Method to Optimize Sensitivity and Throughput on a Hybrid Quadrupole-TOF Instrument for LC/MS-Based Peptide and Proteomic Analysis

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Introduction

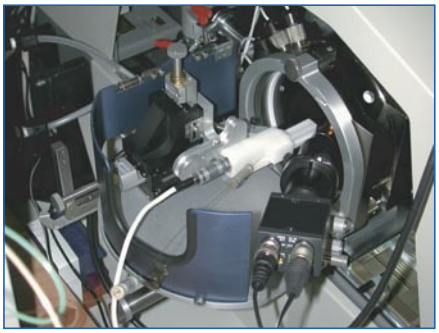
The high mass accuracy and sensitivity or hybrid quadrupole time-of-flight (Q-ToF) type mass spectrometers are well suited for peptide based proteomics. Complex instrumentation affords a large parameter space impacting critical figures of merit such as sensitivity, robustness, and analytical throughput. When coupled to nanobore LC, electrospray ionization (ESI) optimization is complicated by the changes in surface tension and viscosity generated by the mobile phase gradient. Optimizing ESI for nL/min flow rates can be challenging, especially with front-end instrumentation designed and optimized for high-flow (mL/min) operation. Here we endeavor to determine analytical best practices to enable a high level of robustness and throughput without compromising sensitivity. This is accomplished through modification of the instrument front-end and judicious choice of nanobore LC experimental set-up.

Methods

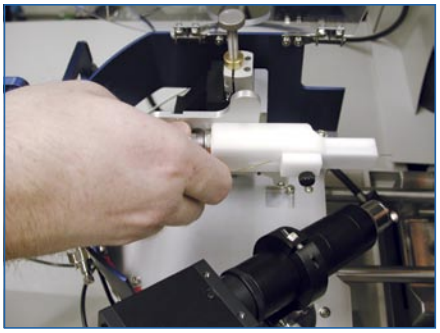
Mass Spectrometer:	QSTAR® (Applied Biosystems)
Nanospray Source:	PicoView® 400 (New Objective)
LC System:	UltiMate™ Plus (LC Packings/Dionex)
Mobile Phase A:	3% ACN with 0.01% TFA
Mobile Phase B:	100% ACN with 0.007% TFA
Trap Column Mobile Phase:	3% ACN with 0.01% TFA
Column flow rate:	1.2 µL / min
Trap flow rate:	50 µL / min
Column:	Swift Armor 150 µm x 250 mm C18 5 µm 180Å (Analytical Sales & Service)
Trap column:	300 µm x 1 mm, 5 µm C18 100Å (Dionex)
Tip:	FS360-75-30-CE (New Objective) 30 µm tip showed a long lifetime and low rate of clogging Voltage applied to metal-coated tip; post-column connection
ABI nanospray voltage:	2500V
Curtain gas:	25 units
Tip to inlet distance:	1 cm
Sheath gas:	None
Declustering Potential (DP1):	75
Declustering Potential (DP2):	10
Focusing Potential:	230
Ion release delay:	11
Ion release width	10

Source Configuration

QSTAR® mass spectrometer with the PicoView® 400 source and NanoLC system

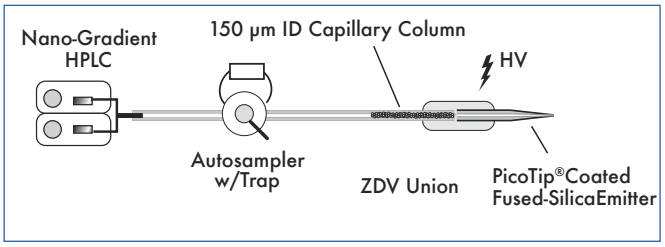


Nanospray source configured to use conventional capillary columns and coated fused-silica tips. HV is applied directly to the tip.



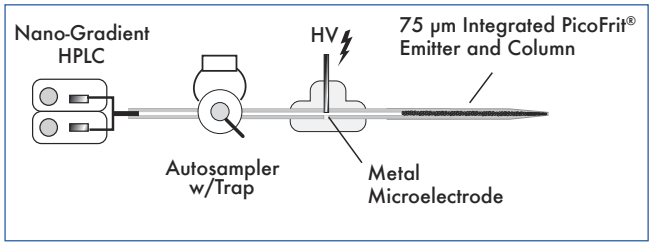
The unique magnetic platform removes for easy tip removal and/or experimental design modification

Separate magnetic stages were used for calibration and analysis. This allowed the analytical column to equilibrate, held magnetically on the side of the LC, while calibration was performed. Upon completion, the stages were quickly exchanged (< 30s) to perform the analysis increasing the over all duty cycle of the instrument.

