DPA-ENRICHED COMPOSITIONS OF OMEGA-3 POLYUNSATURATED FATTY ACIDS IN FREE ACID FORM

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application nos. 61/583,796, filed January 6, 2012; 61/664,047, filed June 25, 2012; 61/669,940, filed July 10, 2012; 61/680,622, filed August 7, 2012; 61/710,517, filed October 5, 2012; and 61/713,388, filed October 12, 2012, the contents of all of which are incorporated herein by reference in their entireties.

2. BACKGROUND

[0002] Pharmaceutical compositions rich in omega-3 ("ω-3" or "n-3") polyunsaturated fatty acids ("PUFAs") are being developed to treat a variety of clinical indications.

[0003] These products, which are derived from natural sources, typically fish oils, are heterogeneous compositions, and comprise various species of omega-3 PUFAs, omega-6 PUFAs, and other minor components, including mono-unsaturated and saturated fatty acids. The observed clinical effects are typically attributed to the composition as a whole, although the most prevalent of the PUFA species present in the mixture, usually EPA and DHA, are believed to contribute a substantial portion of the observed clinical effect. Because they are heterogeneous compositions, the products are defined to include certain obligate polyunsaturated fatty acid species, each within a defined percentage tolerance range. The compositions are further defined to limit certain undesired components, both those originating in the natural source, such as certain environmental contaminants, and those potentially created in the refining process.

[0004] The optimal composition likely differs as among intended clinical indications. Even for the first approved clinical indication, however, treatment of severe hypertriglyceridemia (TGs > 500 mg/dl), the optimal composition has not yet been defined.

[0005] Thus, the first-approved pharmaceutical composition for treatment of severe hypertriglyceridemia comprises the omega-3 PUFA species eicosapentaenoic acid ("EPA") and docosahexaenoic acid ("DHA") in the form of ethyl esters in weight percentages of

approximately 46:38 (EPA:DHA), with EPA and DHA together accounting for approximately 84% of all PUFA species in the composition. By contrast, the more recently approved product, Vascepa® (previously known as AMR101), which is approved for the same clinical indication, is >96% pure EPA in the ethyl ester form, with substantially no DHA. The nutraceutical product, OMAX3, sold as a dietary supplement and promoted in part to lower triglyceride levels, comprises EPA and DHA in a weight ratio of about 4.1:1, wherein the EPA and DHA are likewise in the ethyl ester form, the formulation being more than 84% EPA and DHA by weight and more than 90% omega-3 fatty acids by weight.

[0006] These wide variations in composition reflect continuing uncertainty as to the optimal composition for this clinical indication.

[0007] The uncertainty is due, in part, to competing clinical goals. For example, the omega-3 PUFA species, DHA, is known to be more potent in lowering serum triglycerides than is EPA, but is known to have a greater tendency to increase LDL levels, Mori *et al.*, *Am. J. Clin. Nutr.* 71:1085–94 (2000), Grimsgaard *et al.*, *Am. J. Clin. Nutr.* 66:649 –59 (1997); elevation of LDL has been thought to be clinically disfavored in subjects with elevated cardiovascular risk. Although decrease in platelet aggregation and thrombogenesis by omega-3 PUFAs is often clinically desired, the potential increase in bleeding time has prompted some to propose adding a certain amount of the omega-6 PUFA species, arachidonic acid ("AA"), to pharmaceutical compositions that are rich in omega-3 PUFAs. *See* US pre-grant publication no. 2010/0160435.

[0008] The difficulty in defining an optimal composition is also due in part to enzymatic interconversion among certain omega-3 PUFA species, and to competition between omega-3 and omega-6 polyunsaturated fatty acids for shared enzymes in their respective biosynthetic pathways from medium chain dietary PUFAs (*see* FIG. 1).

[0009] A further challenge in designing an optimal composition is variation in bioavailability of orally administered PUFA compositions. Absorption of PUFAs in the form of ethyl esters is known, for example, to depend on the presence of pancreatic lipase, which is released in response to ingested fats. Absorption of PUFA ethyl esters is therefore inefficient, and is

subject to substantial variation, both among subjects and in any individual subject, depending on dietary intake of fat. *See* Lawson *et al.*, "Human absorption of fish oil fatty acids as triacylglycerols, free acids, or ethyl esters," *Biochem Biophys Res Commun.* 152:328-35 (1988); Lawson *et al.*, *Biochem Biophys Res Commun.* 156:960-3 (1988). Absorption is particularly reduced in subjects on low-fat diets, a diet advocated for subjects with elevated serum triglyceride levels or cardiovascular disease.

[0010] For any specifically desired PUFA pharmaceutical composition, the refining process is designed to produce a final product having the obligate fatty acid components within pre-defined percentage tolerance ranges and to limit certain undesired components to levels below certain pre-defined tolerance limits, with sufficient yield to make the process commercially feasible and environmentally sustainable. Differences in the desired final composition dictate differences in the refining process.

[0011] Various known process steps present trade-offs that make composition-specific adaptation and optimization of the refining process difficult, however. For example, urea inclusion complexation (clathration) in the presence of ethanol is often used to remove saturated and mono-unsaturated long chain fatty acids, increasing the relative proportion of desired long chain omega-3 polyunsaturated fatty acids in the resulting composition. Too little urea reduces long chain omega-3 PUFA enrichment. Excess urea, however, can lead to concentration of unwanted components, and has the potential to lead, at any given temperature and reaction time, to increased production of ethyl carbamate, a carcinogen that is impermissible above certain defined low limits. Existing alternatives to urea complexation, however, present other difficulties.

[0012] There is, therefore, a need for improved pharmaceutical compositions rich in omega-3 polyunsaturated fatty acids, especially for treatment of hypertriglyceridemia and mixed dyslipidemias, and for improved processes for refining such compositions from fish oil.

3. SUMMARY

[0013] In a first aspect, the present disclosure provides DPA-enriched pharmaceutical compositions of omega-3 polyunsaturated fatty acids in free acid form. Enrichment in DPA

content was an unintended and unexpected consequence of the commercial-scale production process. These DPA-enriched pharmaceutical compositions have been demonstrated to have exceptional pharmacological and clinical efficacy in *in vitro* experiments and in human clinical trials.

[0014] Accordingly, in another aspect, methods of treatment are provided. In one series of treatment embodiments, methods of treating severe hypertriglyceridemia (TGs > 500 mg/dL) are provided. In another series of treatment embodiments, methods of treating hypertriglyceridemia (200 mg/dL - 500 mg/dL) by adjunctive administration of a statin and the pharmaceutical compositions described herein are provided. Further treatment methods include, *inter alia*, treatments to increase plasma EPA:AA ratios, treatments to decrease ApoCIII levels, and treatments to reduce or prevent resistance to platelet aggregation inhibitors.

[0015] Also disclosed herein are methods of making the pharmaceutical compositions at commercial scale, including methods that include a urea complexation step in which compositionally-constrained batches of transesterified intermediate feedstock are subjected to a urea complexation step using urea amounts within ranges determined by a new process algorithm.

4. BRIEF DESCRIPTION OF THE FIGURES

[0016] FIG. 1 shows the known human pathways for biosynthesis of omega-3 and omega-6 long-chain polyunsaturated fatty acids from intermediate (medium) chain length essential fatty acids.

[0017] FIG. 2 is flow chart of an exemplary process for preparing an intermediate feedstock of PUFA ethyl esters.

[0018] FIG. 3A plots the average relative purification of classes of fatty acids by a urea complexation step in which algorithmically-determined amounts of urea are added to compositionally-defined intermediate feedstock of PUFA ethyl esters.

[0019] FIG. 3B illustrates the average differential purification of individual species of omega-3 and omega-6 PUFA ethyl esters when algorithmically-determined amounts of urea are added to compositionally-defined intermediate feedstock of PUFA ethyl esters.

[0020] FIG. 4 is a treatment flow diagram illustrating the design of the ECLIPSE clinical study further described in Example 7.

[0021] FIG. 5 compares the bioavailability of total EPA + DHA (baseline-adjusted change) following a single dose (4 g) of Lovaza® during the high-fat and low-fat diet periods.

[0022] FIG. 6 compares the bioavailability of total EPA+DHA (baseline-adjusted change) following a single dose (4g) of Lovaza® ("EE-FA") or Epanova®, a DPA-enriched composition of omega-3 PUFAs in free acid form ("FFA"), during the high-fat diet period.

[0023] FIG. 7 compares the total plasma EPA+DHA concentrations (baseline-adjusted change) following a single dose (4g) of Lovaza® or Epanova® during the low-fat diet period.

[0024] FIG. 8 compares the total plasma EPA concentrations (baseline-adjusted change) following a single dose (4 g) of Lovaza® or Epanova® during the low-fat diet period.

[0025] FIG. 9 compares the total plasma DHA concentrations (baseline-adjusted change) following a single dose of (4g) of Lovaza® or Epanova® during the low-fat diet period.

[0026] FIGS. 10A and 10B present individual subject AUC_{0-t} responses during the low-fat and high-fat diets expressed as the ratio (%) of low-fat AUC_{0-t} to high-fat AUC_{0-t}. Negative ratios were not plotted.

[0027] FIG. 11 is a treatment flow diagram illustrating the design of the 14 day comparative bioavailability trial further described in Example 8 (timeline not to scale).

[0028] FIG. 12A plots the mean unadjusted total EPA+DHA concentrations versus time (linear scale) for treatment with Lovaza® *vs.* treatment with Epanova® in the 14 day comparative bioavailability trial further described in Example 8.

[0029] FIG. 12B is a histogram showing the difference in unadjusted EPA+DHA (nmol/mL) for the points bracketed in FIG. 12A.

[0030] FIG. 13 plots EPA+DHA mean base-line adjusted plasma total EPA+DHA concentrations versus time (linear scale) for treatment with Lovaza® vs. treatment with Epanova® in the 14 day comparative bioavailability study.

[0031] FIG. 14A is a histogram that plots the increases from baseline to steady state in unadjusted blood levels for EPA+DHA in the Lovaza® and Epanova® arms of the 14 day comparative bioavailability study.

[0032] FIG. 14B is a histogram that plots the increases from baseline to steady state in unadjusted C_{avg} for EPA+DHA in the Lovaza® and Epanova® arms of the 14 day comparative bioavailability study.

[0033] FIG. 15A is a histogram that plots the increases from baseline to steady state for total blood levels of DHA in the Lovaza® and Epanova® arms of the 14 day comparative bioavailability study.

[0034] FIG. 15B is a histogram that plots the increases from baseline to steady state for DHA C_{avg} levels in the Epanova® cohort compared to Lovaza® cohort in the 14 day comparative bioavailability study.

[0035] FIG. 16A is a histogram that plots the increases from baseline to steady state for total EPA levels in blood in the Lovaza® and Epanova® arms of the 14 day comparative bioavailability study.

[0036] FIG. 16B plots the increases from baseline to steady state for EPA C_{avg} levels in the Epanova® and Lovaza® cohorts in the 14 day comparative bioavailability study.

[0037] FIG. 17 provides a treatment flow diagram illustrating the design of the EVOLVE study, further described in Example 10.

[0038] FIG. 18 summarizes the EVOLVE trial design in greater detail, further identifying the timing of study visits.

[0039] FIG. 19 shows the disposition of subjects in the EVOLVE trial.

[0040] FIGS. 20A - 20D display average baseline and end-of-treatment ("EOT") plasma levels (in μg/mL) for EPA (FIG. 20A), DHA (FIG. 20B), DPA (FIG. 20C) and AA (FIG. 20D), for each of the treatment arms in the EVOLVE trial.

[0041] FIG. 20E compares average baseline and EOT EPA levels for the ECLIPSE trial described in Example 7, the 14-day bioavailability study described in Example 8, a statin drug-drug interaction study ("STATIN DDI") described in Example 11, each treatment arm as well as the control arm of the EVOLVE trial described in Example 10, and values earlier reported in the literature for the unrelated JELIS trial ("JELIS"), which used a different omega-3 composition.

[0042] FIGS. 21A - 21D plot median baseline and end-of-treatment ("EOT") plasma levels (in μg/mL) for EPA (FIG. 21A), DHA (FIG. 21B), DPA (FIG. 21C), and AA (FIG. 21D) in the EVOLVE trial.

[0043] FIGS. 22A and 22B plot change from baseline to EOT in absolute plasma levels (in μg/mL) of AA, DHA, EPA, and DPA, for each of the treatment arms of the EVOLVE trial. FIG. 22A plots average changes; FIG. 22B plots median changes.

[0044] FIG. 23A plots average change from baseline to EOT, as percentage of baseline value, for AA, DHA, EPA, and DPA in each of the treatment arms of the EVOLVE trial. FIG. 23B plots median percent change from baseline to EOT.

[0045] FIGS. 24A - 24I plot average baseline and EOT plasma levels (in mg/dL, with the exception of LpPLA2, shown in ng/mL) in the EVOLVE trial for triglycerides (FIG. 24A), Non-HDL-C (FIG. 24B), HDL-C (FIG. 24C), V-LDL-C (FIG. 24D), LDL-C (FIG. 24E), ApoB (FIG. 24F), ApoCIII (FIG. 24G), RLP (FIG. 24H), LpPLA2 (FIG. 24I).

[0046] FIGS. 25A - 25I plot median baseline and EOT plasma levels (in mg/dL, with the exception of LpPLA2, shown in ng/mL) in the EVOLVE trial for triglycerides (FIG. 25A), Non-HDL-C (FIG. 25B), HDL-C (FIG. 25C), V-LDL-C (FIG. 25D), LDL-C (FIG. 25E), ApoB (FIG. 25F), ApoCIII (FIG. 25G), RLP (FIG. 25H), LpPLA2 (FIG. 25I).

[0047] FIGS. 26A and 26B plot change from baseline to EOT in absolute plasma levels (in mg/dL) in the EVOLVE trial of triglycerides ("TG"), Non-HDL-C ("NHDL-C"), HDL-C, VLDL-C, and LDL-C for each of the treatment arms of the EVOLVE trial, with FIG. 26A plotting average change and FIG. 26B showing median change.

[0048] FIG. 27 plots the percentage of subjects in the EVOLVE trial, given by the Y-axis, for whom triglyceride levels were reduced by the indicated percentage, given by the X-axis, for 2g dose and 4g dose of Epanova®.

[0049] FIG. 28A plots average change from baseline to EOT, as percentage of baseline value, for TG, non-HDL-c ("NHDL-C"), HDL-C, VLDL-C, LDL-C, ApoB, ApoCIII, LpLPA2, and RLP in each of the treatment arms of the EVOLVE trial, with FIG. 28B plotting median percent change from baseline to EOT.

[0050] FIG. 29 plots the rate of change (absolute value) of the median percentage change from baseline in plasma levels of EPA, DHA, DPA, AA, TG, NHDL-C, and HDL-C between 2g and 4g doses of Epanova® in the EVOLVE trial .

[0051] FIG. 30 illustrates comparative data for Epanova® , as measured in the EVOLVE trial, and data reported by others for AMR-101 (Vascepa), at the indicated doses, with respect to TG levels.

[0052] FIG. 31 illustrates comparative data for Epanova®, as measured in the EVOLVE trial, and AMR-101 (Vascepa), with respect to various blood lipid parameters. Data for AMR-101 were reported by others. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0053] FIG. 32 illustrates comparative data for Epanova® 2g and 4g doses, as determined in the EVOLVE trial, and Lovaza® 4 g dose, with respect to various blood lipid parameters. Data for Lovaza® were reported by others. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0054] FIG. 33 illustrates comparative data for Epanova® 2g and 4g doses, as assessed in the EVOLVE trial, and Lovaza® 4 g dose, as reported by others, with respect to TG levels. The

superscripts indicate data sourced from (1) EVOLVE trial, (2) a meta-analysis from the Lovaza® New Drug Application ("NDA") (3) Lovaza® FDA-approved product Label and (4) Takeda study. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0055] FIG. 34 plots the correlation between percent change in LDL and percent change in ApoCIII, as measured in the EVOLVE trial.

[0056] FIG. 35 plots the least squares (LS) mean percentage change from baseline for the subset of EVOLVE trial subjects having TG baseline levels greater than or equal to 750 mg/dL, for the indicated treatment arms of the EVOLVE study, as further described in Example 10. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0057] FIG. 36 plots the least squares (LS) mean percentage change from baseline for the subset of subjects having Type II diabetes, for the indicated treatment arms of the EVOLVE study, as described in Example 10. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0058] FIG. 37 plots the least squares (LS) mean percentage change from baseline for the subset of subjects undergoing concurrent statin therapy, for the indicated treatment arms of the EVOLVE study, as described in Example 10. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0059] FIG. 38 plots the least squares (LS) mean percentage difference relative to control for triglycerides ("TG"), non-HDL-cholesterol ("NHDL-C"), HDL-C, LDL-C, TC, VLDL-C, and TC/HDL-C, comparing subjects from the EVOLVE study described in Example 10 who either received (STATIN) or did not receive (NON-STATIN) statin therapy concurrent with treatment with the 2g dose of Epanova®. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0060] FIG. 39 plots the median percent change from baseline for TG, NHDL-C, HDL-C, LDL-C, TC, VLDL-C, and TC/HDL-C for the subset of subjects undergoing concurrent statin therapy, in the indicated treatment arms of the EVOLVE study, further described in

Example 10. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0061] FIG. 40 provides a treatment flow diagram illustrating the design of the ESPRIT study, further described in Example 12.

[0062] FIG. 41 shows the disposition of subjects in the ESPRIT trial.

[0063] FIGS. 42A and 42B plot the median LS percentage change from baseline for EPA (FIG. 42A) and DHA (FIG. 42B) from the ESPRIT study, further described in Example 12. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0064] FIG. 43 plots mean LS percentage change from baseline for TG, Non-HDL-C, and HDL-C. Data shown are from the ESPRIT study, further described in Example 12. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0065] FIG. 44 plots mean LS percentage change from baseline for ApoB, LDL-C, VLDL-C, and TC/HDL-C. Data shown are from the ESPRIT study, further described in Example 12. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0066] FIG. 45 plots median percentage change from baseline for TG, with subjects grouped into tertiles by baseline TG levels, for subjects in the ESPRIT trial.

[0067] FIG. 46 plots median percentage change from baseline for Non-HDL-C, with subjects grouped into tertiles by baseline Non-HDL-C levels, for subjects in the ESPRIT trial.

[0068] FIG. 47 plots median percentage change from baseline for LDL-C, with subjects grouped into tertiles by baseline LDL-C levels, for subjects in the ESPRIT trial.

[0069] FIG. 48 plots median percentage change from baseline for TG for each of the treatment arms of the ESPRIT trial, with subjects grouped according to the identity of the statin taken in concurrent therapy.

[0070] FIG. 49 plots median percentage change from baseline for TG for each of the treatment arms of the ESPRIT trial, with subjects grouped into two groups according to low or high potency concurrent statin therapy.

[0071] FIG. 50 plots median percentage change from baseline for Non-HDL-C for each of the treatment arms of the ESPRIT trial, with subjects grouped according to low or high potency concurrent statin therapy.

[0072] FIG. 51 plots median percentage change from baseline for LDL-C for each of the treatment arms of the ESPRIT trial, with subjects grouped into two groups according to low or high potency concurrent statin therapy.

[0073] FIG. 52 plots median percentage change from baseline for TG, with subjects in each treatment arm of the ESPRIAT trial grouped into three groups according to high baseline TG, high baseline EPA, or concurrent rosuvastatin therapy.

[0074] FIG. 53 plots mean LS percentage change in particle size distribution from baseline for V-LDL particles grouped by size, as determined in the ESPRIT trial. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0075] FIG. 54 plots mean LS percentage change in particle size distribution from baseline for LDL particles grouped by size for each of the treatment arms of the ESPRIT trial. (*) indicates a p value of less than 0.05, (**) indicates a p value of less than 0.01, and (***) indicates a p value of less than 0.001.

[0076] FIG. 55 plots LS median percentage change in LDL particle size, with subjects grouped into three groups according to ESPRIT EOT triglyceride levels.

[0077] FIG. 56A depicts baseline arachidonic acid (AA) plasma levels (in μg/mL) for subjects in the clinical trial further described in Example 11, grouped according to genotype at the rs174546 SNP. FIG. 56B depicts percent change from baseline in AA plasma levels at day 15 of treatment with Epanova®, grouped according to genotype at the rs174546 SNP. For each genotype, the interquartile range is indicated by a box, the median is indicated by a

horizontal line in the interior of the interquartile box, and the mean is represented by a diamond. Outliers are represented by open circles. The whiskers extend to the minimum and maximum non-outlier value. Score 1 identifies subjects who are homozygous at the major allele; Score 3 identifies subjects homozygous at the minor allele; and Score 2 represents heterozygotes.

5. DETAILED DESCRIPTION

5.1. Overview: Pharmaceutical Compositions Of Omega-3 Polyunsaturated Fatty Acids In Free Acid Form That Are Unexpectedly Enriched In DPA Have Exceptional Clinical Efficacy

[0078] Urea inclusion complexation (clathration) is a standard step often used in the refining of fish oils to remove saturated and mono-unsaturated long chain fatty acids, thus enriching for desired long chain omega-3 polyunsaturated fatty acids in the resulting composition. Despite long usage, however, and studies designed to characterize the effects of various physiochemical parameters on the process, the degree to which urea complexation enriches individual species of long chain polyunsaturated fatty acids remains unpredictable. This residual unpredictability in the urea complexation procedure, coupled with the potential for generating impermissibly high levels of ethyl carbamate, which would obligate additional processing, initially militated in favor of omitting urea complexation from the commercial scale refining process to be used for producing pharmaceutical grade compositions of omega-3 PUFAs in free acid form meeting certain desired compositional specifications.

[0079] However, as further described in Example 1, early efforts to develop a urea-free commercial scale process made clear that such processes could not reliably produce batches with a composition that met required specifications. Accordingly, a process using urea complexation was sought, and it was discovered that strict compositional control on the PUFA species present in the intermediate ethyl ester feedstock, coupled with use of an algorithmically-determined amount of urea, could reliably produce batches meeting the required specifications, and without exceeding acceptable ethyl carbamate limits.

[0080] As described in Example 2, four exemplary production batches of polyunsaturated fatty acids in free acid form were prepared using a urea complexation step. Strict

compositional controls were applied to the ethyl ester intermediate feedstock, using only batches in which specified species of polyunsaturated fatty acids fell within defined range limits, and urea amounts were used that fell within the range required by the urea calculator algorithm. All four production batches of the pharmaceutical composition were determined to meet the desired compositional specifications.

[0081] As expected, the urea complexation step substantially decreased the percentage of saturated fatty acids and mono-unsaturated fatty acids in the resulting composition, thereby substantially enriching for polyunsaturated fatty acids. *See* FIG. 3A. Unexpectedly, however, performing urea complexation using urea amounts falling within the algorithmically-determined range had differential effects on enrichment of particular species of omega-3 polyunsaturated fatty acids and omega-6 polyunsaturated fatty acids.

[0082] As described below in Example 3, the omega-3 docosapentaenoic acid species, DPA (C22:5 n-3), was found to be enriched, whereas the corresponding omega-6 species, with identical chain length and degree of unsaturation, docosapentaenoic acid (C22:5 n-6), was reduced in prevalence. The divergent effect of urea complexation on enrichment of these two isomers – in conjunction with differences in their relative concentrations in the ethyl ester intermediate feed stock – resulted in a log order difference in their concentrations in the final, free acid, pharmaceutical composition ("API").

[0083] Further production batches were prepared, and as described in Example 4, compositional analysis of 10 batches of API demonstrated reproducibly elevated levels of DPA in the final composition. As described in Example 5, compositional analysis of 21 batches prepared using urea complexation demonstrated a reproducible 10-fold difference in the concentration of the omega-3 species, DPA, as compared to its omega-6 isomer, docosapentaenoic acid (C22:5 n-6).

[0084] At an average concentration of 4.44% (a/a) across 21 production batches, DPA is the third most prevalent species of polyunsaturated fatty acid in the API, exceeded only by EPA and DHA. At this level, the DPA concentration is also approximately 10-fold greater than that reported for an earlier pharmaceutical composition of omega-3 polyunsaturated fatty

acids in free acid form, termed Purepa, in which DPA was reported to be present at a level of 0.5%. *See* Belluzzi *et al.*, *Dig. Dis. Sci.* 39(12): 2589-2594 (1994).

[0085] Although DPA is an intermediate in the biosynthetic pathway from EPA to DHA (*see* FIG. 1), surprisingly little is known about the DPA's specific biological effects. To clarify the potential contribution of DPA to clinical efficacy of the pharmaceutical composition, gene expression profiling experiments were conducted using HepG2 hepatocarcinoma cells.

[0086] As further described in Example 6, DPA's effects on hepatic cell gene expression predict greater clinical efficacy of DPA-enriched compositions.

[0087] The gene expression profiling experiments demonstrated that DPA has significant biological activity at relevant *in vitro* concentrations. These effects are markedly different from those seen with EPA and with DHA.

[0088] At relevant concentration, DPA was observed to affect expression of genes in multiple metabolic pathways, including genes in categories known to be relevant to the clinical effects of omega-3 polyunsaturated fatty acids: genes involved in lipid metabolism, genes involved in cardiovascular physiology, and genes involved in inflammation. Significant second-order effects are also predicted, given the changes observed in the expression of genes that encode proteins that themselves affect gene expression, and in genes encoding proteins that affect post-transcriptional modification.

[0089] Specific effects on expression of several genes involved in lipid metabolism suggest that DPA, at an analogous *in vivo* concentration, should contribute to improvement in various clinically-relevant lipid parameters. In particular, the observed DPA-driven upregulation of **ACADSB**, the short/branched chain acyl-CoA dehydrogenase, predicts lower serum triglyceride levels; DPA-driven downregulation of **HMGCR**, analogous to inhibition of the encoded HMG-CoA-reductase enzyme by statins, would be predicted to lead to favorable decreases in the total cholesterol:HDL ratio; and DPA downregulation of **SQLE**, a rate-limiting step in sterol synthesis, analogously predicts reductions in total cholesterol levels.

[0090] The expression profiling experiments also demonstrated a dose threshold for DPA's effects. The lower concentration tested, chosen to mimic the 10-fold lower concentration of DPA in the earlier free acid omega-3 formulation, Purepa, affected the expression of 10-fold fewer genes than the higher DPA concentration, chosen to mimic the exposure expected from the pharmaceutical compositions described herein, demonstrating that the lower DPA concentration provides subthreshold exposure, and would be expected to provide a subtherapeutic dose *in vivo*.

[0091] Human clinical trials confirmed the exceptional clinical efficacy of the DPA-enriched pharmaceutical composition of omega-3 polyunsaturated fatty acids in free acid form.

