

Overview

Purpose

Develop extraction and LC-MS/MS methods for the determination of DM1 and MCC-DM1 from human blood dried on Mitra® Microsampling Devices (MMD).

Methods:

Human blood containing DM1 and MCC-DM1 was collected and allowed to dry. The dried blood was extracted and analyzed by LC/MS/MS.

Results:

The developed methods resulted in Accuracy/Precision better than 15% for both compounds with a dynamic range of 0.500-50.0 ng/mL



Figure 1: DM1 Extracted from Dried Human Blood (0.500 ng/mL)

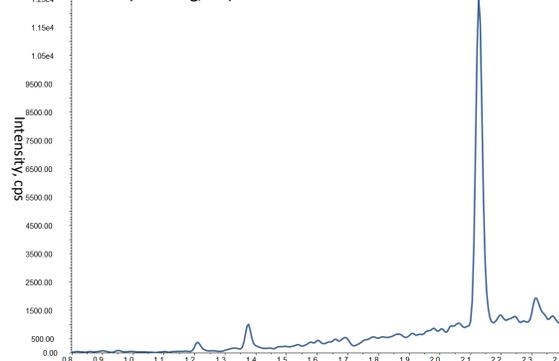
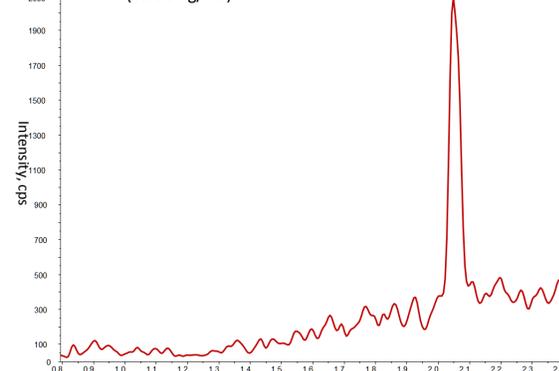


Figure 2: M1-MCC Extracted from Dried Human Blood (0.500 ng/mL)



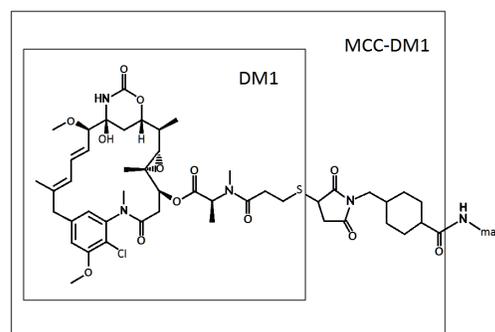
Introductions

ADCs are potent and specific biopharmaceuticals. 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 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

Dried Blood Extraction:

- Sample volume: 20 µL absorbed on MMD
- Device allowed to dry for 2 hours
- Add 10 µL TCEP to 96 DWP
- Add 100 µL MeOH containing IS
- Soak Device in solution for 30 minutes
- Remove Device and derivatize with 10 µL NEM
- Incubate at 37°C for 15 minutes
- Add 100 µL water



LC-MS/MS:

- Shimadzu Binary LC Systems
- Gradient using acetonitrile and water with formic acid
- Flow rate: 700 µL/minute
- Column: Supelco HS C18 (50 X 2.1 mm, 3µm)
- Column temperature: 50°C
- ABSciex 5500/6500 QTRAP operating in MRM mode
- ESI
- Positive ion mode

Table 1: Accuracy/Precision Data

n=6 QC Samples (ng/mL)	DM1		DM1-MCC	
	AVG %Bias	CV	AVG % Bias	CV
1.50	3.3	11.5	8.9	4.8
5.00	-1.8	4.8	-4.3	4.6
40.0	5.7	6.7	5.0	3.8

Conclusions

- Developed methods for the simultaneous extraction and analysis of DM1 and MCC-DM1 from human blood dried on MMD.
- Method is accurate/precise and can be validated for use in clinical study samples collected by a single finger prick.