

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

Chad Christianson, Alturas Analytics, 1324 Alturas Dr., Moscow ID 83843

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. Additionally, the internal standard was aliquotted prior to denaturing the ADC in order to compensate for any preparation variability. Analysis of digested large molecules can be very expensive and time consuming. The pellet digestion method developed allows for accurate, precise and selective quantitative analysis of the ADC via MFLC/MS/MS in only a few hours using cost-effective materials.

Methods

SigmaMAb (Sigma-Aldrich), a commercially available recombinant monoclonal IgG1 human antibody linked to dansyl-fluorophores was the ADC and SILuMAb, an isotope labeled antibody was used as the internal standard. SigmaMAb was spiked into rat plasma and 50 μ L was aliquotted into a 96 DWP. Internal standard was then added to the plate followed by acetonitrile precipitation. The plate was vortexed/centrifuged and the supernatant was removed. The pellet was re-suspended and denatured/reduced in Rapigest and DTT. The plate was incubated at 60°C for 30 minutes and alkylated with iodoacetamide and digested with porcine trypsin by incubating the plate at 37°C for 2.5 hours. Water containing 10% TCA was added to each well and the supernatant was injected onto the HPLC/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

(continued on back)



most selective peptide sequences were LMIDATK (light chain) for quantitation and ALPAPIEK (heavy chain) for confirmation. The most selective internal standard sequence was YAS-ESMSGIPSR (arginine13C6,15N2). 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 -d-glucopyranoside 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. It was observed that a longer digestion period resulted in a higher concentration of interfering peptides. The analysis was performed using an API-6500+ mass spectrometer operating in positive ESI mode. The HPLC systems were Shimadzu LC-30AD pumps operating with binary gradient methods and a flowrate of 0.100 mL/min. Separation was achieved using a Luna C18 column (5 cm x 1.0 mm, 3 μ m). For comparison purposes, the analysis was also performed at a flowrate of 0.700 mL/min on a 2.00 mm ID column. The preliminary data indicated that the 100 μ L/min flowrate resulted in a >40% increase in mass spectrometer signal. The LLOQ for the method is 100 ng/mL with an ULOQ of 10,000 ng/mL and accuracy/precision within 20%.

Novel Aspect

Development of an HPLC/MS/MS method to quantify novel commercially available ADC in rat plasma using MFLC-MS/MS.

Contact Us:

(208) 883-3400

ahoffman@alturasanalytics.com

1324 Alturas Drive, Moscow, ID 83843