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Targeted Peptide Quantification with Small Foot-print Capillary LC-MS/MS

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Introduction

Quantification of low-abundance proteins remains a challenge for proteomics research. Alternatives to standard-flow LC-MS/MS are often sought-after, when sample limited or sensitivity driven, to detect the crucial players in pathways, cellular processes, or biopharma manufacturing. Capillary LC-MS/MS emerges as a compelling solution, offering exceptional sensitivity and minimal sample volume requirements. This technique not only boasts superior detection limits but also dramatically reduces hazardous organic solvent consumption. Lower flow rates also synergistically enhance sensitive mass spectrometry detection due to droplet size reduction.

We present a solid method for peptide quantification using capillary LC-MS/MS, emphasizing three key analytical parameters: (1) limit of detection (LOD) and limit of quantification (LOQ), (2) linear range for multiple peptides, and (3) precision. Sensitivity comparison between capillary LC/MS and standard (std)-flow LC/MS was also conducted. Sensitivity for all peptides surpassed std-flow LC-MS significantly.

Figure 1 Axcend Focus LC/Agilent Ultivo LC/MS/MS

Experimental

Sample Preparation

Sample information: IgG1 mAb (an R&D product from a partner) was produced from Chinese Hamster Ovary (CHO) cells and purified with protein A. Heavy stable isotope-labeled (SIL) peptide standards originating from distinct host cell proteins (HCP) were custom synthesized and provided by a third-party vendor.

The mAb digest was prepared by denaturation, reduction, alkylation, and trypsin digestion. SIL peptides were mixed at equimolar concentration and spiked into mAb digest at seven different levels (62.5, 125, 250, 6250, 12500, 25000, and 50000 amol for each SIL peptide) for standard curve analysis.

Experimental

LC/MS Analysis

Samples were analyzed by Axcend Focus LC coupled with Agilent Ultivo Triple Quadrupole LC/MS system in multiple reaction monitoring (MRM) mode using a five-minute LC gradient. The LC-MRM method was automatically optimized using the Agilent Automation tool integrated with Skyline and Agilent MassHunter workstation software.

Table 1 LC and Ultivo Triple Quadrupole parameters

	Capillary LC/MS Conditions	Std-flow LC/MS Conditions
Column	Reversed-phase C18, 150 µm X 100 mm, 1.8µm	Reversed-phase C18, 2.1 mm X 50 mm, 1.8 µm
Column temperature	40 ºC	40 ºC
Injection volume	250 nL	250 nL
Gradient	0 min 0.5% B 0.5 min 0.5% B 3 min 20% B 5 min 85% B 8 min 85% B	0 min 0.5% B 0.5 min 0.5% B 3 min 15% B 5 min 85% B 7 min 85% B 7.1 min 0.5%B
Mobile phase	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Flow rate	2 µL/min	500 µL/min
Gas temperature	200 ºC	300 ºC
Drying gas flow	6 L/min	13 L/min
Nebulizer gas	13 psi	40 psi
Sheath gas temp (AJS)	NA	250 ºC
Sheath gas flow (AJS)	NA	11 L/min

Method Optimization

Four peptides were selected for targeted quantification. All of them are from host cell proteins (HCP). MRM transitions were optimized. All transitions, peptide sequence and affiliated proteins are listed in Table 2.

Table 2 Targeted proteins, peptides, and transitions.

Targeted Protein	Peptide Sequences	MRM transitions (m/z)
G3HC31_CRIGR	LQDAEIAR	463.3 → 369.2 463.3 → 569.3 463.3 → 684.4 463.3 → 812.4 458.3 → 674.4
Protein S100-A11 (G3HUU6)	DPGVLDR	386.2 → 559.3 391.2 → 569.3 391.2 → 512.3 391.2 → 413.2
CO5_HUMAN	AFTECCVVASQLR	775.9 → 513.3 775.9 → 782.5 775.9 → 942.5 775.9 → 1102.5 770.9 → 1092.5
SUMO1_HUMAN	LLLEYLEEK	579.3 → 1044.6 579.3 → 931.5 579.3 → 818.4 579.3 → 689.4 575.3 → 923.5

Capillary LC/MS-ESI *vs* Std-flow LC/MS-ESI: Higher Sensitivity; Ultra Low Solvent Usage

Peptide abundance in protein digest matrix was compared between capillary LC/MS and std-flow LC/MS with ESI source. Capillary LC/MS shows 10× higher intensity than std-flow LC/MS on all four SIL peptides and uses 250 times less solvent.