[0092] Example 7 presents the results of the ECLIPSE clinical trial, an open-label, single dose, randomized 4-way-crossover study comparing the bioavailability of a 4g dose of Lovaza® to bioavailability of a 4g dose of the DPA-enriched pharmaceutical composition of omega-3 PUFA in free acid form described herein (hereinafter, "Epanova®"), under both high fat and low fat dietary conditions. According to the FDA-approved product label, each 1-gram capsule of Lovaza® contains at least 900 mg of the ethyl esters of omega-3 fatty acids sourced from fish oils, predominantly a combination of ethyl esters of eicosapentaenoic acid (EPA - approximately 465 mg) and docosahexaenoic acid (DHA - approximately 375 mg). The batch of Epanova® used in the trial comprised 57.3% (a/a) EPA, 19.6% (a/a) DHA, and 6.2% (a/a) DPA, each substantially in free acid form.

[0093] The baseline-adjusted change in total EPA+DHA and individual EPA and DHA absorption profiles (AUC) with Epanova® (omega-3 PUFAs in free acid form) were significantly greater than with Lovaza® (omega-3-PUFA ethyl esters) during the high-fat diet period and dramatically better during the low-fat diet period. Furthermore, there was a profound impact of fat content of the meals on the bioavailability of Lovaza®, whereas the bioavailability of Epanova® was much more predictable, due to only modest food effect.

[0094] The superior fat-independent bioavailability of Epanova® over Lovaza® is clinically important, in view of the NCEP ATP III recommendation that subjects with hypertriglyceridemia and dyslipidemias adhere to a low-fat diet during adjunct therapy.

[0095] Example 8 presents results from a 14-day bioavailability study, which demonstrated that the increase in bioavailability observed in the single-dose ECLIPSE trial is maintained, even enhanced, over 2 weeks of dosing. In addition, disaggregated subject-specific data demonstrated that the subject with least response to Epanova® still had a greater day-14 EPA+DHA Cmax than the subject with best response to Lovaza®.

[0096] Example 10 presents the results of the EVOLVE trial, a 12-week, double-blind, olive oil-controlled study of patients selected on the basis of high triglyceride levels, in the range of 500–2,000 mg/dL (severe hypertriglyceridemia). The primary study endpoint was percent change in plasma triglyceride levels from baseline to end-of-treatment ("EOT"). The secondary endpoint was percent change in plasma non-HDL cholesterol ("non-HDL-C") from baseline to EOT.

[0097] As can be seen from FIGS. 20 - 23, 12 week treatment with Epanova® caused dramatic increases in plasma levels of EPA, DHA, and DPA.

[0098] Increases in plasma levels of EPA, DHA, and DPA were accompanied by significant reductions in plasma AA levels, with the 4g dosage regimen effecting an average reduction of 18%, a median reduction of 25.9%, and a least squares ("LS") mean reduction of 23.2%. These decreases in plasma arachidonic acid levels were observed despite the administration of exogenous arachidonic acid, which was present at 2.446% (a/a) in the Epanova® batch used in this trial.

[0099] The increase in EPA plasma levels with concomitant reduction in AA plasma levels caused a significant improvement in the EPA/AA ratio, from approximately 0.10 at baseline to approximately 0.67 (average) and 0.62 (median) at end-of-treatment ("EOT") at the 4g dose. The EPA/AA ratio has been reported to constitute an independent risk factor for coronary atherosclerosis, Nakamua & Maegawa, *Endocrine Abstracts* (2012) 29 OC19.1, with lower ratios associated with progression in coronary atherosclerosis in statin-treated patients with coronary artery disease, Nozue *et al.*, *Am J Cardiol.* 2013 Jan 1;111(1):6-1 (ePub ahead of print).

[0100] Furthermore, treatment with Epanova® resulted in substantial reductions in triglyceride levels (see FIGS. 26A and 26B), reductions in non-HDL-C and VLDL-C, and increase in HDL-C. LDL-C levels were elevated, an observation that may be attributed to an increase in LDL particle size upon treatment (discussed further in Example 12).

[0101] The EVOLVE trial also demonstrated that Apolipoprotein CIII (ApoCIII) was significantly reduced by Epanova® treatment. Elevated levels of ApoCIII have been found to be an independent predictor for cardiovascular heart disease (CHD) risk, whereas genetically reduced levels of ApoCIII have been associated with protection from CHD, and have also been correlated with increase in longevity.

[0102] The extremely high bioavailability of the omega-3 PUFAs in Epanova® revealed previously unknown, and unexpected, differences in pharmacokinetic response among the various PUFA species.

[0103] FIG. 29 plots the rate of change in the median percentage change from baseline in plasma levels of EPA, DHA, DPA, AA, TG, non-HDL-C, and HDL-C (absolute value) between 2g and 4g doses of Epanova®. With little or no increase in plasma levels of DHA and DPA upon doubling of the Epanova® dose from 2g to 4g per day, the rate of change (slope) in the median percentage change from baseline is near zero, predicting little further increase in DHA and DPA plasma levels would be achieved if dose is further increased. Similar plateauing of response was seen in triglyceride levels, HDL-C levels, and non-HDL-C levels (data not shown).

[0104] By contrast, the rate of change for EPA remains high, with a slope of 0.59; further increase in EPA plasma levels is expected to be obtained by increasing Epanova® dosage above 4g per day. Significantly, the rate of change (decrease) in AA levels upon doubling the Epanova® dose from 2g to 4g per day is even higher than that for EPA; further reductions in AA plasma levels are expected as Epanova® dosage is increased above 4g/day. Epanova® thus exhibits unprecedented potency in ability to elevate EPA levels, reduce AA levels, and improve the EPA:AA ratio.

[0105] As shown in FIG. 38, a subset of subjects in the 2g treatment arm of the EVOLVE trial who were receiving concurrent statin therapy displayed greater magnitudes of percentage changes (mean LS difference), relative to control, for TG, non-HDL-C, HDL-C, LDL-C, TC, VLDL-C, and TC/HDL-C, when compared to those subjects in the 2g treatment arm who did not receive concurrent statin therapy. Subjects receiving concurrent statin therapy showed a dose-dependent response to Epanova®, as shown in comparative data for Epanova® 2g and Epanova® 4g displayed in FIG. 39.

[0106] Example 12 describes the ESPRIT clinical trial, which was conducted to study patients on baseline statin therapy with triglyceride levels between 200-500 mg/dL, lower than the patients with severe hypertriglyceridemia enrolled in the EVOLVE study described in Example 10.

[0107] Dose-dependent reductions in triglycerides, reductions in non-HDL-C, and increases in HDL-C, were observed, when compared to olive oil placebo (*see* FIG. 43). Furthermore, dose-dependent reductions in VLDL-C and TC/HDL-C were observed (see FIG. 44). Taken together, the results (summarized in FIGS. 42 - 44) demonstrate efficacy of Epanova® as an add-on to statin therapy in patients with triglyceride levels between 200 -500 mg/dL.

[0108] FIGS. 45 - 52 illustrate that Epanova® is efficacious as an add-on to both low-potency and high-potency statins, in a range of baseline patient conditions. As seen from FIG. 48, the reductions in TG levels were observed for patients who received concurrent rosuvastatin, atorvastatin, and simvastatin therapy. Statistically significant effects on triglycerides, non-HDL-C, and LDL-C levels were observed regardless whether low potency or high potency statins were co-administered, as shown in FIGS. 49 - 51.

5.2. DPA-Enriched Omega-3 Compositions In Free Acid Form

[0109] Accordingly, in a first aspect, improved compositions of polyunsaturated fatty acids ("PUFAs") in free acid form are provided. In various embodiments, the composition is a pharmaceutical composition suitable for oral administration. In a variety of embodiments, the composition is a neutraceutical composition suitable for oral administration.

5.2.1. Typical embodiments

[0110] The composition comprises a plurality of species of omega-3 PUFA, each present substantially in free acid form.

[0111] The composition comprises eicosapentaenoic acid (C20:5 n-3) ("EPA," also known as timnodonic acid), docosahexaenoic acid (C22:6 n-3) ("DHA," also known as cervonic acid), and docosapentaenoic acid (C22:5 n-3) ("DPA", also known as clupodonic acid), each substantially in free acid form.

[0112] The composition comprises EPA in an amount, calculated as a percentage by area on GC chromatogram of all fatty acids in the composition, of at least about 45% ("45% (a/a)"). In various embodiments, the composition comprises EPA in an amount of at least about 46% (a/a) 47% (a/a), 48% (a/a), 49% (a/a), or at least about 50% (a/a). In certain embodiments, the composition comprises EPA in an amount of at least about 51% (a/a), at least about 52% (a/a), at least about 53% (a/a), at least about 54% (a/a), at least about 55% (a/a), at least about 56% (a/a), at least about 57% (a/a), at least about 58% (a/a), even at least about 59% (a/a), at least about 60% (a/a), at least about 61% (a/a), 62% (a/a), 63% (a/a), 64% (a/a), or 65% (a/a).

[0113] In certain embodiments, the composition comprises EPA in an amount of about 45 to about 65% (a/a). In particular embodiments, EPA is present in an amount of about 50 to about 60% (a/a). In various embodiments, EPA is present in an amount of about 52 to about 58.0 % (a/a). In some embodiments, EPA is present in an amount of about 55% (a/a) to about 56% (a/a). In some embodiments, EPA is present in an amount of about 55% (a/a).

[0114] In various embodiments, the composition comprises EPA in an amount, calculated as a percentage by mass of all fatty acids in the composition ("% (m/m)"), of about 50% (m/m) to about 60 % (m/m). In certain embodiments, EPA is present in an amount of about 55% (m/m).

[0115] The composition comprises DHA in an amount of at least about 13% (a/a). In various embodiments, the composition comprises DHA in an amount of at least about 14% (a/a), at least about 15% (a/a), at least about 16% (a/a), at least about 17% (a/a), at least about 18%

(a/a), at least about 19% (a/a), or at least about 20% (a/a). In selected embodiments, the composition comprises DHA in an amount of at least about 21% (a/a), at least about 22% (a/a), at least about 23% (a/a), at least about 24% (a/a), even at least about 25% (a/a).

[0116] In various embodiments, the composition comprises DHA in an amount of about 13% (a/a) to about 25 % (a/a). In certain embodiments, DHA is present in an amount of about 15% (a/a) to about 25% (a/a). In several embodiments, DHA is present in an amount of about 17% (a/a) to about 23 % (a/a). In certain embodiments, DHA is present in an amount of about 19% (a/a) to about 20% (a/a).

[0117] In various embodiments, the compositions comprise DHA in an amount of about 15% (m/m) to about 25% (m/m). In certain embodiments, DHA is present in an amount of about 17% (m/m) to about 23% (m/m). In certain embodiments, DHA is present in an amount of about 20% (m/m).

[0118] The composition comprises DPA in an amount of at least about 1% (a/a). In various embodiments, the composition comprises DPA in an amount of at least about 1.5% (a/a), 2% (a/a), 2.5% (a/a), 3% (a/a), 3.5% (a/a), 4% (a/a), 4.5% (a/a), even at least about 5% (a/a). In selected embodiments, the composition comprises DPA in an amount of at least about 6% (a/a), at least about 7% (a/a), at least about 8% (a/a), or at least about 9% (a/a).

[0119] In a variety of embodiments, the composition comprises DPA in an amount of about 1% (a/a) to about 8% (a/a). In certain embodiments, the composition comprises DPA in an amount of about 2% (a/a) to about 7% (a/a). In selected embodiments, the composition comprises DPA in an amount of about 3% (a/a) to about 6% (a/a). In particular embodiments, the composition comprises DPA in an amount of about 4% (a/a) to about 5% (a/a).

[0120] In various embodiments, the composition comprises DPA, calculated as a percentage by mass of all fatty acids in the composition, in an amount of no less than about 1% (m/m). In various embodiments, the composition comprises DPA in an amount of about 1% (m/m) to about 8% (m/m). In particular embodiments, the composition comprises DPA in an amount of no more than about 10% (m/m).

[0121] The composition comprises EPA and DHA in a total amount of at least about 60% (a/a). In various embodiments, the composition comprises EPA and DHA in a total amount of at least about 61% (a/a), 62% (a/a), 63% (a/a), 64% (a/a), 65% (a/a), 66% (a/a), 67% (a/a), 68% (a/a), 69% (a/a), or at least about 70% (a/a). In particular embodiments, the composition comprise EPA and DHA in a total amount off at least about 71% (a/a), 72% (a/a), 73% (a/a), 74% (a/a), 75% (a/a), 76% (a/a), 77% (a/a), 78% (a/a), 79% (a/a), even at least about 80% (a/a). In certain embodiments, the composition comprises EPA and DHA in total amount of at least about 81% (a/a), 82% (a/a), at least about 83% (a/a), 84% (a/a), even at least about 85% (a/a).

[0122] In various embodiments, the composition comprises EPA and DHA in an amount of about 70.0 % (m/m) to about 80.0% (m/m). In certain embodiments, the composition comprises about 75% (m/m) EPA plus DHA.

[0123] The composition comprises EPA, DHA, and DPA in a total amount of at least about 61% (a/a). In typical embodiments, the composition comprises EPA, DHA, and DPA in a total amount of at least about 62% (a/a), 63% (a/a), 64% (a/a), 65% (a/a), 66% (a/a), at least about 67% (a/a), at least about 68% (a/a), at least about 69% (a/a), or at least about 70% (a/a). In certain embodiments, the composition comprises EPA, DHA, and DPA in a total amount of at least about 71% (a/a), 72% (a/a), 73% (a/a), 74% (a/a), 75% (a/a), 76% (a/a), 77% (a/a), 78% (a/a), 79% (a/a), 80% (a/a), even at least about 81% (a/a), 82% (a/a), 83% (a/a), 84% (a/a), 85% (a/a), 86% (a/a), 87% (a/a), even at least about 88% (a/a).

[0124] In various embodiments, the composition comprises EPA, DHA, and DPA in a total amount of between about 70% (a/a) to about 90% (a/a).

[0125] In a particular series of embodiments, EPA is present in an amount of about 55% (a/a) to about 56% (a/a); DHA is present in an amount of about 19% (a/a) to about 20% (a/a); and DPA is present in an amount of about 4% (a/a) to about 5 % (a/a).

[0126] In certain embodiments, the composition further comprises one or more omega-3 polyunsaturated fatty acid species selected from the group consisting of: α-linolenic acid

(C18:3 n-3), moroctic acid (C18:4 n-3, also known as stearidonic acid), eicosatrienoic acid (C20:3 n-3), eicosatetraenoic acid (C20:4 n-3), and heneicosapentaenoic acid (C21:5 n-3).

[0127] In particular embodiments, the composition comprises EPA, DHA, DPA, and moroctic acid, each substantially in the free acid form. In a variety of embodiments, the composition comprises EPA, DHA, DPA, moroctic acid, and heneicosapentaenoic acid, each substantially in the free acid form. In specific embodiments, the composition comprises EPA, DHA, DPA, moroctic acid, heneicosapentaenoic acid, and eicosatetraenoic acid, each substantially in the free acid form. In selected embodiments, the composition comprises EPA, DHA, DPA, α-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatrienoic acid (C20:3 n-3), eicosatetraenoic acid (C20:4 n-3), and heneicosapentaenoic acid (C21:5 n-3).

[0128] In various embodiments, total omega-3 fatty acids – defined as the sum of alpha-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatrienoic acid (C20:3 n-3), eicosatetraenoic acid (C20:4 n-3), eicosapentaenoic acid (EPA) (C20:5 n-3), heneicosapentaenoic acid (C21:5 n-3), docosapentaenoic acid (C22:5 n-3) and docosahexaenoic acid (DHA) (C22:6 n-3) – constitute from about 80% (a/a) to about 95% (a/a) of all fatty acids in the composition. In a variety of embodiments, total omega-3 fatty acids constitute from about 80 – about 95% (m/m) of all fatty acids in the composition.

[0129] In various embodiments, the composition further comprises one or more species of omega-6 PUFA, each present substantially in the free acid form.

[0130] In certain embodiments, the composition comprises one or more species of omega-6 PUFA selected from the group consisting of linoleic acid (C18:2 n-6), gamma-linolenic acid (C18:3 n-6), eicosadienoic acid (C20:2 n-6), dihomo-gamma-linolenic acid (C20:3 n-6) ("DGLA"), arachidonic acid (C20:4 n-6) ("AA"), and docosapentaenoic acid (C22:5 n-6, also known as osbond acid).

[0131] In particular embodiments, the composition comprises linoleic acid (C18:2 n-6), gamma-linolenic acid (C18:3 n-6), eicosadienoic acid (C20:2 n-6), dihomo-gamma-linolenic

acid (C20:3 n-6) ("DGLA"), arachidonic acid (C20:4 n-6) ("AA"), and docosapentaenoic acid (C22:5 n-6), each present substantially in the free acid form.

[0132] In various embodiments, AA is present in an amount of no more than about 5% (a/a) of the fatty acids in the composition. In certain embodiments, AA comprises no more than about 4.5% (a/a) of the fatty acids in the composition. In particular embodiments, AA is present in an amount of no more than about 4% (a/a) of the fatty acids in the composition.

[0133] In certain embodiments, AA is present in an amount of no more than about 5% (m/m) of the fatty acids in the composition. In certain embodiments, AA comprises no more than about 4.5% (m/m) of the fatty acids in the composition. In particular embodiments, AA is present in an amount of no more than about 4% (m/m) of the fatty acids in the composition.

[0134] In certain embodiments, total omega-6 polyunsaturated fatty acids – defined as the sum of linoleic acid (C18:2 n-6), gamma-linolenic acid (C18:3 n-6), eicosadienoic acid (C20:2 n-6), dihomo-gamma-linolenic acid (C20:3 n-6), arachidonic acid (C20:4 n-6) and docosapentaenoic acid (C22:5 n-6) – comprise no more than about 10% (a/a) of the fatty acids in the composition. In certain embodiments, total omega-6 polyunsaturated fatty acids – defined as the sum of linoleic acid (C18:2 n-6), gamma-linolenic acid (C18:3 n-6), eicosadienoic acid (C20:2 n-6), dihomo-gamma-linolenic acid (C20:3 n-6), arachidonic acid (C20:4 n-6) and docosapentaenoic acid (C22:5 n-6) – comprise no more than about 10% (m/m) of the fatty acids in the composition.

[0135] In specific embodiments, the composition is given by Table 11, with each species of PUFA identified therein falling within the range of about -3SD to about +3 SD of the respectively recited average. In certain embodiments, each species of PUFA identified therein falls within the range of about -2SD to about +2 SD of the respectively recited average. In certain embodiments, each species falls within the range of about -1SD to about +1SD of the respectively recited average.

[0136] In selected embodiments, the composition is given by Table 13, with each species of PUFA identified therein falling within the range of about -3SD to about +3 SD of the respectively recited average. In certain embodiments, each species falls within the range of

about -2SD to about +2 SD of the respectively recited average. In certain embodiments, each PUFA species falls within the range of about -1SD to about +1SD of the respectively recited average.

[0137] In certain embodiments, polyunsaturated fatty acids other than omega-3 and omega-6 polyunsaturated fatty acids are present in an amount of no more than about 5% (a/a). In various embodiments, polyunsaturated fatty acids other than omega-3 and omega-6 polyunsaturated fatty acids are present in an amount of no more than about 5% (m/m).

[0138] In a variety of embodiments, at least 90% of each of the plurality of species of omega-3 PUFA in the composition is in the free acid form. In certain embodiments, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, even at least 99% of each species of omega-3 PUFA in the composition is present in the free acid form. In exemplary embodiments, at least 90% of the total omega-3 polyunsaturated fatty acid content in the composition is present in the free acid form. In certain embodiments, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, even at least 99% of the total omega-3 polyunsaturated fatty acid content in the composition is present in the free acid form.

[0139] In various embodiments, at least 90% of each of the plurality of species of omega-6 PUFA in the composition is in the free acid form. In certain embodiments, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, even at least 99% of each species of omega-6 PUFA in the composition is present in the free acid form. In exemplary embodiments, at least 90% of the total omega-6 polyunsaturated fatty acid content in the composition is present in the free acid form.

[0140] In various embodiments, at least 90% of the total polyunsaturated fatty acid in the composition is present in the free acid form. In certain embodiments, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, even at least 99% of the total polyunsaturated fatty acid in the composition is present in the free acid form.

[0141] The composition comprises, in typical embodiments, no more than about 3.0% (a/a) saturated fatty acids and no more than about 5.0% (a/a) mono-unsaturated fatty acids. In various embodiments, the composition comprises no more than about 3.0% (m/m) saturated fatty acids and no more than about 5.0% (m/m) mono-unsaturated fatty acids.

[0142] In typical embodiments, the composition usefully further comprises an antioxidant. In certain embodiments, the antioxidant is butylated hydroxyanisole (BHA). In some embodiments, the antioxidant is alpha-tocopherol. In some embodiments, alpha-tocopherol is present in an amount of about 0.20 – about 0.40% (m/m). In various embodiments, alpha-tocopherol is present in an amount of about 0.25 – about 0.35% (m/m). In particular embodiments, alpha-tocopherol is present in an amount of about 0.27 – about 0.33% (m/m).

[0143] In typical embodiments, the composition comprises no more than about 0.1 ppm ethyl carbamate. In some embodiments, the composition comprises no more than 0.1 ppm ethyl carbamate. In various embodiments, the composition comprises less than 0.1 ppm ethyl carbamate.

5.2.2. Additional embodiments

[0144] In certain additional embodiments, the composition comprises EPA in an amount, calculated as a percentage by area on GC chromatogram of all fatty acids in the composition, of about 45.0 - about 65.0% (a/a). In certain embodiments, EPA is present in an amount of about 50.0 – about 60.0 % (a/a). In various embodiments, EPA is present in an amount of about 52.0 – about 58.0 % (a/a). In some embodiments, EPA is present in an amount of about 55.0% (a/a).

[0145] In certain embodiments, EPA is present in an amount of about 55.0 – about 58.4 % (a/a). In various embodiments, EPA is present in an amount of about 55.6 – about 57.9 % (a/a). In some embodiments, EPA is present in an amount of about 56.7% (a/a).

[0146] In various embodiments, the composition comprises EPA in an amount, calculated as a percentage by mass of all fatty acids in the composition, of about 50.0 – 60.0% (m/m). In certain embodiments, EPA is present in an amount of about 55% (m/m).

[0147] In certain additional embodiments, the composition comprises DHA in an amount, calculated as a percentage by area on GC chromatogram of all fatty acids in the composition, of about 13.0 – about 25.0 % (a/a). In certain embodiments, DHA is present in an amount of about 15.0 – about 23.0% (a/a). In various embodiments, DHA is present in an amount of about 17.0 – about 21.0% (a/a). In certain embodiments, DHA is present in an amount of about 19.0 – about 20.0% (a/a).

[0148] In certain embodiments, DHA is present in an amount of about 17.7 – about 22.2% (a/a). In various embodiments, DHA is present in an amount of about 18.4 – about 21.4% (a/a). In certain embodiments, DHA is present in an amount of about 19.9% (a/a).

[0149] In various embodiments, the compositions comprises DHA, calculated as a percentage by mass of all fatty acids in the composition, in an amount of about 15.0 – 25.0% (m/m). In certain embodiments, DHA is present in an amount of about 20.0% (m/m).

[0150] In various additional embodiments, the composition comprises EPA and DHA in a total amount, calculated as a percentage by area on GC chromatogram of all fatty acids in the composition, of about 67.0 - about 81.0 % (a/a). In certain embodiments, the composition comprises a total amount of EPA and DHA of about 69.0 – about 79.0% (a/a). In various embodiments, the composition comprises a total amount of EPA plus DHA of about 71.0 – about 77.0% (a/a). In certain embodiments, the composition comprises a total amount of EPA+DHA of about 74.0 – about 75.0% (a/a).

[0151] In various embodiments, the composition comprises EPA+DHA in a total amount, calculated as a percentage by mass of all fatty acids in the composition, of about 70.0-80.0% (m/m). In certain embodiments, the composition comprises about 75.0% (m/m) EPA plus DHA.

[0152] In those additional embodiments that further comprise α-linolenic acid (C18:3 n-3), α-linolenic acid is present in an amount of about 0.07 – about 1.10% (a/a). In certain embodiments, α-linolenic acid is present in an amount of about 0.24 – about 0.91% (a/a). In various embodiments, α-linolenic acid is present in an amount of about 0.40 – about 0.80 %

(a/a). In certain embodiments, α-linolenic acid is present in an amount of about 0.50 to about 0.600% (a/a).

[0153] In those additional embodiments that further comprise moroctic acid (C18:4 n-3), moroctic acid is present in an amount of about 0.01 – about 7.90% (a/a). In certain embodiments, moroctic acid is present in an amount of about 0.25 – about 6.40% (a/a). In certain embodiments, moroctic acid is present in amount of about 1.70% - about 4.90% (a/a). In particular embodiments, moroctic acid is present in an amount of about 3.25% (a/a).

[0154] In those additional embodiments that further comprise eicosatrienoic acid (C20:3 n-3), eicosatrienoic acid is present in an amount of about 0.75 – about 3.50% (a/a). In certain embodiments, eicosatrienoic acid is present in an amount of about 1.20 – about 3.00% (a/a). in various embodiments, eicosatrienoic acid is present in an amount of about 1.60 – about 2.60% (a/a). In certain embodiments, eicosatrienoic acid is present in an amount of about 2.10% (a/a).

[0155] In those additional embodiments that further comprise eicosatetraenoic acid (C20:4 n-3), eicosatetraenoic acid is present in an amount of about 0.01 – about 0.40% (a/a). In certain embodiments, eicosatetraenoic acid is present in an amount of about 0.01 – about 0.30% (a/a). In some embodiments, eicosatetraenoic acid is present in an amount of about 0.03 – about 0.22% (a/a). In some embodiments, eicosatetraenoic acid is present in an amount of about 0.12% (a/a).

[0156] In those additional embodiments that further comprise heneicosapentaenoic acid (C21:5 n-3), heneicosapentaenoic acid is present in an amount of about 0.01 – 4.10% (a/a). In certain embodiments, heneicosapentaenoic acid is present in an amount of about 0.01 – about 3.20% (a/a). In particular embodiments, heneicosapentaenoic acid is present in an amount of about 0.60 – about 2.35% (a/a). In various embodiments, heneicosapentaenoic acid is present in an amount of about 1.50% (a/a).

[0157] In various additional embodiments, DPA is present in an amount of about 0.90 – about 7.60% (a/a). In a variety of embodiments, DPA is present in an amount of about 2.00 – about 6.50% (a/a). In certain embodiments, DPA is present in an amount of about 3.10 –

about 5.40% (a/a). In various embodiments, DPA is present in an amount of about 4.25% (a/a).

[0158] In various additional embodiments, DPA is present in an amount of about 2.13 – about 8.48% (a/a). In certain embodiments, DPA is present in an amount of about 3.19 – about 7.42% (a/a). In some embodiments, DPA is present in an amount of about 4.25 – about 6.37% (a/a). In various embodiments, DPA is present in an amount of about 5.31% (a/a).

[0159] In various embodiments, total omega-3 fatty acids comprise from about 80.0 – about 95% (m/m) of all fatty acids in the pharmaceutical composition.

