APPLICATION NOTE

Advancing *in vitro-in vivo* relationships: Steps in establishing an *in vitro* response for GLP-1 receptor agonists

Introduction

Following the major success of Novo Nordisk, many pharmaceutical companies are revisiting their GLP-1 agonist drug pipelines in hopes of launching competitors to the blockbuster weight-loss medications Wegovy® and Ozempic®.[1] Eli Lilly is close behind with its own version, Monjaro, while others are progressing through early testing stages. Bringing a drug to market is a lengthy and complex process, with numerous milestones to navigate. Early steps include lead candidate selection and formulation design, decisions made with *in vitro* data that predict *in vivo* effects. This makes highly accurate and biorelevant *in vitro* assays essential. In this study, we use our SCISSOR (Subcutaneous Injection Site Simulator) platform to assess the peptide GLP-1 analogue Semaglutide—marketed as Wegovy® and Ozempic®—by comparing its *in vitro* behavior with reported *in vivo* performance.

Methods

Two peptide GLP-1 receptor agonists were tested using the subcutaneous injection site simulator (SCISSOR N3™ (Pion Inc.)) (Figure 1),[2, 3] Wegovy® (3.2 mg/mL) and Ozempic® (1.33 mg/mL), both marketed by Novo Nordisk (Figure 2). SCISSOR N3 cartridges contain an artificial extracellular matrix (ECM) that mimics an *in vitro* subcutaneous environment. Each formulation was injected using a hypodermic needle, a bolus formed within the ECM and API diffused through the ECM before releasing through custom dialysis membranes into a biorelevant bicarbonate buffer, simulating systemic uptake of the drug. The assays ran up to 24 hours to compare release profiles. API concentrations were monitored *in situ* using the Rainbow™ UV-Vis fiber optic Pion dip probes.

Figure 1. SCISSOR - Subcutaneous Injection Site Simulator (right), with the Rainbow UV-Vis fiber optic system (left).

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Results: Absorbable fraction

In vivo absolute bioavailability values for GLP-1 were extrapolated from a paper by Overgaard et al.[4] which reported an *in vivo* absolute bioavailability of 84%. When reviewing the release curves generated by the SCISSOR and Rainbow systems, the GLP-1 analogs achieved maximum % Release values comparable to that of Overgaard, with Wegovy® averaging 78.5% and Ozempic® 79.2% (Figure 2). These data suggest that the fraction of semaglutide that was able to leave the injection site was not subject to any significant degradation before reaching the blood stream, a primary concern for GLP-1 analog development, and therefore the maximum % Release value generated by SCISSOR serves as a good indicator of *in vivo* bioavailability in this case.

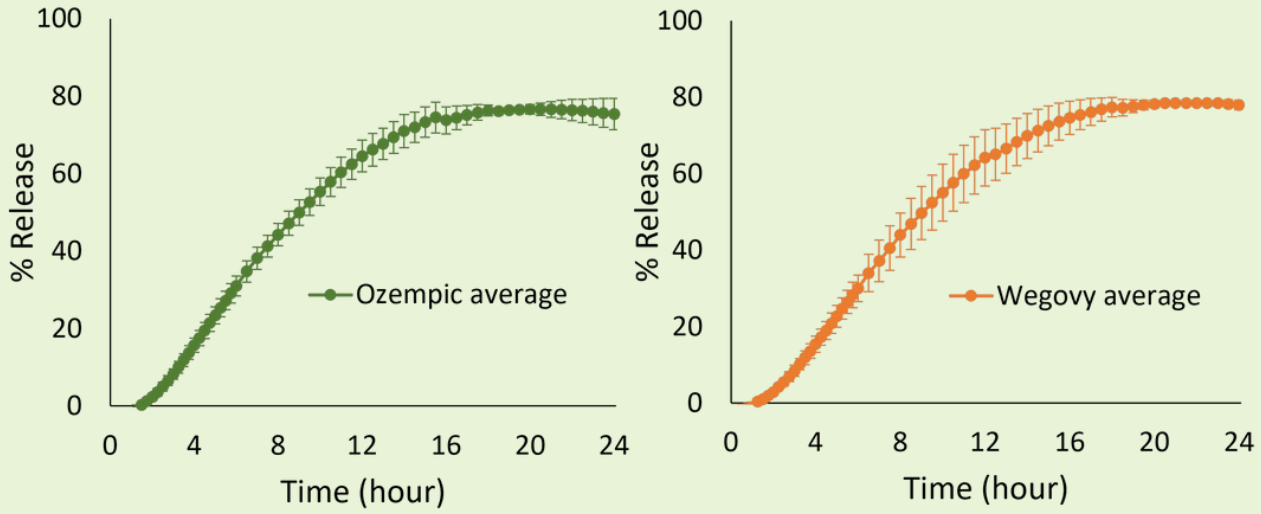

Figure 2: SCISSOR % Release curves generated via Rainbow UV-Vis quantification for Ozempic (left) (n=4), and Wegovy (right)(n=3). It should be noted that evidence of possible sample decomposition was observed during some of the *in vitro* assays run on SCISSOR, which caused the UV data to become unreliable. In these cases, only the portion of data supported with reliable UV analysis is reported.

Results: Absorption rate

To further investigate, the SCISSOR release data was converted to mass (µg) released per unit time. The first derivative of data generated with Rainbow analysis software AuPro® was then used to calculate the average release rate, which was normalized by dividing by the initial mass (figure 3). These calculated release rates from SCISSOR were then compared with the *in vivo* absorption rate constant reported in the literature.[4] Table 1 presents the SCISSOR-derived release rates alongside the literature absorption constants, showing the similarity between those calculated from the SCISSOR vs. reported.

Table 1: Release rate constant calculated for Wegovy® and Ozempic® using the SCISSOR release data as inputs compared against the absorption rate constant referenced in the literature[4].

Sample	Release Rate Constant / Absorption Rate Constant
Wegovy (SCISSOR)	0.0328 h^{-1}
Ozempic (SCISSOR)	0.0310 h^{-1}
GLP-1 (in vivo)	0.0253 h^{-1}

Conclusions

This study demonstrated SCISSOR's capability to effectively evaluate two GLP-1 analogs, Wegovy® and Ozempic®, in an *in vitro* environment that yielded results that closely aligned to *in vivo* data. Specifically, the maximum % Release values generated by SCISSOR and Rainbow were consistent with the *in vivo* absolute bioavailability reported by Overgaard et al. [4]. Together, these findings support the reproducibility and biological relevance of SCISSOR-generated data, reinforcing the platform's value as a predictive *in vitro* tool for assessing subcutaneous drug release of GLP-1 candidates as well as other injectable peptide pharmaceuticals. By providing data that reflects *in vivo* behavior, SCISSOR offers an advanced alternative to early-stage animal testing, helping to bridge the gap between *in vitro* and *in vivo* methodologies and supporting more ethical, efficient, and scientifically robust drug development.

References

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Pion

+1 978.528.2020 | +44 (0) 1342 820720
sales@pion-inc.com
www.pion-inc.com