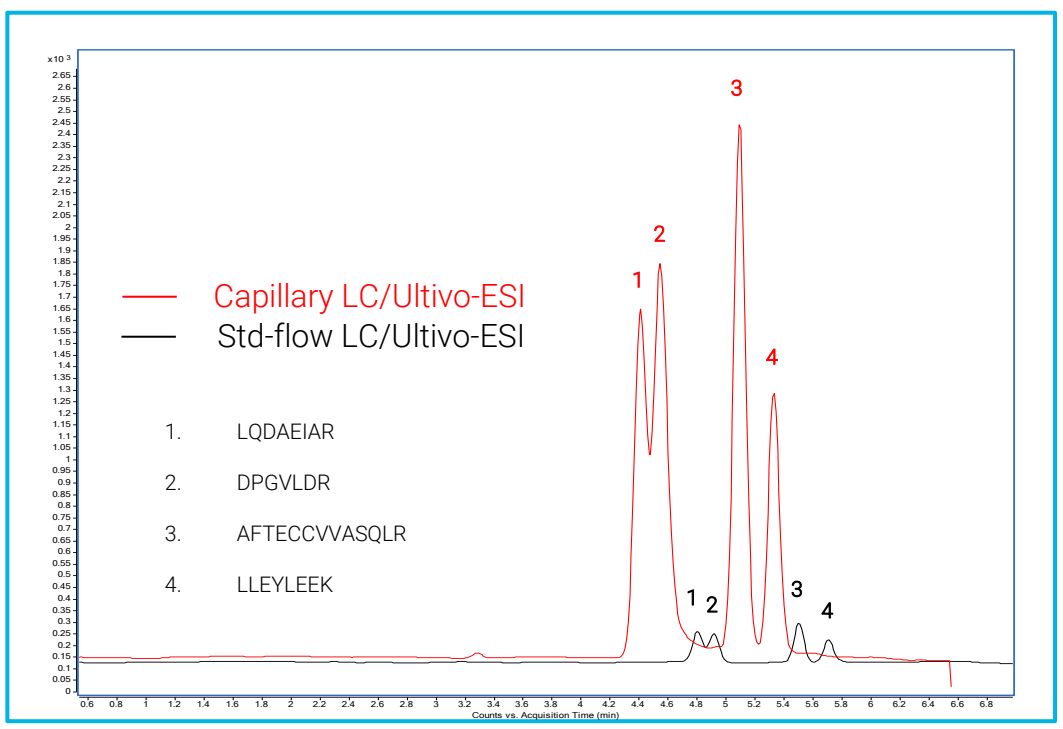

Figure 2 Abundance comparison between capillary LC/MS and std-flow LC/MS with ESI (25 fmol on column)

Capillary LC/MS-ESI *vs* Std-flow LC/MS-AJS: Comparable Sensitivity; Reduced Gas Usage; Ultra Low Solvent Usage

Peptide abundance in protein digest matrix was also compared between capillary LC/MS and std-flow LC/MS with Agilent Jet Stream (AJS) ion source which has high sensitivity for most analytes. As shown in Figure 3 below, capillary LC/MS still leads, with 2x higher peptide intensity on most of SIL peptides. Other than that, capillary LC/MS uses significantly less gas compared to std-flow LC/MS, typically around one-quarter (1/4) the amount.

