

## **Development of a Green and Validated UHPLC-MS/MS Method for Assessing the Pharmacokinetics and Safety of PA-PEG<sub>12</sub>-PA in MCF-7 Cells**

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**Table S1.** Summary of validation parameters for the analytical method of PA-PEG<sub>12</sub>-PA.

Parameters	Description
Selectivity	The analytical method is capable of differentiating PA-PEG <sub>12</sub> -PA from endogenous components in the matrix or other constituents present in the sample.
Recovery	Comparing peak areas of QC samples with those of post-extraction blank matrix spiked at corresponding concentrations.
Matrix effect	Compare the peak areas of PA-PEG <sub>12</sub> -PA and IS in the precipitated protein spiked sample with those in standard solutions.
Accuracy	The mean concentration should be within 15% of the nominal values for the QC samples, except for the LLOQ which should be within 20% of the nominal values.
Precision	The CV value should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%.
Stability	Including room temperature storage stability (4 h at room temperature), autosampler stability (4 h at 4 °C), repeated freeze/thaw stability (3 cycles) and long-term stability (-30 °C for 15 d).
Calibration curve	At least six, non-zero calibrator levels covering the expected range, including LLOQ per analytical run.

**Table S2.** The accuracies and precisions of PA-PEG<sub>12</sub>-PA (Mean  $\pm$  SD , n=18).

	Low QC	Medium QC	High QC
Mean $\pm$ SD (ng/mL)	11.02 $\pm$ 0.34	193.8 $\pm$ 7.90	396.39 $\pm$ 19.91
R.E. (%)	10.22	-3.08	-0.90
Intra-day CV (%)	1.87	2.49	1.40
Inter-day CV (%)	7.41	9.73	14.13

**Table S3.** Recoveries and matrix effects of PA-PEG<sub>12</sub>-PA (Mean  $\pm$  SD , n=3).

	Low QC	Medium QC	High QC
Recoveries (%)	99.21 $\pm$ 2.17	103.05 $\pm$ 2.83	106.61 $\pm$ 5.96
Matrix effects (%)	107.27 $\pm$ 4.13	109.19 $\pm$ 1.96	103.67 $\pm$ 5.83

**Table S4.** Stability of PA-PEG<sub>12</sub>-PA under different conditions (Mean  $\pm$  SD , n=3).

Nominal Concentration (ng/mL)	Storage at room temperature for 4 h (%)	Storage at		
		Storage at autosampler vials (4 °C) for 4 h (%)	Freeze-thaw (3 cycles) (%)	Storage at -30 °C for 15 days (%)
10	10.70 $\pm$ 0.66	10.40 $\pm$ 0.88	10.18 $\pm$ 0.74	10.44 $\pm$ 0.89
200	183.00 $\pm$ 10.15	195.67 $\pm$ 4.04	194.00 $\pm$ 16.46	192.33 $\pm$ 2.08
400	381.00 $\pm$ 3.61	391.00 $\pm$ 6.56	389.33 $\pm$ 3.79	382.33 $\pm$ 10.26

