Technical Report:

Characterization of the percutaneous absorption of diclofenac sodium 10% w/w from multiple human skin donors into human trunk skin. A comparative study using PCCA PLO-Diclogel™ versus PLO transdemal base.

Abstract: PCCA PLO-Diclogel is a proprietary transdermal base utilized in compounded formulations. It contains phospholipids, poloxamer and Pracaxi oil complex, which aid in its penetrating properties. PLO-Diclogel is a new version of the traditional PLO that exhibits non-ionic characteristics that will tolerate the addition of acidic actives (low pH) and high salt loads. Formulation stability has been shown within a pH range of 3 to 10. A common problem with traditional PLO is that the vehicle separates in cold temperatures or low pH ranges, rendering it unusable. PLO-Diclogel is stable in extreme temperature and pH variances. This study was designed to evaluate the percutaneous absorption pharmacokinetics of diclofenac sodium in PLO-Diclogel versus PLO as the vehicle, through human torso skin from multiple donors. Modified Franz cell systems were used for the determination of diclofenac sodium in receptor medium (transdermal flux). Skin tissue samples were analyzed with Ultra Performance Liquid Chromatography (UPLC). The data demonstrates that diclofenac sodium 10% penetrated into and through human skin from both of the compounding vehicles evaluated. The rate of absorption and the total percentage permeation is consistently higher with PCCA PLO-Diclogel than with PLO.

Introduction: PCCA PLO-Diclogel is a proprietary transdermal base utilized in compounded formulations. It contains phospholipids, poloxamer and Pracaxi oil complex, which aid in its penetrating properties. This base has non-ionic characteristics which will tolerate the addition of acidic actives (low pH) and actives with high salt concentrations, allowing for formulation stability within a pH range of 3 to 10. A common problem with traditional PLO is that the vehicle separates in cold temperatures or low pH ranges, rendering it unusable. PLO-Diclogel is stable in extreme temperature and pH variances. PLO-Diclogel has minimal impact on the lipid structure of the skin compared to PLO, and is therefore suitable for patients with dry and sensitive skin.

This skin permeation study was designed to evaluate the percutaneous absorption pharmacokinetics of diclofenac sodium in two different vehicles. Each formulation was evaluated on three replicate sections from three *ex vivo* human torso skin donors. Modified Franz cell systems were used for the determination of diclofenac sodium in receptor medium (transdermal flux). Skin tissue samples were analyzed with Ultra Performance Liquid Chromatography (UPLC). UPLC was chosen due to its high speed and sensitivity compared to HPLC (high performance liquid chromatography).

Methodology: Percutaneous absorption and penetration was measured in *ex vivo* human skin *in vitro* using static Franz diffusion cells. The study was performed with human torso skin samples from the three donors, with three replicates per donor.

The skin samples were all carefully checked for any significant visible damage, such as cuts, holes or epidermal surface damage. The skin samples were prepared to fit the experimental cell as follows: the skin tissue was cut to 3 cm^2 with the removal of the subcutaneous fat and tissue.

After visual examination and preparation, the skin patches were mounted on a receptor compartment with the stratum corneum side facing upward for the integrity check test and further IVPT (*in vitro* permeation test) measurements.

Since skin barrier integrity of each skin section is crucial for the experiment, the skin transcutaneous electrical resistance was determined before application of the drug products, after mounting skin to the diffusion cells.

Formulations containing diclofenac sodium were applied to human torso skin from three donors. The percutaneous absorption of diclofenac sodium was determined over a 24-hour period with receptor solution sample collection at 2, 4, 6, 8, 10, 12, and 24 hours, withdrawing 0.5 mL of sample with medium replacement. The receptor medium was comprised of phosphate buffered saline (PBS), pH 7.2, and had sufficient solubility to ensure skin conditions for *in vitro* studies.

In vitro percutaneous absorption of diclofenac sodium and deposition in skin layers were measured using method DTM-169-R00. The amount of the active, the percent of applied dose in the receptor fluid for each time point, and the drug distribution in the skin layers were determined by UPLC quantitative method. The sampling of the receptor medium, skin surface wash, epidermis and dermis isolation, and collection procedures are specified in the method DTM-169-R00.

