

Title: Measurement and Mathematical Characterization of Cell-level Pharmacokinetics for Antibody-Drug Conjugates: A Case Study with Trastuzumab-vc-MMAE (Supplementary Data)

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Supplementary Tables:

Supplementary Table 1: A list of back-calculated Quality control (QC) samples for ELISA assay developed to quantify total trastuzumab in media and cell lysate matrix.

ELISA Type		QC_2.5 ng/mL	QC_25 ng/mL	QC_250 ng/mL
Media	Predicted (ng/mL)	5.9	23.75	235.8
	Standard Deviation	0.437	1.17	6.78
	CV%	7.4	4.9	2.8
Cell Lysate	Predicted (ng/mL)	3.29	23.94	363.126
	Standard Deviation	0.469	1.25	70.34
	CV%	14.2	5.4	19.31

Supplementary Table 2: A list of back-calculated Quality control (QC) samples for LC-MS/MS assay developed to quantify MMAE in media and cell lysate matrix.

LC-MS/MS Sample Type		QC_1.25 ng/mL	QC_25 ng/mL	QC_90 ng/mL
Media	Predicted (ng/mL)	1.38	25.99	91.50
	Standard Deviation	0.13	0.99	1.50
	CV%	10.40	3.96	1.67
Cell Lysate	Predicted (ng/mL)	1.41	27.60	95.10
	Standard Deviation	0.16	2.60	5.10
	CV%	12.80	10.40	5.67

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Supplementary Table 3: Sensitivity Indices obtained after Global Sensitivity Analysis (GSA) performed using a) **SOBOL** and b) **PRCC** method.

A) Sobol Method

Global Sensitivities	V_Cell	MV	Kin_MMAE	Kout_MMAE	Tubulin	Kon_Tub	Koff_Tub	Kon_ADC	Koff_ADC	Kint_ADC	Kdeg_ADC	AgEx	DT_Cell
Media Unconjugated MMAE	0.10	0.15	0.00	0.19	0.00	0.01	0.00	0.14	0.00	0.14	0.31	1.63	0.00
Cell Unconjugated MMAE	0.59	0.17	0.00	0.02	0.00	0.00	0.00	0.05	0.00	0.11	0.21	1.67	0.04
Media Total MMAE	0.08	0.43	0.04	0.04	0.00	0.00	0.00	0.04	0.00	0.09	0.01	0.29	0.40
Cell Total MMAE	0.56	0.17	0.00	0.01	0.00	0.00	0.00	0.04	0.00	0.02	0.01	2.04	0.05
Media Total Antibody	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.17	0.00	0.39	0.22
Cell Total Antibody	2.19	0.12	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.48	3.89	1.67	0.03

