STUDIES
PCCA LIPODERM® FAMILY BASE STUDIES

Check Out the NEW Lorazepam in Lipoderm Study!
PCCA Lipoderm® Study
A Validated Transdermal Vehicle With Superior Results Over PLO

PCCA is dedicated to providing the very best products for your patients. PCCA is the only company that has a PROVEN transdermal delivery vehicle on the market today. Promethazine HCl in PCCA Lipoderm base was successfully transported through human skin, in vitro, and performed better than PLO.

Study Highlights
Evaluation of the percutaneous absorption of promethazine Hcl, in vitro, using the human cadaver skin model (Study performed by PRACS Institute, Ltd., an independent contract research facility.)

The study was designed to evaluate the percutaneous absorption pharmacokinetics of Promethazine HCl in PCCA Lipoderm versus PLO (Pluronic Lecithin Organogel). Absorption was measured in human cadaver skin, in vitro, using the finite dose technique and Franz Diffusion Cells.

The products were tested on a minimum of triplicate sections from three different cadaver skin donors, for the percutaneous absorption of Promethazine HCl 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 Promethazine HCl content by High Performance Liquid Chromatography (HPLC-UV-MS).

All samples contained Promethazine HCl 25 MG/GM, 1.6% W/W Vitamin E Acetate, 0.2% BHT and 0.2% EDTA for stability purposes. 10% W/W Pentylene Glycol was used as a wetting agent/solvent for the Promethazine HCl. When PCCA studied the ability of PCCA Lipoderm to transport drugs through the skin, a molecule was intentionally chosen that would have difficulty penetrating the skin. Promethazine HCl, due to its molecular weight and polarity, presents a challenge in delivery across the skin.

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 cryo-preservation, sealed in a water impermeable plastic bag, and stored at ≤ -70°C until the day of the experiment. Prior to use it was thawed in ~37°C water, then rinsed in water to remove any adherent blood or other material from the surface.

### Donor Demographics

<table>
<thead>
<tr>
<th>DONOR ID</th>
<th>AGE</th>
<th>RACE</th>
<th>SEX</th>
<th>INTEGRITY TEST RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>RN****</td>
<td>63</td>
<td>Caucasian</td>
<td>Male</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>LS****</td>
<td>71</td>
<td>Caucasian</td>
<td>Male</td>
<td>0.57 ± 0.14</td>
</tr>
<tr>
<td>FB****</td>
<td>65</td>
<td>Caucasian</td>
<td>Male</td>
<td>0.33 ± 0.07</td>
</tr>
</tbody>
</table>

The FIRST Transdermal Base in the Compounding Profession to Undergo Rigorous Transdermal Testing!

Accept No Substitutes!
This study was conducted using PCCA Lipoderm!
Skin from a single donor was cut into multiple smaller sections large enough to fit on static 1.0 cm$^2$ Franz diffusion cells. 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° ± 1.0°C. To assure the integrity of each skin section, its permeability to tritiated water was determined before application of the test products.

All formulations were then applied to the skin sections using a positive displacement pipette set to deliver 5 µL 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 a predetermined volume aliquot saved for subsequent analysis.

**Results**

The data indicate that Promethazine HCl does penetrate into and through human cadaver skin in vitro. Based on total penetration (through the skin into the reservoir solution), the data rank orders the test formulations as: PCCA Lipoderm® > PLO

**PCCA #30-3338**

Epidermal levels were found to be greater for PCCA Lipoderm® formulations (16.8 – 22.8 µg) than the other formulations (8.0 – 10.1 µg).

The percent of applied dose that penetrated past the Stratum Corneum with PCCA Lipoderm® was 2.24 times more than PLO.
### Study Highlights

**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 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.

*Figure 1*: PCCA Lipoderm® demonstrates a superior ability to deliver ketoprofen transdermally versus PLO.
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° C until the day of the experiment. Prior to use, it was thawed in ~37° 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 cm² 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° ± 1.0°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, ³H₂O (NEN, Boston, MA, sp. Act. ~ 0.5 µCi/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 ³H₂O 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 ³H₂O was less than 1.56 µL-equ/cm² 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 µL formulation/cm². 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.

