UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

AKER BIOMARINE AS
Petitioner

v.

NEPTUNE TECHNOLOGIES AND BIORESSOURCES, INC.
Patent Owner

Case IPR2014-00003
Patent 8,278,351 B1

Declaration of Richard B. Van Breemen, Ph.D.

I, Richard B. van Breemen, Ph.D., hereby declare as follows:

1. I have been retained by counsel for Petitioner Aker BioMarine AS to provide expert opinions in connection with this *inter partes* review.

2. My background and qualifications are set forth in the declaration I submitted previously in connection with this IPR, dated September 27, 2013 (Exhibit 1040).

3. Attached hereto as Exhibit A are excerpts from the November 6, 2013 witness statement I submitted in USITC Investigation No. 337-TA-877, *In the Matter of Certain Omega-3 Extracts from Marine or Aquatic Biomass and Products Containing the Same.*

4. I hereby incorporate the statements and information contained in Exhibit A into this declaration and reaffirm their truthfulness and accuracy.

Dated: September 18, 2014

Richard B. van Breemen

Richard B. van Breemen, Ph.D.

EXHIBIT A

UNITED STATES INTERNATIONAL TRADE COMMISSION
Washington, D.C.

Before the Honorable Theodore R. Essex
Administrative Law Judge

In the Matter of	
CERTAIN OMEGA-3 EXTRACTS FROM MARINE OR AQUATIC BIOMASS AND PRODUCTS CONTAINING THE SAME	**Investigation No. 337-TA-877**

WITNESS STATEMENT OF RICHARD B. VAN BREEMEN, PH.D

I. INTRODUCTION

Q.1. Would you please state your name for the record?

A.1. Richard Bruce van Breemen.

Q.2. Where are you employed?

A.2. I am a Professor of Medicinal Chemistry and Pharmacognosy at the University of Illinois-Chicago, or "UIC," College of Pharmacy in Chicago, Illinois.

Q.3. How long have you been with UIC?

A.3. I joined the faculty of UIC in 1994 as an Associate Professor of Medicinal Chemistry at the College of Pharmacy. In 2000, I was promoted to the position I hold today.

[redacted]

[redacted]

Q.41. In RDX-0504, you refer to samples you received from others and then tested. Did you test samples in addition to the extractions you repeated?

A.41. I was also asked to test certain krill extracts that were prepared by others, including samples I received from Dr. Suzanne Budge at Dalhousie University in Halifax, Canada, and samples of material provided to Respondents in this litigation by Dr. Earl L. White, to determine whether they contain the Claimed Phospholipids. All six of the samples I was asked to test from Dr. Budge contained the Claimed Phospholipids, as did the three samples produced by Dr. White. While I do not have personal knowledge regarding how these nine extracts were made, it is my understanding that all of them were made according to the prior art Beaudoin references – WO 00/23546 (referred to as "Beaudoin I") and/or CA 2,251,265 (referred to as "Beaudoin II").

[redacted]

[redacted]

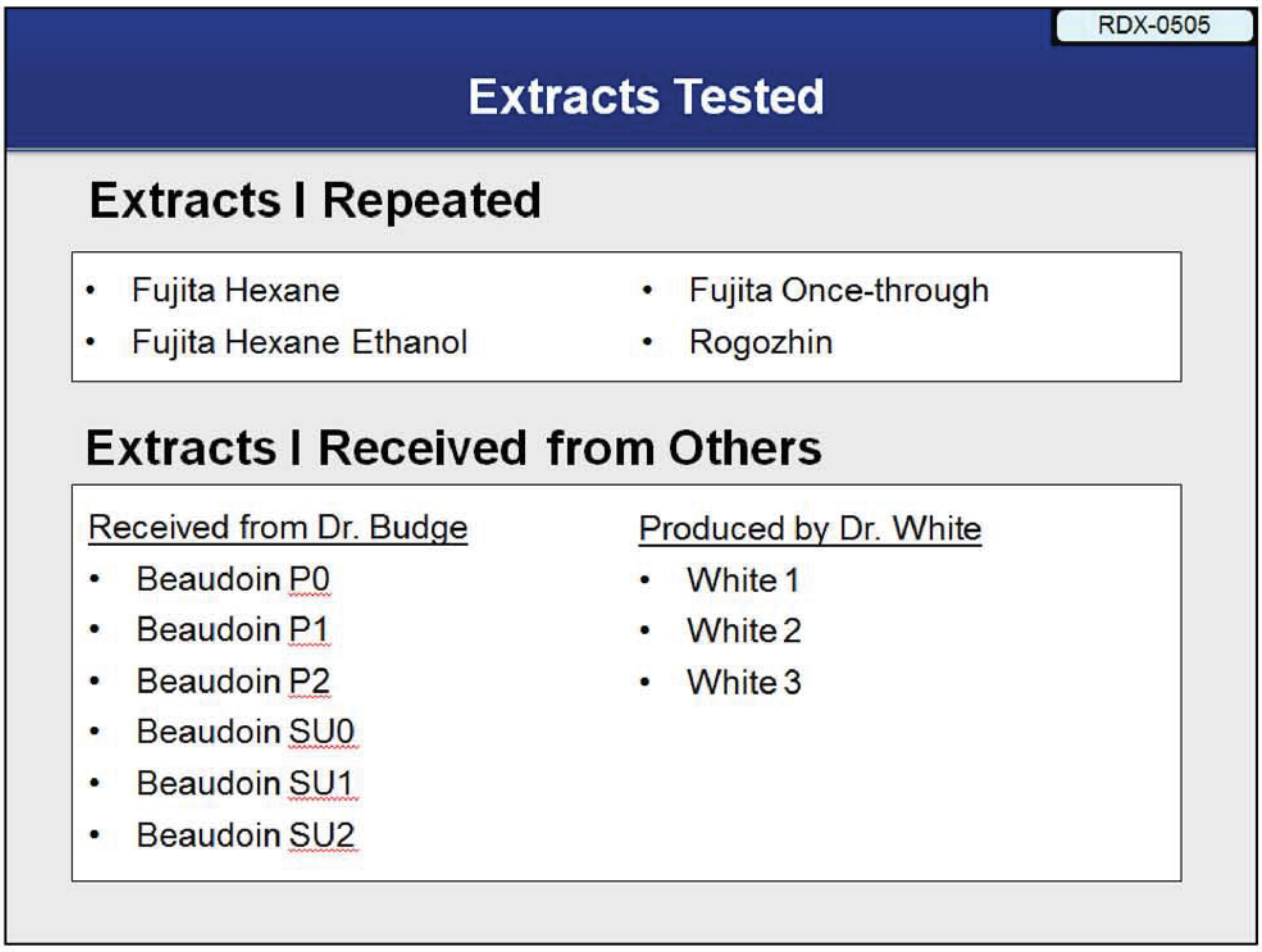
IV. INVALIDITY UNDER 35 U.S.C. § 102

A. Claimed Phospholipids are Present in Krill Extracts Made According to Certain Prior Art References

1. The Extracts Tested

Q.46. I'd like to focus now on the repeat extractions and testing work you performed in reaching your opinions. Would you please identify all the extracts that you tested?

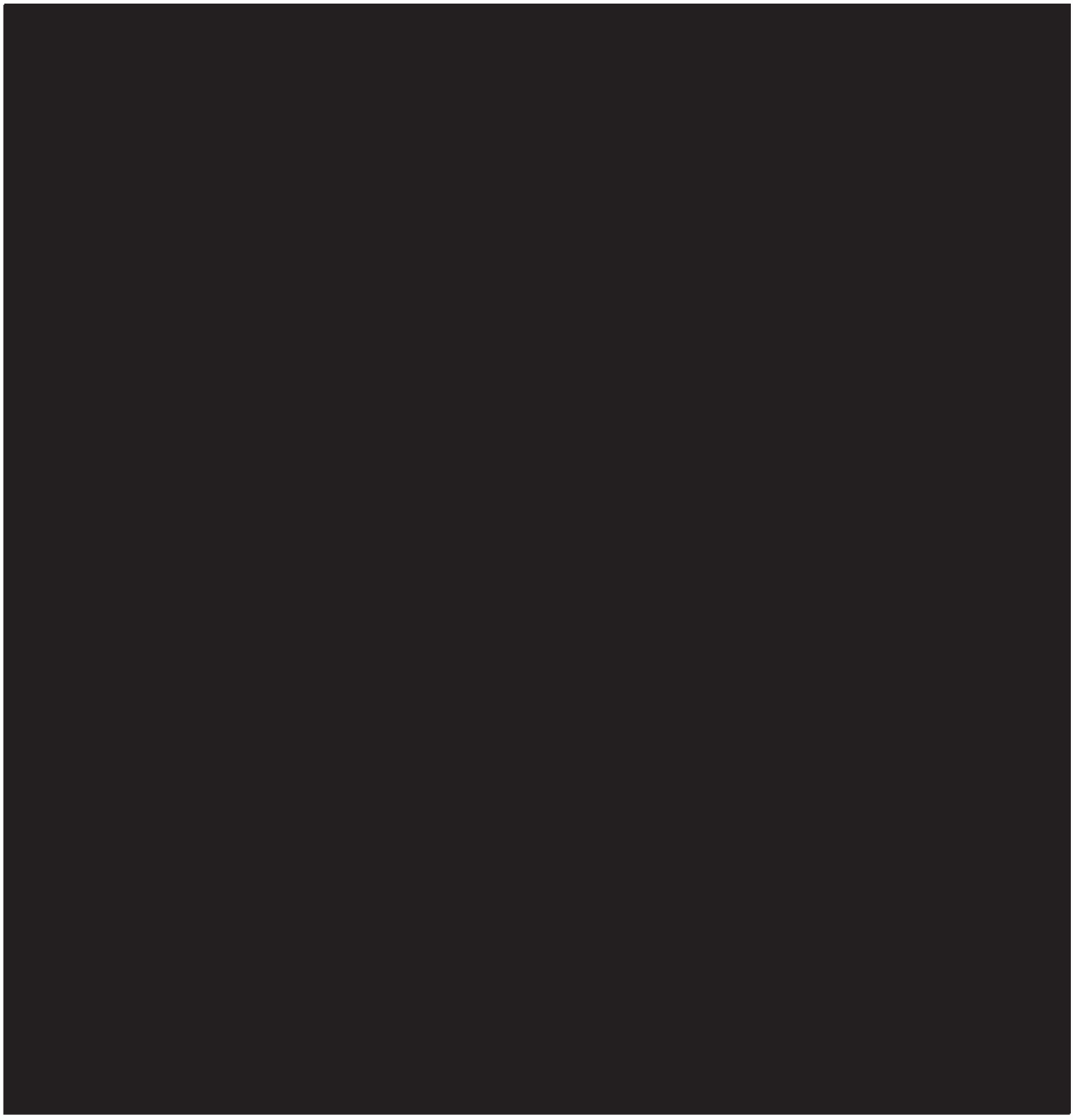
A.46. Yes. RDX-0505 lists the thirteen prior art krill extracts that I tested.



As I mentioned before, I repeated three different extracts by following three different procedures in the Fujita Reference: Fujita Hexane, Fujita Hexane Ethanol, and Fujita Once-through. I also repeated one extract by following the procedure in the Rogozhin patent.

The nine remaining extracts that I tested were extracts that I received from others. Six came from Dr. Budge: Beaudoin P0, Beaudoin P1, Beaudoin P2, Beaudoin SU0, Beaudoin SU1, and Beaudoin SU2. The other three were provided to Respondents in this litigation by Dr. White: White 1, White 2, and White 3. While I do not have personal knowledge regarding how these nine extracts were made, it is my understanding that all of them were made according to the prior art Beaudoin references.

[redacted]



Q.160. Would you please walk us through the setup for your testing, starting with the equipment you used?

A.160. UHPLC-MS analyses of krill oils were carried out using a high resolution Shimadzu IT-TOF mass spectrometer equipped with a Shimadzu Prominence XR HPLC system.

Chromatographic separations were obtained using a Waters Acquity CSH C18 UHPLC column (2.1 mm x 150 mm; 1.7 µm).

Q.161. How were your instruments configured?

A.161. The initial composition of the mobile phase was 80% methanol and 20% water containing 5 mM ammonium formate for 2 min followed by a 5 min gradient from 80% to 100% methanol. The column was re-equilibrated at 80% methanol for 3 min between analyses. The UHPLC mobile phase flow rate was 0.3 mL/min for the IT-TOF mass spectrometer and 0.4 mL/min for the LCMS-8040 triple quadrupole mass spectrometer.

Q.162. Did you use the standard of PC-DHA/DHA that you had purchased as part of your setup?

A.162. The mass spectrometers and UHPLC system were optimized for the analysis of the Claimed Phospholipids using a PC-DHA/DHA standard. The positive ion electrospray mass spectrum of standard PC-DHA/DHA is shown in Figure 1 on RDX-0534. (RX-0580, Fig. 1.)

