International Journal of Pharmaceutics 379 (2009) 125–130

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmacopeial compliance of fish oil-containing parenteral lipid emulsion mixtures: Globule size distribution (GSD) and fatty acid analyses☆

David F. Driscoll*,[1], Pei-Ra Ling, Bruce R. Bistrian

Nutrition/Infection Laboratory, Department of Medicine, B.I. Deaconess Medical Center and Harvard Medical School, Boston, MA, USA

ARTICLE INFO

Article history:
Received 13 November 2008
Received in revised form 15 June 2009
Accepted 18 June 2009
Available online 25 June 2009

Keywords:
Fish oil-containing emulsions
Globule size distribution
n3-Fatty acids
Pharmacopeial compliance

ABSTRACT

Recently, the United States Pharmacopeia (USP) has established Chapter ⟨729⟩ with GSD limits for all lipid emulsions where the mean droplet size (MDS) must be <500 nm and the percent of fat larger than 5 μm ($PFAT_5$) must be <0.05%, irrespective of the final lipid concentration. As well, the European Pharmacopeia (EP) Monograph no. 1352 specifies n3-fatty acid (FA) limits (EPA + DHA ≥ 45%; total n3 or T-n3 ≥ 60%) for fish oil. We assessed compliance with USP physical and EP chemical limits of two fish oil-containing lipid emulsion mixtures. All lipid emulsions passed USP ⟨729⟩ limits. No samples tested had an MDS >302 nm or a $PFAT_5$ value >0.011%. Only one product met EP limits while the other failed. All emulsions tested were extremely fine dispersions and easily met USP ⟨729⟩ GSD limits. The n3-FAs profiles were lower in one, despite being labeled to contain 50% more fish oil than the other product. This latter finding suggests the n3-FA content of the fish oil source and/or the applied manufacturing processes in these products is different.

1. Introduction

In modern times, pharmacopeias around the world have variably functioned as a liaison between government regulatory authorities and the pharmaceutical industry. In America, for example, the United States Pharmacopeia (USP) works directly with industry, and in particular drug innovators to assist in the creation of new drug monographs, usually within five years of its approval by the Food and Drug Administration (FDA) (Driscoll, 2006) The USP also works closely with the European Pharmacopeia (EP) and Japanese Pharmacopeias (JP) in a concerted effort to harmonize its standards (www.usp.org/USPNF/pharmacoepialHarmonization/). Drug monographs contain essential information about the pharmaceutical product, such as the active ingredient(s), acceptable concentration range and limits, packaging and storage requirements, labeling, assay requirements, etc. Thus, this system works well, since industry, as the drug innovator, has the greatest insights into the important physical and chemical issues of the newly developed product. Ultimately, in the U.S. such information will apply to future drug applications to the FDA to ensure generically equivalent products that are both pharmaceutically equivalent and bioequivalent. For the USP, the development of pharmacopeial monographs, as well as relevant chapters (usually detailing important assay methods of monographs), can be used at the discretion of the FDA for drug enforcement purposes.

In the clinical setting, pharmacopeias have generally had little to no impact on practitioners (i.e., physician, nurse) who uses (prepares, administers) the pharmaceutical dosage form to treat the patient. Recently, however, there have been attempts to link pharmacopeial specifications to compatibility and stability issues involving parenteral nutrition admixtures as a patient safety goal (Driscoll, 2005). Practitioners, for their part, assume the dosage form contains the active ingredient(s) and that the labeled amount(s) will provide the correct dose when prepared as directed and administered to the patient. Recently, certain lipid emulsions from one manufacturer have been shown to fail the globule size limits (mean droplet size: <500 nm; percent of fat globules >5 μm: <0.05%) of USP Chapter ⟨729⟩ (Globule Size Distribution in Lipid Injectable Emulsions, 2008) coincident with a change in their packaging containers from glass bottles to plastic bags (Driscoll, 2007a). The abnormal globule size distribution (GSD) of the lipid emulsion has been shown to produce less stable all-in-one (AIO) admixture (Driscoll et al., 2007b, 2009) and less stable lipids when pre-packaged in a syringe (Driscoll et al., 2007c), as applied in neonatal use, compared to similar lipids that pass the globule size limits of ⟨729⟩. In addition, these abnormal fat globule profiles have also been associated with poorer plasma clearance and significantly higher incidence

☆ Presented, in part, at the 2008 European Society of Parenteral and Enteral Nutrition Meeting in Florence, Italy, September 14, 2008.

* Corresponding author. Tel.: +1 774 281 0018.
E-mail address: d.driscoll.ssi@gmail.com (D.F. Driscoll).

[1] At the time of this study, Dr. Driscoll was at BI Deaconess Medical Center and Harvard Medical School. He is now at Stable Solutions, Easton, MA, USA.

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.06.021

Table 1
Fish oil-mixed lipid emulsions studied.[a]

Oil	Lipoplus/lipidem 20%	SMOFlipid 20%
Soy	40%	30%
MCT	50%	30%
Olive	0%	25%
Fish	10%	15%

[a] Oil ratios for each product came from the manufacturer's label and package insert for each product.

of hypertriglyceridemia in critically ill premature infants, than those receiving the same emulsion product, but in formulations which meet ⟨729⟩ limits (Martin et al., 2008). Thus, it appears that failure to comply with pharmacopeial specifications of intravenous lipid emulsions may have clinically important consequences.

