



Ketoprofen Transdermal Study

Ketoprofen in PCCA Lipoderm® Outperforms PLO in Transdermal Testing!

PCCA Lipoderm® now PROVEN to Deliver the NSAID Ketoprofen Through Human Skin *In Vitro*



As part of an ongoing effort to create the highest quality products, PCCA has aggressively studied the ability of Lipoderm® and PLO to deliver ketoprofen across human skin. PCCA teamed up with the highly regarded dermatologic laboratory **PRACS Institute – Cetero Research** to conduct this study. Using PCCA's Special Micronized Ketoprofen USP, PCCA Lipoderm performed better than PLO.

Study

Evaluation of the Percutaneous Absorption of Ketoprofen, *In Vitro*, Using the Human Cadaver (*Ex Vivo*) Skin Model

The study was designed to evaluate the percutaneous absorption pharmacokinetics of PCCA's Special Micronized Ketoprofen. Absorption was measured in human cadaver skin, *in vitro*, using the finite dose technique and Franz Diffusion Cells.

The products were tested on replicate sections from three different cadaver skin donors, for the percutaneous absorption of PCCA's Special Micronized Ketoprofen over a 48-hour dose period. At pre-selected times after dose application, the dermal receptor solution was removed in its entirety, replaced with fresh receptor solution, and an aliquot saved for subsequent analysis. In addition, the epidermis and dermis were recovered and evaluated for drug content. The samples were analyzed for ketoprofen content by High Performance Liquid Chromatography (HPLC).

The *in vitro* human cadaver skin model has proven to be a valuable tool for the study of percutaneous absorption and the determination of the pharmacokinetics of topically

applied drugs. The model uses human cadaver skin mounted in specially designed diffusion cells that allow the skin to be maintained at a temperature and humidity that match typical *in vivo* conditions. A finite dose of formulation is applied to the outer surface of the skin and drug absorption is measured by monitoring its rate of appearance in the receptor solution bathing the inner surface of the skin. Data defining total absorption, rate of absorption, as well as skin content can be accurately determined in this model. The method has historic precedent for accurately predicting *in vivo* percutaneous absorption kinetics.

Results

The data indicate that PCCA's Special Micronized Ketoprofen did penetrate into and through human cadaver skin, *in vitro*, from the test formulations provided. The absorption profiles indicate a rapid penetration to a peak flux occurring at approximately 7-8 hours after dose application followed by a steady decline thereafter. PCCA Lipoderm performed significantly better than PLO at delivering PCCA's Special

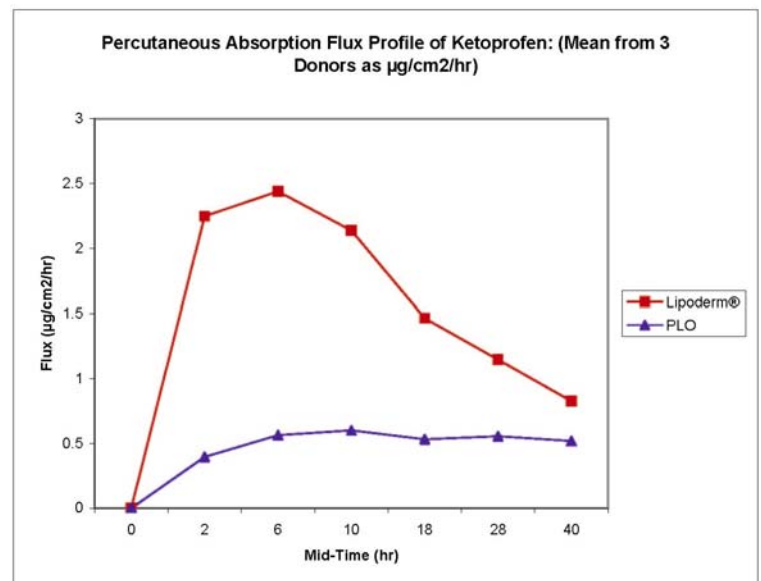


Figure 1: PCCA Lipoderm® demonstrates a superior ability to deliver ketoprofen transdermally versus PLO.



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continued

Micronized Ketoprofen through human skin (see **Figure 1**). This formulation also delivered PCCA's Special Micronized Ketoprofen more rapidly than all other formulations. Lipoderm with pentylene glycol as the wetting agent showed an even better total permeation result versus PLO, with the difference being statistically significant ($p < 0.01$, see **Figure 2**). While this formulation delivered the most ketoprofen across the skin (total absorption), it was not as quick as the other Lipoderm formula.

