Applying online nano-UHPLC to proteomics

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Overview
Online nUHCPL allows larger, smaller IDs improving chromatographic performance. Flow rate should be considered carefully.

Introduction
Ultra-High Performance Liquid Chromatography (UHPLC) pushes the limits of feasible column designs through higher pressure operation. Moving from nano-HPLC to nUHCPL and scouting directly to a mass spectrometer requires attention to the setups to allow the increase in pressure. We systematically evaluate experimental parameters such as column dimensions and various stationary phases in how they apply to peptide analysis.

Factors affecting the chromatographic pair of Online UHPLC performance include:
- Column length
- Column inner diameter
- Flow rate
- Back-pressure material phase
- Bead size

Methods
An Easy-nano LC (Thermo, Odense, Denmark) was used to deliver up to 100 bars was equipped with a 1.7×75 mm, ID 2.1 μm column. A 25 cm column setup was used in parallel with花生配制的螺柱的立体结构, valency and prope

Results
Individually a wide range of dimensional and solid-phase gradient were tested looking for a golden guidance for the high pressure of instrumentation. With aromaticity or a more polar (though because of the nUHCPL the UV detection was chosen at a wavelength specific for UV free flow cell, and short gradients on a lower end specific polar, at 214 nm). A fast analysis of gradient (FAG) is depicted in figure 1, with a few selected peptides under CID detected.

For acquisitions of Boc-Ser-Benzalkonium chloride (BSB) a Thermo LTQ ETD was used with a gradient from 10% to 33% B in 30 minutes, then to 100% B and back to 2% for a certain amount of time depending on mass spectrometer used to allow peptides to accumulate.

For complex samples a Thermo Orbitrap XL was used with a gradient going from 15% to 33% B in 120 minutes. The Orbitrap was set to acquire full scan spectra of m/z 500-6500 (BSB target of 14 Torr) and a maximum excitation time of 500 ms, in parallel 1024 mass scans were acquired in the setting the 10.1 million ion interface, dynamic exclusion was enabled with an exclusion period of 45 seconds.

Data processing
Data was processed using MASCOT version 1.5.0 and (Koehler et al., 1998). The ratio was determined to be the most effective means of isolation. The mass isolation window was set to ±20.000 ppm (an isolation of 10 ppm), and the mass tolerance for all fragments was set to ±0.002 Da. The MS/MS product ions were extracted on the basis of a fixed charged state. The fragmentation percentage of the fragment ions was used to optimize the parameters. The identified peptides were allowed to vary up to two charges.

Conclusion
UHPLC broadens freedom of column selection and flow rates. All at column packed porous. Work in progress: The ability of native radiolabeling.

Online Nano-UHPLC To Proteomics

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For the preparation of goat anti-Bovine albumin (BSA) a Thermo LTQ ETD was used with a gradient from 10% to 33% B in 20 minutes, then to 100% B and back to 2% for a certain amount of time depending on mass spectrometer used to allow peptides to accumulate.

For complex samples a Thermo Orbitrap XL was used with a gradient going from 15% to 33% B in 120 minutes. The Orbitrap was set to acquire full scan spectra of m/z 500-6500 (BSB target of 14 Torr) and a maximum excitation time of 500 ms, in parallel 1024 mass scans were acquired in the setting the 10.1 million ion interface, dynamic exclusion was enabled with an exclusion period of 45 seconds.

Data processing
Data was processed using MASCOT version 1.5.0 and (Koehler et al., 1998). The ratio was determined to be the most effective means of isolation. The mass isolation window was set to ±20.000 ppm (an isolation of 10 ppm), and the mass tolerance for all fragments was set to ±0.002 Da. The MS/MS product ions were extracted on the basis of a fixed charged state. The fragmentation percentage of the fragment ions was used to optimize the parameters. The identified peptides were allowed to vary up to two charges.

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