# Evaluation of the Absorption of a Sublingual Sermorelin Acetate and Ketamine HCl Compounded Formulations in SubOral™ SL HMW using the Caco-2 *In Vitro* Cell Line Model

**Tom Babineau[1], Ajay Uppal CPhT[1], Matt Wlodyga Pharm. D.[2]**

**1** SpecializedRx, Circle Pines, Minnesota, USA **2** Chet Johnson Drug, Amery, Wisconsin, USA

## Overview

Sublingual peptide permeation relies on transport across the dense, highly vascularized epithelial tissue beneath the tongue. With the consideration that native peptides are large, hydrophilic chains, they face major physiological barriers—including salivary washout, enzymatic degradation by peptidases, and tight cellular junctions—that strictly limit passive diffusion into the bloodstream. To address this limitation, a novel compounded sublingual formulation—SubOral™ SL HMW—has been developed. In vitro tissue models demonstrate that the patent-pending buccal delivery vehicle, SubOral™ SL HMW, successfully delivers the peptide into and through human tissues, offering a promising alternative delivery route when compared to and extremely low gastrointestinal absorption of peptides in oral tablets (less than 1% per the labeling for Rybelsus®)[1] and anxiety, pain, and fear associated with subcutaneous injections.

## Introduction

The human intestinal carcinoma cell line, Caco-2, is a widely utilized in vitro model for evaluating the absorption, transport mechanisms, and bioactivities—including antioxidant, anticancer, and anti-inflammatory properties—of drugs and functional therapeutic ingredients. These cells structurally and functionally mimic human small intestinal enterocytes, expressing microvillar brush borders, tight junctions, and phase III efflux and uptake transporters that dictate systemic bioavailability. Monolayer integrity is verified via transepithelial electrical resistance (TEER), typically maintained between 200 and 1000 Ω • cm2. Notably, specific functional formulations can modulate tissue permeability by regulating tight junction paracellularly as well as crossing cell membranes transcellularly, thereby utilizing multiple permeability mechanisms to help improve payload bioavailability. This Caco-2 cell line study was designed to demonstrate the permeability coefficient (Papp) of the 29 amino acid peptide amide, Sermorelin Acetate ($C_{149}H_{246}N_{44}O_{42}S$ • $xC_2H_4O_2$ - MW ~3,418 g/mol) and Ketamine HCl ($C_{13}H_{16}ClNO$ • HCl – MW 274.19 g/mol) in the sublingual vehicle, SubOral™ SL HMW, and utilize dimethyl sulfoxide (DMSO) as the comparative control solvent.

## Methodology

The Caco-2 permeability assay is an in vitro method performed by Eurofins® North America, using human colon carcinoma cells grown on semipermeable inserts to measure the directional flux of a compound. It predicts oral drug absorption, epithelial transport, and active efflux mechanisms across the intestinal barrier. The compounded formulations of sermorelin acetate (10 µM) and ketamine HCl (10 µM) in SubOral™ SL HMW were selected for evaluation for this in-vitro permeability assay. The vehicle control medium utilized throughout the assay consisted of the potent membrane-penetrating solvent dimethyl sulfoxide (DMSO). Several reference standard compounds were used for control absorption reference with the main goal to validate the assay using high permeable molecules, and low permeability compounds for each group.

**Table 1.** In-Vitro Absorption Reference

| Compound I.D. | Test Concentration | Mean Permeability / Permeability | Mean Recovery / Percent Recovery |
|---|---|---|---|
| Colchicine | 10 µM | 0.4 x $10^{-6}$ cm/s | 75% |
| Labetalol | 10 µM | 8.2 x $10^{-6}$ cm/s | 98% |
| Propranolol | 10 µM | 16.6 x $10^{-6}$ cm/s | 88% |
| Ranitidine | 10 µM | 0.2 x $10^{-6}$ cm/s | 82% |
| Antipyrine | 10 µM | 38.6 x $10^{-6}$ cm/s | 85% |
| Digoxin | 10 µM | 0.4 x $10^{-6}$ cm/s | 61% |
| Nadolol | 10 µM | 0.1 x $10^{-6}$ cm/s | 88% |

The test solutions were evaluated for compound permeability from the permeation rate and compound concentration at t=0h and t=2h.

$$P_{app} = \frac{dQ}{dt} x \frac{1}{A \times C_0}$$

Where:
dQ/dt: amount of product present in the basal (A-B) or apical (B-A) compartment as a function of time (nmol/s).
A: area of transwell ($cm^{app}$).
C0: initial concentration of product applied in the apical (A-B) or basal (B-A) compartment (nmol/ml).

