Determination of Fluticasone Furoate in serum-free media using LC-MS/MS to support Lung Microphysiological System Assessment of inhaled Drugs

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Abstract

An accurate and validated LC-MS/MS was developed for the determination of Fluticasone Furoate (FF) in serum-free media (PneumaCult™-ALI Medium and Endothelial Cell Growth Basal Medium) used for lung MPS studies. The analyte and a stable-labeled internal standard were separated on a reversed phase C18 column. Excellent linearity was obtained over a concentration range of 50-2000 nM. FF was stable for 3 freeze/thaw cycles and for 8 hours at RT in low-binding tubes, extracted samples were stable for 20 hours at 2-8 ◦C. No light sensitivity or degradation were observed after incubation at 37 ◦C for 180 minutes in the study media. The method was applied to measure drug adsorption in two different lung MPS and to evaluate the applicability of using lung MPS to study the permeability and solubility of inhaled drugs with different physicochemical properties. Significant intracellular concentrations were observed for human epithelial and endothelial cells using different MPS; however, 60-98% drug loss was also observed due to NSB to polypropylene containers and/or MPS chip materials. These results demonstrate that drugs with similar properties may have significant binding to some MPS chips, suggesting that NSB may impact the accuracy of in-vitro models used to study inhaled drug biology.

Introduction

Fluticasone Furoate (FF) is an inhaled corticosteroid used to treat seasonal and perennial allergic rhinitis. Compared with other corticosteroids, FF has high cellular accumulation, a slower rate of efflux and shows enhanced affinity to glucocorticoid receptor requiring a lower daily dose [1]. FF is water insoluble (logP of 4.13). The limited solubility and lipophilicity of FF could present a challenge, not only for accurate quantification of the drug, but also for invitro applications such as microphysiological systems (MPS) due to potential non-specific binding (NSB) to plastic chips and/or polypropylene containers resulting in significant drug loss. HPLC-UV and spectrofluorimetric methods for simultaneous determination of fluticasone furoate and vilanterol in rabbit plasma [1]. The aim of this study is to develop and validate an accurate and sensitive LC-MS/MS for quantification of FF in the study media and to evaluate drug loss due to degradation or non-specific binding to containers and/or MPS.

References

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Materials and Methods

The analyte and a stable-labeled internal standard were separated on a reversed phase C_{18} column, using 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 cleanup.

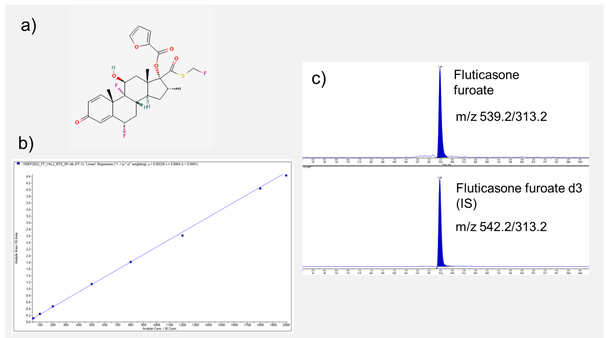
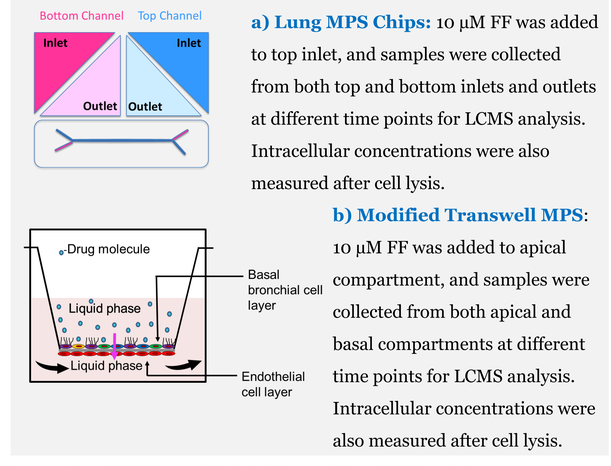


Figure 1. (a) Fluticasone furoate Chemical Structure
(b) A calibration curve (50-2000 nM) and (c) LCMS chromatogram of 50 nM



a) Lung MPS Chips: 10 µM FF was added to top inlet, and samples were collected from both top and bottom inlets and outlets at different time points for LCMS analysis. Intracellular concentrations were also measured after cell lysis.

b) Modified Transwell MPS: 10 µM FF was added to apical compartment, and samples were collected from both apical and basal compartments at different time points for LCMS analysis. Intracellular concentrations were also measured after cell lysis.

