IVPT – Full vs. Aliquot Sampling Techniques Study for Diclofenac Sodium and [^{14}C]-Testosterone Using the Phoenix DB-6 Diffusion Testing System

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Introduction

In October 2022, The US Food and Drug Administration (FDA) published its updated draft guidance on establishing bioequivalence between topical formulations containing the same active pharmaceutical ingredient (API) using in vitro permeation test (IVPT).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) is 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 However, the FDA draft guidance advises full volume replacement for the purposes of maintaining sink conditions and avoiding negative flux.

It is our understanding that negative flux happens due to inadequate mixing, as it means the receptor media just below the membrane becomes overly saturated with API and thus diffusion through the skin slows down. By replacing all the media, it ensures sink conditions throughout the entire cell, even with poor mixing. Or, by using an improved cell and mixer design, like the Phoenix, the entire receptor chamber is equally homogeneous which doesn't slow down/impact the diffusion through the skin. And finally, the long, narrow sampling arms on the traditional Franz cells contain less-concentrated solution because their mixers can't get good flow up inside the arm. Our Phoenix arms are wide and fully homogeneous with the body of the cell, which helps maintaining solubility sink conditions throughout the extended run.

The collection of full volume sample can also be technically challenging compared to aliquoting. Choosing to use an aliquot sampling method for IVPT can be simpler, and can allow for less use of media and the use of less sensitive and expensive equipment because the concentration of API remains high enough during the study.

The objectives of the experiment were to determine if there were differences between these two receptor fluid sampling techniques using Teledyne Hanson Phoenix DB-6 static diffusion cells and whether analytical sensitivity influenced the profiles that the sampling techniques generated.

This research study demonstrates that aliquot sampling is suitable for IVPT studies of Diclofenac Sodium Gel and [^{14}C]-Testosterone Gel when using modern diffusion apparatus. This is likely due to diffusion cell design innovations mentioned above, which ensures receptor chamber and arm stem homogeneity, eliminating "apparent negative flux," a phenomenon that industry hasn't been able to explain. Although this study used Diclofenac Sodium Gel and [^{14}C] -Testosterone Gel as the test articles, partial media replacement can be used with other topical formulations.

Experiment

The entire study was conducted at Charles River Laboratories UK (www.criver.com) by a well-trained scientist under the supervision of a very experienced professional using their internal standard operating procedures and policies. Gel formulations (containing Diclofenac Sodium (1%, w/w) or [^{14}C]-Testosterone (0.1%, w/w)) were prepared and applied to skin samples mounted in cells set to maintain a skin surface temperature of 32 ± 1 °C. Absorption was assessed by sampling at defined timepoints over a 48 h period for Diclofenac Sodium and over a 24 h period for [^{14}C]-Testosterone. For aliquoting technique, a single 250 µL aliquot was taken using a positive displacement pipette. For full replacement technique, the entire contents were removed using a syringe. Pre-warmed receptor fluid was used to replenish the receptor chamber volume after each timepoint (except the terminal timepoint) for both sampling techniques. Receptor fluid samples were analysed by liquid chromatography with tandem mass spectrometry (Diclofenac Sodium) or liquid scintillation counting (LSC) ([^{14}C]-Testosterone). The receptor fluid used was phosphate buffered saline (PBS) containing bovine serum albumin (BSA, ca 5%, w/v) and sodium azide (ca 0.01%, w/v), with the pH confirmed and adjusted to 7.4 ± 0.1.

Dosing Regimen

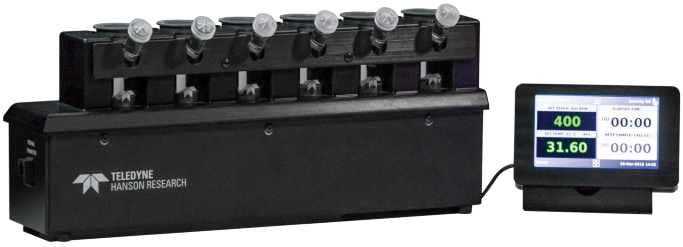
A blank sample of receptor fluid was taken prior to application of the test preparations. A single 10 µL dose of the test preparation (10 µL/cm2) was applied to a minimum of 3 samples of skin obtained from at least 3 different donors for each receptor fluid collection method on each dosing occasion listed in the table below. (24 test samples were taken in total). The test preparation was then applied to the stratum corneum surface of the skin using a positive displacement pipette. The donor chamber of the cells was not occluded; instead, it was kept open to the atmosphere. The same donors were used for each scenario. Following dosing occasion 2, an aliquot of radiolabeled test preparation was taken and diluted as appropriate to determine the radiochemical purity of the test item by HPLC analysis. The samples were stored in a freezer (-20 °C) until analysis was carried out for assay.