RDX-0534

Figure 1

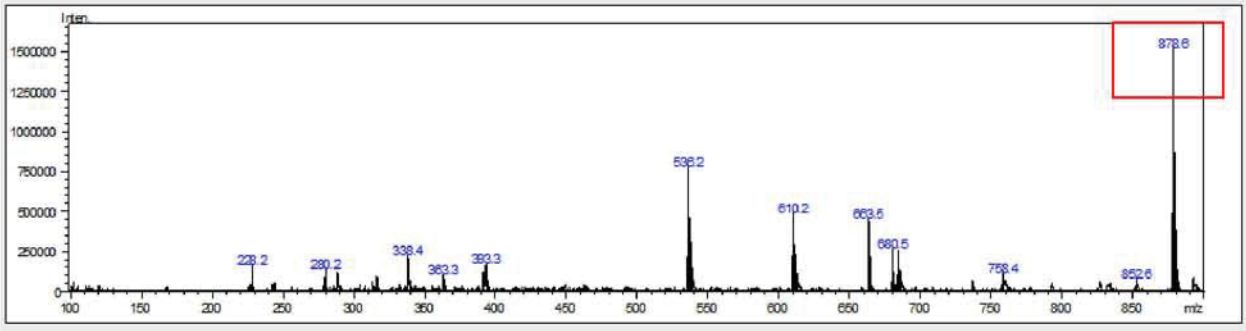


Figure 1. Positive ion electrospray mass spectrum of PC-DHA/DHA standard (from Sigma/Aldrich).

RX-0580, Fig. 1

The theoretical mass of PC-DHA/DHA is 878.5699. As I've indicated with a red box, the expected intact PC-DHA/DHA ion of *m/z* 878.6 was observed as the base peak of the mass spectrum, a result consistent with the presence of PC-DHA/DHA. (RX-0580, Fig. 1.)

Q.163. Did you do any further analysis on this ion of *m/z* 878.6?

A.163. Yes. Turning to Figure 2 on RDX-0535, you'll see that, using *m/z* 878.6[1] as a precursor ion, collision induced dissociation was used and product ion tandem mass spectrometry was used to obtain the tandem mass spectrum of PC-DHA/DHA. (RX-0580, Fig. 2.)

RDX-0535

Figure 2

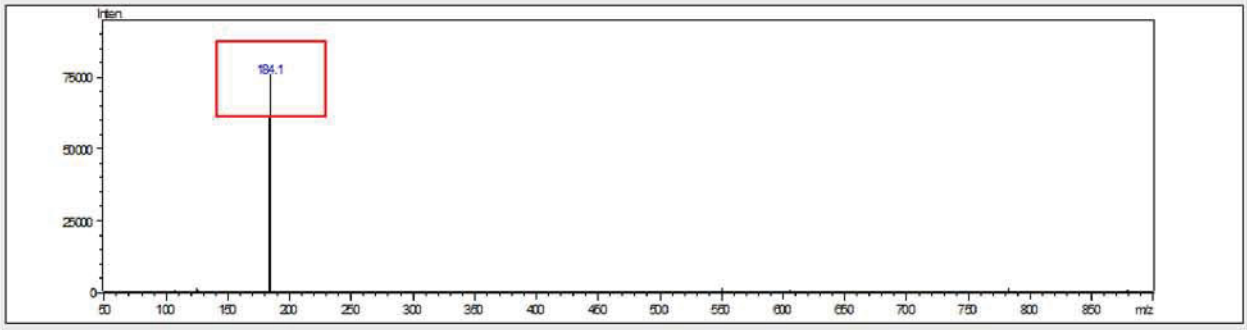


Figure 2. Positive ion electrospray product ion tandem mass spectrum of PC-DHA/DHA ion of *m/z* 878.[6].

RX-0580, Fig. 2

As expected, PC-DHA/DHA fragmented to form an abundant product ion of *m/z* 184.1 containing the PC moiety without the fatty acids, as I've indicated with a red box. This product ion of *m/z* 184.1 is common to all PCs.

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[1] As indicated with brackets in the caption of Figure 2 on RDX-0535, I corrected a typographical error that appeared in the caption of the same figure in my opening expert report (RX-0580). Unless otherwise noted, use of brackets in the captions of figures that appeared in RX-0580 and RX-0585 indicate such typographical error corrections.

Q.164. Is there a peak corresponding to DHA in this mass spectrum?

A.164. No.

Q.165. Is it surprising that there are no peaks corresponding to DHA in this mass spectrum?

A.165. No.

Q.166. Why is that?

A.166. Positive ion electrospray detects positive ions, but fatty acids like DHA form negative ions more readily than positive ions and would not be detected with positive ion electrospray.

Q.167. How were you able to use the data in Figures 1 and 2 for your extract testing?

A.167. Turning to Figure 3 on RDX-0536, you'll see that, based on the data in Figures 1 and 2, an assay based on UHPLC-MS/MS was developed that utilized reversed phase UHPLC separation, positive ion electrospray for ionization of the phospholipids, collision-induced dissociation and selected reaction monitoring to record the transition from the intact phospholipids ions to their common product ion of *m/z* 184. (RX-0580, Fig. 3.)

Figure 3 is a chromatogram showing this transition. This chromatogram plots the chromatography dimension along the X-axis and the tandem mass spectrometry dimension along the Y-axis. The one peak in Figure 3 indicates that the phospholipid was detected when it eluted at 6.9 minutes, and that it was measured at m/z 878, and as was the structurally informative ion of *m/z* 184. Accordingly, the phospholipid is identified as PC-DHA/DHA.

RDX-0536

Figure 3

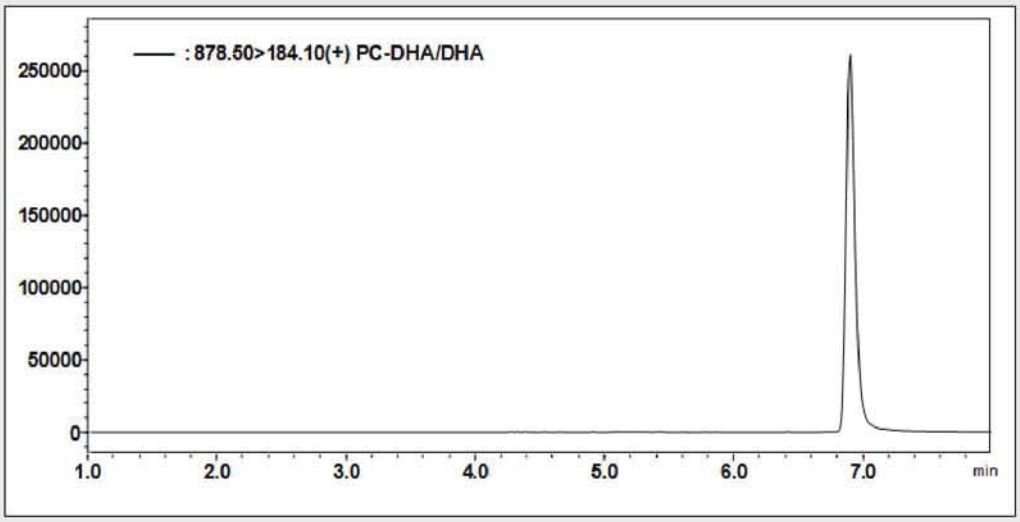


Figure 3. UHPLC-MS/MS triple quadrupole analysis of a PC-DHA/DHA standard of 250 ng/mL obtained using positive ion electrospray with collision-induced dissociation and selected reaction monitoring of the transition *m/z* 878 to *m/z* 184.

RX-0580, Fig. 3

UHPLC was used to separate PC-DHA/DHA from PC-EPA/EPA and from PC-DHA/EPA as well as from other compounds in the krill oil extracts.

Q.168. Did you do anything between the analyses of the extracts you tested to make sure your equipment was getting accurate results?

A.168. Before each analysis of a krill oil sample, a blank analysis was carried out to ensure that there was no carryover of phospholipids from one analysis to the next.

Q.169. How did you confirm that you were actually detecting each of PC-DHA/DHA, PC-EPA/EPA and PC-DHA/EPA in the samples you tested?

A.169. In addition to UHPLC-MS/MS, high resolution accurate mass measurements of intact PC-DHA/DHA, PC-EPA/EPA and PC-DHA/EPA in each of the krill oil samples were used to confirm that their measured elemental compositions were identical to their corresponding theoretical elemental compositions. The standard practice for analyses such as these is that, when a high resolution measurement of an unknown molecule is within 10 ppm of a theoretical value, then the elemental composition of that molecule is confirmed. Accordingly, I used a 10 ppm window as the threshold for determining whether the detected ion mass matched that of the target compound.

[redacted]

Q.266. What were the results of your testing of White 1?

A.266. RDX-0571 through RDX-0573 include Figures 39-41 respectively, and reflect the results of my analysis of White 1, produced in this litigation by Dr. White. (RX-0580, Figs. 39-41.) As shown in these figures, each of the three Claimed Phospholipid species PC-DHA/DHA, PC-EPA/EPA, and PC-DHA/EPA was detected in White 1. (RX-0580, Figs. 39-41.)

Q.267. What is shown in Figure 39?

A.267. Figure 39 shows the positive ion electrospray high resolution IT TOF UHPLC-MS computer-reconstructed mass chromatograms of the White 1. (RX-0580, Fig. 39.) The top chromatogram of Figure 39 shows 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 5.3, 5.5 and 5.7 minutes, respectively. (RX-0580, Fig. 39.) The bottom chromatogram of Figure 39 shows the results of an additional test where White 1 was spiked with a PC-DHA/DHA standard and then reanalyzed. (RX-0580, Fig. 39.)

RDX-0571

Figure 39

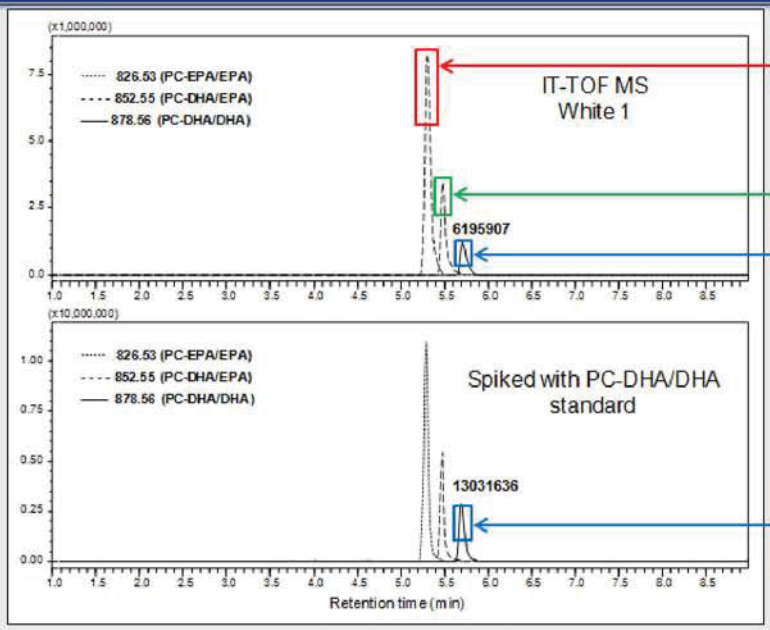


Figure 39. Positive ion electrospray high resolution IT TOF UHPLC-MS computer-reconstructed mass chromatograms of the White 1 sample showing the detection of peaks corresponding to PC-EPA/EPA (m/z 826.53), PC-DHA/EPA (*m/z* 852.55) and PC-DHA/DHA (*m/z* 878.56) eluting at approximately 5.3, 5.5 and 5.7 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.

RX-0580, Fig. 39

Q.268. What do the peaks labeled in red, green, and blue indicate in the top chromatogram of Figure 39?

A.268. The peaks correspond to the retention times of the three Claimed Phospholipids I was testing for. PC-EPA/EPA, indicated in red, eluted first at approximately 5.3 minutes. (RX-0580, Fig. 39.) PC-DHA/EPA, indicated in green, eluted next from the UHPLC-MS system at a retention time of approximately 5.5 minutes. (RX-0580, Fig. 39.) PC-DHA/DHA, indicated in blue, eluted last at a retention time of approximately 5.7 min. (RX-0580, Fig. 39.)

Q.269. What is shown in Figure 40?

A.269. Figure 40 shows the three high resolution accurate mass measurements of the PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA peaks respectively, previously shown in Figure 39. (RX-0580, Figs. 39-40.)