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.

* Results are reported as µL-equ 3H₂O; Acceptance ≤ 1.56 µL-equ/cm²

---

**Ketoprofen Transdermal Study cont’d**

Ketoprofen in PCCA Lipoderm Outperforms PLO in Transdermal Testing!

---

**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 cm²)
E. Heated Water Jacket
F. Magnetic Stir Bar
G. Sampling Port

---

**Donor Demographics**

<table>
<thead>
<tr>
<th>DONOR ID</th>
<th>AGE</th>
<th>RACE</th>
<th>SEX</th>
<th>INTEGRITY TEST RESULT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GN112705</td>
<td>55</td>
<td>Caucasian</td>
<td>Male</td>
<td>0.45 ± 0.13</td>
</tr>
<tr>
<td>IS122705</td>
<td>64</td>
<td>Caucasian</td>
<td>Male</td>
<td>0.24 ± 0.17</td>
</tr>
<tr>
<td>OV071007</td>
<td>59</td>
<td>Hispanic</td>
<td>Male</td>
<td>0.49 ± 0.34</td>
</tr>
</tbody>
</table>

* Results are reported as µL-equ 3H₂O; Acceptance ≤ 1.56 µL-equ/cm²
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%

**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.
Summary
Cetero Research in Fargo, N.D., conducted this study for PCCA, located in Houston, Texas. The study was designed to evaluate the percutaneous absorption pharmacokinetics of Tramadol. Absorption was measured in inner ear feline skin, \textit{in vitro}, using the finite dose technique and Franz Diffusion Cells.

Tramadol 100 MG/GM in Lipoderm was tested on duplicate sections from two different feline inner ear skin donors, for the percutaneous absorption of Tramadol 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 intact skin was recovered and evaluated for drug content. The samples were analyzed for Tramadol content by High Performance Liquid Chromatography (HPLC).

Introduction
The \textit{in vitro} Franz skin finite dose 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 \textit{ex vivo} animal, human cadaver or surgical skin mounted in specially designed diffusion cells that allow the skin to be maintained at a temperature and humidity that match typical \textit{in vivo} conditions.\textsuperscript{1} A finite dose (e.g. 4-7 MG/cm\textsuperscript{2}) 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 \textit{in vivo} percutaneous absorption kinetics.\textsuperscript{2,3}

Objective
To characterize the percutaneous absorption pharmacokinetics of Tramadol in Lipoderm on feline inner ear skin using the \textit{in vitro} finite dose model.

Methods and Procedures

\textbf{Study Skin Preparation:}
Percutaneous absorption was measured using the \textit{in vitro} Franz skin finite dose technique. \textit{Ex vivo}, feline ventral inner ear skin without obvious signs of skin disease was used in this study. The feline ear skin was provided by the study sponsor, via an approved outside laboratory. Prior to use it was thawed in \textsuperscript{\textdegree}C water. The skin was then rinsed in tap water to remove any adherent blood or other material from the surface. The ear skin was transected to separate the ventral (inner surface) from the dorsal aspect of the ear. Any subcutaneous and cartilage tissue, if present, was removed during transection.

Skin from a single donor was cut into multiple smaller sections large enough to fit on nominal 0.8 cm\textsuperscript{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\textsuperscript{\textdegree} ± 1.0\textsuperscript{\textdegree}C.