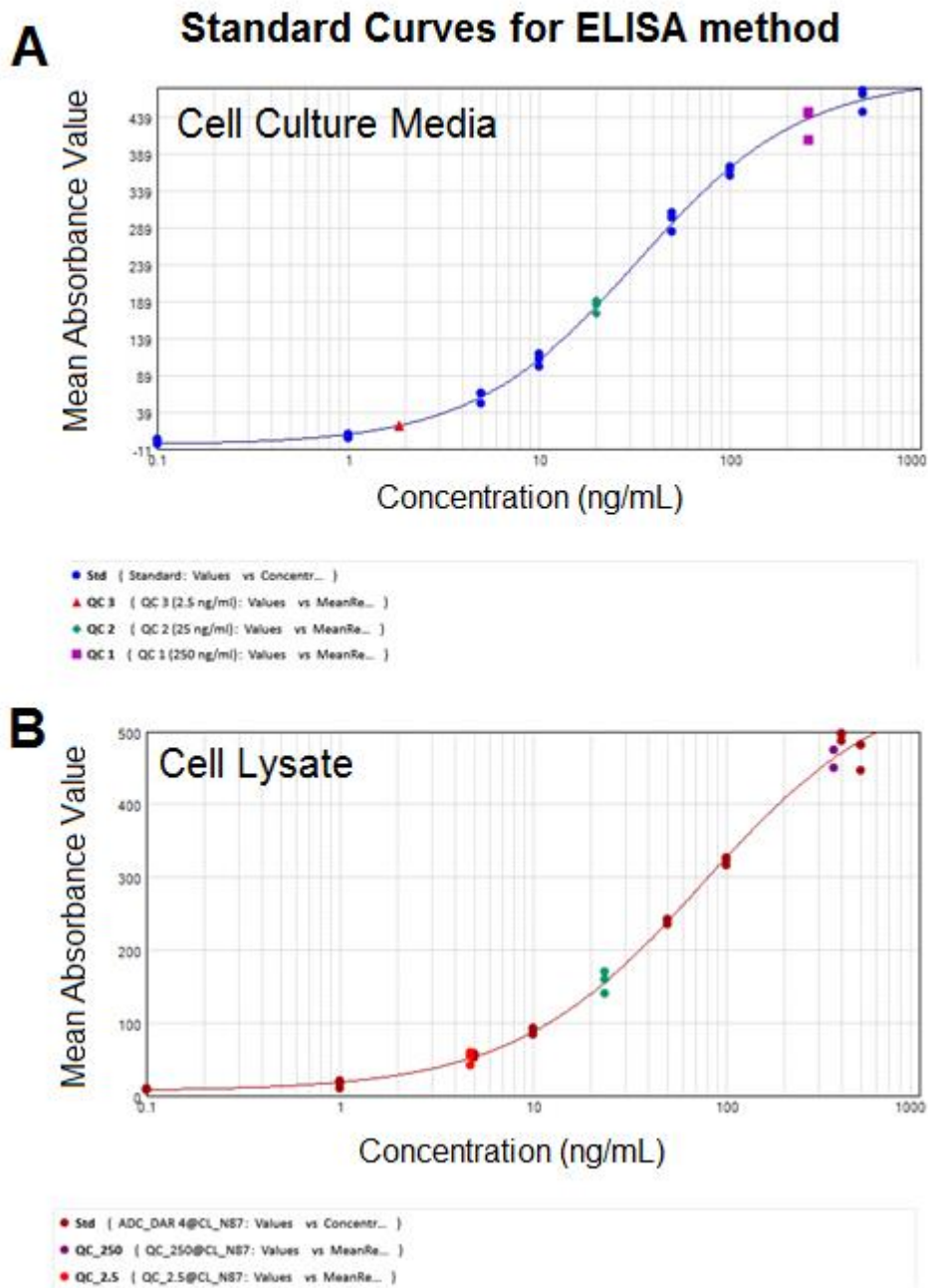
B) PRCC Method

Global Sensitivities	V_Cell	MV	Kin_MMAE	Kout_MMAE	Tubulin	Kon_Tub	Koff_Tub	Kon_ADC	Koff_ADC	Kint_ADC	Kdeg_ADC	AgEx	DT_Cell
Media Unconjugated MMAE	0.08	-0.20	0.00	0.16	0.01	0.01	-0.02	0.13	0.06	0.15	0.19	0.16	-0.05
Cell Unconjugated MMAE	-0.20	0.01	0.03	-0.02	0.03	0.03	0.00	0.10	0.02	0.18	0.16	0.18	-0.02
Media Total MMAE	0.03	-0.40	0.05	-0.11	0.01	-0.02	-0.01	0.11	-0.03	0.17	-0.07	0.40	-0.39
Cell Total MMAE	-0.31	0.04	0.05	-0.06	0.10	-0.01	0.03	0.06	0.00	0.05	0.02	0.28	-0.03
Media Total Antibody	-0.02	-0.42	0.01	-0.04	0.00	-0.02	-0.01	0.13	-0.02	0.22	-0.05	0.46	-0.30
Cell Total Antibody	-0.22	0.00	0.03	0.05	0.07	-0.01	-0.01	0.07	-0.06	0.22	-0.10	0.18	0.02

Supplementary Figures

Supplementary Figure S1. ELISA standard curves generated for quantification of total trastuzumab in (A) media and (B) cell lysate samples.

