

# Evaluation of the Percutaneous Absorption of Tramadol, In Lipoderm<sup>®</sup>, Into Inner Ear Feline Skin, *In Vitro*, Using the Franz Skin Finite Dose Model

Lipoderm<sup>®</sup> Performs Well in Feline Inner Ear Test

Study Summary Report  
Study Sponsor: PCCA, Inc., Houston, Texas

## Summary

Cetero Research in Fargo, North Dakota, 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, *in vitro*, using the finite dose technique and Franz Diffusion Cells.

Tramadol 100mg/gm in Lipoderm<sup>®</sup> 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 *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 *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 *in vivo* conditions.<sup>1</sup> A finite dose (e.g. 4-7 mg/cm<sup>2</sup>) 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.<sup>23</sup>

## Objective

To characterize the percutaneous absorption pharmacokinetics of Tramadol in Lipoderm<sup>®</sup> on feline inner ear skin using the *in vitro* finite dose model.

---

<sup>1</sup> Franz, TJ: Percutaneous absorption: on the relevance of *in vitro* data. *J Invest Dermatol*, 1975, 64:190-195.

<sup>2</sup> Franz TJ: The cadaver skin absorption mode and the drug development process. *Pharmacoepial Forum* 34(5): 1349-1356, 2008.

<sup>3</sup> 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

## **Methods and Procedures**

### *Study Skin Preparation:*

Percutaneous absorption was measured using the *in vitro* Franz skin finite dose technique. *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 ~ -37°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<sup>2</sup> 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.<sup>4</sup> Following a brief (0.5-1 hour) equilibrium period, <sup>3</sup>H<sub>2</sub>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 <sup>3</sup>H<sub>2</sub>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<sup>2</sup>. 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

---

<sup>4</sup> Franz TJ, Lehman PA: The use of water permeability as a means of validation for skin integrity in *in vitro* percutaneous absorption studies. *Abst. J Invest Dermatol* 1990, 94:525.

extracted in 80:20 Ethanol:Water. Extractions were conducted over night 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 (3  $\mu$ , 50 x 3 mm) at a flow rate of 0.4 mL/min.

### *Donor Demographics*

<b>Donor ID</b>	<b>Animal</b>	<b>Sex</b>	<b>Integrity Test Result*</b>
C4625	Domestic Short Hair Feline	Female	2.14 $\pm$ 2.28
C4628	Domestic Short Hair Feline	Male	0.94 $\pm$ 0.33

\*Results are reported as  $\mu$ L-equ  $^3$ H<sub>2</sub>O

### *Formulation*

Tramadol HCl, in an amount necessary to result in a concentration of 100mg/gm, was added to Lipoderm<sup>®</sup> along with 10% propylene glycol as a wetting agent, and mixed with the aid of an electronic mortar and pestle (EMP). The formulation was sheared using an ointment mill and remixed with EMP to achieve accurate content uniformity. Potency was confirmed through the use of a High Performance Liquid Chromatograph (HPLC) with a photo diode array detector. It should be noted that the density of the final formulation was 0.726gm/mL, thus the final tramadol HCl concentration in weight/volume terms was 72.6mg/mL.

### **Results and Discussion**

The data show that Tramadol in Lipoderm<sup>®</sup> 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 hrs 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.

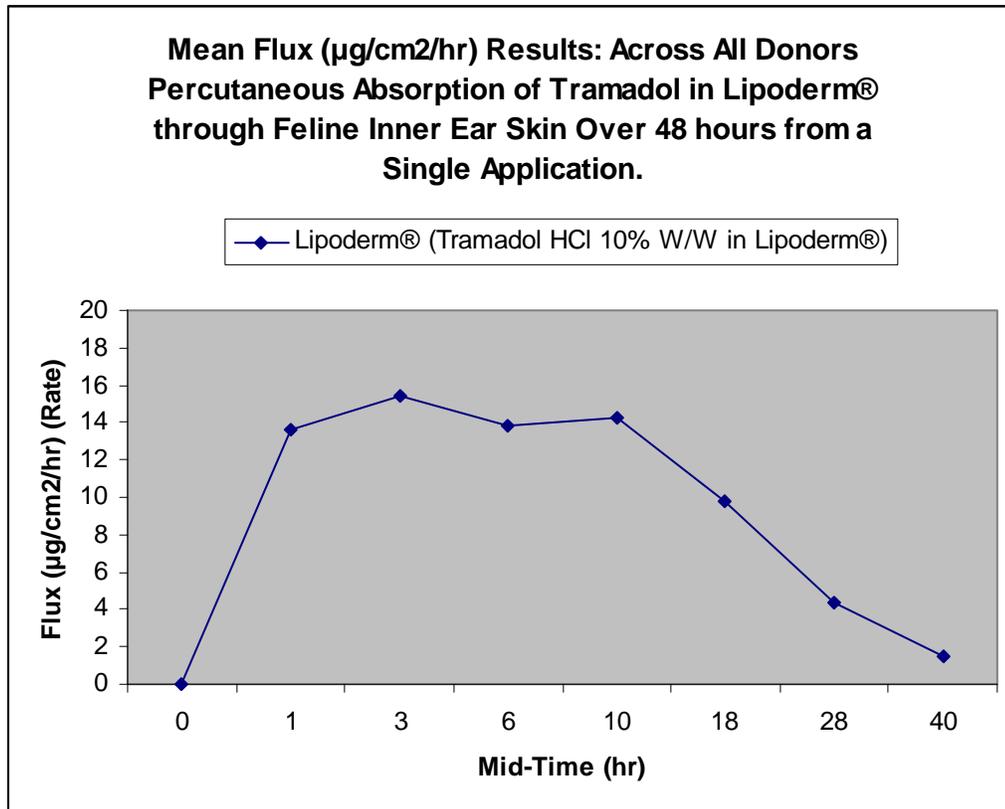
Incredibly, the majority of the applied dose penetrated through the skin into the reservoir solution over the 48 hour study period. Less than 2% of the applied dose was found in the skin, and less than 4% was remaining on the surface. Overall mass balance was very good with approximately 100% of applied dose accounted for in analysis.