Dosing occasion (Analysis Type)	Test parameter	Number of dosed samples	Number of donors	Number of samples with aliquoting	Number of samples with full replacement
LCMS	1	6	3	3	3
LCMS	1	6	3	3	3
LSC	2	6	3	3	3
LSC	2	6	3	3	3

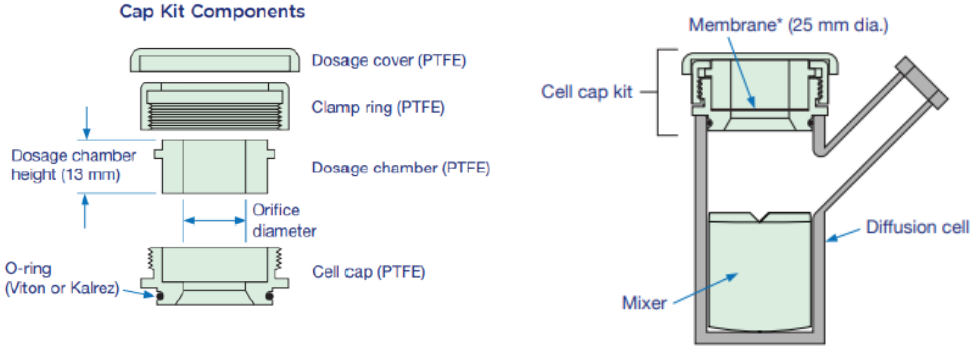
Ethical approval for receipt and use of human skin was obtained from the Lothian Local Research Ethics Committee (REC Reference No. 06/S1101/19) and the Glasgow Royal Infirmary REC (REC Reference No. 08/S0704/30). Tissue was regularly sourced from hospitals and tissue banks. Full thickness skins were obtained, cleaned of subcutaneous fat and muscle, and stored in a freezer set to maintain a temperature of -20 °C until required. The skin was removed from the freezer and allowed to thaw at ambient temperature. The skin is then cut at *ca* 200-400 µm depth with a Zimmer electric dermatome. The thickness of the split-thickness skin was confirmed using a micrometer.

Diffusion

In vitro release studies were performed using six vertical cells (1.0 cm^2 diffusional surface area and receptor chamber volume was nominally 10 mL.) mounted on a 6-station dry-heat diffusion apparatus provided by Teledyne Hanson, USA. The diffusion cells were assembled with donor and receptor chambers separated by the skin membrane.



Phoenix DB-6 Dry Heat Diffusion System



A diagram of the Phoenix vertical diffusion cell and cell cap (donor and dosage chambers)

Test Parameters for In Vitro Permeation Test (IVPT) of Diclofenac Sodium Gel (1% w/w)

Gel formulations containing Diclofenac sodium (1%, w/w) were prepared and applied to skin samples mounted in cells set to maintain a skin surface temperature of 32 ± 1 °C. Absorption was assessed by sampling at defined timepoints over a 48 h period. For aliquoting technique, a single 250 µL aliquot was taken using a positive displacement pipette. For full replacement technique, the entire contents were removed using a syringe. Pre-warmed receptor fluid was used to replenish the receptor chamber volume after each timepoint (except the terminal timepoint) for both sampling techniques. Receptor fluid samples were analysed by liquid chromatography with tandem mass spectrometry.

Diffusion Test Parameters for Diclofenac Sodium IVPT study	
Cell Size	Small, 10 mL volume
Mixer Size	30 mm
Cell Cap	Cell Cap 11.3 mm orifice x 13mm, Threaded
Temperature	32.0 ± 1 °C
Stirring Speed	400 rpm
Membrane	skin
Sampling Time Points	0, 12, 18, 24, 30, 36, and 48 hours
Sample Volume	250 µL for aliquoted sampling and 10 mL full replacement
Replacement Volume	250 µL for aliquoted sampling and 10 mL for full replacement
Average Diffusional Surface Area	1.0 cm2

Test Parameters for In Vitro Permeation Test (IVPT) of [^{14}C]-Testosterone (0.1%, w/w)

Gel formulation of [^{14}C]-Testosterone (0.1%, w/w) was prepared and applied to skin samples mounted in cells set to maintain a skin surface temperature of 32 ± 1°C. Absorption was assessed by sampling at defined timepoints over a 24 h period. For aliquoting technique, a single 250 µL aliquot was taken using a positive displacement pipette. For full replacement technique, the entire contents were removed using a syringe. Pre-warmed receptor fluid was used to replenish the receptor chamber volume after each timepoint (except the terminal timepoint) for both sampling techniques. Receptor fluid samples were analysed by liquid scintillation counting.