RDX-0572

Figure 40

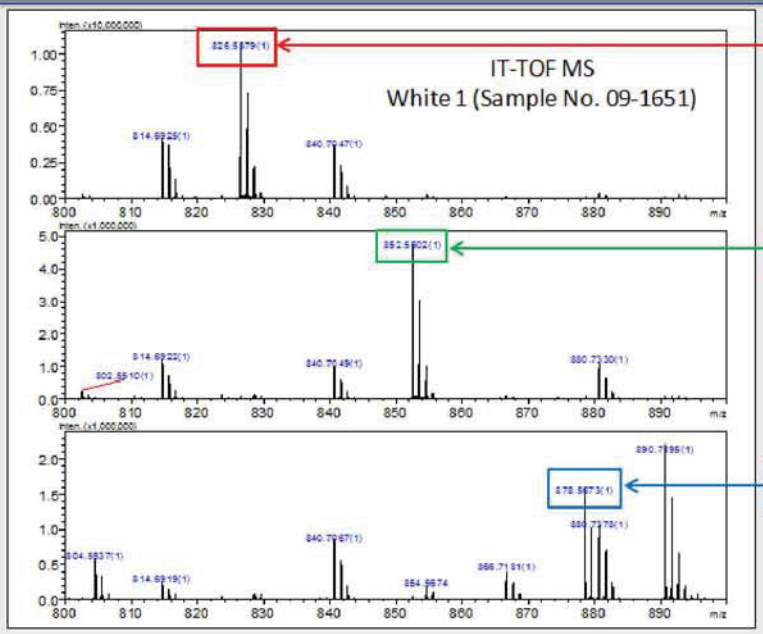


Figure 40. High resolution accurate mass measurements of the peaks corresponding to PC-EPA/EPA (top) eluting at a retention time of approximately 5.3 min (Figure 39), PC-EPA/DHA (middle) eluting at a retention time of approximately 5.5 min (Figure 39), and PC-DHA/DHA (bottom) eluting at a retention time of approximately 5.7 min (Figure 39).

RX-0580, Fig. 40

Q.270. What do the peaks labeled in red, green, and blue indicate in Figure 40?

A.270. As indicated in red at the top of Figure 40, PC-EPA/EPA was measured at *m/z* 826.5379 when it eluted. (RX-0580, Fig. 40.) Because the theoretical mass of PC-EPA/EPA is 826.5386 (Δm = -5 ppm), the elemental composition of this phospholipid in White 1 was determined to be identical to that of PC-EPA/EPA. As indicated in green in the middle of Figure 40, PC-DHA/EPA was measured at *m/z* 852.5502 when it eluted next. (RX-0580, Fig. 40.) Because the theoretical mass of PC-DHA/EPA is 852.5543 (Δm = -4 ppm), the elemental composition of this phospholipid in White 1 was determined to be identical to that of PC-DHA/EPA. Lastly, as indicated in blue at the bottom of Figure 40, PC-DHA/DHA was measured at *m/z* 878.5673 when it eluted. (RX-0580, Fig. 40.) Because the theoretical mass of PC-DHA/DHA is 878.5699 (Δm = -3 ppm), the elemental composition was determined to be identical to that of PC-DHA/DHA.

Q.271. Would you please explain the results of your spiking test in Figure 39?

A.271. As shown in the bottom chromatogram of Figure 39 on RDX-0571, White 1 was spiked with the PC-DHA/DHA standard and reanalyzed using UHPLC-MS. (RX-0580, Fig. 39.) The standard coeluted with one of the phospholipids in the extract – the peak for

PC-DHA/DHA increased and no new peak appeared – thereby identifying this phospholipid in White 1 as PC-DHA/DHA, indicated in blue. (RX-0580, Fig. 39.)

Q.272. What is shown in Figure 41?

A.272. Figure 41 shows the Positive ion electrospray UHPLC-MS/MS analysis of the Claimed Phospholipids in the White 1. (RX-0580, Fig. 41.)

RDX-0573

Figure 41

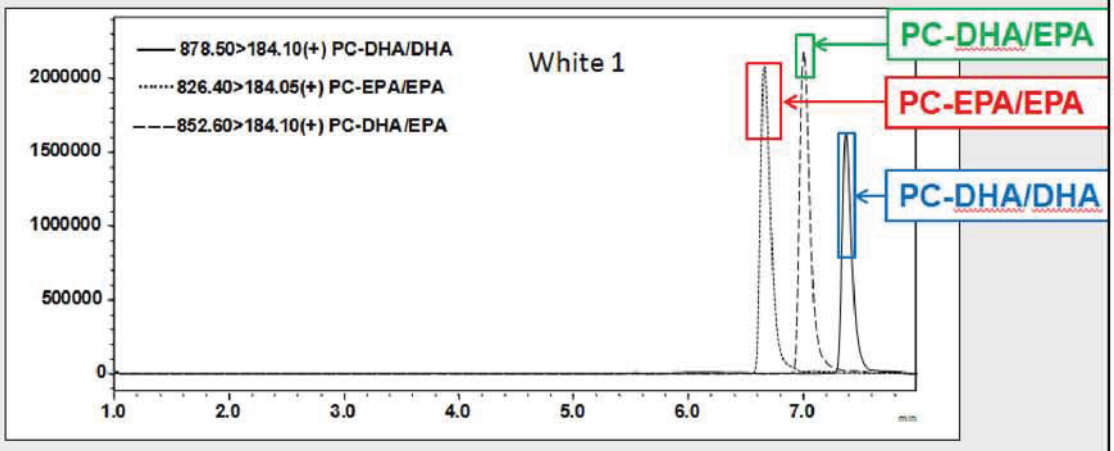


Figure 41. Positive ion electrospray UHPLC-MS/MS analysis of the Claimed Phospholipids in the White 1 sample. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0580, Fig. 41

Q.273. What do the peaks labeled in red, green, and blue indicate in Figure 41?

A.273. Further analysis of the Claimed Phospholipids in White 1 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, indicated in red; *m/z* 852 to *m/z* 184 for PC-DHA/EPA, indicated in green; and *m/z* 878 to *m/z* 184 for PC-DHA/DHA, indicated in blue. (RX-0580, Fig. 41.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in White 1. (RX-0580, Fig. 41.)

Q.274. What were the results of your testing of White 2 and White 3?

A.274. RDX-0574 and RDX-0575 include Figures 50-51 respectively, and reflect the results of my analysis of White 2 and White 3. (RX-0585, Attachment K, Figs. 50-51.) As shown in these figures, each of the three Claimed Phospholipid species PC-DHA/DHA, PC-EPA/EPA, and PC-DHA/EPA was detected in each of White 2 and White 3. (RX-0585, Attachment K, Figs. 50-51.)

The testing I performed on White 2 and White 3 differs from the testing I performed on the other samples described so far.

Q.275. How so?

A.275. In all of the analyses described so far, all krill oil samples were dissolved in chloroform/methanol (60:40, v/v) and then diluted with methanol. However, I found that White 2 and White 3 contained solids that did not dissolve in chloroform/methanol, unlike the other krill oils. Therefore, I did not measure samples White 2 and White 3 after attempting to dissolve them in chloroform/methanol, so as not to damage my UPHPLC-MS-MS system.

Q.276. When did you analyze White 2 and White 3?

A.276. I had previously performed a series of preliminary qualitative analyses designed to identify a suitable solvent for dissolving and then diluting krill oils appropriately for mass spectrometric analysis. In these preliminary studies, I tried dissolving and diluting krill oils in methanol. The oils did not completely dissolve in methanol, so I centrifuged each diluted sample before mass spectrometric analysis of each supernatant. During this time, I analyzed White 2 and White 3 and detected PC-DHA/DHA, PC-EPA/EPA, and PC-DHA/EPA, as shown in Figures 50-51. (RX-0585, Attachment K, Figs. 50-51.)

Q.277. What is shown in Figure 50?

A.277. Figure 50 shows the positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in White Sample 2. (RX-0585, Attachment K, Fig. 50.)

RDX-0574

Figure 50

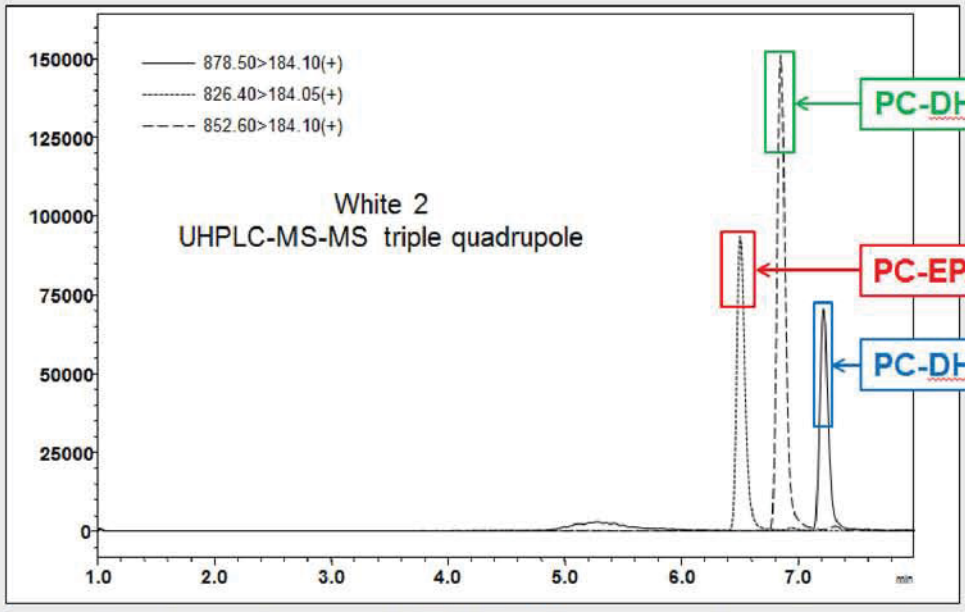


Figure 50. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the White 2 sample. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 50

Q.278. What do the peaks labeled in red, green, and blue indicate in Figure 50?

A.278. Analyses of the Claimed Phospholipids in White 2 were 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, indicated in red; *m/z* 852 to *m/z* 184 for PC-DHA/EPA, indicated in green; and *m/z* 878 to *m/z* 184 for PC-DHA/DHA, indicated in blue. (RX-0585, Attachment K, Fig. 50.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in White 2. (RX-0585, Attachment K, Fig. 50.)

Q.279. What is shown in Figure 51?

A.279. Figure 51 shows the positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in White Sample 3. (RX-0585, Attachment K, Fig. 51.)

RDX-0575

Figure 51

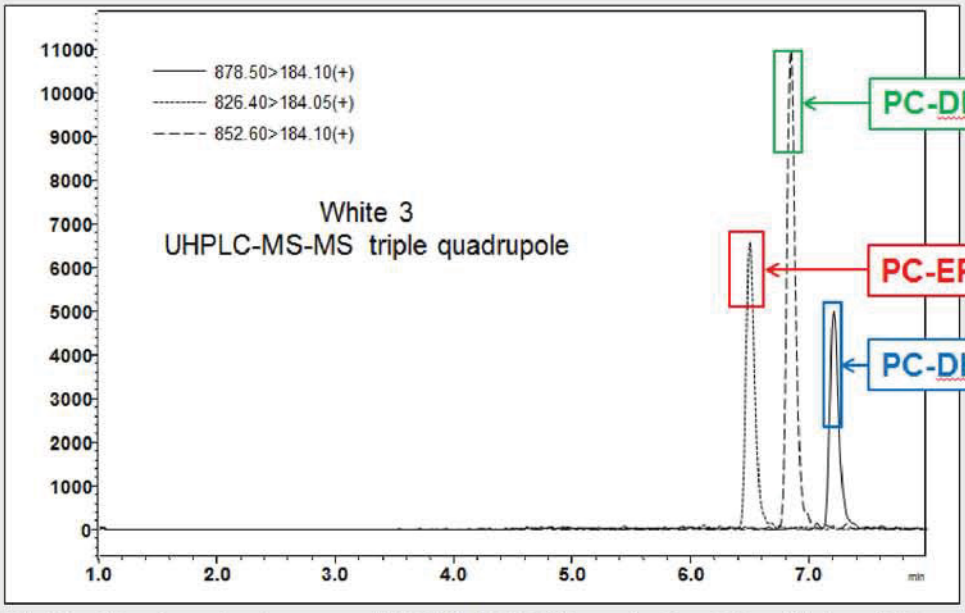


Figure 51. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the White 3 sample. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 51

Q.280. What do the peaks labeled in red, green, and blue indicate in Figure 51?

A.280. Analyses of the Claimed Phospholipids in White 3 were 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, indicated in red; *m/z* 852 to *m/z* 184 for PC-DHA/EPA, indicated in green; and *m/z* 878 to *m/z* 184 for PC-DHA/DHA, indicated in blue. (RX-0585, Attachment K, Fig. 51.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in White 3. (RX-0585, Attachment K, Fig. 51.)

Q.281. Did you analyze any other krill extracts along with White 2 and White 3 during these preliminary qualitative analyses?

A.281. Yes, I also analyzed White 1 and the Fujita Hexane, Fujita Hexane Ethanol, Fujita Once-through, Rogozhin, Beaudoin P0, Beaudoin P1, and Beaudoin P2 extracts.

Q.282. Did you detect the Claimed Phosphlipids in these extracts?