![Franz Diffusion Cell Diagram]

A. Chamber Chimney (open to environment)
B. Skin (nominal 1.0 or 2.0 cm\textsuperscript{2})
C. O-ring Seal
D. Sampling Port
E. Receptor Solution Compartment
F. Water Jacket
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, $^3$H$_2$O (NEN, Boston, MA, sp. Act. ~ 0.5 µCi/ML) was layered across the top of the skin so that the entire exposed surface was covered (approximately 250 - 500 µL). After 5 minutes the $^3$H$_2$O aqueous layer was removed. At 30 minutes the receptor solution was collected and analyzed for radioactive content by liquid scintillation counting.

Just prior to dosing, a pre-dose sample was taken and the reservoir solution was replaced with a fresh solution of 0.1x PBS with 0.1% Volpo. 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 µL formulation/cm$^2$. The dose was spread across the surface with a glass rod. Five to ten minutes after application the chimney portion of the Franz Cell was replaced. At pre-selected times after dosing (2, 4, 8, 12, 24, 32, and 48 hours) the reservoir solution was removed in its entirety, replaced with fresh reservoir solution, and a predetermined volume aliquot saved for subsequent analysis.

After the last sample was collected, the surfaces were washed twice (0.5 ML volume each) with 80:20 Ethanol/Water to collect un-absorbed formulation from the surface of the skin. Following the wash, the intact skin was removed from the chamber and extracted in 80:20 Ethanol/Water. Extractions were conducted overnight at room temperature.

**Analytical Methods:**
Quantification of Tramadol was by High Performance Liquid Chromatography (HPLC/UV). Briefly, HPLC was conducted on a Hewlett-Packard 1100 Series HPLC system with a diode array detector. A solvent system consisting of A) 70% water (pH 9.5) with 10 mM Ammonium formate and B) 30% Methanol was run through a Phenomenex Gemini C18 column (3µ, 50 x 3 mm) at a flow rate of 0.4 ML/min.

**Results and Discussion**
The data shows that Tramadol in Lipoderm does penetrate into and through ex vivo feline inner ear skin using the Franz finite dose model. Time course of penetration demonstrated a rapid rise to a peak rate of penetration within 2.5 hours of dose application, followed by a slow decline in flux thereafter. The rapid absorption is most likely attributable to the thin stratum corneum found in feline inner ear skin.

Incredibly, the majority of the applied dose penetrated through the skin into the reservoir solution over the 48-hour study period. Less than 2% of the applied dose was found in the skin, and less than 4% was remaining on the surface. Overall mass balance was very good with approximately 100% of applied dose accounted for in analysis. See chart at right and tables on next page for synopsis.
Table 1 (Below):
Mean Flux (µg/cm²/hr) Results:
Across Donor Summary
Percutaneous Absorption of Tramadol in Lipoderm Through Feline Inner Ear Skin Over 48 Hours from a Single Application. (Mean ± SE, n=2 Donors).

<table>
<thead>
<tr>
<th>Time (hr)*</th>
<th>Tramadol in Lipoderm®</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>13.57 ± 3.194</td>
</tr>
<tr>
<td>3.0</td>
<td>15.40 ± 0.705</td>
</tr>
<tr>
<td>6.0</td>
<td>13.80 ± 0.537</td>
</tr>
<tr>
<td>10.0</td>
<td>14.21 ± 1.731</td>
</tr>
<tr>
<td>18.0</td>
<td>9.795 ± 1.021</td>
</tr>
<tr>
<td>28.0</td>
<td>4.358 ± 1.053</td>
</tr>
<tr>
<td>40.0</td>
<td>1.496 ± 0.207</td>
</tr>
</tbody>
</table>