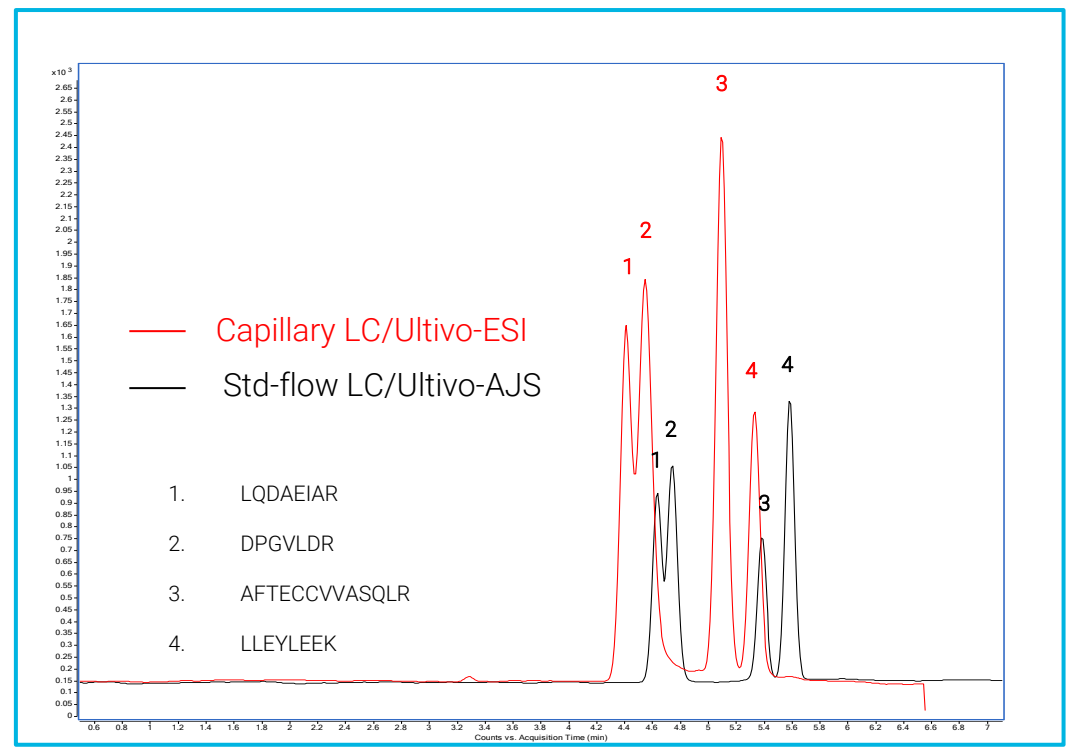

Figure 3 Abundance comparison between capillary LC/MS-ESI and std-flow LC/MS with AJS source

Linearity

Good linearity (>0.998) with nearly three orders of dynamic range (62.5 - 50,000 amol) was achieved for all four SIL peptides in protein digest matrix.

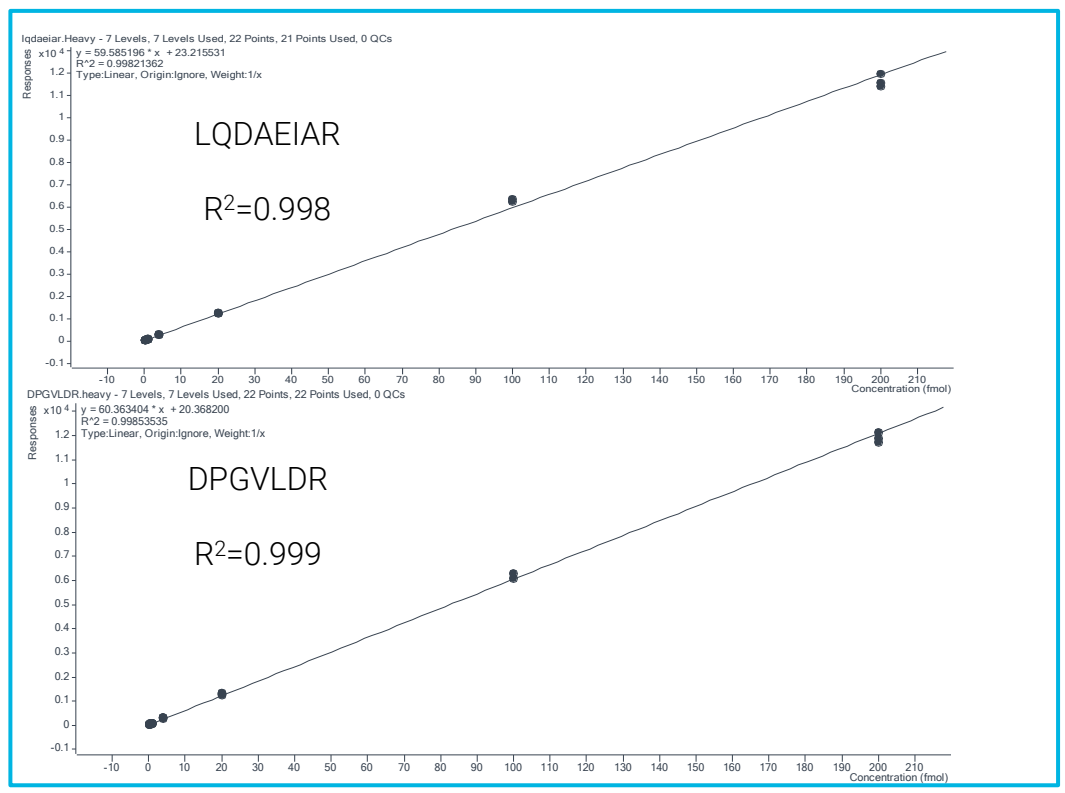

Figure 4a Standard curves for peptides LQDAEIAR and DPGVLDR from 62.5 to 50,000 amol.

