An Immunoaffinity Coupled High Resolution-MS Workflow for Quantifying Biotherapeutics in Rat Plasma

Featuring SCIEX X500B QTOF System Coupled with SCIEX ExionLC™ UHPLC System

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Quantification of monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) in biological matrix is required during multiple stages of the biotherapeutic development process. LC-MS is serving as an orthogonal methodology to ligand binding assays (LBA) for biotherapeutics quantification. Increased interest has been observed in simplifying LC-MS method development and exploring new quantification workflows including high resolution accurate mass spectrometry (HRAMS). The HRAMS technology provides quantification based on the precursor ion of the target analyte, which requires minimum MS optimization compared to MRM based quantification. Herein, we report a HRAMS method to quantify SILuLite SigmaMAb and trastuzumab emtansine in rat plasma, using SCIEX X500B QTOF system with ExionLC™ UHPLC system.

Key Feature of the SCIEX Immunoaffinity-HRAMS Solution

- SCIEX X500B QTOF System with ExionLC UHPLC system (Figure 1) provides:
  - High sensitivity, robustness and throughput for biotherapeutics quantitation
  - Easy method setup with minimum MS optimization
  - Protein quantification and characterization on a single instrument
- Optimized immunoaffinity sample preparation provides:
  - Decreased sample complexity and matrix interference;
  - Desired assay linear dynamic range;
  - Shortened sample preparation time.

Methods

Beads Preparation for Immuno-Capture: As shown in Figure 1, an immunoaffinity bead slurry coated with streptavidin was aliquoted and washed with 1x PBS buffer three times and incubated with Biotinylated Goat Anti-Human IgG Antibody at 100:1 ratio for 1 hour at room temperature with a shaking speed of 1200 RPM. The conjugated beads were subsequently washed and re-suspended with 1x PBS buffer.

Immuno-Capture of Target Analytes: The calibration standard solutions were prepared through serial dilutions and mixed with 100 µL rat plasma. The final concentrations of SILuLite SigmaMAb in plasma are 25, 50, 100, 1000, 5000, 10000, 20000, 50000, 100000 ng/mL, while the final concentrations of trastuzumab emtansine are 10, 25, 50, 100, 500, 5000, 10000, 20000, 50000 ng/mL. The mixtures were subsequently mixed with SILuMab internal standards and incubated with 50 µL conjugated bead slurry for 1 hour at room temperature with a

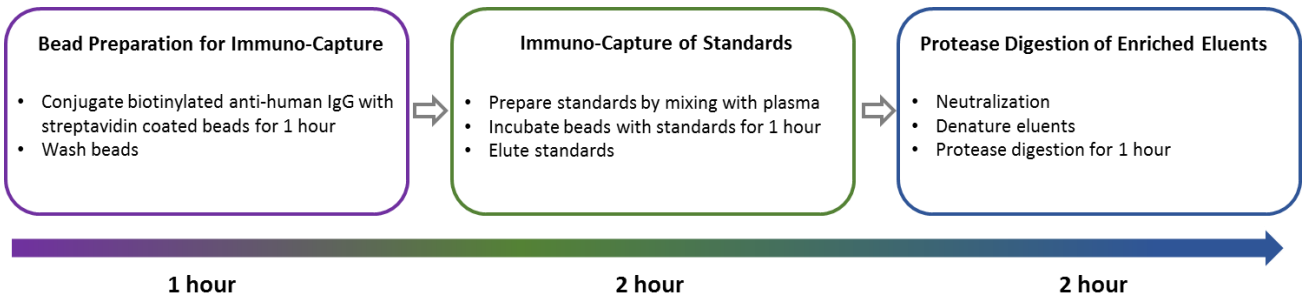

Figure 1. Sample Preparation Workflow.

shaking speed of 1200 RPM. Beads were then washed with 1x PBS buffer and 10 mM ammonium bicarbonate and eluted with 100 µL water with 0.1% TFA.

Protease Digestion of Immuno-Enriched Eluents: The eluents were neutralized with 1 mM calcium chloride in 500 mM ammonium bicarbonate and incubated at 95 °C for 10 mins with shaking. The samples were digested with 1 µg of trypsin for 1 hour at 50 °C after being cooled to room temperature, with a shaking speed of 300 RPM. The digestion was terminated by adding 3 µL formic acid to the samples. The supernatants from the samples were subjected to LC-MS analysis.