Table 2 (Below):
Total Absorption and Mass Balance Results Across Skin Donors:
Arithmetic Mean
Percutaneous Absorption and Penetration of Tramadol in Lipoderm Into and Through Intact Feline Inner Ear Skin Over 48 Hours from a Single Application. Mean ± SE as Percent of Applied Dose and Total Mass (µg/cm²).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tramadol in Lipoderm®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Absorption (µg)</td>
<td>277.1 ± 11.39</td>
</tr>
<tr>
<td>Surface Wash (µg)</td>
<td>9.859 ± 0.149</td>
</tr>
<tr>
<td>Skin (µg)</td>
<td>2.966 ± 0.278</td>
</tr>
<tr>
<td>Total Absorption (%)</td>
<td>100.4 ± 3.239</td>
</tr>
<tr>
<td>Surface Wash (%)</td>
<td>3.648 ± 0.138</td>
</tr>
<tr>
<td>Skin (%)</td>
<td>1.092 ± 0.074</td>
</tr>
<tr>
<td>Total Recovery (%)</td>
<td>105.1 ± 3.175</td>
</tr>
</tbody>
</table>

REFERENCES

Study Summary
Evaluation of the Percutaneous Absorption of Ketamine HCl, Gabapentin, Clonidine HCl and Baclofen in Lipoderm® and Lipoderm® ActiveMax™, Into Human Trunk Skin, In Vitro, Using the Franz Skin Finite Dose Model.

The study was designed to evaluate the percutaneous absorption pharmacokinetics of ketamine HCl, gabapentin, clonidine HCl and baclofen. Absorption was measured in human cadaver skin, in vitro, using the finite dose technique and Franz Diffusion Cells. These four drugs were selected due to their frequent use in topical pain formulations. Additionally, it is common for compounders to put multiple actives in a transdermal vehicle, and it is important to know that their vehicle is actually capable of delivering more than one active. This study resoundingly demonstrates that Lipoderm and Lipoderm ActiveMax have the power and reliability compounders are looking for.

The products were tested on replicate sections from three different cadaver skin donors, for the percutaneous absorption of ketamine HCl, gabapentin, clonidine HCl and baclofen 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 ketamine HCl, gabapentin, clonidine HCl and baclofen content by High Performance Liquid Chromatography (HPLC)/MS.

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. Skin from three donors was cut into multiple smaller sections large enough to fit on static 1.0 cm² Franz diffusion cells. To assure the integrity of each skin section, its permeability to tritiated water was determined before application of the test products. All formulations were then applied to the skin sections using a positive displacement pipette set to deliver 5 μL formulation/cm². The dose was spread across the surface with the Teflo® tip of the pipette. At pre-selected times after dosing (2, 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.

Results
The data indicate that PCCA’s Lipoderm and Lipoderm ActiveMax delivered ketamine HCl, gabapentin, clonidine HCl and baclofen, simultaneously (and intact), into and through human cadaver skin, in vitro. The absorption profiles indicate a rapid penetration to a peak flux for gabapentin and baclofen occurring approximately one (1) hour after dose application, and approximately four (4) hours for ketamine HCl. Clonidine HCl exhibited a rapid penetration to an initial peak flux occurring one (1) hour after dose application, but also a secondary peak at approximately 40 hours, possibly due to a depot of some of the applied dose in the epidermis. (See Figures 1 through 5.)

PCCA is proud to offer Lipoderm™ ActiveMax™, a much anticipated line extension of the popular and trusted base Lipoderm®. PCCA engineered a way to improve the active ingredient carrying capacity of Lipoderm, which is often needed when transdermally treating conditions such as neuropathic pain.

When to Use Lipoderm® ActiveMax™
Lipoderm ActiveMax was developed to show resiliency in the presence of high salt loads. For example, 30% Ketamine HCl in Lipoderm ActiveMax demonstrated superior physicochemical attributes, remaining stable and elegant for six (6) months. If a prescriber desires a formulation with active ingredient concentrations exceeding 20% of the total formulation weight, or if you are having separation issues with your current formulations, Lipoderm ActiveMax is your “go-to” base.

When you want the quality that PCCA’s Lipoderm is known for, and need increased stability in the presence of high active percentages...look no further than Lipoderm ActiveMax!
skin. This information is of great value for pharmacists and physicians utilizing topical preparations for various pain syndromes.