Methods and Procedures

Percutaneous absorption was measured using the *in vitro* cadaver skin finite dose technique. Human cadaver trunk skin without obvious signs of skin disease, obtained within ~24-48 hours of death, was used in this study. It was dermatomed, prepared for cryopreservation, sealed in a water impermeable plastic bag, and stored at $< -70^{\circ}\text{C}$ until the day of the experiment. Prior to use, it was thawed in $\sim 37^{\circ}\text{C}$ water, then rinsed in tap water to remove any adherent blood or other material from the surface.

Skin from a single donor was cut into multiple smaller sections large enough to fit on static 1.0 cm^2 Franz diffusion cells. The dermal chamber was filled to capacity with a reservoir solution of phosphate-buffered isotonic saline (PBS), pH 7.4 ± 0.1 , and the epidermal cell (chimney) left open to ambient laboratory conditions. All cells were mounted in a diffusion apparatus in which the dermal bathing solution was stirred magnetically at approximately 600 RPM and the skin surface temperature maintained at $32.0^{\circ} \pm 1.0^{\circ}\text{C}$.

To assure the integrity of each skin section, its permeability to tritiated water was determined before application of the test products. Following a brief (0.5-1 hour) equilibrium period, $^3\text{H}_2\text{O}$ (NEN, Boston, MA, sp. Act. $\sim 0.5\ \mu\text{Ci}/\text{mL}$) was layered across the top of the skin by dropper so that the entire exposed surface was covered (approximately 200-500 μL). After 5 minutes, the $^3\text{H}_2\text{O}$ aqueous layer was removed. At 30 minutes, the receptor solution was collected and analyzed for radioactive content by liquid scintillation counting. Skin specimens in which absorption of $^3\text{H}_2\text{O}$ was less than $1.56\ \mu\text{L-equ}/\text{cm}^2$ were considered acceptable.

Just prior to dosing, the reservoir solution was replaced with a fresh solution of 1x PBS and an aliquot retained for subsequent analysis (pre-dose sample). The chimney was removed from the Franz Cell to allow full access to the epidermal surface of the skin. All formulations were then applied to the skin sections using a positive displacement pipette set to deliver $5\ \mu\text{L}$ formulation/ cm^2 . The dose was spread across the surface with the Teflon® tip of the pipette. At pre-selected times after dosing (4, 8, 12, 24, 32, and 48 hours), the reservoir solution was removed in its entirety, replaced with fresh reservoir solution, and an aliquot saved for subsequent analysis.



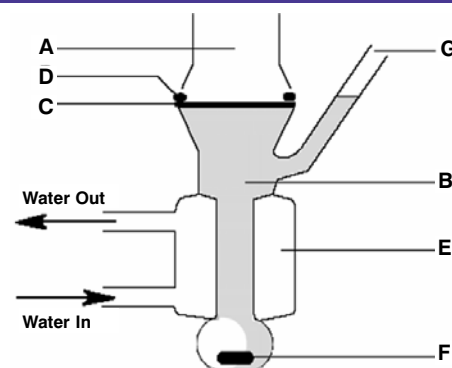
PCCA

compound with confidence®

DONOR DEMOGRAPHICS				
DONOR ID	AGE	RACE	SEX	INTEGRITY TEST RESULT*
GN112705	55	Caucasian	Male	0.45 ± 0.13
IS122705	64	Caucasian	Male	0.24 ± 0.17
OV071007	59	Hispanic	Male	0.49 ± 0.34

* Results are reported as $\mu\text{L-equ } ^3\text{H}_2\text{O}$; Acceptance $\leq 1.56\ \mu\text{L-equ}/\text{cm}^2$

FRANZ DIFFUSION CELL



- A. Chamber Chimney (open to environment)
- B. Receptor Solution Compartment (4.5-6.5 mL)
- C. O-ring seal
- D. Skin (1.0 cm^2)
- E. Heated Water Jacket
- F. Magnetic Stir Bar
- G. Sampling Port

In each study, if spare cells were available they were not dosed but used to evaluate for the appearance of substances diffusing out of the skin that might interfere with the analytic method.



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continued

After the last sample was collected, the skin surface was washed twice (0.5 mL volume each) with equal parts ethanol and water to collect un-absorbed formulation from the surface of the skin. Following the wash, the skin was removed from the chamber, split into epidermis and dermis, and were extracted overnight in equal parts ethanol and water.

Samples were either concentrated or diluted as necessary to fit within the standard curve range prior to analysis. Quantification of PCCA's Special Micronized Ketoprofen was by High Performance Liquid Chromatography (HPLC/UV). Briefly, HPLC was conducted on a Hewlett-Packard 1100 Series HPLC system with an Agilent 1100 Series LC with a diode array detector.

