High sensitivity MRM workflow for signature peptide quantification

Featuring the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready, powered by SCIEX OS Software

Ian Moore[1], Lei Xiong[2]
[1]*SCIEX, Canada;* [2]*SCIEX, USA*

Quantification of peptide/protein therapeutics in biological matrices is crucial for therapeutic development. Serving as an orthogonal technology to the traditional ligand binding assays (LBAs), LC-MS has been routinely adopted for quantitative measurement of protein levels in bioanalytical laboratories. The capability of a triple quadrupole system to quantify low concentration analytes from small sample volumes, in line with current LBAs, has been a main stay of analytical analysis in this area. However, further sensitivity, improved %CV and linear dynamic range (LDR) operating down to lower concentrations would greatly benefit this analytical methodology.

While different MS approaches have been investigated by researchers, quantification using peptides as surrogates (bottom-up proteomic workflows) using targeted LC-MRM strategies remains the most common. It offers not only high sensitivity, but also a wide linear dynamic range (LDR) combined with high reproducibility to provide reliable quantitative measurements.

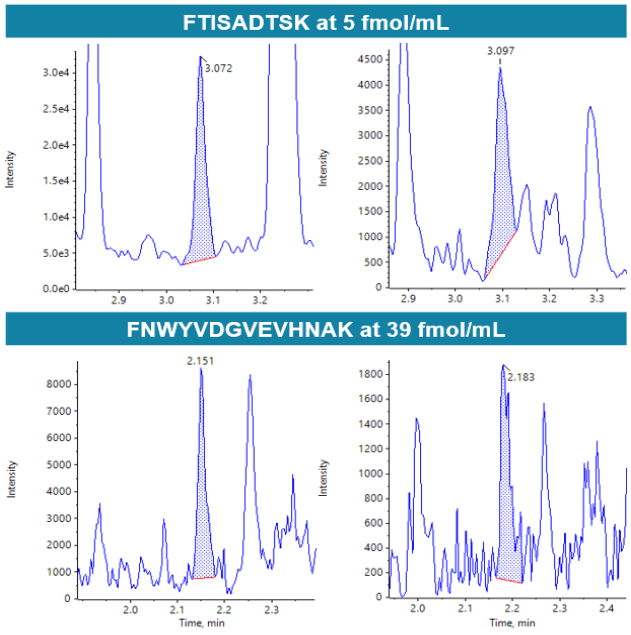

Figure 1. Sensitivity gains for peptide quantification. MRM XIC comparison between SCIEX 7500 System (left) and QTRAP 6500+ LC-MS/MS System (right) for mAb signature peptides at 5 or 39 fmol/mL in rat plasma.

Here, a SCIEX 7500 System was used to quantify a series of surrogate peptides in rat plasma. Multiple hardware improvements on the ion source and the front end of the mass analyzer significantly boost the systems sensitivity and low-level %CV values. Ultra-low LLOQs, ranging from 5 to 39 fmol/mL are achieved. The assay shows high reproducibility, precision, accuracy, and linearity, proving the robustness and performance of the developed method.

Key features of peptide quantification workflows

- Hardware improvements on the SCIEX 7500 System provide significant gains in sensitivity for peptide quantification: the OptiFlow® Pro Ion Source with E Lens™ Technology provides improvements in ion generation and the D Jet™ Ion Guide improves ion sampling[1]
- An average of 3-fold improvement in S/N was observed when analyzing surrogate peptides in biological matrices with high reproducibility, accuracy, and linearity
- SCIEX OS Software—an easy to use, compliance ready and single platform for acquisition, processing and data management

Methods

Sample preparation: Plasma proteins were precipitated with cold methanol. Upon centrifugation, supernatant was discarded. The pellet was solubilized in 200 mM ammonium bicarbonate in 10/90 (v/v) methanol/water. Digestion was performed using trypsin. After one hour at 60°C, the solution was acidified by adding formic acid.[1] The digested plasma was diluted by 200x using 5:1:94 (v/v/v) acetonitrile/formic acid/water. Synthesized peptides (Table 1) were spiked into the digested plasma solution and followed by serial dilution in matrix. Final injection volume was 10 µL.

Table 1. List of peptide targets.

