Ultra-performance convergence chromatography tandem mass spectrometry strategy for quantification of monodisperse polyethylene glycol polymers coupled with multiple fragmentation to enhance sensitivity

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Table S1. Parameters evaluated for assay validation.

Items	Description
Specificity	Method selectivity was verified by analyzing blank cell lysates to confirm the absence of endogenous interferences at the retention time corresponding to HO-PEG_6-OH and its internal standard.
Lower Limit of Quantification	The LLOQ was established as the lowest concentration yielding a signal-to-noise ratio $\geqslant$10 with RSD $\leqslant$15% and accuracy within $\pm$20% over six replicate determinations.
Linearity	Calibration curves were constructed using $1/X^2$ weighted linear regression of peak area ratios (analyte/IS) versus nominal concentrations. The method demonstrated linearity across the validated range (5–500 ng/mL) with correlation coefficients (R^2) $\geqslant$0.99.
Precision and Accuracy	Within-run and between-run variations were assessed through six replicate analyses of QC samples at three concentration levels across three consecutive days. The acceptance criteria required relative standard deviation (RSD) $\leqslant$15% and relative error (RE) within $\pm$15% of nominal values.
Recovery and Matrix Effects	Extraction efficiency was evaluated by comparing peak areas of pre-spiked versus post-spiked samples in triplicate at low, medium, and high concentrations. Matrix effects were quantified as [(post-extraction spiked sample response)/(neat solution response)]$\times$100%.
Stability	Sample stability was investigated under four conditions: ambient temperature (4 h), autosampler storage (4 h), three freeze-thaw cycles (-30 ℃), and long-term storage (-30 ℃/15 days). Analytes were considered stable when concentration deviations remained within $\pm$15% of initial values.

Table S2. The accuracies and precisions of HO-PEG_8-OH (Data are for analysis of 6 replicates on 3 different days).

Concentration (ng/mL)		Accuracy	Precision	
Nominal conc. (ng/mL)	Mean ± SD	RE (%)	Intra-day (%)	Inter-day (%)
5	5.08±0.41	1.60	8.07	7.15
15	14.97±0.70	-0.22	4.87	3.22
50	49.69±2.39	-0.62	4.79	4.93
300	303.22±12.02	1.07	3.81	4.98

Table S3. The extraction recoveries and matrix effects of HO-PEG_8-OH (Mean ± SD, n=4).

Nominal conc. (ng/mL)	Recoveries (%)	Matrix Effects (%)
5	97.52 ± 5.12	105.34 ± 6.18
15	99.92 ± 2.27	111.89 ± 7.72
50	98.79 ± 4.34	96.42 ± 4.82
300	99.27 ± 4.13	100.96 ± 4.40

Table S4. Stability of HO-PEG_8-OH under various storage conditions (Mean ± SD, n=3).

Nominal Conc. (ng/mL)	Long term -30 ℃ for 15 days	Freeze-thaw -30 ℃ (3 cycles)	Autosampler vials (4℃) for 4 h	Room temperature for 4 h
5	5.05 ± 0.44	4.82 ± 0.38	5.11 ± 0.29	4.93 ± 0.41
15	15.07±0.40	14.97±0.80	15.33±1.14	15.10±0.62
50	50.57±2.68	47.87±1.78	50.77±2.87	49.90±1.80
300	303.00±22.91	310.67±8.50	295.67±6.66	299.33±11.59

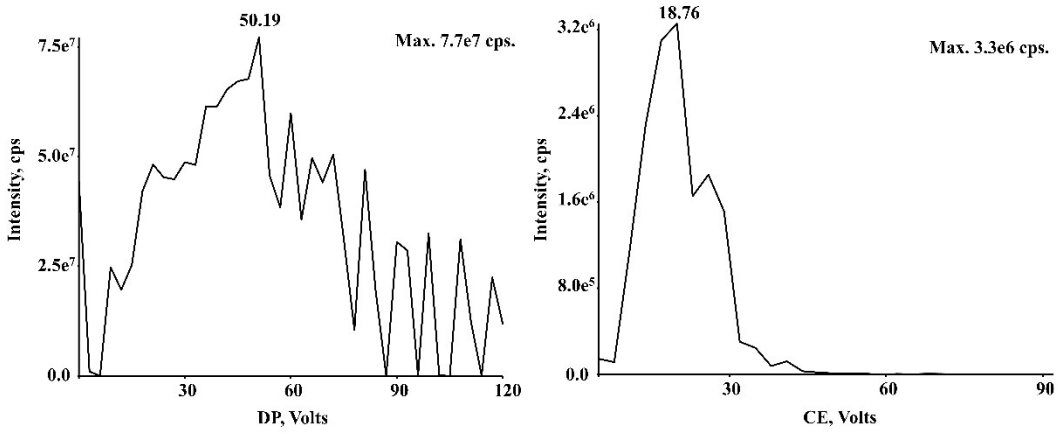

Fig. S1. Optimization of declustering potential (DP) (A) and collision energy (CE) (B) for HO-PEG$_8$-OH in UPC^2-MS^3 analysis.

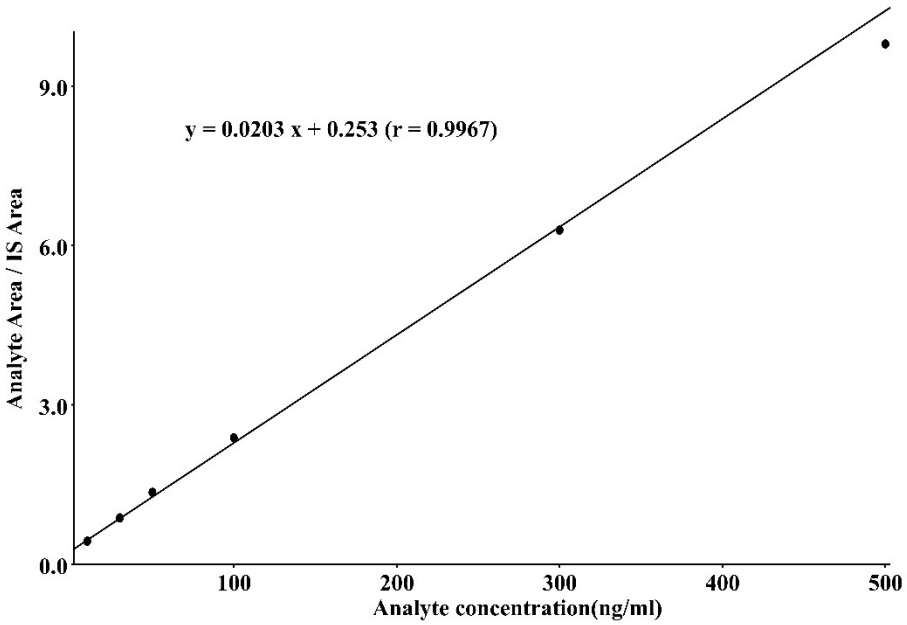

Fig. S2. Typical calibration curve of HO-PEG$_8$-OH with the concentration range of 5-500 ng/mL in cell lysate sample.