Effect of using bio-relevant media in the Dissolv*It*® system to measure dissolution of fluticasone propionate from Flixotide 50 µg Evohaler

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Introduction

- There are various methods for *in vitro* dissolution developed for OIPs. However, most systems require large volumes of dissolution medium and the media currently used have been simple aqueous fluids, or with the addition of various surfactants[1].
- FP is a poorly soluble inhaled drug[2] and represents a challenge in dissolution tests since it is difficult to maintain sink conditions and it is not easy to assay FP in low concentrations. Therefore, a highly sensitive assay is required with an efficient extraction method[3, 4].
- The Dissolv*It*® from Inhalation Sciences was developed as an *in vitro* dissolution tool for OIPs that utilises low volume of dissolution medium and allows particle disintegration to be studied visually while drug dissolution is quantified chemically in a dynamic flow-past model[5].
- The development of a physiologically representative methodology, including a bio-relevant medium, is required to address the current unmet need for a system that is more characteristic of the *in vivo* environment.

Aims

- To validate a new rapid and sensitive LC-MS/MS method to quantify FP in samples from an investigation into the use of bio-relevant media in the Dissolv*It* system.
- To investigate the effect of dissolution medium on FP aerosol particle dissolution, using three different media (Table 1): (i) 1.5% polyethylene oxide including 0.4% L-alphaphosphatidyl choline (PEO), (ii) Survanta®, and (iii) an in house developed simulated lung lining fluid (sLLF), synthesised based on accurate measurements of human lung fluid composition.

Table 1. Protein and lipid concentrations in polyethylene oxide in phosphate buffer solution (PEO), simulated lung lining fluid (sLLF) and Survanta®

Media	Protein concentration (mg/mL)	Lipid concentration (mg/mL)
PEO	-	4.0
sLLF	12.9	5.4
Survanta®[a]	0.01-0.16	4.0

[a] Diluted with water to obtain a lipid concentration of 4.0 mg/mL

Methods

➢ Validation of the LC-MS/MS assay

Calibration standards (156, 313, 625, 1250, 2500, 5000 and 10,000 pg/mL) were prepared by serial dilution of a 1 ug/mL FP working solution with acetonitrile. Validation was conducted in terms of linearity, intra-day & inter-day precision (%CV), accuracy, limit of detection (LOD) & limit of quantification (LOQ), based on FDA guidance.

➢ Deposition and dissolution of FP aerosol in the Dissolv*It*® system

The Flixotide pMDI was connected to the PreciseInhale® aerosol generator (Figure 1). The inhaler was dosed into an airflow of 15 L/min and the aerosol particles were deposited onto glass cover slips. The bio-relevant media, 5.7uL, was applied to one side of the polycarbonate membrane of a single-use dissolution chamber. The simulant, with the membrane, provide diffusion barrier (Figure 1). On the other side of the membrane, the perfusate was streamed past at a flow rate 0.4 mL/min. Particle disintegration was studied using optical microscopy and chemical analysis of FP dissolved in flow-past perfusion medium.

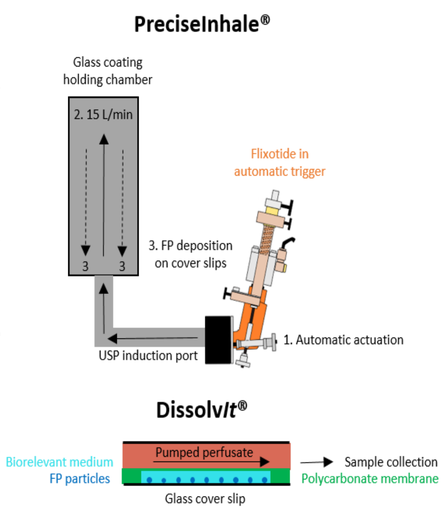


Figure 1. Schematic of the PreciseInhale® and DissolvIt®

➢ FP quantification

Samples were prepared for analysis using SPE. Briefly, 325uL of sample was loaded into a deep-well plate followed by 50uL of internal standard (FP-d5), 300uL of 0.1M zinc sulphate & 75uL of 10% ammonium hydroxide and mixed. They were centrifuged at 3700rpm and transferred to an Evolute® Express 96-well plate. Samples were reconstituted with 30uL of 55% v/v acetonitrile in water and injected into the LC-MS/MS.

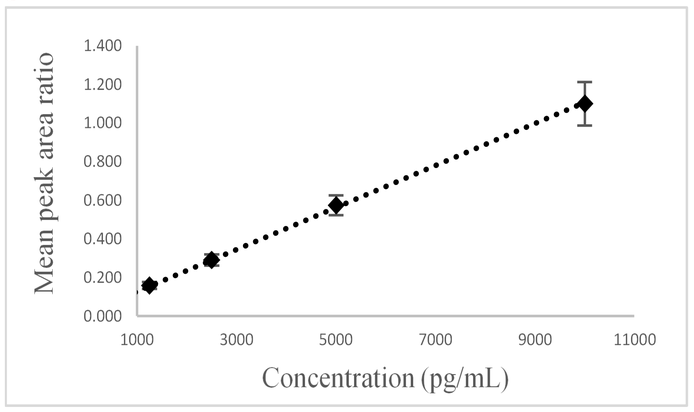
➢ Data analysis

Peak integration was performed using MassLynx 4.1 software. Data was expressed as mean ± standard deviation. For FP dissolution, the %FP in perfusate was expressed as % of amount deposited on the glass slide. One-Way ANOVA was applied and statistically significant when $p \leq 0.05$.