Peptide Sequence	Description
FTISADTSK	*trastuzumab CDR region peptide*
FNWYVDGVEVHNAK	*conserved sequence in human immunoglobulin G (IgG)*
*AGLIVAEGVTK**	*synthetic peptide with C terminal K heavy isotope labeled ($C^{13}N^{15}$)*
*LGLDFDSFR**	*synthetic peptide with C terminal R heavy isotope labeled ($C^{13}N^{15}$)*

LC-MS conditions: Samples were analyzed in triplicate by a SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready, coupled with an ExionLC system. The method details are summarized in Tables 2 and 3. The same sample set was also injected into a SCIEX Triple Quad 6500+ LC-MS/MS System, coupled with the same HPLC system, to characterize the performance difference between the two mass spectrometers. All MRM parameters are optimized on both mass spectrometers for accurate performance comparison

Data processing: Data are processed using Analytics in SCIEX OS Software 2.0.

Table 2. Chromatographic conditions.

Parameter	Value
Column	*Phenomenex bioZen Peptide XB-C18 50×2.1 mm; 2.6 µm*
Mobile Phase A	*Water with 0.1 % formic acid*
Mobile Phase B	*Acetonitrile with 0.1 % formic acid*
Flow Rate	*500 µL/min*
Column Temperature	*40 °C*
Injection Volume	*10 µL*

Table 3. Gradient conditions.

Time [min]	Mobile Phase A [%]	Mobile Phase B [%]
0	*95*	*5*
5.5	*75*	*40*
5.6	*10*	*90*
6.0	*10*	*90*
6.1	*95*	*5*
7.0	*95*	*5*

Table 4. MS parameters on the SCIEX 7500 System.

Parameter	Value	Parameter	Value
Curtain gas	*42 psi*	*Source temperature*	450 °C
Ion source gas 1	*50 psi*	*Ion source gas 2*	70 *psi*
CAD gas	*9 psi*	*Ion spray voltage*	1500 V

Signature peptide quantification

The SCIEX 7500 System integrates innovations that provide improvements in both ion generation and ion sampling. The OptiFlow Pro Ion Source with E Lens Technology provides improvement in ion generation and the D Jet Ion Guide efficiently captures and transmits the ions in the high gas flow behind the orifice plate.

To identify the sensitivity improvements provided by these innovations, the same peptide sample sets were analyzed on both the SCIEX 7500 System and the SCIEX 6500+ System. On average, a 6-fold difference in peak area and a 3-fold difference in S/N were observed (Figure 1).

The peptide serial dilution samples were injected onto a SCIEX 7500 System to evaluate the overall quantification performance. As shown in Figures 2, 3 and 4, the LLOQs of the target peptides range from 5 to 40 fmol/mL, with LDR up to 4 orders of magnitude without internal standard normalization. For all quantified samples, the %CV was within 10% and the accuracy was within 94-106%.

Conclusions

- A highly sensitive peptide quantification workflow using the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready has been developed
- When combining the OptiFlow Pro Ion Source with E Lens Technology and D Jet Ion Guide, an average 3-fold improvement in sensitivity over the previous generation of instrumentation was observed when quantifying surrogate peptides in biological matrix

References

1. Enabling new levels of quantification. SCIEX technical note RUO-MKT-02-11886-A.
2. Ouyang Z. *et al.* (2012) *Bioanalysis* **4(1):** 17-28.

