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Submitted by Chad Christianson

Quantitative MFLC-MS/MS Analysis of the Antibody Drug Conjugate SigmaMAb Extracted from Rat Plasma using Thermo Scientific MSIA microcolumns

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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 ADC is an alternative approach that provides better selectivity. In order to increase the selectivity/sensitivity of the method, analysis was conducted using microflow HPLC (MFLC) and immunocapture sample preparation. Typical immunocapture techniques require the use of labor intensive magnetic beads. Thermo Scientific MSIA sample preparation is simple and can be fully automated, greatly increasing the sample throughput. The resulting MSIA data results in lower limits of quantitation due to the selectivity and extraction efficiency improvements when compared to other non-immunocapture methods such as pellet digestion.

Methods

SigmaMab (Sigma-Aldrich), an IgG1 human antibody was the ADC and SILuMAb, an isotope labeled antibody was the internal standard. SigmaMab was spiked into rat plasma and 25 µL was aliquotted into a 96 DWP. Internal standard was then added to the plate and it was vortexed/centrifuged. The ThermoFisher MSIA streptavidin microcolumns were rinsed with PBS buffer and linked with CaptureSelect Biotin anti-IgG-Fc. The Sigmamab and IS were then captured on the microcolumns, rinsed and eluted with 2% formic acid into a 96 DWP. After denaturing with heat and TCEP reduction the ADC and IS were digested with trypsin by incubating at 50°C for 1 hour. A 40% formic acid solution was added to each well and injected onto the MFLC-MS/MS.

Preliminary Data or Plenary Speakers Abstract

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

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ALPAPIEK (heavy chain) for confirmation. The most selective internal standard sequence was YASESMSGIPSR (arginine$^{13}C_6$,$^{15}N_2$). Initial data was generated using a pellet digest. The lowest limit of quantitation (LLOQ) possible was only 100 ng/mL due to poor recovery (<10%) and selectivity. Recovery was determined by comparing the peak area of the peptide extracted and digested from the ADC in plasma to non-extracted peptide digested from the ADC in a buffer solution. In order to lower the limit of quantitation an immunoaffinity capture method was employed using ThermoFisher MSIA streptavidin microcolumns. The analysis was performed using an API-6500+ mass spectrometer operating in positive ESI mode. The MFLC system was a Waters M Class operating with binary gradient method and a flowrate of 50 µL/min. Separation was achieved using a Phenomenex Kinetex Biphenyl column (5 cm x 1.0 mm, 3 µm). The immunocapture extraction resulted in a 10X improvement from 100 to 10 ng/mL of the LLOQ with only 25 µL of sample volume. Additionally the selectivity was greatly improved with no interfering peaks present in the chromatograms. An eight point calibration curve (analyzed in duplicate) extracted from rat plasma using the MSIA method resulted in a %Bias of <15% from the nominal concentration and a linear r value of 0.9959. The linear dynamic range for the assay was 10.0-10000 ng/mL.

Novel Aspect

Development of a sensitive/selective MSIA immunocapture extraction method coupled with MFLC-MS/MS analysis to quantify an ADC from rat plasma.