Achieving higher sensitivity for LC-SRM MS quantification of low abundance biomarkers using advanced ESI source and interface technologies

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Overview

- Multicapillary/tandem ion funnel triple quadruple mass spectrometry (MS) technology combined with a novel antibody-free strategy significantly increases the sensitivity of biomarker detection and quantification.
- Low ng/mL limit of quantitation (LOQ) for target proteins in non-depleted human female serum can be achieved with as little as 2 µL serum.
- Addition of IgY14 depletion step in the sample analysis workflow further decreased the LOQ down to 50 pg/mL range.

Introduction

Selected reaction monitoring (SRM) MS coupled with liquid chromatography (LC) and stable isotope dilution is a powerful technique for high throughput quantitative analyses of protein biomarkers in biofluids. The major challenge of LC-SRM MS-based assays are insufficient sensitivity and specificity in detecting low abundance disease-related protein biomarkers (<1ng/mL) that are often "masked" by non-significant species present in orders of magnitude higher concentrations.[1]

A high sensitivity LC-ESI MS instrument that combines advanced ESI interface technology with a novel antibody-free strategy, referred to as high-Pressure high-Resolution Intelligent Selection and Multiplexing (PRISM), can greatly improve SRM MS sensitivity. Targeted proteins in human serum at low ng/mL level concentrations can be reliably quantified.

Excellent correlation has been obtained between PRISM-SRM MS and antibody-based immunoassay (ELISA) measurements in the quantification of PSA in a set of clinical serum samples from prostate cancer patients. The addition of IgY14 depletion further improved the LOQ of PRISM-SRM MS assay to ~ 50 pg/mL.

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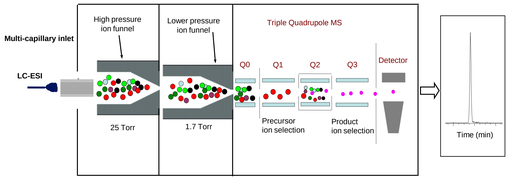
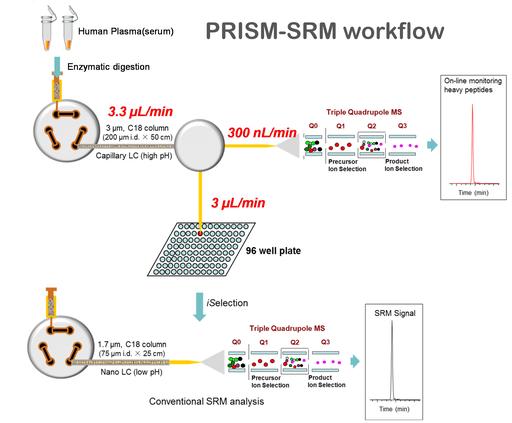
Methods

High sensitivity multicapillary/ tandem ion funnel SRM MS instrument





Target proteins and their surrogate peptides

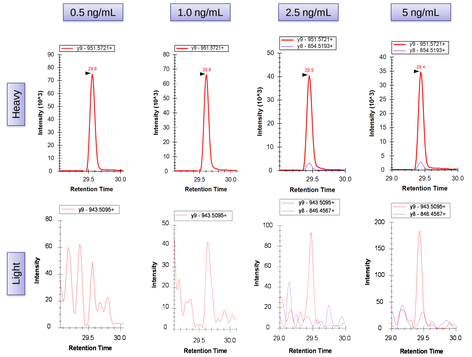
Proteins	MW (kDa)	Peptides	Precursor Ion (m/z)	Product Ion[b] (m/z)
Bovine carbonic anhydrase	29	DFPIANGER	509.9^{2+}	378.7^{+}, 756.4^{+}
		DGPLTGTYR	490.3^{2+}	404.2^{+}, 496.3^{+}
Bovine beta-lactoglobulin	20	VLVLDTDYKK	597.4^{2+}	882.5^{+}, 981.5^{+}
		VYVEELKPTPEGDLEILLQK	772.1^{2+}	976.5^{+}, 1026.2^{+}
Prostate-specific antigen (PSA)	30	IVGGWEC$_{amc}$EK[a]	539.4^{2+}	213.1^{+}, 865.4^{+}
		LSEPAELTDAVK	636.9^{2+}	943.5^{+}, 846.5^{+}

[a] Cysteine was synthesized as carbamidomethyl cysteine.
[b] Two transitions were used for plotting calibration curves for target proteins and correlation curves between calculated and expected target protein concentrations in plasma.

