

# The 6490 Triple Quad LC/MS Enables the Highest Sensitivity for Peptide Quantitation in Plasma



## Objective

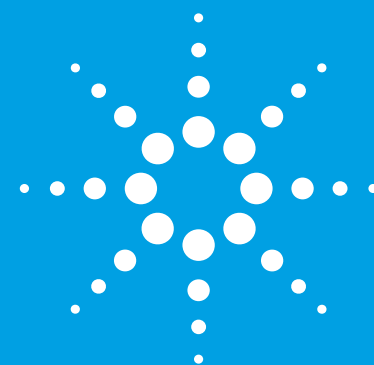
To show the high sensitivity of the 6490 Triple Quadrupole LC/MS System with iFunnel technology for quantitation of peptide samples in plasma.

## Background

The Agilent 6490 Triple Quadrupole mass spectrometer incorporates iFunnel technology to achieve new levels of sensitivity and dynamic range for detection of target peptides in complex matrices such as human plasma. The innovative design of the ion funnel reduces contamination and neutral molecules thus improving overall signal and reducing system noise.

Sensitivity is the key requirement for assays that are both specific and quantitative for target proteins. These assays are critical for clinical research of putative biomarkers, which may have low concentrations in commonly used biofluids such as serum and plasma. Often hundreds of target peptides need to be monitored in thousands of biological samples.

This note describes the results achieved for the analysis of stable isotope-labeled standard peptides spiked into digested non-depleted plasma using the 6490 Triple Quadrupole LC/MS System with iFunnel technology.



## Compound

- Stable isotope-labeled standard peptides spiked into non-depleted human plasma

## Key Benefits

- The results show attomole level sensitivity for quantitation of peptide samples in plasma using standard flow LC
- 110 replicate injections of peptides in plasma demonstrated robustness and reproducibility for these sample types
- The 6490 with iFunnel technology is ideal for the detection of target peptides in complex matrices such as human plasma



## The Approach

The enhanced sensitivity of the 6490 Triple Quad makes it feasible to use standard flow LC systems for many common target proteins. Interfacing the Agilent 1290 Infinity LC with a ZORBAX Rapid Resolution High Definition (RRHD) column to the 6490 LC/MS system provided the excellent retention time reproducibility and high peak capacity required for the analysis of peptides in non-depleted human plasma. Figure 1 shows the linearity and sensitivity achieved for one of the twelve stable isotope-labeled standard (SIS)<sup>1</sup> peptides spiked into digested non-depleted plasma. The accuracy ranged from 97 to 105% for the 11 levels of the calibration curve and the precision across all levels was 2.30 %RSD. The limit of detection was 50 amol on-column (Figure 2).

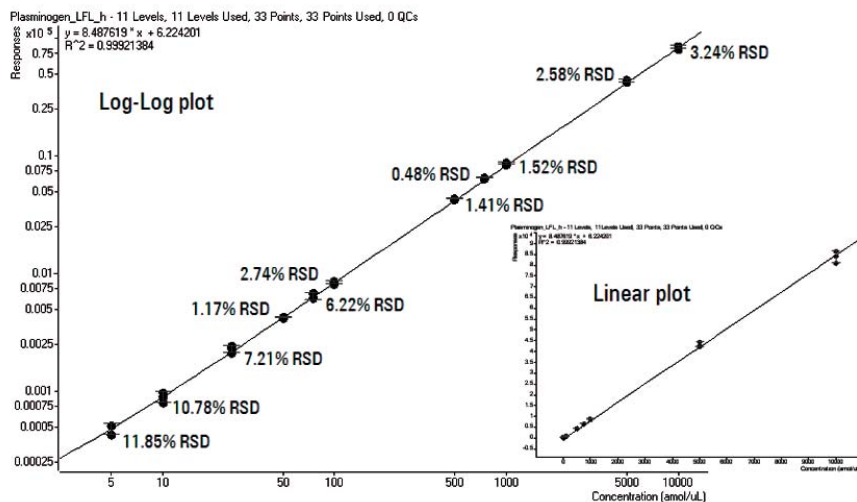


Figure 1: Log-log and linear (inset) calibration curves for the plasminogen SIS peptide (LFLEPTR) from 5 – 10000 amol/μL in 250 ng/μL plasma digest. Area reproducibility noted for each level (n=3).

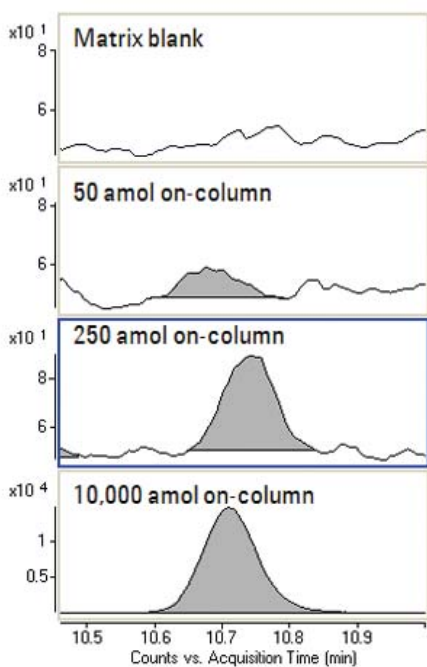


Figure 2: MRM chromatograms (441.3 → 621.4) for the plasminogen SIS peptide spiked at different levels in plasma matrix.

The robustness and reproducibility of the system were assessed by analyzing 110 replicate injections of digested non-depleted plasma spiked with the 12 SIS peptides (2.5 μg plasma protein and 10 fmol of SIS peptides on-column) on a 2.1 mm x 150 mm ZORBAX Eclipse Plus RRHD C18, 1.8 μm column at 0.4 mL/min. For these 110 QC analyses, the response reproducibility ranged from 2.2 %RSD (plasminogen peptide) to 9.8%RSD (adiponectin peptide) and the retention time reproducibility was less than 0.2%RSD. After these analyses, the sensitivity of the system remained unchanged, thus demonstrating robust performance for analysis in complex matrices.

## Summary

The Agilent 6490 Triple Quadrupole LC/MS System with iFunnel technology achieves excellent sensitivity and linearity for SIS peptides spiked into a plasma sample. Analyzing 110 replicate injections of this sample demonstrated robustness and reproducibility.

## Acknowledgment

The samples were kindly provided by Derek Smith and Christoph H. Borchers from the UVic-Genome BC Proteomics Centre.

## Reference

1. M.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.

## Learn more:

[www.agilent.com/chem/qqq](http://www.agilent.com/chem/qqq)

## Find a customer center in your country:

[www.agilent.com/chem/contactus](http://www.agilent.com/chem/contactus)

## U.S. and Canada

1-800-227-9770  
[agilent\\_inquiries@agilent.com](mailto:agilent_inquiries@agilent.com)

## Europe

[info\\_agilent@agilent.com](mailto:info_agilent@agilent.com)

## Asia Pacific

[inquiry\\_isca@agilent.com](mailto:inquiry_isca@agilent.com)

For research use only. Not for use in diagnostic procedures.

Information is subject to change without notice.

© Agilent Technologies, Inc. 2010  
Published in the U.S.A. September 15, 2010  
5990-6513EN



Agilent Technologies