

Enhanced Sensitivity for Protein Quantitation Using a Triple Quadrupole with a Dual Ion Funnel

Christine A. Miller¹, Yanan Yang¹, Derek Smith² and Christoph H. Borchers²

¹Agilent Technologies, Santa Clara, CA and ²UVic-Genome BC Proteomics Centre



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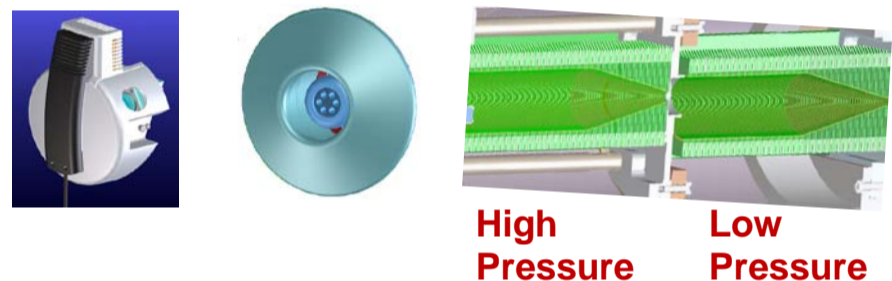
Introduction

Assays that are both specific and quantitative for target proteins are critical for preclinical validation of putative biomarkers. Such assays are typically multiplexed, multiple reaction monitoring (MRM) analyses which can provide the high-throughput required. Sensitivity is a key requirement for such assays as protein biomarker concentrations may be quite low in commonly used biofluids such as serum and plasma. Improving the sensitivity of LC/MS can be achieved by enhancing the sampling and transmission of ions in the mass spectrometer. This study investigates the sensitivity gains achieved for peptides using a triple quadrupole mass spectrometer modified with a dual ion funnel.

6490 QQQ with iFunnel Technology

The Agilent 6490 QQQ incorporates iFunnel technology which is a combination of three fundamental innovations:

- Agilent Jet Stream technology – ESI with thermal gradient ion focusing confinement
- Hexabore sampling capillary with 6 independent parallel bores to enable sampling a much larger fraction of the ions
- Dual-stage ion funnel for efficient removal of large gas volumes and ion transfer to Q1 optics

