Ligand Binding Mass Spectrometric Immunoassay (LB-MSIA) Workflow for Therapeutic Antibodies: A Universal Pre-Clinical Solution for the Bio-analysis of Fully Human Therapeutic Monoclonal Antibodies in Plasma

Amanda Ribar, Kwasi Antwi , Urban Kiernan, and Eric Niederkofler, Thermo Fisher Scientific, Tempe, AZ

Goal:

To illustrate a pre-clinical bioanalytical solution specific for fully human therapeutic monoclonal antibodies using adalimumab in rodent plasma as a model biological system with subsequent analysis by intact mass spectrometric detection.

Introduction

A systematic study was performed to develop a universal workflow solution for the targeted analysis of fully human therapeutic mAbs that provides characterization information necessary to keep pace with new mAb therapeutic innovation and increased biological complexity.Focusing on the enablement of preclinical discovery and development research, the resultant automated and high throughput Ligand Binding-Mass Spectrometric Immunoassay (LB-MSIA™) combines the robust nature of traditional ligand binding assays with HRAM (High Resolution/Accurate Mass) mass spectrometric detection of intact mAbs. This hybrid bioanalytical workflow is specifically enabled by Streptavidin MSIA D.A.R.T.'S technology; a proprietary product that contains molecular trapping micro-columns covalently derivatized with streptavidin within a pipette housing. When the MSIA D.A.R.T.'S are coupled with a high affinity reagent, such as biotinylated anti-human IgG Fc affinity ligands, the workflow is able to selectively analyze for fully human therapeutic mAbs within rodent model plasma. This LB-MSIA demonstrated % CVs and sensitivity akin to a traditional ligand binding assay. Specifically, the reproducible detection of the therapeutic mAb, adalimumab, was achieved at concentrations as low as 125 ng/mL directly from mouse plasma.

Materials

- Thermo Scientific™ Streptavidin MSIA D.A.R.T.'S, PN: 991STR12
- Thermo Scientific Finnpipette™ Novus i multichannel electronic pipette, PN: 991SP12
- Alternative high-throughput option: Thermo Scientific Versette™ automated liquid handler
- Thermo Scientific Finnpipette F1 adjustable-volume pipettes, PN: 4700850
- CaptureSelect™ biotin anti-IgG-Fc (human) conjugate, PN: 7103262100

- Abbvie™ Humira® (adalimumab)
- Mouse plasma (K2 EDTA)
- Thermo Scientific BupH™ modified Dulbecco's Phosphate Buffered Saline (PBS) packs PN: 28374
- MSIA elution buffer
- Fisher Chemical™ Optima™ LC/MS-grade water, PN: W6
- Fisher Chemical Optima LC/MS-grade formic acid, PN: A117
- Fisher Chemical Optima LC/MS-grade Acetonitrile, PN: A955
- Thermo Scientific Nunc™ 500µL 95-well plates, polypropylene, PN: 12-565-368
- Thermo Scientific Nunc 1.3 and 2.0mL deep well plates with shared-wall technology, 96 deep well, polypropylene, PN: 12-565-650
- Thermo Scientific ProSwift™ RP-4H monolith column, 1.0 x 250 mm, PN: 066640
- Thermo Scientific Dionex™ UltiMate® 3000 UHPLC system
- Thermo Scientific Q Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer
- Thermo Scientific XCalibur™ software, version 2.2
- Thermo Scientific Protein Deconvolution™ software, version 3.0 with the ReSpect™ algorithm