This study was undertaken to investigate two novel lipid emulsion oil mixtures containing fish oil, rich in the "bioactive" omega-3 (or n3) fatty acids, in meeting existing pharmacopeial specifications. With respect to the USP, physical compliance with Chapter ⟨729⟩ was assessed, while chemical compliance with the n3-fatty acid requirements of EP Monograph no. 1352 (Omega-3-Säuren-Triglyceride, 2005), were evaluated between these emulsions.

2. Materials and methods

2.1. Lipid emulsions studied

Two novel 20% (w/v) lipid emulsion mixtures containing fish oil were evaluated and the various oil fractions contained in the dispersed phase appears in Table 1. In one product, 10% of the oil phase was fish oil (Lipidem™ or Lipoplus™ 20%[a–d]) or "LPLUS" and in the other, 15% of the oil phase was fish oil (SMOFlipid™ 20%[e–f]) or "SMOF". Four lots of each product and three bottles per lot were evaluated for pharmacopeial compliance. The order in which the samples were analyzed was randomized by the individual bottle (12/product or $n = 24$). Three different analytical methods were applied in this study for the physical measurements of (1) mean droplet size or MDS; and (2) large-diameter tail expressed as percent fat >5 μm or $PFAT_5$; and (3) chemical measurements of the fatty acid profiles of each emulsion, with particular attention to the concentrations of the n3-fatty acids, EPA and DHA, and in this latter analysis, the technician was blinded as to the identity of final sample. For each of the three methods applied in this study, all samples were run in duplicate (24 samples per product per method). Sample preparation for each method was standardized in every step for both 20% emulsions immediately prior to formal instrumental analysis, and hence, treatment of samples from both emulsions was identical.

2.2. USP ⟨729⟩ Method I: mean droplet size (nm)

For the qualitative determination of MDS, a Nicomp™ 370 Submicron Particle Analyzer instrument with Autodilution (Particle Sizing Systems, Santa Barbara, CA) was used (range: 5–2000 nm). It applies the principle of dynamic light scattering, based on the temporal fluctuations in light intensity due to the Brownian motion or diffusion of the particles or droplets in the optical path, using the intensity autocorrelation function to semi-quantitatively determine the average or mean droplet size (MDS) for each sample in duplicate. From these intensity fluctuations and resulting autocorrelation function, the intensity-weighted distribution of diffusion coefficients is calculated. From the latter, the intensity-weighted distribution of droplet radii, or diameters, is obtained, using the Stokes–Einstein relation. The DLS instrument, using a scattering angle of 90 degrees, was calibrated with polystyrene calibrator nanospheres of known diameters (54–993 nm). Approximately 50 μL of each lipid emulsion sample was added to 20 mL of double-filtered (0.22 μm) water in clean glass scintillation vials. The sample was gently swirled, producing a final suspension that was slightly turbid, but translucent. From this mixture, approximately 3 mL was injected into the DLS instrument, whereby it underwent further dilution by a proprietary Autodilution procedure to optimize the final concentration of the sample for analysis. During this time, there was an approximate 100-fold increase in the mean scattering intensity (i.e., photopulse rate) above baseline (no sample) light intensity of between 5 and 10 kHz. Once a stable light scattering photopulse rate was achieved (250–350 kHz), and the sample temperature equilibrated to 23 °C, collection of the scattered intensity data began over 5–10-min intervals. Emulsion samples passed the limits of Method I if the average intensity-weighted MDS result was <500 nm.

2.3. USP ⟨729⟩ Method II: $PFAT_5$ (%)

For the quantitative large-diameter tail assessments, an AccuSizer™ 780/APS™ with Autodilution (Particle Sizing Systems, Santa Barbara, CA), applying the principle of light obscuration, or light extinction, and employing a single-particle (globule) optical sensing (LE/SPOS) technique, was used to determine the population of large-diameter fat globules (> 5 μm) for each sample in duplicate. As a large-diameter fat globule passes through a defined optical sensing zone, a portion of the incident light beam is effectively blocked, causing a momentary decrease in the light intensity reaching the extinction detector (baseline 10 V). The magnitude of the change (reduction) in the resulting signal pulse height is, ideally, proportional to the cross-sectional area of the globule, i.e., to the square of its diameter (for globule diameters smaller than the thin, ribbon-like beam of light that crosses the optical flow cell), thereby defining the optical sensing zone of the sensor). The resulting individual pulse heights are converted to droplet diameters from a previously determined standard calibration curve constructed by using a series of polystyrene calibrator microspheres of known diameters (1.33–200 μm), recognizing interference from (light) extinction efficiency issues between sizes of 1.33–4.9 μm (Driscoll, 2004). In order to avoid coincidence errors (appearance of more than one droplet at a time in the optical sensing zone) during measurement, the final droplet concentration of the sample for analysis was optimized by applying an Autodilution procedure for each emulsion. Using a Teflon sample-capture loop tared to 1.0 mL, each concentrated emulsion sample was first injected into a first-stage diluter, consisting of a 28.5 mL mixing vessel (magnetically stirred) containing double 0.22 μm-filtered water. The sample was further automatically diluted by a second-stage in the instrument using a static mixer with fixed dilution (preset), performed by a computer-controlled syringe pump with micro-stepping (1 mL glass syringe). Data collection by the LE/SPOS instrument was carried out using a size detection range of 1.8–50 μm. The final sample for analysis of the 20% lipid emulsions resulted in dilutions of 1710:1 and 3420:1 (water:sample) for each replicate, respectively. The sample flow rate was set at 1 mL/s and the resolution of the globule size distribution was reported using 128 individual size channels. Once the count rate stabilized following a 45–60 s equilibration period, particle counting and data collection commenced over a period of 120 s for each sample. Data on fat globules sizes above 5 μm were extracted by summing the volume of the globules sized from each size channel (i.e., Σ(#/mL/channel) ($\pi D^3/6$). The sample passed the limits of Method II if the volume-weighted percentage of fat residing in globules >5 μm, or $PFAT_5$, was <0.05%.

