The Integration of Nanoscale Separation and Ionization for the Analysis of Complex Proteomes

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Introduction

Nanoflow LC-MS has been used widely in proteomics research. However, the need for specialized training and in-depth knowledge of instrumentation can become a bottleneck to implementing nanoflow LC-MS for high-throughput experiments of complex proteomes. The integration of a nanospray source with chip-based nanobore columns maximizes performance at low flow rates and ensures reproducibility while simplifying implementation of nanoflow LC-MS capabilities. This new system facilitates the employment of nanoflow LC-MS in qualitative and quantitative proteomic workflows, delivering efficiency while preserving performance. Presented here is an integrated chip-based-nanospray source and nanobore column enabling high-throughput analysis of simple and complex mixtures for qualitative and quantitative workflows.

Methods

Instrumentation

- Mass Spectrometer: Q-Exactive (Thermo)
  - Scan Settings: Resolution: FS-7000; MS/MS – 17500; Mass Range; 400 – 2000 Da; Isolation Width: 2; NCE: 27; Dynamic Exclusion: 30 sec.
  - Source: chip-based nanospray source with self-guided emitter positioning (PicoChip, New Objective)
- Column: 75 µm ID packed-tip (15 µm) column, 10.5 cm bed length with an embedded high-voltage contact (PicoChip, New Objective)
  - Stationary Phase: 3 µm 120 Å Reprosil-PUR C18-AQ (Dr. Maisch)
  - Spray Voltage: 2.2 kV, positive mode
- HPLC: Easy-nLC 1000 (Thermo)
  - Flow Rate: 300 nl/min.
  - Gradient: 5-35% B, 53 min. (6-protein digest), 112 min. (HeLa digest)

Reagents

- Mobile Phase A: 0.1% formic acid in water (Milli-Q)
- Mobile Phase B: 0.1% formic acid in acetonitrile (Merck)
- HeLa Proteome: Human HeLa cell lysate, trypsin digested, 50 µg
- 6-Protein Standard Mixture: trypsin digested, equimolar, 50 fmol/µL (Sigma-Aldrich)

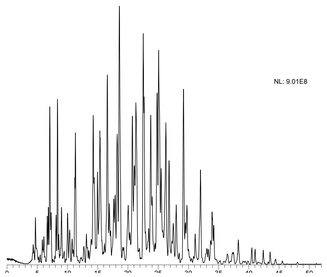
Software

- Mascot (Matrix Science)

ABOVE: PicoChip nanospray columns with source

RIGHT: PicoChip column

Chromatographic Performance



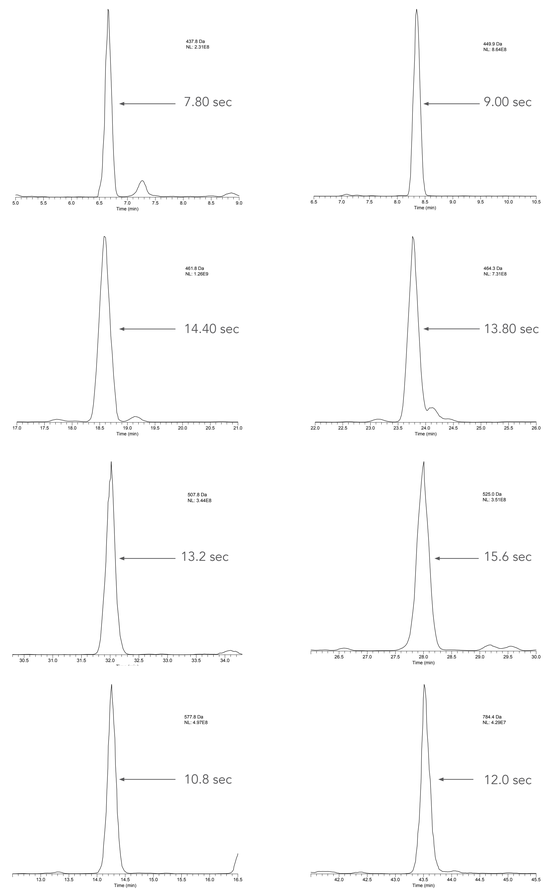
m/z (Da)	Calculated at 13.5% Above Baseline					FWHM (min.)	FWHM (sec.)	Peak Capacity (13.5%)	Asymmetry (10%)
	Apex RT (min.)	Start RT (min.)	End RT (min.)	Area	Height				
437.8	6.65	6.54	6.78	1.79E+09	2.31E+08	0.13	7.80	217.7	0.9
449.9	8.35	8.24	8.47	7.04E+09	8.64E+08	0.15	9.00	227.1	0.9
577.8	14.26	14.10	14.42	4.90E+09	4.97E+08	0.18	10.80	163.5	1.0
461.8	18.58	18.39	18.80	1.71E+10	1.26E+09	0.24	14.40	127.8	1.2
464.3	23.76	23.59	23.97	8.97E+09	7.31E+08	0.23	13.80	137.8	1.4
525.0	28.01	27.76	28.19	4.85E+09	3.49E+08	0.26	15.60	121.9	0.8
507.8	32.01	31.81	32.20	3.66E+09	3.01E+08	0.22	13.20	134.3	1.1
784.4	43.53	43.37	43.71	4.36E+08	3.91E+07	0.20	12.00	153.9	1.1

