PHYTOSTEROLS, PHYTOSTANOLS AND THEIR ESTERS

New specifications prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008). An ADI of 0-40 mg/kg bw, expressed as the sum of phytosterols and phytostanols in their free form, was established at the 69th JECFA (2008).

SYNONYMS

Plant sterols/stanols, Plant sterol/stanol esters, Phytosterol/Phytostanol esters

DEFINITION

Phytosterols, phytostanols and their esters are a group of steroid alcohols and esters that occur naturally in plants. The B-ring of the steroidal moiety of phytosterols is unsaturated in the 5-6 position and is saturated in phytostanols. Phytosterols and phytostanols are isolated from deoderizer distillate (a by-product of edible oil production), or derived from tall oil (a by-product of wood pulp manufacture). They are purified by distillation, extraction, crystallization and washing resulting in products of high purity. Phytosterol blends derived from either vegetable oils or tall oil may be converted to the corresponding phytostanols by catalytic saturation. Some phytosterols and phytostanols may be extracted as esters of fatty acids. Esters are also produced by reacting the sterol/stanols with fatty acids derived from food grade vegetable oils. The fatty acid ester chain may be saturated, mono- or polyunsaturated depending on the source of the vegetable oil. Commercial products may be mixtures of phytosterols, phytostanols and their esters. The production process may include the use of hexane, 1-propanol, ethanol and methanol.

Chemical names

The major free phytosterols and phytostanols are listed below. In some preparations they are esterified with vegetable oil fatty acids.

Sitosterol: (3β)-Stigmast-5-en-3-ol
Sitostanol: (3β,5α)-Stigmastan-3-ol
Campesterol: (3β)-Ergost-5-en-3-ol
Campestanol: (3β,5α)-Ergostan-3-ol
Stigmasterol: (3β)-Stigmasta-5,22-dien-3-ol
Brassicasterol: (3β)-Ergosta-5,22-dien-3-ol

Esters of sitostanol: for example, sitostanyl oleate
Esters of campesterol: for example, campesteryl oleate

C.A.S numbers

The major free phytosterols and phytostanols are listed below. In some preparations they are esterified with vegetable oil fatty acids. Esterified forms have not been assigned C.A.S numbers

Sitosterol: 83-46-5
Sitostanol: 83-45-4
Campesterol: 474-62-4
Campestanol: 474-60-2
Stigmasterol: 83-48-7
Brassicasterol: 474-67-9

Chemical formula

The major free phytosterols and phytostanols are listed below. In

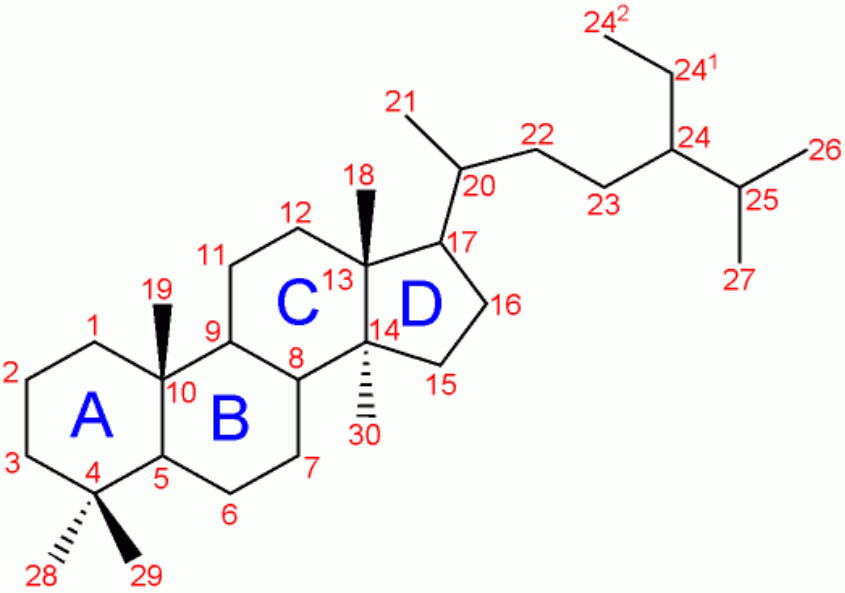
some preparations they are esterified with vegetable oil fatty acids ranging in chain-length from C14 to C18.