2.4. EP Monograph no. 1352: n3-fatty acid profile (wt%)

For the quantitative determination of the fatty acid composition of the emulsions, and in particular the bioactive n3-fatty acids, eicosapentaenoic acid or EPA (20:5n3) and docosahexaenoic acid or DHA (22:6n3), a gas chromatography–mass spectroscopy (GC/MS) technique was applied. Emulsion samples, aseptically taken from freshly opened manufacturer's bottles, were prepared beforehand as fatty acid methyl esters (FAME). Lipids from diluted fat emulsion (9 μL of fat emulsion plus 491 μL of phosphate buffered saline or PBS) solution were extracted by liquid–liquid extraction method with 6 volumes of chloroform–methanol (2:1, v/v). Before the extraction, 30 μL of a 1 mg/mL of heptadecanoic acid (Sigma, St. Louis, MO) in chloroform/methanol (1:1, v/v) were added into each sample as an internal standard. The mixtures were vortexed for 5 min (min), and then centrifuged at 800 g for 10 min. The lower phase was aspirated into a new tube. The chloroform–methanol extracts were evaporated to dryness under nitrogen. Samples were reconstituted by adding 0.5 mL of sodium methoxide (Sigma Chemical, St. Louis, MO) and mixed well. Samples were then placed in a heating block at 100 °C for 3 min and removed to cool down to room temperature. Then, 0.5 mL of methanol base-Boron trifluoride reagent (Acros Organics USA, Morris Plains, NJ) was added, mixed well and incubated at 100 °C for 1 min. After cooling down the tubes to room temperature, 0.5 mL of hexane was added and mixed, then 6.5 mL of a saturated NaCl solution was added and mixed well. The mixture was centrifuged at 800 g for 3 min. The upper hexane layer was aspirated. The FAME mixture was ready for gas chromatography–mass spectrometry. The fatty acid analyses were performed with a Hewlett–Packard 6890N gas chromatograph (GC) coupled to an HP-5875B mass spectrometer (MS) equipped with a Supelcowax SP-10 capillary column (Hewlett–Packard, Palo Alto, CA). The oven temperature was maintained at 110 °C for 5 min, ramped at 10 °C/min to 200 °C and held for 4 min, ramped again at 5 °C/min. to 240 °C and held for 3 min, and then finally ramped to 270 °C at 10 °C/min. and maintained for 5 min. The injector and detector were maintained at 260 °C and 280 °C, respectively. Carrier gas flow rate was maintained at a constant 0.8 mL/min throughout. Total ion monitoring was performed, encompassing mass ranges from 50 to 550 atomic mass units (amus). Peak identification was based upon comparison of both retention time and mass spectra of the unknown peak to that of known standards within the GC–MS database library. FAME mass was determined by comparing areas of unknown FAMEs to that of a fixed concentration of 17:0 internal standard. Response factors were determined for each individual FAME to correct for GC–MS total ion chromatograph discrepancies in quantification. These factors were determined through the use of a gas–liquid chromatography or GLC reference standard which contained known masses of FAMEs ranging from 14 to 24 carbons. The response ratio of each FAME was corrected to a fixed amount ratio for each FAME relative to 17:0.

Linearity was determined using 10 samples each of EPA and DHA concentrations ranging from 0 to 4000 μM. EPA and DHA recoveries were determined at 50, 200, 400 and 800 μM. EPA recoveries ranged from 91.5 to 107.1% (mean: 101.3%) and for DHA, the range was 90.9–108.1% (mean: 99.7%). Functional sensitivity was the lowest fatty acid concentration at which the coefficient of variation (CV) was less than 15%, and was 10 μM for EPA and 20 μM for DHA.