[0160] In additional embodiments that comprise DGLA, DGLA is present in an amount, calculated as a percentage by area on GC chromatogram of all fatty acids in the composition, of about 0.01 – about 4.40% (a/a). In some embodiments, DGLA is present in an amount of about 0.01 – about 3.30% (a/a). In certain embodiments, DGLA is present in an amount of about 0.01 – about 2.20% (a/a). In some embodiments, DGLA is present in an amount of about 1.10% (a/a).

[0161] In some embodiments, DGLA is present in an amount of about 0.28 – about 0.61% (a/a). In certain embodiments, DGLA is present in an amount of about 0.33 – about 0.56% (a/a). In some embodiments, DGLA is present in an amount of about 0.44% (a/a).

[0162] In additional embodiments that comprise AA, AA is present in an amount, calculated as a percentage by area on GC chromatogram of all fatty acids in the composition, of about 0.01 – about 6.90% (a/a). In various embodiments, AA is present in an amount of about 0.01 – about 5.40% (a/a). In certain embodiments, AA is present in an amount of about 0.70 – about 3.80% (a/a). In a number of embodiments, AA is present in an amount of about 2.25% (a/a).

[0163] In various embodiments, AA is present in an amount of about 1.41 – about 4.87% (a/a). In certain embodiments, AA is present in an amount of about 1.99 – about 4.30% (a/a). In a number of embodiments, AA is present in an amount of about 2.57 – about 3.72% (a/a).

[0164] In various embodiments, the composition comprises AA in an amount of no more than about 4.5% (a/a). In some embodiments, the composition comprises AA in an amount of no more than about 3.14% (a/a).

[0165] In those of the additional embodiments that further comprise linoleic acid (C18:2 n-6), linoleic acid is present in an amount of about 0.25% - about 1.30 % (a/a). In certain embodiments, linoleic acid is present in an amount of about 0.40% – about 1.20% (a/a). In various embodiments, linoleic acid is present in an amount of about 0.60% - about 0.95% (a/a). In particular embodiments, linoleic acid is present in an amount of about 0.80% (a/a).

[0166] In those additional embodiments that further comprise gamma-linolenic acid (C18:3 n-6), gamma-linolenic acid is present in an amount of about 0.01 – about 0.65% (a/a). In various embodiments, gamma-linolenic acid is present in an amount of about 0.03 – about 0.51% (a/a). In certain embodiments, gamma-linolenic acid is present in an amount of about 0.15 – about 0.40% (a/a). In several embodiments, gamma-linolenic acid is present in an amount of about 0.27% (a/a).

[0167] In those additional embodiments that further comprise eicosadienoic acid (C20:2 n-6), eicosadienoic acid is present in an amount of about 0.01– about 0.30 (a/a). In various embodiments, eicosadienoic acid is present in an amount of about 0.04 – about 0.24% (a/a). In certain embodiments, eicosadienoic acid is present in an amount of about 0.09 – about 0.20% (a/a). In some embodiments, eicosadienoic acid is present in an amount of about 0.14% (a/a).

[0168] In those additional embodiments that further comprise docosapentaenoic acid (C22:5 n-6), docosapentaenoic acid is present in an amount of about 0.01 – 0.95% (a/a). In various embodiments, docosapentaenoic acid (C22:5 n-6)is present in an amount of about 0.01 – about 1.05% (a/a). In some embodiments, docosapentaenoic acid (C22:5 n-6)is present in an amount of about 0.05 – about 0.71% (a/a). In certain embodiments, docosapentaenoic acid (C22:5 n-6) is present in an amount of about 0.01 – about 0.48% (a/a). In particular embodiments, docosapentaenoic acid (C22:5 n-6) is present in an amount of about 0.24% (a/a).

[0169] In some embodiments, docosapentaenoic acid (C22:5 n-6) is present in an amount of about 0.01 – about 1.19% (a/a). In certain embodiments, docosapentaenoic acid (C22:5 n-6) is present in an amount of about 0.16 – about 0.98% (a/a). In particular embodiments, docosapentaenoic acid (C22:5 n-6) is present in an amount of about 0.57% (a/a).

[0170] In certain embodiments, the composition comprises no more than about 10.0% (a/a) total omega-6 fatty acids.

[0171] The composition comprises, in typical embodiments amongst these additional embodiments, no more than about 3.0% (a/a) saturated fatty acids, no more than about 5.0% (a/a) mono-unsaturated fatty acids, and no more than about 0.1 ppm ethyl carbamate. In some embodiments, the composition comprises no more than 0.1 ppm ethyl carbamate. In various embodiments, the composition comprises less than 0.1 ppm ethyl carbamate.

5.3. Unit Dosage Forms

[0172] In another aspect, the pharmaceutical or neutraceutical composition of DPA-enriched omega-3 PUFAs in free acid form described in Section 5.2 above is usefully packaged in unit dosage forms for oral administration.

[0173] In particular embodiments, the dosage form is a capsule. In certain embodiments, the dosage form is a gelatin capsule. In particular embodiments, the gelatin capsule is a hard gelatin capsule. In other embodiments, the dosage form is a soft gelatin capsule.

[0174] In various embodiments, the capsule comprises Type A gelatin. In some embodiments, the capsule comprises both Type A and Type B gelatin. Sources of collagen for the production of either type A or type B gelatin include, but are not limited to, cows, pigs and fish.

[0175] In various embodiments, the capsule is a soft gelatin capsule comprising sufficient porcine Type A gelatin such that the capsule disintegrates within a time period of not more than 30 minutes in purified water at 37°C after storage for at least 3 months at 40°C. In certain embodiments, the capsule is a soft gelatin capsule comprising sufficient porcine Type A gelatin such that the capsule disintegrates within a time period of not more than 30 minutes in purified water at 37°C after storage for 6 months at 40°C. In certain

embodiments, the capsule is a soft gelatin capsule comprising sufficient porcine Type A gelatin such that the capsule disintegrates within a time period of not more than 30 minutes in purified water at 37°C after storage for 12 months at 40°C.

[0176] In various embodiments, the capsule is a soft gelatin capsule comprising sufficient porcine Type A gelatin such that the capsule disintegrates within a time period of not more than 30 minutes in purified water at 37°C after storage for at least 3 months at 30°C. In certain embodiments, the capsule is a soft gelatin capsule comprising sufficient porcine Type A gelatin such that the capsule disintegrates within a time period of not more than 30 minutes in purified water at 37°C after storage for 6 months at 30°C. In some embodiments, the capsule is a soft gelatin capsule comprising sufficient porcine Type A gelatin such that the capsule disintegrates within a time period of not more than 30 minutes in purified water at 37°C after storage for 12 months at 30°C.

[0177] In certain embodiments, the capsule is a soft gelatin capsule comprising a mixture of porcine type A gelatin and a type B gelatin. In various such embodiments, at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 40% even at least about 50% (w/w) of the gelatin is porcine type A gelatin. In selected embodiments, at least about 55%, 60%, 65%, 70%, 75% (w/w) of the gelatin is porcine type A gelatin. In particular embodiments, at least 80%, 85%, 90%, even 95% (w/w) of the gelatin is porcine type A gelatin.

[0178] In various embodiments, the capsule is a soft gelatin capsule in which the gelatin consists essentially of porcine type A gelatin.

[0179] In some embodiments, the capsule is a reduced cross-linked gelatin capsule, such as those described in U.S. Pat. No. 7,485,323, incorporated herein by reference in its entirety.

[0180] In certain embodiments, the capsule comprises succinylated gelatin.

[0181] In a variety of embodiments, capsules are made from substances that are not animal by-products, such as agar-agar, carrageenan, pectin, konjak, guar gum, food starch, modified corn starch, potato starch, and tapioca. Non-animal sources of materials that can be used to make capsules useful in the oral unit dosage forms described herein are described in U.S.

Patent Publication No. 2011/0117180, incorporated herein by reference. In some embodiments, Vegicaps® Capsules (Catalent) are used.

[0182] In certain capsular oral unit dosage form embodiments, the capsule is uncoated.

[0183] In other capsular oral unit dosage form embodiments, the capsule is coated.

[0184] In certain coated capsule embodiments, the fatty acid composition is released in a time-dependent manner. In various embodiments, there is no substantial release of the PUFA composition for at least 30 minutes after ingestion. In certain embodiments, there is no substantial release of the PUFA composition for at least 30 minutes when release is tested *in vitro*. In certain embodiments, no more than about 20% of the PUFA composition is released within the first 30 minutes when tested *in vitro*. In selected embodiments, no more than about 25%, 30%, even no more than about 35% of the PUFA composition is released within the first 30 minutes, when tested *in vitro*. In particular embodiments, *in vitro* release properties are assessed according to the procedures described in provisional patent application no. 61/749,124, filed January 4, 2013, titled "Method of release testing for omega-3 polyunsaturated fatty acids," by Bharat Mehta, the disclosure of which is incorporated herein by reference in its entirety.

[0185] In particular embodiments, substantial quantities of the PUFA composition are released by about 60 minutes after ingestion. In certain embodiments, substantial quantities of the PUFA composition are released by about 60 minutes when tested *in vitro*. In selected embodiments, at least about 40% of the PUFA composition is released by about 60 minutes, when tested *in vitro*. In various embodiments, at least about 45%, 50%, 55%, 60%, even at least about 65% of the PUFA composition is released by about 60 minutes, when tested *in vitro*. In particular embodiments, *in vitro* release properties are assessed according to the procedures described in provisional patent application no. 61/749,124, filed January 4, 2013, titled "Method of release testing for omega-3 polyunsaturated fatty acids," by Mehta, the disclosure of which is incorporated herein by reference in its entirety.

[0186] In certain embodiments, capsules are coated as described in U.S. Pat. Nos. 5,792,795 and 5,948,818, the disclosures of which are incorporated herein by reference. In various

coated embodiments, the coating is a poly(ethylacrylate-methylacrylate) copolymer. In some embodiments, the coating is Eudragit NE 30-D (Evonik Industries AG), which has an average molecular weight of about 800,000.

[0187] In other coated capsule embodiments, the capsule is coated with an enteric coating that protects the capsule from dissolution or disintegration in the stomach but dissolves at pH values encountered in the small intestine.

[0188] In various embodiments, the oral unit dosage form contains from about 100 mg to about 2000 mg of the PUFA composition. In some embodiments, the oral dosage form contains about 250 mg of the PUFA composition. In some embodiments, the oral dosage form contains about 500 mg of the PUFA composition. In certain embodiments, the oral dosage form contains about 750 mg of the PUFA composition. In some embodiments, the oral dosage form contains about 1000 mg of the PUFA composition. In other embodiments, the oral dosage form contains about 1500 mg of the PUFA composition. In certain embodiments, the unit dosage form contains nonintegral weight amounts of PUFA composition between 100 mg and 2000 mg.

5.4. Dosage Kits

[0189] In another aspect, a plurality of unit dosage forms as above-described may usefully be packaged together in a dosage kit to increase ease of use and patient compliance.

[0190] In certain embodiments, the dosage kit is a bottle. In other embodiments, the plurality of dosage forms is packaged in blister packs, a plurality of which blister packs may optionally be packaged together in a box or other enclosure. Typically, whether in a bottle or one or more blister packs, the plurality of unit dosage forms is sufficient for 30 days, 60 days, or 90 days of dosing. Thus, in selected embodiments, the unit dosage form is a capsule containing approximately one gram of pharmaceutical composition as described above, and the dosage kit comprises 30, 60, 90, 120, 150, 180, 240, 270, or 300 such capsules.

[0191] In various embodiments, the plurality of unit dosage forms is packaged under an inert gas, such as nitrogen or a noble gas, or is packaged under vacuum.

5.5. Methods of Treatment

[0192] In another aspect, methods of treatment are provided.

5.5.1. Treatment of Severe Hypertriglyceridemia (> 500 mg/dL)

[0193] In a first series of treatment embodiments, methods of treating severe hypertriglyceridemia are provided.

[0194] The methods comprise orally administering the pharmaceutical composition described in Section 5.2 above to a patient having pre-treatment serum or plasma triglyceride levels ≥500 mg/dL, in an amount and for a duration sufficient to reduce serum or plasma triglyceride levels below pre-treatment levels. In typical embodiments, each dose of the pharmaceutical composition is administered as one or as a plurality of the unit dosage forms described in Section 5.3, above.

[0195] In various embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce serum or plasma triglyceride levels by at least about 5%, 6%, 7%, 8%, or at least about 9% below pre-treatment levels. In certain embodiments, the composition is administered in an amount and for a duration effective to reduce serum or plasma triglyceride levels by at least 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18% or 19% below pre-treatment levels. In particular embodiments, the composition is administered in an amount and for a duration effective to reduce serum or plasma triglyceride levels by at least about 20% below pre-treatment levels. In various embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce serum or plasma triglycerides by at least about 25%, 30%, 35%, 40%, 45%, even at least about 50% below pre-treatment levels.

[0196] In a series of embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce serum or plasma triglyceride levels by at least about 50 mg/dL, 60 mg/dL, 70 mg/dL, 80 mg/dL, 90 mg/dL, even at least about 100 mg/dL. In certain embodiments, the composition is administered in an amount and for a duration effective to reduce serum or plasma triglyceride levels by at least about 110 mg/dL, 120 mg/dL,130 mg/dL, 140 mg/dL, even at least about 150 mg/dL. In specific embodiments,

the pharmaceutical composition is administered in an amount and for a duration effective to reduce serum or plasma triglyceride levels by at least about 160 mg/dL, 170 mg/dL, 180 mg/dL, even at least about 190 mg/dL or 200 mg/dL.

[0197] In some embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to decrease non-HDL-c levels by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, even at least about 10% below pre-treatment levels.

[0198] In various embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase HDL-c levels by at least about 1% above pre-treatment levels. In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to increase HDL-c by at least about 2%, 3%, 4%, even at least about 5%, 6%, 7%, 8%, 9%, or 10% above pre-treatment levels.

[0199] In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce the total cholesterol:HDL-c ("TC/HDL") ratio by at least about 1% below pre-treatment levels. In some embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to reduce the TC/HDL ratio by at least about 2%, 3%, 4%, 5%, 6%, 7%, 8%, even at least about 9% or at least about 10% below pre-treatment levels.

[0200] In some embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to decrease VLDL-c levels by at least about 5%, 6%, 7%, 8%, 9%, or at least about 10% below pre-treatment levels. In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to decrease VLDL-c levels by at least about 11%, 12%, 13%, 14%, 15%, 16%, 17%, even at least about 18%, 19%, or 20% below pre-treatment levels. In particular embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to decrease VLDL-c levels by at least about 21%, 22%, 23%, 24%, even at least about 25% below pre-treatment levels.

[0201] In a variety of embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to decrease ApoCIII levels. In certain embodiments, the

pharmaceutical composition is administered in an amount and for a duration sufficient to decrease ApoCIII levels by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, even at least about 8%, 9% or 10% below pre-treatment levels.

[0202] In some embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma EPA levels by at least 100% above pre-treatment levels. In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma EPA levels by at least about 200%, 250%, 300%, even at least about 350%, 400%, 450% or at least about 500% above pre-treatment levels. In selected embodiments, the pharmaceutical composition is administered for a time and in an amount effective to increase plasma EPA levels by at least about 550%, 600%, 650%, even at least about 700% above pre-treatment levels.

[0203] In various embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DHA levels by at least about 50% above pre-treatment levels. In particular embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DHA levels by at least about 55%, 60%, 65%, 70%, even at least about 75%, 80%, 85%, or 90% above pre-treatment levels.

[0204] In a series of embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DPA levels by at least about 50% above pre-treatment levels. In some embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DPA levels by at least about 55%, 60%, 65%, 70%, 75%, even at least about 80%, 85%, 90%, 95%, or 100% above pre-treatment levels. In selected embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DPA levels by at least about 110%, 120%, even at least about 125% above pre-treatment levels.

[0205] In a series of embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce arachidonic acid (AA) concentration in plasma by at least about 5% below pre-treatment levels. In certain embodiments, the pharmaceutical

composition is administered in an amount and for a duration effective to reduce arachidonic (AA) concentration in plasma by at least about 6%, 7%, 8%, 9%, 10%, even at least about 11%, 12%, 13%, 14%, even at least about 15%, 16%, 17%, 18%, 19%, 20%, or 21%, 22%, 23%, 24% even at least about 25% below pre-treatment levels.

[0206] In certain embodiments, the pharmaceutical composition is administered in an amount, and for a duration, effect to reduce plasma arachidonic acid concentration by at least about 25 μg/mL. In some embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to reduce plasma AA levels by at least about 50 μg/mL, 55 μg/mL, 60 μg/mL, 65 μg/mL, even at least about 70 μg/mL, 75 μg/mL, 80μg/mL, 85 μg/mL, 90 μg/mL, even at least about 95 μg/mL or 100 μg/mL.

[0207] In certain embodiments, the effective amount is at least about 2g per day. In various embodiments, the effective amount is at least about 3g per day. In particular embodiments, the effective amount is at least about 4g per day. In typical embodiments, the effective amount is about 2g per day. In certain embodiments, the effective amount is about 4g per day.

[0208] In typical embodiments, the pharmaceutical composition is administered for at least 30 days. In certain embodiments, the pharmaceutical composition is administered for at least 60 days. In particular embodiments, the pharmaceutical composition is administered for at least 90 days, 120 days, 180 days, 240 days, or at least 360 days. In certain embodiments, the pharmaceutical composition is administered indefinitely.

[0209] In some embodiments, the pharmaceutical composition is administered daily. In other embodiments, the pharmaceutical composition is administered every other day.

[0210] In particular embodiments, the daily dosage of pharmaceutical composition is administered in a single daily dose. In other embodiments, the pharmaceutical composition is administered in divided doses, with the daily dose divided into two administrations, three administrations, or even four administrations, over the course of the day.

[0211] In certain embodiments, the pharmaceutical composition is administered with food. In certain embodiments, the pharmaceutical composition is administered with a low fat meal.

In other embodiments, the pharmaceutical composition is administered without food. In certain embodiments, the pharmaceutical composition is administered in the fasting state.

[0212] The methods, in certain embodiments, further comprising administering a statin. In particular embodiments, the statin is selected from the group consisting of: pravastatin, lovastatin, simvastatin, atorvastatin, fluvastatin, rosuvastatin, tenivastatin, and pitavastatin.

5.5.2. Treatment of hypertriglyceridemia (200 - 500 mg/dL)

[0213] In another series of treatment embodiments, methods of treating patients who have pre-treatment serum or plasma triglyceride levels of about 200 mg/dL to about 500 mg/dL are provided. In certain embodiments, the patients are already on statin therapy; in these patients, the pre-treatment serum or plasma triglyceride levels are those measured during statin treatment, prior to administration of the pharmaceutical compositions described in Section 5.2 above.

[0214] The method comprises orally administering an effective amount of a statin, and further administering the pharmaceutical composition described in Section 5.2 herein, orally, in an amount and for a duration sufficient to lower serum or plasma triglyceride levels below levels measured prior to treatment with the pharmaceutical composition described herein. The pharmaceutical composition described in Section 5.2 and the statin need not be administered at the same time, with the same dosage schedule, or even on the same days. It is sufficient that the two be administered in sufficient temporal proximity that the patient receives therapeutic benefit concurrently from both.

[0215] In certain embodiments, the pharmaceutical composition described in Section 5.2 is administered in an amount and for a duration sufficient to reduce serum or plasma triglyceride levels by at least about 5% below pre-treatment levels. In various embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to reduce serum or plasma triglyceride levels by at least about 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, even at least about 16%, 17%, 18%, 19%, or at least about 20% below pre-treatment levels.

[0216] In some embodiments, the pharmaceutical composition described in Section 5.2 herein is administered in an amount and for a duration sufficient to reduce non-HDL-cholesterol by at least about 1%, at least about 2%, at least about 3%, 4%, 5%, even at least about 7%, 8%, 9%, or at least about 10% below pre-treatment levels.

[0217] In a series of embodiments, the pharmaceutical composition described in Section 5.2 herein is administered in an amount and for a duration sufficient to raise HDL-c levels by at last about 1%, 2%, 3% or more above pre-treatment levels.

[0218] In some embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma EPA levels by at least 100% above pre-treatment levels. In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma EPA levels by at least about 200%, 250%, 300%, even at least about 350%, 400%, 450% or at least about 500% above pre-treatment levels. In selected embodiments, the pharmaceutical composition is administered for a time and in an amount effective to increase plasma EPA levels by at least about 550%, 600%, 650%, even at least about 700% above pre-treatment levels.

[0219] In various embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DHA levels by at least about 50% above pre-treatment levels. In particular embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DHA levels by at least about 55%, 60%, 65%, 70%, even at least about 75%, 80%, 85%, or 90% above pre-treatment levels.

[0220] In a series of embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DPA levels by at least about 50% above pre-treatment levels. In some embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DPA levels by at least about 55%, 60%, 65%, 70%, 75%, even at least about 80%, 85%, 90%, 95%, or 100% above pre-treatment levels. In selected embodiments, the pharmaceutical composition is

administered in an amount and for a duration effective to increase plasma DPA levels by at least about 110%, 120%, even at least about 125% above pre-treatment levels.

[0221] In a series of embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce arachidonic acid (AA) concentration in plasma by at least about 5% below pre-treatment levels. In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce arachidonic (AA) concentration in plasma by at least about 6%, 7%, 8%, 9%, 10%, even at least about 11%, 12%, 13%, 14%, even at least about 15%, 16%, 17%, 18%, 19%, 20%, or 21%, 22%, 23%, 24% even at least about 25% below pre-treatment levels.

[0222] In certain embodiments, the pharmaceutical composition is administered in an amount, and for a duration, effect to reduce plasma arachidonic acid concentration by at least about 25 μg/mL. In some embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to reduce plasma AA levels by at least about 50 μg/mL, 55 μg/mL, 60 μg/mL, 65 μg/mL, even at least about 70 μg/mL, 75 μg/mL, 80μg/mL, 85 μg/mL, 90 μg/mL, even at least about 95 μg/mL or 100 μg/mL.

[0223] In various embodiments, the pharmaceutical composition described in Section 5.2 herein is administered in unit dosage forms as described in Section 5.3 above.

[0224] In various embodiments, the pharmaceutical composition is administered in an amount of at least about 1g per day. In some embodiments, the pharmaceutical composition is administered in an amount of at least about 2g/day. In certain embodiments, the pharmaceutical composition is administered in an amount of at least about 3g/day. In particular embodiments, the pharmaceutical composition is administered in an amount of at least about 4g/day. In typical embodiments, the pharmaceutical composition is administered in an amount of about 2g/day. In certain embodiments, the pharmaceutical composition is administered in an amount of about 3g/day or about 4g per day.

5.5.3. Treatment To Increase Plasma EPA:AA Ratios

[0225] Methods are also provided for increasing the EPA:AA ratio, without regard to the patient's pre-treatment plasma triglyceride levels. The methods comprise administering the

pharmaceutical composition described in Section 5.2 herein to a patient having an EPA:AA ratio below about 0.25, in an amount and for duration sufficient to increase the patient's EPA:AA ratio to at least about 0.25. In some embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to increase the patient's EPA:AA ratio to at least about 0.3, at least about 0.35, at least about 0.40, at least about 0.45, at least about 0.50, even to a level of at least about 0.55, 0.60, 0.61, 0.62, 0.63, 0.64, or 0.65.

[0226] In certain embodiments, the method comprises administering the pharmaceutical composition in an amount and for a duration effective to increase plasma EPA levels by at least 100% above pre-treatment levels. In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma EPA levels by at least about 200%, 250%, 300%, even at least about 350%, 400%, 450% or at least about 500% above pre-treatment levels. In selected embodiments, the pharmaceutical composition is administered for a time and in an amount effective to increase plasma EPA levels by at least about 550%, 600%, 650%, even at least about 700% above pre-treatment levels.

[0227] In various embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DHA levels by at least about 50% above pre-treatment levels. In particular embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DHA levels by at least about 55%, 60%, 65%, 70%, even at least about 75%, 80%, 85%, or 90% above pre-treatment levels.

[0228] In a series of embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DPA levels by at least about 50% above pre-treatment levels. In some embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DPA levels by at least about 55%, 60%, 65%, 70%, 75%, even at least about 80%, 85%, 90%, 95%, or 100% above pre-treatment levels. In selected embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to increase plasma DPA levels by at least about 110%, 120%, even at least about 125% above pre-treatment levels.

[0229] In a series of embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce arachidonic acid (AA) concentration in plasma by at least about 5% below pre-treatment levels. In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration effective to reduce arachidonic (AA) concentration in plasma by at least about 6%, 7%, 8%, 9%, 10%, even at least about 11%, 12%, 13%, 14%, even at least about 15%, 16%, 17%, 18%, 19%, 20%, or 21%, 22%, 23%, 24% even at least about 25% below pre-treatment levels.

[0230] In certain embodiments, the pharmaceutical composition is administered in an amount, and for a duration, effect to reduce plasma arachidonic acid concentration by at least about 25 μg/mL. In some embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to reduce plasma AA levels by at least about 50 μg/mL, 55 μg/mL, 60 μg/mL, 65 μg/mL, even at least about 70 μg/mL, 75 μg/mL, 80μg/mL, 85 μg/mL, 90 μg/mL, even at least about 95 μg/mL or 100 μg/mL.

[0231] In various embodiments, the pharmaceutical composition described in Section 5.2 herein is administered in unit dosage forms as described in Section 5.3 above.

[0232] In various embodiments, the pharmaceutical composition is administered in an amount of at least about 1g per day. In some embodiments, the pharmaceutical composition is administered in an amount of at least about 2g/day. In certain embodiments, the pharmaceutical composition is administered in an amount of at least about 3g/day. In particular embodiments, the pharmaceutical composition is administered in an amount of at least about 4g/day. In typical embodiments, the pharmaceutical composition is administered in an amount of about 2g/day. In certain embodiments, the pharmaceutical composition is administered in an amount of about 3g/day or about 4g per day.

5.5.4. Treatment to lower serum or plasma ApoCIII levels

[0233] Methods are also provided for increasing a patient's serum or plasma ApoCIII levels, without regard to the patient's pre-treatment plasma triglyceride levels. The methods comprise administering the pharmaceutical composition described in Section 5.2 herein to a patient in need of lower ApoCIII levels, in an amount and for duration sufficient to decrease

the patient's serum or plasma ApoCIII levels. In typical embodiments, the patient is at risk for cardiovascular heart disease.

[0234] In certain embodiments, the pharmaceutical composition is administered in an amount and for a duration sufficient to decrease ApoCIII levels by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, even at least about 8%, 9% or 10% below pre-treatment levels.

5.5.5. Other Methods of Treatment

[0235] In another aspect, the pharmaceutical compositions described herein is used to treat other disorders, including one or more of nonalcoholic steatohepatitis (NASH), hyperlipoproteinemia, including type III hyperlipoproteinemia, and metabolic syndrome.

[0236] In certain embodiments, the pharmaceutical composition is used to reduce resistance to platelet aggregation inhibitors, such as Plavix, including use in the methods described in U.S. Patent Application No. 13/620,312 , the disclosure of which is incorporated herein by reference in its entirety.