Results and Discussion

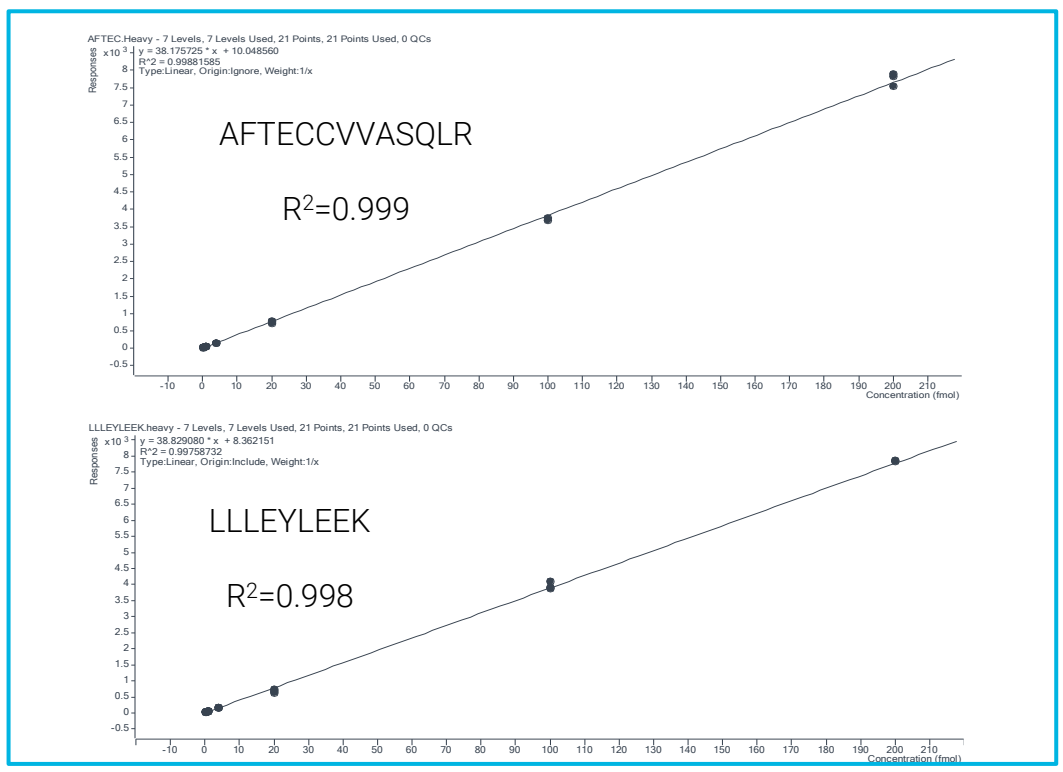

Figure 4b Standard curves for peptides AFTECCVVASQLR and LLLEYLEEK from 62.5 to 50,000 amol.

Sensitivity

LODs are as low as 62.5 amol for all 4 peptides with Axcend capillary LC/MS which are much lower than std-flow LC/MS with either ESI or AJS.

