Coupling a Nanoflow Liquid Chromatography Column to Automated Chip-Based Nanospray Emitters for Tandem Mass Spectrometric Protein Identification

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Overview

Purpose:

Development of a real-time automated nanospray emitter changing system for 75 µm i.d. nanoLC columns.

Methods:

- A low dead volume nanoLC column-to-chip interface was developed.
- A 75 µm i.d. nanoLC system was interfaced with the TriVersa NanoMate.
- Automated robotics allowed for “on-the-fly” emitter changes in real time during a chromatographic run.

Results:

- Fast, automated nanospray emitter changing was demonstrated.
- Real time nanospray emitter changes were conducted in less than three seconds during a chromatographic run without disturbing the chromatographic analysis.

Introduction

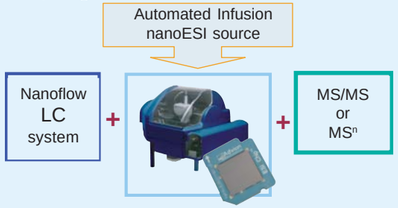
NanoLC-MS with 75 µm i.d. columns and flow rates of 100’s nL/min is gaining in popularity due to lower sample requirements, and better ionization efficiency. Because of the nanoLC requirements for low flow rates, small internal diameter nanospray emitters are required for interfacing the column to the mass spectrometer (MS). These nanospray emitters inherently have issues with robustness as clogging/failure of the emitter is eminent. Currently, column and emitter integration is a permanent configuration and changing an emitter requires skilled human intervention and time. Here we describe automated emitter changes utilizing chip-based nanospray and robotic manipulation. This low dead volume column-to-chip interface allows for an emitter to be changed within seconds without disturbing the chromatography, offering a robust nanoLC-MS interface.

Methods

A low dead volume column-to-chip mandrel was fabricated from a 15 µm i.d. fused silica capillary combined with a pipette tip.

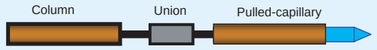
This coupling interface was integrated with an automated chip-based nanospray system (TriVersa NanoMate). The mobile phase for gradient elution through the 75 µm i.d. reversed-phase LC column was transferred to the chip-based ESI system for sheathless nanoflow ESI.

Coupling nanoLC to Chip-based ESI

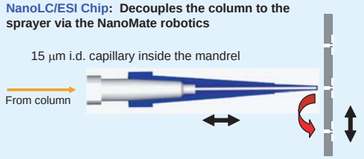


Pulled-capillary vs. ESI Chip

Conventional Technique: Permanent connection via union or one piece construction



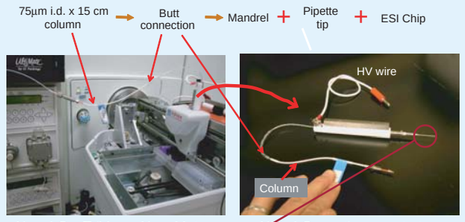
NanoLC/ESI Chip: Decouples the column to the sprayer via the NanoMate robotics



Experimental:

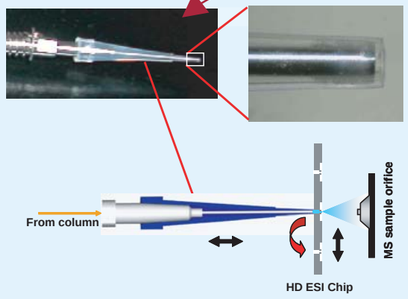
Sample:	BSA digest (Michrom Bioresources)
RP LC column:	Waters Atlantis dC18, 75 µm i.d. x 150 mm; 3 µm ODS, 100 Å
LC System:	UltiMate (LC Packings)
Mobile phase (v/v):	A = Water with 0.1% HAc + 0.01% HFBA B = ACN with 0.1% HAc + 0.01% HFBA
Gradient program:	Injection, followed by a 10 min desalt t = 10 min: gradient and MS data acquisition start t = 10- 55 min, 5% - 50% B
Flow Rate:	280 nL/min
Scan Mode:	1 full MS scan (m/z 465~2000) + 1 full MS/MS scan;
Dynamic exclusion:	Enabled for data-dependent analysis; repeat count = 2, repeat duration = 0.4 min, exclusion duration = 1.0 min
Detector:	LCQ Deca (Thermo)