5.6. Process

[0237] In another aspect, an improved process is presented for refining fish oil into pharmaceutical compositions comprising PUFAs in free acid form, and particularly for refining fish oil into the pharmaceutical compositions described in Section 5.2 herein.

5.6.1. Preparation of Intermediate Feedstock

[0238] The intermediate feedstock is prepared by transesterification of the body oil obtained from fish, for example fish from families Engraulidae, Clupeidae and Scombridae, by standard techniques well-known in the art, with process parameters adjusted so as to achieve a composition falling within the tolerances described in section 5.6.2 immediately below.

[0239] Suitable standard process steps are described, *e.g.*, in U.S. Pat. Nos. 5,656,667; 5,719,302; 5,945,318; 6,204,401; 6,518,049; 6,528,669; 7,491,522; 7,550,613; 7,678,930; 7,718,698; 7,732,488 and in U.S. Pat. Nos. 5,472,705; 5,750,572; 5,776,978; 5,869,714; 7,541,480; 7,553,870; and 7,619,002, incorporated herein by reference.

[0240] In an exemplary process, a crude triglyceride oil is extracted from fish, such as anchovy, sardine, mackerel and menhaden. The crude triglyceride oil is then alkali refined,

e.g. using sodium hydroxide, and deodorized, polished, and dried. The PUFAs are then converted to esters, such as methyl esters or ethyl esters, by transesterification. Transesterification can be performed, for example, by ethanolysis in the presence of ethanol and sodium ethoxide to produce ethyl esters. Transesterification is followed by at least one round, typically a plurality of rounds, of distillation.

[0241] In another exemplary process, triglyceride oil is alkali refined and deodorized, transesterified with ethanol, such as by ethanolysis in the presence of ethanol and sodium ethoxide, and then subject to one or more rounds of fractional distillation.

[0242] FIG. 2 presents a flow chart of an exemplary process for producing the intermediate feedstock. In this process, fish are cooked in water and the resulting mixture of liquids and solids are filtered and the liquid portion centrifuged to remove the aqueous phase. The oily fraction remaining from the preceding step is treated with alkali to neutralize any free fatty acids present, followed by water washing. Thereafter, alkali refined fish oil in the triglyceride form is deodorized and environmental pollutants reduced, *e.g.* by distillation. The dried deodorized fish oil is converted to the ethyl ester form using reaction with ethanol, catalyzed by the use of sodium ethoxide. After completion of the reaction, the excess ethanol is removed by distillation and the ethyl esters washed with a citric acid solution and then with water. In this exemplary process, the ethyl esters are distilled to achieve the required concentration of EPA ethyl ester (EPA-EE) and DHA ethyl ester (DHA-EE) for use as an intermediate feedstock. In some embodiments, multiple rounds of distillation are performed. The exact conditions used are adjusted depending on the composition of the input ethyl ester composition in order to achieve the required concentration of EPA-EE and DHA-EE for the intermediate feedstock, as detailed in section 5.6.2 immediately below.

[0243] Alternatives to these process steps are well known, and may be used as appropriate so long as the resulting intermediate feedstock composition falls within the tolerances defined in section 5.6.2 immediately below.

5.6.2. Intermediate Feedstock Composition

5.6.2.1. Typical embodiments

[0244] The intermediate feedstock composition comprises a plurality of species of omega-3 PUFAs, each present substantially in the form of an ethyl ester.

[0245] The intermediate feedstock composition comprises EPA, DHA, and DPA, each substantially in the form of an ethyl ester.

[0246] In various embodiments, the intermediate feedstock composition comprises EPA ethyl ester (EPA-EE), DHA-EE, and DPA-EE, in an amount, calculated as a percentage by area on GC chromatogram of all fatty acid ethyl esters in the composition, falling within the range of -3SD to +3 SD of the averages respectively recited in Table 9. In certain embodiments, each of EPA-EE, DHA-EE, and DPA-EE falls within -2SD to +2 SD of the respectively recited average. In certain embodiments, each of EPA-EE, DHA-EE, and DPA-EE falls with -1SD to +1SD of the respectively recited average. In certain embodiments, the intermediate feedstock composition comprises EPA-EE, DHA-EE, and DPA-EE within the range set by their respective minima and maxima area percentages among the batches described in Table 8.

[0247] In certain embodiments, the composition further comprises one or more omega-3 polyunsaturated fatty acids, each substantially in the form of the ethyl ester, selected from the group consisting of: α-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatrienoic acid (C20:3 n-3), eicosatetraenoic acid (C20:4 n-3), and heneicosapentaenoic acid (C21:5 n-3). In various embodiments, the one or more further species of omega-3-EE, if present, is present in an amount, calculated as a percentage by area on GC chromatogram of all fatty acid ethyl esters in the composition, falling within the range of -3SD to +3 SD of the averages respectively recited in Table 9. In certain embodiments, each species falls within -2SD to +2 SD of the respectively recited average. In certain embodiments, each species falls with -1SD to +1SD of the respectively recited average. In certain embodiments, the one or more further species of omega-3-EE, if present, is present in an amount, calculated as a percentage by area on GC chromatogram of all fatty acid ethyl esters in the composition,

falling within the range set by their respective minima and maxima area percentages among the batches described in Table 8.

[0248] In certain embodiments, the intermediate feedstock composition also comprises at least one species of omega-6 PUFA. In various embodiments, the composition comprises ethyl esters of one or more omega-6 polyunsaturated fatty acid selected from the group consisting of: linoleic acid (C18:2 n-6), gamma-linolenic acid (C18:3 n-6), eicosadienoic acid (C20:3 n-6), dihomo-gamma-linolenic acid ("DGLA") (C20:3 n-6), arachidonic acid (C20:4 n-6) ("AA"), and docosapentaenoic acid (C22:5 n-6). Each species of omega-6 PUFA is present substantially in ethyl ester form.

[0249] In various embodiments, the one or more species of omega-6-EE, if present, is present in an amount, calculated as a percentage by area on GC chromatogram of all fatty acid ethyl esters in the composition, falling within the range of -3SD to +3 SD of the averages respectively recited in Table 9. In certain embodiments, each species falls within -2SD to +2 SD of the respectively recited average. In certain embodiments, each species falls with -1SD to +1SD of the respectively recited average. In certain embodiments, the one or more further species of omega-3-EE, if present, is present in an amount, calculated as a percentage by area on GC chromatogram of all fatty acid ethyl esters in the composition, falling within the range set by their respective minima and maxima area percentages among the batches described in Table 8.

5.6.2.2. Additional embodiments

[0250] In various additional embodiments, the intermediate feedstock composition comprises EPA ethyl ester (EPA-EE) in an amount, calculated as a percentage by area on GC chromatogram of all fatty acid ethyl esters in the composition, of about 45.0 – about 53.0% (a/a). In certain embodiments, EPA-EE is present in an amount of about 48.40 – about 50.04% (a/a). In various embodiments, EPA-EE is present in an amount of about 48.67 – about 49.77% (a/a). In some embodiments, the intermediate feedstock composition comprises EPA-EE in an amount of about 48.95 – about 49.49% (a/a). In certain embodiments, the intermediate feedstock composition comprises EPA-EE in an amount of about 49.22% (a/a).

[0251] In various embodiments, EPA-EE is present in an amount of about 44.20% (a/a) – about 46.92% (a/a). In some embodiments, EPA-EE is present in an amount of about 45.56% (a/a).

[0252] In various embodiments, EPA-EE is present in an amount of about 425 – 460 mg/g.

[0253] In various additional embodiments, the intermediate feedstock composition comprises DHA ethyl ester (DHA-EE) in an amount, calculated as a percentage by area on GC chromatogram of all fatty acid ethyl esters in the composition, of about 13.0 – about 20.0% (a/a). In various embodiments, DHA-EE is present in an amount of about 16.43 – about 18.28% (a/a). In various embodiments, the feedstock comprises DHA-EE in an amount of about 16.74 – about 17.98% (a/a). In some embodiments, the intermediate feedstock composition comprises DHA-EE in an amount of about 17.05 % - about 17.67% (a/a). In certain embodiments, the intermediate feedstock comprises DHA-EE in about 17.4% (a/a).

[0254] In some embodiments, the intermediate feedstock comprises DHA in an amount of about 14.77% (a/a) – about 17.87% (a/a). In some embodiments, the intermediate feedstock comprises DHA in an amount of about 16.32% (a/a).

[0255] In some embodiments, the intermediate feedstock comprises DHA in an amount of 150 – 170 mg/g.

[0256] In various additional embodiments, DPA-EE is present in an amount of about 4.10 to about 6.74 % (a/a). In some embodiments, DPA-EE is present in an amount of about 4.54 to about 6.30% (a/a). In various embodiments, DPA-EE is present in an amount of about 4.98 to about 5.86% (a/a). In certain embodiments, DPA-EE is present in an amount of about 5.42% (a/a).

[0257] In various embodiments, DPA-EE is present in an amount of about 0.41 to about 0.70% (a/a). In certain embodiments, DPA-EE is present in an amount of about 0.56% (a/a).

[0258] In various embodiments, the intermediate feedstock composition further comprises the ethyl ester of arachidonic acid (C20:4 n-6). In various embodiments, AA-EE is present in an amount of about 1.72 to about 2.81% (a/a). In some embodiments, arachidonic acid-EE is

present in an amount of about 1.9 to about 2.63% (a/a). In various embodiments, arachidonic acid-EE is present in an amount of about 2.09 to about 2.45% (a/a). In some embodiments, arachidonic acid-EE is present in an amount of about 2.27% (a/a).

[0259] In certain embodiments, arachidonic acid-EE is present in an amount of no more than 3.0% (a/a). In some embodiments, arachidonic acid-EE is present in an amount of no more than 4.0% (a/a).

[0260] In those embodiments of the intermediate feedstock that further comprise α-linolenic acid ethyl ester, α-linolenic acid-EE is, in certain embodiments, present in an amount of about 0.3 – about 0.45% (a/a). In some embodiments, α-linolenic acid-EE is present in an amount of about 0.4% (a/a).

[0261] In some embodiments, α-linolenic acid-EE is present in an amount of about 0.24% (a/a) – 0.62% (a/a).

[0262] In some embodiments, α-linolenic acid-EE is present in an amount of about 0.43% (a/a).

[0263] In certain of those embodiments of the intermediate feedstock that further comprise moroctic acid (C18:4 n-3) ethyl ester, moroctic acid-EE is present in an amount, calculated as a percentage by area on GC chromatogram of all fatty acid ethyl esters in the composition, of about 0.60 – about 2.03% (a/a). In some embodiments, moroctic acid-EE is present in an amount of about 0.84 to about 1.80% (a/a). In various embodiments, moroctic acid-EE is present in an amount of about 1.10 to about 1.60% (a/a). In particular embodiments, moroctic acid-EE is present in an amount of about 1.32 % (a/a).

[0264] In various embodiments, moroctic acid-EE is present in an amount of about 0.64% to about 1.93% (a/a). In particular embodiments, moroctic acid-EE is present in an amount of about 1.28 % (a/a).

[0265] In a variety of those embodiments of the intermediate feedstock that further comprise eicosatrienoic acid (C20:3 n-3) ethyl ester, eicosatrienoic acid-EE is present in an amount of

less than about 0.1% (a/a). In some embodiments, eicosatrienoic acid-EE is present in an amount of less than about 0.4% (a/a).

[0266] In embodiments of the intermediate feedstock that further comprise eicosatetraenoic acid (C20:4 n-3) ethyl ester, eicosatetraenoic acid-EE is, in certain embodiments, present in an amount of about 1.57 to about 2.10% (a/a). In some embodiments, eicosatetraenoic acid-EE is present in an amount of about 1.66 to about 2.02% (a/a). In certain embodiments, eicosatetraenoic acid-EE is present in an amount of about 1.75 to about 1.93% (a/a). In specific embodiments, eicosatetraenoic acid-EE is present in an amount of about 1.84% (a/a).

[0267] In certain embodiments, eicosatetraenoic acid-EE is present in an amount of about 1.42 to about 2.49% (a/a). In some embodiments, eicosatetraenoic acid-EE is present in an amount of about 1.95% (a/a).

[0268] In a variety of embodiments of the intermediate feedstock that further comprise heneicosapentaenoic acid (C21:5 n-3) ethyl ester, the heneicosapentaenoic acid-EE is present in an amount of about 2.25 to about 2.36% (a/a). In various embodiments, the heneicosapentaenoic acid-EE is present in an amount of about 2.27 – about 2.34% (a/a). In some embodiments, the heneicosapentaenoic acid-EE is present in an amount of about 2.29 to about 2.32 % (a/a). In particular embodiments, the heneicosapentaenoic acid-EE is present in an amount of about 2.31% (a/a).

[0269] In some embodiments, the heneicosapentaenoic acid-EE is present in an amount of about 1.42 to about 2.76 % (a/a). In particular embodiments, the heneicosapentaenoic acid-EE is present in an amount of about 2.09% (a/a).

[0270] In a series of embodiments of the intermediate feedstock that further comprise the ethyl ester of the omega-6 PUFA, linoleic acid (C18:2 n-6), linoleic acid-EE is present in an amount of about 0.53 to about 0.56% (a/a). In some embodiments, linoleic acid-EE is present in an amount of about 0.53 to about 0.55% (a/a). In some embodiments, linoleic acid-EE is present in an amount of about 0.54 to about 0.55% (a/a). In certain embodiments, linoleic acid-EE is present in an amount of about 0.54% (a/a).

[0271] In some embodiments, linoleic acid-EE is present in an amount of about 0.38 to about 0.83% (a/a). In certain embodiments, linoleic acid-EE is present in an amount of about 0.60% (a/a).

[0272] In embodiments of the intermediate feedstock that further comprise the ethyl ester of gamma-linolenic acid (C18:3 n-6), gamma-linolenic acid-EE is present, in exemplary embodiments, in an amount less than 0.1% (a/a). In embodiments of the intermediate feedstock that further comprise the ethyl ester of gamma-linolenic acid (C18:3 n-6), gamma-linolenic acid-EE is present in an amount less than 0.4% (a/a).

[0273] In embodiments of the intermediate feedstock that further comprise eicosadienoic acid (C20:2 n-6) ethyl ester, eicosadienoic acid-EE is present in an amount of about 0.00 to about 0.63% (a/a) in various exemplary embodiments. In some embodiments, eicosadienoic acid-EE is present in an amount of about 0.00 to about 0.45% (a/a). In certain embodiments, eicosadienoic acid-EE is present in an amount of about 0.00 to about 0.27% (a/a). In particular embodiments, eicosadienoic acid-EE is present in an amount of about 0.09% (a/a).

[0274] In certain embodiments, eicosadienoic acid-EE is present in an amount of about 0.00 to about 0.54% (a/a). In particular embodiments, eicosadienoic acid-EE is present in an amount of about 0.25% (a/a).

[0275] In a plurality of embodiments of the intermediate feedstock that further comprise the ethyl ester of dihomo-gamma-linolenic acid (C20:3 n-6), DGLA-EE is present in an amount of about 0.35 to about 0.68% (a/a). In some embodiments, dihomo-gamma-linolenic acid-EE is present in an amount of about 0.41 to about 0.63% (a/a). In some embodiments, dihomo-gamma-linolenic acid-EE is present in an amount of about 0.46 to about 0.57% (a/a).

[0276] In some embodiments, dihomo-gamma-linolenic acid-EE is present in an amount of about 0.26 to about 0.55% (a/a). In some embodiments, dihomo-gamma-linolenic acid-EE is present in an amount of about 0.40% (a/a).

[0277] Certain additional embodiments of the intermediate feedstock further comprise docosapentaenoic acid (C22:5 n-6), in the form of the ethyl ester. In a variety of these embodiments, docosapentaenoic acid-EE is present in an amount of about 0.54 to about

0.80% (a/a). In some embodiments, docosapentaenoic acid-EE is present in an amount of about 0.59% to about 0.76% (a/a). In various embodiments, docosapentaenoic acid-EE is present in an amount of about 0.63% to about 0.72% (a/a). In particular embodiments, docosapentaenoic acid-EE is present in an amount of about 0.67% (a/a).

[0278] In some embodiments, docosapentaenoic acid (C22:5 n-6)-EE is present in an amount of about 1.45% to about 7.21% (a/a). In some embodiments, docosapentaenoic acid (C22:5 n-6)-EE is present in an amount of about 4.33% (a/a).

5.6.3. Urea Complexation

[0279] Intermediate transesterified feedstock having a composition as above-defined is subjected to urea inclusion complexation. In typical embodiments, the amount of urea used for complexation falls within an algorithmically-determined range.

[0280] Thus, in another aspect, an improved process is presented for refining fish oil into pharmaceutical compositions comprising PUFAs in free acid form, particularly for refining fish oil into the pharmaceutical compositions described herein. The improvement comprises subjecting an intermediate feedstock of transesterified fish oil comprising the ethyl esters of various omega-3 and omega-6 PUFA species in defined percentage ranges to a step of urea inclusion complexation, wherein the amount of urea used for complexation is within the range calculated according to (*i*) formula I(a), or (*ii*) according to formula I(b), or (*iii*) according to both formula I(a) and formula I(b) with the urea amount set to a value within the range set by, and inclusive of, the results of formulae I(a) and I(b), such as an average thereof, wherein the formulae are as follows:

$$[\text{Urea}] = F_{\text{enrichment-DHA}} * ((\text{DHA}_{\text{target}} - \text{DHA-EE}_{\text{input}})/\text{DHA-EE}_{\text{input}})) \quad \text{(Ia)}$$

$$[\text{Urea}] = F_{\text{enrichment-EPA}} * ((\text{EPA}_{\text{target}} - \text{EPA-EE}_{\text{input}})/\text{EPA-EE}_{\text{input}})) \quad \text{(Ib).}$$

[0281] The DHA and EPA target values are selected based on the desired final composition. The enrichment factors, $F_{\text{enrichment-DHA}}$ and $F_{\text{enrichment-EPA}}$, can be the same or different. In a typical embodiment, $F_{\text{enrichment-DHA}}$ and $F_{\text{enrichment-EPA}}$ are the same, with a value of about 100/0.34, or about 300.

[0282] Using the algorithmically determined amount of urea, complexation is performed according to standard techniques. *See, e.g.*, U.S. Pat. Nos. 4,377,526; 5,106,542; 5,243,046; 5,679,809; 5,945,318; 6,528,669; 6,664,405; 7,541,480; 7,709,668; and 8,003,813, the disclosures of which are incorporated herein by reference.

[0283] In an exemplary embodiment, the intermediate feedstock is mixed with a solution of urea in ethanol. The complexation is carried out at 60°C – 80°C, the mixture is then cooled, and the mixture is thereafter filtered or centrifuged to remove urea complexes. Ethanol is removed by distillation and the oil washed several times with water.

5.6.4. Post-complexation Finishing

[0284] Following removal of urea complexes, the uncomplexed PUFA esters are hydrolyzed to free fatty acids by standard techniques. The composition is further purified by distillation, either before or after hydrolysis, and further finished using one or more of the following standard techniques: treatment with active carbon, chromatographic purification, solvent removal, bleaching, *e.g.* with bleaching earth, and supercritical extraction. Antioxidants, such as BHA or α-tocopherol, are added.

6. EXAMPLES

6.1. Example 1: Urea Complexation Is Required For Reliable Production Of Omega-3 PUFA Compositions in Free Acid Form That Meet Specification Requirements

[0285] Urea inclusion complexation (clathration) is a standard step often used in the refining of fish oils to remove saturated and mono-unsaturated long chain fatty acids, thus enriching for desired long chain omega-3 polyunsaturated fatty acids in the resulting composition. Despite long usage, however (*see, e.g.*, U.S. Pat. No. 4,377,526), and studies designed to characterize the effects of various physiochemical parameters on the process (*see, e.g.*, Hayes *et al.*, "Triangular Phase Diagrams To Predict The Fractionation Of Free Fatty Acid Mixtures Via Urea Complex Formation," *Separation Sci. Technol.* 36(1):45–58 (2001) and Hayes, "Purification of Free Fatty Acids via Urea Inclusion Compounds," in Handbook of Functional Lipids (Taylor & Francis Group) (2005)), the degree to which urea complexation enriches individual species of long chain polyunsaturated fatty acids, including species of

both omega-3 PUFAs, and omega-6 PUFAs, remains unpredictable. This residual unpredictability in the urea complexation procedure, and the potential for urea complexation to generate impermissible levels of ethyl carbamate, which would obligate further processing, initially militated in favor of omitting urea complexation from the commercial scale refining process to be used for producing pharmaceutical grade compositions of omega-3 PUFAs in free acid form meeting the specifications set forth in Table 1, below.

Table 1
Initial Target Specifications

Parameter (all species to be present as free acid)	**Specification limit**
EPA	50.0-60.0% (m/m)
DHA	15.0-25.0% (m/m)
EPA + DHA	70.0-80.0% (m/m)
Total omega-3 fatty acids	80.0-90.0% (m/m)
Arachidonic Acid	nmt 4.5% (a/a)
Saturated fatty acids	nmt 3.0% (a/a)
Mono-unsaturated fatty acids	nmt 5.0% (a/a)
Omega-6 fatty acids	nmt 10.0% (a/a)
Other unsaturated fatty acids	nmt 5.0% (a/a)
Total unidentified above 0.1%	nmt 2.0% (a/a)

"**(m/m)**" -- percentage, by weight, of all fatty acids in the composition
"**(a/a)**" -- percentage by area on gas chromatogram of all fatty acids in the composition
"**nmt**" -- "no more than".

[0286] Early efforts to develop a urea-free process, however, demonstrated that such processes could not reliably produce pharmaceutical compositions on a commercial scale that met the required target compositional specification. Table 2, below, presents data on two such lots. Values that fell outside of the desired specification range are underlined.

Table 2

	Batch 1 (non-winterized)	**Batch 2 (winterized)**

Parameter (all species to be present as free acid)	Specification limit	(m/m)	(a/a)	(m/m)	(a/a)
EPA	50.0-60.0% (m/m)	**49.0**	51.5	**49.8**	53.0
DHA	15.0-25.0% (m/m)	19.6	20.3	19.6	20.9
EPA + DHA	70.0-80.0% (m/m)	**68.3**	71.8	**69.4**	73.9
Total omega-3 fatty acids	80.0-90.0% (m/m)	**78.0**	81.2	**79.3**	83.7
Arachidonic Acid	nmt 4.5% (a/a)	2.8	2.8	2.9	2.8
Saturated fatty acids	nmt 3.0% (a/a)	1.9	2.6	0.6	0.5
Mono-unsaturated fatty acids	nmt 5.0% (a/a)	**6.6**	5.1	**6.7**	5.1
Omega-6 fatty acids	nmt 10.0% (a/a)	4.2	5.3	4.3	5.2
Other unsaturated fatty acids	nmt 5.0% (a/a)	2.7	0.3	2.1	0.4
Total unidentified above 0.1%	nmt 2.0% (a/a)	**5.4**	**5.5**	**6.3**	**5.4**

[0287] Accordingly, a process using urea complexation was sought, and it was discovered that strict compositional control on the PUFA species present in the intermediate ethyl ester feedstock, coupled with use of urea amounts within ranges set algorithmically, could reliably produce pharmaceutical compositions meeting the specifications set forth in Table 1 without exceeding acceptable ethyl carbamate limits.

[0288] The compositional requirements for the intermediate ethyl ester feedstock are presented in Section 5.6.2 and Examples 2 and 4. *See* Tables 3 - 6, 8 - 9.

[0289] The optimal amount of urea required to be used was found to be determined by (*i*) formula I(a), or (*ii*) according to formula I(b), or (*iii*) according to both formula I(a) and formula I(b), with the urea amount set to a value within the range set by, and inclusive of, the results of formulae I(a) and I(b), such as the average of the two results, wherein the formulae are as follows:

$$[\text{Urea}] = F_{\text{enrichment-DHA}} * ((\text{DHA}_{\text{target}} - \text{DHA-EE}_{\text{input}})/\text{DHA-EE}_{\text{input}})) \quad \text{(Ia)}$$

$$[\text{Urea}] = F_{\text{enrichment-EPA}} * ((\text{EPA}_{\text{target}} - \text{EPA-EE}_{\text{input}})/\text{EPA-EE}_{\text{input}})) \quad \text{(Ib).}$$

The enrichment factors, $F_{enrichment\text{-}DHA}$ and $F_{enrichment\text{-}EPA}$, can be the same or different. A typical value, using the intermediate feedstock batches described in Examples 2 and 4, has been found to be about 100/0.34 (*i.e.*, about 300) for both.

6.2. Example 2: Compositional Analysis Of Four Exemplary Production Batches Produced Using Controlled Urea Complexation Confirm That Specification Requirements Were Met

[0290] Four exemplary production batches of polyunsaturated fatty acids in free acid form were prepared. Strict compositional controls were applied to the ethyl ester intermediate feedstock, using only batches in which specified species of polyunsaturated fatty acids fell within defined range limits. Urea amounts to be used for complexation at production scale were first determined empirically at lab scale, using small test batches of the ethyl ester intermediate feedstock and varying the concentration of urea, thereby varying the oil:urea:ethanol ratio. The optimal concentration suggested by the test scale determinations was confirmed to fall within the range required by the algorithm described in Example 1, and used for production scale manufacture.

[0291] The composition of the intermediate transesterified feedstock and the final pharmaceutical composition ("active pharmaceutical ingredient", or "API"), was determined by gas chromatography. Results are compiled in Tables 3 – 6, below.