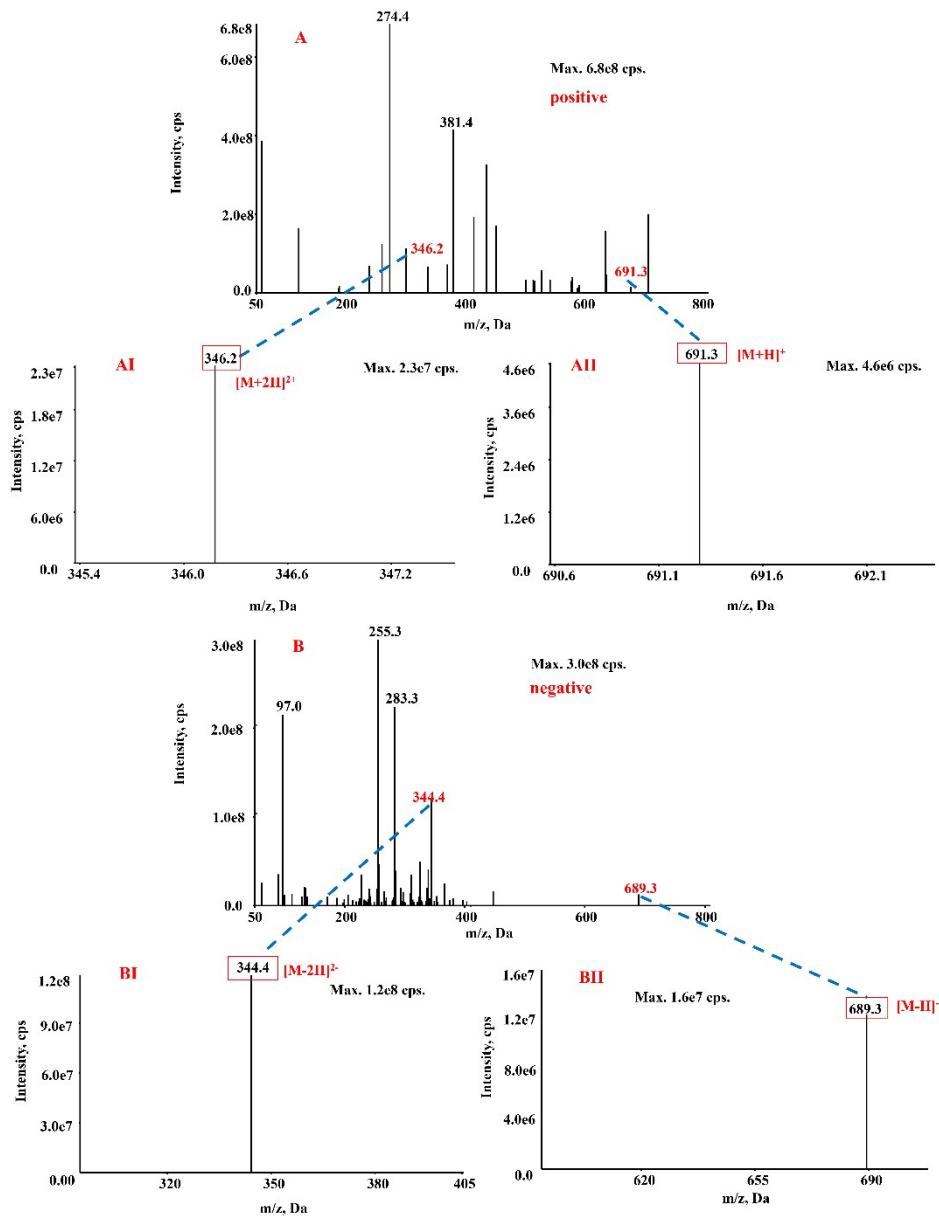
**Table S5.** The quantities of PA-PEG<sub>12</sub>-PA in MCF-7 cells. The quantities of PA-PEG<sub>12</sub>-PA were calculated by this formula:  $W (\%) = C_1 * V_1 * 10^{-6} / (1 * V_2) * 100\%$ .

Time (h)	PA-PEG <sub>12</sub> -PA concentration in cells				Quantity of PA-PEG <sub>12</sub> -PA in cell (%)			
	C <sub>1</sub> (ng/mL)				W=C <sub>1</sub> *V <sub>1</sub> *10 <sup>-6</sup> /(1*V <sub>2</sub> ) *100			
	I	II	III	mean	I	II	III	mean
0.5	6.54	6.07	5.19	5.93	1.64E-04	1.52E-04	1.30E-04	1.48E-04
1	9.91	10.20	8.43	9.51	2.48E-04	2.55E-04	2.11E-04	2.38E-04
2	12.00	9.44	13.20	11.55	3.00E-04	2.36E-04	3.30E-04	2.89E-04
4	13.40	16.30	13.40	14.37	3.35E-04	4.08E-04	3.35E-04	3.59E-04
8	11.90	19.40	16.80	16.03	2.98E-04	4.85E-04	4.20E-04	4.01E-04
12	41.00	22.30	24.70	29.33	1.03E-03	5.58E-04	6.18E-04	7.33E-04
24	28.60	38.70	39.80	35.70	7.15E-04	9.68E-04	9.95E-04	8.93E-04
36	43.40	22.00	34.60	33.33	1.09E-03	5.50E-04	8.65E-04	8.33E-04
48	25.40	17.90	35.50	26.27	6.35E-04	4.48E-04	8.88E-04	6.57E-04