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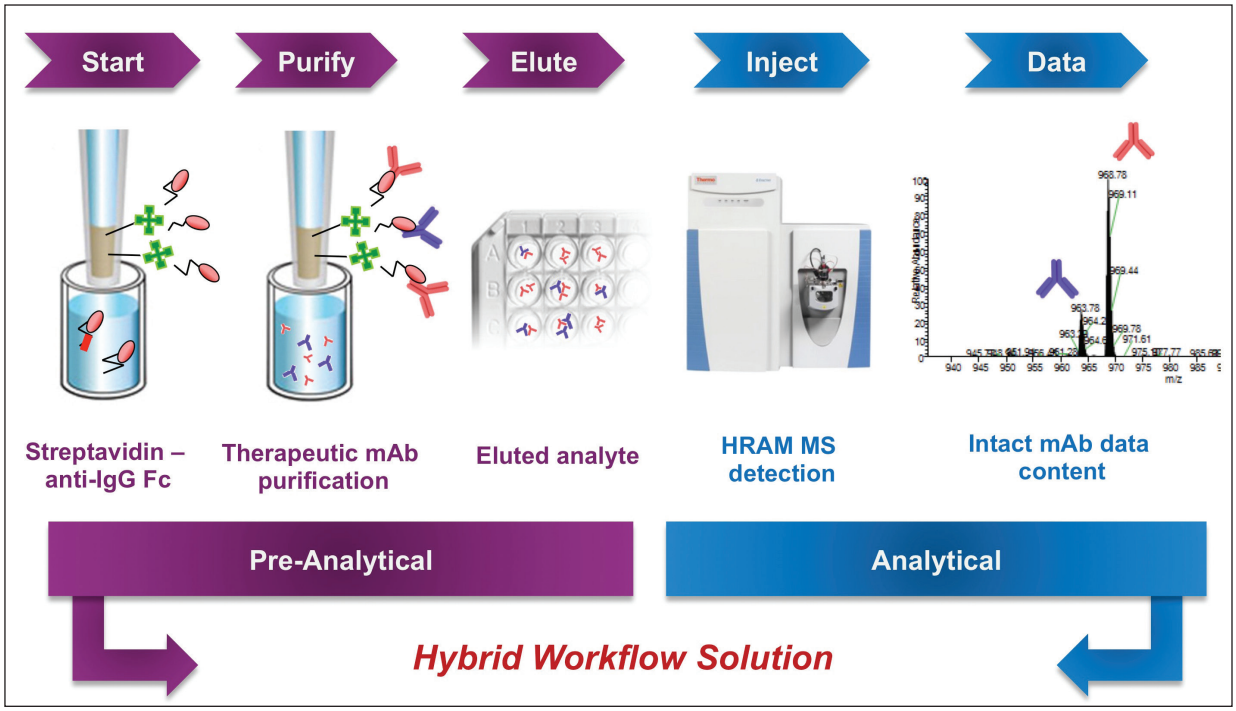

Methods

The LB-MSIA work flow for the bio-analysis of therapeutic antibodies may be broken down into five major steps as illustrated in Figure 1. A Thermo Scientific Novus i electronic pipette was used to provide the repetitive pipetting (aspirating and dispensing cycles) necessary to pass all solutions over the micro-column housed within each of the MSIA D.A.R.T.'S. The Streptavidin MSIA D.A.R.T.'S are first derivatized with a biotin-conjugated affinity ligand that specifically binds to the Fc portion of all four human IgG subclasses. The next step is to assay for the fully human therapeutic monoclonal antibody (adalimumab) from rodent plasma samples by incubating the samples with the anti-IgG-Fc-derivatized Streptavidin MSIA D.A.R.T.'S. The affinity bound adalimumab is subsequently released from the micro-column by treatment with the elution buffer. The ensuing eluate containing intact adalimumab is then detected by HRAM LC-MS. Utilizing Thermo Scientific's XCalibur (Version 2.2) and Protein Deconvolution (Version 3.0) Software the resulting raw HRAM MS data is processed to provide high content qualitative data.

Pre-Analytical

Derivatization of MSIA Streptavidin D.A.R.T.'S with Affinity Ligand

To enable the Streptavidin MSIA D.A.R.T.'S to have a specific affinity for fully human or humanized mAbs, each of the micro-columns were loaded with 125 µL of 4 µg/mL CaptureSelect biotin anti-IgG-Fc (Human) conjugate, a single domain antibody (Life Technologies), prepared in PBS (BupH™ Modified Dulbecco's PBS). This was accomplished by following the steps provided in Table 1 utilizing a Thermo Scientific Novus i electronic pipette equipped with Streptavidin MSIA D.A.R.T.'S.

	Assay Step	Assay Solution	Total Well Volume (µL)	Asp/Disp Volume (µL)	Asp/Disp Cycles	▲ / ▼ Speed
1	Buffer Pre-Rinse	PBS	200	150	10x	4
2	Immobilization of anti-IgG-Fc to D.A.R.T.'S	Biotin anti-IgG Fc conjugate antibody	125	70	500x	1
3	Buffer Rinse	PBS	200	150	10x	4
4	Buffer Rinse	PBS	200	150	10x	4

Table 1 – Derivatization of Streptavidin MSIA D.A.R.T.'S with Biotinylated Anti-Human IgG Fc; Novus i Protocol in Descending Order

Sample Preparation

All samples prepared consisted of 200 µL of mouse plasma supplemented with varying concentrations of adalimumab within the range of 125-8000 ng/mL. Prior to incubation of the samples with the anti-IgG-Fc-derivatized Streptavidin MSIA D.A.R.T.'S each sample was further diluted with 200 µL PBS. Using the Novus i, the following steps outlined in Table 2 were performed to capture adalimumab from the samples.