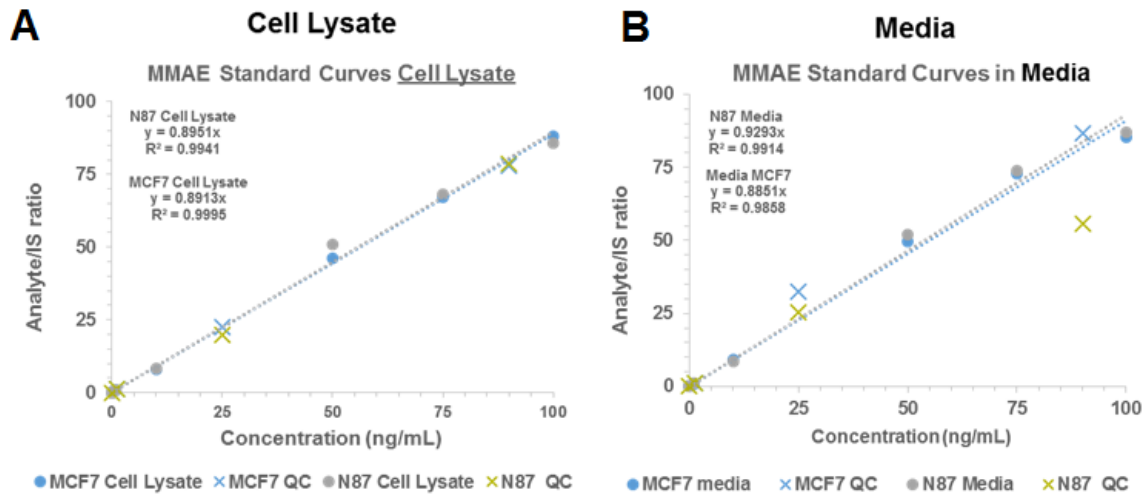
Supplementary Figure 1



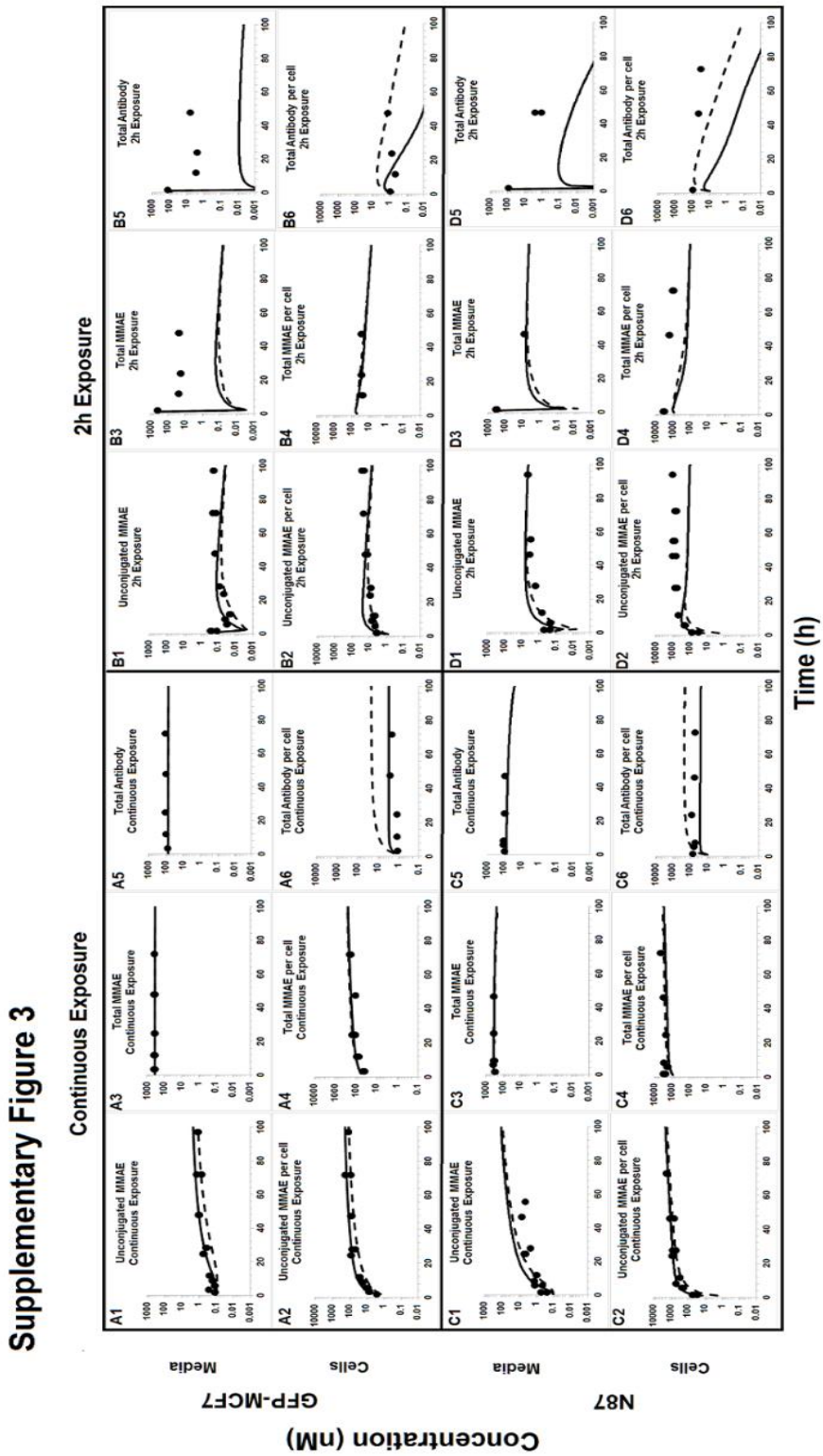
Supplementary Figure S2. LC-MS/MS standard curves generated for quantification of unconjugated MMAE in (A) cell lysate and (B) media.

Supplementary Figure 2

Standard Curves for LC-MS/MS Method for MMAE Quantification



Supplementary Figure S3. Model generated and observed media and intracellular PK profiles of different ADC analytes after continuous exposure (panels A and C) and 2h exposure (panels B and D) of 75 nM T-



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vc-MMAE in GFP-MCF7 cells (panels A and B) and N87 cells (panels C and D). Here 3% carryover of ADC after 2h washing was not incorporated. Solid circles represent observed data, solid lines represent model fitted profiles, and dashed lines represent model simulated profiles generated using a slower kdeg value obtained from (Maass et al., 2016).