

EPA/EPA eluted first at a retention time of approximately 2.5 min, and PC-DHA/DHA eluted at approximately 2.8 min.

Figure 38. Positive ion electrospray mass spectra of the phosphatidyl choline standards PC-DHA/DHA and PC-EPA/EPA. Protonated molecules of each phospholipid were detected as the base peaks of each spectrum at m/z 878 and m/z 826, respectively.

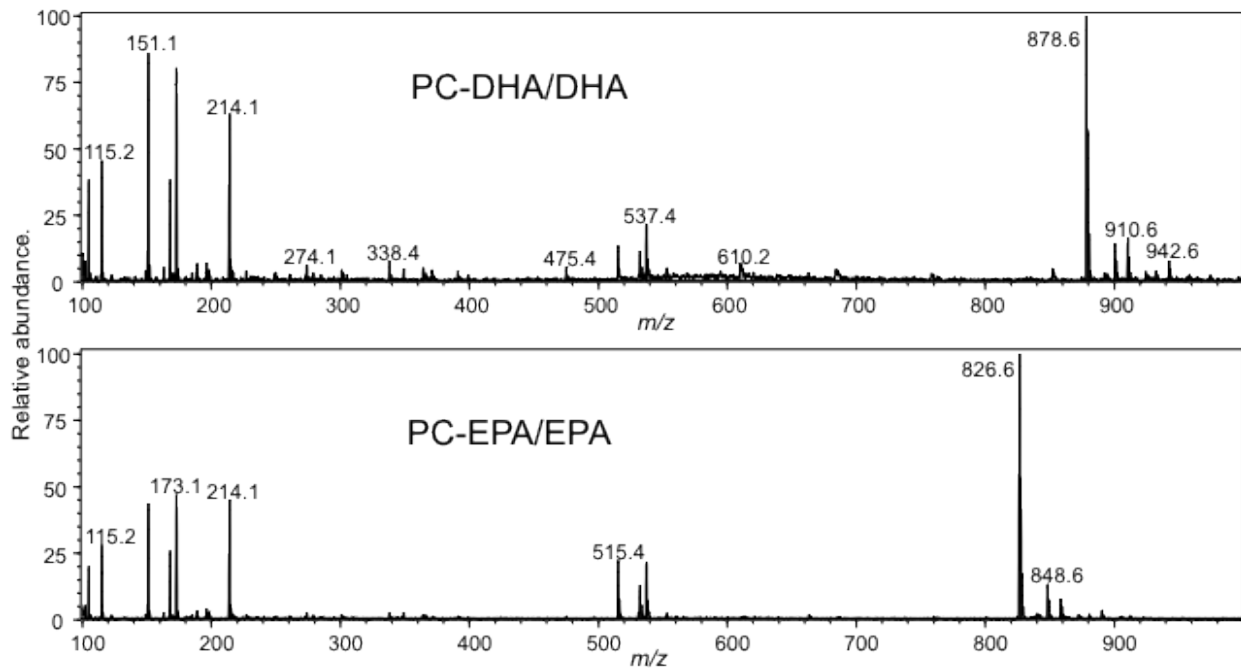


Figure 39. Positive ion electrospray product ion tandem mass spectra of PC-DHA/DHA and PC-EPA/EPA. The protonated molecules of m/z 878 and m/z 826 corresponding to PC-DHA/DHA and PC-EPA/EPA, respectively, were used as precursor ions. Based on these tandem mass spectra, the abundant product ion of m/z 184 was used for subsequent measurements of these phospholipids during SRM.