Table 3			
PUFA species		**Intermediate Feedstock**	**API**
(common name)	(identity)	(transesterified ethyl esters) (% a/a)	(final free fatty acid composition) (% a/a)
linoleic acid	18:2 n-6	0.54	0.55
gamma-linolenic acid	18:3 n-6	0.00	0.15
α-linolenic acid	18:3 n-3	0.45	0.39
moroctic acid	18:4 n-3	1.52	1.70
eicosadienoic acid	20:2 n-6	0.00	0.10
dihomo-gamma-linolenic acid	20:3 n-6	0.47	0.35
arachidonic acid **(AA)**	20:4 n-6	2.11	2.43
eicosatrienoic acid	20:3 n-3	0.00	0.15
eicosatetraenoic acid	20:4 n-3	1.78	2.18
eicosapentaenoic acid **(EPA)**	20:5 n-3	49.42	57.25
heneicosapentaenoic acid	21:5 n-3	2.32	2.79
docosapentaenoic acid	22:5 n-6	0.71	0.83
docosapentaenoic acid **(DPA)**	22:5 n-3	5.80	6.23
docosahexaenoic acid **(DHA)**	22:6 n-3	17.09	19.58
Aggregate Metrics			
	Total %	99.41	98.43
	PUFAs %	82.77	96.30
	Total Omega 3	78.37	90.26

Table 3			
PUFA species		**Intermediate Feedstock**	**API**
(common name)	(identity)	(transesterified ethyl esters) (% a/a)	(final free fatty acid composition) (% a/a)
	Total Omega 6	3.83	4.41
	Remaining PUFAs	0.57	1.63
	Saturates %	4.34	0.35
	Mono-unsaturates %	12.30	1.34
	Unknowns %	0.60	0.42

Table 4			
PUFA species		**Intermediate Feedstock**	**API**
(common name)	(identity)	(transesterified ethyl esters) (% a/a)	(final free fatty acid composition) (% a/a)
linoleic acid	18:2 n-6	0.54	0.49
gamma-linolenic acid	18:3 n-6	0.00	0.14
α-linolenic acid	18:3 n-3	0.45	0.34
moroctic acid	18:4 n-3	1.52	1.67
eicosadienoic acid	20:2 n-6	0.00	0.13
dihomo-gamma-linolenic acid	20:3 n-6	0.47	0.39
arachidonic acid **(AA)**	20:4 n-6	2.11	2.45
eicosatrienoic acid	20:3 n-3	0.00	0.25
eicosatetraenoic acid	20:4 n-3	1.78	2.02
eicosapentaenoic acid **(EPA)**	20:5 n-3	49.42	57.64
heneicosapentaenoic acid	21:5 n-3	2.32	2.75
docosapentaenoic acid	22:5 n-6	0.71	0.79
docosapentaenoic acid **(DPA)**	22:5 n-3	5.80	6.22
docosahexaenoic acid **(DHA)**	22:6 n-3	17.09	19.65
Aggregate Metrics			
	Total %	99.41	98.60
	PUFAs %	82.77	96.35

Table 4			
PUFA species		**Intermediate Feedstock**	**API**
(common name)	(identity)	(transesterified ethyl esters) (% a/a)	(final free fatty acid composition) (% a/a)
	Total Omega 3	78.37	90.54
	Total Omega 6	3.83	4.38
	Remaining PUFAs	0.57	1.43
	Saturates %	4.34	0.31
	Mono-unsaturates %	12.30	1.25
	Unknown %	0.60	0.69

Table 5			
PUFA species		**Intermediate Feedstock**	**API**
(common name)	(identity)	(transesterified ethyl esters) (% a/a)	(final free fatty acid composition) (% a/a)
linoleic acid	18:2 n-6	0.54	0.59
gamma-linolenic acid	18:3 n-6	0.00	0.12
α-linolenic acid	18:3 n-3	0.38	0.38
moroctic acid	18:4 n-3	1.09	1.16
eicosadienoic acid	20:2 n-6	0.00	0.12
dihomo-gamma-linolenic acid	20:3 n-6	0.57	0.45
arachidonic acid	20:4 n-6	2.42	2.84
eicosatrienoic acid	20:3 n-3	0.00	0.22
eicosatetraenoic acid	20:4 n-3	1.97	2.11
eicosapentaenoic acid **(EPA)**	20:5 n-3	49.20	55.81
heneicosapentaenoic acid	21:5 n-3	2.30	2.72
docosapentaenoic acid	22:5 n-6	0.64	0.13
docosapentaenoic acid **(DPA)**	22:5 n-3	5.06	5.46
docosahexaenoic acid **(DHA)**	22:6 n-3	17.64	19.45
Aggregate Metrics			
	Total %	99.60	98.76

Table 5			
PUFA species		**Intermediate Feedstock**	**API**
(common name)	(identity)	(transesterified ethyl esters) (% a/a)	(final free fatty acid composition) (% a/a)
	PUFAs %	82.48	93.77
	Total Omega 3	77.64	87.31
	Total Omega 6	4.36	4.24
	Remaining PUFAs	0.48	2.22
	Saturates %	5.15	0.24
	Mono-unsaturates %	11.98	2.97
	Unknowns %	0.40	1.79

Table 6			
PUFA species		**Intermediate Feedstock**	**API**
(common name)	(identity)	(transesterified ethyl esters) (% a/a)	(final free fatty acid composition) (% a/a)
linoleic acid	18:2 n-6	0.55	0.55
gamma-linolenic acid	18:3 n-6	0.00	0.12
α-linolenic acid	18:3 n-3	0.38	0.37
moroctic acid	18:4 n-3	1.13	1.26
eicosadienoic acid	20:2 n-6	0.36	0.00
dihomo-gamma-linolenic acid	20:3 n-6	0.56	0.42
arachidonic acid **(AA)**	20:4 n-6	2.43	2.86
eicosatrienoic acid	20:3 n-3	0.00	0.16
eicosatetraenoic acid	20:4 n-3	1.82	2.09
eicosapentaenoic acid **(EPA)**	20:5 n-3	48.84	57.08
heneicosapentaenoic acid	21:5 n-3	2.28	2.78
docosapentaenoic acid	22:5 n-6	0.63	0.10
docosapentaenoic acid **(DPA)**	22:5 n-3	5.02	5.49
docosahexaenoic acid **(DHA)**	22:6 n-3	17.61	20.00
Aggregate Metrics			
	Total %	99.49	98.74

Table 6			
PUFA species		**Intermediate Feedstock**	**API**
(common name)	(identity)	(transesterified ethyl esters) (% a/a)	(final free fatty acid composition) (% a/a)
	PUFAs %	82.11	94.94
	Total Omega 3	77.09	89.22
	Total Omega 6	4.53	4.05
	Remaining PUFAs	0.49	1.67
	Saturates %	5.42	0.40
	Mono-unsaturates %	11.96	1.57
	Unknowns %	0.51	1.84

[0292] All four production batches of API met the compositional specifications set forth in Table 1, above.

6.3. Example 3: Controlled Urea Complexation Differentially Enriches Selected Omega-3 and Omega-6 Species

[0293] As expected, the urea complexation step substantially decreased the percentage of saturated fatty acids and mono-unsaturated fatty acids in the resulting composition, thereby substantially enriching for polyunsaturated fatty acids. See Tables 3 - 6, and FIG. 3A. Unexpectedly, however, performing urea complexation using urea amounts falling within the algorithmically-determined range had a differential effect on enrichment of individual species of omega-3 polyunsaturated fatty acids and omega-6 polyunsaturated fatty acids.

[0294] Table 7 provides a qualitative assessment of enrichment of various species of polyunsaturated fatty acid, comparing prevalence in the ethyl ester intermediate feedstock to that in the free acid API, averaged across the four production batches described in Tables 3 - 6. *See also* FIG. 3B.

Table 7

Polyunsaturated fatty acid species	Qualitative effect of controlled urea complexation
linoleic acid (C18:2 n-6)	neutral
gamma-linolenic acid (C18:3 n-6)	enriched
α-linolenic acid (C18:3 n-3)	reduced
moroctic acid (C18:4 n-3)	enriched
eicosadienoic acid (C20:2 n-6)	neutral
dihomo-gamma-linolenic acid (C20:3 n-6)	reduced
arachidonic acid (C20:4 n-6) **(AA)**	enriched
eicosatrienoic acid (C20:3 n-3)	enriched
eicosatetraenoic acid (C20:4 n-3)	enriched
eicosapentaenoic acid (C20:5 n-3) **(EPA)**	enriched
heneicosapentaenoic acid (C21:5 n-3)	enriched
docosapentaenoic acid (C22:5 n-6)	reduced
docosapentaenoic acid (C22:5 n-3) **(DPA)**	enriched
docosahexaenoic acid (C22:6 n-3) **(DHA)**	enriched

[0295] Although omega-3 polyunsaturated fatty acids, as a class, are substantially enriched, the effect of urea complexation on omega-6 PUFAs, as a class, is not as predictable. On average, the omega-6 species DGLA and docosapentaenoic acid are reduced in prevalence; gamma-linolenic acid and arachidonic acid are increased; and there is little or no effect on linolenic acid and eicosadienoic acid.

[0296] We noted, in particular, that the omega-3 docosapentaenoic acid species, DPA (C22:5 n-3), is enriched, whereas the corresponding omega-6 species, with identical chain length and degree of unsaturation, docosapentaenoic acid (C22:5 n-6), is reduced in prevalence. The divergent effect of urea complexation on enrichment of these two isomers – in conjunction with differences in their relative concentrations in the ethyl ester intermediate feed stock – results in a log order difference in their concentrations in the final, free acid, API. Averaging across the four batches of API presented in Tables 3 - 6, the omega-3 docosapentaenoic acid species, DPA, is present in the final API at 5.85% (a/a), whereas the omega-6 docosapentaenoic acid species is present at an average concentration of 0.46% (a/a).

[0297] At an average concentration of 5.85% (a/a), DPA is the third most prevalent species of polyunsaturated fatty acid in the API, exceeded only by EPA and DHA. At this level, the DPA concentration is also 10-fold greater than that reported for an earlier pharmaceutical composition of omega-3 polyunsaturated fatty acids in free acid form, termed Purepa, in which DPA was reported to be present at a level of 0.5%. *See* Belluzzi *et al.*, *Dig. Dis. Sci.* 39(12): 2589-2594 (1994).

6.4. Example 4: Compositional Analysis Of Ten (10) Exemplary Production Batches Of API Demonstrates Reproducibly Elevated Levels Of DPA

[0298] Further production batches were prepared according to the methods described in Example 2.

[0299] Data from ten (10) batches of API, inclusive of the four batches described in Tables 3-6 in Example 2, produced from eight (8) different batches of intermediate transesterified (ethyl ester) feedstock, are presented in the tables below. The composition of each of the intermediate feedstock batches is shown in Table 8. Table 9 presents the average

("AVG"), standard deviation ("STDEV", "SD"), and Delta ("Δ", the absolute difference between +1 SD and -1 SD, +2 SD and -2 SD, *etc.*) across the 8 batches of intermediate feedstock for each of the listed (ethyl ester) species. The composition of each of the ten batches of final API is shown in Table 10, below; Table 11 presents the average, standard deviation, and Delta values for each of the listed (free acid) species across the 10 batches of API.

Table 8
Intermediate (Ethyl Ester) Feedstock Batches

		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
	BV No.	319021	319553	319554	320613	320766	320941	320824	320862
	ONC No.	22581	24876	24906	27008	27824	27824	28069	28139
Identity	**Common name**	**area%**	**area%**	**area%**	**area%**	**area%**	**area%**	**area%**	**area%**
C18:2(n-6)	Linoleic acid	0.552	0.516	0.522	0.571	0.689	0.712	0.657	0.611
C18:3(n-6)	Gamma-linolenic acid	0.166	0.146	0.141	0.157	0.253	0.218	0.283	0.159
C18:3(n-3)	α-Linolenic acid	0.379	0.368	0.351	0.422	0.516	0.498	0.419	0.491
C18:4(n-3)	Moroctic acid	1.403	0.991	1.008	1.100	1.432	1.462	1.372	1.505
C20:2(n-6)	Eicosadienoic acid	0.156	0.181	0.194	0.167	0.423	0.366	0.274	0.212
C20:3(n-6)	Dihomo-gamma-linolenic acid	0.314	0.384	0.421	0.376	0.415	0.473	0.446	0.398
C20:4(n-6)	Arachidonic acid	1.977	2.362	2.316	2.805	2.867	2.884	3.306	2.152
C20:3(n-3)	Eicosatrienoic acid	0.171	0.200	0.216	0.181	0.270	0.223	0.220	0.245
C20:4(n-3)	Eicosatetraenoic acid	1.855	1.908	1.870	1.653	2.159	2.142	1.896	2.132
C20:5(n-3)	EPA	46.131	45.698	44.908	45.317	45.131	45.675	45.416	46.185
C21:5(n-3)	Heneicosapentaenoic acid	2.239	2.105	2.156	2.165	1.763	1.761	2.140	2.407
C22:5(n-6)	Docosapentaenoic acid	0.658	0.575	0.556	0.508	0.535	0.524	0.509	0.572
C22:5(n-3)	DPA	5.341	4.634	4.598	5.178	2.858	2.874	4.324	4.834
C22:6(n-3)	DHA	15.875	16.102	15.997	15.700	16.861	17.046	16.128	16.852

Table 9
Intermediate (Ethyl Ester) Feedstock
8 batch statistics

Identity	Common name	AVG	SD	−3SD	−2SD	−1SD	+1SD	+2SD	+3SD	1SD Δ	2SD Δ	3SD Δ
C18:2(n-6)	Linoleic acid	0.60	0.08	0.38	0.45	0.53	0.68	0.76	0.83	0.15	0.30	0.45
C18:3(n-6)	Gamma-linolenic acid	0.19	0.05	0.03	0.08	0.14	0.24	0.30	0.35	0.11	0.22	0.32
C18:3(n-3)	α-Linolenic acid	0.43	0.06	0.24	0.30	0.37	0.49	0.56	0.62	0.13	0.26	0.38
C18:4(n-3)	Moroctic acid	1.28	0.21	0.64	0.86	1.07	1.50	1.71	1.93	0.43	0.86	1.28
C20:2(n-6)	Eicosadienoic acid	0.25	0.10	-0.05	0.05	0.15	0.35	0.45	0.54	0.20	0.40	0.60
C20:3(n-6)	Dihomo-gamma-linolenic acid	0.40	0.05	0.26	0.31	0.36	0.45	0.50	0.55	0.10	0.19	0.29
C20:4(n-6)	Arachidonic acid	2.58	0.45	1.23	1.68	2.13	3.03	3.48	3.93	0.90	1.80	2.70
C20:3(n-3)	Eicosatrienoic acid	0.22	0.03	0.12	0.15	0.18	0.25	0.28	0.31	0.07	0.13	0.19
C20:4(n-3)	Eicosatetraenoic acid	1.95	0.18	1.42	1.60	1.77	2.13	2.31	2.49	0.36	0.71	1.07
C20:5(n-3)	EPA	45.56	0.45	44.20	44.65	45.10	46.01	46.46	46.92	0.91	1.81	2.72
C21:5(n-3)	Heneicosapenta-enoic acid	2.09	0.22	1.42	1.64	1.87	2.32	2.54	2.76	0.45	0.90	1.34
C22:5(n-6)	Docosapentaenoic acid	0.56	0.05	0.41	0.46	0.51	0.60	0.65	0.70	0.10	0.20	0.30
C22:5(n-3)	DPA	4.33	0.96	1.45	2.41	3.37	5.29	6.25	7.21	1.92	3.84	5.76
C22:6(n-3)	DHA	16.32	0.52	14.77	15.29	15.80	16.84	17.36	17.87	1.04	2.07	3.10

Table 10
Final (free acid) API Batches

		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9	Batch 10
	API Batch #	36355	36395	37225	37289	38151	38154	38157	38300	38303	38306
	Intermediate Batch #	1	1	2	3	4	4	5	7	8	6
Identity	**Common name**	**area %**	**area %**	**area %**	**area %**	**area %**	**area %**	**area %**	**area %**	**area %**	**area %**
C18:2(n-6)	Linoleic acid	0.55	0.49	0.59	0.55	0.60	0.61	0.78	0.62	0.53	0.72
C18:3(n-6)	Gamma-linolenic acid	0.15	0.14	0.12	0.12	0.17	0.16	0.16	0.22	0.15	0.15
C18:3(n-3)	α-Linolenic acid	0.39	0.34	0.38	0.37	0.45	0.45	0.55	0.41	0.44	0.50
C18:4(n-3)	Moroctic acid	1.70	1.67	1.16	1.26	1.37	1.37	1.87	1.65	1.77	1.81
C20:2(n-6)	Eicosadienoic acid	0.10	0.13	0.12	0.09	0.10	0.10	0.27	0.12	0.11	0.12
C20:3(n-6)	Dihomo-gamma-linolenic acid	0.35	0.39	0.45	0.42	0.42	0.45	0.52	0.51	0.42	0.51
C20:4(n-6)	Arachidonic acid	2.43	2.45	2.84	2.86	3.50	3.50	3.64	4.02	2.57	3.60
C20:3(n-3)	Eicosatrienoic acid	0.15	0.25	0.22	0.16	0.20	0.17	0.25	0.18	0.17	0.23
C20:4(n-3)	Eicosatetraenoic acid	2.18	2.02	2.11	2.09	1.96	1.90	2.64	2.13	2.34	2.54
C20:5(n-3)	EPA	57.25	57.64	55.81	57.08	56.25	56.38	56.88	56.30	56.72	57.15
C21:5(n-3)	Heneicosapentaenoic acid	2.79	2.75	2.72	2.78	2.68	2.60	2.15	2.57	2.88	2.18
C22:5(n-6)	Docosapentaenoic acid	0.20	0.17	0.72	0.71	0.61	0.62	0.66	0.63	0.71	0.66
C22:5(n-3)	DPA	6.23	6.22	5.46	5.49	6.12	5.97	3.41	5.15	5.59	3.43
C22:6(n-3)	DHA	19.58	19.65	19.45	20.00	19.16	18.79	20.60	20.10	20.97	21.01

Table 11
Final (free acid) API
10 batch statistics

Identity	Common name	AVG	SD	−3 SD	−2 SD	−1 SD	+1 SD	+2 SD	+3 SD	1SD Δ	2SD Δ	3SD Δ
C18:2(n-6)	Linoleic acid	0.61	0.09	0.34	0.43	0.52	0.69	0.78	0.87	0.18	0.35	0.53
C18:3(n-6)	Gamma-linolenic acid	0.15	0.03	0.07	0.10	0.13	0.18	0.21	0.24	0.06	0.11	0.17
C18:3(n-3)	α-Linolenic acid	0.43	0.06	0.23	0.30	0.36	0.49	0.56	0.62	0.13	0.26	0.39
C18:4(n-3)	Moroctic acid	1.56	0.25	0.81	1.06	1.31	1.81	2.06	2.31	0.50	1.00	1.50
C20:2(n-6)	Eicosadienoic acid	0.13	0.05	-0.03	0.02	0.07	0.18	0.23	0.29	0.11	0.21	0.32
C20:3(n-6)	Dihomo-gamma-linolenic acid	0.44	0.06	0.28	0.33	0.39	0.50	0.56	0.61	0.11	0.22	0.33
C20:4(n-6)	Arachidonic acid	3.14	0.58	1.41	1.99	2.57	3.72	4.29	4.87	1.15	2.30	3.46
C20:3(n-3)	Eicosatrienoic acid	0.20	0.04	0.08	0.12	0.16	0.24	0.28	0.32	0.08	0.16	0.24
C20:4(n-3)	Eicosatetraenoic acid	2.19	0.24	1.46	1.71	1.95	2.43	2.68	2.92	0.49	0.97	1.46
C20:5(n-3)	Eicosapentaenoic acid **(EPA)**	56.74	0.56	55.07	55.63	56.19	57.30	57.86	58.42	1.12	2.23	3.34
C21:5(n-3)	Heneicosapentaenoic acid	2.61	0.25	1.85	2.11	2.36	2.86	3.12	3.37	0.51	1.01	1.52
C22:5(n-6)	Docosapentaenoic acid	0.57	0.21	-0.05	0.16	0.36	0.78	0.98	1.19	0.41	0.83	1.24

Table 11
Final (free acid) API
10 batch statistics

Identity	Common name	AVG	SD	−3 SD	−2 SD	−1 SD	+1 SD	+2 SD	+3 SD	1SD Δ	2SD Δ	3SD Δ
C22:5(n-3)	Docosapentaenoic acid **(DPA)**	5.31	1.06	2.13	3.19	4.25	6.37	7.42	8.48	2.12	4.23	6.35
C22:6(n-3)	Docosahexaenoic acid **(DHA)**	19.93	0.75	17.68	18.43	19.18	20.68	21.43	22.18	1.50	2.99	4.49

[0300] As is evident from Table 11, the log order difference in relative concentration in the API of the omega-3 docosapentaenoic acid species, DPA (C22:5 n-3), and the omega-6 docosapentaenoic acid isomer (C22:5 n-6), is maintained – at 5.31% (a/a) for DPA (C22:5 n-3) *vs.* 0.57% (a/a) for docosapentaenoic acid (C22:5 n-6) – as is the 10-fold increase in concentration of DPA as compared to the earlier omega-3 free acid Purepa formulation reported in Belluzzi *et al.* (5.31 *vs.* 0.5%).

6.5. Example 5: Compositional Analysis Of 21 Exemplary Production Batches Demonstrates Reproducibly Elevated Levels Of DPA

[0301] The high absolute and relative concentration of the omega-3 docosapentaenoic acid species, DPA, has now been observed across 21 batches of API produced using urea complexation, as summarized in Tables 12 and 13, below.

Table 12
Final (free acid) API
21 batch statistics

Identity	Common name	Min % (a/a)	Max % (a/a)	AVG % (a/a)
C18:2(n-6)	Linoleic acid	0.49	1.00	0.74
C18:3(n-6)	Gamma-linolenic acid	0.12	0.52	0.24
C18:3(n-3)	a-Linolenic acid	0.34	0.83	0.54
C18:4(n-3)	Stearidonic (moroctic) acid	1.16	5.83	2.83
C20:2(n-6)	Eicosadienoic acid	0.10	0.27	0.15
C20:3(n-6)	Dihomo-gamma-linolenic acid	0.24	0.52	0.40
C20:4(n-6)	Arachidonic acid (**AA**)	2.32	4.02	3.17
C20:3(n-3)	Eicosatrienoic acid	0.10	0.25	0.16
C20:4(n-3)	Eicosatetraenoic acid	1.40	2.82	2.13
C20:5(n-3)	Eicosapentaenoic (timnodonic) acid (**EPA**)	48.61	57.64	55.40
C21:5(n-3)	Heneicosapentaenoic acid	1.81	2.88	2.33
C22:5(n-6)	Docosapentaenoic acid	0.17	0.73	0.58
C22:5(n-3)	Docosapentaenoic acid (**DPA**)	2.77	6.23	4.44
C22:6(n-3)	Cervonic acid (**DHA**)	15.99	21.78	19.35

Table 13
Final (free acid) API
21 batch statistics

Identity	Common name	AVG % (a/a)	SD	−3SD	−2SD	−1SD	+1SD	+2SD	+3SD	1SD Δ	2SD Δ	3SD Δ
C18:2(n-6)	Linoleic acid	0.74	0.16	0.26	0.42	0.58	0.90	1.07	1.23	0.32	0.65	0.97
C18:3(n-6)	Gamma-linolenic acid	0.24	0.11	-0.09	0.02	0.13	0.35	0.46	0.58	0.22	0.44	0.66
C18:3(n-3)	α-Linolenic acid	0.54	0.15	0.09	0.24	0.39	0.69	0.84	0.99	0.30	0.60	0.90
C18:4(n-3)	Stearidonic (moroctic) acid	2.83	1.49	-1.63	-0.15	1.34	4.31	5.80	7.28	2.97	5.94	8.92
C20:2(n-6)	Eicosadienoic acid	0.15	0.04	0.02	0.07	0.11	0.20	0.24	0.28	0.09	0.17	0.26
C20:3(n-6)	Dihomo-gamma-linolenic acid	0.40	0.07	0.18	0.25	0.32	0.47	0.55	0.62	0.15	0.30	0.45
C20:4(n-6)	Arachidonic acid	3.17	0.51	1.65	2.16	2.67	3.68	4.19	4.70	1.01	2.03	3.04
C20:3(n-3)	Eicosatrienoic acid	0.16	0.05	0.01	0.06	0.11	0.21	0.26	0.31	0.10	0.20	0.31
C20:4(n-3)	Eicosatetraenoic acid	2.13	0.41	0.92	1.32	1.73	2.54	2.94	3.35	0.81	1.62	2.43
C20:5(n-3)	Timnodonic acid **(EPA)**	55.40	2.13	49.00	51.13	53.27	57.53	59.66	61.80	4.26	8.53	12.79
C21:5(n-3)	Heneicosapenta-enoic acid	2.33	0.34	1.29	1.64	1.98	2.67	3.02	3.36	0.69	1.38	2.07
C22:5(n-6)	Docosapentaenoic acid	0.58	0.16	0.11	0.27	0.43	0.74	0.90	1.06	0.31	0.63	0.94
C22:5(n-3)	Docosapentaenoic acid **(DPA)**	4.44	1.16	0.98	2.13	3.29	5.60	6.75	7.91	2.31	4.62	6.93
C22:6(n-3)	Cervonic acid **(DHA)**	19.35	1.69	14.28	15.97	17.66	21.04	22.73	24.42	3.38	6.76	10.14

6.6. Example 6: DPA's Effects On Hepatic Cell Gene Expression Predict Greater Clinical Efficacy Of DPA-Enriched Compositions

[0302] DPA is the third most prevalent species of polyunsaturated fatty acid in the pharmaceutical compositions analyzed in the examples above, and is present at a concentration 10-fold that in Purepa, an earlier pharmaceutical composition of omega-3 polyunsaturated fatty acids in free acid form. Although DPA is an intermediate in the biosynthetic pathway from EPA to DHA (*see* FIG. 1), surprisingly little is known about the DPA's specific biological effects. *See* Kaur *et al.*, "Docosapentaenoic acid (22:5n-3): a review of its biological effects," *Prog. Lipid Res.* 50:28-34 (2011). To clarify the potential contribution of DPA to clinical efficacy of the pharmaceutical composition, gene expression profiling experiments were conducted.

6.6.1. Methods

[0303] Cell culture and treatment - Hep G2 hepatocarcinoma cells were cultured in serum-free Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich) with 4.5 g/l glucose, l-glutamine, $NaHCO_3$ and pyridoxine HCl supplemented with 1% (vol/vol) nonessential amino acids, 1% Na-pyruvate, 1% penicillin/streptomycin, and 10% (vol/vol) fatty acid-free bovine serum albumin (BSA), all purchased from Gibco BRL.

[0304] Cell cultures were transferred weekly by trypsinization and incubated at 37°C in a humidified incubator containing 5% CO_2. After 5 weeks of cell culture, EPA (eicosapentaenoic acid, lot #0439708-2, Cayman Chemicals), DPA (docosapentaenoic acid, lot 163481-26, Cayman Chemicals), and DHA (docosahexaenoic acid, lot 0437083-5, Cayman Chemicals), diluted immediately before use in serum free DMEM, were added to triplicate wells (250,000 cells/well) at the final effective concentrations set forth in Table 14, below.

[0305] Ratios of EPA (at 100 μM), DHA (at 40 μM), and DPA (at 11 μM) were chosen to approximate the ratios of EPA, DHA, and DPA in the pharmaceutical compositions (API) described in Section 5.2 and Example 5 (*see* Tables 12 and 13). Absolute concentrations were chosen to best approximate – within the constraint imposed by the desired compositional ratios and constraints imposed by the culture conditions – the plasma ranges observed in the 2g and 4g treatment arms of the EVOLVE trial (*see* Example 10). The lower

DPA concentration (1 μM) was chosen to approximate the systemic exposure that would be expected from use of the earlier pharmaceutical composition of omega-3 polyunsaturated fatty acids in free acid form, termed Purepa, in which DPA was reported to be present at a level 1/10 that seen in the current pharmaceutical composition.

[0306] The HepG2 cells were incubated with the identified fatty acid (EPA, DHA, DPA, or specified mixtures) for a total of 48 hours prior to cell harvest and RNA extraction.

