Abstract

Quantitative HPLC-MS/MS Analysis of DM1 and DM1-MCC Extracted From Whole Blood Dried Onto a Mitra® Microsampling Device

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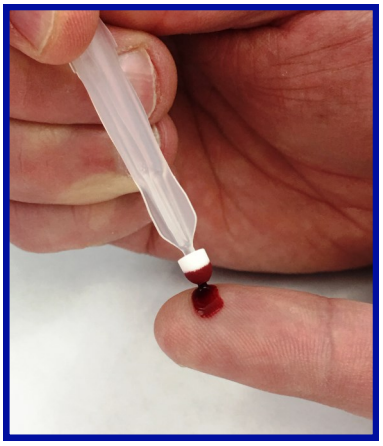

Introduction:

Antibody drug conjugates (ADCs) are a potent and specific biopharmaceutical. ADCs combine the specific targeting abilities of an antibody with a cytotoxin that attacks only the cancer cells that contain a unique marker. Many ADCs utilize DM1 as the warhead linked with an antibody by SMCC. Typically plasma is collected for ADC bioanalysis which requires the subject to spend hours at a clinic. This regime is often inconvenient for stage three subjects affected by aggressive cancer. Convenient and accurate sample collection at home is possible using the Mitra® Microsampling Device (MMD). MMD allows for accurate and precise self-collection of a blood sample with only a finger prick. DM1 and DM1-MCC collected on MMD were extracted and analyzed by HPLC-MS/MS.

Methods:

DM1 and MCC-DM1 were spiked into whole blood and absorbed onto a Mitra® Microsampling Device. Ten microliters of blood was absorbed onto the device and was allowed to dry at ambient temperature for 2 hours. Once dried the device was placed into a 96 DWP containing internal standard, TCEP, and 500 microliters of acetonitrile. The plate was placed into an incubator set at 25ºC and gently vortexed for 15 minutes. The device was removed from the well, evaporated to dryness and reconstituted with 1/1 water/acetonitrile. Ten microliters of NEM was added to each well and the plate was incubated at 37ºC for thirty minutes. The extract was then analyzed on an API6500 coupled with Shimadzu HPLC pumps.

Preliminary Data:

The analysis was performed using an API-6500 mass spectrometers operating in positive ESI mode. The HPLC systems were Shimadzu LC-20AD pumps operating with binary gradient methods and a flowrate of 0.700 mL/min. Separation was achieved using a Discovery HS C18 column (5 cm x 2.1 mm, 3 mm). An eight point calibration curve in human blood ranging from 0.5-50 ng/mL was absorbed onto the device, extracted and analyzed in duplicate. The corresponding regression line was used to quantify the quality control samples prepared at three concentrations 1.5, 5.0 and 40.0 ng/mL. The data indicates that DM1 and MCC-DM1 can be accurately quantified from human blood dried on the Mitra® Microsampling Device. The accuracy and precision of the assay was within 15% of the nominal concentrations.

Novel Aspect:

DM1 and MCC-DM1 can be accurately quantified from blood collected using Mitra® Microsampling Devices allowing subjects to accurately self-collect samples.

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