A.282. Yes, I detected the Claimed Phospholipids in every sample I analyzed, every time I analyzed the sample.

Q.283. Would you please walk us through the chromatograms associated with these analyses?

A.283. RDX-0576 through RDX-0583 include Figures 42-49, and are the chromatograms associated with these analyses. (RX-0585, Attachment K, Figs. 50-51.) Analysis of the Claimed Phospholipids in each sample 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-DHA/EPA, and *m/z* 878 to *m/z* 184 for PC-DHA/DHA.

A chromatogram for this analysis for White 1 is shown in Figure 49 on RDX-0576. (RX-0585, Attachment K, Fig. 49.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in White 1. (RX-0585, Attachment K, Fig. 49.)

RDX-0576

Figure 49

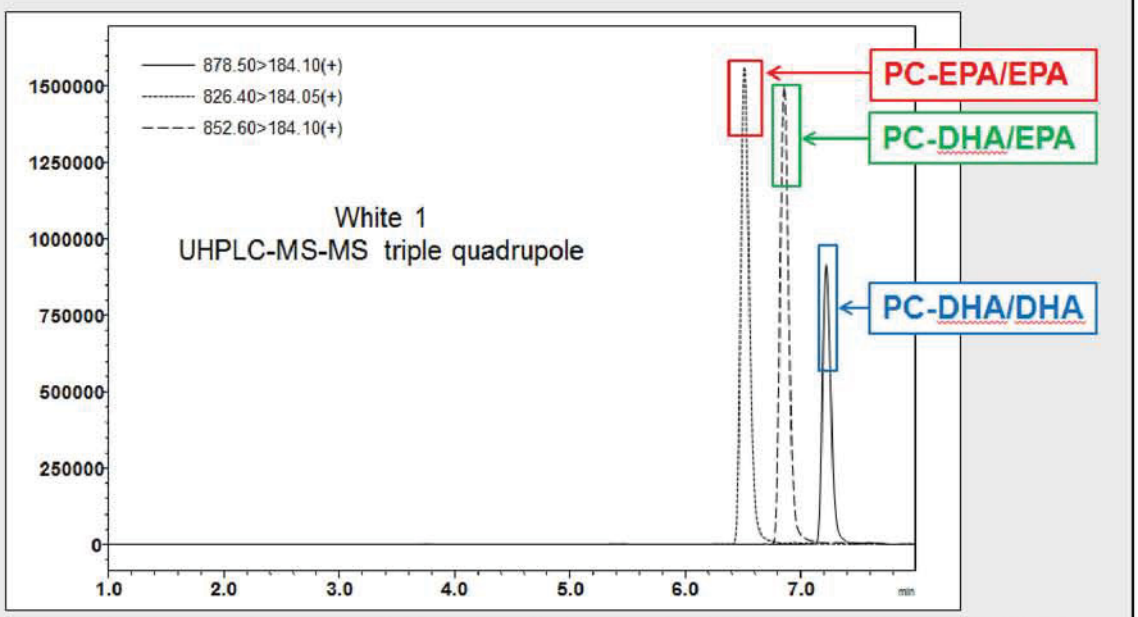


Figure 49. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the White 1 sample. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 49

A chromatogram for this analysis for the Fujita Hexane extract is shown in Figure 42 on RDX-0577. (RX-0585, Attachment K, Fig. 42.) As you can see, PC-EPA/EPA, PC-

DHA/EPA, and PC-DHA/DHA were all detected in the Fujita Hexane extract. (RX-0585, Attachment K, Fig. 42.)

RDX-0577

Figure 42

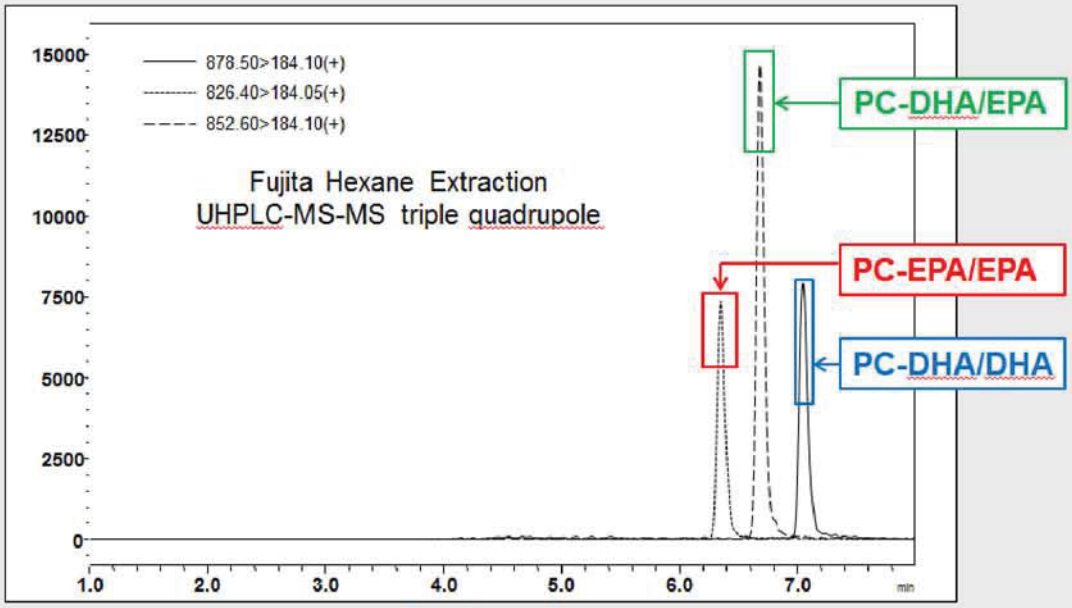


Figure 42. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the Fujita hexane extract. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 42

A chromatogram for this analysis for the Fujita Hexane Ethanol extract is shown in Figure 43 on RDX-0578. (RX-0585, Attachment K, Fig. 43.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in the Fujita Hexane Ethanol extract. (RX-0585, Attachment K, Fig. 43.)

RDX-0578

Figure 43

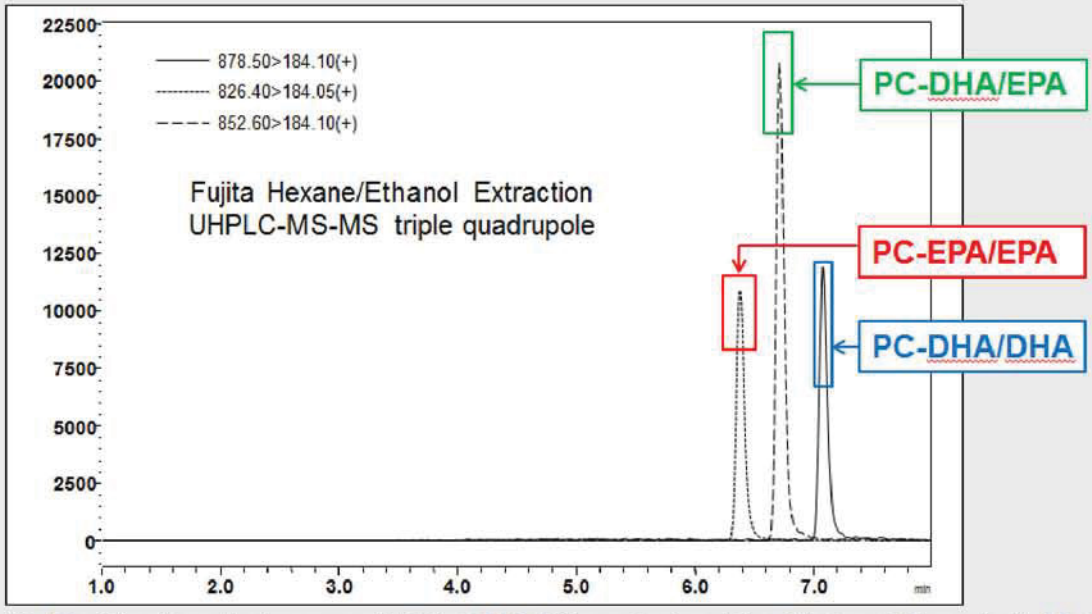


Figure 43. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the Fujita hexane/ethanol extract. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 43

A chromatogram for this analysis for the Fujita Once-through extract is shown in Figure 44 on RDX-0579. (RX-0585, Attachment K, Fig. 44.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in the Fujita Once-through extract. (RX-0585, Attachment K, Fig. 44.)

RDX-0579

Figure 44

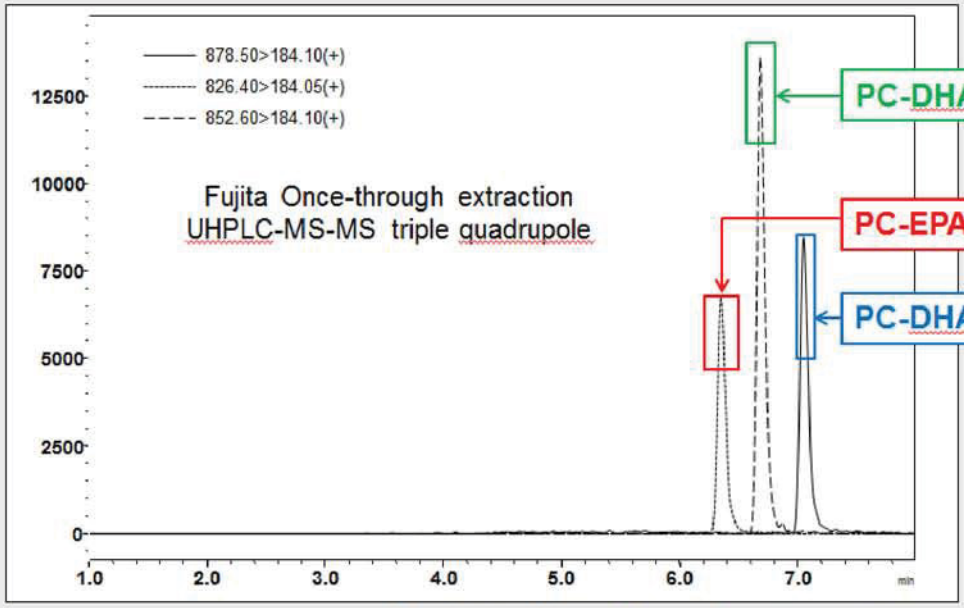


Figure 44. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the Fujita once-through extract. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 44

A chromatogram for this analysis for the Rogozhin extract is shown in Figure 45 on RDX-0580. (RX-0585, Attachment K, Fig. 45.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in the Rogozhin extract. (RX-0585, Attachment K, Fig. 45.)

RDX-0580

Figure 45

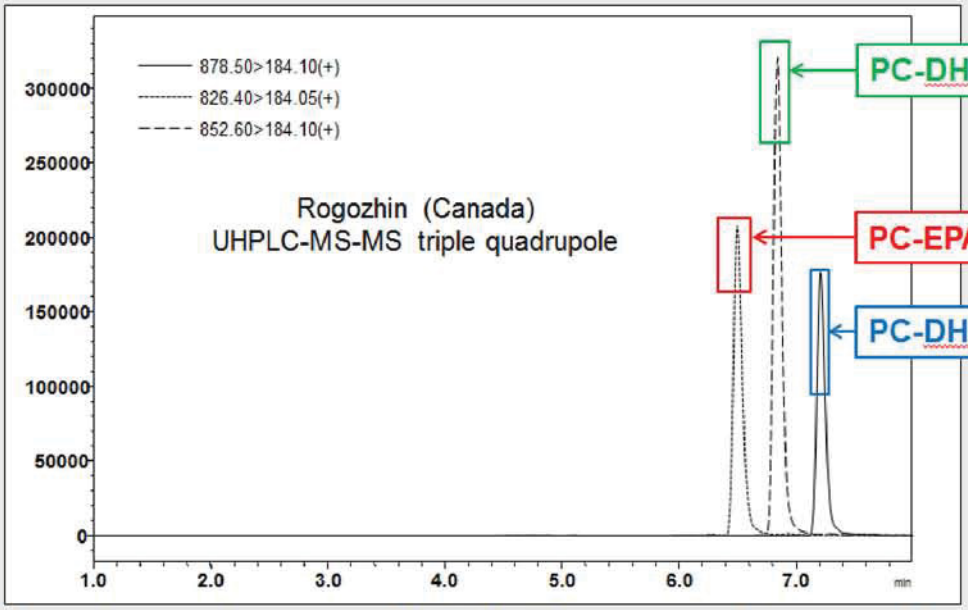


Figure 45. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the Rogozhin extract. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 45

A chromatogram for this analysis for the Beaudoin Extract P0 is shown in Figure 46 on RDX-0581. (RX-0585, Attachment K, Fig. 46.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in Beaudoin Extract P0. (RX-0585, Attachment K, Fig. 46.)