Results

- Excellent linearity between the mean peak area ratio of FP/FP-d5 and the concentration of FP in the samples was observed (R^2 value = 0.999).
- The inter-day and intra-day precision data complied with the validation guidance, with all CV being <20%, except for 156 pg/mL and 313 pg/mL.
- The accuracy data for all FP standard concentrations passed the accepted criteria of 85-115% (Figure 2).
- The LOD and LOQ were 106 pg/mL and 312 pg/mL respectively.

a



b

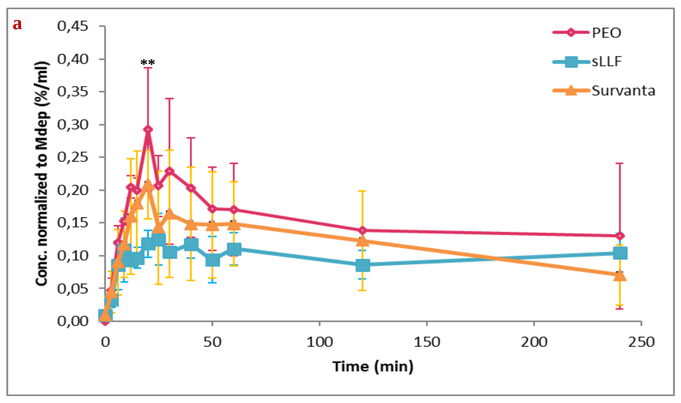
FP (pg mL-1)							
Theoretical concentration	156	313	625	1250	2500	5000	10000
Mean measured concentration[a]	142.6 ± 66.5	288.1 ± 71.9	645.6 ± 93.3	1404.1 ± 166.9	2732.2 ± 277.3	5557.4 ± 493.7	10808.5 ± 1110.2
CV (%)	46.6	24.9	14.5	11.9	10.15	8.8	10.3
Accuracy[b] (%)	91.4	92.0	103.3	112.3	109.3	111.1	108.1

[a] n=9
[b] accepted range = 85-115%

Figure 2. Validation of the solid phase extraction and LC-MS/MS assay of fluticasone propionate (FP): a) Linearity of the mean peak area ratio vs concentration; b) FP concentration, precision and accuracy. Data expressed as mean ± SD (n=9).

- FP concentration in perfusate was highest at all time points when FP dissolved in PEO & lowest in sLLF (Figure 3a).
- The FP concentration profile in perfusate was very similar between PEO and Survanta®, both reaching a Cmax at 20min; but the difference in the FP concentration values in PEO and sLLF at 20 min was statistically significant (One-Way ANOVA, $p<0.05$).
- The cumulative percent of FP transferred into the perfusate over time showed similar profiles in each medium (Figure 3b).

a



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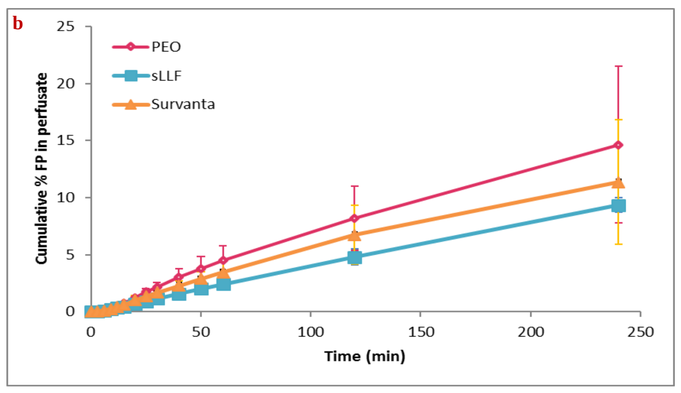

*Figure 3. Dissolution of FP. a) Concentration of FP in the perfusate over time following dissolution in PEO, sLLF and Survanta normalised to mass deposited on glass cover slips. **Difference in FP concentration in PEO and sLLF is statistically significant (One-Way ANOVA, $p<0.05$). b) Cumulative % of FP transferred into the perfusate over time, following the dissolution in PEO, sLLF and Survanta. Data expressed as mean ± SD (n=3).*

Discussion

- SPE offers an improved extraction method over liquid phase extraction since it is less time-consuming and requires minimal sample preparation and solvent use[4].
- The 156pg/mL FP standard fell outside the accepted CV (<20%), attributed to the concentration being close to the LOD (106 pg/mL). However, the FP concentrations in the dissolution experiments fell within the upper range of the assay, which was fit for purpose.
- The PEO medium used as a standard in the Dissolv*It*® system possessed a lower lipid content than in sLLF. It was hypothesised that dissolution of FP in sLLF would be enhanced as the greater lipid content may facilitate drug solubilisation. However, the results showed less FP transfer to the perfusate when sLLF was used compared to PEO, and it is speculated that FP may preferentially reside or become trapped within lipid/lamellar structures in sLLF.
- sLLF contains cholesterol, unlike other lung fluid simulants, and studies have shown that cholesterol can form tight nanodomain complexes with DPPC, stabilising the DPPC in lipid structures such that once the FP is solubilised within, it is less likely to leave such structures[6].

Conclusions

- ✓ A SPE/ LC-MS/MS assay for FP was established successfully and able to quantify low concentrations (pg/mL) of the FP in lung fluids.
- ✓ The hypothesis that the dissolution and transfer of FP in the perfusate would be enhanced by using sLLF was not supported by this study. FP may reside preferably in the lipid structures, limiting the transfer into the perfusate.
- ✓ Further studies are required to evaluate more fully the impact of the medium composition on dissolution profile and whether more bio-relevant media can provide data more predictive of inhaled particle dissolution.

References

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