**Formulations Tested To Make 100 GM**

**Lipoderm Formula (PCCA Formula #7919)**

- Ketamine HCl USP CIII 5 GM
- Gabapentin USP 10 GM
- Clonidine HCl USP 0.2 GM
- Baclofen USP 2 GM
- Propylene Glycol USP 10 GM
- Base, PCCA Lipoderm® q.s. 100 GM

**Lipoderm ActiveMax™ Formula**

- Ketamine HCl USP CIII 5 GM
- Gabapentin USP 10 GM
- Clonidine HCl USP 0.2 GM
- Baclofen USP 2 GM
- Propylene Glycol USP 10 GM
- Base, PCCA Lipoderm® ActiveMax™ q.s. 100 GM

---

**Figure 2: Ketamine Flux versus Time**

**Figure 3: Gabapentin Flux versus Time**
PCCA Lipoderm®/Lipoderm® ActiveMax™

PCCA Lipoderm Breakthrough Study cont’d
Lipoderm and Lipoderm ActiveMax Proven to Deliver Four Drugs Simultaneously Through Human Skin In Vitro.

Figure 4: Baclofen Flux versus Time

Figure 5: Clonidine Flux versus Time

WOW
The WOW Factor

PCCA answers more than 500 calls a day about preparing medicine for patients.
**PCCA Lipoderm/Lorazepam Study**

**Lipoderm and Lipoderm ActiveMax Now Proven to Deliver Lorazepam Into and Through Human Skin**

**Summary**

Cetero Research in Fargo, North Dakota, conducted this study for PCCA, located in Houston, Texas. The study was designed to characterize the percutaneous absorption of Lorazepam from Lipoderm and Lipoderm ActiveMax. Absorption was measured in ex vivo human torso skin in vitro, using the finite dose technique and static Franz Diffusion Cells.

Each formulation was evaluated on 3 replicate sections from 3 ex vivo human torso skin donors. The percutaneous absorption of Lorazepam was determined over a 48-hour period with receptor solution samples collection at 0 hour (pre-dose) and 2, 4, 8, 12, 24, 32 and 48 hours. In addition, the glass rod used for dosing, surface wash, stratum corneum, epidermis and dermis were recovered and assessed for drug content. The samples were analyzed for Lorazepam content using a High Performance Liquid Chromatography (HPLC) analytical method developed by Cetero Research Pre-Clinical Dermatology Laboratory.

**Introduction**

The in vitro Franz human skin finite dose 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 ex vivo human torso skin mounted in specially designed diffusion chambers allowing the skin to be maintained at a temperature and humidity that match typical in vivo conditions. A finite dose (for example, 2 mg/cm² – 10 mg/cm²) 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.

**Objective**

To characterize the percutaneous absorption of Lorazepam from Lipoderm and Lipoderm ActiveMax into and through human torso skin using the Franz Finite Dose In Vitro Permeation Test (IVPT) Model.

**Methods and Procedures**

**Study Skin Preparation:**

Percutaneous absorption was measured using the in vitro Franz human skin finite dose technique. Human, ex vivo, torso skin samples without obvious signs of skin disease were used in this study. Dermatomed, cryopreserved skin had been sealed in a water-impermeable bag and stored at ~-20°C or lower until the day of the experiment. Prior to use the skin was thawed in ~37°C water, then rinsed in water to remove any adherent blood or other material from the surface.

The skin was cut into multiple smaller sections large enough to fit on nominal 2.0 cm² diffusion cells. The dermal (receptor) chamber was filled to capacity with a receptor solution of Phosphate Buffered Saline (PBS) with 0.008% Gentamicin (pH 7.4 ± 0.1), and the epidermal chamber (chimney) was left open to ambient laboratory environment. All diffusion cells were mounted in a diffusion apparatus in which the receptor solution was in contact with the underside of the dermis. The dermal bathing solution was stirred magnetically at approximately ~600 RPM with the water jacket temperature controlled to maintain a skin surface temperature at 32.0 ± 1.0°C (surface temperature determined using a non-contact validated surface infrared temperature indicator). The ambient laboratory conditions were controlled for a target range for relative humidity of 35% - 55%, and for a target range for temperature of 21°C ± 4°C.