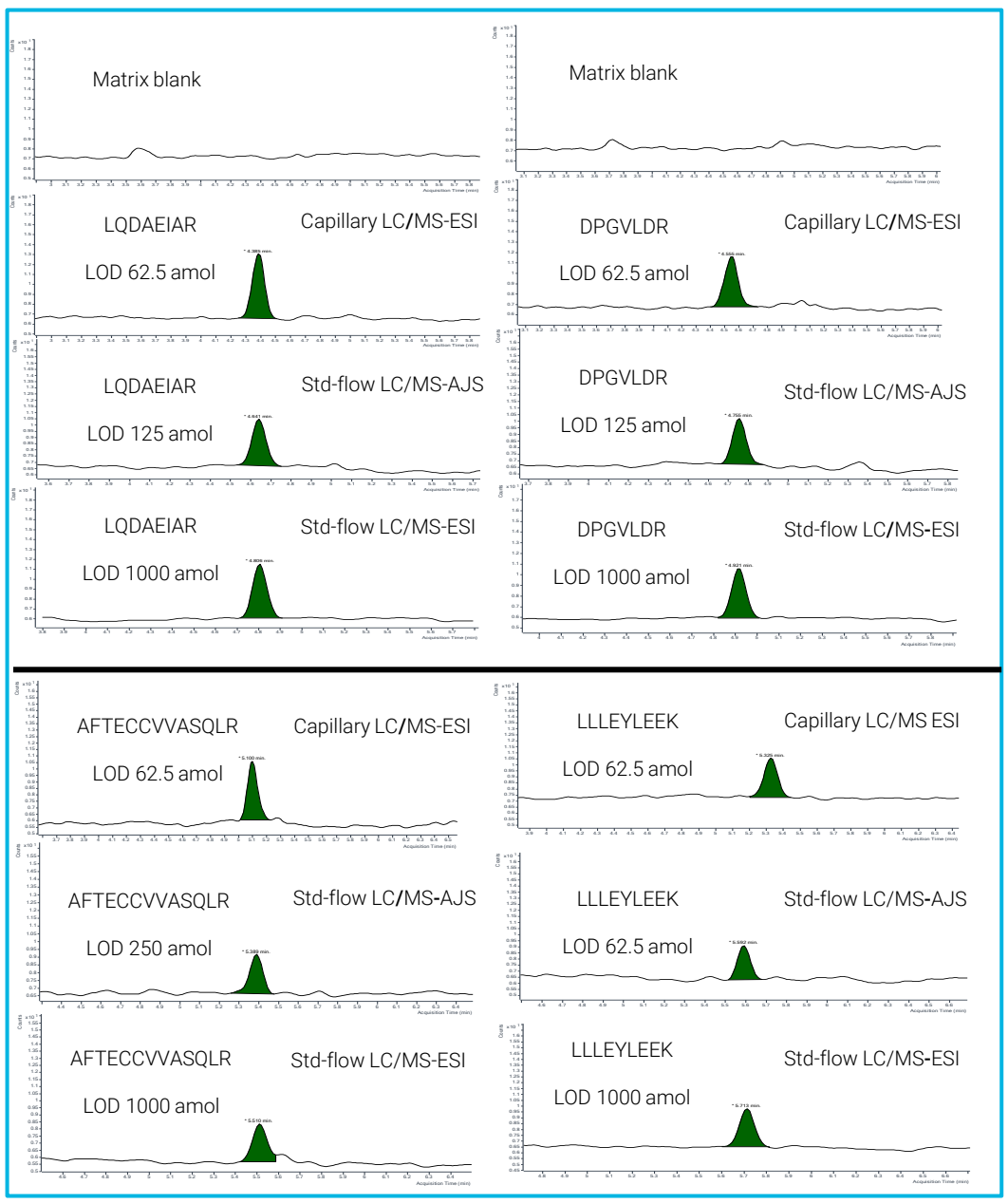

Figure 5 Stacked extracted ion chromatograms showing the LOD

Precision and Accuracy

Precision and accuracy testing on capillary LC/MS was established by running all levels of SIL peptides in three replicates. All peptides show good precision and accuracy at different levels.

High-precision retention times were achieved with all injections.

Table 3 Precision and accuracy for four SIL peptides in mAb matrix.

	LQDAEIAR			DPGVLDR		
On-column amount (amol)	%RSD (n=3)	%Accuracy	%RT RSD	%RSD (n=3)	%Accuracy	%RT RSD
62.5	16.3	79.4		8.2	61.5	
125	8.9	96.1		5.2	117.7	
250	7.1	105.3		7.3	111.7	
1,000	9.9	106.7	0.56	8.22	112.6	0.35
5,000	1.6	103.8		3.4	106.0	
25,000	2.8	103.3		2.7	100.9	
50,000	2.0	97.7		1.8	98.6	

	AFTECCVVASQLR			LLLEYLEEK		
On-column amount (amol)	%RSD (n=3)	%Accuracy	%RT RSD	%RSD (n=3)	%Accuracy	%RT RSD
62.5	17.6	95.1		9.0	136.4	
125	6.4	110.8		12.9	84.4	
250	10.1	106.2		8.3	97.6	
1,000	7.8	88.8	0.19	3.1	93.1	0.16
5,000	3.6	97.5		6.5	87.3	
25,000	1.3	97.5		3.2	101.7	
50,000	2.4	101.3		0.3	100.9	

Conclusions

A Solid Method for HCP Peptide Quantification was Developed for Axcend Capillary LC/Ultivo Triple Quadrupole LC/MS System.

- Axcend capillary LC/Ultivo TQ achieves over 10-fold improvement in sensitivity compared to std-flow LC/MS-ESI and 2-fold improvement to std-flow LC/MS-AJS.
- Capillary LC/MS dramatically cuts solvent use by a factor of 250 and significantly reduces gas consumption.
- Good linearity (>0.998) with nearly three orders of dynamic range was achieved for all peptides in protein digest matrix.
- This small foot-print capillary LC/MS platform shows high sensitivity, great precision and accuracy which is suitable for low level peptide quantification.

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