Table 14

Sample #	PUFA species	Final concentration per well	Well	RNA quality (260/280)
GL 01	EPA	30 μM	a	2.0
GL 02	EPA	30 μM	b	2.1
GL 03	EPA	30 μM	c	2.0
GL 04	EPA	100 μM	a	1.98
GL 05	EPA	100 μM	b	2.05
GL 06	EPA	100 μM	c	2.0
GL 07	DHA	12 μM	a	2.0
GL 08	DHA	12 μM	b	2.04
GL 09	DHA	12 μM	c	2.0
GL 10	DHA	40uM	a	2.0
GL 11	DHA	40uM	b	2.0
GL 12	DHA	40uM	c	2.11
GL 13	DPA	0.3μM	a	2.07
GL 14	DPA	0.3μM	b	2.0
GL 15	DPA	0.3μM	c	2.0
GL 16	DPA	3.0 μM	a	2.09
GL 17	DPA	3.0 μM	b	2.0
GL 18	DPA	3.0 μM	c	1.99
GL 19	DPA	1 μM	a	2.2
GL 20	DPA	1 μM	b	2.03
GL 21	DPA	1 μM	c	2.03
GL 22	DPA	11 μM	a	2.0
GL 23	DPA	11 μM	b	2.08

Table 14

Sample #	PUFA species	Final concentration per well	Well	RNA quality (260/280)
GL 24	DPA	11 μM	c	2.06
GL 25	EPA:DHA:DPA 100:40:1(50:20:0.5)	total 200μM	a	2.05
GL 26	EPA:DHA:DPA 100:40:1(50:20:0.5)	total 200μM	b	2.0
GL 27	EPA:DHA:DPA 100:40:1(50:20:0.5)	total 200μM	c	2.0
GL 28	EPA:DHA:DPA 100:40:11 (50:20:5.5)	total 200μM	a	2.0
GL 29	EPA:DHA:DPA 100:40:11 (50:20:5.5)	total 200μM	b	2.06
GL 30	EPA:DHA:DPA 100:40:11 (50:20:5.5)	total 200μM	c	2.07
GL 31	EPA:DHA:DPA 30:12:0.3 (50:20:0.5)	total 60μM	a	2.07
GL 32	EPA:DHA:DPA 30:12:0.3 (50:20:0.5)	total 60μM	b	2.13
GL 33	EPA:DHA:DPA 30:12:0.3 (50:20:0.5)	total 60μM	c	2.05
GL 34	EPA:DHA:DPA 30:12:3 (50:20:5.5)	total 60μM	a	2.0
GL 35	EPA:DHA:DPA 30:12:3 (50:20:5.5)	total 60μM	b	2.12
GL 36	EPA:DHA:DPA 30:12:3 (50:20:5.5)	total 60μM	c	2.01
GL 37	BSA (fatty acid free)		a	2.03
GL 38	BSA (fatty acid free)		b	2.00
GL 39	BSA(fatty acid free)		c	2.00

[0307] Cell Harvest and RNA Isolation - Total RNA was isolated using TRIzol, according to manufacturer's instructions (Invitrogen). RNA quality was assessed with a Nanodrop 8000 Spectrophotometer (Thermo Scientific). As set forth in Table 14, above, each of the RNA

extractions for each treatment had a 260/280 ratio between 2.0 and 2.2. RNA was then further purified with Qiagen RNeasy columns. From 300 ng of total RNA per prep, the Illumina TotalPrep RNA Amplification kit (Ambion) was used to generate amplified biotinylated cRNA after reverse transcription by the Eberwine procedure. Aliquots of the treated and control RNA samples were sent to a gene expression core lab for analysis. The remainder of the total RNA samples were stored at -70°C.

[0308] Expression Assay and Data Analysis - Specific transcripts within the biotinylated cRNA were measured by fluorescent imaging after direct hybridization to Illumina HT-12 bead arrays, v.4.0. Gene expression data were analyzed using Ingenuity® iReport™ software (Ingenuity Systems, Redwood City, CA).

6.6.2. Results

6.6.2.1. Expression Profiling Demonstrates That DPA Has Biological Effects Different From EPA and DHA

[0309] Although DPA is an intermediate in the biosynthetic pathway from EPA to DHA, and although DPA is known to retroconvert to EPA *in vivo*, Kaur *et al.*, *Prog. Lipid Res.* 50:28-34 (2011), we observed markedly different effects on hepatic cell gene expression after incubating with DPA, as compared to effects seen with EPA and with DHA.

[0310] For a high-level assessment of similarities and differences in effects on gene expression, we used the Ingenuity® iReport™ software to query the gene expression data for the top 5 responses, ranked by the Ingenuity® iReport™ algorithm, seen after exposure to each of EPA (100 μM), DHA (40 μM), and DPA (11 μM), within various curated categories of genes. Results are cumulated in Table 15, below. An analogous assessment, using a different categorization, is presented in Table 16, which follows. Symbols used are: "|" – attribute is unique to the specified fatty acid species; "¶" – attribute is shared with another fatty acid species; and "◆" – attribute was commonly observed with all 3 fatty acid species.

Table 15

	EPA	DHA	DPA
Diseases and Disorders			
Cancer	◆	◆	◆

Table 15			
	EPA	**DHA**	**DPA**
Connective Tissue Disorders	|		
Dermatological Diseases and Conditions		¶	¶
Developmental Disorder		|	
Hematological Disease			|
Immunological Disease	|		
Infectious Disease	◆	◆	◆
Inflammatory Disease	|		
Renal and Urological Disease		|	
Reproductive System Disease			|
Molecular and Cellular Functions			
Cellular Compromise	|		
Cell Death and Survival	¶	¶	
Cellular Development	◆	◆	◆
Cellular Function and Maintenance	|		
Cellular Growth and Proliferation	◆	◆	◆
Cellular Movement		|	
Cell-To-Cell Signaling and Interaction		¶	¶
Gene Expression			|
RNA Post-Transcriptional Modification			|
Physiological System Development and Function			
Connective Tissue Development and Function		|	
Hematological System Development and Function	¶		¶
Hematopoiesis	|		
Immune Cell Trafficking			|
Lymphoid Tissue Structure and Development	|		
Reproductive System Development and Function	¶	¶	
Skeletal and Muscular System Development and Function		|	
Tissue Development		¶	¶
Tissue Morphology			|
Tumor Morphology	◆	◆	◆
Top Canonical Pathways			

Table 15			
	EPA	**DHA**	**DPA**
CD27 Signaling in Lymphocytes			|
IL-17A Signaling in Airway Cells		¶	¶
IL-17A Signaling in Gastric Cells		¶	¶
IL-8 Signaling	|		
IMLP Signaling of Neutrophils	|		
Role of IL-17F in Allergic Inflammatory Airway Diseases		|	
Role of IL-17A in Arthritis	◆	◆	◆
Role of IL-17A in Psoriasis		¶	¶
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	|		
TREM1 Signaling	|		

Table 16			
	EPA	**DHA**	**DPA**
Tox Lists			
Cardiac Hypertrophy	|		
Hepatic Cholestasis	◆	◆	◆
Hepatic Stellate Cell Activation		|	
Increases Transmembrane Potential of Mitochondria and Mitochondrial Membrane			|
Liver Necrosis/Cell Death	|		
Liver Proliferation	¶	¶	
Mechanism of Gene Regulation by Peroxisome Proliferators via PPARα		|	
PPARα/RXRα Activation		¶	¶
Primary Glomerulonephritis Biomarker Panel (Human)			|
Renal Necrosis/Cell Death	¶		¶
Clinical Chemistry and Hematology			
Increased Levels of Albumin			|
Increased Levels of Creatinine	◆	◆	◆
Increased Levels of Hematocrit	|		
Increased Levels of Red Blood Cells	¶	¶	

Table 16			
	EPA	**DHA**	**DPA**
Cardiotoxicity			
Cardiac Arrhythmia			|
Cardiac Damage	|		
Cardiac Dysfunction		|	
Cardiac Fibrosis	¶	¶	
Cardiac Hypertrophy	◆	◆	◆
Cardiac Inflammation	◆	◆	◆
Cardiac Necrosis/Cell Death	¶		¶
Cardiac Proliferation		|	
Congenital Heart Anomaly			|
Hepatotoxicity			
Biliary Hyperplasia			|
Liver Cholestasis	◆	◆	◆
Liver Damage	|		
Liver Enlargement		¶	¶
Liver Hepatitis		¶	¶
Liver Inflammation	◆	◆	◆
Liver Necrosis/Cell Death	|		
Liver Proliferation	¶	¶	
Nephrotoxicity			
Renal Damage	¶	¶	
Renal Hydronephrosis			|
Renal Inflammation	◆	◆	◆
Renal Necrosis/Cell Death	◆	◆	◆
Renal Nephritis	◆	◆	◆
Renal Proliferation	◆	◆	◆

[0311] The data highlight marked differences in the effects of DPA, EPA, and DHA, across multiple categories.

[0312] Differences in the effects on gene expression were also observed using a different analysis, in which the specific genes most significantly up-regulated and down-regulated by each of EPA (100 μM), DHA (40 μM) and DPA (11 μM) were identified. The data are respectively compiled in Tables 17 (up-regulated genes) and 18 (down-regulated genes) below. Symbols used are: "→" –expression affected at both DPA concentrations; "¶" expression regulated in common with another fatty acid species, identified in parentheses; "◆" gene regulated by all three fatty acid species.

Table 17
top genes up-regulated

fatty acid species	μM Conc.	genes	
DPA	11	↑ MST1 (includes EG:15235)	→¶(DHA)
		↑ MGC16121	→
		↑ AMT	→
		↑ AHSA2	→◆
		↑ SRSF1	→¶(EPA)
		↑ HNRNPA2B1	
		↑ ALDOC	
		↑ TOP3B	
		↑ STK36	
		↑ SRSF5	
DHA	40	↑ HIST2H2AA3/HIST2H2AA4	¶(EPA)
		↑ AHSA2	◆
		↑ MT1X	
		↑ SNORA62	
		↑ HIST1H3A (includes others)	
		↑ MST1 (includes EG:15235)	¶(DPA)
		↑ HIST2H2AC	
		↑ LOX	¶(EPA)
		↑ LSMD1	
		↑ MRPS34	
EPA	100	↑ HIST2H2BE (includes others)	
		↑ AHSA2◆	
		↑ SRSF1	¶(DPA)
		↑ RGS2 (includes EG:19735)	
		↑ HIST2H2AA3/HIST2H2AA4	¶(DHA)
		↑ MAT2A	

Table 17 top genes up-regulated		
fatty acid species	**μM Conc.**	**genes**
		↑ ZNF91 ↑ HIST1H3A (includes others) ¶(DHA) ↑ LOX ¶(DHA) ↑ GNAI3

Table 18 top genes down-regulated		
fatty acid species	**μM Conc.**	**Genes**
DPA	11	↓ IL8 ◆ ↓ CDKN2AIPNL ◆ ↓ CATSPER2 →◆ ↓ CCBE1 ¶(DHA) ↓ ALPP ¶(DHA) ↓ CCL20 ◆ ↓ DDX51 ¶(DHA) ↓ QRFPR ↓ ZNF14 ↓ RELB ¶(EPA)
DHA	40	↓ CATSPER2 ◆ ↓ IL8 ◆ ↓ CCL20 ◆ ↓ CDKN2AIPNL ◆ ↓ MAP2K2 ↓ DDX51 ¶(DPA) ↓ CCBE1 ¶(DPA) ↓ JOSD1 ↓ ALPP ¶(DPA) ↓ ZNF652
EPA	100	↓ IL8 ◆ ↓ CCL20 ◆ ↓ NFKBIA ↓ IER3 ↓ RELB ¶(DPA) ↓ CATSPER2 ◆ ↓ CDKN2AIPNL ◆

Table 18 top genes down-regulated		
fatty acid species	**μM Conc.**	**Genes**
		↓ G0S2 ↓ ZFP38 ↓ HERDSPUD1

Differences in the effects of DPA, EPA, and DHA were also readily be seen by comparing the genes whose expression is uniquely affected by each of the species of omega-3 PUFA.

Table 19 Top genes uniquely regulated by DPA (11 μM)			
Gene	**EPA**	**DHA**	**DPA**
1. ALDOC			↑
2. AMT			↑
3. HNRNPA2B1			↑
4. MGC16121			↑
5. PRKCD			←
6. RELA (inhibited)			←
7. SRSF5			↑
8. STK36			↑
9. TLR7 (inhibited)			←
10. TOP3B			↑
11. QRFPR			↓
12. ZNF14			↑

Table 20 Top genes uniquely regulated by **DHA** (40 μM)			
Gene	**EPA**	**DHA**	**DPA**
1. FOXO3		←	
2. LSMD1		↑	
3. MAP2K2		↓	
4. MRPS34		↑	
5. MT1X		↑	

Table 20 Top genes uniquely regulated by **DHA** (40 μM)			
Gene	**EPA**	**DHA**	**DPA**
6. NFKB1		←	
7. SNORA62		↑	
8. TNFSF11		←	
9. ZNF652		↓	

Table 21 Top genes uniquely regulated by EPA (100 μM)			
Gene	**EPA**	**DHA**	**DPA**
1. Beta-estradiol (inhibited)	←		
2. GNAI3	↑		
3. G0S2	↓		
4. HERPUD1	↓		
5. HIST2H2BE (includes others)	↑		
6. IL2 (inhibited)	←		
7. IER3	↓		
8. Lipopolysaccharide (inhibited)	←		
9. MAT2A	↑		
10. NFkB (complex) (inhibited)	←		
11. NFKBIA	↓		
12. RGS2 (includes EG:19735)	↑		

[0313] Differences in the effects of DPA, EPA, and DHA on gene expression can also be seen by comparing the genes whose expression is most significantly affected by at least two of the species of polyunsaturated fatty acid.

Table 22 Top genes commonly regulated by DHA and DPA			
Gene	**EPA**	**DHA**	**DPA**
1. AHSA2	↑	↑	↑

Table 22 Top genes commonly regulated by DHA and DPA			
Gene	**EPA**	**DHA**	**DPA**
2. ALPP		↓	↓
3. CATSPER2	↓	↓	↓
4. CCL20	↓	↓	↓
5. CDKN2AIPNL	↓	↓	↓
6. CCBE		↓	↓
7. DDX51		↓	↓
8. IL8	↓	↓	↓
9. MST1 (includes EG:15235)		↑	↑

Table 23 Top genes commonly regulated by EPA and DPA			
Gene	**EPA**	**DHA**	**DPA**
1. AHSA2	↑	↑	↑
2. CATSPER2	↓	↓	↓
3. CCL20	↓	↓	↓
4. CDKN2AIPNL	↓	↓	↓
5. IL8	↓	↓	↓
6. RELB	↓		↓
7. SRSF1	↑		↑

[0314] These analyses collectively demonstrate that there are marked differences in the effects of EPA, DHA, and DPA across multiple physiological, pharmacological, and biochemical categories. EPA, DHA, and DPA are not identical in effect; the particular species that are present in an omega-3 PUFA composition clearly matter to the physiological effects that the composition will have upon administration.

6.6.2.2. DPA Has Significant Activity At Higher, But Not Lower, Concentration

[0315] Two concentrations of DPA were assessed. As noted above, the higher DPA concentration (at 11 μM), was chosen so that ratios of EPA (at 100 μM), DHA (at 40 μM),

and DPA (at 11 μM) would approximate the ratios of EPA, DHA, and DPA in the pharmaceutical compositions (API) described in Section 5.2 and Example 5, with absolute concentrations chosen to best approximate – within the constraint imposed by the desired compositional ratio and constraints imposed by the culture conditions – the plasma ranges observed in the treatment arms of the EVOLVE trial (*see* Example 10). The lower DPA concentration (1 μM) was chosen to approximate the systemic exposure that would be expected from use of the earlier pharmaceutical composition of omega-3 polyunsaturated fatty acids in free acid form, termed Purepa, in which DPA was reported to be present at a level 1/10 that seen in the current pharmaceutical composition.

[0316] Overall, 310 genes were uniquely responsive to the higher, but not the lower, DPA level. The large number of genes that show statistically significant changes in gene expression predicts that DPA will have meaningful biological effects when the higher concentration is reached *in vivo*. By contrast, the lower DPA concentration is clearly a sub-threshold dose, at least with respect to regulation of these 310 genes, and far less a response would be expected at this lower *in vivo* plasma concentration.

[0317] When effects were assessed on genes that are broadly categorized by the iReport™ software as affecting molecular and cellular function, two subcategories uniquely appear within the top 5, ranked by the Ingenuity® iReport™ algorithm, at the higher, but not lower DPA, concentration – those involved in gene expression, and those affecting RNA post-transcriptional modification. Given the potential for pleiotropic second-order effects caused by changes in the expression of genes that encode proteins that themselves affect gene expression, and in genes encoding proteins that affect post-transcriptional modification, these results suggest that DPA is capable of modulating a large number of metabolic pathways at the higher, but not lower, concentration.

[0318] The threshold dose effect can also be seen by focusing on three categories of genes known to be relevant to the clinical effects of omega-3 polyunsaturated fatty acids: genes involved in lipid metabolism, genes involved in cardiovascular physiology, and genes involved in inflammation (assignment of genes to the identified categories performed automatically by the iReport™ software). Results are tabulated in Table 24, below.

Table 24			
	Total gene responses Low [DPA]	Total gene responses High [DPA]	Gene responses in common
Lipid metabolism	2	22	0
Cardiovascular	10	51	6
Inflammatory	18	22	4

[0319] As shown in Table 24, only 2 genes involved in lipid metabolism were responsive to the 1 μM concentration of DPA, whereas 22 lipid metabolism genes uniquely responded with statistically significant change in expression upon incubation in the presence of 11 μM DPA. Focusing on lipid metabolism, 1 μM DPA is clearly a sub-threshold dose, whereas 11 μM has significant effects.

[0320] A greater number of genes proved responsive to the 1 μM DPA dose in the cardiovascular physiology category, and we observed a five-fold (rather than 10-fold) increase in number of genes affected at 11 μM DPA. An even greater number of genes involved in inflammatory pathways were responsive to 1 μM DPA, with only a minor increase in gene number observed at 11 μM.

[0321] The 11 μM *in vitro* concentration is lower than the ~ 90 μM plasma concentration observed in the 4g/day EVOLVE patients. *See* Example 10. The results thus predict that a clinically-relevant dose of the DPA-enriched compositions described in Section 5.2 and Example 5 (*see* Table 12 and 13) will have significant metabolic effects, including effects on lipid metabolism, cardiovascular physiology, and inflammation. Few, if any, of these DPA-specific effects would be expected at the 10-fold lower DPA levels seen in the earlier Purepa preparation.

6.6.2.3. DPA, At Higher Concentration, Affects Expression Of Multiple Lipid Metabolism Genes

[0322] The 22 lipid metabolism genes that demonstrate statistically significant changes in expression at the 11 μM DPA concentration, but not 1 μM concentration, are identified in Table 25, below.

Table 25
Lipid Metabolism Genes Responsive to 11 μM DPA

Symbol	Entrez Gene Name	Entrez Gene ID for Human	Fold Change	Drug(s) known to affect expression
APOA2	apolipoprotein A-II	336	-1.229	
CD83	CD83 molecule	9308	-1.629	
DGAT1	diacylglycerol O-acyltransferase 1	8694	1.602	omacor
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	3337	-1.489	
FGFR3	fibroblast growth factor receptor 3	2261	1.336	pazopanib
GNAI3	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	2773	1.455	
IL8	interleukin 8	3576	-2.535	
IL32	interleukin 32	9235	-1.629	
IL18 (includes EG:16173)	interleukin 18 (interferon-gamma-inducing factor)	3606	-1.347	
IP6K1	inositol hexakisphosphate kinase 1	9807	-1.242	
IP6K2	inositol hexakisphosphate kinase 2	51447	1.319	
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	3815	1.239	dasatinib, sunitinib, pazopanib, tivozanib, OSI-930, telatinib, tandutinib, imatinib, sorafenib
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	4790	-1.386	
PDPN (includes EG:10630)	podoplanin	10630	-1.307	

Table 25
Lipid Metabolism Genes Responsive to 11 μM DPA

Symbol	Entrez Gene Name	Entrez Gene ID for Human	Fold Change	Drug(s) known to affect expression
PGF	placental growth factor	5228	-1.284	aflibercept
PIP4K2B	phosphatidylinositol-5-phosphate 4-kinase, type II, beta	8396	-1.578	
PLA2G16	phospholipase A2, group XVI	11145	-1.337	
PLIN5	perilipin 5	440503	-1.371	
PTGR2	prostaglandin reductase 2	145482	-1.416	
PTX3	pentraxin 3, long	5806	-1.536	
RGS2 (includes EG:19735)	regulator of G-protein signaling 2, 24kDa	5997	1.289	
STIP1	stress-induced-phosphoprotein 1	10963	-1.469	

[0323] DPA's effects on expression of several of these genes suggest that DPA, at analogous *in vivo* concentration, should lead to improvement in various clinically-relevant lipid parameters.

[0324] For example, DPA at 11 μM upregulates **ACADSB**, the short/branched chain acyl-CoA dehydrogenase. The ACADSB gene product is involved in breakdown of triglycerides; upregulation would be expected to result in lower serum triglyceride levels. **HMGCR**, which is downregulated, encodes HMG-CoA reductase, the rate-limiting enzyme for cholesterol synthesis and the target for statin inhibition. Thus, analogous to statin action, downregulation of expression of the HMGCR gene by DPA should lead to favorable decreases in the total cholesterol:HDL ratio. **SQLE**, which is also downregulated, encodes squalene epoxidase, which catalyzes the first oxygenation step in sterol biosynthesis and is thought to be one of the rate-limiting enzymes in this pathway. Downregulation of SQLE should also lead to reduced total cholesterol levels.

6.6.2.4. Summary of Expression Profiling Results

[0325] Our expression profiling experiments using a hepatic cell line demonstrate that DPA has significant biological activity at a concentration that approximates the plasma levels observed in human patients administered a 4g daily dose of an exemplary batch of the DPA-enriched pharmaceutical composition.

[0326] At this concentration, DPA affects expression of genes in multiple metabolic pathways, including genes in categories known to be relevant to the clinical effects of omega-3 polyunsaturated fatty acids: genes involved in lipid metabolism, genes involved in cardiovascular physiology, and genes involved in inflammation. Significant second-order effects are expected, given the changes we observed in the expression of genes that encode proteins that themselves affect gene expression, and in genes encoding proteins that affect post-transcriptional modification.

[0327] Specific effects on expression of several genes involved in lipid metabolism suggest that DPA, at analogous *in vivo* concentration, should lead to improvement in various clinically-relevant lipid parameters. In particular, we observed DPA-driven upregulation of

ACADSB, the short/branched chain acyl-CoA dehydrogenase, expected to result in lower serum triglyceride levels; downregulation of **HMGCR**, which, like treatment with statins, should lead to favorable decreases in the total cholesterol:HDL ratio; and downregulation in **SQLE**, which should analogously lead to reduced total cholesterol levels.

[0328] These effects are distinguishable from those observed with EPA and DHA.

[0329] Our experiments demonstrated statistically significant dose-dependent effects for DPA, with the lower concentration, chosen to mimic the 10-fold lower concentration of DPA in an earlier free acid omega-3 formulation, affecting 10-fold fewer genes than the higher DPA concentration, chosen to mimic the plasma exposure observed in a clinical trial of the DPA-enriched pharmaceutical compositions described here. At least with respect to the 300 genes uniquely regulated by the higher DPA concentration –notably including genes beneficially affecting lipid metabolism – the lower DPA concentration provides subthreshold exposure, and would be expected to provide a subtherapeutic dose *in vivo*.

6.7. Example 7: ECLIPSE clinical trial

6.7.1. DRUG AGENTS

[0330] Lovaza® – Prescription Lovaza® capsules were acquired through commercial US sources. According to the FDA-approved product label, each 1-gram capsule of Lovaza® contains at least 900 mg of the ethyl esters of omega-3 fatty acids sourced from fish oils, predominantly a combination of ethyl esters of eicosapentaenoic acid (EPA - approximately 465 mg) and docosahexaenoic acid (DHA - approximately 375 mg). Independent compositional analysis was not performed.

[0331] STUDY DRUG (Epanova®) – Type A porcine soft gelatin capsules coated with Eudragit NE 30-D (Evonik Industries AG) were prepared, each containing one gram of a PUFA composition in which the polyunsaturated fatty acids are present in the form of free fatty acids ("API"). The encapsulated API had the composition set forth in Table 26.

Table 26

PUFA species	API

(common name)	(carbon chain length: number double bonds, omega series)	(final free fatty acid composition) (% a/a)
linoleic acid	18:2 n-6	0.55
gamma-linolenic acid	18:3 n-6	0.15
α-linolenic acid	18:3 n-3	0.39
moroctic acid	18:4 n-3	1.70
eicosadienoic acid	20:2 n-6	0.10
dihomo-gamma-linolenic acid (DGLA)	20:3 n-6	0.35
arachidonic acid **(AA)**	20:4 n-6	2.43
eicosatrienoic acid	20:3 n-3	0.15
eicosatetraenoic acid	20:4 n-3	2.18
eicosapentaenoic acid **(EPA)**	20:5 n-3	57.25
heneicosapentaenoic acid	21:5 n-3	2.79
docosapentaenoic acid	22:5 n-6	0.83
docosapentaenoic acid **(DPA)**	22:5 n-3	6.23
docosahexaenoic acid **(DHA)**	22:6 n-3	19.58
	Total %	98.43
	PUFAs %	96.30
	Total Omega 3	90.26
	Total Omega 6	4.41
	Remaining PUFAs	1.63
	Saturates %	0.35
	Mono-unsaturates %	1.35
	Unknowns %	0.42

6.7.2. STUDY DESIGN

[0332] An open-label, single dose, randomized, 4-way crossover study of bioavailability was conducted with two different treatments: 4 grams of Epanova® or 4 g of Lovaza®, each administered with a low-fat and high-fat meal to 54 healthy adults. FIG. 4 provides a treatment flow diagram illustrating the design of the study: briefly, after a washout period, subjects were randomized to one of two treatment sequences:

(i) Epanova® (low fat) → Lovaza® (low-fat) → Epanova® (high-fat) → Lovaza® (high fat), or

(ii) Lovaza® (low-fat) → Epanova® (low-fat) → Lovaza® (high-fat) → Epanova® (high-fat).

[0333] Low-fat period meals (periods 1 and 2): no breakfast (fasting); no-fat lunch (0 g fat; 600 kcal) after the 4-hour blood draw; low-fat dinner (9 g fat; 900 kcal) after the 12-hour blood draw. Low-fat food items were: fat-free yogurt, fruit cup, fat-free Fig Newtons, Lean Cuisine meal. High-fat period meals (periods 3 and 4): high-fat breakfast (20 g fat; 600 kcal) immediately after the 0.5 hour blood draw; high-fat lunch (30 g fat; 900 kcal) after the 4-hour blood draw; and high-fat dinner (30 g fat; 900 kcal) after the 12-hour blood draw. High-fat food items were: breakfast sandwich & powdered mini-donuts; cheese pizza; potato chips; and cheese and ham panini.

