Alturas Analytics, Inc.
The LC/MS Experts™

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Recent Trends with the Transfer from LBA to LC-MS Assays: Microflow LC-MS Applications for Large Biomolecules

Shane Needham, Ph.D.
Co-Founder and Laboratory Director
Alturas Analytics, Inc.
Soup to Nuts of Large Molecule Bioanalysis by LC-MS/MS: From Sequence Selection to Sample Enrichment, to Microflow LC

Shane Needham, Ph.D.
Co-Founder and Laboratory Director
Alturas Analytics, Inc.
Large Molecule Analysis: LBA or LC-MS/MS

Selectivity/Sensitivity?

Signal/Noise!!!
GAINS IN SELECTIVITY AND SENSITIVITY

- Off-line sample preparation
- High resolution mass spectrometer
- MS/MS/MS
- Ion mobility applications
- Derivitization
- Chromatographic improvements
  - Microflow LC-MS – optimized source
  - More resolution
  - Different stationary phase or orthogonal chromatography
GAINS IN SELECTIVITY AND SENSITIVITY

• Sequence Selection
• Sample Preparation
  ▪ Mass Spectrometric Immunoassay (MSIA™)
  ▪ Microelution
• Microflow LC-MS
  ▪ Optimized source design
  ▪ Optimized MFLC
ADC MIMIC:

Sigmamab antibody drug conjugate (ADC) mimic consists of the human universal monoclonal antibody standard (catalog number MSQC4, an IgG1 monoclonal antibody) conjugated to dansyl fluorophores via an LC-SMCC crosslinker.

SILuMAb Infliximab is a recombinant, stable isotope-labeled, monoclonal antibody which incorporates [13C6, 15N4]-Arginine and [13C6, 15N2]-Lysine.
METHODS – SAMPLE PREPARATION

- Sample volume: 50 μL
- Add 25 μL Infliximab internal standard
- Precipitate with acetonitrile 0.1% formic acid, remove supernatant
- Denature pellet with 100 μL of RapiGest™ and incubate
- Reduce with 10 μL of dithiothreitol and incubate
- Derivatize with 10 μL of iodoacetamide and incubate
- Digest with 25 μL of porcine trypsin at 0.200 mg/mL
- Incubate at 37°C for 2.5 hours
- Dilute 1:1 with 10% TCA Solution
METHODS – MFLC-MS/MS

• Waters Acquity M-Class Binary LC Systems
• Gradient using acetonitrile and water with 0.1% formic acid
• Flow rate: 50 μL/min
• Column: Phenomenex Diphenyl (50 X 1.0 mm, 3 μm)
• Column temperature: 50°C
• Sciex 5500/6500+ QTRAP operating in MRM mode
• ESI
• Positive ion mode
SEQUENCE SELECTION: SKYLINE
SKYLINE PREDICTED PEPTIDE SEQUENCES: Q1 AND PRODUCT ION
EXAMPLE MRM OF PREDICTIONS FROM SKYLINE

Improving Selectivity!
MICROFLOW SOURCE: OPTIFLOW VS NORMAL SOURCE

Selectivity or Sensitivity???

Signal/Noise!!
MSIA™
Mass Spectrometric Immunoassay

Photos: thermofisher.com
OFF-LINE SAMPLE PREPARATION: IMMUNOAFFINITY

SIGMA ADC MIMIC 100 ng/mL PELLET DIGESTION

Selectivity or Sensitivity???

Signal/Noise!!

SIGMA ADC MIMIC 10.0 ng/mL MSIA™
GENERIC METHODS FOR SCREENING OF PEPTIDE THERAPEUTICS BY MFLC-MS/MS
NORMAL FLOW (0.7 mL/min) 100 ng/mL
NON EXTRACTED
OPTIMIZED MFLC-MS: 100 ng/mL EXTRACTED FROM 50 μL RAT PLASMA, 50 μL/min

Selectivity or Sensitivity???

Signal/Noise!!
## OFF-LINE SAMPLE PREPARATION: MICROELUTION SPE Signal/Noise!!

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<th>Peptide</th>
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<th>Recovery WCX (%) blowdown and reconstitute</th>
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ACKNOWLEDGEMENTS

Team Alturas
  ▪ Chad Christianson, Jennifer Zimmer, Cody Jensen

SCIEX-LC-MS, Optiflow™
Thermo-MSIA™
Waters-M Class MFLC
LARGE MOLECULE BIOANALYSIS BY LC-MS

Many options to improve selectivity/sensitivity

SIGNAL/NOISE DRIVES ANALYTICAL CHEMISTRY – LLOQ

At Alturas, in the last month we used these methods to transfer a large molecule assay to our lab and improve throughput by >50% and analyze more than 2500 samples to support a clinical study!
Contact Us:
AlturasAnalytics.com

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