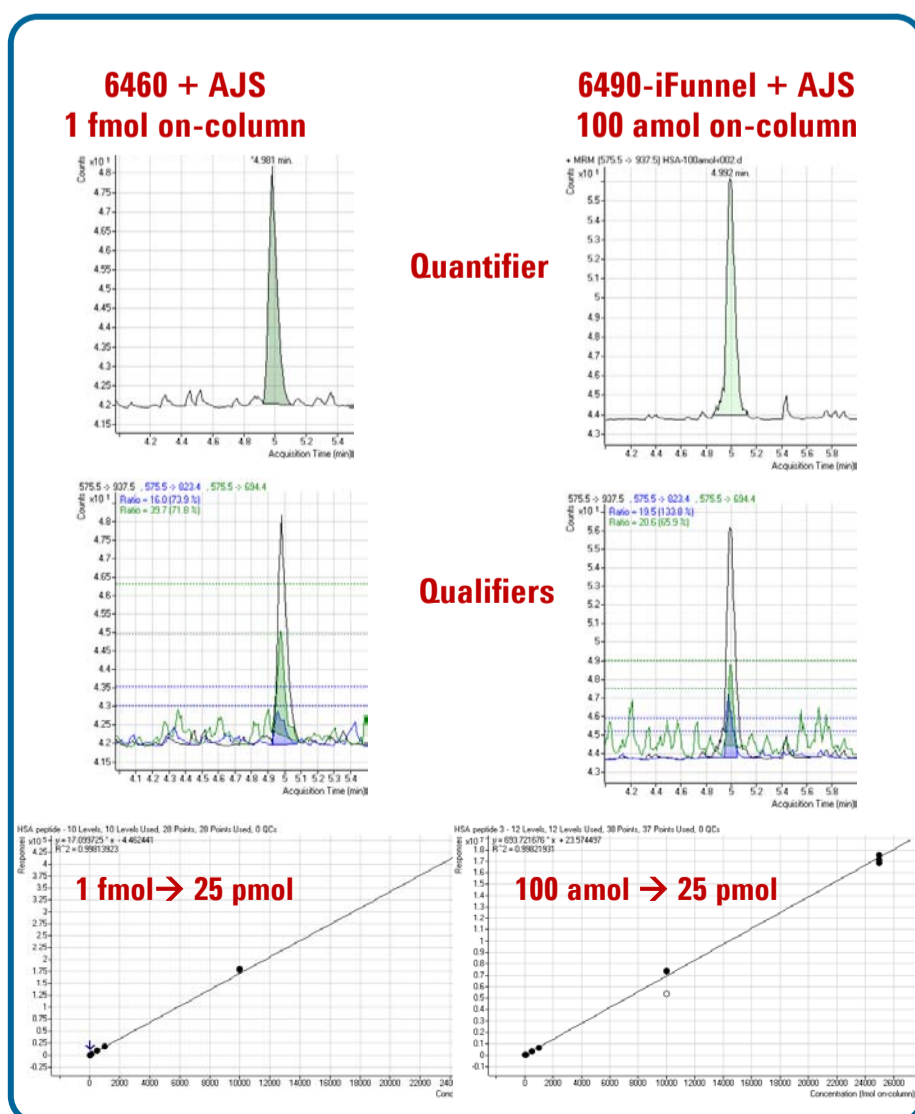


Impact on Sensitivity

6460 QQQ vs. 6490 QQQ Using Standard Flow LC

Instrument: 1290 LC system + 6460 or 6490 QQQ using AJS
Column: Poroshell 120 2.1 x 150 mm
Flow rate: 0.5 mL/min
Sample: HSA synthetic peptide standard LVNEVTEFAK

Results for this peptides showed a 10x improvement in sensitivity for the 6490 QQQ compared to the 6460 QQQ.



Impact of Flow Rate on Sensitivity

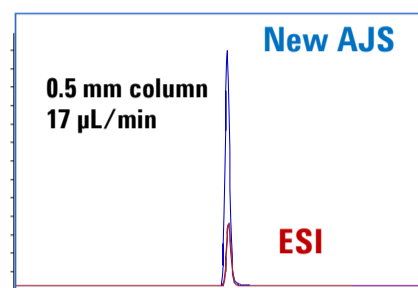
6490 QQQ Using Standard, Capillary and Nano Flow LC

LC System: 1290 Infinity, LC1200 capLC system or HPLC-Chip
LC Column: Zorbax SB-C18 columns 2.1 or 0.5 mm id x 150 mm for standard and cap flow. For nanoflow, HPLC-Chip with 40 nL enrichment column and 75 μm x 43 mm analytical column.

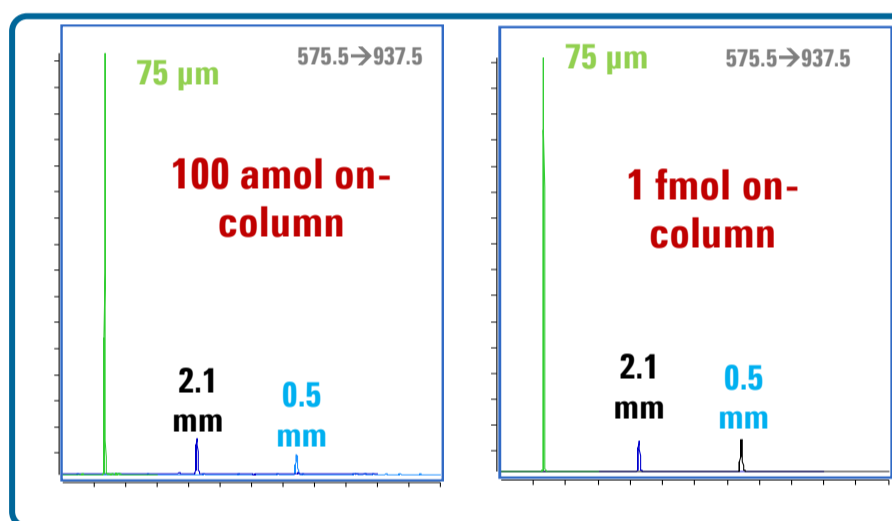
Flow rate: 300, 17 or 0.6 μL/min

Sample: HSA synthetic peptide standard LVNEVTEFAK spiked into trypsinized serotransferrin (10 fmol/μL).