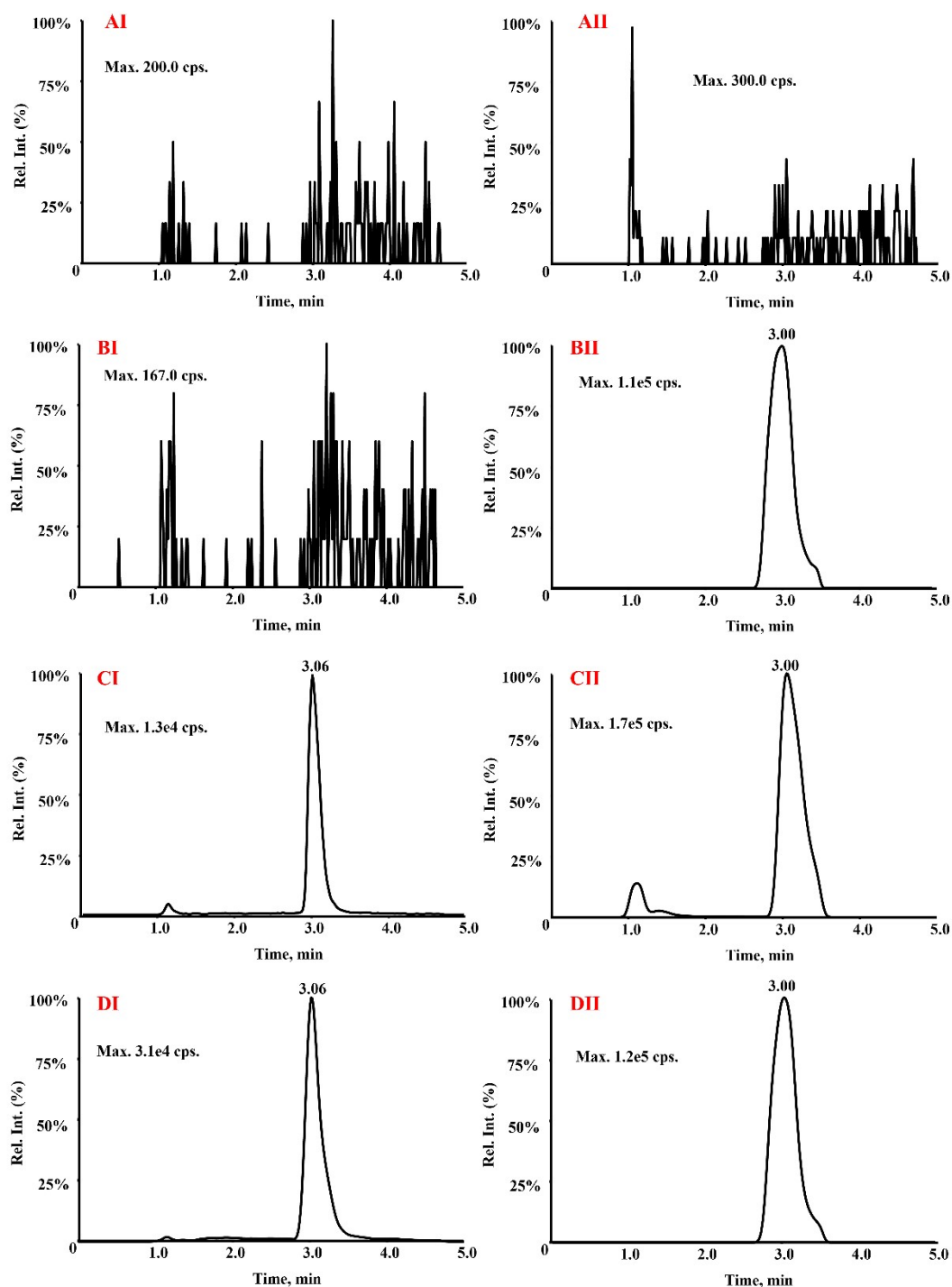
“V<sub>1</sub>” in the table represents the volume of the cell sample, V<sub>1</sub>=0.5 mL;

“V<sub>2</sub>” in the table represents the total volume of PEG polymer administered to MCF-7 cells, V<sub>2</sub>=2 mL;

