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Improving Sensitivity of GLP-1 Analogues Quantitation Using Multiple Spray ESI Technology on a Microflow LC-MS/MS

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1. Introduction

Glucagon-like peptide-1 (GLP-1) receptor agonists drugs, a class of medications utilized to treat type 2 diabetes and obesity, has gained interest in drug development in the past decade. These larger biotherapeutic molecules tend to present analytical challenges compared to small drug molecules. Some challenges include poor ionization, poor MS/MS fragmentation, and carryover.

A multiple spray electrospray ionization (ESI) allows increased sensitivity by enhanced ionization efficiency through multi-nozzle emitter instead of the traditional single-nozzle emitter, **Figure 1**.

Fig. 1. The Newomics M3 Multi-Nozzle Emitter (left) and the Newomics Source mounted on the Shimadzu LCMS (right).

This work will demonstrate a robust microflow LC-MS/MS system to measure commercially available GLP-1 analogs with increased sensitivity compared to conventional semi-microflow system.

2. Methods

Two common GLP-1 analogs, semaglutide and liraglutide, were purchased through Caymen chemicals. Standards were dissolved and further diluted in 6% acetic acid solutions. The analysis was performed on a microflow LC system with trap-and-elute configuration (**Figure 2**) coupled with a triple-quadrupole LC-MS/MS system. A Newomics DuoESI source with a M3 emitter was used as the microflow ionization source. Source parameters, such as probe position, voltages, gas flow, and temperatures were optimized to maximize sensitivity. Method details are summarized in **Table 1**.

Fig. 2. Nexera Mikros trap and elute LC system with additional loading pump rinsing functionality.

Table 1. Acquisition parameters.

Nexera Mikros	
Mobile Phase	A: Water with 0.1% Formic acid
	B: Acetonitrile with 0.1% Formic acid
Trap Column	CERI L-column2 C8 Cartridge
	0.3 mm x 5 mm, 5 µm
Analytical Column	CERI L-column2 C8 Column
	0.2 mm x 50 mm, 3 µm
Trap Gradient	10% (1.0 min) ⇒ 100% (1.01 -3.7 min) ⇒ 10% (3.8 – 5.99 min) ⇒ 100% (6.0-12.0 min)
Analytical Gradient (%B)	35% (1.25 min) ⇒ 80% (5.5-9.0 min) ⇒35% (9.1-9.7 min) ⇒ 95% (11.0-12.0 min) ⇒35% (12.1 min)
Column Oven Temp.	50 ℃
Flow rate	Loading 50 µL/min and Elution 5 µL/min
Injection Volume	5 µL
Rinsing Type	Internal/External
LCMS-8060	
Nebulizer gas flow	1 L/min
Interface Voltage	3 kV
DL Temp.	150 ℃
Heatblock Temp.	150 ℃
CID Gas Pressure	350 kPa

3. Results

The charge distribution and proper precursors were evaluated for both target analytes, semaglutide and liraglutide, using the microflow source. Under the sample preparation and analytical condition, the most sensitive precursor for semaglutide and liraglutide were $[M+4H]^{4+}$ with an m/z of 1029.30 and $[M+4H]^{4+}$ with an m/z of 938.75 respectively (**Figure 3**). The finalized MRM transitions are shown in **Table 2**.

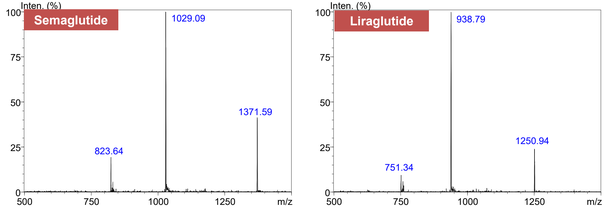

Fig. 3. Mass spectrum of semaglutide and liraglutide under Q3 scan confirming proper precursor selection at 4+ charge state.

Table 2. Selected MRM transition for quantification.

	Quantifier transition	Qualifier transition
Semaglutide	1029.3 > 1238.3	1029.3 > 1302.9
		1372.1 > 1238.3
Liraglutide	938.75 >1064.3	938.75 > 1128.6
		1251.3 > 1245.3

Though microflow ionization source has less parameters than traditional electrospray ionization source, the impact of different parameters on the charge distribution and sensitivity were investigated. By optimizing the source positions, interface voltage, gas flow, and temperature, the signal response was increased from 10 – 200% depending on the parameter settings. After optimization, the microflow ionization source provided 4 – 8 times higher signal response compared to semi-microflow ionization source for the target analytes, **Figure 4**.

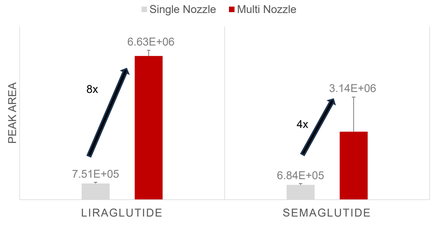

Fig. 4. Sensitivity increase for GLP-1 peptides from single-nozzle emitter to multi-nozzle emitter

A trap-and-elute configuration was utilized to allow flexible injection volumes under microflow analysis as well as flexible autosampler rinsing functionality. GLP-1 peptides are large hydrophobic peptides that often suffer from poor chromatographic peak shapes and carry-over. By incorporating internal and external rinsing, carryover was minimized without compromising the total run time which leads to increased throughput.

Analytical and trap columns were evaluated for their performance with this workflow. Reverse phase columns ranging from C4-C18 functionality were tested for their analyte retention, peak shape, and overall carryover performance. The column set selected in Table 1 provided the best performance and resulted in carryover less than 0.1% at a concentration of 200 ng/mL.

4. Conclusion

These results showcased the capability and possibility of utilizing microflow-LC system with microflow ionization source to achieve increased sensitivity for GLP-1 peptides on existing mass spectrometers.

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