RDX-0581

Figure 46

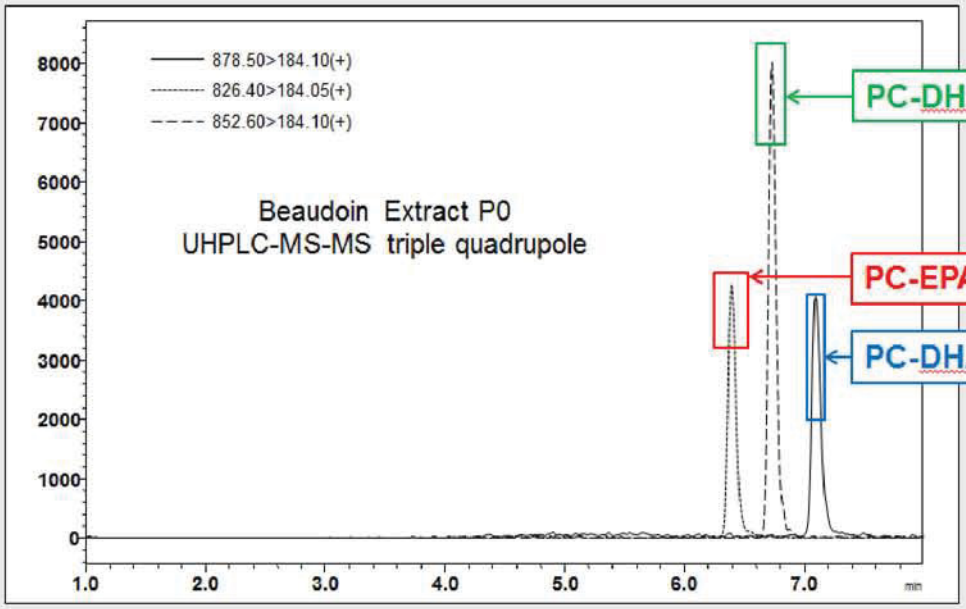


Figure 46. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the Beaudoin extract P0. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 46

A chromatogram for this analysis for the Beaudoin Extract P1 is shown in Figure 47 on RDX-0582. (RX-0585, Attachment K, Fig. 47.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in Beaudoin Extract P1. (RX-0585, Attachment K, Fig. 47.)

RDX-0582

Figure 47

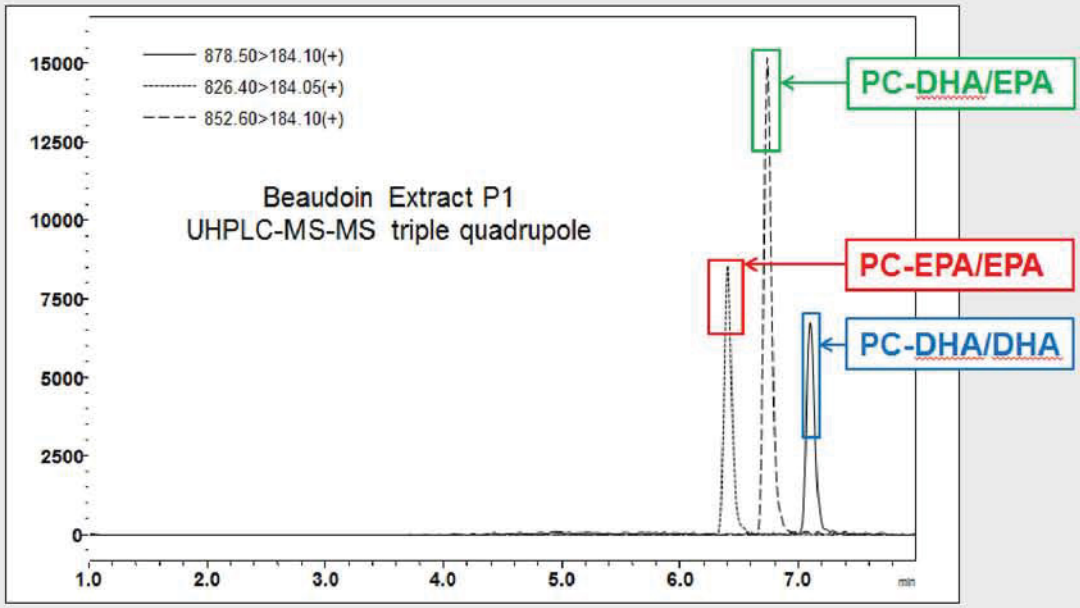


Figure 47. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the Beaudoin extract P1. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 47

A chromatogram for this analysis for the Beaudoin Extract P2 is shown in Figure 48 on RDX-0583. (RX-0585, Attachment K, Fig. 48.) As you can see, PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA were all detected in Beaudoin Extract P2. (RX-0585, Attachment K, Fig. 48.)

RDX-0583

Figure 48

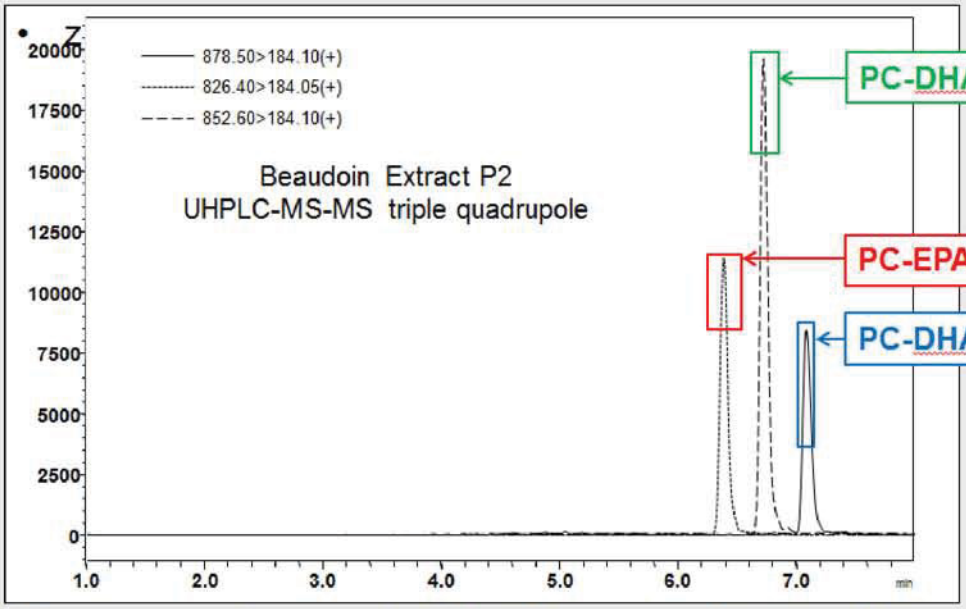


Figure 48. Positive ion electrospray UHPLC-MS-MS analysis of the Claimed Phospholipids in the Beaudoin extract P2. A triple quadrupole mass spectrometer was used with collision-induced dissociation and selected reaction monitoring (SRM) of the transitions indicated.

RX-0585, Attachment K, Fig. 48

[redacted]

Q.318. Have you reviewed any analyses Neptune and its retained experts have relied on previously with respect to testing for the Claimed Phospholipids?

A.318. I have.

Q.319. How does your analysis compare to those Neptune analyses?

A.319. My analysis relating to the presence of the Claimed Phospholipids in the prior art krill extracts I tested is more reliable and comprehensive than similar analyses Neptune and its retained experts have relied on previously. In other words, my analysis presents even more evidence that the Claimed Phospholipids are present than the reports and studies Neptune and its experts commissioned and relied upon.

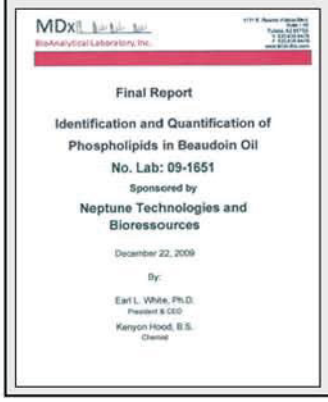
Q.320. Would you please give an example of why that is the case?

A.320. As I've shown on RDX-0589C, according to pages WHITEITC-00000419 and WHITEITC-00000421 of Dr. White's 2009 report, Dr. White analyzed a "Beaudoin Oil Sample" for Neptune for identity and relative concentrations of "phospholipids containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the sn-1 and sn-2 positions of the glycerol backbone." "The PLs of interest included phosphatidylcholine (PC), phosphophatidylethanolamine [sic] (PE) and phosphatidylinositol (PI)."

RDX-0589C

Dr. White Analyzed a Beaudoin Oil Sample in 2009 with Tandem Mass Spectrometry, Finding Molecules having the Same Molecular Weights as the Theoretical Molecular Weights for Intact PC-EPA/EPA (826), PC-DHA/EPA (852), and PC-DHA/DHA (878)

A sample labeled Beaudoin Oil No. Lab: 09-1651 was submitted in a brown opaque 250 mL bottle to MDx BioAnalytical Laboratory for LC/MS and LC-MS/MS analysis to determine the identify of three classes of phospholipids (PL) present and to obtain relative concentrations of each lipid with the hope of obtaining quantitative information about phospholipids containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the sn-1 and sn-2 positions of the glycerol backbone. The PLs of interest included phosphatidylcholine (PC), phosphophatidylethanolamine (PE) and phosphatidylinositol (PI). These PL classes were believed to be present at 85%, 7% and 8% for PC, PE and PL, respectively.



MDx BioAnalytical Laboratory, Inc.

Final Report

Identification and Quantification of Phospholipids in Beaudoin Oil

No. Lab: 09-1651

Sponsored by

Neptune Technologies and Bioressources

December 22, 2009

By:

Earl L. White, Ph.D.
President & CEO

Kenyon Hood, B.S.
Chemist

Table 1 Phospholipids Detected in Beaudoin Oil Sample

Phospholipid	PL Class	RT	m/z	Area	Relative%
C20:5/C20:5	PC w/EPA & EPA	18.9	826	543	2.19
C14:0/C20:5	PC	19.4	752	904	3.64
C22:6/C20:5**	PC w/DHA & EPA	19.5	852		
C21:0/C20:5**	PC w/EPA	19.5	852	452	1.82
C12:0/22:4*	PC	20.5	754	849	3.42
C22:6/C13:0**	PC w/DHA	20.5	764	61	0.25
C16:0/C20:5**	PC w/EPA	20.9	764	69	0.28
C22:6/C16:0**	PE w/DHA	21.3	764	19	0.08
C20:5/C18:1**	PE w/EPA	21.9	764	18	0.07
C20:5/C16:0	PC w/EPA	21.0	780	3861	15.54
C22:6/C16:1**	PS w/DHA	21.5	806	656	2.64
C22:6/C16:0**	PC w/DHA	22.0	806	1610	6.48
C18:0/C18:0**	PC	20.9	790	63	0.25
C22:6/C18:1**	PE w/DHA	22.1	790	41	0.17
C22:6/C22:6	PC w/DHA & DHA	20.1	878	162	0.65

RX-0503C, WHITEITC-00000419, 421

As shown in Table 1 of Dr. White's 2009 report on RDX-0503C, Dr. White concluded that he detected the following Claimed Phospholipids in the "Beaudoin Oil Sample" he analyzed: PC-EPA/EPA, PC-DHA/EPA, and PC-DHA/DHA. He claims, however, that he could not differentiate PC-DHA/EPA.

Q.321. What did Dr. White base his conclusions on?

A.321. Dr. White based his conclusions on his findings, through tandem mass spectrometry, of molecules having the same molecular weights as the theoretical molecular weights for intact PC-EPA/EPA (826), PC-DHA/EPA (852), and PC-DHA/DHA (878).

Q.322. How is your analysis more reliable or comprehensive than Dr. White's?

A.322. My analysis achieves an even higher level of confidence than Dr. White's, at least for the reasons that I used an authentic standard as a positive control, and each of my spectra shows not only the parent (826, 852, and 878) ions associated with the species of interest but also shows product ions of *m/z* 184 containing the PC moiety without the fatty acids. I also confirmed that the compounds I detected contained the exact same elemental composition as the target species.

[redacted]

Q.331. Are there any other examples showing your analyses to be more reliable and comprehensive than Neptune's?

A.331. As previously mentioned, Neptune submitted a May 31, 2011 declaration by Dr. White attaching his May 29, 2011 "Final Report" to the USPTO in connection with the reexamination of the '348 Patent. As shown on RDX-0591, Dr. White stated on page NEP877ITC-00003353 of his declaration that he detected compounds in the Beaudoin oil he analyzed having the same molecular weight as intact PC-EPA/EPA (826) and PC-EPA/DHA (852).