	Assay Step	Assay Solution	Total Well Volume (µL)	Asp/Disp Volume (µL)	Asp/Disp Cycles	▲ / ▼ Speed
1	Adalimumab Capture by Anti-IgG-Fc MSIA D.A.R.T.'S	Sample Solution	400	300	500x	1
2	Buffer Rinse	PBS	200	150	10x	4
3	Buffer Rinse	PBS	200	150	10x	4
4	Water Rinse	Water	200	150	10x	4
5	Water Rinse	Water	200	150	10x	4

Table 2 – Adalimumab Affinity Purification; Novus i Protocol in Descending Order

Sample Elution

Following the selective capture of adalimumab with the anti-IgG-Fc-derivatized Streptavidin MSIA D.A.R.T.'S, each device was treated with 50 µL of the MSIA Elution Buffer liberating the adalimumab.

> The MSIA elution buffer is a proprietary product recommended for all high throughput applications. An alternative elution buffer option is to use 2% formic acid with 10% methanol, however different from the MSIA elution buffer the alternative elution buffer does not prevent loss in signal due to absorption of the protein to the plastics, which worsens with time. If utilizing the alternative elution buffer ensure the samples are loaded onto the LC-MS within one hour of eluting.

Reference Table 3 for the specifics of the repetitive pipetting used to pass the elution buffer over the micro- column within the D.A.R.T.'S. HRAM LC-MS was then used to detect intact adalimumab contained with the eluate.

	Assay Step	Assay Solution	Total Well Volume (µL)	Asp/Disp Volume (µL)	Asp/Disp Cycles	▲ / ▼ Speed
1	Elution	Elution Buffer	50	30	20x	4

Table 3 – Elution of Affinity-Trapped Adalimumab from Anti-IgG-Fc-Derivatized D.A.R.T.'S ; Novus i Protocol in Descending Order

Analytical Detection

Liquid Chromatography

The affinity-purified adalimumab eluate were separated on a Thermo Scientific Dionex UltiMate 3000 RSLC (Rapid Separation Liquid Chromatography) system utilizing a Thermo Scientific ProSwift RP-4H (1 x 250 mm) column heated to 60 °C. Separation was performed utilizing a gradient of 10% - 32% of 0.2% formic acid in acetonitrile over 12 minutes at a flow rate of 200 µL/min.

Mass Spectrometry

For all samples, full-scan MS data were acquired over the range of m/z 2000-4,500 in positive-ion mode on a Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer with a resolving power of 17,500 (FWHM) at m/z 200 and the AGC (Automatic Gain Control) set to a target value of 3.00E6.

Data Analysis

All LC-MS raw data was collected using Thermo Scientific's XCalibur Software, Version 2.2. From the raw MS data an extracted ion chromatogram was generated for the five most abundant charge states of adalimumab, which were then integrated to obtain the AUC (Area Under the Curve) value for each sample analyzed.

Further characterization of adalimumab, specifically in reference to the presence of glycosylation, was obtained from processing the MS raw data using Thermo Scientific's Protein Deconvolution Software Version 3.0 with the ReSpect algorithm.

