INTRODUCTION

Due to their heterogeneous nature, ADCs require multiple bioanalytical assays to quantify the various forms. One important assay is the total antibody measurement. The total antibody measurement is the sum of all the conjugated antibody species plus the unconjugated antibody. Different protein sample preparation techniques can be combined with LC-MS/MS analysis to make a total antibody measurement. By combining immunocapture sample preparation with LC-MS/MS detection more selective assays with lower LLOQs are possible over a direct plasma or pellet digest. The purpose of this study was to develop an immunocapture LC-MS/MS method for the selective quantitation of the ADC, ado-trastuzumab emtansine using the BioBA sample prep kit.

MATERIALS AND METHODS

Sample Preparation:
Ado-trastuzumab emtansine spiking solutions (10x) were serially diluted from a stock solution (20 mg/mL) and used to spike rat plasma (KEDTA). Immunocapture beads were prepared with goat anti-human IgG (Southern Biotech) following the BioBA kit procedure. Each calibration standard and QC sample was processed with a 25 µL aliquot of prepared beads. A 50 µL aliquot of plasma was diluted 2-fold with internal standard solution (BLUAMAB, Sigma-Aldrich) and added to the beads. Samples were incubated with beads for 1 hr with mixing. Samples were incubated with beads for 1 hr with mixing. Samples were eluted from the beads for 10 minutes at 35 °C, then neutralized, reduced, alkylated and digested using the components of the BioBA kit following the example protocol. The reaction times and temperatures were as follows: reduction for 1 hour at 50 °C, alkylolation for 30 minutes at room temperature and digestion at 37 °C for 3.5 hours.

HPLC Conditions:

**System** Stmatzu LC-20
**Column** Phenomenex 2.6 µm, Kinetex C18 50 x 2.1 mm, 1.0 µL/min
**Flow rate** 350 µL/min
**Run Time** 7 minutes
**Rinsing Solution** 60:20:20 IPA: Methanol: Acetonitrile

**System** SCIEX M3 MicroLC System
**Column** Ekaigen, HALO Peptide-ES, C18, 0.3 x 50 mm, 2.7 µm
**Flow rate** 8 µL/min
**Column temperature** 40 °C
**Injection volume 5 µL**
**Run Time** 7 minutes
**Rinsing Solution** 60:20:20 IPA: Methanol: Acetonitrile

**MS/MS Conditions:**
The MRM analysis was performed on a SCIEX QTRAP 6500® system equipped with an IonDrive™ Turbo V source. The source parameters plus probe and electrode positions were optimized prior to the analytical run.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Q1</th>
<th>Q3</th>
<th>DP (V)</th>
<th>CE (V)</th>
<th>CXP (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTLMISR</td>
<td>Heavy</td>
<td>423.2</td>
<td>518.3</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td>DTLMISR</td>
<td>418.5</td>
<td>506.2</td>
<td>40</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>FTISADTSK</td>
<td>485.2</td>
<td>721.3</td>
<td>90</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>IYPTNGYTR</td>
<td>542.8</td>
<td>688.4</td>
<td>60</td>
<td>16</td>
<td>11</td>
</tr>
</tbody>
</table>

RESULTS

**Signature Peptide Selection and Digest Optimization**

The signature peptides IYPTNGYTR and DTLMISR from the CDR region of trastuzumab and the total antibody DTLMSIR were chosen for quantitation due to the absence of a lysine residue. Due to the heterogeneous nature of trastuzumab conjugates, the calibration standard and QC sample concentrations analyzed in this study.

Digest Surfactant Comparison

The BioBA sample preparation kit includes an anionic mass spec compatible surfactant to improve digest efficiency and signature peptide yield. The signature peptide yield from digests using the BioBA surfactant (0.02%) was compared to a neutral mass spec compatible surfactant OGS (0.6%) and a second commercially available anionic surfactant (0.9%).

Immunocapture

Immunocapture from plasma was performed with goat anti-human IgG antibody coated BioBA magnetic beads. This antibody will capture all ADC species including those without payload attachment and gives a total antibody measurement.

After immunocapture from plasma the samples were processed following the BioBA protocol which included washing, elution (pH ~2.2), neutralization, reduction (TCEP), alkylolation (IAM), and digestion with trypsin/inuss and surfactant. Samples were prepared for LC injection by stopping the digestion with formic acid and diluting 5-fold with water. Figure 4 shows the steps in the BioBA sample processing workflow and the calibration standard and QC samples analyzed.

**CONCLUSIONS**

1. Robust bioanalysis of ADCs
   Achieved accurate quantitation of ado-trastuzumab emtansine. BioBA sample prep kits provide the necessary reagents and protocols for enrichment and detection of a wide range of large molecules.
2. Gold Standard & Proven LC-MS performance
   Integrated LC/MS capabilities for both standard flow and microflow. M3 MicroLC System offers sensitivity increase.

<table>
<thead>
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<th>Figure 1</th>
<th>Figure 2</th>
<th>Figure 3</th>
<th>Figure 4</th>
<th>Figure 5A</th>
<th>Figure 5B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deamidation and rearrangement of the signature peptide DTLMISR signature peptide yield from three different mass spec compatible surfactants.</td>
<td>Signature peptide yield from three different mass spec compatible surfactants.</td>
<td>Total immunocapture strategy to capture all ADC species including those without payload attached and gives a total antibody measurement.</td>
<td>The steps of the BioBA sample processing workflow and the calibration standard and QC sample concentrations analyzed in this study.</td>
<td>The calibration curves and accuracy and precision statistics for the signature peptide DTLMISR from the total antibody assay of ado-trastuzumab emtansine.</td>
<td>The calibration curves and accuracy and precision statistics for the signature peptide IYPTNGYTR from the total antibody assay of ado-trastuzumab emtansine.</td>
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</tbody>
</table>

**TRADEMARKS/LICENSES**

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