The fatty acid profile of each emulsion covered a range of 8–24 carbons, and the method was modeled from previous method (Moser and Moser, 1991). Data on selected fatty acids, particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, and others within detection limits were expressed as a weight-in-volume percentage (g/100 mL) or wt%, as a fraction of the total lipids present of each emulsion formulation (20 g/L) were evaluated. The sample passed the EP limits if the sum of EPA + DHA was ≥45%, and total omega-3 fatty acids or T-n3 was ≥60%.

2.5. Statistical analysis

All data are presented as the mean ± SD. If any product failed pharmacopeial specifications, then a formal statistical analysis was performed between formulations that included a one-way analysis of variance (ANOVA) assessment with lipid product, LPLUS and SMOF, as the independent variable, and physical assessments (MDS, $PFAT_5$) and/or chemical assessments (selected long-chain fatty acids) as the dependent variable(s). Additional statistical assessments, such as comparing n3/n6 ratios between products were also performed. An ***a priori*** level of statistical significance was set at a p-value of <0.05.

3. Results

3.1. Mean droplet size

The intensity-weighted mean droplet sizes of the dispersions are shown in Table 2. The SMOF products had a higher MDS (298 ± 2 nm) than the LPLUS products (279 ± 7 nm). Nonetheless, both products passed the globule size limit of Method I of USP ⟨729⟩ (<500 nm). No sample tested (n = 24/lipid emulsion) had an MDS >302 nm.

3.2. Percent fat >5 μm

The volume-weighted percent of fat greater than 5 μm or $PFAT_5$ levels are also shown in Table 2. The number of fat globules sized per sample run for the SMOF replicates (range: 229,836–524,254) and LPLUS (range: 110,767–472,793) were similar. The SMOF and LPLUS products were comparable (0.006 ± 0.001% vs. 0.008 ± 0.002%), and both products passed the globule size limit of Method II of USP ⟨729⟩ ($PFAT_5$ <0.05%). No sample tested (n = 24/lipid emulsion) had a $PFAT_5$ value >0.011%.

3.3. Wt% of selected fatty acids

The weight-in-volume percentages (g/100 mL) or wt% of 8–23 carbon fatty acids measured are shown in Table 3. Most notably, the EPA and DHA levels were higher in the LPLUS formulation compared to those measured in the SMOF emulsion (EPA: 3.69 ± 0.14% vs. 3.03 ± 0.14%; DHA: 2.53 ± 0.14% vs. 2.00 ± 0.15%, respectively), despite the latter product containing 50% more fish oil in the oil phase of the emulsion (15% vs. 10%). In terms of meeting EP requirements, Table 4 shows the results for the various lots of emulsion studied for each product and the percentage of EPA and DHA present in the fish fraction of the oil phase. Clearly, the SMOF product failed EP specifications for EPA and DHA contents in fish oil (minimum: 45%) for each batch studied, averaging

Table 2
Globule size analyses of lipids studied.

Lipid	Lot	MDS (nm)	$PFAT_5$ (%)
Lipidem 20%	7062A182	268 ± 2	0.005 ± 0.001
Lipoplus 20%	7243A182	285 ± 3	0.008 ± 0.001
Lipidem 20%	7364A182	285 ± 3	0.010 ± 0.001
Lipidem 20%	7484A182	277 ± 1	0.010 ± 0.001
Mean ± SD		279 ± 7	0.008 ± 0.002
SMOFlipid 20%	WF1578	298 ± 2	0.005 ± 0.001
SMOFlipid 20%	WG1526	299 ± 1	0.006 ± 0.001
SMOFlipid 20%	16AK0106	296 ± 2	0.007 ± 0.001
SMOFlipid 20%	16AL0040	300 ± 1	0.005 ± 0.001
Mean ± SD		298 ± 2	0.006 ± 0.001

Table 3
Medium- and long-chain fatty acid profiles expressed as weight percent (wt%) from total lipids (20 g/100 mL).

Fatty acid	Carbon#	Notation	LPLUS 20%	SMOF 20%
Caprylic	8	8:0	24.18 ± 0.86	13.85 ± 0.59
Capric	10	10:0	16.13 ± 0.48	9.85 ± 0.31
Lauric	12	12:0	0.15 ± 0.02	0.20 ± 0.05
Myristic	14	14:0	0.13 ± 0.01	1.15 ± 0.04
Pentadecanoic	15	15:0	0.01 ± 0.00	0.08 ± 0.00
Palmitic	16	16:0	6.56 ± 0.10	9.83 ± 0.19
Palmitoleic	16	16:1	0.20 ± 0.02	1.58 ± 0.01
Stearic	18	18:0	1.29 ± 0.08	1.50 ± 0.21
Oleic	18	18:1n9	13.44 ± 0.31	30.77 ± 0.72
Linoleic	18	18:2n6	25.72 ± 0.71	21.42 ± 0.19
α-Linolenic	18	18:3n3	3.41 ± 0.46	2.50 ± 0.10
Stearidonic	18	18:4n3	0.14 ± 0.06	0.42 ± 0.03
Arachidic	20	20:0	0.22 ± 0.01	0.28 ± 0.02
Eicosenoic	20	20:1n9	0.25 ± 0.01	0.28 ± 0.03
Dihomo-γ-linolenic	20	20:3n6	0.05 ± 0.00	0.03 ± 0.00
Arachidonic	20	20:4n6	0.52 ± 0.03	0.43 ± 0.02
Eicosapentaenoic	**20**	**20:5n3**	**3.69 ± 0.14**	**3.03 ± 0.12**
Behenic	22	22:0	0.31 ± 0.17	0.17 ± 0.03
Erucic	22	22:1n9	0.17 ± 0.01	0.10 ± 0.03
Adrenic	22	22:4n6	0.02 ± 0.01	0.02 ± 0.01
Docosapentaenoic	**22**	**22:5n3**	**0.70 ± 0.06**	**0.36 ± 0.02**
Osbond	22	22:5n6	0.14 ± 0.00	0.11 ± 0.01
Docosahexaenoic	**22**	**22:6n3**	**2.53 ± 0.14**	**2.00 ± 0.15**
Tricosanoic	23	23:0	0.03 ± 0.01	0.02 ± 0.01