To ensure the integrity of each skin section, its permeability to tritiated water was determined before application of the test formulations. Following a brief equilibrium period, [3H₂O] (PerkinElmer, Boston, MA, sp. Act. ~ 0.5 μCi/mL) was layered across the top of the skin by repeater pipette so that the entire exposed surface was covered (approximately 500 μL). At 5 minutes after application the [3H₂O] aqueous layer was removed by blotting with laboratory tissue. At 30 minutes after application the receptor solution was collected and analyzed for radioactive content by liquid scintillation counting. Torso skin specimens in which absorption of [3H₂O] were less than 1.56 μL/cm² were considered acceptable. Skin sections that failed the water barrier integrity test may still have been used as non-dosed control diffusion cells if needed. Donor demographics are presented below.

**Donor Demographics:**

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>Age</th>
<th>Race</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC082411</td>
<td>56</td>
<td>Hispanic</td>
<td>Male</td>
</tr>
<tr>
<td>MY051710</td>
<td>65</td>
<td>Hispanic</td>
<td>Female</td>
</tr>
<tr>
<td>JG111808</td>
<td>63</td>
<td>Caucasian</td>
<td>Male</td>
</tr>
</tbody>
</table>
PCCA Lipoderm®/Lorazepam Study cont’d
Lipoderm and Lipoderm ActiveMax Now Proven to Deliver Lorazepam Into and Through Human Skin

Analytical Methods:
Quantification of Lorazepam from study samples was by High Performance Liquid Chromatography (HPLC/UV) from an analytical method developed by the Cetero Research Pre-Clinical Dermatology Laboratory.

For the Receptor Solution and Tape Strips:
Briefly, a gradient solvent system consisting of Solvent A – 0.1% Formic acid in ddH₂O and Solvent B – Methanol was run through a Zorbax Eclipse XDB-C18, 3.5μ (2.1 x 50 mm) column at a flow rate of 0.400 mL/min. Eluting peaks were quantified using a diode array detector (DAD) set at 254 nm referenced to 360 nm.

For the Glass Rod, Surface Wash, Dermis and Epidermis:
Briefly, an isocratic solvent system consisting of 45% Solvent A – 0.1% Formic acid in ddH₂O and 55% Solvent B – Methanol was run through a Zorbax Eclipse XDB-C18, 3.5μ (2.1 x 50 mm) column at a flow rate of 0.400 mL/min. Eluting peaks were quantified using a Mass Spectrometer Detector with a positive polarity with Ions monitored at 321.10 m/z.

Study Methods:
The study consisted of the evaluation of Lorazepam 5 mg/gm in Lipoderm and Lipoderm ActiveMax, dosed to 2 cm² skin sections mounted in static Franz diffusion cells. Prior to administration of the topical test formulations to the skin sections, a pre-dose receptor solution sample was collected and the entire receptor compartment was refilled with 0.1x-Phosphate Buffered Saline (PBS) containing 0.1% Oleth-20 and 0.008% Gentamicin pH (7.4 ± 0.1) receptor solution. The chimney was then temporarily removed from the diffusion cell to allow full access to the epidermal surface of the skin.

Subsequently, each of the formulations was applied to 3 replicate skin sections from the same torso skin donor. One non-dosed control cell, with skin, was included per donor. 5 A nominal volume equivalent of 5 mg formulation/ cm²/skin section was drawn up and dispensed onto the skin surface using a calibrated positive displacement pipette and evenly dispersed and rubbed into the skin surface using a glass rod (new rod for each skin section). Each glass rod was retained and extracted in 80:20 Ethanol:Water for residual formulation. The difference between the pipette application and rod recovery provides the actual amount of test formulation that was applied.