LEFT: Base peak chromatographic separation of 6-protein standard digest on a 10.5 cm PicoChip column

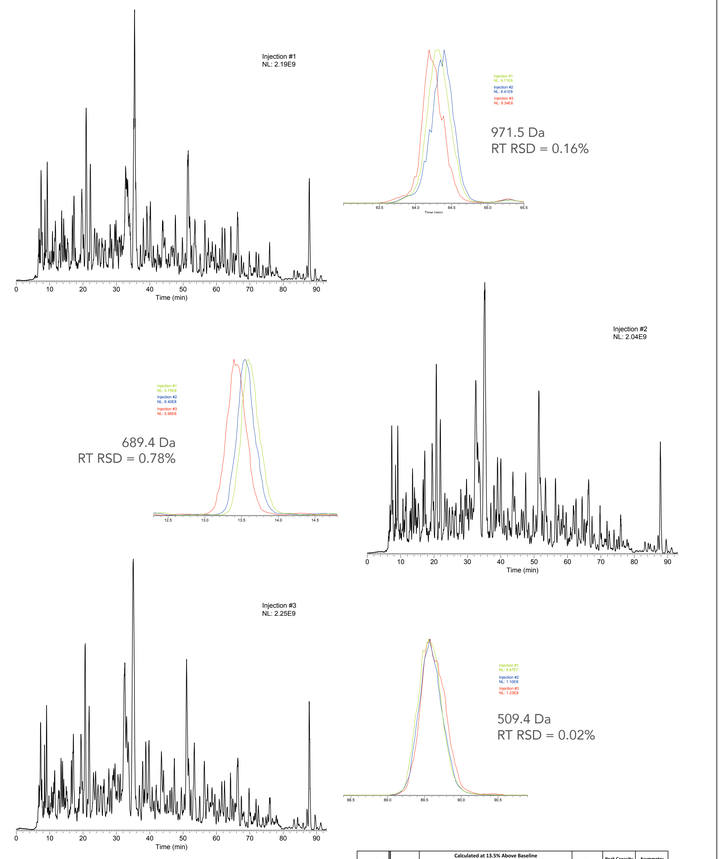
ABOVE: Peptide specific peak capacity, peak width and asymmetry data calculated for different 6 protein peptides separated on a 10.5 cm Picochip column

BELOW: Extracted ion chromatograms of different 6 protein peptides

Narrow Peaks Across the Gradient



Chromatographic Reproducibility Using a Complex Sample



ABOVE: Base peak and extracted ion chromatograms of HeLa cell digest for three replicate injections on a 10.5 cm PicoChip column

RIGHT: Peptide specific peak capacity, peak width and asymmetry data calculated for 7 different HeLa peptides separated on 10.5 cm PicoChip columns

