

## OVERVIEW

### Purpose:

Demonstrate an accurate, precise, and selective method to analyze Adalimumab in human plasma samples using MSIA™ capture and microflow LC-MS/MS.

### Methods:

Streptavidin MSIA™ (Mass Spectrometric Immunoassay) D.A.R.T.™ were treated with biotinylated human TNF- $\alpha$  in order to capture Adalimumab in human plasma samples. The Adalimumab was eluted from the MSIA™ D.A.R.T.™, denatured, reduced, and digested. A signature peptide was measured by microflow LC-MS/MS.

### Results:

- 1000-20,000 ng/mL dynamic range
- A/P 90%  $\pm$  5%
- Blanks <15% of LLOQ Response
- 2x faster than magnetic bead immunocapture method

## INTRODUCTION

Humira® (Adalimumab) is the top selling prescription drug in the world. It is FDA approved for the treatment of ten autoimmune diseases including rheumatoid arthritis, Crohn's disease, and ulcerative colitis. Given the number of biosimilars pending Humira® patent expiration in 2023, there is a need for robust and accurate methods for Adalimumab analysis. LC-MS/MS analysis offers a solution to traditional ligand binding assay selectivity challenges. MSIA™ is a precise and accurate immunocapture method that can be fully automated for fast sample preparation.



## MATERIALS

SigmaMAB Adalimumab – MilliporeSigma

Internal Standard: SiLu™MAB Adalimumab Stable-Isotope Labeled Monoclonal Antibody [13C6, 15N4] - Arginine and [13C6, 15N2] - Lysine - MilliporeSigma

300  $\mu$ L Mass Spectrometric Immunoassay (MSIA™) Streptavidin D.A.R.T.™ - Thermo Scientific™

Biotinylated Human TNF- $\alpha$  Protein - ACROBiosystems

Water, PBS Buffer, TCEP and Sequencing Grade Modified Trypsin - Promega

Waters ACQUITY UPLC® M-Class Binary LC System

Sciex 6500+ operating in MRM mode

## METHODS

### Extraction:

- Plate Preparation
    - Aliquot plasma and internal standard
    - Aliquot rinses (PBS buffer and water)
    - Aliquot Biotinylated TNF- $\alpha$
    - Aliquot Elution Solvent
  - Capture Biotinylated TNF- $\alpha$  with MSIA™ D.A.R.T.™, rinse
  - Capture Adalimumab with TNF- $\alpha$ -treated MSIA™ D.A.R.T.™, rinse
  - Elute Adalimumab
  - Adjust pH 2M Tris buffer
  - Denature/Reduce with Heat (80°C) & 0.1 M TCEP 15 minutes
  - Add Ammonium Bicarbonate/Calcium Chloride
  - Digest with 10  $\mu$ L of trypsin (0.8 mg/mL)
  - Incubate at 50°C for 1 hour
  - Stop digestion with 40% formic acid solution
- ### Microflow LC-MS/MS:
- Determined sensitive and selective signature peptide using Skyline predictions
  - Quantitation peptide: EVQLVESGGGLVQPGR (2X Charge)
  - Adalimumab: 812.9  $\rightarrow$  1056.5
  - Internal Standard: 818.1  $\rightarrow$  1066.6
  - Waters ACQUITY UPLC® M-Class Binary LC Systems
  - Gradient using acetonitrile and water with 0.1% formic acid
  - Flow rate: 10  $\mu$ L/min
  - Column: HALO® Biphenyl (50 X 0.3 mm, 3  $\mu$ m)
  - Column temperature: 50°C
  - Sciex 6500+ operating in MRM mode
  - ESI
  - Positive ion mode
  - Optiflow™ Turbo V Source (1-50 mL/min probe)

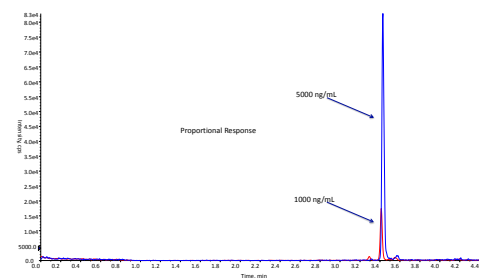


Figure 1: 1,000 and 5,000 ng/ML Adalimumab Extracted from Human Plasma

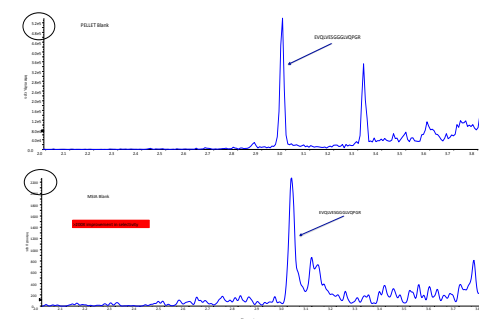


Figure 2: Pellet Extraction vs MSIA™ (blank plasma).

## CONCLUSION

- An accurate, precise, and selective method was developed to analyze Adalimumab using MSIA™ and Microflow LC-MS/MS. The method is faster than using streptavidin treated beads. This method could also be validated to support clinical studies.
- Next Step: Microsampling using MITRA® (Neoteryx) microsampling device, which would be ideal for pediatric or at home sampling.