Boldface: Major marine n3-fatty acids in fish oil.

33.4% (range: 31.5–36.2%), where all batches of LPLUS passed averaging 62.1% (range: 60.6–63.4%). These differences were also statistically significant ($p < 0.001$). As well, there were also higher amounts of docosapentaenoic acid or DPA in LPLUS than SMOF (DPA: 0.70 ± 0.06% vs. 0.36 ± 0.02%, respectively). Although not shown, adding the remaining omega-3 fatty acid DPA to assess compliance with the EP specifications for total n3-fatty acids in fish oil (minimum: 60%) showed similar results between formulations (SMOF: 35.8%; LPLUS: 69.1%).

As LPLUS contains approximately 1/3 more soybean oil than SMOF (40% vs. 30%), it has higher levels of the essential fatty acids linoleic and linolenic acids (18:2n6: 25.72 ± 0.71% vs. 21.42 ± 0.19%; 18:3n3: 3.41 ± 0.46% vs. 2.5 ± 0.10%, respectively). Similarly, since SMOF uniquely contains olive oil, the oleic acid concentration was higher than LPLUS (30.77 ± 0.72% vs. 13.44 ± 0.31%, respectively) as well as higher palmitic acid concentrations (SMOF: 9.83 ± 0.19% vs. LPLUS: 6.56 ± 0.10%). In addition, SMOF also had notably higher concentrations of myristic acid (14:0: 1.15 ± 0.04% vs. 0.13 ± 0.01%, respectively) and palmitoleic acid (16:1: 1.58 ± 0.01% vs. 0.20 ± 0.05%, respectively) than LPLUS formulations.

The LPLUS formulations also contain more MCTs than does SMOF (50 vs. 30%, respectively), and the higher levels are shown in Table 3. The measured concentrations of the medium-chain fatty acids for both emulsions are proportionately lower than expected (LPLUS by 9.5%; SMOF by 6.1%), and this is likely due to their higher volatility during measurement compared to the long-chain fatty acids.

4. Discussion

The current emulsion oil mixtures that contain fish oil were shown to be in compliance with the physical requirements for globule size limits of USP Chapter ⟨729⟩ for both mean droplet size and the percent of fat >5 μm that represents the large-diameter tail, and thus would be considered "fine" (vs. "coarse") emulsions. Hence, although these standards are only applicable in United States at the present time, both formulations meet pharmacopeial specifications, and from a physical standpoint, these emulsions would be considered pharmaceutically equivalent.

The results showed that SMOF did not meet EP Monograph no. 1352 chemical specifications for the minimum amounts of omega-3 fatty acid concentrations for the fish oil fraction of the dispersed oil phase of the emulsion whereas LPLUS did. Moreover, despite containing 50% more fish oil than LPLUS, the omega-3 fatty acid concentrations were almost 50% lower in the SMOF product. Put another way, to deliver 1 g (total) of the bioactive omega-3 fatty acids EPA and DHA, 25% more SMOF lipid emulsion would be needed than that delivered from the LPLUS product (20% emulsion: 100 mLs vs. 80 mLs, respectively).

From a clinical perspective, the purpose of prescribing fish oil-containing emulsions is to provide a less pro-inflammatory mixture than conventional lipid emulsions rich in omega-6 fatty acids. This may be a particularly important issue, since the omega-3 fatty acids from fish oil, particularly, EPA and DHA, are considered important and favorable modulators of the inflammatory response in patients with severe metabolic stress (Bistrian, 2003; Wanten and Calder, 2007). The other major n3-fatty acid present in fish oil is DPA. The EP Monograph no. 1352 identifies two limits for n3-fatty acids in fish oil. The first limit states a minimum concentration of EPA and DHA of 45%, while the second limit is minimum "total omega-3 acids" concentration of 60%.