Based on the *in vitro* correlation coefficient, $P_{app}$ values were obtained from Caco-2 barrier studies and were used to define and predict the intestinal cellular permeability of the tested compounds.

## Discussion and Results

Caco-2 permeability and transport assay results show how well test compounds cross the intestinal epithelial barrier, with data evaluated using Apparent Permeability ($P_{app}$) values, Transepithelial Electrical Resistance (TEER), and mass balance recovery calculations. The goal of this study was to assess the Apparent Permeability values of both Sermorelin Acetate and Ketamine Hydrochloride in the sublingual base, SubOral™ SL HMW by SpecializedRx. The Caco-2 cell line is significantly more difficult to penetrate than the human buccal TR146 cell line for paracellularly transported compounds, primarily due to its much tighter intercellular junctions. Consequently, this Caco-2 model serves as a rigorous, high-baseline benchmark; given that the sublingual vehicle SubOral™ SL HMW demonstrated a high apparent permeability coefficient ($P_{app}$) across these restrictive tight junctions, it is highly probable to exhibit even greater permeation within the more permissive, open paracellular spaces of the buccal TR146 cell line model.

**Table 2.**

| Caco-2 Permeability Class | $P_{app}$ Range |
|---|---|
| High Permeability | $P_{app} \geq 10 \times 10^{-6}$ cm/s<br>(Correlates to ($F_a$) ≥ 85% oral absorption in humans) |
| Moderate Permeability | $P_{app} = 1$ to $10 \times 10^{-6}$ cm/s<br>(Correlates to ($F_a$) 50% to 84% oral absorption in humans) |
| Low Permeability | $P_{app} < 1 \times 10^{-6}$ cm/s<br>(Correlates to ($F_a$) < 50% oral absorption in humans) |

(Where $F_a$ = Fractional Oral Absorption)

