M0930-03-017

Optimized Extraction and MFLC-MS/MS Analysis of the Antibody Drug Conjugate SigmaMAb Extracted from Rat Plasma

Emily Witthuhn, Chad David Christianson, Jennifer Zimmer, Sharon DeChenne, Shane Needham

Alturas Analytics, Inc., 1324 Alturas Drive, Moscow, ID 83843

CONTACT INFORMATION: Ann Hoffman, ahoffman@alturasanalytics.com

aaps PharmSci 360 ADVANCING PHARMACEUTICAL SCIENCES, CAREERS, AND COMMUNITY

PURPOSE

Antibody drug conjugates (ADCs) are potent and specific biopharmaceuticals. These proteins are typically analyzed using costly immunoassay methods, which do not have the selectivity to accurately quantify the ADC alone. HPLC-MS/MS analysis of the digested antibody is an alternative approach that provides better selectivity with less method development and cost when compared to magnetic bead immunoassay analysis. Additionally, coupling the mass spectrometer with a microflow LC platform (MFLC) results in a significant increase in the analyte signal. In this poster we describe an accurate, precise, cost effective extraction and MFLC-MS/MS analysis of the ADC SigmaMAb that can be applied to the extraction and analysis of other ADCs.

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

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
- ABSciex 5500/6500+ QTRAP operating in MRM mode
- ESI
- Positive ion mode

RESULTS

Sample preparation and instrument parameters were optimized in order to obtain the highest signal for the ADC with optimal chromatographic resolution. For SigmaMAb, the most selective peptide sequences were LMIDATK (light chain) for quantitation and ALPAPIEK (heavy chain) for confirmation. The most selective internal standard sequence was YASESMSGIPSR (arginine $^{13}C_6$, $^{15}N_2$). The data indicated that a pellet digested using acetonitrile 0.1% formic acid removed a substantial amount of interfering proteins. Urea, Rapigest, and Octyl-β-dglucopyranoside were evaluated to provide optimal antibody denaturing. It was found that Rapigest provided superior denaturing to Octyl-β-d-glucopyranoside and is also faster to denature than urea and doesn't require further dilution of the sample to reduce the concentration prior to the trypsin digestion. The digestion time required and the trypsin concentration were also evaluated. The data suggests that the digestion reaches completion after 2.5 hours at 37°C with a trypsin concentration of 0.2 mg/mL. Further clean-up of the sample was evaluated using various SPE phases and it was determined that a simple TCA crash provided the highest recovery of the target peptide. A 16 point calibration curve was extracted and analyzed in duplicate using the optimized methods. The concentration range was 100-10,000 ng/mL. The results of the analysis indicate the method is accurate and precise. Using a 1/X*X linear regression, the r value was found to be 0.9979 with the percent difference of the standard points less than 15% from the nominal concentrations. Using the MFLC system instead of the conventional HPLC (flowrate of 0.700 mL/min) resulted in a >40% increase in peptide signal. Additional experiments were conducted in order to increase the sensitivity and selectivity of the assay using MSIA Microcolumns packed with Streptavidin. The data indicates that an LLOQ of 10.0 ng/mL is achievable.

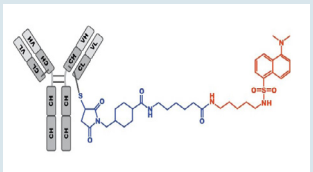

Figure 1: SigmaMAb Antibody Drug Conjugate Mimic (Recombinant Monoclonal IgG1 Human Antibody Linked to dansyl-fluorophores) 100 ng/mL

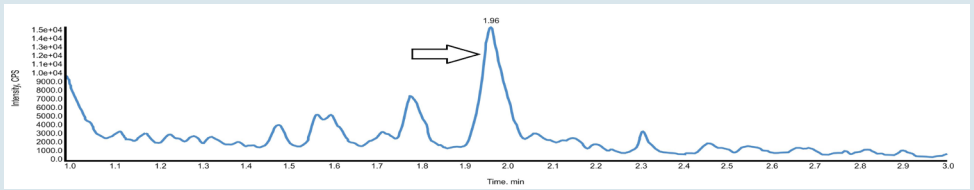

Figure 2: SigmaMAb ADC Mimic (Digested to peptide) 100 ng/mL Extracted from Rat Plasma Chromatogram