Some have advocated a more favorable n3/n6 ratio for parenteral lipid emulsions (Grimm et al., 2006). The n3/n6 ratios observed in this study, which included all of the anti-inflammatory 20 and 22-carbon n3-fatty acids found in fish oil, against the major pro-inflammatory 18-carbon n6 fatty acid linoleic acid, the LPLUS had a significantly better ratio than SMOF (EPA + DHA + DPA/LA: 0.27 vs. 0.25, respectively, $p = 0.002$). If only EPA and DHA are included, however, the favorable n3/n6 ratio for LPLUS vs. SMOF is no longer significant (EPA + DHA/LA: 0.24 vs. 0.23, respectively, $p = 0.062$). A more recent assessment of the value of the overall n3/n6 ratio, which includes the 18 carbon n3 and n6 fatty acids as well, as a marker of its beneficial effects, concluded that the absolute intakes of n3-fatty acids are more clinically important (Stanley et al., 2007).

Table 4
EPA and DHA analyses and EP specifications.

Lipid	Lot	EPA[a] (%)	DHA[a] (%)	EPA + DHA/fish oil (100)[b,c]
Lipidem 20%	7062A182	3.69 ± 0.01	2.64 ± 0.06	63.3%
Lipoplus 20%	7243A182	3.59 ± 0.06	2.61 ± 0.02	62.0%
Lipidem 20%	7364A182	3.57 ± 0.13	2.49 ± 0.24	60.6%
Lipidem 20%	7484A182	4.03 ± 0.27	2.31 ± 0.09	63.4%
Mean ± SD		**3.69 ± 0.14**	**2.53 ± 0.14**	**62.1%**
SMOFlipid 20%	WF1578	2.99 ± 0.06	2.10 ± 0.10	33.9%
SMOFlipid 20%	WG1526	2.90 ± 0.19	1.82 ± 0.18	31.5%
SMOFlipid 20%	16AK0106	3.22 ± 0.10	2.21 ± 0.07	36.2%
SMOFlipid 20%	16AL0040	3.00 ± 0.05	1.93 ± 0.07	33.3%
Mean ± SD		**3.03 ± 0.12**	**2.00 ± 0.15**	**33.4%**

[a] As wt% of total lipids (20 g/100 mL).
[b] As wt% of fish oil fraction of dispersed phase (LPLUS, 10% or 2 g/100 mL; SMOF, 15% or 3 g/100 mL).
[c] Sum of EPA + DHA in fish oil as percent, EP Monograph no. 1352 limit, ≥45%.

Table 5
European Pharmacopeia Monograph No. 1352: Omega-3-acid triglycerides (recognized fish species).

Examples from recognized sources[a]	EPA, wt%	DHA, wt%	DPA, wt%	Sum, wt%
Carangidao family				
Mackerel, Atlantic	7.4	11.6	1.7	20.7
(Jack) Mackerel, Pacific	7.4	13.6	1.8	22.8
Clupeidae family				
Herring, Atlantic	8.9	10.8	0.6	20.3
Herring, Pacific	7.7	5.4	1.3	14.4
Engraulidae family				
Anchovies, European	13.1	22.2	0.7	36.0
Osmeridae family				
Smelt, Rainbow	13.9	21.1	0.9	35.9
Salmonidae family				
Salmon, Atlantic	5.7	19.8	5.1	30.6
(Sockeye) Salmon Pacific	6.9	8.7	0.5	16.1
Scombridge family				
Tuna, Bluefin	6.5	20.7	2.9	30.1
Tuna, Yellowfin	5.4	26.8	1.9	34.1
Average (SD)	8.3% (2.8)	16.1% (6.6)	1.7 (1.6)	26.1% (7.8)

[a] Exler, 1987.

However, in any case, these formulations would not be considered bioequivalent with respect to the delivery of the bioactive omega-3 fatty acids, EPA and DHA.

The failure of SMOF to comply with pharmacopeial specifications for the omega-3 fatty acid requirements of fish oil suggests either the source and/or methods of processing the fish oil component of the emulsion are responsible. According to EP Monograph no. 1352, "the origin of the omega-3 acids is the body oil from fatty fish species" (Omega-3 Acid Triglycerides, 2005), and identifies six common families of fish. To assess the EPA and DHA contents in the aforementioned native marine sources of fish oil, we tabulated data on representative species from each family of fish in Table 5 from a U.S. government database (Exler, 1987). The data sample shows that the contents of omega-3 fatty acids in the various fish families are variable, and on average, approach 30%, recognizing that there is a high degree of inter-species variability and intra-species seasonal variations (Soriguer et al., 1997). As commercial fish oil is a mixture of fatty fish species, the amounts found in the SMOF product parallel those found in native fish. Hence, the EP minimums for EPA and DHA (45%) and total omega-3 fatty acids (60%) can only be achieved by additional processing of the native fish oil.