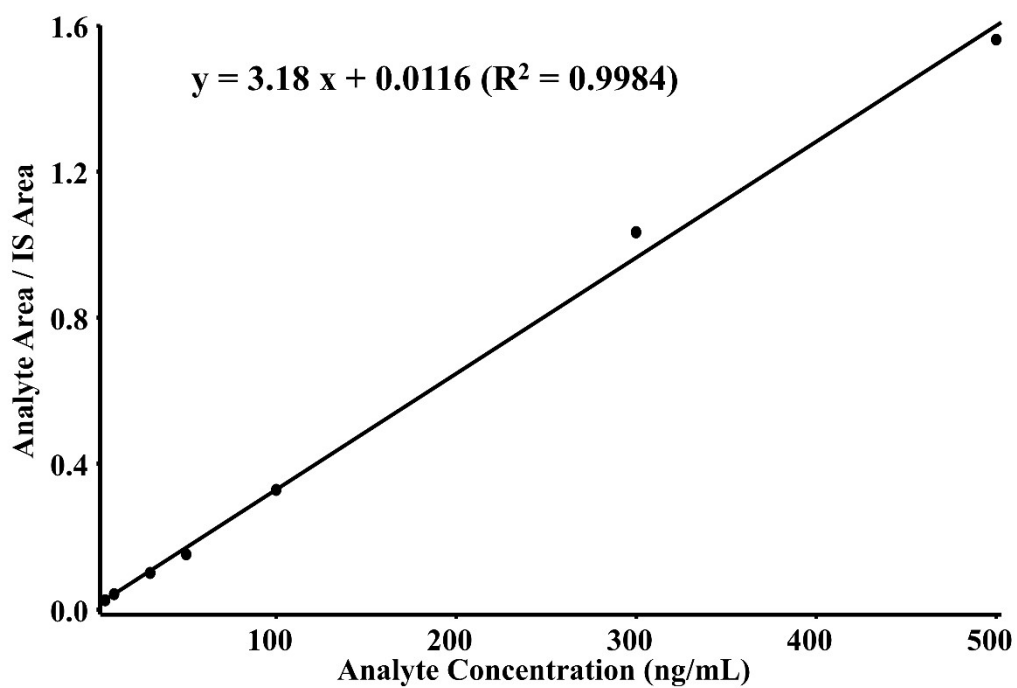
“1” in the table represents the concentration of PEG polymer administered to MCF-7 cells at 1 mg/mL ;



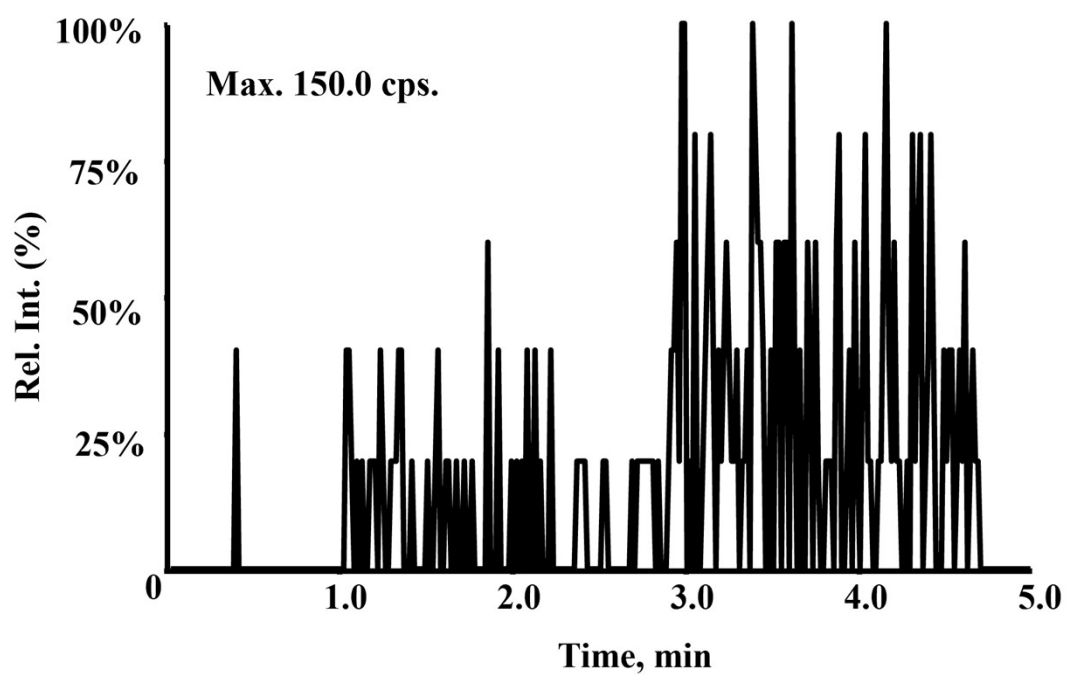
**Figure S1.** Comparison of the mass spectrometric response of PA-PEG<sub>12</sub>-PA in positive (A) and negative (B) ion modes. The key ions are as follows: Panel A (positive ion mode): (I) Doubly charged precursor ion [M+2H]<sup>2+</sup>, *m/z* 346.2; (II) Singly charged precursor ion [M+H]<sup>+</sup>, *m/z* 691.3. Panel B (negative ion mode): (I) Doubly charged precursor ion [M-2H]<sup>2-</sup>, *m/z* 344.4; (II) Singly charged precursor ion [M-H]<sup>-</sup>, *m/z* 689.3.