Diffusion Test Parameters for [^{14}C] -Testosterone (0.1%, w/w) IVPT study	
Cell Size	Small, 10 mL volume
Mixer Size	30mm
Cell Cap	Cell Cap 11.3 mm orifice x 13mm, Threaded
Temperature	32.0 ± 1 °C
Stirring Speed	400 rpm
Membrane	skin
Sampling Time Points	0, 1, 2, 4, 8, 12, and 24 hours
Sample Volume	250 µL for aliquoted sampling and 10 ml full replacement
Replacement Volume	250 µL for aliquoted sampling and 10 mL for full replacement
Average Diffusional Surface Area	1.0 cm2

Calculation of Absorption and Flux profile: Calculation of the results were performed based on the guidance provided in the *USP General Chapter <1724> Semisolid Product—Performance Tests*.[4]

Comparative IVPT data of Diclofenac Sodium and Testosterone using Two Sampling Procedures

1. The results obtained during the study of Diclofenac Sodium cumulative absorption are presented in Table 1 to compare data obtained with partial sampling (aliquoting) and full replacement of the receptor medium from the vertical diffusion cells.

Time (hours)	Partial Replacement	Full Replacement
	Average Absorption	Average Absorption
0	0.00	0.00
12	168.53	191.44
18	499.07	577.58
24	1460.17	1702.49
30	4494.60	5072.89
36	6827.51	7218.38
48	8438.94	8982.53

Table 1. Cumulative absorption of Diclofenac Sodium Gel 1%.

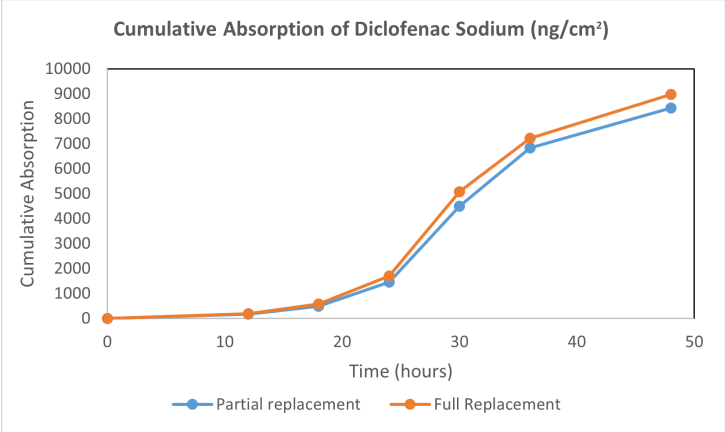


Figure 1. Graph of cumulative absorption of Diclofenac Sodium Gel 1%.

2. The results obtained during the study of Diclofenac Sodium cumulative absorption are presented in Table 2 to compare data obtained with partial sampling (aliquoting) and full replacement of the receptor medium from the vertical diffusion cells.

Time (hour)	Partial Replacement	Full Replacement
	Average Flux	Average Flux
0	0	0
12	14.04	15.95
18	55.09	64.36
24	160.18	187.48
30	505.74	561.73
36	388.82	357.58
48	160.25	147.01

Table 2. Cumulative absorption of Diclofenac Sodium Gel 1%.

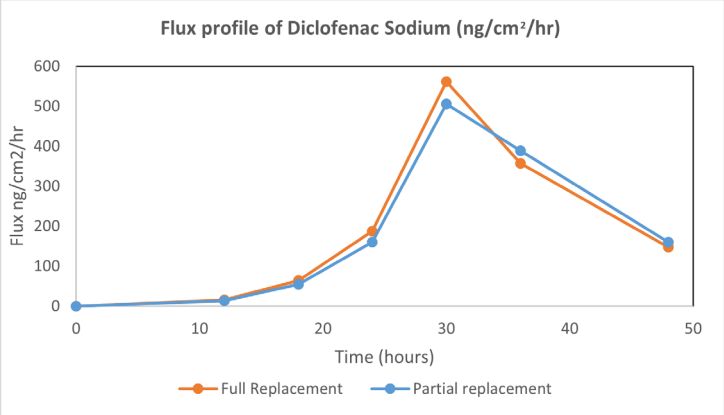


Figure 2. Flux profile of Diclofenac Sodium Gel 1%.