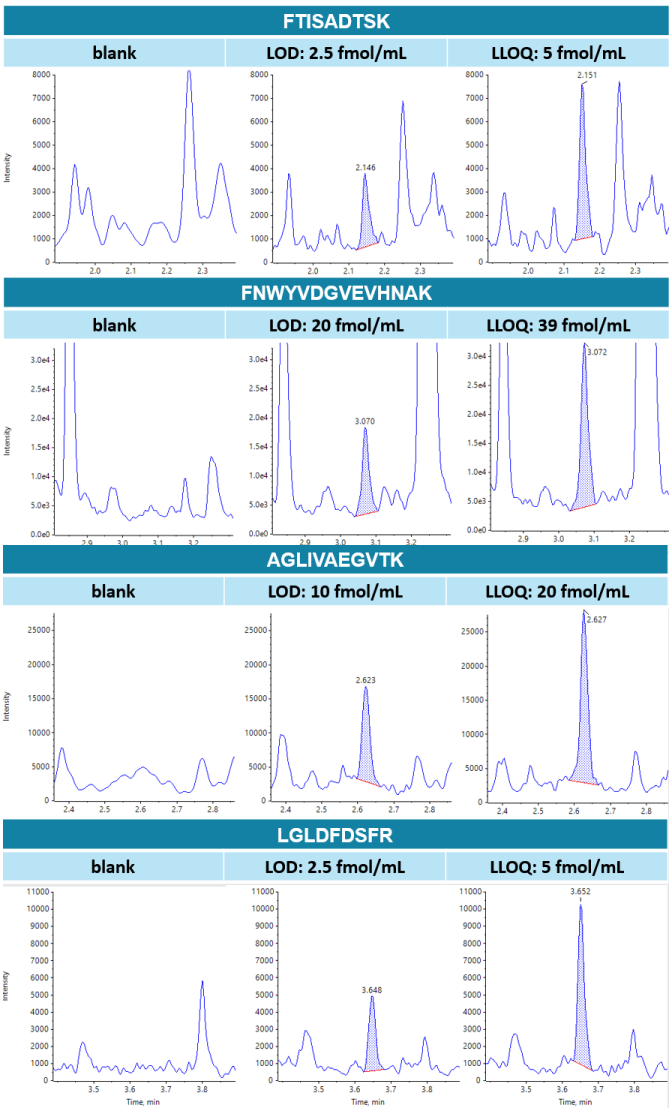

Figure 2. XICs of target peptide quantification in rat plasma. From left to right: in matrix blank, at LODs and LLOQs.

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
FTISADTSK	5.00	3 of 3	4.868e0	3.894e-1	8.00	97.36
FTISADTSK	10.00	3 of 3	9.419e0	1.550e-1	1.65	94.19
FTISADTSK	20.00	3 of 3	1.951e1	6.531e-1	3.35	97.56
FTISADTSK	39.00	3 of 3	3.967e1	5.048e-1	1.27	101.73
FTISADTSK	78.00	3 of 3	7.901e1	3.394e0	4.30	101.29
FTISADTSK	156.00	3 of 3	1.601e2	3.603e0	2.25	102.66
FTISADTSK	313.00	3 of 3	3.206e2	5.314e0	1.66	102.44
FTISADTSK	625.00	3 of 3	6.381e2	2.658e1	4.17	102.10
FTISADTSK	1250.00	3 of 3	1.272e3	5.123e1	4.03	101.75
FTISADTSK	2500.00	3 of 3	2.497e3	5.252e1	2.10	99.89
FTISADTSK	5000.00	3 of 3	4.930e3	1.015e2	2.06	98.60
FTISADTSK	10000.00	3 of 3	1.016e4	1.551e2	1.53	101.61
FTISADTSK	20000.00	3 of 3	1.967e4	3.129e2	1.59	98.34
FTISADTSK	40000.00	3 of 3	4.020e4	9.680e2	2.41	100.49

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
FNWYVDGVEVHNAK	39.00	3 of 3	3.943e1	2.157e0	5.47	101.10
FNWYVDGVEVHNAK	78.00	3 of 3	7.691e1	2.047e0	2.66	98.60
FNWYVDGVEVHNAK	156.00	3 of 3	1.536e2	5.674e0	3.69	98.45
FNWYVDGVEVHNAK	313.00	3 of 3	3.104e2	2.079e1	6.70	99.16
FNWYVDGVEVHNAK	625.00	3 of 3	6.216e2	1.164e1	1.87	99.45
FNWYVDGVEVHNAK	1250.00	3 of 3	1.244e3	1.977e1	1.59	99.49
FNWYVDGVEVHNAK	2500.00	3 of 3	2.594e3	3.973e1	1.53	103.76
FNWYVDGVEVHNAK	5000.00	3 of 3	4.949e3	1.497e1	0.30	98.98
FNWYVDGVEVHNAK	10000.00	3 of 3	1.025e4	1.141e2	1.11	102.46
FNWYVDGVEVHNAK	20000.00	3 of 3	1.989e4	2.022e2	1.02	99.45
FNWYVDGVEVHNAK	40000.00	3 of 3	3.943e4	8.714e2	2.21	98.58
FNWYVDGVEVHNAK	80000.00	3 of 3	8.041e4	1.874e3	2.33	100.51