See chart and table below for synopsis.

**Table 1: Mean Flux ( $\mu\text{g}/\text{cm}^2/\text{hr}$ ) Results: Across Donor Summary**  
 Percutaneous Absorption of Tramadol in Lipoderm<sup>®</sup> through Feline Inner Ear Skin  
 Over 48 hours from a Single Application (Mean  $\pm$  SE, n=2 Donors).

Time (hr)*	Tramadol in Lipoderm <sup>®</sup>
1.0	13.57 $\pm$ 3.194
3.0	15.40 $\pm$ 0.705
6.0	13.80 $\pm$ 0.537
10.0	14.21 $\pm$ 1.731
18.0	9.795 $\pm$ 1.021
28.0	4.358 $\pm$ 1.053
40.0	1.496 $\pm$ 0.207

\* Time as midpoint between samples.

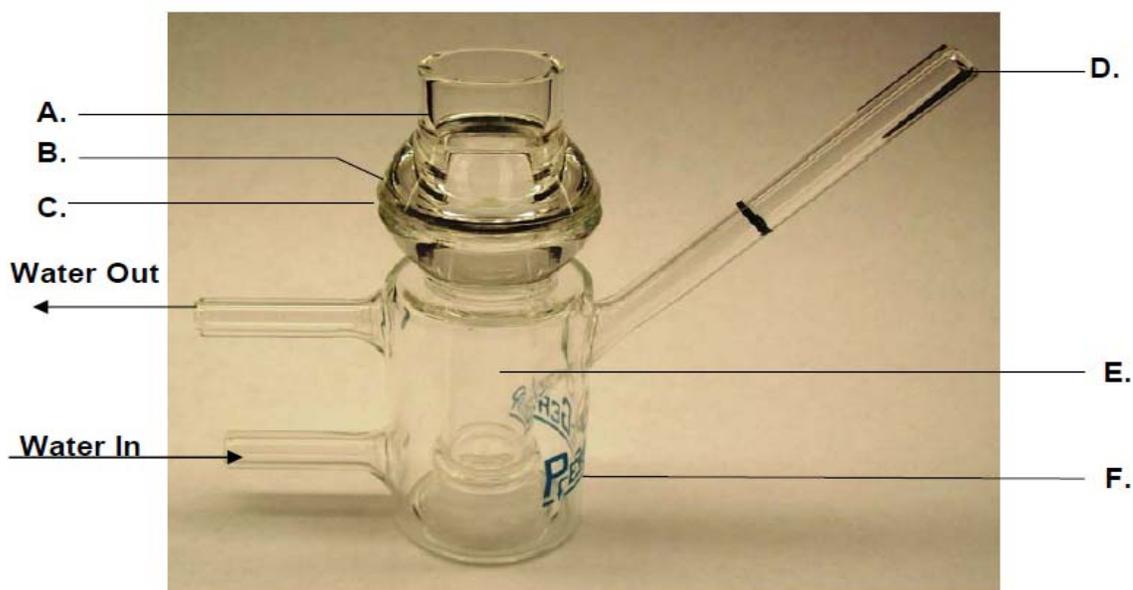


**Table 2: Total Absorption and Mass Balance Results**  
**Across Skin Donors: Arithmetic Mean**

Percutaneous Absorption and Penetration of Tramadol in Lipoderm<sup>®</sup> into and through Intact  
 Feline Inner Ear Skin  
 Over 48 hours from a Single Application.

Mean  $\pm$  SE as Percent of Applied Dose and Total Mass ( $\mu\text{g}/\text{cm}^2$ ).

Parameter	Tramadol in Lipoderm®
Total Absorption ( $\mu\text{g}$ )	277.1 $\pm$ 11.39
Surface Wash ( $\mu\text{g}$ )	9.859 $\pm$ 0.149
Skin ( $\mu\text{g}$ )	2.966 $\pm$ 0.278
Total Absorption (%)	100.4 $\pm$ 3.239
Surface Wash (%)	3.648 $\pm$ 0.138
Skin (%)	1.092 $\pm$ 0.074
Total Recovery (%)	105.1 $\pm$ 3.175



- A. Chamber Chimney (open to environment)
- B. Skin (nominal 1.0 or 2.0  $\text{cm}^2$ )
- C. O-ring seal
- D. Sampling Port
- E. Receptor Solution Compartment
- F. Water Jacket