**Assay**—Prepare a filtered and degassed mixture of water and acetonitrile (65:35) that contains 2 mL of trifluoroacetic acid in each 1000 mL of solution. Make adjustments if necessary (see System Suitability under Chromatography (621)).

0.01 M Methanolic sodium hydroxide—Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, add 500 mL of methanol, and mix.

Levothyroxine stock solution—Dissolve an accurately weighed quantity of USP Levothyroxine RS in 0.01 M Methanolic sodium hydroxide to obtain a solution having a known concentration of about 0.4 mg of levothyroxine per mL.

Liothyronine stock solution—Dissolve an accurately weighed quantity of USP Liothyronine RS in 0.01 M Methanolic sodium hydroxide to obtain a solution having a known concentration of about 0.4 mg of liothyronine per mL. Make a 1:10 dilution of this solution using Mobile phase.

Standard preparation—Transfer appropriate volumes of Levothyroxine stock solution and Liothyronine stock solution to a suitable container, and dilute quantitatively and stepwise, if necessary, with Mobile phase to obtain a solution having known concentrations of about 10 µg of levothyroxine per mL and 2.5 µg of liothyronine per mL.

Assay preparation—Transfer 20 Tablets to a 200-mL volumetric flask, add 180 mL of Mobile phase, and sonicate for 15 minutes, occasionally swirling the flask to accelerate the disintegration of the Tablets. Cool to room temperature, and dilute with Mobile phase to volume. Transfer a portion of the solution to a centrifuge tube, and centrifuge for 10 minutes at 5000 rpm. Quantitatively dilute a portion of the clear supernatant with 0.01 M Methanolic sodium hydroxide in 500 mL of water. Cool, add 500 mL of methanol, and mix.

**Lipid Injectable Emulsion**

Lipid Injectable Emulsion used in total parenteral nutrition is a sterile 10 (0.10 g per mL), 20 (0.20 g per mL), or 30 (0.30 g per mL) percent w/v emulsion in an aqueous vehicle. The aqueous phase contains 0.6 percent to 1.8 percent w/v parenteral Egg Phospholipids in Water for Injection and contains, if necessary, an osmotic agent, such as glycerin in amounts of 1.7 percent to 2.5 percent w/v, or a suitable stabilizer, such as a fatty acid salt. The most frequently used oil present is Soybean Oil, which provides an ample supply of the essential fatty acids: linoleic acid and linolenic acid. Other oils, such as Safflower Oil, Medium-Chain Triglycerides, Olive Oil, Fish Oil, or other suitable oils, can be mixed with Soybean Oil. Hence, Soybean Oil can be the only oil or be part of a mixture of these other oils. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of the total oil(s). It contains no antimicrobial agents. The final products are terminally sterilized.

**Packaging and storage**—Preserve in an appropriate container (see Injections (1)). Use elastomeric closures that are compatible with both the oil and water phases of the Emulsion. Store at a temperature not below 4° (protect from freezing) or above 30° (protect from excessive heat).

**Labeling**—The label states the identity and the quantities of the specific oils in the Emulsion. The label states the total osmotic concentration (or osmolarity) in mOsm per L. The labeling contains the following information: do not use if there is evidence of excessive creaming or aggregation, if excessive free oil droplets are visible, or if there are other indications of compromised integrity, such as microbial growth, present in the product.

**USP Reference standards** (11)—

USP Endotoxin RS

**Fatty acid composition**—Transfer a volume of the Emulsion, equivalent to about 200 mg of lipids, to a stopped extraction vessel, add 10 mL of ether, and mix. Add 5 g of anhydrous sodium sulfate, mix, and allow the mixture to stand until separation of the layers is complete. Wet the packing of a chromatographic silica cartridge with a few mL of ether, transfer about 5 mL of the ether layer from the extraction vessel to the column reservoir, and elute at a rate of between 5 and 10 drops per minute into a suitable vessel. Evaporate the ether from the eluant, and dissolve the residue in 5.0 mL of toluene. Transfer 1.0 mL of the toluene solution to a reaction vial, and add 0.4 mL of (m-trifluoromethylphenyl) trimethylammonium hydroxide in methanol. Cover, mix, and allow to stand for 30 minutes. Inject about 1 µL of this solution into a gas chromatograph equipped with a 0.53-mm × 50-m wide-bore, fused-silica capillary column coated with a 2.0-µm thickness of liquid phase G16 and maintained at a temperature of 200°. The column is connected to a flame-ionization detector. Helium is used as the carrier gas at a flow rate of about 10 mL per minute. Measure the main peak areas of the methyl esters of the fatty acids. The relative peak areas expressed as a percentage of the main peaks are in the known ranges for the oil (e.g., Soybean Oil, USP; Safflower Oil, USP) as specified on the label. For oil mixtures, analysis of each oil should be performed to identify known peaks prior to emulsification as specified on the label.

**Bacterial endotoxins** (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.
pH (791): between 6.0 and 9.0.

Globule size limits—The Injectable Emulsion meets the requirements of the limits specified in both Method I and Method II as directed under Globule Size Distribution in Lipid Injectable Emulsions (729).

