standard and reanalyzed using UHPLC-MS/MS (Figure 8). The standard coeluted with the phospholipid in the extract thereby identifying this phospholipid in the Fujita hexane/ethanol extract as PC-DHA/DHA.

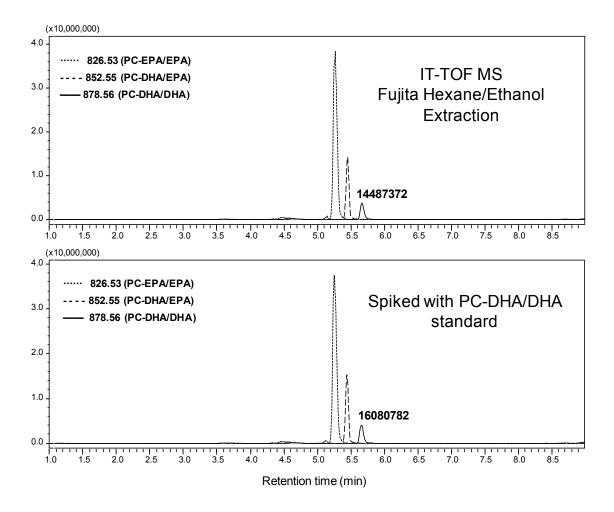


Figure 8. Positive ion electrospray high resolution IT-TOF UHPLC-MS computer-reconstructed mass chromatograms of the Fujita hexane ethanol extract showing the detection of peaks corresponding to PC-EPA/EPA (*m/z* 826.53), PC-DHA/EPA (*m/z* 852.55) and PC-DHA/DHA (*m/z* 878.56) eluting at approximately 5.2, 5.4 and 5.6 minutes, respectively (top). The



extract was spiked with a PC-DHA/DHA standard and then reanalyzed (bottom). Note that the area of the peak corresponding to PC-DHA/DHA increased confirming the identity of PC-DHA/DHA in the extract.

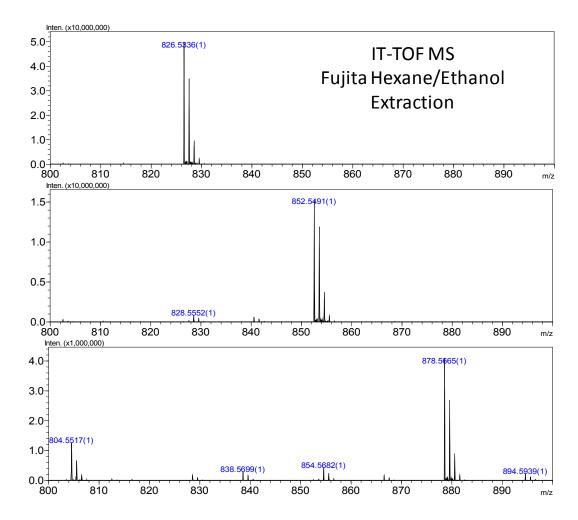


Figure 9. High resolution accurate mass measurements of the peaks corresponding to PC-EPA/EPA (top) eluting at a retention time of approximately 5.2 min (Figure 8), PC-EPA/DHA (middle) eluting at a retention time of approximately 5.4 min (Figure 8), and PC-DHA/DHA (bottom) eluting at a retention time of approximately 5.6 min (Figure 8).



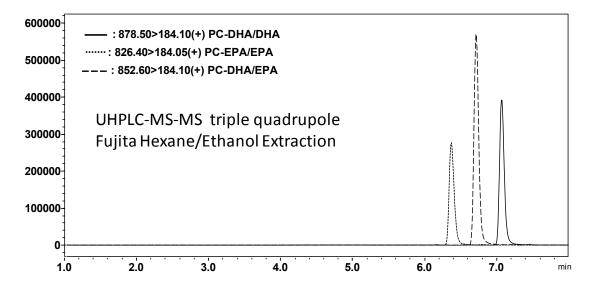


Figure 10. Positive ion electrospray UHPLC-MS/MS analysis of the Claimed Phospholipids in the Fujita Hexane/Ethanol Extract. A triple quadrupole mass spectrometer was used with collision-induced dissocation and selected reaction monitoring (SRM) of the transitions indicated.