[0334] Pre-trial screening washout requirements were: 60 days for fish oil, EPA or DHA supplements or fortified foods; 7 days for fish, flaxseed, perilla seed, hemp, spirulina, or black currant oils, statins, bile acid sequestrants, cholesterol absorption inhibitors or fibrates. The crossover washout period was at least 7 days.

[0335] The evening before the in-clinic visit, subjects consumed a low-fat dinner 12 hours before time 0 of each treatment period (9 g fat; 900 kcal). Investigational product (Epanova® or LOVAZA®) was administered in the morning after the pre-dose blood draws (time 0). Pharmacokinetic blood sampling for each 2-day treatment period at -1.0, -0.5 and 0 hours (pre-dosing) and post-dosing at 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours (+/- 5 minutes) for the 1st day and at 24 hours (+/- 15 min) for the 2nd day.

6.7.3. PHARMACOKINETIC AND STATISTICAL ANALYSES

[0336] The following pharmacokinetic parameters for EPA and DHA plasma concentrations were calculated for the baseline-adjusted change in total and individual EPA and DHA concentrations by standard noncompartmental methods: AUC_{0-t}, AUC_{0-inf}, C_{max}, and T_{max}.

[0337] The primary determinants of bioavailability: ln-transformed area under the plasma concentration versus time curve (AUC_t) and maximum measured plasma concentration

(C_{max}) over a 24-hour interval for the baseline-adjusted change in total and individual EPA and DHA concentrations.

[0338] Plasma concentrations were baseline-adjusted prior to the calculation of pharmacokinetic parameters. Figures are plotted for the baseline-adjusted change in geometric means (ln-transformed).

[0339] Analysis of variance (ANOVA) was used to evaluate the ln-transformed pharmacokinetic parameters for differences due to treatments, period, dosing sequence and subjects within sequence.

[0340] Ratios of means were calculated using the least square means for ln-transformed AUC_{0-t}, AUC_{0-inf}, and C_{max}.

[0341] The ratios of means and their 90% confidence intervals are to lie above the upper limit of 125.00% for AUC_{0-t}, AUC_{0-inf} and C_{max} in order to show Epanova® has superior relative bioavailability compared to Lovaza® with regards to diet.

6.7.4. RESULTS

[0342] Study population - The study enrolled 54 healthy adults, 41 males (75.9%) and 13 females (24.1%), aged 21 to 77. All of the treatment periods were completed by 51 subjects (94.4%), with 53 subjects (98.1%) completing the low fat portion of the study. The population was predominantly Black or African-American (66.7%) with 31.5% White and 1.8% Asian.

[0343] Bioavailability - FIG. 5 compares the bioavailability of total EPA+DHA (baseline-adjusted change) following a single dose (4g) of Lovaza® during the high-fat and low-fat periods (fasted dose conditions), confirming that the bioavailability of Lovaza® is significantly decreased with the low-fat diet. The baseline-adjusted change in total plasma EPA+DHA levels show that the AUC_t for Lovaza® in the low-fat meal period is decreased by 83.3% compared to Lovaza® in the high-fat meal period: 661.6 vs 3959.5 nmol-h/mL, respectively ($p<0.0001$) (LS mean data in Table 27, below). C_{MAX} of Lovaza® in the low-fat period decreased by 80.6% compared to the high-fat period ($p<0.0001$) and the T_{MAX}

increased 62% in the low-fat period compared to the high-fat period (10.2 vs. 6.3 hrs, respectively; p=0.0001).

Table 27

Bioavailability Parameter	Least Square Mean		Ratio of Means (%)	P-value [a]	Intra Subject C.V. % [b]	90% Confidence Interval Limits (%)	
	Low-Fat	High-Fat				Lower	Upper
Baseline-Adjusted Change							
AUC_t (nmol•hr/mL)	661.63	3959.52	16.7	<0.0001	69.1	3.47	29.95
C_{max} (nmol/mL)	86.89	448.63	19.4	<0.0001	70.7	5.50	33.23
T_{max}(hr)	10.19	6.28	162.3	0.0001	54.5	138.32	186.24
Baseline-Adjusted Change (Ln-transformed) Data (Geometric Means)							
Ln AUC_t (nmol•hr/mL)	652.06	3468.17	18.8	<0.0001	55.3	15.72	22.49
Ln C_{max} (nmol/mL)	60.61	398.07	15.2	<0.0001	69.2	12.35	18.78

N = 53

[a] p-value is for the Least Square (LS) Mean Difference between Epanova® and Lovaza® from the ANOVA model
[b] covariance %

[0344] FIG. 6 compares the bioavailability of total EPA+DHA (baseline-adjusted change) during the high-fat period following a single dose (4g) of Lovaza® versus a single dose (4g) of Epanova®, demonstrating that in the high-fat meal periods, in which the bioavailability of Lovaza® was confirmed to be greatest, the bioavailability EPA + DHA was nonetheless significantly greater when administered in free fatty acid form (Epanova®) than as the corresponding ethyl ester omega-3 composition (Lovaza®) ($p<0.0007$).

[0345] FIG. 7 compares the bioavailability of total EPA+DHA (baseline-adjusted change) following a single dose of Epanova® vs. Lovaza® during the low-fat diet period, demonstrating that the baseline-adjusted change in total plasma EPA+DHA levels show a

4.6-fold greater AUC_t for Epanova® than Lovaza® during low-fat meal periods: 3077.8 *vs.* 668.9 nmol-h/mL, respectively ($p<0.0001$) (LS mean data in Table 28, below). C_{max} of Epanova® is 3.2-fold greater than Lovaza® ($p < 0.0001$) and T_{max} is 20% shorter than LOVAZA® (8 vs 10 hrs, respectively; $p=0.0138$).

Table 28

Bioavailability Parameter	Least Square Mean		Ratio of Means (%)	P-value [a]	Intra Subject C.V. % [b]	Inter Subject C.V. % [b]	90% Confidence Interval Limits (%)	
	Epanova®	Lovaza®					Lower	Upper
Baseline-Adjusted Change								
AUC_t (nmol•hr/mL)	3077.83	668.95	460.10	<0.0001	62.9	253	402.77	517.42
C_{max} (nmol/mL)	277.58	86.35	321.46	<0.0001	71.6	48.9	27236	370.56
T_{max}(hr)	8.08	10.21	79.23	0.0138	45.8	24.6	65.60	92.86
Baseline-Adjusted Change (Ln-transformed) Data (Geometric Means)								
Ln AUC_t (nmol•hr/mL)	2651.41	658.09	402.90	<0.0001	63.9	243	329.71	492.33
Ln C_{max} (nmol/mL)	225.79	60.70	371.95	<0.0001	66.3	42.7	304.37	454.53

N = 53

[a] p-value is for the Least Square (LS) Mean Difference between Epanova® and Lovaza® from the ANOVA model
[b] covariance %

[0346] FIG. 8 compares the bioavailability of EPA (baseline-adjusted change) following a single dose of Epanova® vs. Lovaza® during the low-fat diet period, showing a 13.5-fold greater AUC_t for Epanova® than Lovaza® during low-fat meal periods: 578.2 vs. 42.7 μg·h/mL, respectively ($p<0.0001$) (LS mean data are presented in Table 29, below). C_{MAX} of Epanova® is 5.6-fold greater than Lovaza® ($p<0.0001$) and TMAX is 12% shorter than Lovaza® (8 vs. 9 hours, respective; $p=0.2605$).

Table 29

Bioavailability Parameter	Least Square Mean		Ratio of Means (%)	P-value [a]	Intra Subject C.V. %[b]	Inter Subject C.V. %[b]	90% Confidence Interval Limits (%)	
	Epanova®	Lovaza®					Lower	Upper
Baseline-Adjusted Change								
AUC_t (µg•hr/mL)	578.22	42.67	1355.1	<0.0001	80.8	18.2	1163.8	1546.4
C_{max} (µg /mL)	52.64	9.45	557.0	<0.0001	83.9	49.8	467.32	646.68
T_{max}(hr)	8.06	9.13	88.28	02605	54.7	25.8	71.02	105.54
Baseline-Adjusted Change (Ln-transformed) Data (Geometric Means)								
Ln AUC_t (µg •hr/mL)	495.66	48.65	457.09	<0.0001	93.0	23.5	713.46	1283.9
Ln C_{max} (µg /mL)	39.02	4.66	837.53	<0.0001	102.1	52.3	630.85	1111.9

N = 53
[a] p-value is for the Least Square (LS) Mean Difference between Epanova® and Lovaza® from the ANOVA model
[b] Covariance %

[0347] FIG. 9 compares the bioavailability of DHA (baseline-adjusted change) following a single dose of Epanova® *vs.* Lovaza® during the low-fat diet period, showing a 2.2-fold greater AUC_t for Epanova® than Lovaza® during low-fat meal periods: 383.1 vs 173.4 μg·hr/mL, respectively ($p<0.0001$) (LS mean data presented in Table 30, below). C_{max} of Epanova® is 1.9-fold greater than Lovaza® ($p<0.0001$) and T_{MAX} is 21% shorter than Lovaza® (8 *vs.* 11 hours, respectively ; $p=0.0148$). The 2.2-fold greater DHA bioavailability in Epanova® vs Lovaza® occurred despite there being 42% less DHA in the Epanova® formulation.

Table 30

	Least Square Mean						90% Confidence Interval Limits (%)	
Bioavailability Parameter	Epanova®	Lovaza®	Ratio of Means (%)	P-value[a]	Intra Subject C.V. %[b]	Inter Subject C.V. %[b]	Lower	Upper
Baseline-Adjusted Change								
AUC_t (µg•hr/mL)	383.06	173.40	220.91	<0.0001	55.2	32.1	192.10	249.72
C_{max} (µg /mL)	35.50	19.19	185.02	<0.0001	66.0	48.3	154.43	215.61
T_{max}(hr)	8.45	10.72	78.84	0.0148	47.3	24.0	64.82	92.87
Baseline-Adjusted Change (Ln-transformed) Data (Geometric Means)								
Ln AUC_t (µg •hr/mL)	337.09	162.19	207.84	<0.0001	61.3	21.4	171.98	251.17
Ln C_{max} (µg /mL)	30.17	15.00	201.14	<0.0001	52.5	42.2	170.73	236.96

N = 53

[a] p-value is for the Least Square (LS) Mean Difference between Epanova® and Lovaza® from the ANOVA model

[b] covariance %

[0348] FIGS. 10A and 10B present individual subject AUC_{0-t} responses during the low-fat and high diets expressed as the ratio (%) of low-fat AUC_{0-t} to high-fat AUC_{0-t}. Negative ratios were not plotted. The data show that during the low-fat diet period, 30 of 54 (56%) subjects on Epanova® (free fatty acids) versus 3 of 52 (6%) on Lovaza® (ethyl esters) maintained an AUC_t that was $\geq$50% of the respective high-fat diet period AUC_t.

[0349] A total of 51 adverse events were reported by 29 subjects. The most common adverse events were headaches (10 subjects) and loose stools or diarrhea (9 subjects). All adverse events were mild in severity, and none were serious. There were no clinically significant changes in laboratory, vital sign or physical assessments.

6.7.5. CONCLUSIONS

[0350] The baseline-adjusted change in total EPA+DHA and individual EPA and DHA absorption profiles (AUC) with Epanova® (omega-3 PUFAs in free acid form) were significantly greater than with Lovaza® (omega-3-PUFA ethyl esters) during the high-fat diet period and dramatically better during the low-fat diet period. Furthermore, there was a very profound impact of fat content of the meals on the bioavailability of Lovaza®, whereas the bioavailability of Epanova® was much more predictable due to only a modest food effect. The superior fat-independent bioavailability of Epanova® over Lovaza® is clinically important as subjects with severely elevated triglycerides require a very low-fat diet. These findings demonstrate a significant therapeutic advantage of free fatty acid Omega-3 composition for treatment of severe hypertriglyceridemia in view of the NCEP ATP III recommendation to have these subjects adhere to a low-fat diet during adjunct therapy.

6.8. Example 8: 14 Day Comparative Bioavailability Trial

[0351] To determine whether the effects observed after a single dose were maintained after repeat dosing, a longer term study was performed. FIG. 11 is a treatment flow diagram illustrating the design of the 14 day comparative bioavailability trial, in which study drug (Lovaza® or Epanova®) was consumed with a low fat breakfast. In contrast, doses were given fasting in the low fat arm of the original ECLIPSE trial described in Example 7.

[0352] Changes from baseline to steady state in EPA and DHA levels in the Lovaza® arm of the 14 day comparative bioavailability were consistent with prior studies, as shown in Table 31, which presents the mean percentage change in EPA and DHA in the identified prior studies.

Table 31
Prior third party studies

Drug	Study	No. Subjects	No. Weeks	Δ EPA (%)	Δ DHA (%)
omega-3 ethyl ester	CK85-013	17	8	276	34
	CK85-014	54	12	300	50
	CK85-017	29	12	300	50
	CK85-019	26	12	200	29
	CK85-022	30	12	233	23
	CK85-023	28	12	139	11
	CK85-95014	30	24	260	54
	CK85-95009	22	16	173	-10*
	CK85-94010	20	6	202	77**
	CK85-95011	49	12	361**	59
	CK85-95012	6	6	156*	40

EPA range (%Δ): (156*----**209**----361**)
DHA range (%Δ): (-10*------**34**------77**)

[0353] FIG. 12A plots the mean un-adjusted total EPA+DHA concentrations versus time (linear scale), both for treatment with Lovaza® and treatment with Epanova®. FIG. 12B is a histogram showing the difference in unadjusted EPA+DHA (nmol/mL) for the time points bracketed in FIG. 12A. FIGS. 12A and 12B demonstrate that after 14-days of dosing, accumulation of EPA+DHA from Epanova® was 2.6 fold higher than Lovaza® in subjects maintained on a low-fat diet.

[0354] FIG. 13 plots mean baseline-adjusted plasma total EPA+DHA concentrations versus time (linear scale) for treatment with Lovaza® vs. treatment with Epanova® in the 14 day comparative bioavailability study, demonstrating that after 14-days of dosing with a low-fat

meal, EPA+DHA levels (AUC_{0-24}) from Epanova® were 5.8 fold higher than Lovaza® in subjects maintained on a low-fat diet.

[0355] FIG. 14A is a histogram that plots the increases from baseline to steady state in unadjusted blood levels for EPA+DHA in the Lovaza® and Epanova® arms of the 14 day comparative bioavailability study, demonstrating that blood levels of EPA+DHA increased 316% from baseline to steady-state in the Epanova® cohort compared to 66% in the Lovaza® cohort. FIG. 14B is a histogram that plots the increases from baseline to steady state in unadjusted C_{avg} for EPA+DHA in the Lovaza® and Epanova® arms of the 14 day comparative bioavailability study, demonstrating that average concentration (C_{avg}) levels of EPA+DHA increased 448% from baseline in the Epanova® cohort compared to 90% in the Lovaza® cohort.

[0356] FIG. 15A is a histogram that plots the increases from baseline to steady state for total blood levels of DHA in the Lovaza® and Epanova® arms of the 14 day comparative bioavailability study, demonstrating that levels of DHA increased 109% from baseline to steady-state in the Epanova® cohort compared to 34% in the Lovaza® cohort. FIG 15B plots the increases from baseline to steady state for DHA C_{avg} levels in the Epanova® cohort compared to Lovaza® cohort in the 14 day comparative bioavailability study, and demonstrates that average concentration (C_{avg}) levels of DHA increased 157% from baseline in the Epanova® cohort compared to 47% in the Lovaza® cohort.

[0357] FIG. 16A is a histogram that plots the increases from baseline to steady state for total EPA levels in blood in the Lovaza® and Epanova® arms of the 14 day comparative bioavailability study, and demonstrates that Levels of EPA increased 1021% from baseline to steady-state in the Epanova® cohort compared to 210% in the Lovaza® cohort. FIG. 16B plots the average concentration increases from baseline to steady-state, and demonstrates that C_{avg} levels of EPA increased 1,465% from baseline in the Epanova® cohort compared to 297% in the Lovaza® cohort.

[0358] The data demonstrate that the increase in bioavailability observed after single dosing in the ECLIPSE trial is maintained, even enhanced, over the longer term (2 weeks). In

addition, disaggregated subject-specific data (not shown) demonstrate that the subject with least response to Epanova® still had a greater day 14 EPA+DHA C_{max} than the subject with best response to Lovaza®.

[0359] The increased C_{avg} and total blood levels of clinically relevant omega-3 PUFA species achieved with Epanova® as compared to Lovaza® predicts significantly improved efficacy in lowering serum triglyceride levels and in reducing cardiovascular risk.

6.9. Example 9: 13 week rat study

[0360] This study compared omega-3 exposure and its effects on serum lipid levels in rats treated with equivalent doses of Epanova® or LOVAZA® for 13 weeks.

[0361] The Sprague Dawley rat was selected for this study because it was the rat strain used in the toxicology program conducted with Lovaza®, and thus permitted direct comparison of the data from the present study with Epanova® to publicly available rat toxicity data in the Lovaza® Summary Basis of Approval. The study design provided a robust toxicology evaluation of Epanova® with dose selections based upon the publicly available rat toxicity data for Lovaza® (maximum tolerated dose = 2000 mg/kg). The Sprague Dawley rats provide a model that is recognized to predict the effects of omega-3 PUFAs on lipid changes for triglycerides and total cholesterol in human subjects. Results at 13 weeks are shown in Table 32, below.

Table 32

Treatment	Sex	DHA exposure [a]		EPA exposure [a]		TGs (% difference from control)	Cholesterol (% difference from control)
		$AUC_{(0-t)}$ (μg·hr/mL)	C_{max} (μg/mL)	$AUC_{(0-t)}$ (μg·hr/mL)	C_{max} (μg/mL)		
Epanova®	M	15.56	1.82	14.02	2.15	-32	-45
	F	13.00	2.05	9.50	1.38	-53	-41
	Both	14.28	1.94	11.76	1.77	-43	-43
Lovaza®	M	6.55	0.47	7.57	0.73	-14	-25
	F	4.97	0.40	6.04	0.55	-38	-36
	Both	5.76	0.43	6.81	0.64	-26	-31

$AUC_{(0-t)}$ Area under plasma concentration time curve to the last sample time. Measure of systemic exposure

C_{max} Maximum plasma concentration

[a] Dose normalized values based on estimated doses of DHA and EPA

[0362] As shown in Table 32, Epanova® provided not only markedly higher maximum plasma concentrations (C_{max}) of DHA and EPA than Lovaza®, but also provided markedly higher $AUC_{(0-t)}$ for the two omega-3 species; $AUC_{(0-t)}$ is a measure of systemic exposure. The greater bioavailability and long term systemic exposure of these two omega-3 PUFA species with Epanova® therapy resulted in long term differences in lipid lowering efficacy, with Epanova® effecting substantially greater reductions in plasma triglycerides and in total cholesterol than was seen with LOVAZA®. The compositions described herein thus provide greater efficacy with respect to two clinically important cardiovascular parameters.

6.10. Example 10: EVOLVE Trial

6.10.1. DRUG AGENTS

[0363] STUDY DRUG (Epanova®) – Type A porcine soft gelatin capsules were prepared, each containing one gram (1g) of a PUFA composition comprising omega-3 PUFAs in free acid form ("API"). The capsules were coated with Eudragit NE 30-D (Evonik Industries AG). The API had the composition given in batch 2 of Table 10 (*see* Example 4, above).

[0364] PLACEBO – Capsules were prepared containing olive oil for use as a control.

6.10.2. STUDY DESIGN

[0365] A 12-week double-blind, olive oil-controlled, study was performed in the United States, Denmark, Hungary, India, Netherlands, Russia, and Ukraine. Subjects were selected on the basis of high triglyceride levels, in the range of 500–2,000 mg/dL. Subjects were randomly selected to receive 2, 3, or 4 grams of Epanova®, or 4 grams of olive oil as placebo. The general trial design is illustrated in FIG. 17, with FIG. 18 providing a more detailed treatment flow diagram further identifying the timing of study visits. The primary study endpoint was percent change in plasma triglyceride levels from baseline to end-of-treatment ("EOT"). The secondary endpoint was percent change in plasma non-HDL-cholesterol ("Non-HDL-C") from baseline to EOT.

6.10.3. RESULTS

[0366] FIG. 19 shows the disposition of all subjects, with "AE" abbreviating "adverse event" and "SAE" abbreviating "serious adverse event."

[0367] A total of 1,356 subjects were initially screened, and of these, 399 were selected to participate in the study. Of the 399 subjects, 99 received olive oil placebo, 100 received Epanova® 2 g/day; 101 received Epanova® 3 g/day; and 99 received Epanova® 4 g/day. Table 33 shows average triglyceride (TG) and cholesterol measurements for the subjects at randomization (prior to treatment), in comparison to desirable levels as described by the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), produced by the National Heart Lung and Blood Institute.

Table 33

Parameter	Desirable (mg/dL) [1]	Patients randomized for trial			
		4 g/day	3 g/day	2 g/day	Olive Oil
TG	<150	655	715	717	686
HDL-C	>40	29	28	27	29
LDL-C	<100	90	81	77	78
Non-HDL-C	<130	225	215	205	215
VLDL-C	<30	126	124	123	125

[1] NCEP ATP III, September 2002

[0368] Of the patients receiving olive oil, five total were withdrawn from the study due to the following reasons: withdrawn consent (1), lost to follow-up (1), and other reasons (3). Of the patients receiving Epanova® 2 g/day, seven total were withdrawn due to the following reasons: adverse effects (5), withdrawn consent (1), and other reasons (1). Of the patients receiving Epanova® 3 g/day, 14 total were withdrawn due to: adverse effects (7), noncompliance (2), withdrawn consent (1), lost to follow-up (3), and other reasons (1). Of the patients receiving Epanova® 4 g/day, 9 were withdrawn, due to: adverse effects (5), noncompliance (1), withdrawn consent (2), and other reasons (1).

[0369] Epanova® achieved the primary endpoint of triglyceride reduction and the secondary endpoint of reduction of non-HDL cholesterol (total cholesterol level minus the level of HDL-cholesterol) ("non-HDL-C") at all doses, and produced statistically significant reductions in multiple established markers of atherogenicity: Apo B, Apo CIII, RLP, and LpPLA2. In patients on concomitant statin therapy, Epanova® provided additive efficacy on key lipid parameters: TG; non-HDL-C; HLD-c; total cholesterol (TC); and TC/HDL-C.

[0370] Plasma levels of EPA, DHA, and DPA – the three species of omega-3 lc-PUFA in greatest abundance in Epanova® – were measured at baseline and at end-of treatment (EOT), as were plasma levels of the omega-6 lc-PUFA, arachidonic acid (AA). Table 34, below, separately tabulates average baseline, median baseline, average end-of-treatment (EOT), and median EOT plasma levels for EPA, DHA, DPA, and AA, as well as TG, NHDL-C, HDL-C, VLDL-C, and LDL-C.

[0371] Baseline plasma levels of EPA, DHA, DPA, and AA indicate effective randomization of subjects among the treatment arms. EPA:AA ratios at baseline were about 0.10 (*see* Table 37, below).

[0372] FIGS. 20A - 20E plot the average baseline and end-of-treatment ("EOT") plasma levels (in μg/mL) for EPA (FIG. 20A), DHA (FIG. 20B), DPA (FIG. 20C) and AA (FIG. 20D), for each of the treatment arms in the EVOLVE trial. FIG. 20E compares average baseline and EOT EPA levels for each treatment arm and the control (olive oil) arm to values earlier reported for ECLIPSE (*see* Example 7), 14-day bioavailability study(see Example 8), a statin drug-drug-interaction study (Statin DDI), and the unrelated JELIS trial conducted by others with a different omega-3 PUFA formulation ("JELIS"). Note that the Japanese subjects in the JELIS trial had higher baseline EPA levels. FIGS. 21A - 21D plot median baseline and end-of-treatment (EOT) plasma levels (in μg/mL) for EPA (FIG. 21A), DHA (FIG. 21B), DPA (FIG. 21C), and AA (FIG. 21D).

Table 34
(Baseline and EOT absolute plasma levels)

		Baseline (Average)	EOT (Average)	Baseline (Median)	EOT (Median)
EPA (µg/mL)	2g	36.6	126.8	26.7	104
	3g	41.4	174.7	30.7	141.9
	4g	38.9	199.7	25.7	170
DHA (µg/mL)	2g	106.6	159.9	93.5	148.3
	3g	113.7	183.6	97.4	156.9
	4g	104.8	188.8	91.8	169.1
DPA (µg/mL)	2g	37.6	61.77	35.23	54.59
	3g	38.71	69.36	34.71	58.56
	4g	36.84	69.73	32.53	66.03
AA (µg/mL)	2g	377.9	327.4	358.4	279.2
	3g	394.9	344	368.8	313.8
	4g	393.9	298.1	363.4	274.2
TG (mg/dL)	2g	760.1	608.7	669	554
	3g	766.9	754.5	612	560.8
	4g	730.5	557.2	631	511
Non-HDL-C (mg/dL)	2g	219.6	208.2	205.3	209.3
	3g	223.4	221.5	215.3	197
	4g	230.7	214.1	225	211
HDL-C (mg/dL)	2g	28	30.7	27.3	29
	3g	29.1	30.4	28	28
	4g	29.9	32.2	28.7	29
V-LDL-C (mg/dL)	2g	138.1	106.9	123.3	98
	3g	143	121.5	124	93.8
	4g	143.9	100.7	126	87
LDL-C (mg/dL)	2g	83.1	101.3	77.3	93.3
	3g	84.9	99.7	81	95
	4g	90.4	113.4	90.3	109.5
ApoB (mg/dL)	2g	115.6	121.1	114	120
	3g	114.5	116	112	115

Table 34
(Baseline and EOT absolute plasma levels)

		Baseline (Average)	EOT (Average)	Baseline (Median)	EOT (Median)
	4g	119.3	126.6	118	121.5
ApoCIII (mg/dL)	2g	26.5	24.3	22	21
	3g	27.8	25	27	21
	4g	27.5	22.7	27	21
RLP (mg/dL)	2g	55.5	49.7	49.7	37
	3g	62.7	54.4	54.4	34.5
	4g	58.1	43.4	43.4	33
LpPLA2 (ng/mL)	2g	270.6	236.7	266	225
	3g	271.2	241.4	244.5	223.5
	4g	266.9	223.2	249	208

[0373] Table 35, below, tabulates the average change and the median change in absolute plasma levels from baseline to EOT for EPA, DHA, DPA, and AA, as well as TG, NHDL-C, HDL-C, VLDL-C, and LDL-C.