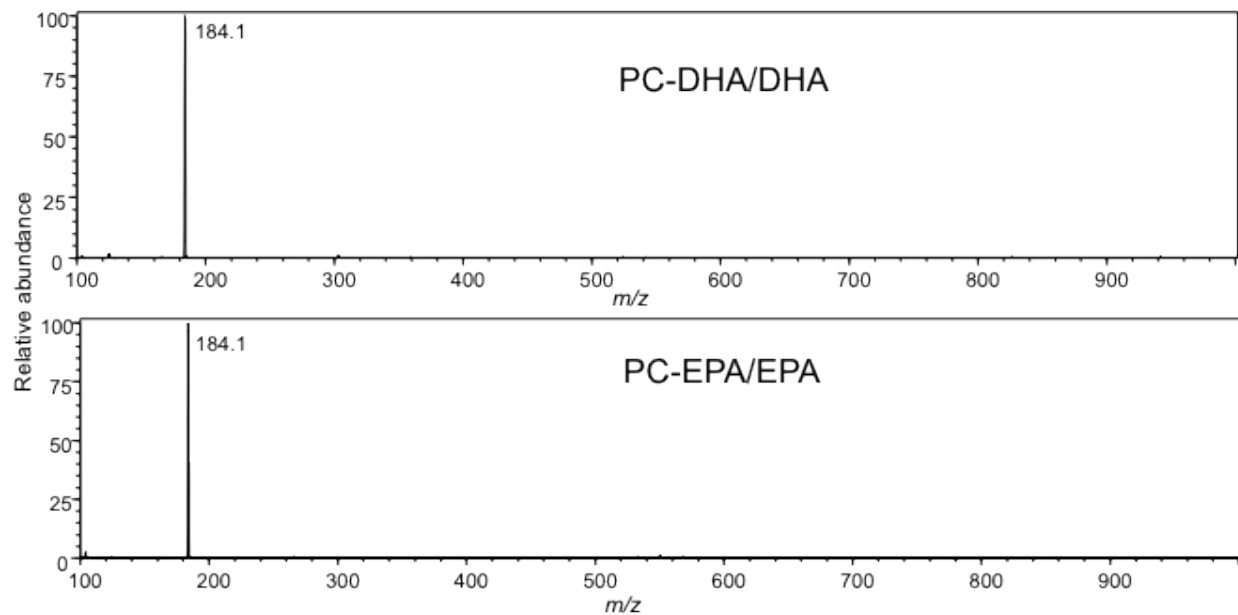
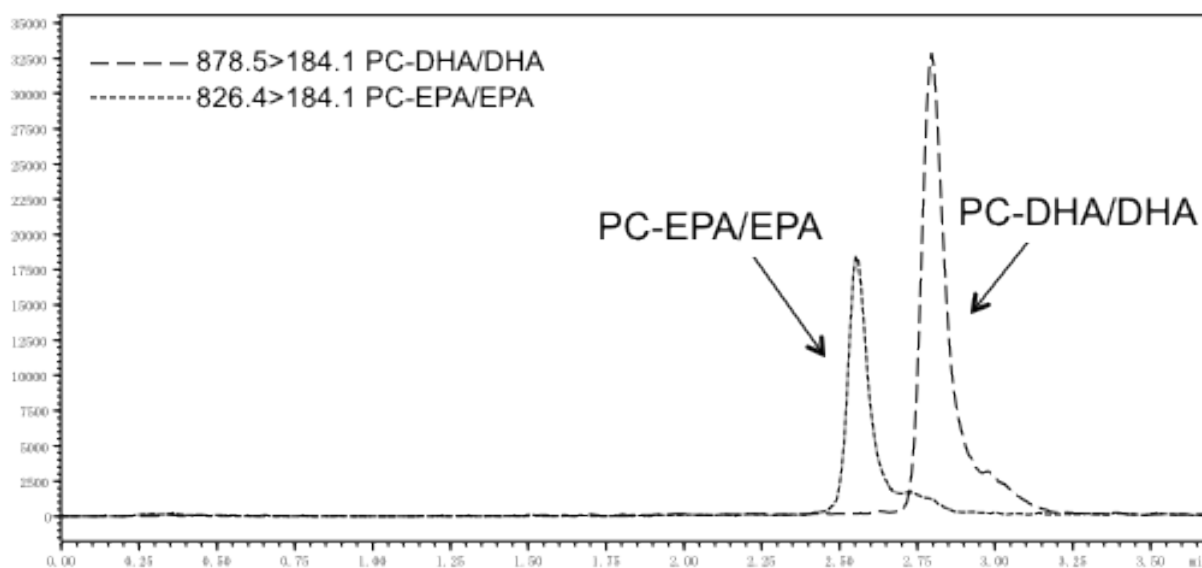