**Figure 1.** Permeability By Caco-2 Assay

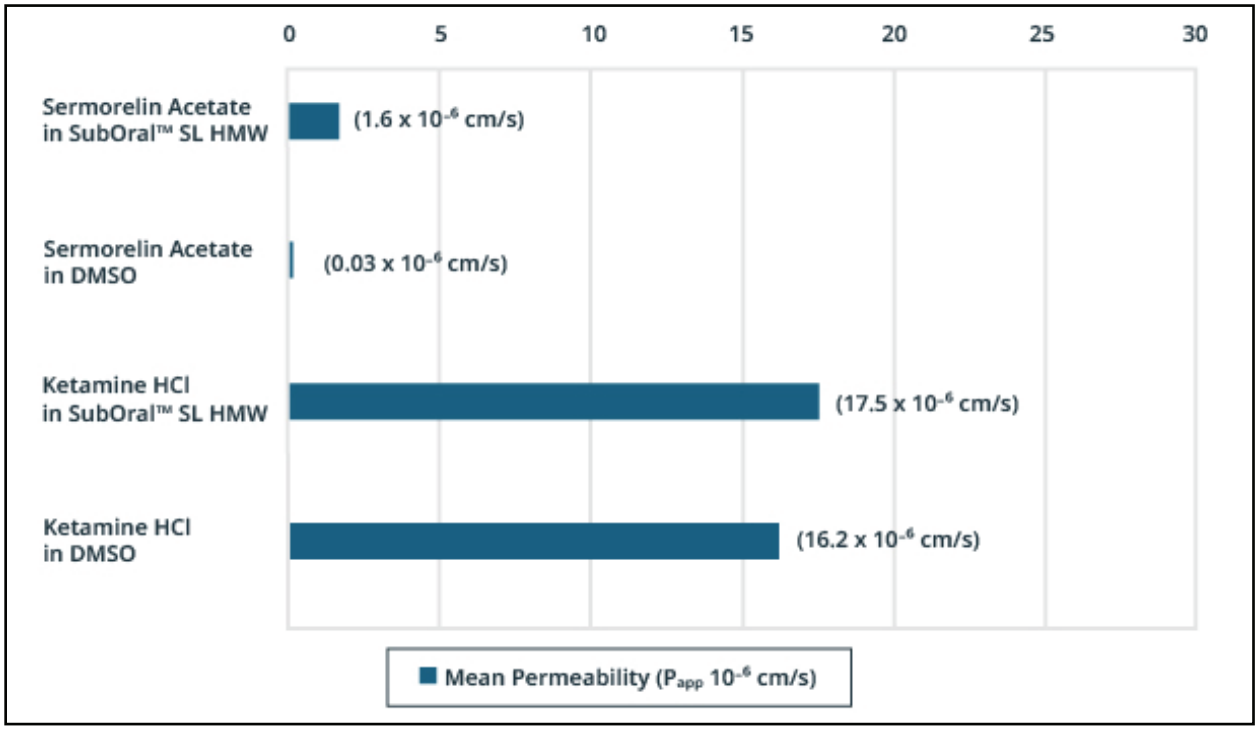



**Table 3.**

| Compound | Results ($P_{app}$) | Permeability Classification |
|---|---|---|
| Sermorelin Acetate in SubOral™ SL HMW (Cool Mint) | $1.6 \times 10^{-6}$ cm/s<br>(Correlates to ($F_a$) = 55% to 65% in humans) | Moderate |
| Sermorelin Acetate in DMSO | $0.03 \times 10^{-6}$ cm/s<br>(Correlates to ($F_a$) < 1% to 5% in humans) | Low |
| Ketamine HCl in SubOral™ SL HMW (Cool Mint) | $17.5 \times 10^{-6}$ cm/s<br>(Correlates to ($F_a$) = 90% to 100% in humans) | High |
| Ketamine HCL in DMSO | $16.2 \times 10^{-6}$ cm/s<br>(Correlates to ($F_a$) = 90% to 100% in humans) | High |

## Conclusion

Sublingual and buccal drug delivery provides a distinct clinical advantage over traditional oral administration by bypassing gastrointestinal degradation and hepatic first-pass clearance. The structural properties of the buccal mucosa—specifically its permissive barrier, neutral pH microenvironment, and low enzymatic density—facilitate efficient peptide transport. Once across the epithelium, therapeutics enter the highly vascularized mucosal tissue, draining directly into the superior vena cava via the systemic venous network [2]. Consequently, the sublingual route presents a compelling strategy to overcome the notoriously low bioavailability seen in commercial oral semaglutide tablets or many patients' preference to not perform self-administered injections. Data from this in vitro study validates that SubOral™ SL HMW achieves robust large molecule peptide and small molecule drug delivery into and through the tighter human intestine tissue as compared to the wider and looser intercellular junctions in human gingival and oral tissues. The Caco-2 cell line is significantly more difficult to penetrate than the human buccal TR146 cell line for paracellularly transported compounds, primarily due to its much tighter intercellular junctions.

In this penetration study, both sermorelin acetate and ketamine HCl formulated in SubOral™ SL HMW demonstrated superior cellular permeation (sermorelin acetate: 5,233% increase, ketamine HCl: 8% increase) compared to the control samples in dimethyl sulfoxide (DMSO), despite the latter's well-established efficiency as a potent biological membrane penetration enhancer.

Therefore, this study confirms that the SubOral™ SL HMW vehicle significantly enhances the in vitro permeability of Sermorelin Acetate and Ketamine HCl, overcoming significant epithelial barriers using a stringent Caco-2 cell model. Results indicate the formulation drives both paracellular and transcellular transport, validating its potential to improve bioavailability for both small molecules and high-molecular-weight peptides, including GLP-1 receptor agonists. Further confirmatory studies are currently underway, please contact your SpecializedRx representative for current study publications.

**References**


1. U.S. Food and Drug Administration. (2022). *Rybelsus (semaglutide) tablets, for oral use [Prescribing Information].* fda.gov
2. Hearnden, V., Sankar, V., Hull, K., Vidović Juras, D., Greenberg, M., Kerr, A. R., Lockhart, P. B., Patton, L. L., Porter, S., & Thornhill, M. H. (2012). New developments and opportunities in oral mucosal drug delivery for local and systemic disease. *Advanced Drug Delivery Reviews*, 64(1), 16–28. doi.org