For capillary flow LC, a new AJS source was used with active cooling of the nebulizer. This source gave a significant improvement in sensitivity compared to the ESI source with the same microflow nebulizer (right).



- Results (below) showed no significant difference between standard flow and capillary flow LC → AJS behaves as a mass detector not a concentration detector!
- Nanoflow LC still provides a significant increase in sensitivity most likely due to better sampling of the ions



Quantitation of Peptides in Plasma

6490 QQQ Using Standard Flow LC

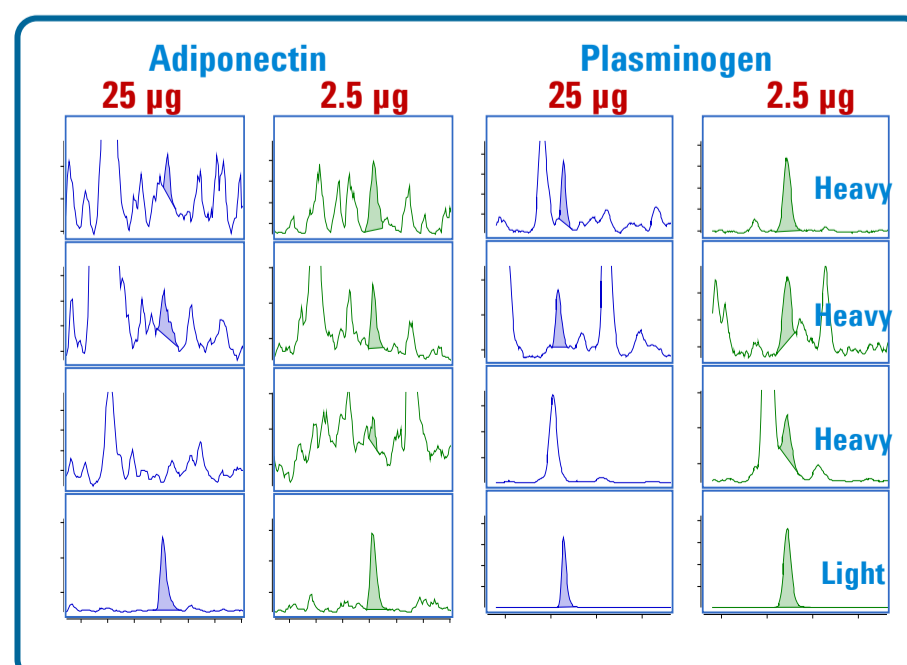
Instrument: 1290 LC system + 6460 or 6490 QQQ using AJS
Column: ZORBAX Eclipse Plus RRHD C18, 2.1x150mm, 1.8 μm, 95Å at 50 C

Flow rate: 0.4 mL/min

Sample: 12 stable isotope-labeled standard (SIS)¹ peptides spiked into a non-depleted plasma digest
MRM: 3 transitions for each heavy and light peptide
Injection volume: 10 μL

Impact of matrix: 10x dilution improves LOD!

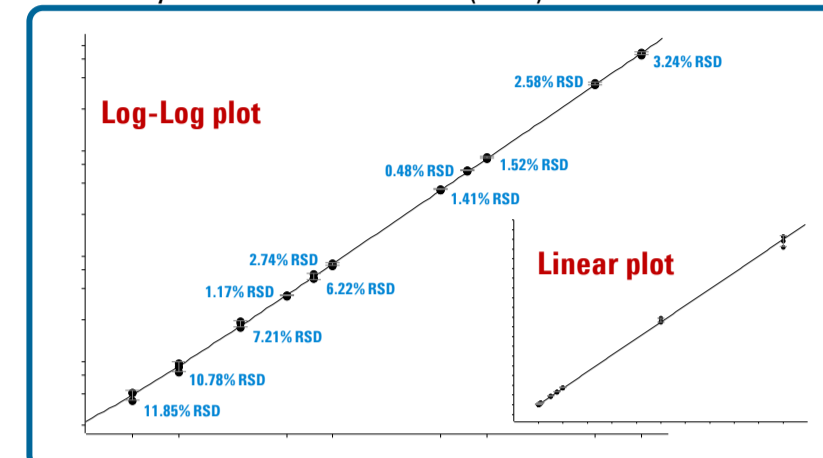
Initial analyses were done using 25 μg of plasma digest per injection. Diluting the sample 10-fold reduced matrix interference and improved the signal (below for 75 amol/μL sample).



