is a highly water-soluble drug (log P of < 0.4). SS was chosen as a model inhaled drug to understand how lung microphysiological systems can be used to evaluate the permeability and epithelial solubility of clinically relevant inhaled drugs. Development of a sensitive method is necessary to accurately measure low intracellular concentrations and to evaluate drug permeability.

Albuterol Sulfate permeability was evaluated using two different lung microphysiological systems. The amount of drug that permeates within various tissues of the lungs can provide a better outlook for pharmacokinetic studies. The results from evaluation of the MPS may aid future studies of new and existing inhaled medications.

References:
1. S. Erram, et al. Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 864–874
2. D. Zhang, et al, Biomed. Chromatogr. 2012; 26: 1176–1182 .

Materials and Methods

Instrument: SCIEX: QTRAP 4500 with Agilent UPLC system

Column: Phenomenex Kinetex XB-C_{18}, 50 x 2.1 mm, 2.6 µm, 100 Å

Mobile Phases: (A) 0.05:2:98 Formic Acid/Methanol/Water, v/v/v
(B) Methanol with 0.1% Formic Acid, v/v

Stock solution: 20mM was prepared in LCMS grade water.

Matrix: PneumaCult™-ALI Medium.

Sample dilution: 20 µL sample was diluted with 80 µL of 20 ng/mL of Salbutamol D-9 in 1:1 Methanol/water, v/v. Samples were prepared and stored in low binding tubes.

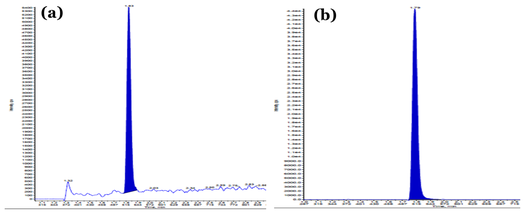
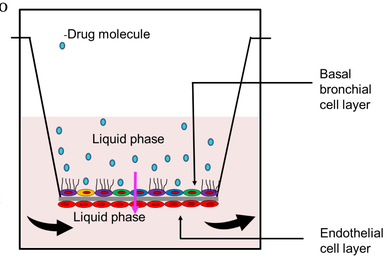


Figure 1. LC-MS/MS Chromatogram of (a) Albuterol Sulfate LLOQ (10 nM), (b) Albuterol sulfate-d9

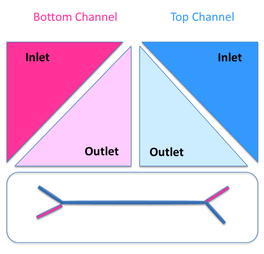
Microphysiological Systems:

(A) Modified Transwell-based Lung MPS model

- 10 µM drug was added to apical compartment
- At different time points samples were collected from apical and basal compartments
- Cell lysate samples were collected after cell lysis



(B) Lung MPS Chip



- 10 µM drug was added to top inlet.
- At different time points samples were collected from top and bottom inlets and outlets.
- After 180 minutes cell lysate samples were collected after cell lysis

Results and Discussion

Evaluation of Nonspecific Binding

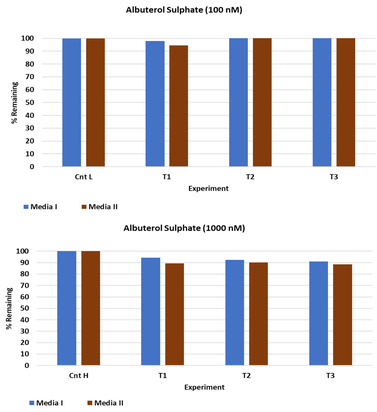


Figure 3. Evaluation of NSB for 100 nM and 1000 nM Albuterol Sulfate in propylene tubes after several mixing and transfer cycles.

Cnt L (100nM), H (1000nM): Control samples in Low binding tube

Transfer 1 (**T1**), Transfer 2 (**T2**), Transfer 3 (**T3**)

Media I: Pneumacult basal media

Media II: Endothelial cell basal media

No Significant Non Specific Binding to polypropylene or MPS materials was Observed

Method Validation

Table 1: Albuterol Sulfate Method Validation Results

Validation Item	Summary
Linearity	10-2000 nM
Precision and Accuracy	3 acceptable runs
Freeze/Thaw Stability	3 cycles (-80 ◦C /RT) in Low Binding (LB) tubes
Bench Top stability	8 hours at RT in LB tubes without treatment
Dilution Linearity	20-fold dilution
Autosampler Stability	29 hours at RT
Long Term stability	75 days at -80 C in LoBind tubes

Evaluation of Lung Cell Permeability using MPS

(A) Modified Transwell Based MPS:

Table 2. Measured SS concentrations in Transwell Based MPS: Co-culture Perfused Samples (CP)

C-P	APICAL		LYSATE APICAL	LYSATE BASAL	BASAL
	Time point (min)	Conc (nM)	Conc (nM)	Conc (nM)	Conc (nM)
	0	10448.00	< 10 nM	No Peak	< 10 nM
	30	9708.30	12.78	12.37	47.78
	60	10071.00	10.68	10.78	77.72
	180	8960.00	25.92	11.37	356.55

❖ Consistent increase in basal compartment drug concentrations over time for modified Transwell MPS was observed

(B) Lung MPS Chips:

Table 3. Measured SS concentrations using Lung MPS Chips

CHIP-3	Top Inlet	Bottom Outlet	Top Outlet	Bottom Inlet
Time point	Conc (nM)	Conc (nM)	Conc (nM)	Conc (nM)
0	10686.52	NA	NA	No Peak
30	11018.04	48.72	8170.80	No Peak
60	10503.74	60.51	10519.55	No Peak
120	10954.88	55.72	10753.76	No Peak
180	11183.13	57.32	10872.43	No Peak
Cell Lysate Top	< 10.00			
Cell Lysate Bottom	< 10.00			

❖ Preliminary results display permeability of the drug from the top inlet into the bottom outlet

Conclusions

- ✓ No significant non-specific binding to polypropylene or MPS materials was observed.
- ✓ Consistent increase in basal compartment drug concentrations over time was observed for modified Transwell MPS, and low intracellular concentrations were observed for both epithelial and endothelial cells.
- ✓ Low and consistent concentrations were observed in bottom outlet for Lung MPS chip over different time points. Six different Lung MPS chips showed comparable results.
- ✓ The overall preliminary results were consistent with drug properties and clinical information.