In Vitro Permeation Testing: Assessment of Sampling Techniques and Analytical Methods

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1 INTRODUCTION

In October 2022, the FDA published its updated draft guidance on establishing bioequivalence between topical formulations containing the same active pharmaceutical ingredient (API) using *in vitro* permeation test (IVPT). A systematic series of development and validation studies (for both IVPT and analytical methods) are needed before performing a final pivotal study that determines whether there is bioequivalence between a blinded reference listed drug (RLD) formulation and test formulation.

Qualification of the receptor fluid sampling technique is an important part of the IVPT method validation stage to ensure appropriate accuracy and precision of sample collection. Historically, receptor fluid sampling uses the aliquoting technique for static vertical diffusion cells (VDCs). However, the FDA draft guidance advises full volume replacement for the purposes of maintaining sink conditions and avoiding negative flux. The collection of full volume sample can be technically challenging compared to aliquoting which is dependent on the type of VDC that is used.

Therefore, the objectives of this study were:

1. To determine whether there were differences between aliquot sampling versus full replacement of receptor fluid, using Teledyne Hanson Phoenix DB6 static VDCs.
2. To determine whether analytical sensitivity (liquid chromatography with tandem mass spectrometry (LC-MS/MS) and liquid scintillation counting (LSC)) influenced the absorption profiles generated by the sampling techniques.

The *in vitro* permeation of Diclofenac sodium or [^{14}C]-Testosterone through *ex vivo* human split-thickness skin was tested to investigate these objectives.

2 EXPERIMENTAL PROCEDURES

Phoenix DB6 static VDCs were qualified and assembled by putting together the dosage chamber and clamp ring on top of the cell cap to form the "cap kit". The diffusion cells were then positioned in a Phoenix Dry Heat System (DB6 manual sampling system) pre-calibrated to maintain a skin surface temperature of 32˚C ± 1˚C. Pre-warmed receptor fluid (phosphate buffered saline containing bovine serum albumin (*ca* 5%, w/v) and sodium azide (*ca* 0.01%, w/v)) was added up to the fill mark on the receptor chamber arm.

Samples of full-thickness human skin from 3 donors were obtained from Tissue Solutions Ltd., thawed, and dermatomed to a thickness of *ca* 400 µm. Sections of split-thickness skin were cut (equivalent to 1.13 cm^2) then mounted onto the cells. Barrier integrity of the skin samples was assessed using electrical resistance with an acceptance criterion of >7.7 kΩ. The underside of the skin samples were checked to be free from air bubbles prior to dosing.

Formulations containing Diclofenac sodium (1%, w/w) or [^{14}C]-Testosterone (0.1%, w/v) were prepared in propylene glycol: water (80:20, v/v) and each applied to the *stratum corneum* surface of 12 skin samples (4 replicates per donor) at an application rate of 10 µL/cm^2.

Absorption was assessed by sampling receptor fluid at timepoints over a 48 h period for Diclofenac sodium and over a 24 h period for [^{14}C]-Testosterone. For aliquoting, a single 250 µL aliquot was taken using a positive displacement pipette. For full replacement, the entire contents were collected by removing the cap kit and pouring from the cell. Pre-warmed receptor fluid was used to replenish the receptor chamber volume after each timepoint (except the terminal timepoint) for both sampling techniques. Samples were analysed by either LC-MS/MS (Diclofenac sodium) or mixed with scintillation fluid then analysed by LSC ([^{14}C]-Testosterone).

Figure 1. Phoenix DB6 from Teledyne Hanson and schematic of Diffusion cell.

3 RESULTS

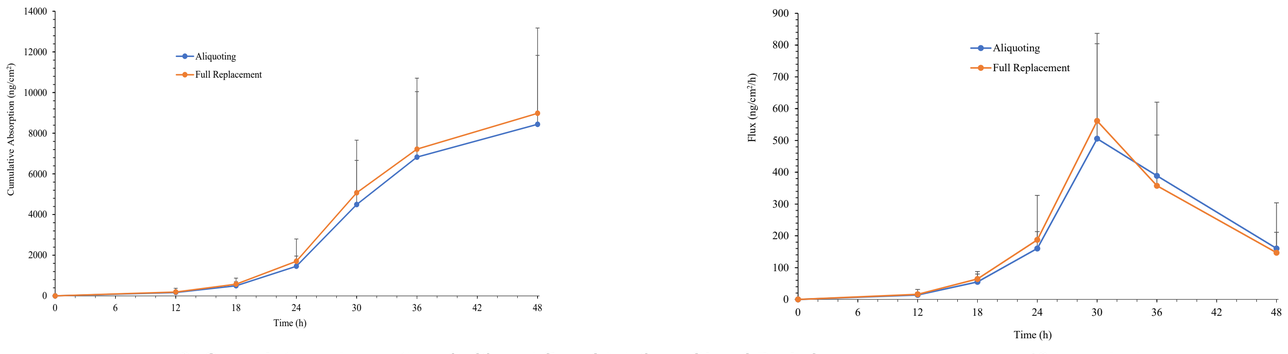

Figure 2. Cumulative absorption *(left)* and flux *(right)* profile of Diclofenac sodium over a 48 h period. Mean + SD are shown (n = 6 cells; 2 cells per donor)