Approximately 5-10 minutes after dose application, the donor compartment (chimney) of the diffusion cell was replaced. At nominal time points 2, 4, 8, 12, 24, 32 and 48 hours, the receptor solution was removed in its entirety, and a 6 mL volume aliquot saved for analysis.

After the last receptor sample was collected, the surface was washed to collect the unabsorbed formulation from the surface of the skin. The washing procedure involved dispensing and refluxing two volumes (1 mL volume each) with 80:20 Ethanol:Water to collect any un-absorbed formulation from the skin surface. Both volumes were combined for each skin section.

Following the surface wash, the skin was tape stripped, up to 10 times, with all tape strips being pooled as a single sample. Tape strip sample were collected using 3M Transpore® surgical tape (manufactured by 3M, St. Paul, MN 55144), and were extracted overnight at room temperature in 3 mL Acetonitrile (ACN).

The skin samples were split into epidermis and dermis and each extracted overnight at room temperature in 1 mL 80:20 Ethanol:Water.

All samples were stored in glass containers and stored at ~-20°C pending analysis. Samples were transferred to LC vials just prior to analyzing.

Formulation:
Lorazepam in an amount necessary to result in a concentration of 5 mg/gm, was added to Lipoderm and Lipoderm ActiveMax, along with 10% propylene glycol as a wetting agent, and mixed with the aid of an electronic mortar and pestle (EMP, 3 minutes at a setting of 7). The formulation was sheared twice using an ointment mill, once at a setting of 2 and once at a setting of 1, then remixed with EMP (1 minute at a setting of 5) to achieve accurate content uniformity. Potency was confirmed through the use of a High Performance Liquid Chromatograph (HPLC) with a photo diode array detector.

Results and Discussion
The rate of percutaneous absorption is presented as the flux of Lorazepam that appears in the receptor solution under the skin. Individual diffusion cell values were calculated then averaged across replicates for a within donor mean. The mean from each donor was averaged to obtain the across donor population (mean). Lorazepam absorption is outlined in Figure 1, below. The distribution of Lorazepam following a dose exposure of 48 hours to ex vivo human skin is presented as a percent of applied dose in figures 2 and 3 on next page.

The data indicates that Lorazepam can penetrate from the test formulations into and through ex vivo human skin using the in vitro Franz finite dose model. Lorazepam’s penetration profile was essentially similar from both formulations (though differing in magnitude) by demonstrating a slow rise in flux to peak at approximately 30 hrs after topical administration.

Topical Lorazepam is commonly prescribed by physicians in settings such as hospice, when the oral route is not viable, to relieve anxiety, restlessness and anticipatory nausea and vomiting. Having data to demonstrate the ability of Lipoderm and Lipoderm Activemax to deliver Lorazepam transdermally is important for physicians who are considering this route of administration. The vehicle being used by the pharmacy making the formulation plays a large and important role in the delivery of the active ingredient. Using a proven methodology will produce better outcomes and generate greater confidence amongst physicians and patients.
PCCA Lipoderm/Lorazepam Study cont’d

Lipoderm and Lipoderm ActiveMax Now Proven to Deliver Lorazepam Into and Through Human Skin

Figure 1: Lorazepam Flux versus time

Figure 2: Percent of applied dose penetrating to the receptor solution and dermis

