INTRODUCTION

The number of possible species that can be generated when considering the metabolic behavior of an Antibody Drug Conjugate (ADC) is vast. One major challenge is to determine species that are present in addition to any conjugate or linker that may be released or present in the drug or reagent, without the drug or reagent in the other formulation. Understanding and identifying the major released drug-containing species is essential as an aspect of ADC drug development. Until recently no specific ADC metabolite work flow existed in a software package making consideration of all these species a difficult process.

METHODS

Sample Preparation:

1. 50 µg ADC suspended in 0.1 M PBS, pH 7.4, 1 mL of a 250 µL of ACN, then centrifuge at 14,000 × g for 15 minutes.
2. Add 400 µL of 3:1 ACN to centrifuge tube and vortex vigorously.
3. Add 200 µL of 3:1 ACN to centrifuge tube to wash for 50 times.
4. Add 200 µL of acetonitrile to wash, vortex and transfer to a clean tube.
5. Centrifuge at 14,000 × g for 15 minutes.
6. Centrifuge supernatant to a clean tube and dry under vacuum at 37 °C.

LC-MS/MS Data Collection:

• SCIEX TripleTOF® 6600 System with WATTHB Emission (Biopolymers Research & Development, Sciex) using HCD fragmentation at q1=8.25 and q1=16 for U and D, respectively. The scan time was 850 ms.

RESULTS

A total of 15 potential metabolites of ADC were found in the 24 hour time point of the incubation. Of the 15 mass ions that were observed in the TOF MS, 7 contained a non-fragmentable linker of the payload species (QuiMe®) plus a payload component. Several mass ions in the triple quadrupole LC/MS/MS analysis contained a mass ion that was not found in the triple quadrupole analysis (Figure 4). The released payload was found in the TOF MS charged under solid state at 0.02-0.05 ppm (1115.7475, 553.2148 and 1125.4575).

The MS/MS spectrum also showed a relatively high abundance of a fragment ion at mass 2145.4784 in the TOF MS, which could be assigned to a doubly charged species. The MS/MS spectrum was highlighted with the metabolitePilot 2.0 software displaying a complete mass range for a fragment which contained a mass of 2145.4784. For research use only. Not for use in diagnostic procedures.