Table 35									
	AA (μg/mL)	**EPA** (μg/mL)	**DPA** (μg/mL)	**DHA** (μg/mL)	**TG** (mg/dL)	**non-HDL-C** (mg/dL)	**HDL-C** (mg/dL)	**VLDL-C** (mg/dL)	**LDL-C** (mg/dL)
average change from baseline to EOT									
2g	-50.5	90.2	24.17	53.3	-183.6	-13.3	2.6	-31.2	18
3g	-50.9	133.3	30.65	69.9	-69	-6.1	1.7	-21.9	15.5
4g	-95.8	160.8	32.89	84	-220.2	-21.9	2.1	-42.5	21.7
median change from baseline to EOT									
2g	-79.2	77.3	19.36	54.8	-172.2	-16	1.7	-31.5	14.8
3g	-55	111.2	23.85	59.5	-150.1	-6.7	1.8	-25.3	11.9
4g	-89.2	144.3	33.5	77.3	-178	-17	1.7	-37.7	22.3

[0374] FIGS. 22A, 22B, 26A, and 26B plot the data in the table above, showing the change from baseline to EOT in absolute plasma levels (in μg/mL) of AA, DHA, EPA, and DPA for each of the treatment arms of the EVOLVE trial, with FIG. 22A plotting average change and FIG. 22B showing median change from baseline.

[0375] Table 36A, below, separately tabulates average, median, and least squares mean percentage change from baseline to EOT in plasma levels of EPA, DHA, DPA, and AA, as well as TG, NHDL-C, HDL-C, VLDL-C, and LDL-C, for each of the treatment arms of the EVOLVE trial.

[0376] Table 21B, below, separately tabulates percentage change from baseline to EOT and LS mean change in the average and median plasma levels of ApoB, ApoCIII, LpPLA2, and RLP, for each of the treatment arms of the EVOLVE trial.

Table 36A

	AA (µg/mL)	EPA (µg/mL)	DPA (µg/mL)	DHA (µg/mL)	TG (mg/dL)	n-HDL-C (mg/dL)	HDL-C (mg/dL)	VLDL-C (mg/dL)	LDL-C (mg/dL)
average percentage change from baseline to EOT									
2g	-10.5	410.8	86.08	69	-21.2	-5.4	10	-21.2	25.6
3g	-11.2	538.1	96.59	88.4	-14.1	-3.4	5.6	-18.6	20
4g	-18	778.3	131.66	106	-25.2	-8	7.2	-27.5	26.2
median percentage change from baseline to EOT									
2g	-15.6	253.9	75.3	61.2	-25.8	-7.7	7	-24.7	21.4
3g	-17.9	317	68.6	61.9	-21.7	-3.2	6.2	-21.5	15.5
4g	-25.9	404.8	74.87	65.5	-30.7	-7.7	5	-34.7	26.2
LS mean percentage change									
2g	-15.14	267.04	--	56.72	-26.47	-7.77	7.46	-27.05	19.35
3g	-15.98	331.86	--	64.07	-24.38	-6.49	3.33	-25.62	13.94
4g	-23.2	406.32	--	71.77	-31.1	-9.76	5.71	-33.23	19.36

Table 36B				
	ApoB	ApoCIII	LpPLA2	RLP
average percentage change from baseline to EOT				
2g	5.9	-8.3	-11.3	-0.9
3g	4.6	-8.5	-8.8	-6.9
4g	5.7	-9.8	-14.1	-10.3
median percentage change from baseline to EOT				
2g	6.3	-8.7	-11.3	-20.2
3g	5.6	-12.8	-9.5	-16.2
4g	5.7	-15	-14.6	-28.2
LS mean change (%)				
2g	3.84	-10.87	-14.93	-20.67
3g	2.28	-12.16	-11.06	-22.63
4g	3.78	-14.39	-17.17	-27.52

[0377] FIG. 23A plots the average change from baseline to EOT, as percentage of baseline value, for AA, DHA, EPA, and DPA in each of the treatment arms of the EVOLVE trial, and FIG. 23B plots the median percent change from baseline to EOT.

[0378] Table 37 below presents EPA/AA ratios at beginning and end-of-treatment for each of the treatment arms of the EVOLVE trial.

Table 37 EPA/AA ratios		
	baseline	**EOT**
average		
2g	0.096851	0.387294
3g	0.104837	0.507849
4g	0.098756	0.669909
median		
2g	0.074498	0.372493

Table 37 EPA/AA ratios		
	baseline	**EOT**
3g	0.083243	0.452199
4g	0.070721	0.619985

[0379] As can be seen from Tables 35 and 36A and FIGS. 20 - 23, 12 week treatment with Epanova® caused dramatic increases in plasma levels of EPA, DHA, and DPA. For example, at the 2g dose, the average percentage change from baseline to EOT in EPA plasma levels was 411%; at the 4g dose, 778%. Median percentage change in EPA plasma levels were respectively 254% and 405%. At the 2g dose, the average percentage change from baseline to EOT in DHA plasma levels was 69%; at the 4g dose, the average percentage change was 106%. Median percentage change in DHA plasma levels appear less dramatic, with a 61.2% change at 2g Epanova®, and 65.5% change at 4g.

[0380] Increases in plasma levels of EPA, DHA, and DPA were accompanied by significant reductions in plasma AA levels, with the 4g dosage regimen effecting an average reduction of 95.8 μg/mL and median reduction of 89.2 μg/mL, which correspond to an average percentage reduction of 18%, a median percentage change of 25.9%, and a LS mean change of 23.2%. It should be noted that the decrease in plasma arachidonic acid levels was observed despite exogenous administration of arachidonic acid, which was present at 2.446% (a/a) in the Epanova® batch used in this trial.

[0381] The increase in EPA plasma levels and concomitant reduction in AA plasma levels cause a significant improvement in the EPA/AA ratio, as shown in Table 37, from approximately 0.10 at baseline to approximately 0.67 (average) and 0.62 (median) at EOT at the 4g dose.

[0382] Furthermore, treatment with Epanova® resulted in substantial reductions in TG levels, as shown in FIG. 26A and FIG. 26B which plot the average and median, respectively, for the absolute change from baseline. FIG. 27 illustrates the percentage of subjects who exhibited 0-10% reduction in TG, 10-20% reduction in TG, 20-30% reduction in TG, 30-40%

reduction in TG, 40-50% reduction in TG, and greater than 50% reduction in TG, for Epanova® 2g and 4g doses.

[0383] FIG. 26A and FIG. 26B also show that non-HDL-C and VLDL-C were reduced, while HDL-C was elevated. LDL-C levels were also elevated, a measurement that is likely due to an increase in LDL particle size upon treatment (discussed further in Example 12). Average and median percentage changes are displayed in FIG. 28A and FIG. 28B, respectively.

[0384] Absolute average baseline and EOT levels are plotted in FIGS. 24A - 24I for TG (FIG. 24A), Non-HDL-C (FIG. 24B), HDL-C (FIG. 24C), V-LDL-C (FIG. 24D), LDL-C (FIG. 24E), ApoB (FIG. 24F), ApoCIII (FIG. 24G), RLP (FIG. 24H), and LpPLA2 (FIG. 24I). Absolute median baseline and EOT levels are plotted in FIGS. 25A - 25I for TG (FIG. 25A), Non-HDL-C (FIG. 25B), HDL-C (FIG. 25C), V-LDL-C (FIG. 25D), LDL-C (FIG. 25E), ApoB (FIG. 25F), ApoCIII (FIG. 25G), RLP (FIG. 25H), and LpPLA2 (FIG. 25I).

[0385] The extremely high bioavailability of the omega-3 PUFAs in Epanova® revealed differences in pharmacokinetic response among the various plasma species. FIG. 29 plots the rate of change in the median percentage change from baseline in plasma levels of EPA, DHA, DPA, AA, TG, non-HDL-C, and HDL-C (absolute value) between 2g and 4g doses of Epanova®. Table 38, below, tabulates the results:

Table 38
(rate of change in median percentage change from baseline)
(absolute value)

EPA	DHA	DPA	AA	TG	non-HDL-C	HDL-C
0.59432847	0.07026143	0.00571049	0.66025641	0.189922	0	0.285714

[0386] Given little or no increase in plasma levels of DHA and DPA upon doubling of the Epanova® dose from 2g to 4g per day, the rate of change (slope) in the median percentage change from baseline is near zero, predicting little further increase in DHA and DPA plasma levels will be seen upon further increase in dose. Similar plateauing of response is seen in triglyceride levels, HDL-C levels, and non-HDL-C levels (data not shown).

[0387] By contrast, the rate of change for EPA remains high, with a slope of 0.59; further increase in EPA plasma levels is expected to be obtained by increasing Epanova® dosage above 4g/day. Significantly, the rate of change in AA levels upon doubling the Epanova® dose from 2g to 4g per day is even higher than that for EPA; further reductions in AA plasma levels are expected as Epanova® dosage is increased above 4g/day. Epanova® thus exhibits unprecedented potency in ability to reduce AA levels.

[0388] Summary of the results of the EVOLVE trial are tabulated in Table 39, below.

Table 39

	OO (n=98)		2 gram (n=99)				3 gram (n=97)				4 gram (n=99)			
	Baseline (mg/dL)	**%A**	**Baseline (mg/dL)**	**%A**	**p-value**	**LSM difference rel. to OO**	**Baseline (mg/dL)**	**%A**	**p-value**	**LSM difference rel. to OO**	**Baseline (mg/dL)**	**%A**	**p-value**	**LSM difference**
TG	686.3	-10.4	717	-25.8	0.003	-22.1	715	-21.7	0.021	-20	655	-30.7	<0.001	-26.7
Non-HDL-C	214.5	-0.9	205.3	-7.7	0.018	-10.3	215.3	-3.2	0.042	-9	225	-7.7	0.002	-12.2
HDL-C	28.7	2.2	27.3	7	0.088	5.5	28	6.2	0.127	1.4	28.7	5	0.127	3.7
TC/HDL-C	8.8	-1.9	8.8	-9.5	0.024	-11.8	8.8	-7.2	0.137	-7.7	9	-14.1	0.004	-12.9
LDL-C	78.2	9.8	77.3	21.4	0.003	16.2	81	15.5	0.092	10.8	90.3	26.2	<0.001	16.3
VLDL-C	124.5	-11.3	123.3	-24.7	0.006	-18.4	124	-21.5	0.017	-17	126	-34.7	<0.001	-24.6
ApoB	110	2.3	114	6.3	0.344	3	112	5.6	0.834	1.4	118	5.7	0.449	2,9

[0389] The EVOLVE trial also demonstrated that Apolipoprotein CIII (ApoCIII) was significantly reduced by Epanova® treatment. ApoCIII inhibits lipoprotein lipase activity and hepatic uptake of triglyceride-rich lipoproteins. Elevated levels of ApoCIII have been found to be an independent predictor for cardiovascular heart disease (CHD) risk while genetically reduced ApoCIII is associated with protection from CHD.

[0390] Omega-3 fatty acid formulations containing DHA have been shown to increase LDL-C in patients with severe hypertriglyceridemia (Kelley et al., 2009, *J. Nutrition*, 139(3):495-501). This effect on LDL-C is postulated to be a result of increased lipoprotein particle size (Davidson et al., 2009, *J. Clin. Lipidology*, 3(5):332-340). Clinical data suggest that eicosapentaenoic acid (EPA) alone, at a dose which lowers triglycerides to a similar extent as EPA + DHA, does not raise LDL-C, but also fails to lower ApoCIII (Homma *et al.*, 1991, *Atherosclerosis*, 91(1):145-153).

[0391] FIG. 34 shows the correlation between percent change in LDL and percent change in ApoCIII for data from the EVOLVE trial. A Pearson correlation coefficient of -0.28 was obtained when these data were fit using a linear regression, demonstrating that increases in LDL correlated with decreases in ApoCIII upon treatment with Epanova®. These results are consistent with previous reports of increased LDL upon administration of DHA, an observation that may be attributed to increased lipoprotein particle size. The effects of Epanova® on lipoprotein particle size are discussed further in Example 12, below.

[0392] A subset of subjects, shown in Table 40, exhibited a greater than 800% increase in EPA with less than 5% decrease in triglyceride levels. This failure to respond can likely be attributed to a deficiency or functional defect in the Type 1 lipoprotein lipase (LPL) enzyme. LPL hydrolyzes triglycerides present in chylomicrons to free fatty acids, and impairment of LPL is known to be associated with severe hypertriglyceridemia (Fojo and Brewer, 1992, *J. of Int. Med.* 231:669-677). Subjects who exhibit a substantial increase in EPA following treatment with Epanova®, accompanied by a minor change in clinical parameters such as triglyceride levels, AA levels, *etc.*, can be classified as non-responders. Such subjects can be removed from treatment with Epanova®.

Table 40

COUNTRY of the subject	Dose	BMI	BASELINE TG (mg/dL)	EOT TG (mg/dL)	TG % change from baseline	BASELINE EPA (mcg/mL)	EOT EPA (mcg/mL)	EPA % change from baseline	Baseline HbA1c	EOT HbA1c
Hungary	2	32.4	778	831	6.8	18.4	171.4	832	7.3	8.1
Hungary	2	33.4	924	937	1.4	11.4	133.6	1067	5.9	6.2
Hungary	2	36.2	782	803	2.6	12.5	173.6	1288	6.0	5.6
Russia	2	31.2	857	1723	101.1	22.1	243.7	1001	5.6	6.2
Netherlands	2	27.1	511	589	15.2	15.4	257.4	1572	5.0	5.0
India	2	25.1	460	544	18.4	4.6	79.9	1632	6.7	6.6
US	3	34.6	1047	1077	2.8	16.7	273.7	1543	6	5.6
US	3	36	622	687	10.3	16.4	193.8	1080	6.6	7.2
US	3	31	838	870	3.8	14.3	151.2	961	5.6	6.1
US	3	40.8	888	995	12	61.8	650.6	953	8	8.7
Hungary	3	36.3	484	463	-4.3	10.1	185.3	1740	6.1	6.0
Hungary	3	29.6	647	627	-3.1	8.6	110.3	1177	6.2	6.3
Hungary	3	36.6	851	1016	19.3	10.4	94.4	811	8.2	8.6
Hungary	3	28.6	707	730	3.2	8.3	408.5	4827	5.8	6
Hungary	3	32.8	2158	2273	5.3	24.5	480.1	1863	5.6	5.1
Hungary	3	31.7	1034	992	-4.1	10.6	280.5	2538	5.3	5.5
Hungary	3	34.8	976	1110	13.7	22.4	224.8	905	7.3	7.9

Table 40

COUNTRY of the subject	Dose	BMI	BASELINE TG (mg/dL)	EOT TG (mg/dL)	TG % change from baseline	BASELINE EPA (mcg/mL)	EOT EPA (mcg/mL)	EPA % change from baseline	Baseline HbA1c	EOT HbA1c
Hungary	3	28.8	728	1210	66.1	22.4	289	1071	7.1	7.9
Ukraine	3	36.2	1664	10317	520.1	91.9	1238.8	1248	8.1	10.8
Hungary	4	30.1	714	702	-1.7	11.1	256.7	2214	5.1	5.4
Hungary	4	31.7	785	1300	65.6	11.7	466	3886	8.5	10.9
Hungary	4	27.3	513	527	2.6	16.6	198.3	1093	7.8	8
Hungary	4	31.9	508	625	23.2	3.5	198.4	5504	6	7.3
Ukraine	4	33.6	563	589	-2.5	34.9	327.9	841	7	7.3
Russia	4	29.7	664	702	5.7	38.2	795.2	1984	5.5	5.1
Russia	4	41.6	483	504	4.3	10.9	231.1	2026	6.2	6.3
India	4	32.8	839	1721	105.0	13.8	1066.6	7624	5.6	5.2

6.11. Example 11: Statin Drug-Drug Interaction Trial

6.11.1. DRUG AGENTS

[0393] STUDY DRUG (Epanova®) – Type A porcine soft gelatin capsules were prepared, each containing one gram (1g) of a PUFA composition comprising omega-3 PUFAs in free acid form ("API"). The capsules were coated with Eudragit NE 30-D (Evonik Industries AG). The API had the composition given in batch 3 of Table 9 (see Example 4, above).

[0394] STUDY DRUG (Zocor®) – 40 mg tablets of simvastatin produced by Merck Sharp & Dohme Ltd. were obtained from a commercial source.

[0395] STUDY DRUG (Aspirin®) – 81 mg enteric-coated tablets produced by Bayer HealthCare Pharmaceuticals were obtained from a commercial source.

6.11.2. STUDY DESIGN

[0396] An open-label, randomized, 2-way crossover study was designed to evaluate the effect of multiple doses of Epanova® on the multiple-dose pharmacokinetics of simvastatin in healthy normal subjects. Low dose aspirin (81 mg) was also be administered daily in both study arms.

[0397] Treatment condition "**A**" consisted of co-administration of an oral dose of 40 mg of simvastatin (1 tablet), 81 mg of aspirin (1 tablet) and 4 g (4 capsules) of Epanova®, once a day (every 24 hours) with 240 mL of water on the mornings of Days 1 to 14, for a total of 14 doses, under fasting conditions. Treatment condition "**B**" consisted of administration of an oral dose of 40 mg of simvastatin (1 tablet) and 81 mg of aspirin (1 tablet) once a day (every 24 hours) with 240 mL of water on the mornings of Days 1 to 14, for a total of 14 doses, under fasting conditions. There was a 14 day washout between treatments.

[0398] A total of 52 subjects were enrolled and randomized with respect to order of treatment. Of these, 46 participants were Hispanic.

[0399] Blood was drawn for plasma fatty acid levels (EPA, DHA, AA) at check-in (day -1) and at check-out (day 15) following the treatment arm with Epanova® (treatment "**A**"). Genotyping was performed at various previously identified SNPs, including SNPs in the

FADS1 gene (*e.g.* rs174546), including a SNP associated with conversion of DGLA to AA (SNP rs174537), the FADS2 gene, and Scd-1 gene.

6.11.3. RESULTS

[0400] Average baseline and end-of-treatment ("EOT") plasma levels (in μg/mL) for EPA levels are shown in FIG. 20E.

[0401] FIG. 56 shows arachidonic acid (AA) plasma levels for subjects grouped according to genotype at the rs174546 SNP, at (**A**) baseline (in μg/mL), and (**B**) day 15 of treatment with Epanova® (in percent change from baseline). For each genotype, the interquartile range is indicated by a box, the median is indicated by a horizontal line in the interior of the interquartile box, and the mean is represented by a diamond. Outliers are represented by open circles. The whiskers extend to the minimum and maximum non-outlier value. Score 1 identifies subjects who are homozygous at the major allele; Score 3 identifies subjects homozygous at the minor allele; and Score 2 represents heterozygotes.

[0402] Prior to treatment, the Hispanic population had a higher prevalence of TT homozygotes (41%) compared to CC homozygotes (24%) for SNP rs174546. This corresponded to significantly different baseline levels of EPA (CC=18 μg/mL; CT=11 μg/mL; TT=7 μg/mL, $p<0.0001$) and arachidonic acid (AA) (CC=266 μg/mL; CT=202 μg/mL; TT=167 μg/mL, $p<0.0001$) across genotypes.

[0403] In response to treatment with Epanova®, a substantial increase in EPA was observed, with the largest percent increase in the TT genotype (TT: 1054%, CT:573%, CC:253%).

6.12. Example 12: ESPRIT Trial

6.12.1. DRUG AGENTS

[0404] STUDY DRUG (Epanova®) – Type A porcine soft gelatin capsules were prepared, each containing one gram (1g) of a PUFA composition comprising omega-3 PUFAs in free acid form ("API"). The capsules were coated with Eudragit NE 30-D (Evonik Industries AG). The API had the composition given in batch 3 of Table 9 (see Example 4, above).

[0405] PLACEBO – Capsules were prepared containing olive oil for use as a control.

6.12.2. STUDY DESIGN

[0406] As shown in FIG. 38, a subset of subjects in the 2g treatment arm of the EVOLVE trial who were receiving concurrent statin therapy displayed greater magnitudes of percentage changes (mean LS difference), relative to control, for TG, non-HDL-C, HDL-C, LDL-C, TC, VLDL-C, and TC/HDL-C, when compared to those subjects in the 2g treatment arm who did not receive concurrent statin therapy. Subjects receiving concurrent statin therapy showed a dose-dependent response to Epanova®, as shown in comparative data for Epanova® 2g and Epanova® 4g displayed in FIG. 39.

[0407] As a follow-on to the enhanced efficacy observed for Epanova® in conjunction with statin therapy, the ESPRIT clinical trial was conducted to study patients on baseline statin therapy. As shown in FIG. 40, patients were selected for the ESPRIT study based on TG levels between 200-500 mg/dL and baseline statin therapy. Of the 660 patients who were selected for the trial, 220 were treated with olive oil placebo, 220 were treated with Epanova® 2g dose, and 220 were treated with Epanova® 4g dose. All placebo and Epanova® treatments were administered in addition to the baseline statin therapy.

[0408] Table 41, below, shows the baseline levels for TG, HDL-C, LDL-C, non-HDL-C, and VLDL-C for subjects in the ESPRIT trial, in comparison to desirable levels as described by the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), issued by the National Heart Lung and Blood Institute.

Table 41
Baseline levels

	Desirable (mg/dL)	**2 g/day**	**4 g/day**	**Olive Oil (OO)**
TG	<150	265	265	269
HDL-C	>40	38	37	38
LDL-C	<100	92	91	87
Non-HDL-C	<130	139	135	132
VLDL-C	<30	42	43	42

6.12.3. RESULTS

[0409] FIG. 41 illustrates the patient disposition for the ESPRIT trial, showing that 6 patients were withdrawn from the placebo arm, 6 patients were withdrawn from the 2g treatment arm, and 12 patients were withdrawn from the 4g treatment arm. The number of patients who experienced adverse effects (AE) was low overall, with 2 in the placebo arm, 3 in the 2g treatment arm, and 7 in the 4g treatment arm.

[0410] Patients in the ESPRIT trial exhibited significant percentage changes in plasma EPA and DHA levels, as shown in FIG. 42A and FIG. 42B, respectively. These patients also demonstrated dose-dependent reductions in TG, reductions in non-HDL-C, and increases in HDL-C, compared to olive oil placebo (see FIG. 43). Furthermore, dose-dependent reductions in VLDL-C and TC/HDL-C were observed (see FIG. 44). Taken together, the results of FIGS. 42-44 demonstrate efficacy of Epanova® as an add-on to statin therapy.

[0411] Further details of the results of the ESPRIT trial are presented in FIGS. 45 - 52, demonstrating that Epanova® is efficacious as an add-on to both low-potency and high-potency statins, in a range of baseline patient conditions. FIG. 45 shows the results for median TG percentage change from baseline for three tertiles of patients, partitioned by baseline TG levels. FIG. 46 shows the results for median non-HDL-C percentage change from baseline for three tertiles of patients, partitioned by baseline non-HDL-C levels. FIG. 47 shows the results for median LDL-C percentage change from baseline for three tertiles of patients, partitioned by baseline LDL-C levels.

[0412] As seen from FIG. 48, the reductions in TG levels were observed for patients who received concurrent rosuvastatin, atorvastatin, and simvastatin therapy. Statistically significant effects on triglycerides, non-HDL-C, and LDL-C levels were observed regardless of whether low potency or high potency statins were co-administered, as shown in FIGS. 49-51.

[0413] FIG. 52 compares median percentage changes from baseline for triglycerides for (A) patients having higher TG baseline levels ($\geq$ 294 mg/dL), (B) patients having high EOT EPA levels ($\geq$ 26.58 μg/mL), and (C) patients receiving concurrent rosuvastatin therapy. The

results show that the Epanova® 2g dose works similarly to the 4g dose in those patient populations shown in FIG. 52.

[0414] The increased LDL-C levels observed upon treatment with Epanova® were consistent with observed increased lipoprotein particle size. Large VLDL, Medium VLDL, small VLDL, VLDL total, and VLDL size were measured for placebo and each of the treatment arms of the ESPRIT trial. The results are displayed in FIG. 53 and show that Epanova® treatment resulted in increased amounts of large VLDL particles and correspondingly decreased amounts of small VLDL particles. Decreased VLDL particles were observed together with increases in LDL particle size, as shown in FIG. 54. Shown in FIG. 55, as end-of-treatment TG levels decreased, percentage increases in LDL-P size were larger. Taken together, FIGS. 53 – 55 demonstrate that Epanova® treatment resulted in increased lipoprotein particle size, an observation that can account for the observed increased LDL-C.

[0415] Table 42, below, summarizes the results of the ESPRIT trial.

Table 42

		Olive Oil (OO)			4 g/day*					2 g/day*				
		Base	EOT	%Δ	Base	EOT	%Δ	p-value	diff	Base	EOT	%Δ	p-value	diff
Non-HDL-C	median	132	134	1	135	129	-6	<0.001	-6	139	133	-3	0.037	-3
	mean	135	136	1	139	132	-5			140	136	-2		
TG	median	269	260	-4	265	215	-21	<0.001	-15	265	222	-15	<0.001	-9
	mean	280	268	-3	287	233	-18			284	244	-14		
HDL-C	median	38	38	2	37	38	3	0.988	1	38	39	2	0.988	0
	mean	39	40	3	39	40	4			39	40	3		
LDL-C	median	87	91	2	91	92	1	0.647	0	92	95	5	0.025	4
	mean	92	93	4	94	94	4			92	97	6		
VLDL-C	median	42	41	-3	43	33	-20	<0.001	-16	42	37	-12	0.008	-8
	mean	46	44	3	47	38	-14			47	40	-10		
TC	median	174	174	1	170	167	-4	<0.001	-4	177	174	-1	0.049	-2
	mean	174	176	1	178	172	-3			179	176	-1		
TC/ HDL-C	median	5	5	-2	5	4	-7	0.001	-5	5	5	-4	0.119	-3
	mean	5	5	-1	5	5	-6			5	5	-3		

[0416] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

[0417] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s).

CLAIMS

1. The use of a pharmaceutical composition comprising Eicosapentaenoic acid (EPA) in a weight percent amount of 50% to 60%;
   Docosahexaenoic acid (DHA) in a weight percent amount of 17% to 23%;
   Docosapentaenoic acid (n-3)(DPA) in a weight percent amount of 1% to 8%;
   wherein at least 90% by weight of the polyunsaturated acid in the composition is present in the free acid form, in the manufacture of a medicament for the treatment of severe hypertriglyceridemia, wherein the treatment, once started, comprises the oral administration of at least about 2g per day of the pharmaceutical composition to a patient having pre-treatment serum or plasma triglyceride levels greater than or equal to 500 mg/dL.

2. The use according to claim 1, wherein the amount of pharmaceutical composition is at least about 3g per day.

3. The use according to claim 1, wherein the amount of pharmaceutical composition is at least about 4g per day.

4. The use according to any one of claims 1-3, wherein at least 95% by weight of the polyunsaturated fatty acid in the composition is present in the free acid form.

5. The use according to any one of claims 1-3, wherein DPA is present in a weight percent amount of at least about 2%.

6. The use according to any one of claims 1-3, wherein DPA is present in a weight percent amount of at least about 3%.

7. The use according to any one of claims 1-3, wherein DPA is present in a weight percent amount of at least about 4%.

8. The use according to any one of claims 1-3, wherein DPA is present in a weight percent amount of at least about 4.5%.

9. The use according to any one of claims 1-3, wherein the pharmaceutical composition comprises EPA present in a weight percent amount of about 55% to about 56%; DHA present in a weight percent amount of about 19% to about 20%; DPA present in a weight percent amount of about 4% to about 5%.