RDX-0591

In a 2011 Declaration Submitted to the USPTO During the '348 Patent Reexamination, Dr. White Stated that He Detected Compounds Having the Same Molecular Weights as the Claimed Phospholipids, but Concludes they are Not the Claimed Phospholipids

11. PLs detected in the Fractions were primarily phosphatidylcholines as confirmed by the characteristic *m/z* 184 ion in the MS/MS spectra. With the exception of PL with molecular weight (MW) of 732.5 Da, PLs in an amount of ≥ 5% were low MW PLs. Six of the PLs detected in the samples (*m/z* 542, 568, 666, 808, 826 and 852) have molecular weights, which, without further analysis, could be considered evidence of a PC containing DHA and EPA, or a combination of DHA and EPA, however MS/MS spectra shown in the figures below confirm that these PLs do not have attached to the sn-1 and sn-2 positions of the glycerol backbone DHA and DHA, EPA and EPA, DHA and EPA, or EPA and DHA.

RX-0020, NEP877ITC-00003353

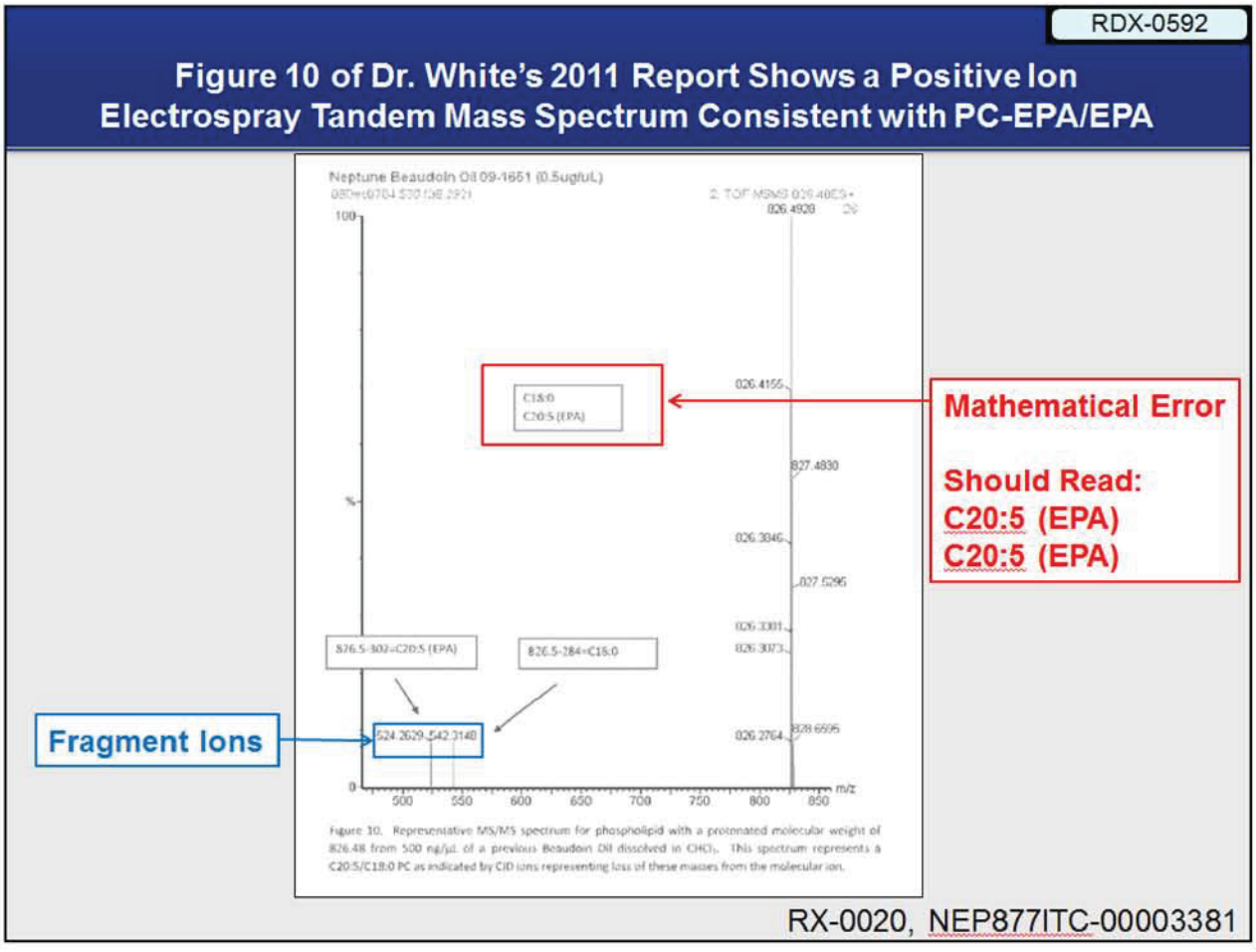
However, he concluded that "MS/MS spectra …confirm that these PLs do not have attached to the sn-1 and sn-2 positions of the glycerol backbone DHA and DHA, EPA and EPA, DHA and EPA, or EPA and DHA."

Q.332. Is Dr. White's conclusion that his MS/MS spectra confirm the phospholipids do not have DHA and/or EPA attached to the sn-1 and sn-2 positions correct?

A.332. No, it is not correct.

Q.333. Would you please explain why Dr. White's conclusion is not correct?

A.333. There is a mathematical error in Dr. White's report. Figure 10 of Dr. White's report, which is found on page NEP877ITC-00003381 of RX-0020 and included on RDX-0592, shows a positive ion electrospray tandem mass spectrum consistent with PC containing EPA at both sn-1 and sn-2 positions, but Dr. White does not label it as such. According to Dr. White, Figure 10 indicates a phospholipid containing EPA (C20:5) and stearic acid (C18:0). However, when calculating the molecular mass of PC with EPA (C20:5) in one position and stearic acid (C18:0) in the other, the molecular mass would be 807.6, which would be detected as a protonated molecule of *m/z* 808.6.



Q.334. Is that what Dr. White's Figure 10 indicates was detected?

A.334. No. Instead, the protonated molecule of the phospholipid in Figure 10 is *m/z* 826.5, which is consistent with a PC containing EPA at both sn-1 and sn-2 positions, *i.e.*, PC-EPA/EPA.

Q.335. Is there any other support for that conclusion?

A.335. Yes. The assignment of the phospholipid PC-EPA/EPA is also supported by fragment ions of *m/z* 524.3 and *m/z* 542.3 in Figure 10, which correspond to the loss of EPA (-302), $[MH-302]^+$, and loss of dehydrated EPA, $[MH-284]^+$, respectively. These fragment ions are indicated in blue on RDX-0592.

Q.336. Is there any other reason why Dr. White's conclusion that his MS/MS spectra confirm the phospholipids do not have DHA and/or EPA attached to the sn-1 and sn-2 positions is incorrect?

A.336. Yes. As shown on RDX-0593, Dr. White indicates on page NEP877ITC-00003369, that his only reported basis for concluding in 2011 that he did not detect the Claimed Phospholipid species was that he did not see fragment ions corresponding to EPA and/or DHA.

RDX-0593

Dr. White's Only Reported Basis for Concluding in 2011 that He Did Not Detect the Claimed Phospholipids was the He Did Not See Fragment Ions Corresponding to EPA and DHA

PLs detected in the eight samples were primarily phosphatylcholines as confirmed by the characteristic *m/z* 184 ion in the MS/MS spectra[1]. With the exception of PL with molecular weight (MW) of 732.5 Da, PLs in an amount of ≥ 5% were low MW PLs. Six of the PLs detected in the samples (*m/z* 542, 568, 666, 808, 826 and 852) all have MWs that could represent PCs with DHA and EPA or a combination of the two, however MS/MS spectra shown in the figures below confirms that these PLs do not have attached to the sn-1 and sn-2 positions of the glycerol backbone DHA and DHA, EPA and EPA, DHA and EPA, or EPA and DHA.

Figure 1 – 3 show representative total ion chromatograms for 500 ng/µL of each Beaudoin oil sample disolved in $CHCl_3$. Peak areas were calculated from the protonated molecular ions for each of the major peaks in the spectra. MS/MS spectra for a representative Beaudoin oil sample are shown in Figures 4-9, and 11. Figure 10 is a representative MS/MS spectrum for a PC with a molecular ion at *m/z* 826.5. Fragment ions detected at *m/z* 524.3 and 542.3 are indicative of 302 and 284 Da losses from the protonated molecular ion which is indicative of the loss of a C20:5 (EPA) and C18:0 fatty acids from the protonated molecular ions. However, there was no ion observed at *m/z* of 499 which would have been indicative of a loss of 327 which is DHA from the protonated molecular ion at *m/z* of 826, which is indicative that there is no DHA attached to the phospholipid.

RX-0020, NEP877ITC-00003369

Dr. White's reliance on the absence of these fragment ions is flawed. There are no product ions showing the individual fatty acids in Dr. White's spectra, and that is expected because the analysis was done in positive ion mode. As I explained earlier, fatty acids like EPA and DHA tend to form negative ions, which would not be detected in positive ion mode.

Q.337. What should Dr. White have done to determine whether the sample he was testing generated any product ions showing the individual fatty acids?

A.337. Dr. White should have used a standard to determine whether it generated any product ions showing the individual FAs. Had he used a standard, he likely would have seen the same spectra I was seeing in Figures 1-2 when I tested my PC-DHA/DHA standard, just the parent ion and the ion of *m/z* 184 corresponding to PC.

Q.338. Did you express your opinion that Dr. White's conclusion was incorrect during the reexamination proceedings involving the '348 Patent?

A.338. Yes. I responded to Dr. White's declaration in my own April 17, 2012 declaration to the USPTO.

Q.339. I'm handing you what's been marked RX-0078. Do you recognize this document?

A.339. Yes. This is my April 17, 2012 declaration to the USPTO during reexamination of the '348 Patent.

Q.340. Would you please show an example from your declaration where you state that Dr. White's conclusion was incorrect?

A.340. An excerpt of page AKER877ITC00737366 of my declaration is shown on RDX-0594. As you can see, I explain that Dr. White's conclusion was incorrect, and that Complainants' own experts agreed with me, including Dr. Yeboah and Dr. Shahidi.

RDX-0594

I Responded to Dr. White's Declaration with my Own April 17, 2012 Declaration, Explaining that Dr. White's Conclusion was Incorrect and that Complainants' Own Experts Agreed

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION OF RICHARD B. VAN BREEMEN, PH.D., IN SUPPORT OF INTER PARTES REEXAMINATION OF U.S. PATENT NO. 8,030,348

3. Tables 1 and 2 of the 2011 White Declaration demonstrate the presence of phospholipids species detected as protonated molecules of *m/z* 826 and *m/z* 852 in fractions from each of the sample sets tested. These masses are consistent with phosphatidylcholines containing two eicosapentainoic acid groups (PC-EPA/EPA) (*m/z* 826) and one EPA group plus one docosahexaenoic acid group (PC-EPA/DHA) (*m/z* 852), respectively. Neptune's experts, Dr. Yeboah and Dr. Shahidi both recognize and acknowledge this fact. As stated by Dr. Yeboah in ¶36 of his Declaration:

> The species detected at *m/z* values of 826 and 852 represent amounts in a range on the order of only 0.1 to 2.8% of the phospholipids of the oil. I understand that phospholipids represent about 40% of the total lipids in krill oil and therefore, the raw data of Tables 1 and 2 of the White Declaration shows that the amount of phospholipids carrying two and EPA and DHA in the total Beaudoin oil is only about 0.05 to 1.1%.

Likewise, Dr. Shahidi stated in ¶22 of his Declaration:

> As Beaudoin reports an oil potentially with a small amount of the phospholipid containing two of EPA and DHA (i.e. about 0.1 to 1%), it is my opinion that this is not a biologically effective amount. As the claims of the' 348 patent are directed to biologically effective amounts of this composition, they are distinct from Beaudoin.

Both Dr. Yeboah and Dr. Shahidi agree that the Beaudoin samples generated by Neptune and analyzed by Dr. White contain the claimed phospholipid species with two of EPA and DHA (PC-EPA/EPA and PC-DHA/DHA).

