

## Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060NX

### A Low Level, Carryover Free and Wide Range, LC-MS/MS Method for Quantitation of Semaglutide from Human Plasma

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#### User Benefits

- ◆ Method for Semaglutide quantitation from human plasma was developed and validated as per ICH M10 guidelines.
- ◆ The developed method achieved a low limit of quantification (LLOQ) level and wide dynamic range.
- ◆ The developed method demonstrates no carryover for the dynamic range covered.

#### ■ Introduction

Semaglutide (Figure 1) is a peptide used as an antidiabetic medication for the treatment of type-2 diabetes. It is also used as an anti-obesity medication for weight loss. Recently, studies have shown that Semaglutide also works on the brain, suggesting its potential utility for various diseases, including Parkinson's disease and Alzheimer's disease. The non-specific adsorption of Semaglutide on column, HPLC flow path and high background noise at low level in complex matrix like human plasma makes quantitation of Semaglutide difficult at low level. To overcome these challenges, we developed an MRM based LC-MS/MS method for quantifying Semaglutide. This method is well-suited for pharmacokinetic studies of Semaglutide because it offers low limit of quantification, no carryover and has a wide dynamic range.

Shimadzu LCMS-8060NX (Figure 2) was used to determine Semaglutide in plasma at low levels.



Figure 1: Amino acid sequence for Semaglutide

#### ■ Experimental

The Semaglutide reference standard was procured from local vendor.

Human plasma was procured from local vendor to prepare calibration standards and quality control (QC) samples. Precursor ion selection, MRM optimization at different collision energies and voltages was done using Shimadzu's "Optimization for method" tool. Optimized MRM for 2 product ions with optimized voltages and collision energies (CE) were developed.

A LC method (Table 1) was developed using UHPLC column (Shim-pack Claris) to elute Semaglutide with no carry over. Using the developed LC method and optimized MRM, LLOQ of 0.2 ng/mL and upper limit of quantification (ULOQ) of 600 ng/mL was achieved with no carry over.

For Quantitation, a wide linearity batch ranging from 0.2 to 600 ng/mL was processed in human plasma. For QC check lower limit quality control (LLQC), lower quality control (LQC), medium quality control (MQC) and higher quality control (HQC) samples were processed in replicates and were quantified against the linearity. The accuracy for the calibration standards and QC samples was found to be within acceptable range (Figure 3).



Figure 2: Nexera™ X3 UHPLC coupled with an LCMS-8060NX

#### ■ Method

Table 1: Analytical conditions

System Configuration	
LC-MS/MS	: LCMS-8060NX
Auto-sampler	: Nexera™ X3 with SIL-40C
Column	: Shim-pack Scepter™ Claris C8-120, 3µm 2.1 x 100 mm P/N: 227-31212-05
Analytical Conditions	
Flow rate	: 0.3 mL/min
Mobile phase A	: 1 % formic acid in water
Mobile phase B	: 1 % formic acid in Methanol : Acetonitrile (1:1 v/v)
Rinsing Type	: Internal and external
Elution mode	: Gradient mode
Run time	: 10 min
Injection volume	: 25 µL
Column oven temp	: 65 °C
MS Conditions	
MS Interface	: ESI (Ion Focus)
Desolvation line temperature	: 180 °C
Heating block temperature	: 300 °C
Interface temperature	: 300 °C
Drying gas	: 11 L/min
Nebulizing gas flow	: 3 L/min
Heating gas flow	: 12 L/min

MRMs and their CEs	Precursor ion	Product ion	Collision energy
	1029.2	1302.5	39
	1029.2	1359.1	36

## ■ Sample Analysis

### Protein Precipitation

Take 0.3 mL pre-spiked plasma, add 0.7 mL precipitating solvent, add 0.1 mL of 2 % ammonia solution

### Layer Separation

Vortex for 2 min, centrifuge at 7000 rpm for 5 min

### SPE

Load the aliquot on pre-conditioned cartridge under positive pressure

### Reconstitution

Evaporate the eluant till dryness under nitrogen stream and reconstitute it with 0.3 mL diluent

## ■ Results and Discussion

Validation parameters such as specificity, linearity, accuracy, precision and carryover were studied as per ICH M10 Guidelines.

### ❖ System precision and specificity

System precision was evaluated by calculating variation of the peak area and retention time (RT) of six replicates of 300 ng/mL processed Semaglutide standard.

The % RSD was found to be less than 5 for peak area, whereas the difference in RTs for 6 replicate injections was found to be within  $\pm 0.1$  min (Table 2). Specificity of the method was determined by comparing the response of blank sample (reagent and matrix) against reporting level. Response in reagent/matrix blank sample was well within <20 % of the reporting limit and met the acceptance criteria.

### ❖ Linearity study

For linearity study, processed calibration standards were used. All calibration standards were found within 85 to 115 % accuracy (Table 3). The linearity is shown in figure 3.

### ❖ Accuracy and Precision study

QC samples at 4 different levels - LLQC, LQC, MQC and HQC were processed in replicates and quantified for accuracy and precision study. The observed results were within acceptance criteria of % RSD  $\pm 15$  % (Table 4).

