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Spirulina Tablets

DEFINITION
Spirulina Tablets are prepared from Spirulina. It contains NLT 100% of the labeled amount of spirulina, represented by a content of beta carotene of NLT 0.15% of the labeled amount of spirulina, a content of total carotenoids of NLT 0.35% of the labeled amount of spirulina, and a content of C-phycocyanin of NLT 6.0% of the labeled amount of spirulina.

IDENTIFICATION

• **A. FATTY ACIDS PROFILE**

Methyl oleate solution: 1 mg/mL of [USP Methyl Oleate RS](#) in *n*-heptane

Standard solution: Dissolve quantities of [USP Methyl Palmitate RS](#), [USP Methyl Palmitoleate RS](#), [USP Methyl Stearate RS](#), [USP Methyl Oleate RS](#), [USP Methyl Linoleate RS](#), [USP Methyl Linolenate RS](#) (methyl alpha linolenate), and methyl gamma linolenate (obtained commercially) in *n*-heptane to obtain concentrations of each methyl ester as given in [Table 1](#).

Table 1

Methyl Ester	Relative Retention Time	Concentration (mg/mL)
Methyl palmitate	0.76	0.8
Methyl palmitoleate	0.79	0.8
Methyl stearate	0.97	0.8
Methyl oleate	1.00	0.8
Methyl linoleate	1.06	0.8
Methyl gamma linolenate	1.12	0.8
Methyl alpha linolenate	1.18	0.8

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer an amount of the powder, equivalent to about 100 mg of spirulina, to a 50-mL round-bottom flask, fitted with a water-cooled reflux condenser and a magnetic stir bar. Add 100 mg of pyrogalllic acid, 10 mL of 0.5 N methanolic sodium hydroxide solution (20 mg/mL of sodium hydroxide in methanol), and heat the flask while stirring and under reflux for 15 min. Add 5 mL of a solution of 14% boron trifluoride–methanol through the reflux condenser into the flask, and continue boiling for 2 more min. Add 5.0 mL of *n*-heptane through the condenser, and continue boiling for an additional 1 min. Cool the flask, remove the condenser, and add 15 mL of saturated sodium chloride solution. While the solution is still tepid, insert a stopper into the flask, shake vigorously, and allow the layers to separate. Add sufficient saturated sodium chloride solution to bring the upper *n*-heptane layer up into the neck of the flask. Transfer the *n*-heptane layer into a suitable container, add a small quantity of anhydrous sodium sulfate (previously washed with *n*-heptane), and shake. [NOTE—Fatty acids methyl esters should be analyzed as soon as possible. They may be kept in an atmosphere of nitrogen at 2° for 24 h.]

Blank: Use *n*-heptane.

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

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused silica capillary; 0.25-µm film of phase G16 coating

Temperatures

Injector: 220°

Detector: 260°

Column: See [Table 2](#).

Table 2

Initial Temperature (°)	Hold Time at 170° (min)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	2	5	240	5

Carrier gas: Helium

Flow rate: 1 mL/min

Split ratio: 100:1. [NOTE—If necessary, adjust the *Split ratio* and/or sample dilution to obtain a tailing factor of 0.8–1.5 for the fatty acid methyl ester peaks.]

Injection volume: 1 µL

System suitability

Samples: *Methyl oleate solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methyl stearate and methyl oleate peaks

Relative standard deviation: NMT 6.0% for the peak responses of each of the methyl palmitate and methyl stearate peaks for replicate injections; NMT 1.0% for the peak response ratio of the methyl palmitate to methyl stearate peaks in replicate injections

Analysis

Samples: *Methyl oleate solution*, *Standard solution*, *Sample solution*, and *Blank*

Identify the fatty acid ester peaks in the chromatogram of the *Sample solution* based on the relative retention times provided in [Table 1](#) and by comparison to the chromatograms of the *Methyl oleate solution* and the *Standard solution*. Measure the peak response for all of the fatty acid ester peaks from the *Sample solution*.

Calculate the percentage of each fatty acid component in the portion of Tablets taken:

$$\text{Result} = (A/B) \times 100$$

A = peak response of individual fatty acid ester from the *Sample solution*

B = sum of responses of all peaks, excluding the solvent, from the *Sample solution*

Acceptance criteria: See [Table 3](#) for individual fatty acids.

Table 3

Fatty Acids	Percentage of Total (%)
Palmitic acid	35–60
Palmitoelcic acid	2–8
Stearic acid	1–5
Oleic acid	1–7
Linoleic acid	13–25
Gamma linolenic acid	13–27
Alpha linolenic acid	<0.5

Analysis: Proceed as directed in the test for *Content of Beta Carotene and Total Carotenoids*, except analyze the *Sample solution* and *Standard solution* at 432 nm.

Acceptance criteria: The major peak in the *Sample solution* chromatogram is due to chlorophyll A, at a relative retention time of about 0.8 to the peak of all-*trans*-beta carotene.

• **C. HPLC FOR CAROTENOIDS**

Analysis: Proceed as directed in the test for *Content of Beta Carotene and Total Carotenoids*.

Acceptance criteria: The major peak from the *Sample solution* corresponds to that of all-*trans*-beta carotene from the *Standard solution*. The *Sample solution* chromatogram exhibits additional peaks due to oscillol rhamnoside, myxol rhamnoside, zeaxanthin, alloxanthin, echinenone, beta cryptoxanthin, and *cis*-isomers of beta carotene as shown in [Table 5](#). [NOTE—The *cis*-beta carotene peaks may partially be resolved from the all-*trans*-beta carotene peak.]

STRENGTH

• **CONTENT OF BETA CAROTENE AND TOTAL CAROTENOIDS**

[NOTE—It is important to have all glassware ready before starting the test. Minimize exposure of the *Standard solution* and *Sample solution* to light. Proceed under subdued light using low-actinic glassware at a room temperature not to exceed 25°. The *Standard solution* and *Sample solution* are stable for 12 h.]

Solution A: A mixture of a solution of 0.05% butylated hydroxytoluene in methanol and water (9:1). Allow the mixture to equilibrate to room temperature before adjusting to final volume with the solution of 0.05% butylated hydroxytoluene in methanol.

Solution B: A mixture of a solution of 0.05% butylated hydroxytoluene in methanol and methylene chloride (88:12). Allow the mixture to equilibrate to room temperature before adjusting to final volume with the solution of 0.05% butylated hydroxytoluene in methanol.

Mobile phase: See [Table 4](#).

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1.0	95	5
2.5	0	100
10.75	0	100
11.00	95	5
16	95	5

Diluent: 50 µg/mL of butylated hydroxytoluene in methanol

Standard stock solution: 500 µg/mL of [USP Beta Carotene RS](#) in methylene chloride. [NOTE—Prepare fresh at the time of use, and minimize the time before it is used to prepare the *Standard solution*.]

Standard solution: 2.5 µg/mL of [USP Beta Carotene RS](#) in *Diluent* from *Standard stock solution*.

Determine the concentration of the *Standard solution* as follows.

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Mode: Visible

Analytical wavelength: 450 nm

Cell path: 1 cm

Blank: *Diluent*

Take three measurements using three different portions of the *Standard solution*.

Calculate the concentration of beta carotene (µg/mL) in the *Standard solution*.

$$C_s = (A/F) \times (P/100)$$

A = average absorbance of the three portions of *Standard solution*

F = one thousandth of the absorptivity value of beta carotene in *Diluent*, 0.25 mL · µg⁻¹ · cm⁻¹

