

Application Note

Improved GLP-1 Receptor Agonist Peptide Recovery Using a QuanRecovery™ With MaxPeak™ High Performance Surfaces (HPS) Collection Plate

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Este é um Resumo de aplicações e, por isso, não inclui uma seção de experimento detalhada.

Abstract

GLP-1 (glucagon-like peptide-1) receptor agonist therapeutics are used to treat metabolic diseases like type 2 diabetes and obesity. However, analyzing them with liquid chromatography-mass spectrometry (LC-MS) can be challenging due to their large size and the hydrophobicity of their fatty acid conjugate moieties. A major issue with these peptides is non-specific binding (NSB), where the peptides interact with and adhere to surfaces or materials used during sample preparation, resulting in peptide loss and unreliable results. In this study, we demonstrate that the use of a Waters QuanRecovery with MaxPeak HPS 700 µL Collection Plate effectively reduces non-specific binding and improves recovery of GLP-1 peptides such as semaglutide, exenatide, liraglutide, and tirzepatide. Ultimately, this is an improvement in analyte sample handling that can help improve assay sensitivity and reliability.

Experimental

A stock solution mix of the GLP-1 (10 µg/mL) receptor agonist therapeutic peptides (Table 1) was prepared from individual peptide solutions (1 mg/mL) of semaglutide, liraglutide, exenatide, and tirzepatide. The organic composition of this stock solution mix was 80/20/1 water/acetonitrile/formic acid. LC-MS analysis samples of the combined peptides were prepared directly in the QuanRecovery with MaxPeak HPS 700 µL Collection Plates or standard polypropylene plates at 1 and 10 ng/mL concentrations in an organic composition of 80/20/1 water/acetonitrile/formic acid. A 4 minute reversed-phase LC analysis was performed using an ACQUITY™ UPLC Peptide CSH C₁₈ Column and ACQUITY UPLC I-Class PLUS System with MS detection on a Xevo™ TQ-XS Tandem Quadrupole Mass Spectrometer operating with multiple reaction monitoring (MRM) scans. Peptide transitions are listed in Table 2. A 10 µL volume of the 1 and 10 ng/mL GLP-1 therapeutic peptide mixes were injected for analysis.

| Pharmaceutical trade name | Active pharmaceutical ingredient | MWT (g/mol) | pI | No. of residues | HPLC index* |
|---------------------------|----------------------------------|-------------|------|-----------------|-------------|
| Ozempic® | Semaglutide | 4113.6 | 5.74 | 31 | 89.7 |
| Rybelsus® | | | | | |
| Wegovy® | | | | | |
| Victoza® | Liraglutide | 3751.2 | 5.01 | 31 | 91 |
| Byetta® | Exenatide | 4186.6 | 4.74 | 39 | 90 |
| Mounjaro® | Tirzepatide | 4810.5 | 4.52 | 39 | 141 |

*Table 1. Physiochemical properties of the GLP-1 receptor agonist therapeutic peptides, illustrating their large, hydrophobic, and acid nature. *higher number=more hydrophobic; †Contains a fatty acid conjugate.*

LC Conditions

UPLC:

ACQUITY UPLC I-Class PLUS System , FL with
Column Manager (CMA)

| | |
|---------------------|---|
| Mobile phase A: | 0.1% Formic acid in water |
| Mobile phase B: | 0.1% Formic acid in acetonitrile |
| Column: | ACQUITY UPLC Peptide CSH C18 Column, 130 Å, 1.7 µm 2.1 mm x 50 mm (p/n: 186006936) |
| Column temperature: | 65 °C |
| Sample temperature: | 10 °C |
| Injection volume: | 5–10 µL |
| Analysis time: | 4 min |
| WNW: | 90:10 Water:Acetonitrile+0.1% Formic Acid |

Gradient Table

| Time (min) | Flow rate (mL/min) | %A | %B | Curve |
|------------|--------------------|------|------|---------|
| Initial | 0.300 | 80.0 | 20.0 | Initial |
| 0.05 | 0.300 | 80.0 | 20.0 | 6 |
| 1.50 | 0.300 | 15.0 | 85.0 | 6 |
| 2.00 | 0.300 | 15.0 | 85.0 | 6 |
| 2.20 | 0.300 | 85.0 | 15.0 | 6 |
| 3.00 | 0.300 | 85.0 | 15.0 | 6 |

MS Conditions

| | |
|-----|------------------------------|
| MS: | Xevo TQ-XS Mass Spectrometer |
|-----|------------------------------|

Capillary (kV): 2

Cone voltage: 32 V

Desolvation temperature: 500 °C

Desolvation flow: 1100 L/Hr

| GLP-1 receptor agonist peptide | Precursor (m/z) | Fragment (m/z) | Collision energy (eV) |
|--------------------------------|-----------------|----------------|-----------------------|
| Semaglutide | 823.3 | 1018.5 | 20 |
| | 1029.3 | 689.9 | 30 |
| | | 1238.1 | 30 |
| Liraglutide | 938.7 | 1064.2 | 22 |
| | | 1128.4 | 35 |
| Exenatide | 838 | 396.0 | 20 |
| | 1605.5 | 948.0 | 10 |
| Tirzepatide | | 396.0 | 50 |
| | 1204.4 | 298.9 | 50 |
| | | 396.0 | 40 |

Table 2. MRM transitions and collision energy for the GLP-1 receptor agonist therapeutic peptides.