Sitosterol: $C_{29}H_{50}O$
Sitostanol: $C_{29}H_{52}O$
Campesterol: $C_{28}H_{48}O$
Campestanol: $C_{28}H_{50}O$
Stigmasterol: $C_{29}H_{48}O$
Brassicasterol: $C_{28}H_{46}O$

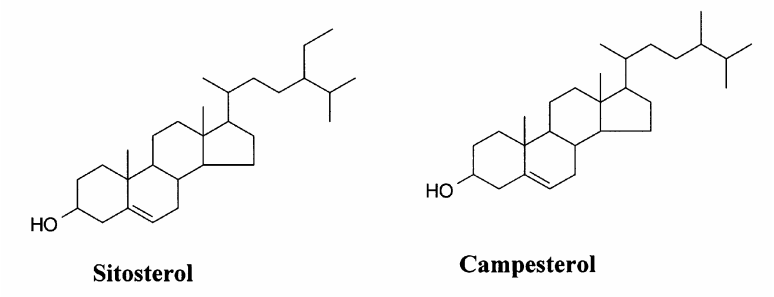
Examples of phytosteryl and phytostanyl esters:
Campesteryl oleate: $C_{46}H_{81}O_2$
Sitostanyl oleate: $C_{47}H_{85}O_2$

Structural formulae

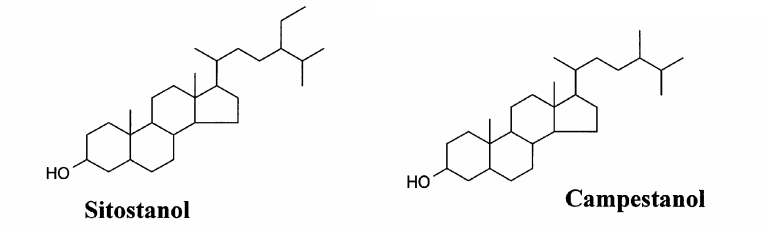
Steroid skeleton



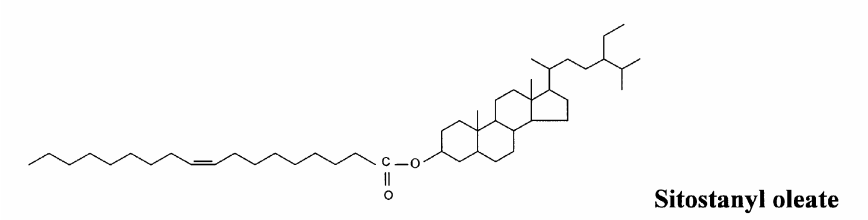
Some examples of phytosterols, phytostanols and a phytostanyl ester



Sitosterol **Campesterol**



Sitostanol **Campestanol**



Sitostanyl oleate

Formula weight

Sitosterol: 414.72
Sitostanol: 416.73
Campesterol: 400.69
Campestanol: 402.70
Stigmasterol: 412.67
Brassicasterol: 398.67

Examples of phytosteryl and phytostanyl esters:
Campesteryl oleate: 683.19
Sitostanyl oleate: 699.19

Assay

Products containing only free sterols and stanols: not less than 95% on a total free sterol/stanol basis.
Products containing only esterified sterols and stanols: not less than 55% sterol/stanol on a saponifed sample.
Products that are mixtures of free and esterified sterols and stanols: the content of stanols/sterols ranges between 55 and 95% as determined by measurement of free sterols/stanols in a native and saponified sample.
Difference between 55% and 95% is attributable to the fatty acid ester component.

DESCRIPTION

Free-flowing, white to off-white powders, pills or pastilles; colourless to pale yellow liquids

FUNCTIONAL USE

This preparation serves no technological purpose in food. It is added to food as a source of phytosterols and phytostanols.