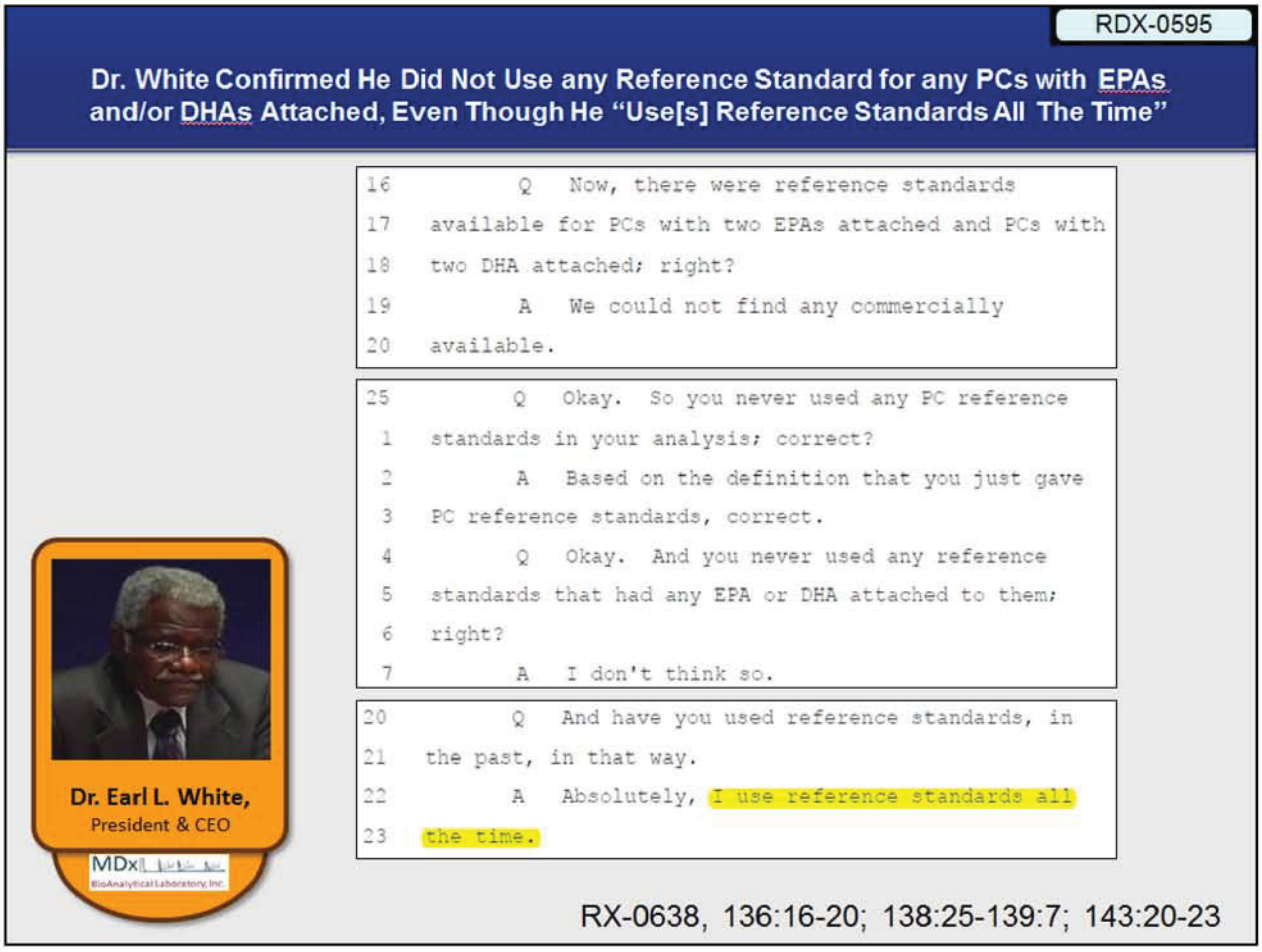
RX-0078, AKER877ITC00737366

Q.341. Do you still hold your opinion that Dr. White's conclusion in his 2011 report and declaration was incorrect?

A.341. Yes. Since my April 17, 2012 Declaration was submitted, Dr. White has given a deposition and provided documents to Respondents. Dr. White's documents and testimony further support my opinions expressed herein and in my April 17, 2012 Declaration.

Q.342. Would you please provide an example of how these materials further support your opinions?

A.342. For example, Dr. White confirmed at pages 135-143 of his deposition that he did not use any reference standard for any PCs with EPAs and/or DHAs attached with the sn-1 or sn-2 positions, even though he "use[s] reference standards all the time." He also stated that, had his spectra looked like spectra for reference standards for the Claimed Phospholipid species, that would have been "good evidence" such Claimed Phospholipid species were present. Excerpts of this testimony (RX-0638 (E. White Dep. Tr.), 136:16-20; 138:25-139:7; 143:6-23) are shown on RDX-0595. This testimony supports my opinions that Dr. White's opinions are unreliable because he did not use any reference standard.



Q.343. Are there other examples from Dr. White's deposition that further support your opinion that his conclusion regarding the absence of the Claimed Phospholipids was incorrect?

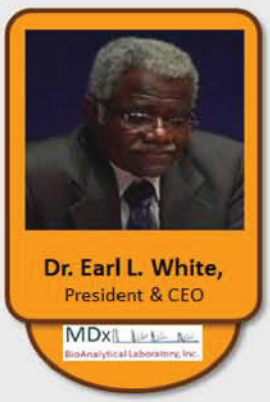
A.343. Dr. White confirmed at pages 63-67 of his deposition that, in 2009, he concluded the Beaudoin 09-1651 oil contained Claimed Phospholipid PC species based on his findings of compounds with molecular weights of 826, 852, and 878. Excerpts of this testimony (RX-0638 (E. White Dep. Tr.), 67:19-25; 65:6-13; 63:25-64:8) are included on RDX-0596 through RDX-0597.

RDX-0596

Dr. White Further Confirmed that in 2009 He Concluded that the Beaudoin 09-1651 Oil Contained the Claimed Phospholipid PC Species Based on his Findings of Compounds with Molecular Weights of 826, 852, and 878

19 Q And in this 2009 report you concluded that
20 the Beaudoin oil 09-1651 contained PC with EPA and EPA
21 attached at the sn-1 and sn-2 positions based on your
22 findings of a compound with a molecular weight of 826;
23 correct?
24 MS. CUNNINGHAM: Objection to form.
25 A Correct.

6 Q And according to the caption these figures
7 represent "Ion chromatograms with phospholipids with
8 molecular weights including 852"; correct?
9 A Correct.
10 Q Looking back at table one of your report,
11 you identified the molecule having molecular weight
12 852 as being PC with DHA and EPA attached; correct?
13 A Correct.



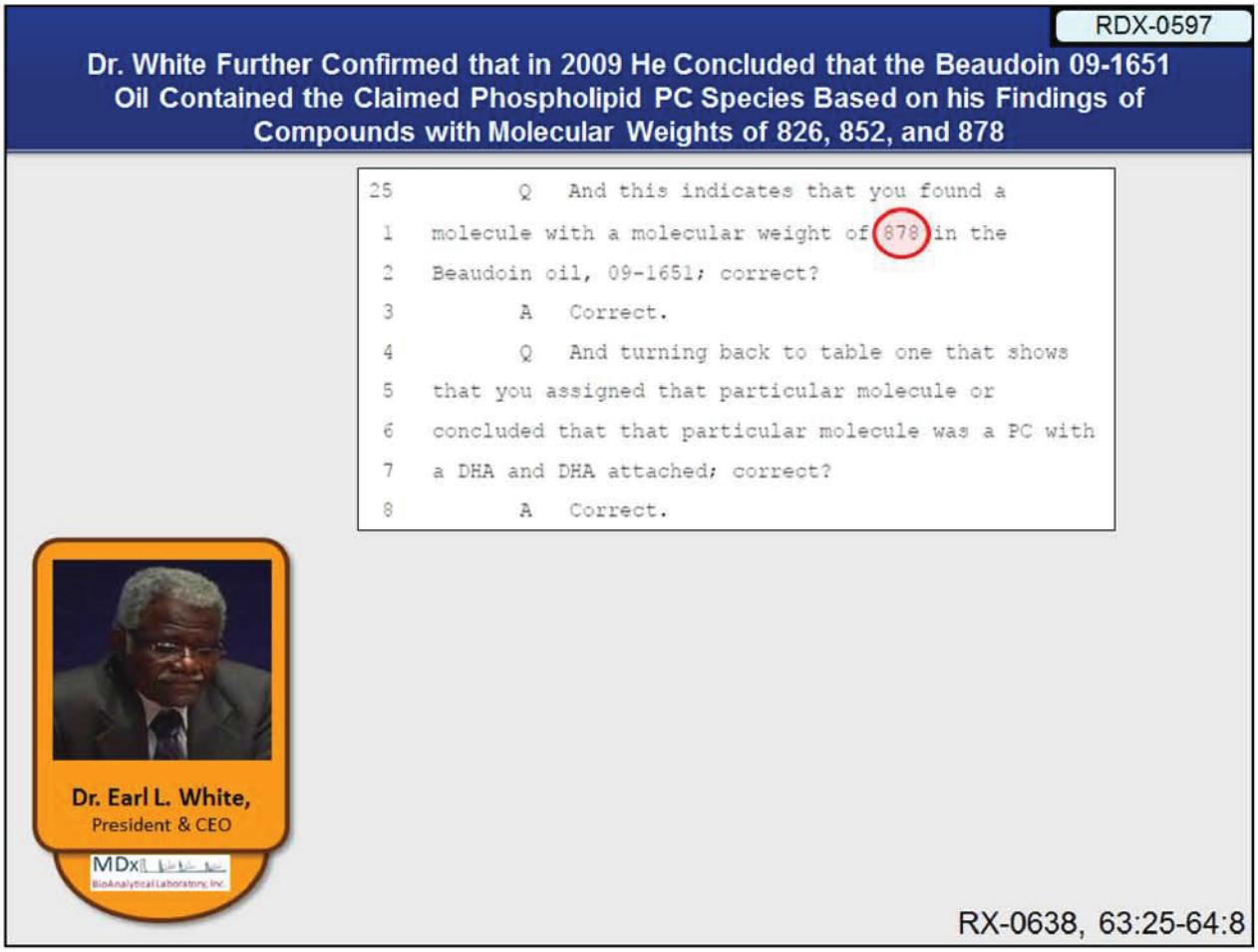
Dr. Earl L. White,
President & CEO

MDx BioAnalytical Laboratory, Inc.

RX-0638, 67:19-25; 65:6-13

AKBM 1109

RX-0579C.0131



Q.344. Did he detect compounds with those molecular weights in connection with his 2011 report?

A.344. Yes. According to Dr. White's 2011 report, on page NEP877ITC-00003369, "[s]ix of the PLs detected in the samples (*m/z* 542, 568, 566, 808, 826 and 852) all have MWs that could represent PCs with DHA and EPA or a combination of the two."

Q.345. Why did he not reach the same conclusion he had reached in 2009 that the Claimed Phospholipids were present?

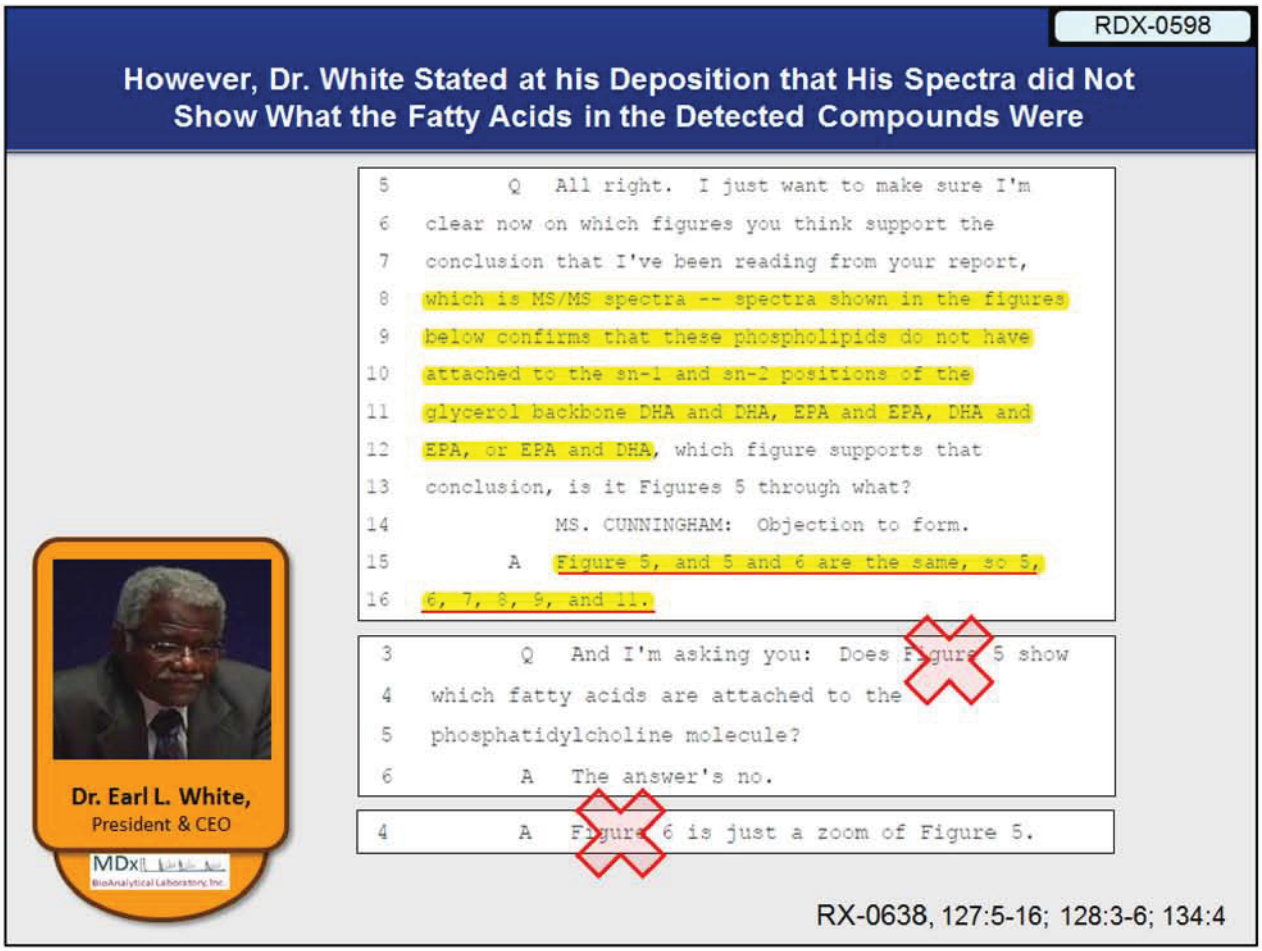
A.345. As I previously stated, Dr. White's only reported basis for concluding in 2011 that he did not detect the Claimed Phospholipid species was that he did not see fragment ions corresponding to EPA and/or DHA.