Materials and Instrumentation:

- Diclofenac Sodium 10% in PLO
- Diclofenac Sodium 10% in PCCA PLO-Diclogel
- Skin Donor 1: Human skin RC140051, Donor ID F140085
- Skin Donor 2: Human skin RC140068, Donor ID S140397
- Skin Donor 3: Human skin RC140098 and RC140099, Donor ID F140217
- FDC-6 Transdermal Diffusion Cell Drive Console, Logan Instruments Corp.
- UPLC/MS/MS System with TQ detector and data acquisition system
- UPLC Column, Acquity UPLC BEH, C18, 1.7 mm, 150 mm x 2.1 mm

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Results: The total absorption, rate of absorption and the skin content (distribution) of diclofenac sodium were determined for both formulations: PLO-Diclogel and PLO gel. Both test formulations contained diclofenac sodium 10% (w/w) and ethyl alcohol 5% (w/w). The absorption results indicate the percutaneous absorption of the active substance through the skin, whereas the distribution results indicate the percutaneous absorption into the skin.

The total absorption and the skin content were determined after 24 hours from a single application of the transdermal compounded formulations in the skin sections. The total absorption corresponded to the total amount of drug recovered in the receptor solutions. The skin content corresponded to the total amount of active substance recovered in the stratum corneum, dermis and epidermis ($\mu g/cm^2$).

The rate of percutaneous absorption, on the other hand, is a time-averaged value and it was determined as the mean flux of the active substances collected at the receptor solutions ($\mu g/cm^2/h$), over the 24-hour period (18 hours mid time). Rate of percutaneous absorption is presented as the flux. Flux (rate of penetration) is not a directly measurable value, such as concentration, but rather is a time-averaged value determined across a sampling period. The rate of percutaneous absorption showed rapid penetration to a peak flux for PLO-Diclogel, occurring approximately one hour after dose application, but also a secondary peak at approximately nine hours and third peak at 18 hours, possibly due to a depot of some of the applied dose in the epidermis. In contrast, PLO showed only a gradual rise in rate of penetration, reaching maximum values at a time point of 24 hours. Mass accountabilities ranged from 90% to 100% of the applied doses across all test formulations.

Conclusions: This *in vitro* study was conducted to evaluate the performance of two topical compounding vehicles containing 10% diclofenac sodium when applied to *ex vivo* human skin, using the Franz finite dose model, which has been demonstrated to correlate with clinical study results.

The data demonstrates that diclofenac sodium 10% penetrated into and through human skin, from both of the compounding vehicles evaluated. The rate of absorption and the total percentage permeation is consistently higher with PLO-Diclogel than PLO, which translates to quicker onset and potentially increased efficacy.

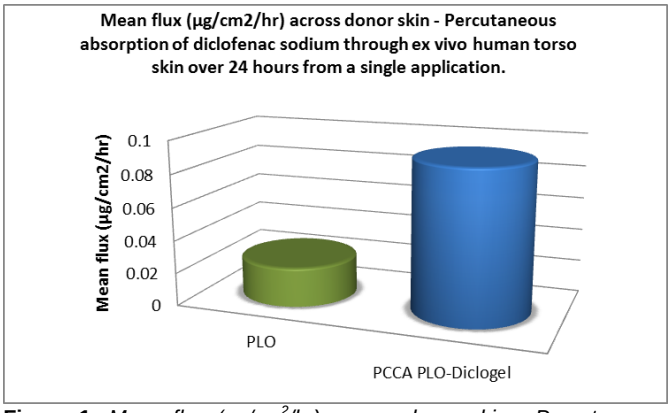

Figure 1: *Mean flux ($\mu g/cm^2/hr$) across donor skin - Percutaneous absorption of diclofenac sodium through ex vivo human torso skin over 24 hours from a single application. (Mean ± SD, n= 3 Donor)*

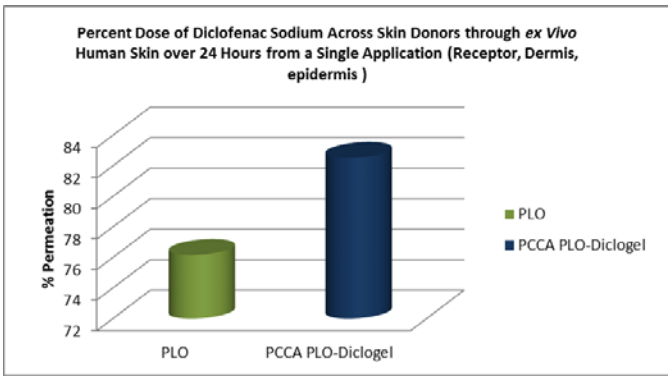

Figure 2: *Total percentage permeation of applied dose beyond stratum corneum after a single dose application (receptor, dermis, epidermis and stratum corneum).*

References:

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2. Franz, TJ: The cadaver skin absorption model and the drug development process. Pharmacopeial Forum 34 (5): 1349-1356, 2008.
3. Franz, TJ, Lehman PA, Raney S: Use of excised human skin to assess the bioequivalence of topical products. Skin Pharmacol Physiol 22:276- 286, 2009