Formulas Tested

Lipoderm® Formula

Ketoprofen USP, PCCA Special Micronized	10% W/W
Diethylene Glycol Mono Ethyl Ether, NF	2% W/W
Propylene Glycol	8% W/W
PCCA Lipoderm®	q.s. to 100%

PLO Formula

Ketoprofen USP, PCCA Special Micronized	10% W/W
Diethylene Glycol Mono Ethyl Ether, NF	2% W/W
Propylene Glycol	8% W/W
Lecithin Isopropyl Palmitate Solution	22% W/W
Poloxamer 407 20% Solution	q.s. to 100%

A third formula, using Pentylene Glycol as a wetting agent, also was evaluated:

Lipoderm® Formula with Pentylene Glycol

Ketoprofen USP, PCCA Special Micronized	10% W/W
Pentylene Glycol	10% W/W
PCCA Lipoderm®	q.s. to 100%

PCCA PRODUCT INFORMATION

PCCA Lipoderm® PCCA #30-3338

Ketoprofen USP,
PCCA Special Micronized PCCA #50-3849

Pentylene Glycol PCCA #30-3811

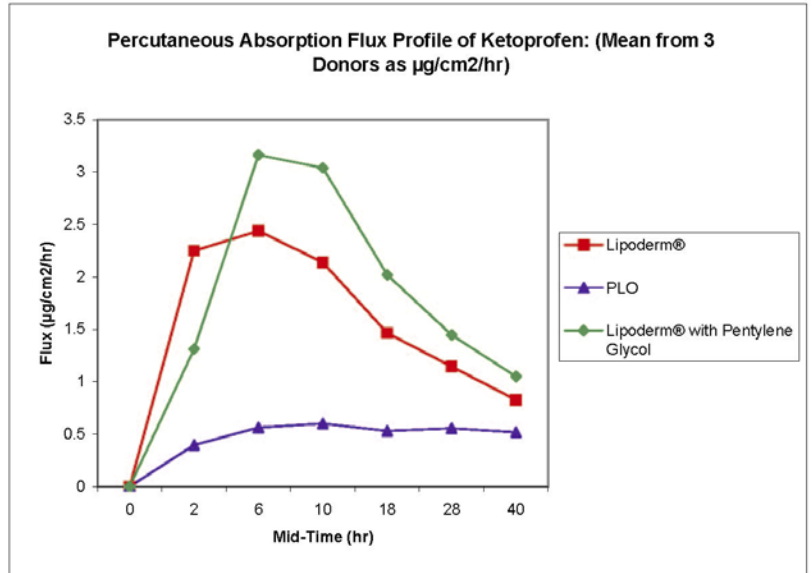


Figure 2: The use of pentylene glycol 10% in PCCA Lipoderm® as wetting agent increases extent of percutaneous absorption, but is a little slower.

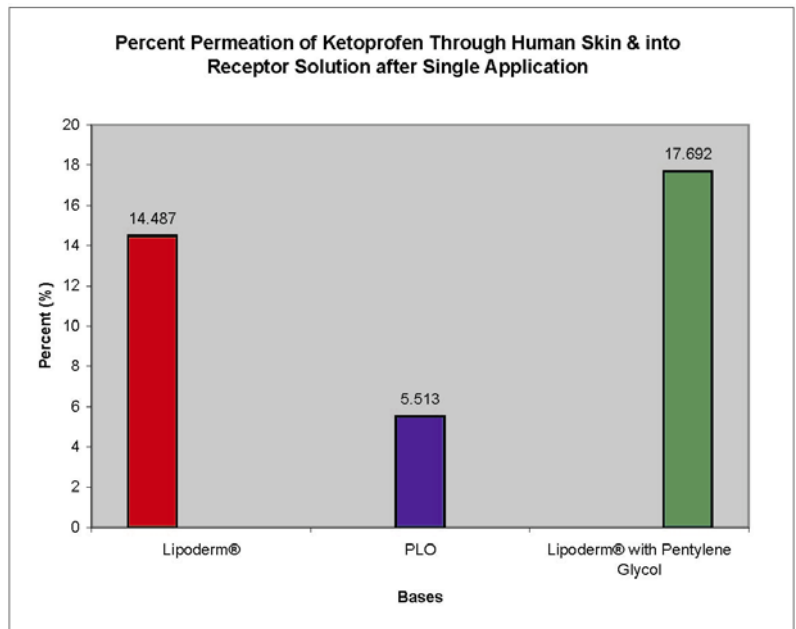


Figure 3: Percent of applied ketoprofen dose that was delivered completely through human skin *in vitro* was significantly better with PCCA Lipoderm® versus PLO.