LC-MS Conditions: Samples were injected and analyzed in triplicate by SCIEX X500B QTOF system coupled with SCIEX ExionLC UHPLC system. The chromatographic condition is described in Table 1. Samples along with the LC flow were eluted into the mass spectrometer from 1 to 5 minutes and diverted out to waste out of the time period through a divert valve.

The source/gas parameters and MS parameters were set up to the values shown in Table 2, and the data were processed and analyzed using SCIEX OS 1.3 software.

Table 2: Source/Gas Parameters for Analytical Flow Analysis.

Source/Gas Parameter	Value	Source/Gas Parameter	Value
Curtain gas:	*30*	*CAD gas:*	*7*
Ion source gas 1:	*65*	*Ion spray voltage:*	*5500*
Ion source gas 2:	*65*	*Source temperature:*	*600*
TOF start mass:	*400*	*Declustering potential:*	*80*
TOF stop mass:	*700*	*Collision energy:*	*10*
Accumulation time:	*0.25s*	*Method duration:*	*7 min*

Results

Unlike a traditional MRM based quantitation workflow, LC-MS analysis was performed with high resolution TOF MS scan mode. TOF MS based workflow utilizes the high resolution and mass accuracy, to deliver low signal to noise high quality quant data without fragmentation. At the same time, the high resolving power of TOF analyzer minimizes the background interference from matrix and allows quantification based on a single isotopic ion for the analytes noted in this work.

Table 1: Chromatographic Conditions for UHPLC.

Parameter	Value
Stationary phase	*Phenomenex Kinetex 2.6 µm, XB-C18 Column, 50 x 0.3 mm*
Mobile phase A	*0.1% formic acid in water*
Mobile phase B	*0.1% formic acid in acetonitrile*
Flow rate	*700 µL/min*
Column temperature	*40 ºC*
Injection volume	*30 µL*

Time	Flow Rate (µL/min)	%A	%B
0	*700*	*95*	*5*
0.70	*700*	*95*	*5*
0.80	*700*	*86*	*14*
3.50	*700*	*75*	*90*
4.00	*700*	*60*	*40*
4.50	*700*	*10*	*90*
6.00	*700*	*10*	*90*
6.10	*700*	*95*	*5*
7.50	*700*	*95*	*5*

With minimal MS method optimization, this assay achieved a LLOQ of 25 ng/mL for SILuLite, and a LLOQ of 10 ng/mL for trastuzumab emtansine in rat plasma. The assay accuracies are within 90%-110% (SILuLite) and 85%-114% (trastuzumab emtansine), while the CV%s are below 15% for all tested sample runs. With the utilization of heavy isotopic labeled mAb as internal standard, the assay covered a wide dynamic range as 3.5 orders of magnitude for both SILuLite (25-100000 ng/mL) and trastuzumab emtansine (10-50000 ng/mL) and displayed regression coefficients (r) of 0.993 using a weighting of 1/x2 for SILuLite, and 1/x for trastuzumab emtansine, respectively.

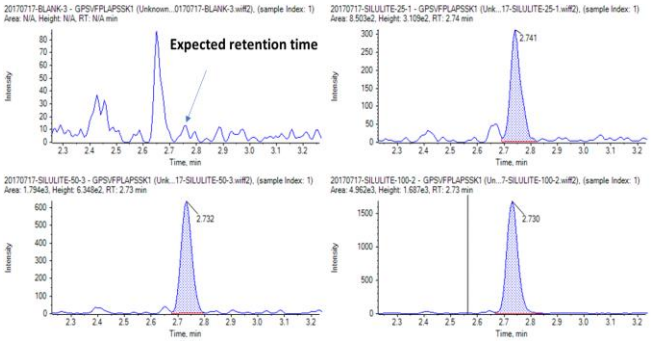

Figure 2. Quantitation of SILuLite. Extracted ion chromatograms (XICs) of selected precursor ion for SILuLite with increasing concentrations (from top left to bottom right: blank, 25, 50, and 100 ng/mL).

