Development of UPLC-MS/MS assay for determination of PA-PEG_8-PA polymers in rat plasma coupled with $[M-H]^-$ to enhance sensitivity

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Supplementary information

The supplementary materials included method validation for detection of PA-PEG_8-PA polymers (Figure S1) and the optimization of mobile phase (aqueous phase) for chromatographic separation of PA-PEG_8-PA and $mPEG_6$-PA (Figure S2).

Method validation

The procedures for method validation are shown in Fig.S1.

Items	Description
Selectivity	The blank matrix samples were individually analyzed for endogenous substances that may interfere with the target analyte.
Accuracy and precision	Accuracy and precision are based on the analysis of six replicate QC samples on three different days.
Matrix effect	Compare the peak areas of PA-PEG8-PA and IS in the precipitated protein spiked sample with those in the pure solution.
Recovery	Comparing peak areas of QC samples with those of post-extraction blank rat plasma spiked at corresponding concentrations.
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 14 d).
Low limit of quantification (LLOQ)	The lowest concentration of the calibration curve that could be determined with accuracy±20% and precision≤15%.
Calibration curve	At least six, non-zero calibrator levels covering the expected range, including LLOQ per analytical run
Dilution methodology	The effect of dilution in the analysis of plasma samples containing PA-PEG8-PA concentrations above the upper limit of the standard curve was validated by analysis of 6 replicates of rat plasma spiked with PA-PEG8-PA 20 μg/mL after dilution with blank rat plasma using a 1:20 dilution factor to 1000 ng/mL.

Fig.S1 The procedures for method validation

Chromatographic separation of PA-PEG$_8$-PA and mPEG$_6$-PA polymers

Formic acid should not be added in the aqueous phase, as its presence can reduce the MS intensity of PA-PEG$_8$-PA and mPEG$_6$-PA. These contents are shown in Figure S2.

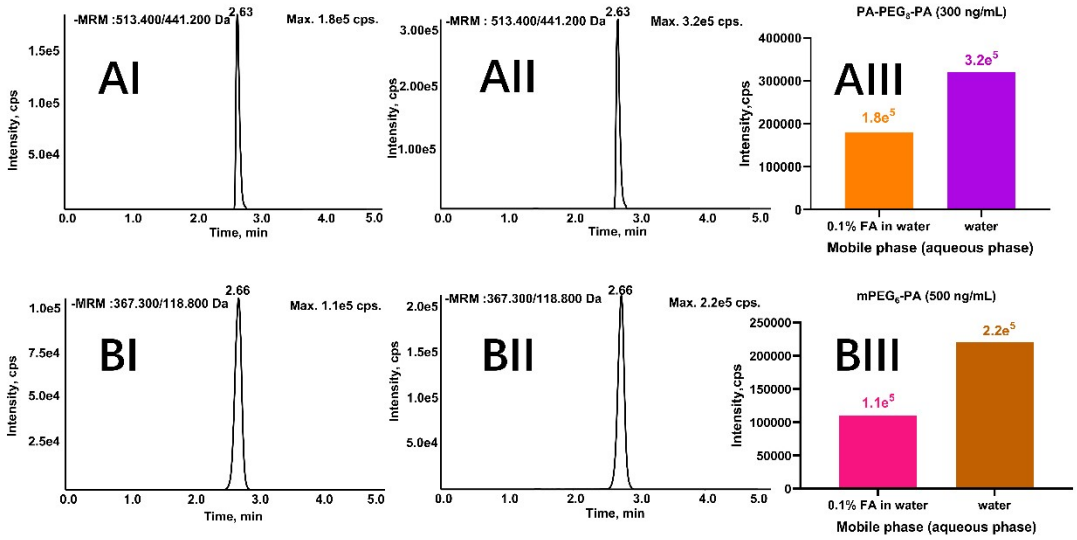

Figure S2. Optimization of UPLC conditions (aqueous phase) for chromatographic separation of PA-PEG$_8$-PA (A) and mPEG$_6$-PA (B). (AI) The aqueous phase(I), 0.1% formic acid in water; The organic phase (II), methanol; 0.0→1.5 min, II runs from 10%→10%; 1.5-2.0 min, II runs from 10%→95%; 2-3.5 min, II runs from 95%→95%; 3.5-3.6 min, II runs from 95%→10%; 3.6-5.0 min, II runs from 10%→10%. (AII) The aqueous phase(I), water; The organic phase (II), methanol; 0.0→1.5 min, II runs from 10%→10%; 1.5-2.0 min, II runs from 10%→95%; 2-3.5 min, II runs from 95%→95%; 3.5-3.6 min, II runs from 95%→10%; 3.6-5.0 min, II runs from 10%→10%. (AIII) Comparison of MS intensity of PA-PEG$_8$-PA with different aqueous phase (0.1% formic acid in water and water); (BI) The aqueous

phase(I), 0.1% formic acid in water; The organic phase (II), methanol; 0.0→1.5 min, II runs from 10%→10%; 1.5-2.0 min, II runs from 10%→95%; 2-3.5 min, II runs from 95%→95%; 3.5-3.6 min, II runs from 95%→10%; 3.6-5.0 min, II runs from 10%→10%. (BII) The aqueous phase(I), water; The organic phase (II), methanol; 0.0→1.5 min, II runs from 10%→10%; 1.5-2.0 min, II runs from 10%→95%; 2-3.5 min, II runs from 95%→95%; 3.5-3.6 min, II runs from 95%→10%; 3.6-5.0 min, II runs from 10%→10%. (BIII) Comparison of MS intensity of $mPEG_6$-PA with different aqueous phase (0.1% formic acid in water and water);