m/z (Da)	Injection #	Calculated at 13.5% Above Baseline					FWHM (min.)	Peak Capacity (13.5%)	Asymmetry (10%)
		Apex RT (min.)	Start RT (min.)	End RT (min.)	Area	Height			
402.0	1	7.41	7.22	7.95	1.94E+10	9.01E+08	0.32	153.05	1.20
	2	7.31	7.12	7.87	2.15E+10	9.77E+08	0.32	149.00	2.80
	3	7.27	7.05	7.89	2.20E+10	9.09E+08	0.32	133.14	2.50
	AVG	**7.33**	**7.13**	**7.90**	**2.10E+10**	**9.29E+08**	**0.32**	**145.07**	**2.17**
	STD	**0.07**	**0.09**	**0.04**	**1.36E+09**	**4.16E+07**	**0.00**	**10.52**	**0.85**
	RSD	**0.98**	**1.20**	**0.53**	**6.49**	**4.48**	**0.00**	**7.25**	**39.25**
689.4	1	13.59	13.38	13.88	9.73E+09	5.07E+08	0.28	223.00	1.50
	2	13.55	13.32	13.82	1.07E+10	6.38E+08	0.27	223.00	1.20
	3	13.39	13.16	13.7	1.08E+10	5.56E+08	0.32	209.56	1.40
	AVG	**13.51**	**13.29**	**13.80**	**1.04E+10**	**5.99E+08**	**0.29**	**217.52**	**1.37**
	STD	**0.11**	**0.11**	**0.09**	**5.74E+08**	**3.57E+07**	**0.03**	**9.49**	**0.15**
	RSD	**0.78**	**0.86**	**0.66**	**5.53**	**5.96**	**9.12**	**4.36**	**11.18**
457.3	1	28.11	27.87	28.48	9.34E+09	4.48E+08	0.37	182.97	1.40
	2	28	27.75	28.34	9.59E+09	4.74E+08	0.34	189.14	1.40
	3	27.73	27.41	28.07	1.13E+10	5.10E+08	0.37	169.18	1.10
	AVG	**27.95**	**27.68**	**28.30**	**1.01E+10**	**4.77E+08**	**0.36**	**180.43**	**1.30**
	STD	**0.20**	**0.24**	**0.21**	**1.09E+09**	**3.11E+07**	**0.02**	**10.22**	**0.17**
	RSD	**0.70**	**0.86**	**0.74**	**10.77**	**6.51**	**4.81**	**5.66**	**13.32**
518.4	1	40.19	39.82	40.54	2.54E+10	1.02E+09	0.41	155.17	0.90
	2	40.04	39.62	40.4	3.10E+10	1.14E+09	0.49	143.31	0.90
	3	39.8	39.38	40.13	3.19E+10	1.19E+09	0.46	149.00	0.80
	AVG	**40.01**	**39.61**	**40.36**	**2.94E+10**	**1.12E+09**	**0.45**	**149.16**	**0.87**
	STD	**0.20**	**0.22**	**0.21**	**3.54E+09**	**8.79E+07**	**0.04**	**5.93**	**0.06**
	RSD	**0.49**	**0.56**	**0.52**	**12.04**	**7.88**	**8.91**	**3.98**	**6.66**
577.8	1	56.54	56.26	56.89	1.05E+10	4.88E+08	0.37	177.19	1.30
	2	56.38	56.11	56.71	1.20E+10	5.64E+08	0.37	186.00	1.20
	3	56.38	56.04	56.68	1.31E+10	5.68E+08	0.40	174.44	0.90
	AVG	**56.43**	**56.14**	**56.76**	**1.19E+10**	**5.40E+08**	**0.38**	**179.21**	**1.13**
	STD	**0.09**	**0.11**	**0.11**	**1.35E+09**	**4.50E+07**	**0.02**	**6.04**	**0.21**
	RSD	**0.16**	**0.20**	**0.20**	**11.35**	**8.33**	**4.56**	**3.37**	**18.37**
971.5	1	64.29	64.02	64.62	1.87E+10	8.68E+08	0.38	186.00	1.20
	2	64.39	64.04	64.67	1.71E+10	8.38E+08	0.37	177.19	1.20
	3	64.18	63.96	64.54	1.75E+10	9.31E+08	0.35	192.38	1.60
	AVG	**64.29**	**64.01**	**64.61**	**1.78E+10**	**8.79E+08**	**0.37**	**185.19**	**1.33**
	STD	**0.11**	**0.04**	**0.07**	**8.19E+08**	**4.73E+07**	**0.02**	**7.63**	**0.23**
	RSD	**0.16**	**0.07**	**0.10**	**4.60**	**5.38**	**4.17**	**4.12**	**17.32**
509.4	1	89.56	89.26	89.91	2.25E+09	9.86E+07	0.42	171.77	1.20
	2	89.57	89.29	89.91	2.31E+09	1.10E+08	0.38	180.03	1.10
	3	89.54	89.3	89.96	2.82E+09	1.28E+08	0.40	169.18	1.80
	AVG	**89.56**	**89.28**	**89.93**	**2.46E+09**	**1.12E+08**	**0.40**	**173.66**	**1.37**
	STD	**0.02**	**0.02**	**0.03**	**3.15E+08**	**1.47E+07**	**0.02**	**5.67**	**0.38**
	RSD	**0.02**	**0.02**	**0.03**	**12.81**	**13.13**	**5.00**	**3.26**	**27.70**

Sequence Coverage

Samples	Number of MS Scans	Number of MS/MS Scans	MS/MS Identified	Peptide Sequences Identified	Protein Group Identified
HeLa1	8590	54378	14144	7327	1933
HeLa2	8597	54430	14044	7247	1929
HeLa3	8661	54178	13630	6966	1885

Peptide and protein identification results for HeLa cell proteome digest

Protein	MW (Da)	Coverage (%)
Serum albumin - Bos taurus (Bovine)	71244	61%
Serotransferrin - Bos taurus (Bovine)	79870	65%
Alpha-2-HS-glycoprotein - Bos taurus (Bovine)	39193	39%
Carbonic anhydrase 2 - Bos taurus (Bovine)	29096	55%
Ovalbumin - Gallus gallus (Chicken)	43196	40%
Lysozyme C - Gallus gallus (Chicken)	16741	29%

Summary of sequence coverage for 6 protein standard

Conclusions

- Integrated chip-based column and source design provided high performance chromatography
  - Observed symmetrical peak shape and baseline separation for 6-protein digest
- Self-guided emitter positioning increases the stability of nanospray for improved detection of low abundance peptides
- Chip-based system enables high-throughput analysis of both simple and complex proteomes through ease-of-use
- Observed good sequence coverage for 6-protein digest
  - 30% For lysozyme C
  - 65% For bovine serotransferrin
- Demonstrated good retention time reproducibility for three replicate injections of a HeLa cell digest

Future Work

- Evaluate performance on longer columns in the PicoChip column format for:
  - Optimized gradient length
  - Increased peptide recovery
  - Improved peak shape
  - Identification of more biomarkers