Maximizing Flexibility: A Gas and Temperature Enabled Chip-Based Solution for Nanoflow and Microflow LC-MS
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Introduction

Chromatography is one of the most powerful tools in the field of biochemical analysis. It is widely used in various areas of research from drug discovery to environmental monitoring. However, traditional chromatographic systems have limitations in terms of cost, size, and speed. Chip-based chromatography offers an alternative solution with potential advantages such as lower cost, smaller size, and faster analysis times. Here, we present a chip-based solution that supports long columns, temperature control, and UPLC compatibility, but is targeted to minimize the need for special expertise in handling. Existing chip-based technologies have limitations such as low distance of the tip from the inlet, lack of ability to control temperature, and limited sheath gas capability.

Methods

- HPLC: Home-brew Thermoshim Full Year A/M
- Pump Range: 150/300/800/1500 psi
- Injection Volume: 100/300/800/1500 µL
- Temperature: 40 °C
- Voltage: 400 V
- PostChip Puriﬁcation with a tandem ESI-QTOF System
- Flow Rate: 4.6 mL/min
- Column: 360 µm OD x 150 µm ID x 150 µm tip column, slurry packed to 10 cm
- Gradient: 1 to 40% B in 10 min
- Flow Rates: 1, 2, 3, 4, and 5 µL/min.

Conclusions

- The data show significant improvement in spray stability when using heated conditions and at ambient temperature.
- The identified proteins are Man5GlcNAc2, Man8GlcNAc2, Lysozyme, BSA-Cysteinylated, and at ambient temperature.
- Future Work
  - Larger BSA samples will be run to further enhance the scalability of the chip-based solution.
  - Further optimization of the high-throughput method using liquid chromatography and electrospray ionization will be performed to improve signal intensity and resolution.
  - Protein analysis will be further pursued and optimized using various types of analyses.

Chip-Based Intact Protein Analysis

- 1. Berg, AL; Dewberry AD; Svobodova H; Valaskovic GA; Wang, P; "Evaluating and Improving the Performance of a Chip-Based Solution for Nanoflow and Microflow LC-MS" (2016). The identified proteins are Man5GlcNAc2, Man8GlcNAc2, Lysozyme, BSA-Cysteinylated, and at ambient temperature.

Efficiency of Gas and Spray Morphology

- Various flow rates were assessed using equal mass standard. 1 min data was collected at 5 µL/min and a flow rate of 1 µL/min. Chromatograms are normalized to the highest area for better comparison.

Gradient Variation Effects

- The figure shows the difference in spray morphology with sheath gas settings 0 and 30. The flow rate was 5 µL/min.

Flow Rate Effects

- The pressure response of the BSA standard was run at 3.25E7, 5.50E6, and 7.68E6 psi.

Gas/Spray Stability/Intensity

- The data show significant improvement in spray stability when using heated conditions and at ambient temperature.

Effect of Gas on Spray Morphology

- The data show significant improvement in spray stability when using heated conditions and at ambient temperature.

Peak capacity and retention time of 8 PicoSure peptides. As the gradient length decreases, the retention times of PicoSure standard. All data was collected at 5 uL/min to 8 uL/min.

Acceleration of multiple peptides of various gradient lengths and cycle time as indicated above. 30 data sets were utilized at 5 µL/min, and a flow rate of 1.5 µL/min. Chromatograms are normalized to the highest area for better comparison.

Chromatographic data collected of nitrogen standards at various flow rates 0.5 µL/min to 10 µL/min. 84 data sets were utilized.

Chromatographic data collected of BSA standard at various flow rates 0.5 µL/min to 10 µL/min. All data sets were utilized.