### ❖ Carryover

Carryover was assessed by analysing blank sample after injecting highest calibration standard, the area response at the retention time of Semaglutide for the blank sample analysed after highest calibration standard was found to be less than 20.0 % of the area response of the LLOQ standard (Figure 4).

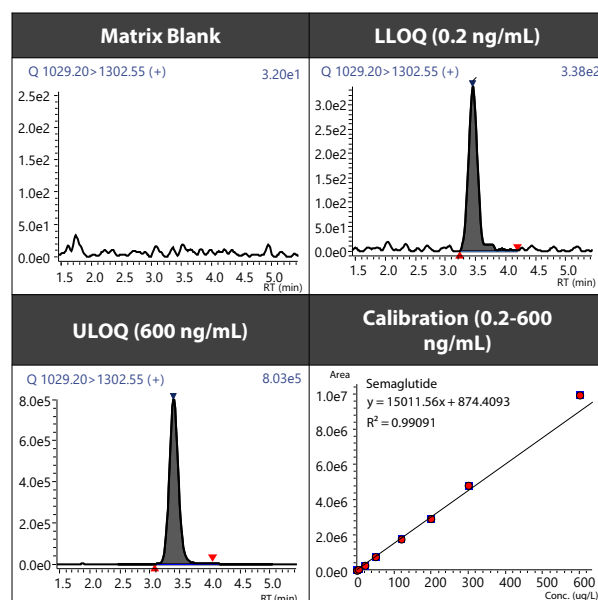


Figure 3: Chromatograms for blank, LLOQ, ULOQ and calibration curve

Table 2: System precision and Specificity

Sample name	RT (min)	Area
SST-1	3.498	4826358
SST-2	3.487	4857930
SST-3	3.490	4930536
SST-4	3.486	5052494
SST-5	3.480	5048113
SST-6	3.474	5174361
Mean	3.486	4981632
SD	0.008	133123
% RSD	0.2	3.0

Table 3: Linearity range

Std. Conc. (ng/mL)	Observed conc. (ng/mL)	Accuracy (%)	S/N
0.2	0.19	96	155
0.4	0.43	109	394
1.0	1.04	94	926
5.0	4.45	89	3135
20.0	18.90	94	4023
60.0	52.71	88	10747
120.0	122.05	102	18396
200.0	202.26	101	22809
300.0	334.10	111	29113
600.0	688.59	114	60416

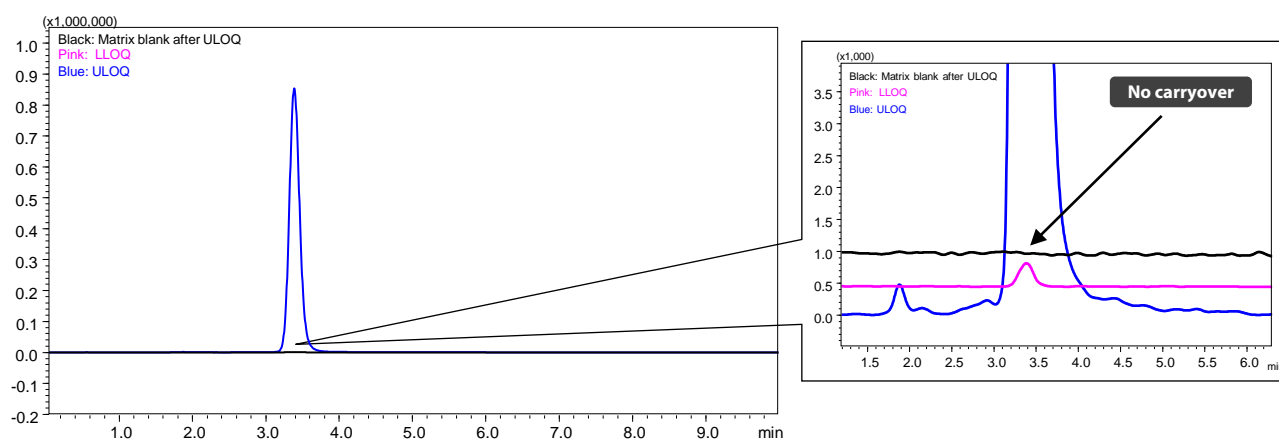


Figure 4: Chromatographic overlay of blank, LLOQ and ULOQ to depict no carryover after ULOQ sample

Table 4: Accuracy and Precision results.

Parameter	Spiked Conc. (ng/mL)	Observed conc. (ng/mL)	Accuracy (%)	Precision (RSD)
LLOQQC	0.2	0.21	105	6.3 %
		0.22	110	
		0.19	95	
		0.20	100	
LQC	0.6	0.61	102	7.9 %
		0.52	87	
		0.55	92	
		0.61	102	
MQC	200.0	194.64	97	4.0 %
		186.78	93	
		178.45	89	
		180.00	90	
HQC	480.0	450.89	90	4.9 %
		484.00	97	
		475.38	95	
		508.00	102	

■ Conclusion

- A highly sensitive and precise method for quantifying GLP-1 peptide in human plasma was developed using the Shimadzu LCMS-8060NX system.
- This method effectively addresses common challenges, including achieving low-level LLOQ, wide dynamic range, and carryover-free detection.
- The results met the accuracy and precision standards of ICH M10 guidelines, confirming the reliability of the method.

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