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Chia Seed Oil

CAS RN[®]: 93384-40-8.

DEFINITION

Chia Seed Oil is derived from the seeds of the Chia plant (*Salvia hispanica* L.). The oil is extracted from the seeds by cold pressing. No solvents or external heat are employed in the extraction process. Tocopherol may be added as an antioxidant.

IDENTIFICATION

- **A.** It meets the requirements in *Specific Tests* for [Fats and Fixed Oils \(401\)](#), [Procedures](#), [Fatty Acid Composition](#).
- **B.** [IDENTIFICATION OF FIXED OILS BY THIN-LAYER CHROMATOGRAPHY \(202\)](#): The R_f values of the principal spots of the *Sample solution* correspond to those of the *Standard solution*.
- **C.** It meets the requirements in *Specific Tests* for *Sterol Composition*.

SPECIFIC TESTS

- [FATS AND FIXED OILS \(401\)](#), [Procedures](#), [Acid Value](#): NMT 2.5
- [FATS AND FIXED OILS \(401\)](#), [Procedures](#), [Peroxide Value](#): NMT 10.0
- [FATS AND FIXED OILS \(401\)](#), [Procedures](#), [Iodine Value](#): 180–210
- [FATS AND FIXED OILS \(401\)](#), [Procedures](#), [Saponification Value](#): 180–230
- [FATS AND FIXED OILS \(401\)](#), [Procedures](#), [Unsaponifiable Matter](#): NMT 1.5
- [FATS AND FIXED OILS \(401\)](#), [Procedures](#), [Fatty Acid Composition](#): Chia Seed Oil exhibits the composition profile of fatty acids in [Table 1](#).

Table 1

Fatty Acid	Shorthand Notation	Area Percentage (%)
Palmitic acid	16:0	5.0–9.0
Stearic acid	18:0	2.0–5.0
Oleic acid	18:1	4.0–9.0
Linoleic acid	18:2	17.0–22.0
Alpha-linolenic acid	18:3 (n–3)	57.0–70.0
Gamma-linolenic acid	18:3 (n–6)	0.0–0.4

• STEROL COMPOSITION

Internal standard solution: 0.3 mg/mL of [USP Cholesterol RS](#) in [2-propanol](#)

Standard solution: Transfer 50 mg of [USP Chia Seed Oil RS](#) to a 25-mL screw-cap test tube, add 2.0 mL of [2-propanol](#), and sonicate to dissolve. Add 3.0 mL of 1 M methanolic potassium hydroxide and 0.8 mL of *Internal standard solution* to the test tube. Cap the test tube and place in a hot water bath at 80° for 60 min. Remove the test tube from the water bath and cool to room temperature, then add 3 mL each of [water](#) and [n-hexane](#). Mix the solution with a vortex mixer or shake vigorously for at least 30 s. Immediately add 5 mL of saturated [sodium chloride](#), cap, and mix with a vortex mixer or shake thoroughly for at least 15 s. Let the solution stand for 5 min, or until the upper layer becomes clear, and transfer to a separate tube. Shake the lower layer once more with 3 mL of [n-hexane](#), and combine the hexane extracts. Evaporate the hexane extracts with the aid of a nitrogen stream to dryness. Add 0.3 mL of [pyridine](#) silylated with 1.0 mL of BSA+TMCS+TMSI mixture (3:2:3)¹ and let it stand at room temperature for 15 min. Inject this solution into a gas chromatograph.

Sample solution: Prepare as directed for the *Standard solution*, except replace [USP Chia Seed Oil RS](#) with Chia Seed Oil.

Chromatographic system

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

Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m fused silica capillary, bonded with a 0.25-µm film of phase [G27](#)
Temperatures
Injection port: 240°
Detector: 325°
Column: See [Table 2](#).

Table 2

Initial Temperature (°)	Hold Time at 240° (min)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
240	3	2	300	7

Carrier gas: Helium
Flow rate: 1.5 mL/min
Split ratio: 2:1
Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between β-sitosterol and Δ5-avenasterol
Relative standard deviation: NMT 2.0% for the ratios of β-sitosterol to internal standard peak responses from replicate injections
Chromatogram similarity: The chromatogram from the *Standard solution* is similar to the reference chromatogram supplied with [USP Chia Seed Oil RS](#). Identify the retention times of six relevant sterol methyl esters by comparing the chromatogram of the *Standard solution* with the reference chromatogram supplied with [USP Chia Seed Oil RS](#). The retention times of the sterols with reference to β-sitosterol are given in [Table 3](#).

Table 3

Identification	Relative Retention Time
Cholesterol (internal standard)	0.73
Campesterol	0.91
Stigmasterol	0.94
β-Sitosterol	1.00
Δ5-Avenasterol	1.02
Cycloartenol	1.06
24-Methylenecycloartenol	1.11

Analysis

Sample: *Sample solution*

Calculate the area percentage of each individual sterol in the portion of Chia Seed Oil taken:

Result = $(R_U/R_T) \times 100$

R_U = peak response ratio of each sterol component to the internal standard (internal standard ratio) from the *Sample solution*

R_T = sum of six internal standard ratios from the *Sample solution*

Acceptance criteria: Chia Seed Oil exhibits six sterol components, each with the normalized area percentage shown in [Table 4](#).

Table 4