Q.346. Are there other examples from Dr. White's deposition and documents that further support your opinion that his conclusion regarding the absence of the Claimed Phospholipids was incorrect?

A.346. Dr. White confirmed during his deposition on pages 128-179 that his analyses do not show what the attached fatty acids in the detected compounds were.

Q.347. Would you please walk us through Dr. White's testimony on this topic?

A.347. Initially, on page 127 of his deposition, Dr. White identified Figures 5-9 and 11 from his report as supporting his conclusion that his spectra confirm the absence of the Claimed Phospholipids. This testimony (RX-0638 (E. White Dep. Tr.), 127:5-16) is found on RDX-0598.



But Dr. White later stated that each of those identified figures fails to show which fatty acids are attached to the PC molecule.

Q.348. Would you please give an example of Dr. White's testimony that the figures he identified do not show which fatty acids are attached to the PC molecule?

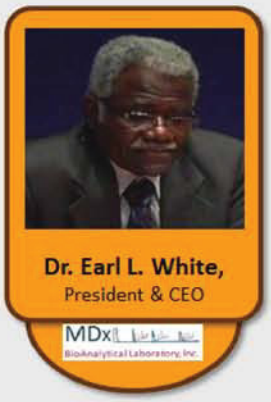
A.348. Examples of such testimony regarding Figures 5 and 6 (RX-0638 (E. White Dep. Tr.), 128:3-6; 134:4) is shown on RDX-0598. Dr. White stated that Figure 5 does not "show which fatty acids are attached to the phosphatidylcholine molecule" and that "Figure 6 is just a zoom of Figure 5." These contradictory statements are labeled with a red "x" symbol on RDX-0598.

Q.349. Did Dr. White make similar statements with respect to the other figures he had initially identified as supporting his conclusion?

A.349. Yes. Dr. White similarly testified that his spectra did not show what the fatty acids in the detected compounds were for Figures 7-8 (RDX-0638 (E. White Dep. Tr., 152:14-22; 179:24-180:8) and Figures 9-11 (RDX-0638 (E. White Dep. Tr., 179:20-23; 178:18-21, 179:11-13). This testimony is shown on RDX-0599 and RDX-0600 respectively.

RDX-0599

However, Dr. White Stated at his Deposition that His Spectra did Not Show What the Fatty Acids in the Detected Compounds Were



Q Let's look at Figure 7 for a moment. Is it your belief that this Figure 7 supports your conclusion that there was not a phosphatidylcholine having an EPA and/or DHA attached at both the sn-1 and sn-2 positions?

A Yes.

Q Does this show what fatty acids were attached to the phosphatidylcholine?

A No.

Q In Figure 8 you describe that as showing the molecule having the molecular weight of 808; right?

A Yes.

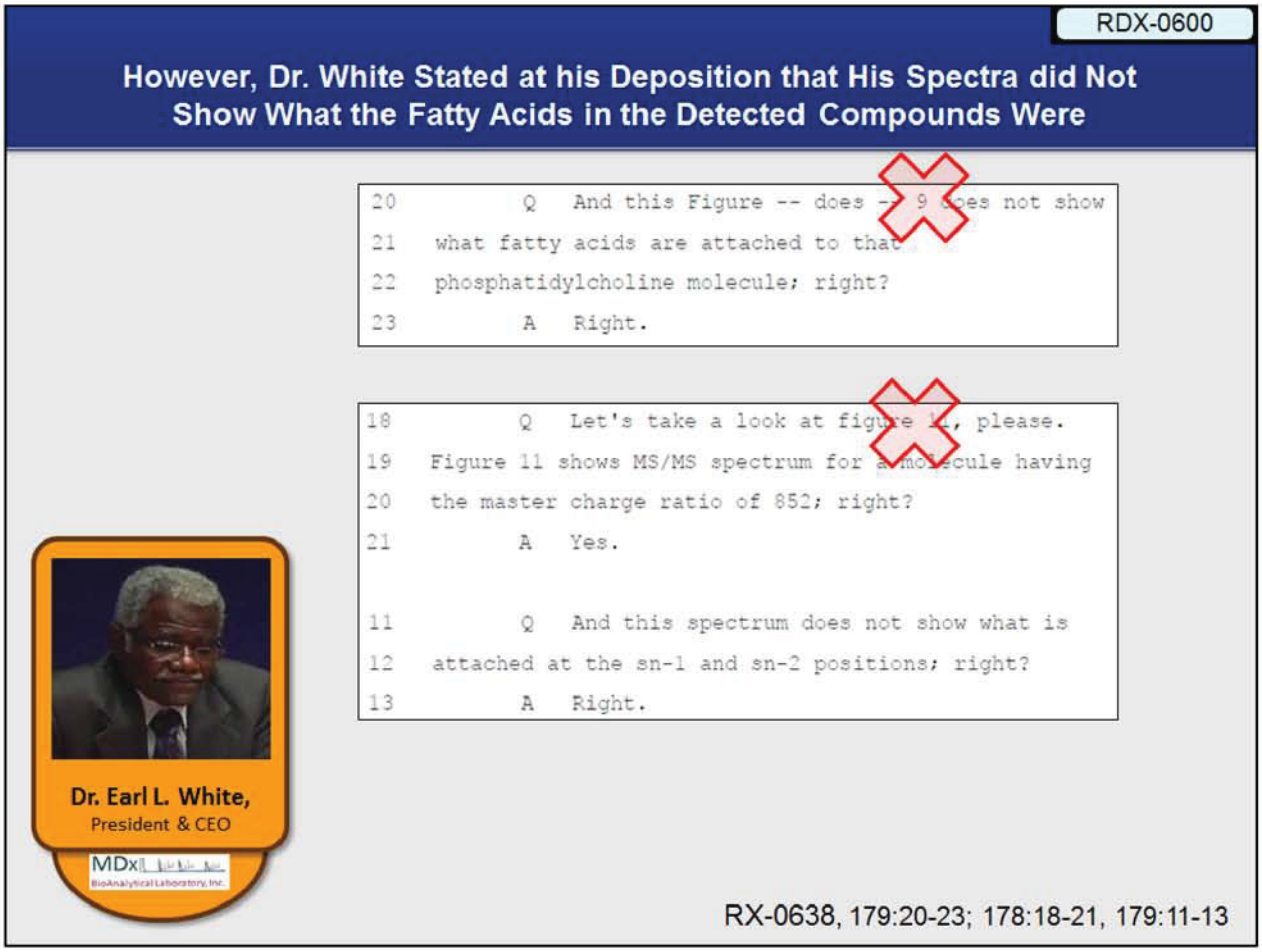
Q And this shows there's a phosphatidylcholine there; right?

A Yes.

Q And this does not show what fatty acids are attached to phosphatidylcholine; right?

A Right.

RX-0638, 152:14-22; 179:24-180:8



Q.350. Did Dr. White identify any molecules corresponding to the detected ions of *m/z* 826 and *m/z* 852 ratios from his 2011 analysis?

A.350. No.

Q.351. What conclusion have you drawn from Dr. White's testimony that none of his figures show what the attached fatty acids are?

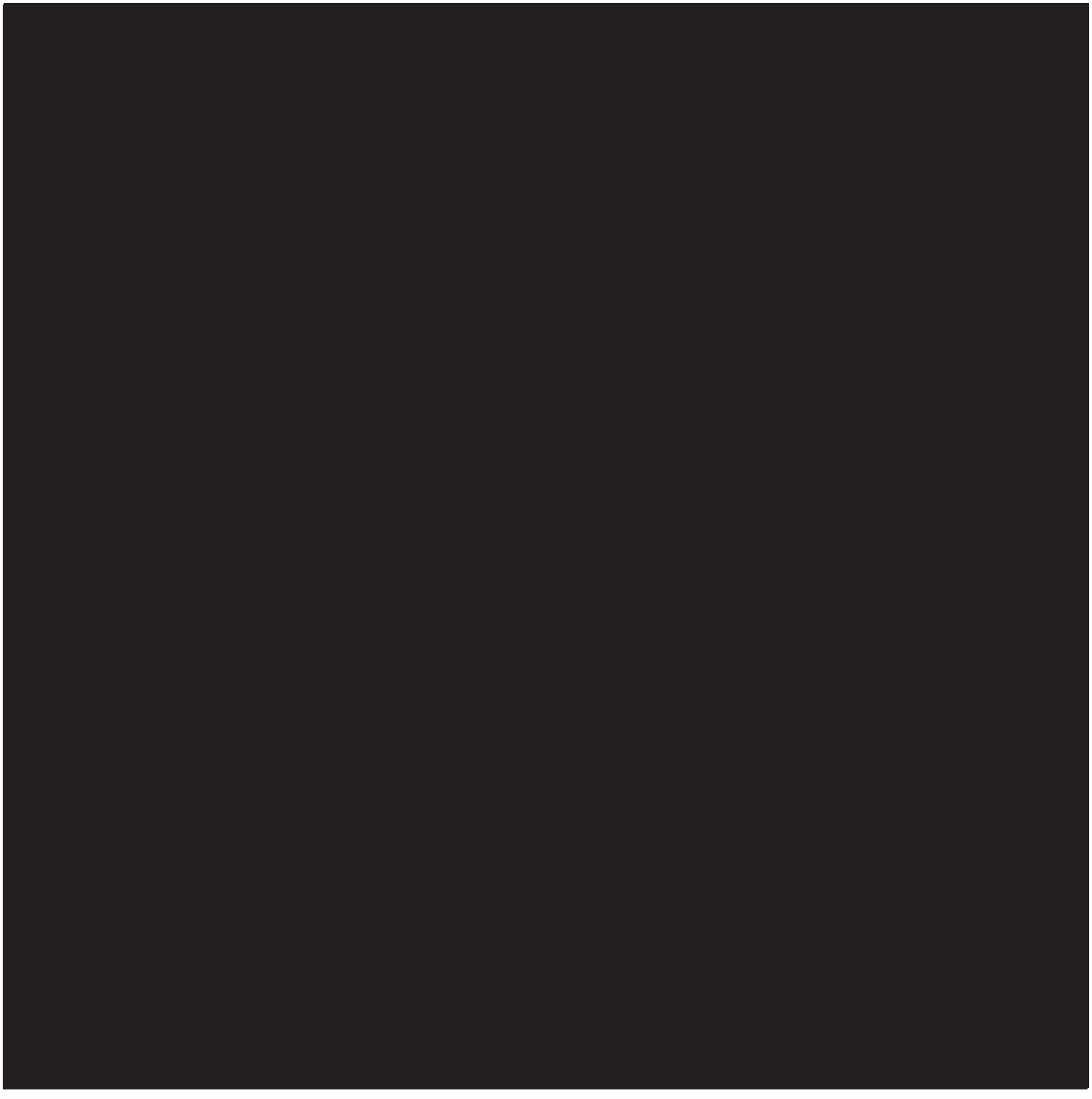
A.351. It is unreasonable for Dr. White to conclude that his analyses show the fatty acids must not be DHA or EPA when he simultaneously concludes that his analyses do not show what the attached fatty acids are, and he didn't test a positive control to see if it reacted differently.

Q.352. To your understanding, was Dr. White's 2009 report submitted to the PTO during any of the prosecutions or reexaminations related to the '351 and '675 Patents?

A.352. Based on my reading of the file histories in this case, Dr. White's 2009 report was never submitted to the PTO in connection with either of the '351 and '675 Patents or the parent '348 patent or the related reexamination proceedings.

Q.353. Do you think Dr. White's 2009 report should have been submitted to the PTO?

A.353. Yes. I think it is entirely inconsistent for Dr. White to report to the PTO that he did not detect the Claimed Phospholipid species in a krill oil extract even though he found *m/z* ratios of at least 826 and 852, when he earlier reported to Neptune that he detected the Claimed Phospholipid species in a krill oil extract based on those same *m/z* findings of 826, 852, and 878.



Executed this 6th day of November 2013.

Respectfully submitted,

Dr. Richard B. van Breemen