Figure 3: Distribution of Lorazepam into and through ex vivo Human Torso Skin over 48 hours from a Single Application. Mean ± SE, n=3 Donors, as Percent of Applied Dose and Total Mass (μg/2-cm²)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lorazepam 5 mg/gm in Lipoderm</th>
<th>Lorazepam 5 mg/gm in Lipoderm ActiveMax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor (μg)</td>
<td>4.54 ± 2.36</td>
<td>3.27 ± 1.30</td>
</tr>
<tr>
<td>Dermis (μg)</td>
<td>0.12 ± 0.01</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Epidermis (μg)</td>
<td>1.99 ± 0.70</td>
<td>2.19 ± 0.31</td>
</tr>
<tr>
<td>Stratum corneum (μg)</td>
<td>2.25 ± 0.84</td>
<td>3.03 ± 1.37</td>
</tr>
<tr>
<td>Surface Wash (μg)</td>
<td>45.10 ± 1.92</td>
<td>43.48 ± 1.93</td>
</tr>
<tr>
<td>Receptor (%)</td>
<td>8.38 ± 4.37</td>
<td>6.48 ± 2.56</td>
</tr>
<tr>
<td>Dermis (%)</td>
<td>0.22 ± 0.02</td>
<td>0.33 ± 0.07</td>
</tr>
<tr>
<td>Epidermis (%)</td>
<td>3.65 ± 1.29</td>
<td>4.36 ± 0.61</td>
</tr>
<tr>
<td>Stratum corneum (%)</td>
<td>4.13 ± 1.53</td>
<td>6.04 ± 2.76</td>
</tr>
<tr>
<td>Surface Wash (%)</td>
<td>83.06 ± 3.73</td>
<td>86.43 ± 3.64</td>
</tr>
<tr>
<td>Total Recovery (%)</td>
<td>99.41 ± 2.80</td>
<td>103.64 ± 2.96</td>
</tr>
</tbody>
</table>
PCCA Lipoderm/Lorazepam Study cont’d
Lipoderm and Lipoderm ActiveMax Now Proven to Deliver Lorazepam Into and Through Human Skin

Franz Diffusion Cell

A. Chamber Chimney (open to environment)
B. Skin (nominal 1.0 or 2.0 cm²)
C. O-ring Seal
D. Sampling Port
E. Receptor Solution Compartment
F. Water Jacket

Figure 4: Photograph of a Franz Diffusion Cell

REFERENCES

5. The blank control skin and diffusion cell is sampled and processed in the same manner as a dosed cell to assess for donor specific analytical interference or evidence of cross contamination.
6. As flux (rate of penetration) is not a discrete directly measurable value (such as concentration), but rather is a time-averaged value determined across a sampling period, by convention it is reported at the mid-point of sample collection for that sample period. For example, if a sample is collected at t=8 hr and the next sample at t=12 hr, the flux is calculated as the amount of compound appearing in the receptor solution between 8-hrs and 12-hrs, divided by that time span, 4 hrs, and plotted to the mid-sample time point of 10 hrs (8 hrs + 4hrs/2).

PCCA PRODUCT INFORMATION

PCCA Lipoderm® ......................... PCCA # 30-3338
Lipoderm® ActiveMax™ ................. PCCA # 30-4482

FYI

Did you know?

PCCA has invested more than $7.7 million since 2000 on formula and base development and ensuring formula compatibility with our APIs. We are the only company in our industry that has developed more than 8,000 proprietary formulas for our Members. Not only have we put more than $7.7 million into making sure PCCA chemicals work in our formulas, we’re the only company in the industry who has invested in clinical studies to ensure our transdermal bases deliver the formula efficaciously to the patient.
Eye spy
We inspect all products for damage or tampering when they arrive and again in repacking.

Clean sweep
We exceed industry standards in our production rooms to prevent cross-contamination.

Return to sender
We reject almost 200 chemical lots per year.

Seeing red
We use high-tech infrared fingerprint analysis for chemical identification.

Twice as nice
We double-check all testing at every stage of QA/QC.

Match game
We match: Certificate of Analysis to current USP standards, manufacturer to our approved list, and product to its repacking label.

A matter of degree
Chemicals are tested only by degreed Chemical Analysts.

Making the grade
Testing also includes:
- Ultraviolet-visible analysis
- Melting point
- Specific gravity
- Solubility

We're Certifiable
FDA, DEA, State of Texas and Good Manufacturing Practices (cGMP)