3. The results obtained during the study of [^{14}C]-Testosterone cumulative absorption is presented in Table 3 to compare data obtained with partial sampling (aliquoting) and full replacement of the receptor medium from the vertical diffusion cells.

Time (hours)	Partial Replacement	Full Replacement
	Mean	Mean
0	0.00	0.00
1	0.113	0.226
2	1.36	2.86
4	12.1	20.9
8	78.6	126
12	201	394
24	2862	3125

Table 3. Cumulative Absorption of [^{14}C]-Testosterone 0.1%.

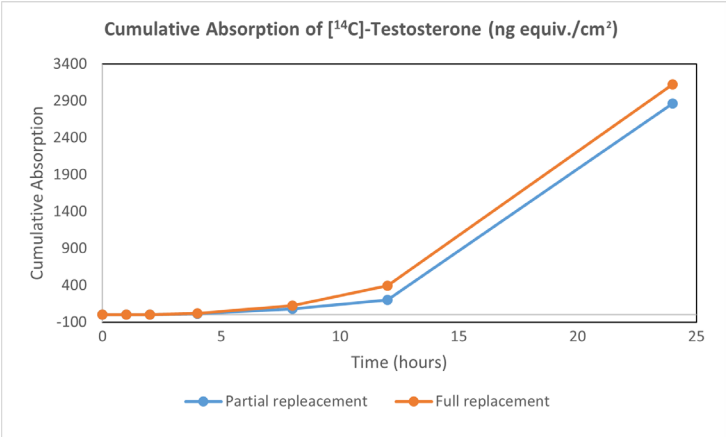


Figure 3. Cumulative Absorption of [^{14}C]-Testosterone.

Time (hour)	Partial replacement	Full Replacement
	Average Flux	Average Flux
0	0.00	0.00
1	0.11	0.23
2	1.26	2.63
4	5.35	9.05
8	16.64	26.37
12	30.52	66.99
24	221.74	227.57

Table 4. Flux of [^{14}C]-Testosterone

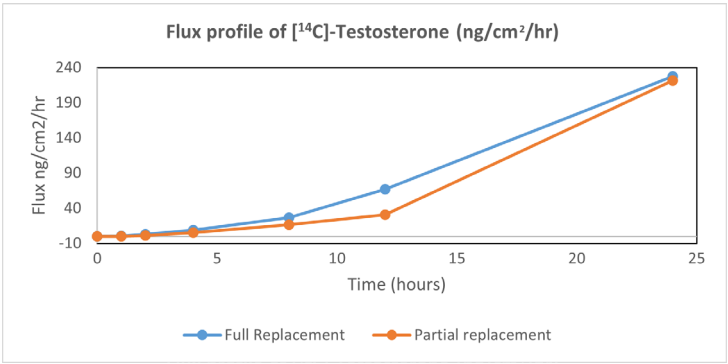


Figure 4. Flux of [^{14}C]-Testosterone (ng/cm^2/hr) .

Conclusion

No significant differences were observed in the cumulative absorption (cumulative amount) and flux profiles for Diclofenac Sodium. Cumulative amount = 8439 ± 3391 ng/cm^2 for aliquoted sampling (partial replacement) and 8983 ± 4195 ng/cm^2 for full replacement was obtained, while J_{max} = 506 ± 299 ng/cm2/h for aliquoted sampling (partial replacement) and 562 ± 275 ng/cm^2/h for full replacement was observed at 30 h post dose. For the study of [^{14}C] Testosterone, cumulative amount = 2862 ± 2019 ng/cm^2 (aliquoting) and 3125 ± 2340 ng/cm2 (full replacement), maximum flux = 222 ± 158 ng/cm^2/h for aliquoted sampling (partial replacement), and 228 ± 164 ng/cm^2/h for full replacement at 24 h post dose was obtained. Based on the similar flux profile observed from both studies, it is believed that if the study for Testosterone would have continued for longer than 24 hours, it would have resulted in decline flux after achieving the J_{max} (maximum flux). The experiments showed that comparable profiles could be generated using both aliquoted sampling (partial replacement) and full replacement technique with Teledyne Hanson's Phoenix DB-6 static diffusion cells. It also confirms that both LC-MS/MS and LSC analytical methods are robust enough to analyse samples obtained by either sampling technique. The full study report has been prepared and archived at Charles River Laboratories.[5]

References

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