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Quantitative Microflow HPLC-MS/MS Analysis of the Antibody Drug Conjugate SigmaMAb Extracted from Rat Plasma

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OVERVIEW

Purpose

Develop extraction and MFLC-MS/MS methods for the determination of SigmaMAb ADC Mimic from rat plasma

Methods

Rat plasma was spiked with SigmaMAb ADC Mimic and depleted using pellet digestion. The pellet was denatured, reduced, derivatized, digested and analyzed by MFLC-MS/MS.

Results

The developed methods resulted in Accuracy/Precision better than %15 with a dynamic range of 100-10,000 ng/mL.

INTRODUCTION

Antibody drug conjugates (ADCs) are potent and specific biopharmaceuticals. These large molecules are typically analyzed using ELISA 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. In order to increase the sensitivity of the method the analysis was conducted using microflow HPLC (MFLC). Additionally, the internal standard was aliquotted prior to denaturing the ADC in order to compensate for any preparation variability.

The extraction of large molecules using immunocapture techniques to remove endogenous plasma proteins or capture the target protein can be very expensive and time consuming. The pellet digestion method developed allows for cost effective, accurate, precise and selective quantitative analysis of the ADC SigmaMAb Mimic from plasma via MFLC-MS/MS in only a few hours.

METHODS

Sample Preparation

- Sample volume: 50 µL
- Add 25 µL Infliximab internal standard
- Precipitate with acetonitrile, 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

Solid Phase Extraction Procedure

- Condition Oasis MAX (Waters strong anion exchange) SPE plate
- Add entire sample volume
- Wash with water and water:methanol 9:1
- Elute with 2% TFA in methanol
- Evaporate solution
- Reconstitute with 100 µL of water containing 0.1% formic acid

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

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

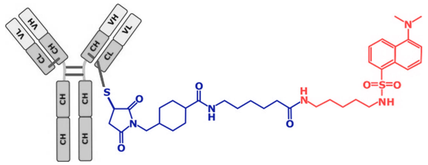


Figure 2: Linear Calibration Curve Generated from the Analysis of 16 Standard Curve Samples (8 Concentrations in Duplicate) Extracted from Rat Plasma; r=0.9979, 100-10,000 ng/mL

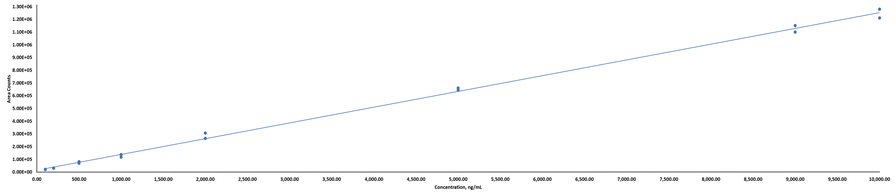


Figure 3: SigmaMAb ADC Mimic (Digested to peptide) 100 ng/mL Extracted from Rat Plasma Chromatogram

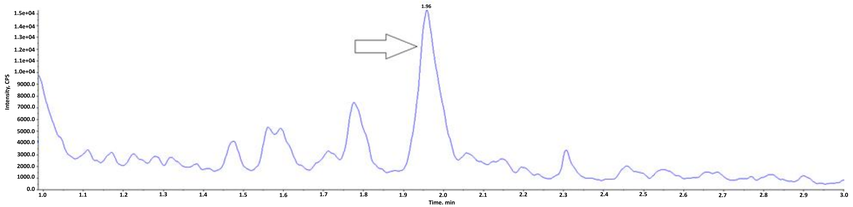
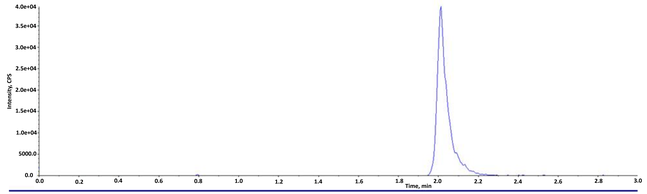


Figure 4: Internal Standard (Digested Infliximab Stable-Isotope Labeled Monoclonal Antibody) Chromatogram



Protein	Peptide Sequence	Molecular Weight	Charge State	Q1	Q3
SigmaMAb ADC Mimic	ALPAPIEK	838	2	420	327
Infliximab Stable-Isotope	*YASESMS GIPSR	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