Supplementary Information

Instrument

Analysis of PEGylated gemcitabine, PEGylated BDP, PEGylated irinotecan was performed on an LC-ESI-MS system. The LC-MS system consisted of an Agilent 1100 Series HPLC (Agilent Technologies, Palo Alto, CA, USA) coupled to a Triple Tof 5600 mass spectrometer (Sciex, Ontario, Canada) equipped with a TurboIonSpray source. Data acquisition and integration were controlled by Analyst Software.

MS^{All} Analysis of precursor ions and product ions of PEGylated gemcitabine, PEGylated BDP, PEGylated irinotecan with low CE and high CE

Characterization of PEGylated gemcitabine, PEGylated BDP, PEGylated irinotecan was conducted with an Q-Q-TOF mass spectrometer.

For PEGylated gemcitabine, the chemical structure of PEGylated gemcitabine was shown in the supplementary figure 1. Supplementary figure 2 illustrates MS^{AII} analysis of the precursor ions of PEGylated gemcitabine. Supplementary figure 3 illustrates MS^{AII} analysis of the product ions of PEGylated gemcitabine at CE 43 eV. Supplementary figure 4 illustrates MS^{AII} analysis of the product ions of PEGylated gemcitabine at CE 80 eV. Supplementary figure 5 illustrates LC-ESI-MS^{AII} analysis of PEGylated gemcitabine (A, XIC of 133.0857+/-0.0039Da; B, XIC of 112.0506+/-0.0035Da; C, XIC of 89.0599+/-0.0029Da;). In our study, dissociation in q2 generated a series of high resolution PEG-related product ions at m/z 89.0599, 133.0869, 177.1102, 221.1366 corresponding to fragments containing various numbers of ethylene oxide subunits and PEGylated gemcitabine specific product ions at m/z 112.0506. PEGylated gemcitabine could be detected by positive ion electrospray ionization followed by high resolution extracted ions based on PEG and the specific compound.



Supplementary figure 1. Chemical structure of PEGylated gemcitabine



Supplementary figure 2. MS^{All} analysis of the precursor ions of PEGylated gemcitabine at CE 5 eV.



Supplementary figure 3. MS^{All} analysis of the product ions of PEGylated gemcitabine at CE 43 eV.



Supplementary figure 4. MS^{All} analysis of the product ions of PEGylated gemcitabine at CE 80 eV.



Supplementary figure 5. XIC of the product ions of PEGylated gemcitabine at CE 80 eV. (A, XIC of

133.0857+/-0.0039Da; B, XIC of 112.0506+/-0.0035Da; C, XIC of 89.0599+/-0.0029Da;).

For MPEG2K-BDP. The chemical structure of MPEG2K-BDP was shown in the supplementary figure 6. Supplementary figure 7 illustrates MS^{All} analysis of the precursor ions of MPEG2K-BDP. Supplementary figure 8 illustrates MS^{All} analysis of the product ions of MPEG2K-BDP at CE 40 eV. Supplementary figure 5 illustrates LC-ESI-MS^{All} analysis of XIC of the product ions of MPEG2K-BDP at CE 40 eV. (A, XIC of 133.0871+/-0.0031Da; B, XIC of 177.1129+/-0.0036Da; C, XIC of 221.1389+/-0.0043Da; D, XIC of 342.141+/-0.0005Da). In our study, dissociation in q2 generated a series of high resolution PEG-related product ions at m/z 89.0599, 133.0869, 177.1102, 221.1366 corresponding to fragments containing various numbers of ethylene oxide subunits and MPEG2K-BDP specific product ions at m/z 342.141. MPEG2K-BDP could be detected by

positive ion electrospray ionization followed by high resolution extracted ions based on PEG and the specific compound.



Supplementary figure 7. MS^{All} analysis of the precursor ions of MPEG2K-BDP at CE 10 eV.



Supplementary figure 8. MS^{All} analysis of the product ions of MPEG2K-BDP at CE 40 eV.



Supplementary figure 9. XIC of the product ions of MPEG2K-BDP at CE 40 eV.

(A, XIC of 133.0871+/-0.0031Da; B, XIC of 177.1129+/-0.0036Da; C, XIC of 221.1389+/-0.0043Da; D, XIC of 342.141+/-0.005Da;).

For PEGylated irinotecan. The chemical structure of PEGylated irinotecan was shown in the supplementary figure 10. Supplementary figure 11 illustrates MS^{All} analysis of the precursor ions of PEGylated irinotecan. Supplementary figure 12 illustrates MS^{All} analysis of the product ions of PEGylated irinotecan at CE 40 eV. Supplementary figure 13 illustrates LC-ESI-MS^{All} analysis of XIC of the product

ions of PEGylated irinotecan at CE 40 eV. (A, XIC of 133.0880+/-0.0027Da; B, XIC of 177.1142+/-0.0036Da; C, XIC of 221.1403+/-0.0043Da; D, XIC of 285.144+/-0.005Da; E, XIC of 569.276+/-0.008Da).

In our study, dissociation in q2 generated a series of high resolution PEGrelated product ions at m/z 89.0599, 133.0869, 177.1102, 221.1366 corresponding to fragments containing various numbers of ethylene oxide subunits and PEGylated irinotecan specific product ions at m/z 285.144, 569.276. PEGylated irinotecan could be detected by positive ion electrospray ionization followed by high resolution extracted ions based on PEG and the specific compound.



Supplementary figure 10. Chemical structure of PEGylated irinotecan



Supplementary figure 11. MS^{All} analysis of the precursor ions of PEGylated irinotecan at CE 10 eV.



Supplementary figure 12. MSAII analysis of the product ions of PEGylated irinotecan at CE 40 eV.



Supplementary figure 13. XIC of the product ions of PEGylated irinotecan at CE 40 eV. (A, XIC of 133.0880+/-0.0027Da; B, XIC of 177.1142+/-0.0036Da; C, XIC of 221.1403+/-0.0043Da; D, XIC of 285.144+/-0.005Da; E, XIC of 569.276+/-0.008Da).