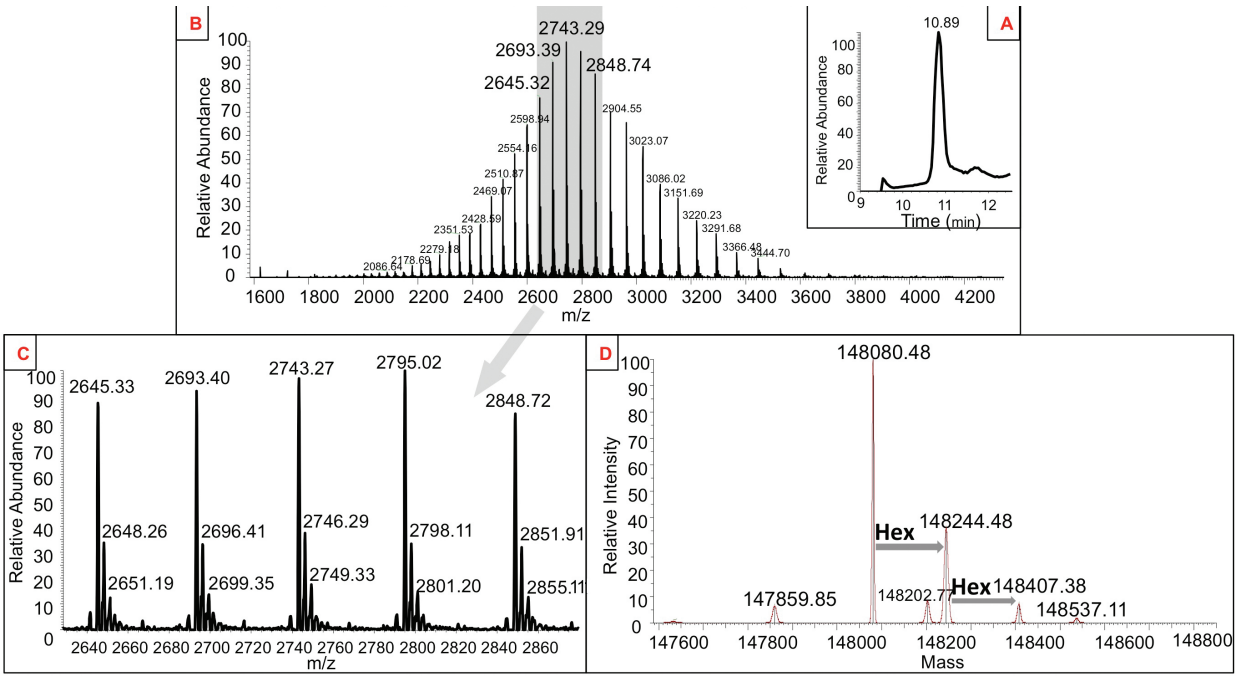

Figure 2. Intact analysis of adalimumab: 500ng adalimumab sample at a concentration of 2.5 µg/mL purified from mouse plasma. A) Base peak chromatogram of adalimumab showing the elution profile of intact adalimumab with all of its sugars. B) and C) Raw MS spectra and corresponding zoom-in of the region m/z 2600-2880 around the five most abundant charge state peaks, respectively. D) The deconvolved average mass (M+H) of intact adalimumab.

Results and Discussion

The MSIA workflow provides a unique push button and automated solution for therapeutic mAb bio-analytics. By combining the performance characteristics of traditional ligand binding assays with the benefits of HRAM MS detection, high value data content is produced that is highly sensitive, robust, and reproducible. The molecular trapping technology of the MSIA D.A.R.T.'S creates an ideal scenario to assay therapeutics from rodent plasma utilizing high affinity anti-IgG-Fc binders specific for fully human mAbs. The integration of the Q Exactive for HRAM detection helps provide additional analytical flexibility over other developing triple quadrupole methods reliant on peptide analysis.

Intact Analysis of Adalimumab

The data presented in Figure 2 shows the analysis of intact adalimumab purified from a sample containing 500 ng of adalimumab at a concentration of 2.5 µg/mL in mouse plasma. The LC elution profile shown in Figure 2A indicates minimal background interference from co-eluting plasma proteins as a result of the cleanliness of the eluate from the Streptavidin MSIA D.A.R.T.'S. Figures 2B and 2C respectively show the resulting mass spectrum of intact adalimumab and the corresponding zoom-in of m/z 2600-2880 to highlight the five most abundant charge state peaks. Figure 2C further shows the ability of the method to resolve and identify the various hexose groups attached to the intact adalimumab.

The mass spectrum was then deconvolved resulting in Figure 2D. The deconvolution data showed four masses, with 148080, 148244, and 148407 representing the addition of hexose groups (162 Da) to adalimumab, while the additional mass, 148203, has a mass difference suggestive of the presence of the additional two sodium and two potassium adducts (~123 Da).

Analytical Detection

To test the sensitivity of the developed universal LB-MSIA workflow for the pre-clinical bio-analysis of therapeutic mAbs (specifically adalimumab) from mouse plasma, samples were run producing a plot akin to a dosing curve represented in Figure 3. Triplicate samples were prepared and analyzed for each concentration. For each sample the AUC from the extracted ion chromatogram for the top five charge states were summed and the average for each concentration was then used to generate the plot in Figure 3. The assay achieved a linear dynamic range of 125 - 8000 ng/mL for the detection of intact adalimumab (native structure preserved) with CVs within the acceptable range for traditional ligand binding assays.