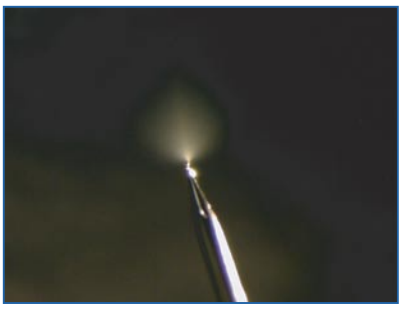


Source configuration for using 150 µm ID capillary columns. The columns is directly coupled to a 30 µm fused-silica PicoTip metal coated for HV conductivity.

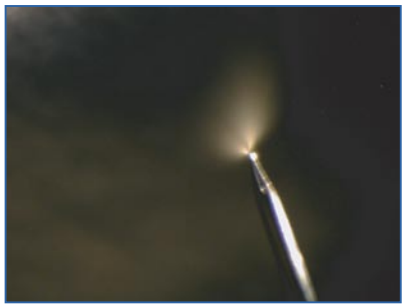
Configuration for using a 75 µm ID PicoFrit® column. The HV is applied directly to the mobile phase with a platinum microelectrode in a pre-column arrangement.



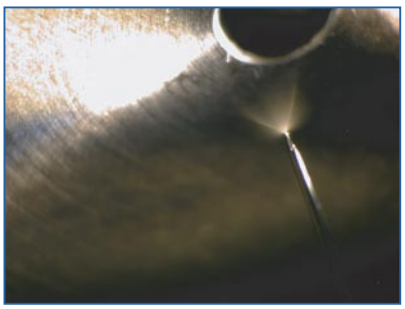
Observation of Spray Stability



Spray pattern with 5% organic at start of separation (taken with PicoView®)



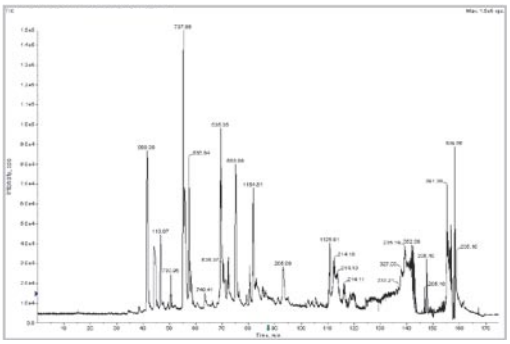
Spray pattern with 35% organic at end of gradient (taken with PicoView®)



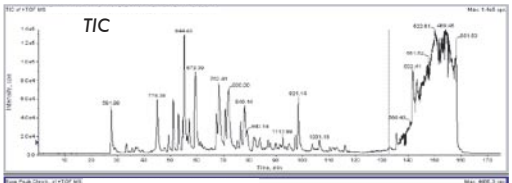
Spray pattern with 35% organic at end of gradient (taken with PicoView®)

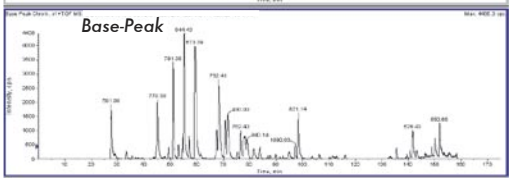
Long Gradient Performance

- **Peptide analysis incorporating a trap column**
- Samples underwent a rapid 10 min microwave enzymatic digest
- Resulted in >50% peptide coverage



Base-peak chromatogram:
- 3-Protein mix digest
- Trap with 150 µm ID column
- 5 - 35% ACN gradient over 170 min
- Full scan MS





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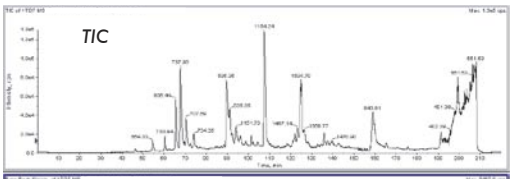
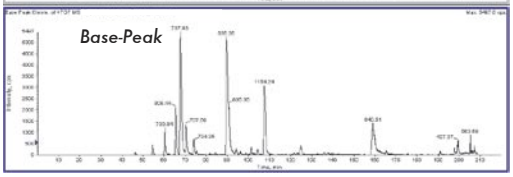