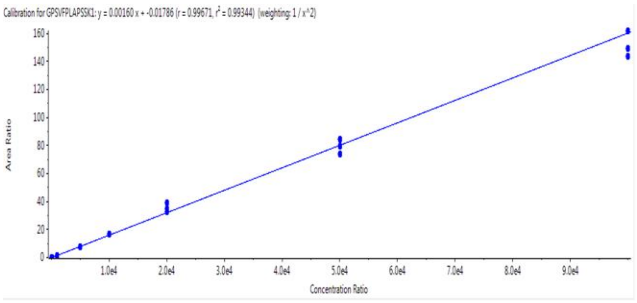

Figure 3. Calibration Curve for Quantitation of SILuLite in Rat Plasma (25 ng/mL to 100000 ng/mL).

Table 4: Quantitation Summary for SILuLite.

Actual Conc. (ng/mL)	Calculated Conc. (ng/mL)	CV% (n = 3)	Accuracy (%)
25	*26*	*2.79*	*105.20*
50	*45*	*3.86*	*90.88*
100	*97*	*5.43*	*97.21*
1000	*1021*	*0.37*	*102.06*
5000	*4848*	*1.62*	*96.95*
10000	*10370*	*1.00*	*103.71*
20000	*22100*	*8.94*	*110.51*
50000	*49460*	*6.20*	*98.91*
100000	*94560*	*6.14*	*94.56*

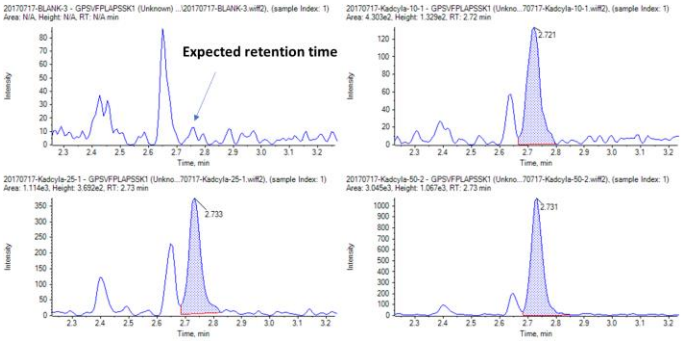

Figure 4. Quantitation of trastuzumab emtansine. Extracted ion chromatograms (XICs) of selected precursor ion for trastuzumab emtansine with increasing concentrations (from top left to bottom right: blank, 10, 25 and 50 ng/mL).

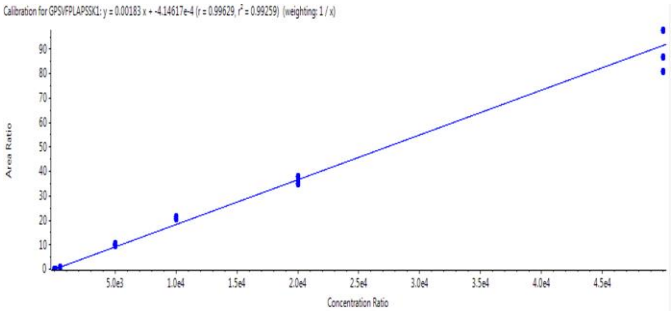

Figure 5. Calibration Curve for Quantitation of trastuzumab emtansine in Rat Plasma (10 ng/mL to 50000 ng/mL).

Table 5: Quantitation Summary for trastuzumab emtansine.

Actual Conc. (ng/mL)	Calculated Conc. (ng/mL)	CV% (n = 3)	Accuracy (%)
10	*9*	*10.85*	*85.77*
25	*22*	*4.73*	*87.16*
50	*47*	*5.73*	*94.20*
100	*111*	*1.77*	*111.21*
500	*509*	*4.40*	*101.81*
5000	*5522*	*2.57*	*110.43*
10000	*11380*	*2.17*	*113.85*
20000	*19810*	*4.15*	*99.03*
50000	*48270*	*9.76*	*96.55*

Conclusion

A hybrid immunoaffinity-HRAMS method for quantifying trastuzumab emtansine and SILuLite in rat plasma was successfully demonstrated. The SCIEX X500B QTOF system coupled with ExionLC UHPLC System provides robust and high-quality quantification of trastuzumab emtansine (10 ng/mL as the LLOQ) and SILuLite (25 ng/mL as the LLOQ). The assay has good reproducibility and wide dynamic range of 3.5 orders of magnitude, as well as high throughput to analyze >180 samples/day, with minimum MS optimization requirement. This method can serve as a generic workflow for quantification of mAbs and ADCs in mammalian matrix without modification.

References

1. Quantification of Trastuzumab in Rat Plasma using an Improved Immunoaffinity-LC-MS/MS Method, SCIEX Technical Note

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