INTRODUCTION

- Thyroglobulin is a 660 kDa dimeric protein proposed as a clinical research biomarker for evaluating treatment efficiency in thyroid cancer recurrence.
- Current research immunoassays yield high false negative rates.
- SISCAPA combined with standard flow LC/MS has been implemented as an alternative approach in clinical research labs.
- Microflow LC/MS operating at <5 µL/min offers substantial analytical sensitivity benefits over standard flow.

METHODS

SISCAPA Enrichment Workflow

- Column: 150 µm x 50 mm PST BEH C18 iKey
- Trap: 300 µm x 50 mm Symmetry C18
- MS: Xevo TQ-S operated in MRM mode
- Inject: 20 µL partial loop in a 22.8 µL loop
- Gradient: 9.9 to 27.5%B in 2.2 min @ 3 µL/min
- Trap Loading: 50 µL/min for 0.8 min
- Cycle Time: 6.75 min

RESULTS

Experiment 1: Serial Dilution of Peptide Standards

<table>
<thead>
<tr>
<th>Amol on Column</th>
<th>Avg Light Area</th>
<th>CV</th>
<th>Avg Heavy Area</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>40000</td>
<td>1343852.3</td>
<td>4.9%</td>
<td>1181870.0</td>
<td>4.4%</td>
</tr>
<tr>
<td>10000</td>
<td>267515.0</td>
<td>1.1%</td>
<td>234487.0</td>
<td>0.5%</td>
</tr>
<tr>
<td>1600</td>
<td>406532.7</td>
<td>4.8%</td>
<td>32011.7</td>
<td>1.5%</td>
</tr>
<tr>
<td>320</td>
<td>8312.3</td>
<td>2.0%</td>
<td>7147.7</td>
<td>2.6%</td>
</tr>
<tr>
<td>64</td>
<td>3161.0</td>
<td>4.9%</td>
<td>1640.0</td>
<td>1.3%</td>
</tr>
<tr>
<td>12.8</td>
<td>246.0</td>
<td>16.3%</td>
<td>254.0</td>
<td>16.4%</td>
</tr>
</tbody>
</table>

Experiment 2: Human Plasma Titration Of Varying Amounts

Experiment 3: Reverse Curve for Heavy FSP

- LLOD is 15 amol FSP on column.
- LLOQ, the point at which CV >20%, is 45 amol FSP on column.

Experiment 4: Titrating Tg Protein in Bovine Plasma

- LLOQ of the assay including the digestion step using 50 µL of sample is estimated to be 0.78 ng/mL.

Figure 1. Analytical workflow employed. Peptides of Tg are derived by digesting plasma using trypsin. A unique peptide specific to Tg, FSPDSSAGASALLR (FSP) and the SIS are selectively enriched by incubation with an anti-FSP antibody conjugated to a magnetic bead. The beads are then washed to remove unbound matrix and the bound peptides released for analysis by microflow LC/MS using acid elution.

Microflow LC/MS Configuration and Method

- Column: 150 µm x 50 mm PST BEH C18 iKey
- Trap: 300 µm x 50 mm Symmetry C18
- MS: Xevo TQ-S operated in MRM mode
- Inject: 20 µL partial loop in a 22.8 µL loop
- Gradient: 9.9 to 27.5%B in 2.2 min @ 3 µL/min
- Trap Loading: 50 µL/min for 0.8 min
- Cycle Time: 6.75 min

CONCLUSION

- Microflow is well suited to analyze SISCAPA extracts.
- A <1 ng/mL quantification limit of Tg is achieved with a cycle time of 6.75 min.
- LLOQ is comparable with the best in literature for standard flow while utilizing a simplified enrichment, 10x less volume of starting plasma, 1/2 the injection volume, and cycle times that are only 0.25 min longer.


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