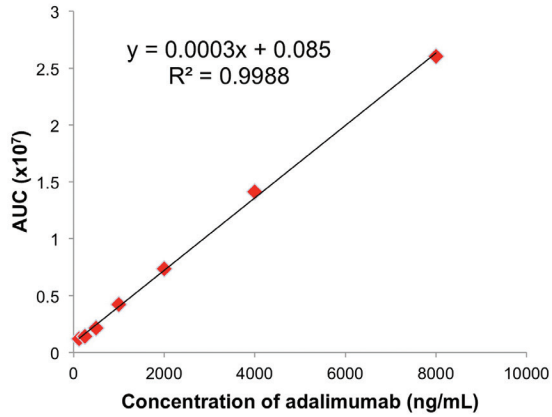

Figure 3. Dynamic range of the LB-MSIA. Results of the intact analysis of adalimumab dosed into mouse plasma spanning the range from 125 – 8000 ng/mL.

Conclusion

A hybrid approach for the universal bio-analysis of fully human therapeutic mAbs in pre-clinical research was demonstrated. By combining the performance characteristics of traditional ligand binding assays with the benefits of HRAM MS detection, a highly sensitive, robust, and reproducible method was create to generate high value data content for bio-analysis. The high selectivity of the CaptureSelect biotin anti-IgG-Fc (human) conjugate with the molecular trapping technology of the MSIA D.A.R.T.'S creates an ideal scenario to assay low abundant (ng/mL) intact human therapeutic mAbs from rodent plasma. As a hybrid approach, the use of the Q Exactive for HRAM detection helps provide additional analytical flexibility and data content over other developing triple quadrupole methods that are reliant on peptide analysis. As shown, the combined benefits of the LB-MSIA enable the characterization of intact native mAb over a wide dynamic range (125 - 8000 ng/mL).

Ordering Information

MSIA D.A.R.T.'S for Immunoaffinity Capture		
Compatible with the Thermo Scientific Versette Automated Liquid Handler and Thermo Scientific Finnpipette® Novus i Multichannel Electronic Pipette		
Cat. No.	**Description**	**Packaging**
991CUS02	300µl MSIA D.A.R.T.'S, Custom	Pack of 96 units
991PRT11	300µl MSIA D.A.R.T.'S, Protein A	Pack of 96 units
991PRT12	300µl MSIA D.A.R.T.'S, Protein A	Pack of 24 units
991PRT13	300µl MSIA D.A.R.T.'S, Protein G	Pack of 96 units
991PRT14	300µl MSIA D.A.R.T.'S, Protein G	Pack of 24 units
991PRT15	300µl MSIA D.A.R.T.'S, Protein A/G	Pack of 96 units
991PRT16	300µl MSIA D.A.R.T.'S, Protein A/G	Pack of 24 units
991STR11	300µl MSIA D.A.R.T.'S, Streptavidin	Pack of 96 units
991STR12	300µl MSIA D.A.R.T.'S, Streptavidin	Pack of 24 units
991001096	300µl MSIA D.A.R.T.'S, Insulin	Pack of 96 units
991001024	300µl MSIA D.A.R.T.'S, Insulin	Pack of 24 units
991R	300 µL MSIA D.A.R.T.'S, Reloadable Rack	1 reloadable rack, D.A.R.T.'S are not included
Automated Liquid Handling Platform		
Cat. No.	**Description**	
650-MSIA	MSIA Versette Automated Liquid Handler	
Multichannel Pipettes and Pipette Stand		
Cat. No.	**Description**	**Packaging**
991S	Finnpipette Novus i Adjustable Pipette Stand	1 pipette stand
991SP12	Finnpipette Novus i Electronic 12-Channel Pipette, 30-300µl and Pipette Stand	1 pipette and 1 pipette stand
Liquid Chromatography		
Cat. No.	**Description**	
	Thermo Scientific™ Dionex™ UltiMate® 3000 UHPLC System	
066640	ProSwift™ RP-4H Monolith Column, 1.0 x 250 mm	
Mass Spectrometry and Software		
Description		
Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer		
Thermo Scientific™ TSQ Vantage Triple Stage Quadrupole Mass Spectrometer		
Thermo Scientific™ Pinpoint Software		
Thermo Scientific™ XCalibur™ Software		
Thermo Scientific™ Protein Deconvolution Software, Version 3.0 with the ReSpect™ algorithm		

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