With respect to achieving the EP standards for omega-3 fatty acids, there is evidence from the fatty acid profile that certain fatty acids were removed to elevate the final concentrations of the omega-3 fatty acids in the native fish oil. For example, the concentrations of myristic (14:0) and palmitoleic (16:1) acids are significantly lower in the LPLUS product compared to the SMOF formulation (14:0: 0.13 ± 0.01 vs. 1.15 ± 0.04; 16:1: 0.20 ± 0.02 vs. 1.58 ± 0.01, $p < 0.001$, respectively). One possible way of fractionating the fish oil is by molecular distillation that separates the oil components by weight grouping, and removes undesirable fractions such as myristic and palmitoleic acids from those present in its natural form, thus allowing the oil to be more concentrated in the desirable omega-3 fatty acids. To illustrate the significance of these fatty acids in the processing of the native fish oil, Table 6 shows the profile of selected fatty acids in fish, soybean and olive oil (Exler, 1987; Lide, 1990). MCT oil is not included since its composition is virtually free of fatty acids >12 carbons (Senior, 1968). From this table, the concentrations of myristic and palmitoleic acids are more than 10 times higher than the residual amounts found in either soybean or olive oils. Thus, the difference between LPLUS and SMOF clearly involved the removal of these fatty acids, among others from the fish oil product. Table 6 also provides data on other fatty acids in fish (e.g., palmitic and oleic acids) that are commonly found in the vegetable oils used in lipid emulsions.

Finally, our data confirms the omega-3 fatty acid contents in these formulations as reported by others. Linseisen et al. (2000) reported EPA plus DHA levels for LPLUS of 54% of the fish oil profile, thus also meeting EP specifications. If the level of EPA plus DHA in the present study are corrected for presumed volatilized MCTs, they would be very close to previously reported concentrations (Linseisen et al., 2007), and nonetheless still well within EP limits (Omega-3 Acid Triglycerides, 2005; Omega-3-Säuren-Triglyceride, 2005). Meanwhile, Wanten and Calder (2007), Waitzberg et al. (2006), Grimm et al. (1994), and DeNardi et al. (2008), all reported the concentration of EPA plus DHA levels in SMOF of between 30 and 33% in the fish oil profile as we did, and hence, not meeting EP specifications, since they would be even lower with correction for MCT concentrations. It should be noted, however, that all these authors reported these levels as provided to them by the manufacturer, whereas those presented here were measured directly.

Table 6
Fatty acid profile of fish and the oil phases of the study emulsions.

Examples from EP-approved[10] sources[16]	Myristic wt%	Palmitic, wt%	Palmitoleic, wt%	Oleic, wt%	Linoleic, wt%	α-Linolenic, wt%
Carangidao family						
Ex. Mackerel, Atl.	5.6	17.6	6.0	18.9	1.8	1.3
Ex. Mackerel, Pac./Jack	5.0	20.4	6.9	19.5	1.7	0.7
Clupeidae family						
Ex. Herring, Atl.	7.0	17.1	7.9	19.2	1.6	1.3
Ex. Herring, Pac.	7.3	16.1	8.4	23.2	1.5	0.5
Engraulidae family						
Ex. Anchovies, Eur.	7.4	17.4	9.8	15.2	2.4	0
Osmeridae family						
Ex. Smelt, Rainbow	3.9	16.6	10.1	20.6	2.3	2.5
Salmonidae family						
Ex. Salmon, Atl	2.4	11.2	4.5	24.0	3.1	5.2
Ex. Sockeye Salmon, Pac.	3.9	13.5	7.0	18.4	5.1	1.2
Scombridge family						
Ex. Tuna, Bluefin	3.2	18.9	3.8	21.5	1.2	0
Ex. Tuna, Yellowfin	1.6	23.2	3.7	16.1	1.2	1.8
Average	4.7	17.2	6.8	19.7	2.2	1.5
Soybean oil	0.1	9.8	0.4	28.9	50.7	6.5
Olive oil	Trace	6.9	0	84.4	4.6	0

5. Conclusions

Two fish oil-containing lipid emulsion mixtures were compared against recent pharmacopeial developments involving certain physical and chemical specifications showing one formulation meets both requirements, while the other only meets the physical limits. Chemical requirements for the bioactive fatty acids EPA and DHA in the fish oil used were met by the LPLUS product while the SMOF product was more than 25% below the pharmacopeial minimum limits. As omega-3 fatty acids are given for reasons beyond nutrition support indications, the products are not bioequivalent. Although SMOF contains 50% more fish oil in its labeled amount, it contains only about half the concentration of the important bioactive omega-3 fatty acids, EPA and DHA of LPLUS.

Acknowledgements

This study was supported by a research grant from B. Braun, Melsungen, Germany. We greatly appreciate the collaboration with the laboratory of Steven D. Freedman, M.D., Ph.D., Associate Dean for Clinical and Translational Research at Harvard Medical School, in measuring the fatty acid concentrations of the samples, and the technical support of his assistant, Joanne Brown, MS.

References

Bistrian, B.R., 2003. Clinical aspects of essential fatty acids metabolism: Jonathan Rhoads Lecture. J. Parenter. Enter. Nutr. 27, 168–175.

Chapter ⟨729⟩, 2008. Globule Size Distribution in Lipid Injectable Emulsions. The United States Pharmacopeia 31/National Formulary, 26, pp. 285–87.

DeNardi, L., Bellinati-Pires, R., Torrinhas, R.S., Bacchi, C.E., Arias, V., Waitzberg, D.L., 2008. Effect of fish oil containing parenteral lipid emulsions on neutrophil chemotaxis and resident-macrophages' phagocytosis in rats. Clin. Nutr. 27, 283–288.