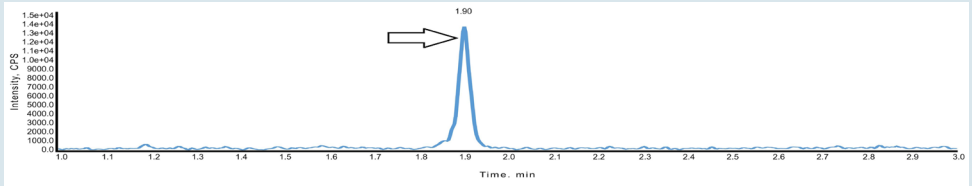

Figure 3: SigmaMAb ADC Mimic (Digested to peptide) 10.0 ng/mL Extracted from Rat Plasma using MSIA Chromatogram

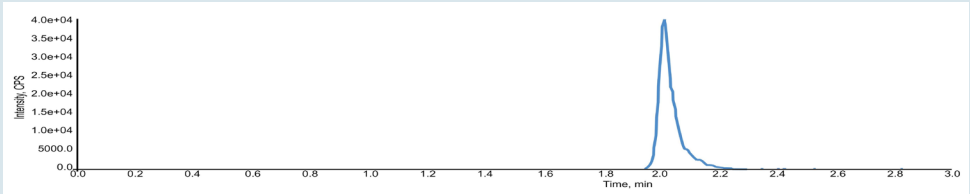

Figure 4: Internal Standard (Digested Infliximab Stable-Isotope Labeled Monoclonal Antibody) Chromatogram

Protein	Peptide Sequence	Molecular Weight	Charge State	Q1	Q3
SigmaMAb ADC Mimic	LMIDATK	791	2	397	711
SigmaMAb ADC Mimic Confirmation	ALPAPIEK	838	2	420	327
Infliximab Stable-Isotope	*YASESMSGIPSR	1294	2	648	845

*arginine $^{13}C_6$, $^{15}N_2$ labeled

CONCLUSIONS

- Developed methods for the extraction and analysis of SigmaMAb ADC Mimic from rat plasma
- Method developed is accurate/precise and is cost effective compared to immunocapture sample purification techniques
- MFLC method resulted in a >40% increase in the peptides instrument response when compared to conventional HPLC
- If lower sensitivity is required, MSIA can be utilized to lower the lower limit of quantitation

Alturas Analytics, Inc.
The LC/MS Experts™

Alturas Analytics, Inc.

Focused on LC-MS/MS Bioanalysis and PK Services

Known as the technology leader of MS/MS bioanalysis, Alturas Analytics delivers results on time with scientific integrity that exceeds industry standards. Privately owned and client focused, in our 18 years of operation Alturas Analytics has earned a reputation for personalized service and innovative MS/MS solutions.

BIOANALYTICAL SERVICES

- Methods Development, Transfer and Validation
- GLP and Non-GLP Sample Analysis
- Antibody Drug Conjugate (ADC) and Other Macromolecule Analysis
- Protein Binding Determination
- Dried Blood Spot (DBS) and Matrix Spot Analysis
- PK and TK Analysis and Reporting

QUALITY SYSTEMS

- Compliant to GLP and FDA, EMA and Crystal City Guidelines
- Onsite QA and IT Departments
- Thermo-Watson LIMS
- 21 CFR Part 11 Compliant
- GLP Validated Analyst Software
- Validated WinNonlin®
- Rush and Next Business Day Data Delivery Available

RESEARCH

- 15% of Capacity Reserved for Research
- New Bioanalytical Techniques Including:
  - Microflow LC-MS/MS
  - Immunocapture
  - PicofuzeTM
  - Creation of Novel Dye to Analyze Translucent Matrices Such As:
    - Tears, Cerebrospinal Fluid, Synovial Fluid

FACILITIES AND EQUIPMENT

- 15,000 Sq. Ft. Purpose-Built Facility
- Sciex 4000, 5500 and 6500 mass spectrometers
- Shimadzu UPLCs
- Waters M-Class Microflow UPLCs
- Watson LIMS system
- HTDialysis Micro-Equilibrium Devices

Alturas Analytics, Inc.
The LC/MS Experts™

1324 Alturas Drive | Moscow, ID 83843 | 208.883.3400 | www.alturasanalytics.com