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
AGLIVAEGVTK	20.00	3 of 3	1.905e1	1.436e0	7.54	95.27
AGLIVAEGVTK	39.00	3 of 3	4.116e1	4.313e-1	1.05	105.53
AGLIVAEGVTK	78.00	3 of 3	8.045e1	1.473e0	1.83	103.15
AGLIVAEGVTK	156.00	3 of 3	1.648e2	6.464e0	3.92	105.62
AGLIVAEGVTK	313.00	3 of 3	3.248e2	1.000e1	3.08	103.78
AGLIVAEGVTK	625.00	3 of 3	6.429e2	3.048e1	4.74	102.86
AGLIVAEGVTK	1250.00	3 of 3	1.276e3	4.018e1	3.15	102.08
AGLIVAEGVTK	2500.00	3 of 3	2.556e3	1.313e2	5.14	102.23
AGLIVAEGVTK	5000.00	3 of 3	4.970e3	5.112e1	1.03	99.40
AGLIVAEGVTK	10000.00	3 of 3	9.883e3	1.213e2	1.23	98.83
AGLIVAEGVTK	20000.00	3 of 3	1.886e4	4.672e2	2.48	94.32
AGLIVAEGVTK	40000.00	3 of 3	3.643e4	1.269e3	3.48	91.07
AGLIVAEGVTK	80000.00	3 of 3	7.669e4	2.622e3	3.42	95.86

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
LGLDFDSFR	5.00	3 of 3	5.058e0	4.337e-1	8.58	101.16
LGLDFDSFR	10.00	3 of 3	1.010e1	3.713e-1	3.68	100.96
LGLDFDSFR	20.00	3 of 3	1.890e1	9.260e-1	4.90	94.51
LGLDFDSFR	39.00	3 of 3	3.883e1	1.325e0	3.41	99.58
LGLDFDSFR	78.00	3 of 3	7.613e1	7.507e-1	0.99	97.60
LGLDFDSFR	156.00	3 of 3	1.530e2	3.346e0	2.19	98.10
LGLDFDSFR	313.00	3 of 3	3.138e2	9.416e0	3.00	100.25
LGLDFDSFR	625.00	3 of 3	6.195e2	9.281e0	1.50	99.12
LGLDFDSFR	1250.00	3 of 3	1.264e3	4.096e1	3.24	101.09
LGLDFDSFR	2500.00	3 of 3	2.506e3	1.276e2	5.09	100.25
LGLDFDSFR	5000.00	3 of 3	4.960e3	8.268e1	1.67	99.21
LGLDFDSFR	10000.00	3 of 3	1.025e4	3.253e2	3.17	102.53
LGLDFDSFR	20000.00	3 of 3	1.998e4	1.481e2	0.74	99.89
LGLDFDSFR	40000.00	3 of 3	4.230e4	1.816e3	4.29	105.76

Figure 3. Quantification result summaries.

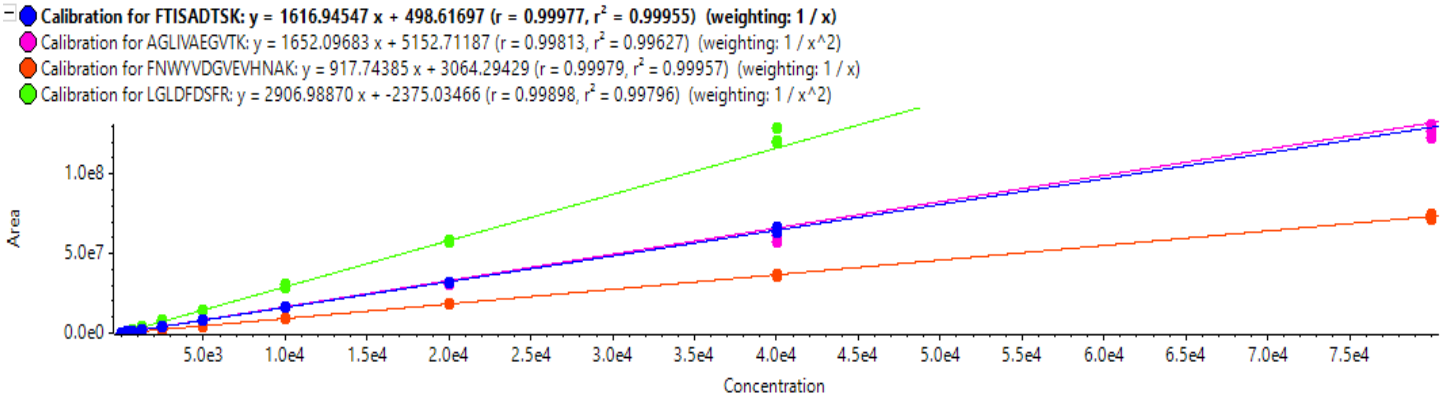

Figure 4. Calibration curves of target peptides with R^2 from 0.996 to 0.999.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices