Development of a UPLC-MS/MS Method for the Simultaneous Determination of Five Monomethyl Auristatin E (MMAE) Metabolites from Human Plasma

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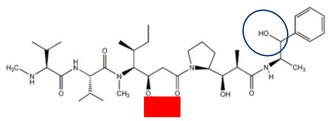
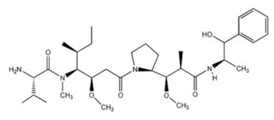
INTRODUCTION

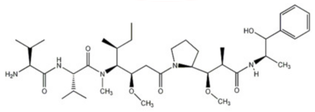
Monomethyl auristatin E (MMAE) is a synthetic toxin that inhibits cell division by blocking the polymerization of tubulin. It has been linked to various monoclonal antibodies (to form antibody-drug-conjugate, ADC) for targeted cancer therapy. The purpose of this work was to develop a UPLC method for the simultaneous determination of five MMAE metabolites, C4, C5, C7, C8, and C13, from sodium citrate human plasma. The assay was challenged by the need to address interferences among analytes and from MMAE, to control the stability/conversion of the analytes, and to reach the low quantification limit (10 pg/mL). C4 and C7 are isomers and need to be either chromatographically separated or differentiated by unique MRM transitions. Meanwhile, due to the cross-talk from C13 to C4, C7 to C5, as well as MMAE to C8, baseline separation on chromatography was needed between the cross-talk pairs. In addition, the conversion from C7 to C5 was observed both in whole blood and plasma, and thus needs to be inhibited during sample collection and processing.



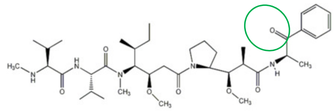
C4
mol. wt. 703.49



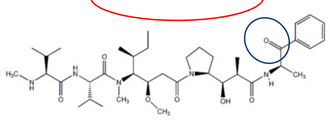
C5
mol. wt. 604.82



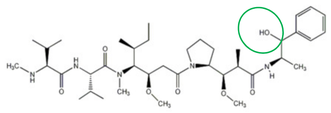
C7
mol. wt. 703.49



C8
mol. wt. 715.96



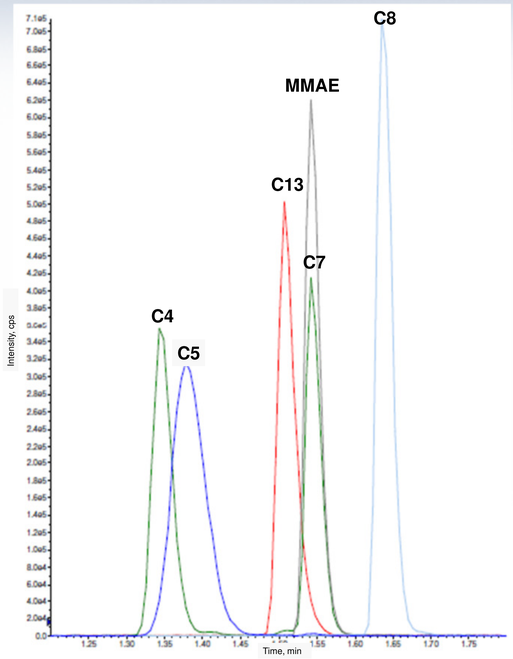
C13
mol. wt. 701.94



MMAE
mol. wt. 717.98

METHODS

The conversion from C7 to C5 in whole blood was minimized by chilling the samples in ice bath immediately after collection and harvesting plasma within 30 min with a refrigerated centrifuge. The plasma samples were stabilized by mixing with formic acid solutions immediately after harvest. The formic acid treated plasma samples were extracted by solid-phase extraction (SPE) with Waters Oasis® MCX 96-Well SPE Plates (30 mg). The extracted samples were then injected onto an Acquity UPLC® BEH C18 column and gradiently eluted with a mixture of methanol, water, and formic acid. The analytes were detected by an AB Sciex API 5000 mass spectrometer. High instrument response variation, caused by insufficient dwell time, at low concentration level was observed while all analytes were monitored in one injection. To have adequate dwell time in order to improve the assay precision, the procedure to have two separate injections was adopted.



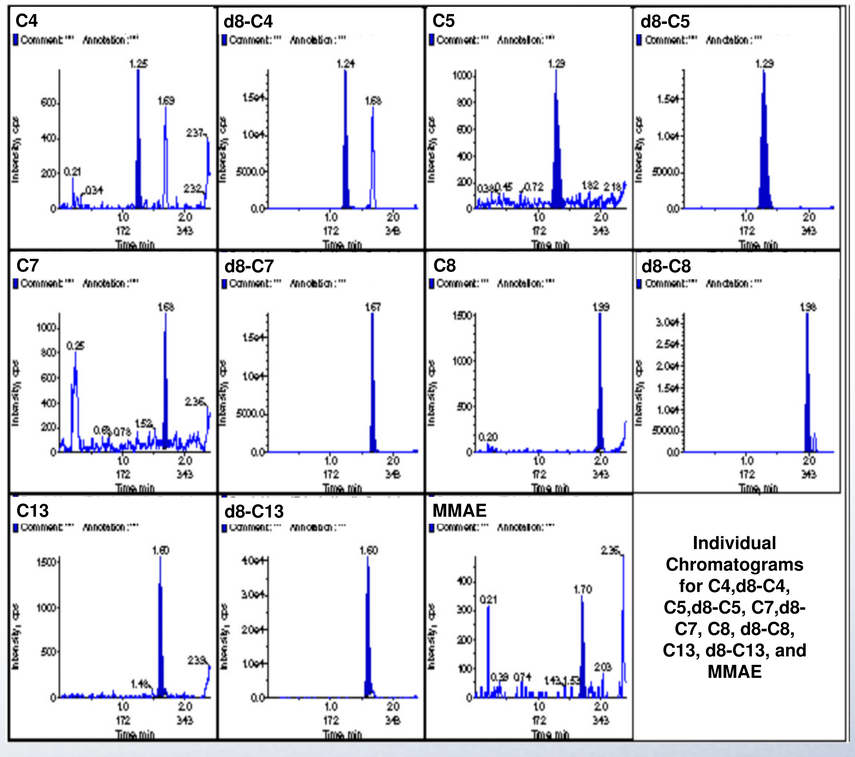
Chromatogram of C4, C5, C7, C8, C13, and MMAE

UPLC Conditions	
Column	BEH C18, 1.7 µm 2.1 x 50 mm, Waters
Mobile Phases	A: Water: Formic Acid @ 100:1 (V:V) B: Methanol: Formic Acid @ 100:1 (V:V)
Temperature	70ºC

- Gradient Elution starting at 40%B

MRM Transitions

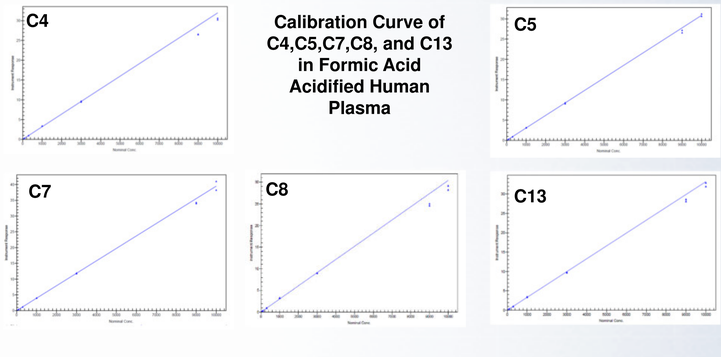
Compound	MS Transition	Dwell Time (msec)	CE (eV)
C4	704.5 > 152.5	35	50
C5	605.5 > 134.2	35	50
C7	704.5 > 506.5	35	30
C8	716.5 > 150.2	35	50
C13	702.5 > 150.2	35	50
d8-C4	712.5 > 152.5	20	50
d8-C5	613.5 > 134.2	20	50
d8-C7	712.5 > 506.5	20	30
d8-C8	724.5 > 150.2	20	50
d8-C13	710.5 > 150.2	20	50



Individual Chromatograms for C4,d8-C4, C5,d8-C5, C7,d8-C7, C8, d8-C8, C13, d8-C13, and MMAE

Results

With the LC-MS/MS conditions developed, no interference was observed among the analytes or from MMAE. An assay range of 10-10,000 pg/mL was established for all five analytes. The method was validated with respect to linearity, sensitivity, accuracy, precision, selectivity, hemolyzed plasma, lipemic plasma, recovery, matrix effect, and carryover; and was successfully applied to clinical sample analysis.



Calibration Curve of C4,C5,C7,C8, and C13 in Formic Acid Acidified Human Plasma

	C4	C5	C7	C8	C13
LLOQ QC Intraday %CV	2.8 to 10.2	5.8 to 10.8	2.7 to 5.9	3.9 to 5.8	2.1 to 10.2
LLOQ QC Intraday %RE	-18.7 to -0.9	-8.9 to -2.0	-16.1 to 1.0	-14.6 to 9.0	-9.6 to 4.0
Analytical QC Intraday %CV	0.7 to 4.6	0.8 to 4.3	1.0 to 5.4	0.7 to 4.9	0.9 to 4.6
Analytical QC Intraday %RE	-11.6 to 8.0	-6.7 to 7.3	-10.3 to 4.7	-10.7 to 11.3	-8.5 to 9.0
LLOQ QC Interday %CV	10.1	9.1	10.3	12.6	8.6
LLOQ QC Interday %RE	-8.6	-5.4	-10.2	-6.0	-2.7
Analytical QC Interday %CV	5.5 to 7.1	5.1 to 6.6	4.1 to 7.8	4.2 to 7.4	5.0 to 6.8
Analytical QC Interday %RE	-4.9 to 1.7	0.0 to 1.0	-4.0 to -0.7	-5.6 to 4.0	-1.9 to 4.0
Average Recovery of Analyte (%)	73.2	75.0	72.4	80.6	76.4
Average IS Normalized Matrix Factor	1.03	1.00	1.02	1.00	0.992

NOVEL ASPECT

An LC-MS/MS method was developed, validated, and successfully applied for simultaneous determination of the five MMAE metabolites.