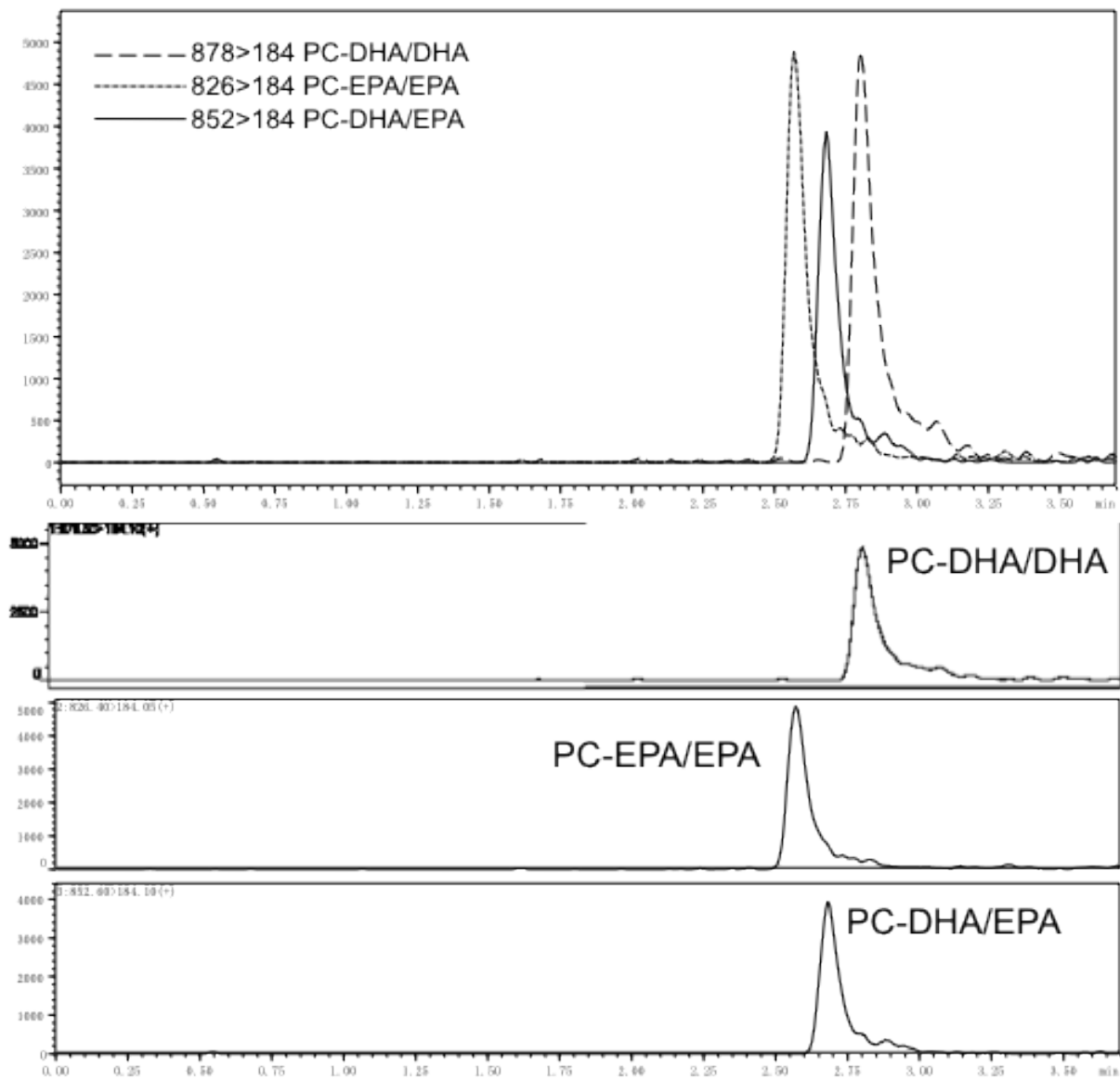
Figure 40. UHPLC-MS-MS chromatogram of a mixture of PC-EPA/EPA and PC-DHA/DHA standards obtained using positive ion electrospray and SRM of the transition m/z 826 to m/z 184 for PC-EPA/EPA and SRM of the transition m/z 878 to m/z 184 for PC-DHA/DHA. The retention times of PC-EPA/EPA and PC-DHA/DHA were ~ 2.5 min and ~ 2.8 min, respectively.



96. UHPLC-MS-MS was used to measure the 18 krill oil samples (Table 1), which included 9 samples from *E. pacifica* and 9 samples from *E. superba* that had been heated to 60 °C, 70 °C or 125 °C, or not heated at all. Solvent blanks were injected and analyzed between UHPLC-MS-MS analyses of each krill oil sample. PC-EPA/EPA and PC-DHA/DHA were detected in all 18 krill oil samples. PC-DHA/EPA was also detected in all 18 krill oil samples and eluted between PC-

DHA/DHA and PC-EPA/EPA during UHPLC-MS-MS. No phospholipid peaks were detected in any of the solvent blank analyses, which proved that there was no sample carry over between analyses. The UHPLC-MS-MS chromatograms showing the detection of all three phospholipids in the 18 krill oil samples are shown in Figures 41 through 58.

Figure 41. Positive ion electrospray UHPLC-MS-MS with SRM analysis of an acetone fraction of krill oil from *E. pacifica* that had been not been heat treated. Note that PC-EPA/EPA was detected at a retention time of ~2.5 min, PC-DHA/DHA was detected eluting at ~2.8 min, and PC-DHA/EPA eluted in between the other phospholipids at a retention time of ~2.7 min.



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