Results and Discussion

Minimizes NSB, increases recovery, sensitivity, and repeatability

Mitigation of GLP-1 receptor agonist NSB using QuanRecovery with MaxPeak 700 μ L plates

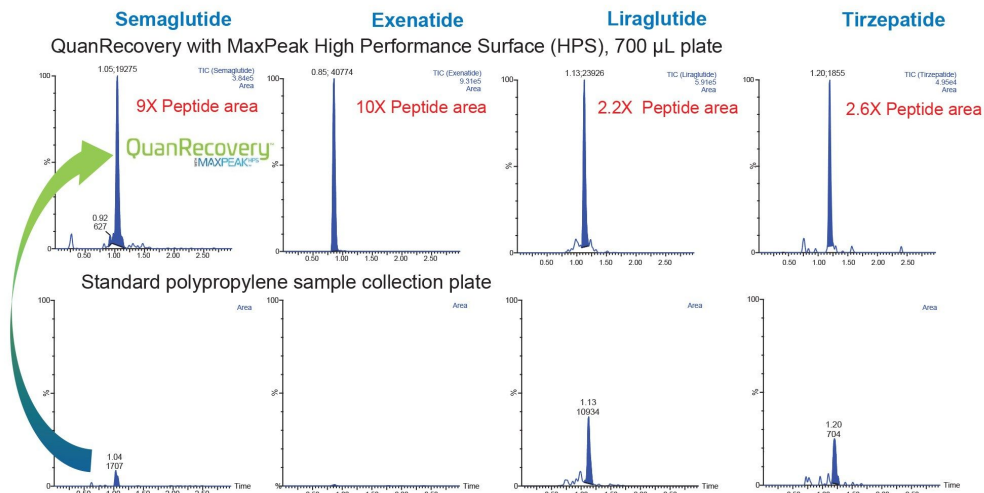


Figure 1. Representative LC-MRM-MS chromatograms demonstrating mitigation of non-specific binding to collection plates for the GLP-1 receptor agonist therapeutic peptides, semaglutide, exenatide, liraglutide, and tirzepatide (1 ng/mL prepared in a solution of 80/20/1 water, acetonitrile, and formic acid) prepared in a QuanRecovery with MaxPeak HPS, 700 μ L Collection Plate vs standard polypropylene sample collection plates.

GLP-1 Receptor agonist NSB mitigation using QuanRecovery with MaxPeak 700 μ L plates vs standard polypropylene plates

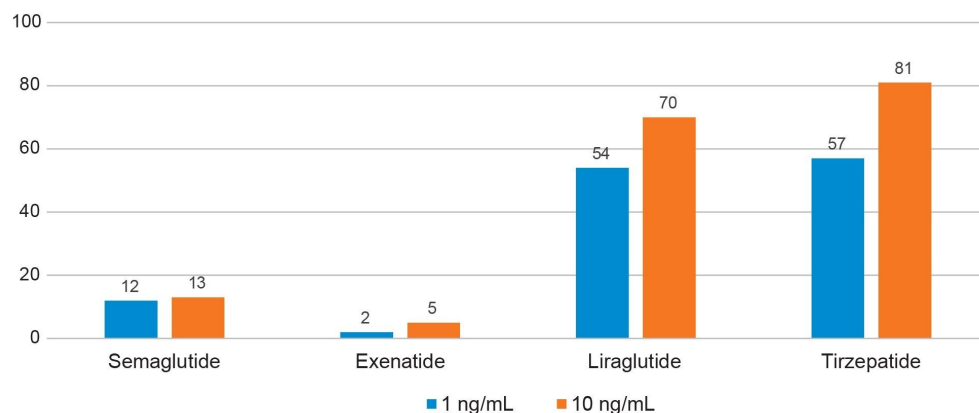


Figure 2. Illustration of improved GLP-1 receptor agonist therapeutic peptide recovery using QuanRecovery with MaxPeak HPS Collection Plates vs standard polypropylene collection plates for 1 and 10 ng/mL mixes (Diluent Composition: 80/20/1 Water/Methanol/Formic Acid. MS response of standard plate was normalized to QuanRecovery with MaxPeak HPS Collection Plates MS response.

Conclusion

Use of QuanRecovery with MaxPeak HPS Collection Plates effectively mitigate NSB of the GLP-1 receptor agonist therapeutic peptides, significantly improving LC-MS detection and robustness of the assay.

Ordering Information

| Description | P/N |
|---|-----------|
| ACQUITY UPLC Peptide CSH C ₁₈ Column, 130Å, 1.7 μ m 2.1 \times 50 mm | 186006933 |
| QuanRecovery™ with MaxPeak, 700 μ L plate 25/pk | 186009184 |
| Polypropylene cap mat round well for 96-well (pk/50) | 186002483 |

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[ACQUITY UPLC I-Class PLUS System <](#)

<https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/acquity-uplc-i-class-plus-system.html>>

[Xevo TQ-XS Triple Quadrupole Mass Spectrometer <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-xs.html](#)>

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