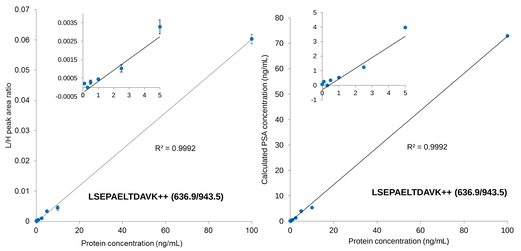
Results

PRISM-SRM MS quantification of targeted peptide LSEPAELTDAVK^{2+} derived from prostate specific antigen (PSA) in non-depleted and depleted female human serum

a) PSA in non-depleted serum sample:



Extracted ion chromatography for selected SRM transitions at different concentrations

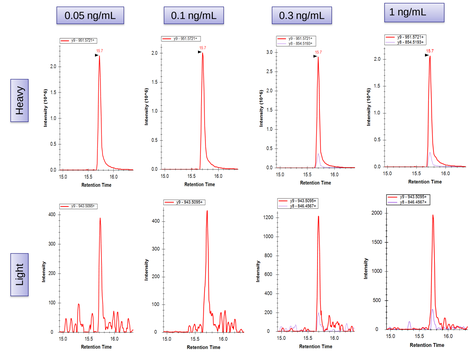


Concentration (ng/mL)	0	0.1	0.3	0.5	1	2.5	5	10	100
Light/Heavy[c]	6.28×10^{-5}	2.09×10^{-4}	0	2.86×10^{-4}	4.44×10^{-4}	1.03×10^{-3}	3.28×10^{-3}	4.39×10^{-3}	6.03×10^{-2}
CV(%)	173	11	0	38	14	19	11	17	3

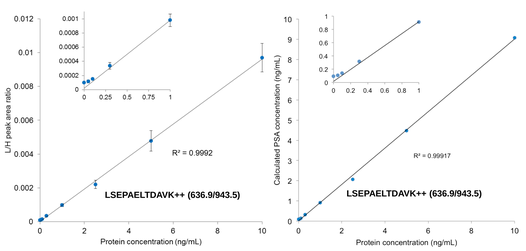
[c] Heavy peptides were spiked in at 0.5 fmol/µL.

Triplicate PRISM-SRM MS quantification of PSA in non-depleted female human serum

b) PSA in IgY-14 depleted serum sample



Extracted ion chromatography for selected SRM transitions at different concentrations

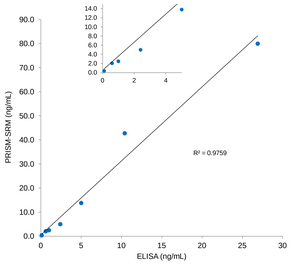


Concentration (ng/mL)	0	0.05	0.1	0.3	1	2.5	5	10
Light/Heavy[d]	9.9×10^{-5}	1.2×10^{-4}	1.5×10^{-4}	3.4×10^{-4}	9.8×10^{-4}	2.2×10^{-3}	4.8×10^{-3}	9.7×10^{-3}
CV(%)	13	18	1.7	14	9	11	13	9

[d] Heavy peptides were spiked in at 2 fmol/µL.

Triplicate PRISM-SRM MS quantification of PSA in IgY-14 depleted female human serum

c) Correlation between PRISM-SRM MS and ELISA PSA measurements of 7 prostate cancer patient serum samples



Conclusions

- PRISM-SRM MS with an advanced ESI-MS interface technology enabled detection and quantification of low abundance proteins in biofluids with good sensitivity and high reproducibility.
- Excellent correlation between PRISM-SRM MS-based and ELISA assays was demonstrated by measuring PSA from cancer patient samples.
- With addition of IgY14 immunoaffinity depletion, the LOQ for a PRISM-SRM MS-based assay can be further improved to 50-100 pg/mL in human plasma/serum.

Acknowledgements

This work was supported by the NIH National Cancer Institute (1R33CA155252). Research used capabilities developed under the support of the NIH National Center for Research Resources (5P41 RR018522-10) and National Institute of General Medical Sciences (8 P41 GM103493-10), and the U.S. Department of Energy Biological and Environmental Research (DOE/BER). Significant portions of the work were performed in the Environmental Molecular Science Laboratory, a DOE/BER national scientific user facility at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. PNNL is operated for the DOE by Battelle under contract DE-AC05-76RLO-1830.

References

1. Keshishian H, Addona T, Burgess M, Kuhn E and Carr SA. Quantitative, multiplexed assays for low abundance proteins in plasma by targeted mass spectrometry and stable isotope dilution. *Mol Cell Proteomics* 6: 2212-2229 (2007).