Quantitation of Peptides in Plasma

Excellent sensitivity at standard LC flow rates

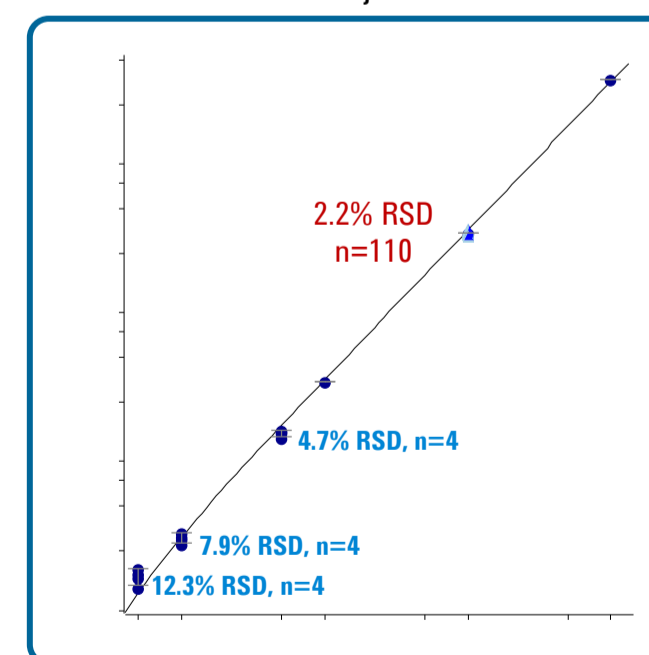
Calibration curves for the plasminogen SIS peptide from 5 – 10000 amol/μL in 250 ng/μL plasma digest. Area reproducibility noted for each level (n=3).



Protein	SIS sequence	Label	LOQ (amol/μL)	
			25 μg plasma	2.5 μg plasma
Adiponectin	IFYNQQNHVDGSGTGK	K+6	100	100
Antithrombin-III	DDLVSDFAFHK	K+6	75	50
Apolipoprotein precursor	A-II SPELQAEAK	K+6	500	100
Apolipoprotein C-III	GWVTDGFSSLK	K+6	25	25
Ceruloplasmin	EYTDASFTNR	R+6	500	500
Heparin cofactor II	TLEAQLTPR	R+6	10	25
Histidine-rich glycoprotein	DGYLFQLLR	R+10	25	10
Kininogen-1	TVGSDTFYSFK	R+6	75	25
L-selectin	AEIEYLEK	K+6	10	25
Plasminogen	LFLEPTR	R+6	10	5
Vitamin D-binding protein	THLPEVFLSK	K+6	10	25
von Willebrand Factor	ILAGPAGDSNVVK	K+6	50	25

Reproducibility of quantitation in plasma samples

- Robustness and reproducibility were assessed by analyzing 110 replicate injections of a QC standard (2.5 μg plasma protein +10 fmol of SIS peptides on-column per injection)
- Response reproducibility ranged from 2.2%RSD (plasminogen peptide) to 9.8%RSD (adiponectin peptide)
- Retention time reproducibility was less than 0.2%RSD for all peptides.
- After these 110 analyses, the sensitivity of the system remained unchanged. The figure below shows the calibration curves for the plasminogen peptide with points from before and after the 110 injections of the QC standard.



Conclusions

- The 6490 QQQ with iFunnel technology shows a 10x improvement in sensitivity compared to the 6460
- With the new AJS source, there was no significant gain in sensitivity from using a smaller column id (lower flows)
- Nanoflow is still significantly more sensitive than capillary or standard flow
- Using standard flow LC/MS on the 6490, we demonstrated robust, sensitivity peptide quantitation in plasma digests

Reference

¹M.A. Kuzyk, D. Smith, J. Yang, T.J. Cross, A.M. Jackson, D.B. Hardie, N.L. Anderson, and C.H. Borchers, "Multiple reaction monitoring-based, multiplexed, absolute quantitation of 45 proteins in human plasma," *Mol. Cell. Proteomics*, 8:1860-1877, 2009.