Post-column connection with 15 µm i.d. x 25 cm capillary



NanoLC + TriVersa NanoMate + LCQ Deca ion trap MS

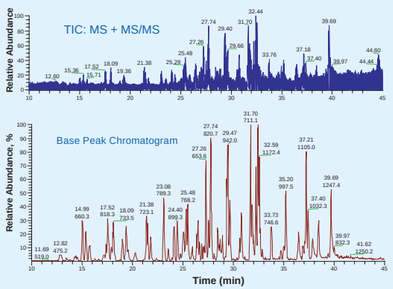
NanoLC coupling mandrel



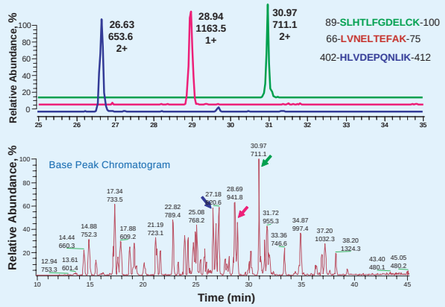
NanoLC Mandrel Coupled to HD ESI Chip

Results

NanoLC-ESI-MS/MS of 100 fmol BSA Digest

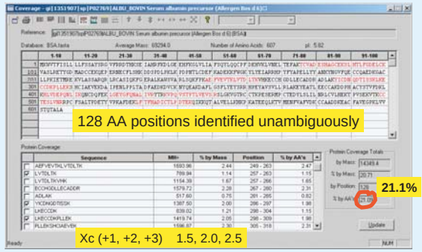


NanoLC-ESI-MS/MS of 10 fmol BSA Digest



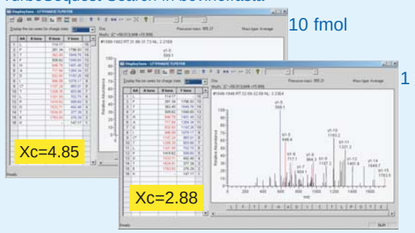
NanoLC-ESI-MS/MS of 1 fmol BSA Digest

TurboSequest Search in *bovine.fasta*



NanoLC-ESI-MS/MS of BSA digest: 10 fmol vs 1 fmol

TurboSequest Search in *bovine.fasta*



Summary of Protein Identification Results

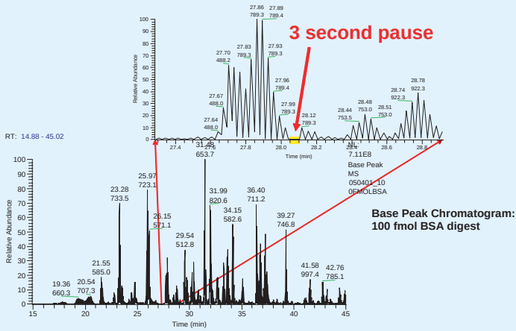
Sample: BSA digest (from Michrom Bioresources)

Injected amount	Identified[a] AA positions	peptides	Sequence coverage	Xc for T58[b] at m/z 955
100 fmol	371	34	61.1%	5.2
10 fmol	298	26	49.0%	4.9
1 fmol	128	11	20.1%	2.9

[a] Only those unambiguous peptides counted; Filter set at Xcorr (+1, +2, +3) ≥ 1.5, 2.0, 2.5

[b] T58 sequence: 529-LFTFHADIC*TLPDTEK-544

“on-the-fly” Emitter Change = 3 Second Pause



Conclusions

- The automated chip-based nanospray emitter changing system for nanoLC provided good LC resolution and peak shapes, as well as high sequence coverage and high sensitivity in protein identification.
- Real time “on-the-fly” emitter changes may be conducted in seconds without disturbing chromatography.
- Future work includes spray sensing to ensure continuous nanospray in the event of an emitter failure.