A Sensitive LC-MS/MS Method for the Determination of DM4 and DM4-Me in Human Plasma

Shashank Gorityala[1], Yunlin Fu[2], Rachel Caminiti[1], Wenkui Li[2], David Humphries[1], Aaron Ledvina[1], Stephanie Cape[1] and Jimmy Flarakos[2]
[1]Covance Laboratories Inc., Madison, WI; [2]Novartis Institutes for BioMedical Research, East Hanover, NJ

Summary

- Quantitative method for unconjugated DM4 and DM4-Me in clinical study
- Choice of matrix (Plasma vs Serum) driven by analyte properties
- Extraction: Protein precipitation followed by chemical reduction and SPE
- Calibration range for DM4 and DM4-Me: 0.100-50.0 ng/mL

Introduction

Antibody-drug conjugates (ADCs) are a novel class of therapeutic agents, in which one or more small cytotoxic molecules (payload) are linked covalently to a monoclonal antibody (mAb). The exposure of unconjugated payload and/or its metabolites in circulation is important for the evaluation of the safety and efficacy of ADCs.

In this work, we describe the development and validation of an LC-MS/MS method for the determination of unconjugated DM4 and its metabolite DM4-Me in human plasma. DM4, a derivative of maytansine, is the cytotoxic payload of an ADC developed at Novartis. DM4 contains a free sulfhydryl group, which reacts with other thiol-containing molecules in biological matrices to form conjugates, leading to underestimation of the free payload concentration. This renders a technical challenge in developing a robust bioanalytical assay in support of clinical development.

Method

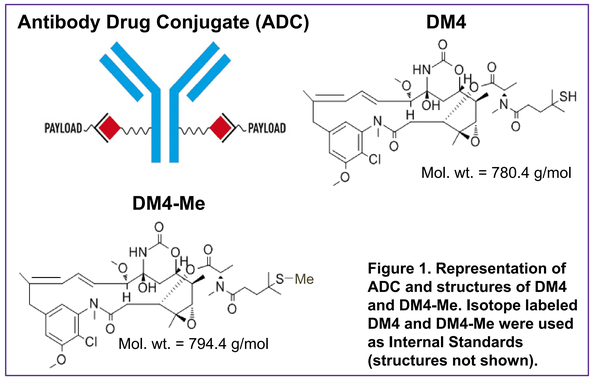

Figure 1. Representation of ADC and structures of DM4 and DM4-Me. Isotope labeled DM4 and DM4-Me were used as Internal Standards (structures not shown).

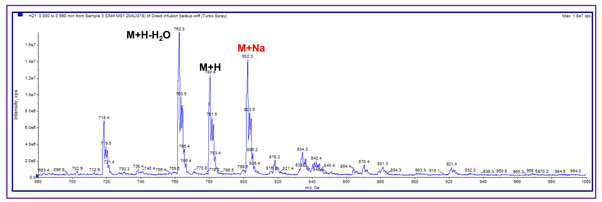

Figure 2. MS1 of DM4 showing the adducts formation.

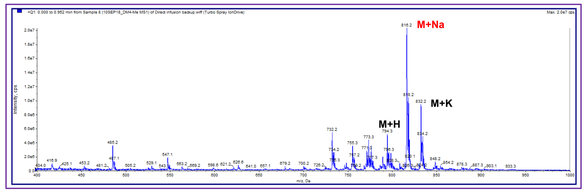

Figure 3. MS1 of DM4-Me showing the adducts formation.

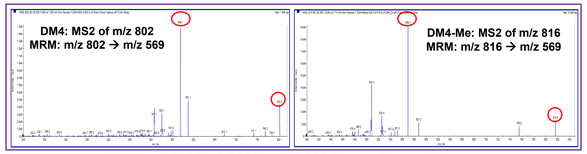

Figure 4. MS2 of DM4 and DM4-Me sodium adducts.

LC-MS/MS

HPLC Parameters	
Parameter	**Details**
LC System	Shimadzu, Prominence, 20 Series
Analytical Column	Waters, XTerra® RP18, 2.1 x 50 mm, 3.5 µm
Column Temp	40°C
Mobile Phase A	[Sodium Formate 20mM (aq)]: Water: Formic Acid (0.05:100:0.1, v:v:v)
Mobile Phase B	[Sodium Formate 20mM (aq)]: Acetonitrile: Methanol: Formic Acid (0.05:50:50:0.1, v:v:v:v)
Gradient Time	3.10 min
Injection Details	4-6 µL
MS/MS System	Sciex API 6500+ in Positive TurboIonSpray® (ESI+)

Mass Spectrometer Parameters		
Compound Name	**Transition**	**Retention Time (min)**
DM4	802.3 → 569.1	1.99
DM4-Me	816.2 → 569.3	2.03
ISTD1	808.2 → 569.3	1.99
ISTD2	820.3 → 569.2	2.03

Sample Preparation Procedure

50.0 µL aliquot of Human Matrix
↓
25.0 µL ISTD mix
↓
Protein Precipitation w/ ACN: MeOH: FA (90:10:0.5)
↓
Transfer supernatant
↓
Reduction using TCEP for 90 min at 60°C
↓
SPE using SOLA HRP
↓
Elute with methanol
↓
Dry and Reconstitute
↓
LC-MS

Significance of Reduction Step: Choice of Matrix

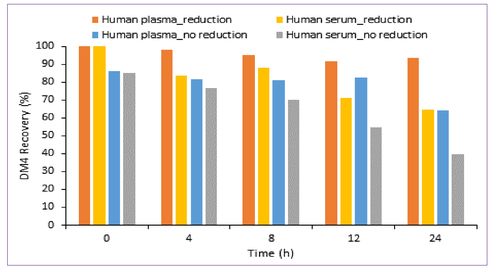

Figure 5. Graphical representation of DM4 recovery in human plasma and serum on wet-ice with and without reduction step.

- Protein precipitation using ACN:MeOH:FA (90:10:0.5, v:v:v) provided the best ADC precipitation efficiency (data not shown). This is a crucial step to remove the ADC and prevent it from undergoing reduction.
- Conjugation is more predominant at room temperature compared to wet-ice conditions in blood or plasma (Data not shown). Sample thawing and processing is on wet ice.
- TCEP, DTT and 2-Mercaptoethanol were evaluated for reduction step.
- Reduction using TCEP recovers the endogenous conjugated DM4 more efficiently in human plasma vs. in human serum, driving the choice of matrix as human plasma for this clinical study.
- Talking point: Why does human serum fail to show similar DM4 recovery as human plasma post-reduction?

Results

- Calibration range for DM4 and DM4-Me: 0.100 to 50.0 ng/mL.

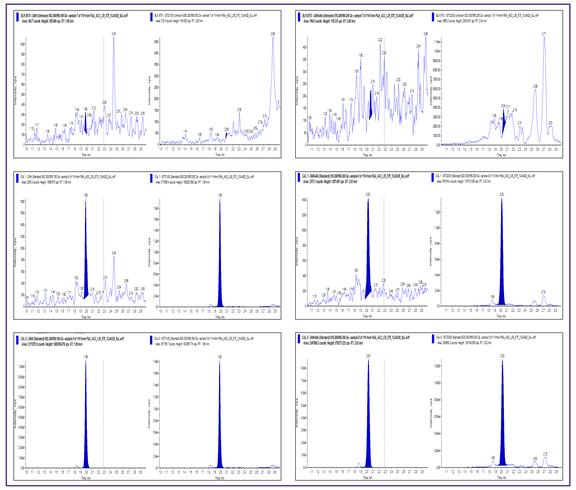

Figure 6. Representative chromatograms for DM4 (left) and DM4-Me (right) including BLK MTX, LLOQ, ULOQ in human plasma.

Accuracy and Precision

	Analyte Nominal Concentration (ng/mL)													
	LLOQ QC (n=6)			LQC (n=6)			LMQC (n=6)			MQC (n=6)			HQC (n=6)		
	0.100	%CV	% Bias	0.300	%CV	% Bias	2.50	%CV	% Bias	20.0	%CV	% Bias	40.0	%CV	% Bias
DM4	0.103	6	3	0.315	5	5	2.48	4	-1	20.3	2	1	41.4	2	3
DM4-Me	0.103	4	3	0.287	5	-4	2.33	4	-7	19.7	3	-1	41.4	3	4

Recovery and Matrix Factor

- The recovery for DM4 ranged from 62-71%, whereas for DM4-Me it ranged from 70-76%.
- The matrix factor for DM4 ranged from 0.94 to 1.06; for DM4-Me ranged from 0.92 to 0.97.

Other Assay Validation Tests

- The following were evaluated in the method development for both DM4 and DM4-Me
  - Whole Blood Stability on wet ice for up to 2h
  - Stability in plasma on wet ice for up to 24h, after 5 freeze-thaw cycles, 9 days in freezer set at <-60°C
  - Matrix effect in hemolysis or hyperlipidemia plasma
- The stability of the linker was evaluated by stressing the ADC spiked QCs prepared in human plasma
  - The linker was stable for the sample preparation steps, on wet ice up to 24h, after 5 freeze-thaw cycles, 9 days in freezer set at <-60°C

Conclusion

- A sensitive and robust method for the quantitation of DM4 and DM4-Me was developed and it is currently being validated according to FDA guidance for bioanalytical method validation.
- Reduction step in the sample preparation aids deconjugation of DM4 from the matrix components to facilitate reliable quantitation of unconjugated DM4 in human plasma.

References

Heudi, O., Barteau, S., Picard, F. & Kretz, O. Quantitative analysis of maytansinoid (DM1) in human serum by on-line solid phase extraction coupled with liquid chromatography tandem mass spectrometry - Method validation and its application to clinical samples. *Journal of Pharmaceutical and Biomedical Analysis* 120, 322-332 (2016).

Wei, D., Sullivan, M., Espinosa, O. & Yang, L. A sensitive LC-MS/MS method for the determination of free maytansinoid DM4 concentrations—Method development, validation, and application to the nonclinical studies of antitumor agent DM4 conjugated hu-anti-Cripto MAb B3F6 (B3F6-DM4) in rats and monkeys. *International Journal of Mass Spectrometry* 312, 53-60 (2012).

Presented at WRIB 2020

COVANCE