Bioanalysis of Adalimumab in Human Plasma Samples Using MSIA™ Capture and Microflow LC-MS/MS

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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.'S™ were treated with biotinylated human TNF-α in order to capture Adalimumab in human plasma samples. The Adalimumab was eluted from the MSIA™ D.A.R.T.'S™, 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% ± 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 in human plasma samples. A signature peptide denatured, reduced, and from the MSIA™ the Adalimumab was measured by microflow digested. A signature peptide

MATERIALS

SigmaMAb Adalimumab – MilliporeSigma
Internal Standard: SILuMAb
Labeled Monoclonal Antibody
Adalimumab Stable-Isotope Labeled Monoclonal Antibody Internal Standard: SILu™MAb
MilliporeSigma
Optiflow™ Turbo V Source (1-50 mL/min probe)

METHODS

Extraction:
- Plate Preparation
  - Aliquot plasma and internal standard
  - Aliquot rinses (PBS buffer and water)
  - Aliquot Biotinylated TNF-α
  - Aliquot Elution Solvent
- Capture Biotinylated TNF-α with MSIA™ D.A.R.T.’S™, rinse
- Capture Adalimumab with TNF-α-treated MSIA™ D.A.R.T.’S™, 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 μL 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 → 1056.5
- Internal Standard: 818.1 →1066.6
- Waters ACQUITY UPLC® M-Class Binary LC Systems
- Gradient using acetonitrile and water with 0.1% formic acid
- Flow rate: 10 μL/min
- Column: HALO® Biphenyl (50 X 0.3 mm, 3 μm)
- Column temperature: 50°C
- Sciex 6500+ operating in MRM mode
- ESI
- Positive ion mode

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.