Diclofenac Sodium	Aliquoting	Full Replacement
AMT (ng/cm^2)	8439 ± 3391	8983 ± 4195
Maximum Flux (ng/cm^2/h)	506 ± 299	562 ± 275
Maximum Flux Timepoint	30 h	30 h

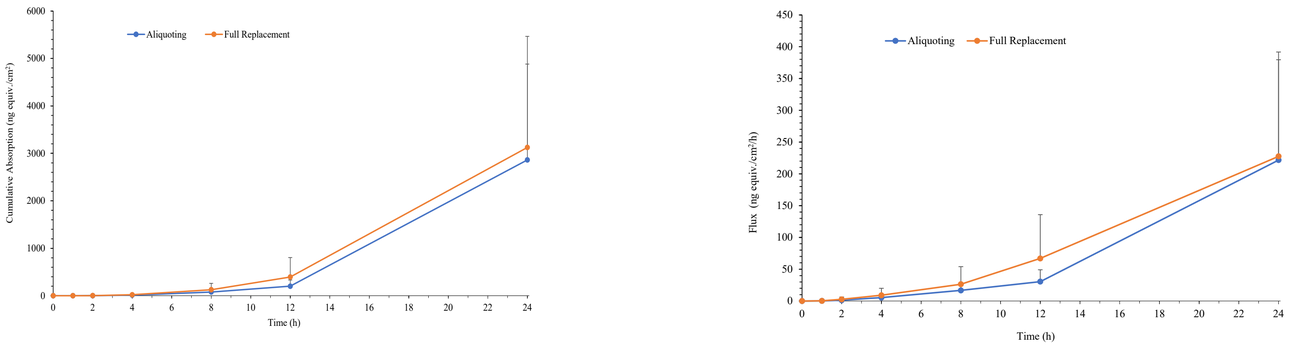

Figure 3. Cumulative absorption *(left)* and flux *(right)* profiles of [^{14}C]-Testosterone over a 24 h period. Mean + SD are shown (n = 6 cells; 2 cells per donor)

[^{14}C]-Testosterone	Aliquoting	Full Replacement
AMT (ng/cm^2)	2862 ± 2019	3125 ± 2340
Maximum Flux (ng/cm^2/h)	222 ± 158	228 ± 164
Maximum Flux Timepoint	24 h	24 h

AMT: total amount permeated by terminal timepoint

4 CONCLUSION

The choice of sampling technique must be robust and consistent for a multi-study project such as IVPT bioequivalence which consists of the following, in addition to analytical method development and validation:

1. **IVPT Method Development**
   Determine type of diffusion cell for use, identify a minimum of 8 non-zero sampling timepoints, and establish an appropriate sampling schedule and duration for subsequent studies.
2. **IVPT Sensitivity**
   Obtain distinct profiles between three different dose amounts/durations.
3. **IVPT Pilot**
   Determine statistical difference between the RLD/Test and Alternative Formulations, estimate sample size for the pivotal study, and validate the experimental procedures.
4. **IVPT Pivotal**
   Determine bioequivalence between the RLD and Test Formulations.

In conclusion, the experiments confirmed comparable profiles could be generated with both aliquoting and full replacement technique when using Teledyne Hanson DB6 static VDCs. No clear differences were observed between the aliquoting and full replacement sampling technique in terms of the cumulative absorption and flux profiles for skin samples dosed with Diclofenac sodium or [^{14}C]-Testosterone. Maximum flux occurred at 30 h post dose (Diclofenac sodium) and 24 h post dose ([^{14}C]-Testosterone) regardless of sampling technique.

Both analytical methods (LC-MS/MS and LSC) were robust enough to analyse samples obtained by either sampling technique. To further examine the influence of analytical sensitivity, a follow-up study would be to spike radiolabelled material into the formulation containing Diclofenac sodium, perform IVPT using the same donors over the same experimental duration as the non-radiolabelled phase of this presented study, then compare the absorption profiles between samples analysed by LC-MS/MS and LSC.