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Evening Primrose Oil Capsules

DEFINITION
Evening Primrose Oil Capsules are prepared with Evening Primrose Oil and contain NLT 95.0% of the labeled amounts of each, γ -linolenic (C18:3 n–6), linoleic (C18:2 n–6), and oleic (C18:1 n–9) acids.

IDENTIFICATION

• **A. FATTY ACID PROFILE**

System suitability solution and **Chromatographic system:** Proceed as directed in *Strength*.

Sample solution: Proceed as directed for the *Sample solution* in *Strength*, beginning with “Transfer 80 mg” but without the addition of the *Internal standard*.

Analysis: Identify the specified fatty acid methyl ester peaks by comparing them to the reference chromatogram provided with the lot of [USP Evening Primrose Oil RS](#) being used. Determine the percentage of each constituent relative to the total integrated area.

Acceptance criteria: The *Sample solution* conforms to the fatty acids composition profile in [Table 1](#).

Table 1

Fatty Acid	Shorthand Notation	Percentage (%)
Palmitic acid	16:0	4.0–10.0
Stearic acid	18:0	1.0–4.0
Oleic acid	18:1 n–9	5.0–12.0
Linoleic acid	18:2 n–6	65.0–85.0
γ -Linolenic acid	18:3 n–6	7.0–14.0

STRENGTH

• **CONTENT OF γ -LINOLENIC, LINOLEIC, AND OLEIC ACIDS**

[NOTE— γ -Linolenic acid is quantitated against [USP Methyl Linolenate RS](#).]

0.5 N methanolic sodium hydroxide solution: Dissolve 2 g of sodium hydroxide in 100 mL of methanol.

Internal standard: Methyl pentadecanoic acid

Sample solution: Weigh NLT 10 Capsules. With a sharp blade, carefully slice open the Capsules, avoiding loss of shell material. Combine the Capsule contents in a suitable container and mix well. Remove any adhering substance from the emptied Capsules by washing with several portions of diethyl ether, and discard the washings. Allow the empty Capsule shells to air-dry over a period of NMT 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the empty Capsule shells and calculate the average fill weight/Capsule (A_F).

Transfer 80 mg of the combined Capsule contents directly into a tared 30-mL screw-top glass centrifuge tube and weigh accurately. Re-tare and accurately weigh about 40 mg of the *Internal standard* into the same tube. Add 2 mL of 0.5 N methanolic sodium hydroxide solution, tightly cap, and transfer to a heating block or another appropriate heating device. Reflux the solution until fat globules disappear (usually 5–10 min). Add 2 mL of 0.14 g/mL boron trifluoride in methanol, cap, and reflux for 2 min. Add 4 mL of chromatographic *n*-heptane, cap, and reflux for 1 min. Cool, add about 8 mL of saturated sodium chloride solution, shake, and centrifuge to separate the layers. Dilute an aliquot of the upper (heptane) layer with chromatographic *n*-heptane (1:8) and mix well.

System suitability solution: Using about 80 mg of [USP Evening Primrose Oil RS](#), proceed as directed for the *Sample solution*, beginning with “Transfer 80 mg” but without the addition of the *Internal standard*.

Standard solution: Accurately weigh about 20 mg of [USP Methyl Oleate RS](#), 70 mg of [USP Methyl Linoleate RS](#), and 20 mg of [USP Methyl Linolenate RS](#) directly into a tared 30-mL screw-top glass centrifuge tube. Proceed as directed for the *Sample solution*, beginning with “Re-tare”.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

- Mode:** GC
- Detector:** Flame ionization
- Column:** 0.53-mm × 30-m fused silica capillary; coated with a 1.0-µm film of G16
- Temperatures**
 - Injection port:** 220°
 - Detector:** 260°
 - Column:** See [Table 2](#).

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	0	70	2
70	5	240	5

- Carrier gas:** Helium
- Linear velocity:** 50 cm/s
- Split mode:** Splitless
- Injection volume:** 1 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

- Chromatogram similarity:** The *System suitability solution* chromatogram is similar to the reference chromatogram provided with the lot of [USP Evening Primrose Oil RS](#) being used.
- Resolution:** NLT 1.5 between methyl stearate and methyl oleate, *System suitability solution*
- Relative standard deviation:** NMT 2% for the peak area ratios of specified analytes to the internal standard, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

[NOTE—The relative retention times for γ-methyl linolenate and α-methyl linolenate are about 0.98 and 1.0, respectively.]

Identify the retention times of the relevant fatty acid methyl esters by comparing the peaks in the chromatogram of the *System suitability solution* with those in the reference chromatogram. Identify the locus for the internal standard peak by comparison of the chromatograms of the *Standard solution* and the *System suitability solution*.
Calculate the content, in mg/g, of γ-linolenic, linoleic, and oleic acids in the portion of Capsule content taken:

Result = $(R_U/R_S) \times (A_U/A_S) \times (m_S/W) \times (M_{r1}/M_{r2})$

- R_U = peak area ratio of the relevant methyl ester peak to the internal standard peak from the *Sample solution*
- R_S = peak area ratio of the relevant methyl ester peak to the internal standard peak from the *Standard solution*
- A_U = weight of the *Internal standard* in the *Sample solution* (mg)
- A_S = weight of the *Internal standard* in the *Standard solution* (mg)
- m_S = weight of the relevant [USP Methyl Ester RS](#) in the *Standard solution* (mg)
- W = sample weight used to prepare the *Sample solution* (g)
- M_{r1} = molecular weight of the relevant fatty acid (g/mol)