Driscoll, D.F., 2004. Examination of selection of light-scattering and light-obscuration acceptance criteria for lipid injectable emulsions. Pharm. Forum 30, 2244–2253.

Driscoll, D.F., 2005. Stability and compatibility assessment techniques for total parenteral nutrition admixtures: setting the bar according to pharmacopeial standards. Curr. Opin. Clin. Nutr. Metab. Care 8, 297–303.

Driscoll, D.F., 2006. Lipid injectable emulsions: pharmacopeial and safety issues. Pharm. Res. 23, 1959–1969.

Driscoll, D.F., 2007a. Globule-size distribution in injectable 20% lipid emulsions: compliance with USP requirements. Am. J. Health-Syst. Pharm. 64, 2032–2036.

Driscoll, D.F., Silvestri, A.P., Mikrut, B.A., Bistrian, B.R., 2007b. Stability of adult-based total nutrient admixtures with soybean oil-based lipid injectable emulsions: the effect of glass versus plastic packaging. Am. J. Health-Syst. Pharm. 64, 396–403.

Driscoll, D.F., Ling, P.R., Bistrian, B.R., 2007c. Physical stability of 20% lipid injectable emulsions via simulated syringe infusion: effects of glass vs. plastic product packaging. J. Parenter. Enter. Nutr. 31, 148–153.

Driscoll, D.F., Thoma, A., Franke, R., Klütsch, K., Nehne, J., Bistrian, B.R., 2009. Fine vs. coarse total nutrient admixtures (TNAs) as 3-chamber plastic (3-C-P) bags over 48 hours. Am. J. Health-Syst. Pharm. 66, 649–656.

Exler, J., 1987. Composition of foods: finfish and shellfish products. In: Agriculture Handbook No. 8–15. U.S. Department of Agriculture, Washington, DC.

Grimm, H., Tibell, A., Norrlind, B., Blecher, C., Wilker, S., Schwemmle, K., 1994. Immunoregulation by parenteral lipids: impact of the n-3 to n-6 fatty acid ratio. JPEN 18, 417–421.

Grimm, H., Mertes, N., Goeters, C., Mayaer, K., Grimminger, F., Fürst, P., 2006. Improved fatty acid and leukotriene pattern with a novel lipid emulsion in surgical patients. Eur. J. Nutr. 45, 55–60, http://www:usp.org/USPNF/pharmacopeialHarmonization/.

Lide DR, 1990. Handbook of Chemistry and Physics, 1990–91, 71st ed. CRC Press, Boston, USA, Fats and Oils, pp. 7–29.

Linseisen, J., Hoffmann, J., Lienhard, S., Jauch, K.W., Wolfram, G., 2000. Antioxidant status of surgical patients receiving TPN with an Ω-3-fatty acid containing lipid emulsion supplemented with α-tocopherol. Clin. Nutr. 19, 177–184.

Martin, C.R., Dumas, G.J., Zheng, Z., MacKinnon, B., Al-Aweel, I., Bistrian, B.R., Pursley, D.M., Driscoll, D.F., 2008. Incidence of hypertriglyceridemia in critically ill neonates receiving lipid injectable emulsions in glass vs. plastic containers: a retrospective analysis. J. Pediatr. 152, 232–236.

Moser, H.W., Moser, A.B., 1991. Measurement of saturated very long chain fatty acids in plasma. In: Techniques in Diagnostic Human Biochemical Genetics: A Laboratory Manual. Wiley-Liss, Inc., New York, pp. 177–191.

Omega-3 Acid Triglycerides, 2005. Monograph No. 1352, European Pharmacopeia, 5.4, pp. 3995–3997.

Omega-3-Säuren-Triglyceride, 2005. Monograph 1352, German Pharmacopeia, Kommentar zur Ph. Eur. 5.0, 22 Lfg. 2005, pp. 1–4.

Senior, J.R., 1968. Physical and Chemical Properties of Medium ChainTriglyceride Oil. Appendix I. University of Pennsylvania Press, Philadelphia, PA, USA, p. 277.

Soriguer, F., Serna, S., Valverde, E., Hernando, J., Martin-Reyes, A., Soriguer, M., Pareja, A., Francisco, T., Esteve, I., 1997. Lipid, protein, and calorie content of different Atlantic and Mediterranean fish, shellfish, and mollusks commonly eaten in the south of Spain. Eur. J. Epidemiol. 13, 451–463.

Stanley, J.C., Elsom, R.L., Calder, P.C., Griffin, B.A., Harris, W.S., Jebb, S.A., Lovegrove, J.A., Moore, C.S., Riemersma, R.A., Sanders, T.A., 2007. UK Food Standards Agency Workshop Report: the effects of the dietary n-6:n-3 fatty acid ratio on cardiovascular health. Br. J. Nutr. 98, 1305–1310.

Waitzberg, D.L., Torrinhas, R.S., Jacintho, T.M., 2006. New parenteral lipid emulsions for clinical use. J. Parenter. Enter. Nutr. 30, 351–367.

Wanten, G.J.A., Calder, P.C., 2007. Immune modulation by parenteral lipid emulsions. Am. J. Clin. Nutr. 85, 1171–1184.