

Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-Aug-2017
Document Type: Dietary Supplement Monographs
DocId: GUID-AE8CF649-9A0B-4F77-81E6-228E3AD9A10B_2_en-US
DOI: https://doi.org/10.31003/USPNF_M9975_02_01
DOI Ref: yvl4q

© 2025 USPC
Do not distribute

Plant Stanol Esters

DEFINITION

Plant Stanol Esters consists mainly of the fatty acid esters of plant sitostanol (24-ethylcholestan-3 β -ol) and plant campestanol (24-methylcholestan-3 β -ol). The sum of campestanol and sitostanol is NLT 56% (w/w). It is produced from esterification of plant stanols with fatty acids from rapeseed oil, sunflower oil, corn oil, soybean oil, and their blends. It can be a liquid or solid depending on the temperature and on the fatty acids with which plant stanols are esterified. A suitable antioxidant may be added.

IDENTIFICATION

- **A.** It meets the acceptance criteria for the *Content of Total Stanols* and *Content of Unesterified Stanols* tests.
- **B.** The retention times of the campestanol trimethylsilyl (TMS) and sitostanol TMS peaks from the *Derivatized sample solution* in the test for *Content of Total Stanols* correspond to the campestanol TMS and sitostanol TMS peaks from the *Derivatized standard solution*.

COMPOSITION

• CONTENT OF TOTAL STANOLS

Silylation reagent: Bis(trimethylsilyl)-trifluoroacetamid (BSTFA) with 1% trimethylchlorosilane (TMCS)¹

Saponification solution: 2 M potassium hydroxide solution in ethanol (11.22 g of [potassium hydroxide](#) dissolved in 100 mL of [ethanol](#))

Internal standard solution: 0.6 mg/mL of [USP Epicoprostanol RS](#) in *n*-propanol

Derivatized internal standard solution: Transfer 500 μ L of the *Internal standard solution* into a 12-mL screw-cap test tube and evaporate to dryness in a vacuum oven or under nitrogen flow. Add 200 μ L of *Silylation reagent* and 100 μ L of [pyridine](#) to the test tube, mix well, and heat for 15 min at 70°. Cool the solution to room temperature before injecting into the gas chromatograph.

Standard solution: Melt a portion of [USP Plant Stanol Esters RS](#) in an oven or microwave oven to obtain a clear and homogenous liquid. Transfer 40–70 mg of the melted [USP Plant Stanol Esters RS](#), accurately weighed, to a 25-mL volumetric flask and dissolve in [tetrahydrofuran](#). Sonicate the solution using an ultrasonic bath for about 15 min. After sonication, allow the flask to cool to ambient temperature (for about 1 h) before filling up to the mark with [tetrahydrofuran](#), and mix well.

Derivatized standard solution: Transfer 500 μ L each of the *Internal standard solution* and the well-mixed *Standard solution* to a 12-mL screw-cap test tube. Evaporate the sample to dryness either under moderate nitrogen flow or in a vacuum oven (temperature not higher than 80°). Add 2.5 mL of *Saponification solution* to the residue, close the test tube cap tightly, mix well on a vortex mixer, and reflux in an ultrasonic bath with a heater at a temperature of 65°–75° for 1 h. Add 2 mL of deionized water and 3 mL of *n*-heptane to the mixture, mix on a vortex mixer for 1 min, and wait until the layers separate. If necessary, centrifuge the test tubes to separate the layers. Transfer the *n*-heptane fraction to another test tube and repeat the extraction of the aqueous phase with 3 mL of *n*-heptane. Collect the *n*-heptane fractions containing plant stanols and plant sterols in the same test tube and evaporate to dryness under moderate nitrogen flow. During the evaporation, test tubes can be warmed (approximately up to 70°). Transfer 200 μ L of *Silylation reagent* and 100 μ L of [pyridine](#) to the test tube, mix well, and heat for 15 min at 70°. Cool the solution to room temperature before injecting into the gas chromatograph.

Sample solution: Prepare as directed for the *Standard solution* except replace the [USP Plant Stanol Esters RS](#) with Plant Stanol Esters.

Derivatized sample solution: Prepare as directed for the *Derivatized standard solution* except replace the *Standard solution* with *Sample solution*.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.32-mm x 30-m fused silica capillary; bonded with a 0.25- μ m film of phase G27

Temperatures

Injection port: 310°

Detector: 310°

Column: 300°

Carrier gas: Helium

Flow rate: 1 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 50:1

System suitability

Samples: *Derivatized internal standard solution* and *Derivatized standard solution*

Suitability requirements

[NOTE—Locate the epicoprostanol (internal standard) peak in the chromatogram of the *Derivatized standard solution* by comparing its retention time with that of the epicoprostanol peak in the chromatogram of the *Derivatized internal standard solution*. The relative retention times of the components in the *Derivatized standard solution* are shown in [Table 1](#).]

Resolution: NLT 1.0 between sitosterol and sitostanol, *Derivatized standard solution*

Relative standard deviation: NMT 2.0% for the epicoprostanol peak, *Derivatized internal standard solution*

Chromatogram similarity: The chromatogram obtained from the *Derivatized standard solution* is similar to the reference chromatogram provided with the lot of [USP Plant Stanol Esters RS](#) being used.

Table 1

Name	Relative Retention Time
Episcoprostanol	1.00
Cholestanol	1.17
Campesterol	1.34
Campestanol	1.37
Sitosterol	1.54
Sitostanol	1.57

Analysis

Sample: *Derivatized sample solution*

[NOTE—The response factor of epicoprostanol is equivalent to that of campestanol or sitostanol.]

Separately calculate the percentage (w/w) of campestanol and sitostanol in the portion of Plant Stanol Esters taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response of campestanol or sitostanol from the *Derivatized sample solution*

r_S = peak response of epicoprostanol from the *Derivatized sample solution*

C_S = concentration of [USP Epicoprostanol RS](#) in the *Derivatized sample solution* (mg/mL)

C_U = concentration of Plant Stanol Esters in the *Derivatized sample solution* (mg/mL)

F = purity factor of [USP Epicoprostanol RS](#) (obtained from the RS label)

Acceptance criteria

Sum of campestanol and sitostanol: NLT 56% (w/w) of total stanols [sum of percentages (w/w) of campestanol and sitostanol]. The percentage of campestanol and sitostanol in total stanols is NMT 32% and NLT 68%, respectively.

• CONTENT OF UNESTERIFIED STANOLS

Silylation reagent, Internal standard solution, Derivatized internal standard solution, and Chromatographic system: Proceed as directed in the test for *Content of Total Stanols*.

Sample solution: Transfer 400 µL of the *Internal standard solution* to a 12-mL screw-cap test tube and evaporate to dryness either under moderate nitrogen flow or in a vacuum oven (temperature not higher than 80°). Melt a portion of Plant Stanol Esters in an oven or microwave oven to obtain a clear and homogenous liquid. Transfer 30–70 mg of the melted Plant Stanol Esters, accurately weighed, to the