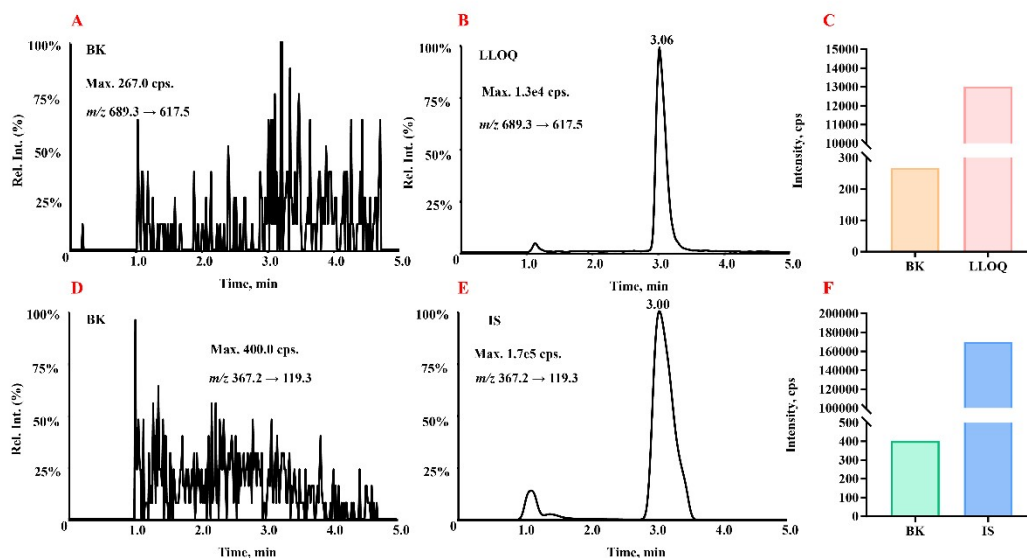
**Figure S2.** Representative UHPLC-MRM chromatograms of PA-PEG<sub>12</sub>-PA (I) and its internal standard M-PEG<sub>6</sub>-PA (II) obtained from (A) blank matrix, (B) blank matrix spiked with the internal standard (500 ng/mL), (C) blank matrix spiked with PA-PEG<sub>12</sub>-PA at the lower limit of quantification (5 ng/mL) and the internal standard (500 ng/mL), and (D) an actual MCF-7 cell sample.



**Figure S3.** Representative standard curve regression of PA-PEG<sub>12</sub>-PA.



**Figure S4.** Representative UHPLC-MS/MS chromatogram of the supernatant from the second PBS rinse following cell washing.



**Figure S5.** Evaluation of carry-over effect for PA-PEG<sub>12</sub>-PA and the internal standard (IS, M-PEG<sub>6</sub>-PA) using the UHPLC-MS<sup>2</sup> method. (A) PA-PEG<sub>12</sub>-PA of a blank matrix (BK) injected immediately after the upper limit of quantification (ULOQ, 500.0 ng/mL) for PA-PEG<sub>12</sub>-PA; (B) PA-PEG<sub>12</sub>-PA of the lower limit of quantification (LLOQ, 5 ng/mL) for PA-PEG<sub>12</sub>-PA; (C) Bar chart comparing the peak area responses of PA-PEG<sub>12</sub>-PA at LLOQ versus the post-ULOQ blank; (D) Chromatogram of a blank matrix injected after ULOQ for the IS; (E) Chromatogram of the IS; (F) Bar chart comparing the peak area responses of the IS versus the post-ULOQ blank.