Limit of oil droplet mean diameters (See Method I—Light Scattering Method under Globule Size Distribution in Lipid Injectable Emulsions (729)—Using the method of light scattering, determine the mean droplet diameter (MDD): the sample meets the requirements. The intensity-weighted mean droplet diameter (MDD) for the Injectable Emulsion must be ≤500 nm, or 0.5 µm, irrespective of the concentration of the dispersed lipid phase.

Limit of large globule volume-diameter (See Method II—Light Obscuration or Extinction Method under Globule Size Distribution in Lipid Injectable Emulsions (729)—Using the method of light obscuration, determine the size distribution of globules in the large-diameter tail of the dispersion (detection threshold ≥2.0 µm). Calculate the volume-weighted mass of lipid in the form of globules with diameters in excess of 5.0 µm per 100 mL of the Injectable Emulsion. The volume-weighted, large-diameter fat globule limits of the dispersed phase, expressed as the percentage of fat residing in globules larger than 5 µm (PFAT5) for a given Injectable Emulsion, is not to exceed 0.05%.

Limit of free fatty acid—

Solvent—Prepare a mixture of heptane, isopropanol, and water (400:400:200) in a separatory funnel. Allow the phases to separate, and discard the lower phase. Filter the upper phase (heptane solution) through 40 g of anhydrous sodium sulfate. Store in a tightly capped glass container, and use within 1 week.

Chromatographic column—Prepare a slurry of heptane and chromatographic silica gel having an average pore size of 6 nm, and activate at a temperature of 110 °C prior to use. Transfer the slurry to a 2.3-cm chromatographic column having an average pore size of 6 nm, and pack to a bed height of between 5 cm and 6 cm. Rinse the column bed before applying the next rinse. Collect a total of 120 mL of eluant; bubble nitrogen through the solution, and titrate with calculated on the anhydrous basis.

Assay—Transfer 20.0 mL of the Injectable Emulsion to a flask, freeze, and lyophilize. Dissolve the residue in 30 mL of Solvent, and transfer the solution to the column. Rinse the flask with three 30-mL portions of Solvent, and transfer the washings to the column, allowing each rinsing to drain to the top of the column bed before applying the next rinse. Collect a total of 120 mL of effluent. Add 10 drops of phenolphthalein TS to the effluent, bubble nitrogen through the solution, and titrate with 0.02 N alcoholic potassium hydroxide VS until the solution remains pale pink after mixing for 10 seconds. Titrate a blank using 120 mL of Solvent. Calculate the quantity, in mEq, of free fatty acids per g of oil in the Injectable Emulsion using the formula:

\[
(V_f - V_b)N / 20C
\]

where \(V_f\) is the volume, in mL, of 0.02 N alcoholic potassium hydroxide consumed by the eluant; \(V_b\) is the volume, in mL, of 0.02 N alcoholic potassium hydroxide consumed by the blank; \(N\) is the normality of the 0.02 N alcoholic potassium hydroxide; and \(C\) is the labeled concentration, in g per mL, of the total oil(s) in the Injectable Emulsion: not more than 0.07 mEq of free fatty acids per g of oil is found.

Other requirements—It meets the requirements under Injections (1).

Assay preparation—Transfer an accurately measured portion of Emulsion, equivalent to about 800 mg of oil, to a 100-mL volumetric flask with the aid of additional portions of Mobile phase. Dilute with Mobile phase to volume, and mix to obtain a solution containing about 8 mg of oil per mL.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a refractive index detector and a 4.1-mm × 25-cm column that contains packing L21. The flow rate is about 1 mL per minute, adjusted so that the peak due to oil elutes at about 6.5 minutes. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the capacity factor, \(k'\), is not less than 1.0; the tailing factor for the oil peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of oil in the portion of Emulsion taken by the formula:

\[
100C(r_0 / r)
\]

in which \(C\) is the concentration, in mg per mL, of Soybean Oil or other relevant oils used in the Emulsion in the Standard preparation; and \(r_0\) and \(r\) are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Lisinopril

\[
\text{C}_{27}\text{H}_{34}\text{N}_{2}\text{O}_{5} \cdot 2\text{H}_{2}\text{O} \quad 441.52
\]

\(\text{L-Proline, 1-[N-((1-carboxy-3-phenylpropyl)-l-lysyl)]-dihydrate, (S)-, 1-[N-[(S)-1-Carb}-\]

\(\text{H}_{2}\text{O} \quad [83915-83-7].\)

It contains not less than 98.0 percent and not more than 102.0 percent of \(\text{C}_{27}\text{H}_{34}\text{N}_{2}\text{O}_{5}\), calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—USP Lisinopril RS

Identification—

A: Infrared Absorption (197M).

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the Standard preparation obtained as directed in the Assay.

Specific rotation (781S): between −115.3° and −122.5° (\(\lambda = 405 \text{ nm.}\))

Test solution: 10 mg per mL, in 0.25 M zinc acetate. Prepare the 0.25 M zinc acetate solution as follows. Mix 600 mL of water with 150 mL of glacial acetic acid and 54.9 g of zinc acetate, and stir to dissolve the zinc acetate. While stirring, add 150 mL of ammonium hydroxide, cool to room temperature, and adjust with ammonium hydroxide to a pH of 6.4. Transfer the solution to a 1000-mL volumetric flask, dilute with water to volume, and mix.