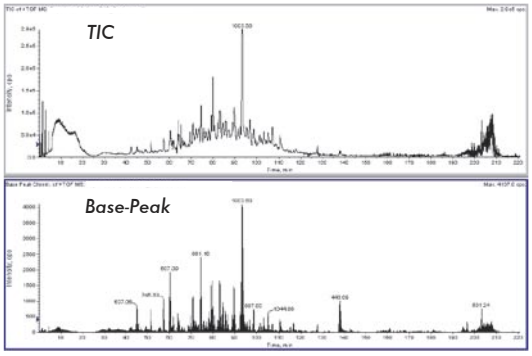


Glycopeptide enriched fetuin:
- 150 µm ID column with trap
- 5 - 45% ACN gradient
- 220 minute run
- 3 Enzyme digest
- 1.2 µL/min flow rate
- 30 µm ID coated tip

Peptide analysis using direct on column sample injection

- Samples underwent a rapid 10 min microwave enzymatic digest
- Resulted in a more complete peptide coverage of > 80%
- Overall better recovery of small peptide fragments

Methionine containing peptides from serum:
- 150 µm ID column w/trap
- 3 - 40% ACN gradient
- 220 minute run
- 1.2 µL/min flow rate
- 30 µm ID coated tip



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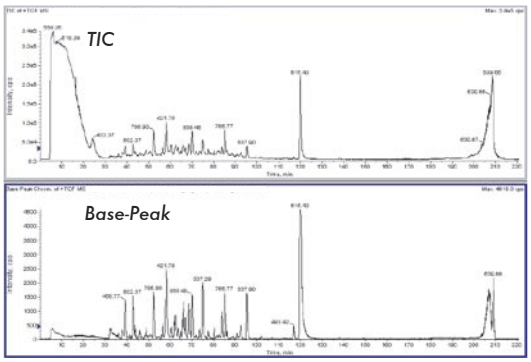
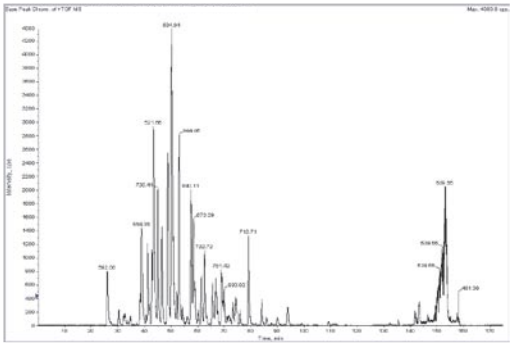


Comparison of a capillary column and a PicoFrit® column

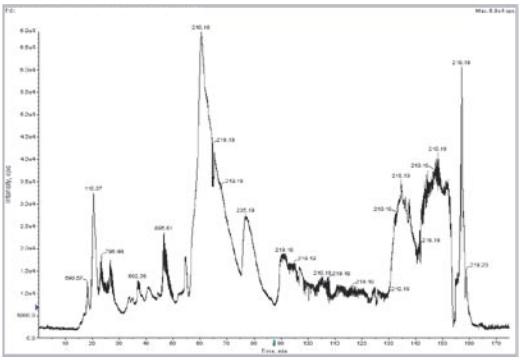
- Dual enzyme digestion was performed overnight for efficient cleavage

AspN/GluC:
150 µm ID x 25 cm C18 1.2 µL/min microflow separation of Sample #2
- 5 - 45% ACN gradient
- 170 minute run
- 3 enzyme digest
- 1.2 µL/min flow rate
- 30 µm ID coated tip



AspN/GluC:
75 µm ID x 5 cm C18 200 nL/min nanoflow separation of Sample #2
- 4X dilution
- 5 - 45% ACN gradient
- 170 minute run
- 200 nL/min flow rate
- 15 µm ID fritted tip

Even with a 4-fold sample dilution, the 75 µm ID column exhibits sample overload



Conclusions

- Spray visualization permits validation of spray performance accross the full range of gradient composition
- Judicious choice of spray voltage, as determined by visualization, enables stability over long periods of time and an extended gradient (3 - 4 hour runs)
- The 150 µm ID column, coupled to a 30 µm ID coated PicoTip®, provides a stable, robust platform for routine operation
- Stability was obtained at 1.2 µL/min at 2500 V without the need for assistance from sheath gas
- The 75 µm ID PicoFrit® column provides a better than 4-fold sensitivity enhancement, making it the optimal choice for challenging samples
- Peptide analysis incorporating a trap column resulted in >50% peptide coverage while direct on-column sample injection resulted in more complete peptide coverage of >80%

Originally presented at the 52nd Conference of the American Society of Mass Spectrometry, 2004