Figure 2. Lung MPS Chip and Modified Transwell-based MPS model

Results and Discussion

Table 1. Fluticasone Furoate Method Validation summary

Validation Item	Summary
Linearity	50-2000 nM
Precision and Accuracy	3 acceptable runs
Freeze/Thaw	3 cycles (-80 ◦C /RT) in Low Bind tubes
Bench Top stability	8 hours at RT in LB tubes without treatment
Stock solution stability (0.5 mM)	150 days at -80 ◦C in DMSO
Dilution Linearity	20-fold dilution
Autosampler Stability	72 hours at 2-8 ◦C
Long Term stability	51 days
Samples were treated (1:1) with methanol, v/v to minimize NSB	

Evaluation of Light sensitivity and Thermal stability:

FF was stable in regular light at room temperature for two hours, and it was stable in study media at 37◦C for 3 hours.

Evaluation of non-specific binding to polypropylene (PP):

Significant non-specific binding (NSB) was observed (~98%) was observed after 3 transfers in PP tubes was observed

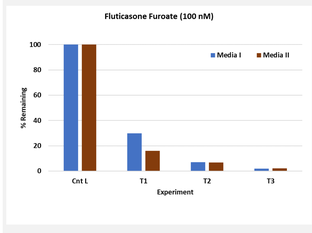


Figure 3. Evaluation of Non-specific binding of FF.

Control (100 nM) sample was prepared in low binding tube and compared to (T_1, T_2, T_3) samples that were mixed and transferred in PP tubes for 1, 2, 3 cycles, respectively

Evaluation of drug adsorption to MPS systems:

Significant drug loss (~ 70-90%) was observed after incubating FF for 3 hours in different MPS models

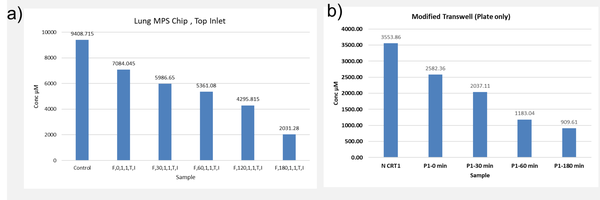


Figure 4. Evaluation of drug adsorption to (a) Lung MPS chip (b) Modified transwell plate

Evaluation of Lung cell permeability using two different MPS systems:

Table 2. Lung MPS Chips-Preliminary Samples

CHIP-1	Top Inlet	Bottom Outlet	Top Outlet	Bottom Inlet
Time point (min)	Conc (nM)	Conc (nM)	Conc (nM)	Conc (nM)
0	7630.71	NA	NA	< 0
30	7439.09	< 50 nM	1140.25	< 0
60	7555.55	193.58	2072.20	< 0
120	8066.04	359.83	2987.04	< 0
180	6520.05	423.45	2453.43	< 0
Cell Lysate Top	6592.97			
Cell Lysate Bottom	803.97			

Table 3. Modified Trans well plates (Co-culture perfused samples)

	APICAL		LYSATE APICAL	LYSATE BASAL	BASAL
C-F-M	Time point (min)	Conc (nM)	Conc (nM)	Conc (nM)	Conc (nM)
	0	6786.30	152.23	< 50 nM	< 50 nM
	30	4536.14	954.10	< 50 nM	< 50 nM
	60	3148.00	656.29	< 50 nM	< 50 nM
	180	2863.88	1117.28	< 50 nM	83.08

MPS Chips and Modified Transwell-based plate, showed comparable results, significant intracellular concentrations were observed, suggested higher cellular uptake.

Conclusion

- ✓ A sensitive, reliable and reproducible LC-MS/MS has been developed and validated for determination of fluticasone furoate in media used for MPS studies.
- ✓ Significant intracellular concentrations were observed for human epithelial and endothelial cells using different MPS
- ✓ 60-98% drug loss was also observed due to NSB to polypropylene containers and/or MPS chip materials.
- ✓ These results demonstrate that drugs with similar properties may have significant binding to some MPS systems, NSB may impact the accuracy of in-vitro models used to study inhaled drug biology.

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