68. Further analysis of the Claimed Phospholipids in the Fujita hexane ethanol extract was carried out using positive ion electrospray UHPLC-MS/MS with collision-induced dissociation and selected reaction monitoring (SRM) on a triple quadrupole tandem mass spectrometer. The SRM transitions that were used for analysis were m/z 826 to m/z 184 for PC-EPA/EPA, m/z 852 to m/z 184 for PC-EPA/DHA, and m/z 878 to m/z 184 for PC-DHA/DHA. A chromatogram for this analysis is shown in Figure 10.



Figures 11-13 below reflect the result of my analysis of the Fujita 69. once-through extract, Sample No. vB O. As shown in the Figures, each of the following three Claimed Phospholipid species was detected in the extract: PC-DHA/DHA, PC-EPA/EPA, and PC-EPA/DHA. Using UHPLC-MS on the high resolution IT-TOF mass spectrometer (Figure 11), PC-EPA/EPA eluted first at approximately 5.2 minutes and was measured at m/z 826.5338 (Figure 12). Since the theoretical mass of PC-EPA/EPA is 826.5386 ( $\Delta m = -6$  ppm), the elemental composition of this phospholipid in the Fujita once-through extract was determined to be identical to that of PC-EPA/EPA. PC-EPA/DHA eluted next from the UHPLC-MS system at a retention time of approximately 5.4 minutes (Figure 11) and was measured at m/z 852.5493 (Figure 12). Since the theoretical mass of PC-EPA/DHA is 852.5543 ( $\Delta m = -6$  ppm), the elemental composition of this phospholipid in the Fujita hexane extract was determined to be identical to that of PC-EPA/DHA. PC-DHA/DHA eluted at a retention time of approximately 5.6 min (Figure 11) and was measured at m/z 878.5668 (Figure 12). Since the theoretical mass of PC-DHA/DHA is 878.5699 ( $\Delta m = -3$  ppm), the elemental composition was determined to be identical to that of PC-DHA/DHA. Furthermore, the Fujita once-through extract was spiked with a PC-DHA/DHA standard and reanalyzed using UHPLC-MS/MS (Figure 11). The standard coeluted with the phospholipid in



the extract thereby identifying this phospholipid in the Fujita once-through extract as PC-DHA/DHA.

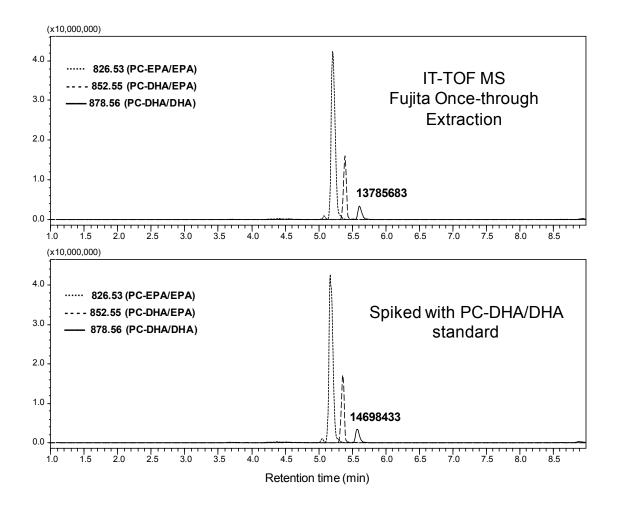


Figure 11. Positive ion electrospray high resolution IT TOF UHPLC-MS computer-reconstructed mass chromatograms of the Fujita once-through extract showing the detection of peaks corresponding to PC-EPA/EPA (*m/z* 826.53), PC-DHA/EPA (*m/z* 852.55) and PC-DHA/DHA (*m/z* 878.56) eluting at approximately 5.2, 5.4 and 5.6 minutes, respectively (top). The extract was spiked with a PC-DHA/DHA standard and then reanalyzed (bottom). Note that the area



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