

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

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### 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.

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### Experimental

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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 C<sub>18</sub> 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 C <sub>18</sub> Column, 130 Å, 1.7 µm 2.1 mm x 50 mm (p/n: 186006933)
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.*

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## Results and Discussion

Minimizes NSB, increases recovery, sensitivity, and repeatability

Mitigation of GLP-1 receptor agonist NSB using QuanRecovery with MaxPeak 700  $\mu$ L plates

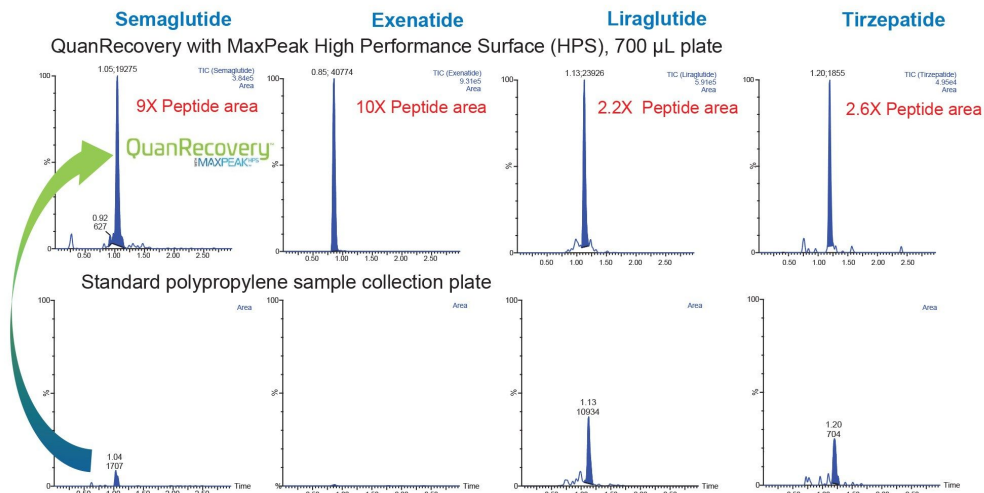


Figure 1. Representative LC-MRM-MS chromatograms demonstrating mitigation of non-specific binding to collection plates for the GLP-1 receptor agonist therapeutic peptides, semaglutide, exenatide, liraglutide, and tirzepatide (1 ng/mL prepared in a solution of 80/20/1 water, acetonitrile, and formic acid)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

### GLP-1 Receptor agonist NSB mitigation using QuanRecovery with MaxPeak 700 $\mu$ L plates vs standard polypropylene plates

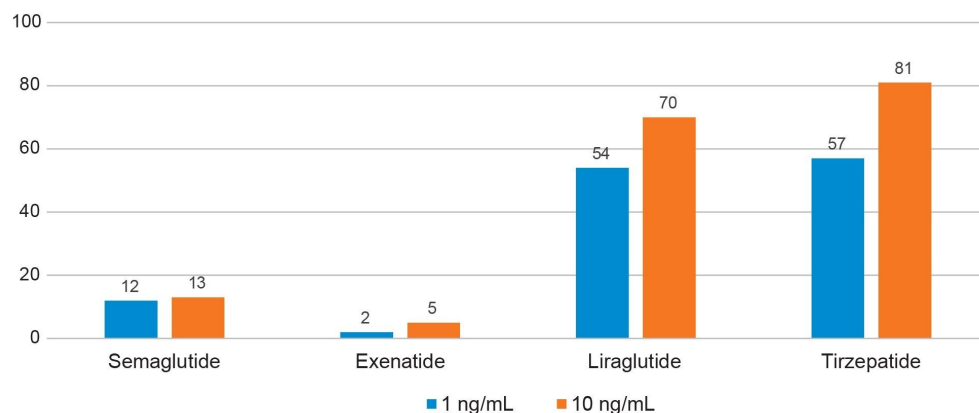


Figure 2. Illustration of improved GLP-1 receptor agonist therapeutic peptide recovery using QuanRecovery with MaxPeak HPS Collection Plates vs standard polypropylene collection plates for 1 and 10 ng/mL mixes (Diluent Composition: 80/20/1 Water/Methanol/Formic Acid. MS response of standard plate was 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 <sub>18</sub> Column, 130Å, 1.7 $\mu$ m 2.1 $\times$ 50 mm	186006933
QuanRecovery™ with MaxPeak, 700 $\mu$ 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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