Overview

- IgY14-SuperMix on-line immunodepletion process was coupled with LC-SRM-MS to analyze human female plasma spiked with five non-human proteins and human PSA at 7 concentration ranges in addition to three endogenous proteins.

- A side-by-side comparison of the limit of detection (LOD) for IgY14 only and IgY14-SuperMix tandem depletion was performed.

- Analytes processed with IgY14-SuperMix tandem depletion provided considerable enhancement in sensitivity for detecting low-abundance spiked-in proteins in human female plasma.

Introduction

With improved capability for high throughput candidate quantification and verification, selected reaction monitoring mass spectrometry (SRM-MS) coupled with liquid chromatography (LC) is playing an increasingly important role in quantitative protein analysis. SRM-MS is a powerful approach for the detection and quantification of specific peptides. The major advantages of SRM-MS over traditional methods include high sensitivity, high selectivity, and the ability to analyze complex mixtures with minimal sample pretreatment.

Evaluation of the loading capacity for immunodepletion columns

- Three different amounts of plasma samples (see below) in triplicate were injected into the IgY14-SuperMix depletion system to assess the loading capacity.

- Trypsin digestion with TFE treatment followed by LC-MS/MS (Thermo LTQ mass spectrometer) analysis for each transition.

- Loading volume of 125 μL was used for the remainder of the experiments.

Selection of peptides, transitions, and Optimization of SRM-MS analysis

- Two heavy synthetic peptides per protein (only one peptide from CRP) were analyzed by LC-SRM-MS, and three transitions were selected for each peptide in the SRM analysis.

- Scaled SRM-MS was employed with five segments.

- Optimized collision energies were used for each transition. For data analysis purposes, only the best transition from each peptide was considered.

Results

Evaluation of the loading capacity for immunodepletion columns

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide</th>
<th>Kinetic energy (eV)</th>
<th>Product ion (m/z)</th>
<th>Precursor ion (m/z)</th>
<th>y</th>
<th>LOD (ng/mL)</th>
<th>%CV</th>
<th>Fold-through</th>
<th>Bound %</th>
<th>Bound LOD (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>YKQVHPTGK</td>
<td>3.1960</td>
<td>536.2372</td>
<td>272.1641</td>
<td>1</td>
<td>52.94</td>
<td>21.79</td>
<td>42.45</td>
<td>4.77</td>
<td>6.8</td>
</tr>
<tr>
<td>HSA bundle</td>
<td>SUVAPKPPK</td>
<td>3.1960</td>
<td>536.2372</td>
<td>272.1641</td>
<td>1</td>
<td>52.94</td>
<td>21.79</td>
<td>42.45</td>
<td>4.77</td>
<td>6.8</td>
</tr>
<tr>
<td>Troponin Tc</td>
<td>TQDENPVVHFFK</td>
<td>3.1960</td>
<td>536.2372</td>
<td>272.1641</td>
<td>1</td>
<td>52.94</td>
<td>21.79</td>
<td>42.45</td>
<td>4.77</td>
<td>6.8</td>
</tr>
<tr>
<td>Lactoglobulin</td>
<td>GYSIFSYATK</td>
<td>3.1960</td>
<td>536.2372</td>
<td>272.1641</td>
<td>1</td>
<td>52.94</td>
<td>21.79</td>
<td>42.45</td>
<td>4.77</td>
<td>6.8</td>
</tr>
<tr>
<td>Chicken ovalbumin</td>
<td>DGPLTGTYR</td>
<td>3.1960</td>
<td>536.2372</td>
<td>272.1641</td>
<td>1</td>
<td>52.94</td>
<td>21.79</td>
<td>42.45</td>
<td>4.77</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Conclusions

- IgY14-SuperMix tandem depletion coupled with scheduled SRM-MS provided ~1–5 ng/mL detection limit with ~10–100 times sensitivity enhancement (without fractionation) in detecting low-abundance spiked proteins in human plasma compared to IgY14 only depletion.

- IgY14-SuperMix tandem depletion not only afforded improved LODs in protein values, but also in the reproducibility for the majority of peptides in this study compared to IgY14 only depletion.

- CRP was detected in both depletion processes, however, the signal intensity was comparatively higher for the IgY14-SuperMix tandem depleted samples.

- Tandem depletion flow-through was found to provide only free PSA whereas the IgY14 only depleted flow-through contained both free and bound PSA (with ACT).

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References