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MP 558

A low level, Carryover Free, Covering Wide Range, LC-MS/MS Method for Quantitation of Semaglutide in Human Plasma

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1. Introduction

Semaglutide (Figure 1) is a GLP-1 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 (LLOQ), no carryover and has a wide dynamic range .
Shimadzu LCMS-8060NX (Figure 2) was used to determine Semaglutide in plasma at low levels.

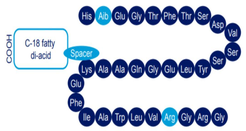

Figure. 1 Amino acid sequence for Semaglutide

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

2. 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 (CE) and voltages was done using Shimadzu's "Optimization for method" tool. Optimized MRM for 2 product ions was developed using optimized voltages and CE.
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 following extraction protocol (Figure 3) and were quantified against the linearity.

3. 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, 3 µm 2.1 mm 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 Interface	: ESI +ive mode (Ion Focus)

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

4. Sample analysis

Due to high protein binding affinity of Semaglutide an extraction protocol was developed, the optimised protocol is mentioned in figure. 3.

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

↓

Solid Phase Extraction
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

Figure. 3 Sample extraction protocol

5. Results & Discussions

Validation parameters such as specificity, linearity, accuracy, precision and carryover were studied as per ICH M10 Guidelines[1]

5-1. 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 ±0.1 min. 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.

5-2. Linearity study

For linearity study, processed calibration standards were used. All calibration standards were found within 85 to 115 % accuracy. The linearity for Semaglutide is shown in figure 5.

5-3. 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 ± 15 %. (Table 2)

5-4. 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)

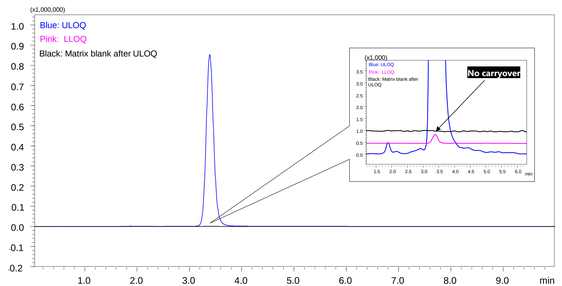

Figure. 4 Chromatographic overlay of ULOQ, Blank and LLOQ depicting no Carryover.

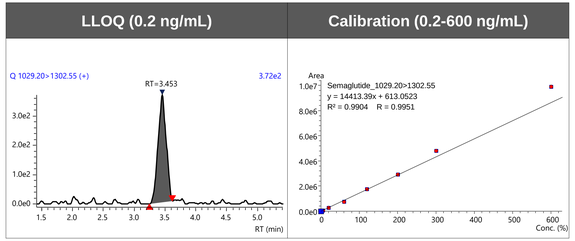

Figure. 5 Chromatogram of LLOQ and calibration curve

Table. 2 Accuracy and Precision results

Parameter	Spiked Conc. (ng/mL)	Observed conc. (ng/mL)	Accuracy (%)	Precision (RSD)
LLQC	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	94	4.9 %
		484.00	101	
		475.38	99	
		508.00	106	

6. 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 [1], confirming the reliability of the method.

7. References

1) ICH harmonized guideline. Bioanalytical method validation and study sample analysis M10