CHARACTERISTICS

IDENTIFICATION

Solubility

Practically insoluble in water.
Phytosterols and phytostanols are soluble in acetone and ethyl acetate.
Phytosterol and phytostanol esters are soluble in hexane, iso-octane and 2-propanol

Gas Chromatography (Vol. 4)

The retention time for the major peak of a saponified sample in a GC chromatogram of the sample corresponds to that of the β-sitosterol/sitostanol standard using the conditions described in the Method of Assay. The relative retention times of β-sitosterol/sitostanol are approximately 1.066 and 1.073, respectively.

PURITY

Total ash (Vol. 4)

Not more than 0.1 %

Residual solvents (Vol. 4)

Hexane, 1-propanol, ethanol or methanol: 50 mg/kg either singly or in combination

Water (Vol. 4)

Not more than 4% (Karl Fischer). The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Water Determination”)

Arsenic (Vol. 4)

Not more than 3 mg/kg
Determine by the atomic absorption hydride technique. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

Lead (Vol. 4)

Not more than 1 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY

Principle

Sterols/stanols are silylated and analysed by gas chromatography with flame ionization detection (Volume 4, “Analytical Techniques, Chromatography”). Esterified sterols/stanols are first saponified and the non-polar components are extracted, dried and silylated. For quantification an internal standard is added to the sample.

Sample preparation

a. Free sterols/stanols

Accurately weigh approximately 15 mg 5α-cholestane and approximately 50 mg sterol concentrate into a reaction vial. Add approximately 1 ml methyl tert-butyl ether (MTBE) to dissolve the sample. Warm to 40 – 50° to improve solubility. Add 4.0 ml hexane

and mix. Transfer 50 µl of the solution to a small test-tube and evaporate to dryness under nitrogen at 50 – 60°. Add 60 µl N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 240 µl pyridine, mix, cap the tube and heat at 60 – 70° for approximately 30 minutes. Mix the solution after 5 – 10 minutes. Add 1.7 ml heptane, mix and transfer the solution to a GC vial.

b. Sterol/stanol esters
Accurately weigh approximately 15 mg 5α-cholestane and approximately 100 mg sterol ester accurately into a reaction vial. Add 2 ml ethanolic potassium hydroxide solution (6.6 g KOH in 50 ml ethanol), mix and heat for 90 minutes at 70°. Mix the solution every 15 minutes during saponification. Add 1 ml water and 4 ml heptane to the saponified solution and mix thoroughly for 15 seconds. Wait until the two layers separate completely and transfer the heptane extract to a test-tube. Repeat the extraction twice with 4 ml heptane, collect all three heptane extracts in the same test tube and mix thoroughly. Transfer 50 µl of the solution to a small test-tube and evaporate to dryness under nitrogen at 70 – 80°. Add 60 µl BSTFA and 240 µl pyridine, mix, cap the tube and heat at 60 – 70° for approximately 30 minutes. Mix the solution after 5 – 10 minutes. Add 1.7 ml heptane, mix and transfer the solution to a GC vial.

Equipment
Gas chromatograph, suitable for capillary columns equipped with:
- flame ionization detector (FID)
- cold on-column injector
- autosampler

Capillary column:
- Precolumn: uncoated fused silica capillary, (apolar deactivated), 1.0 m x 0.53 mm i.d. (e.g. Interscience, HRGC precolumn, code 26060370, or equivalent)
- Analytical column 1: CP SIL 13CB, (length 25 m, 0.25 mm i.d.) 0.2 µm film thickness (the dimensions of the column may be altered to accommodate commercially available columns)
- Analytical column 2: CP SIL 8CB, (length 30 m, 0.25 mm i.d.) 0.25 µm film thickness (the dimensions of the column may be altered to accommodate commercially available columns)
All columns are to be connected together with glass quick-seal connectors.

Suitable GC conditions:
- Helium carrier gas flow: 0.9 ml/min
- Detector Temperature: 325°
- FID flow air: 300 ml/min
- FID flow H_2: 30 ml/min
- FID flow makeup N_2: 30 ml/min

Procedure
Inject 0.5 µl of the sample into the gas chromatograph and run according to the following oven temperature program: 60° (for 1 min), then 15°/min up to 250°, then 2°/min up to 300° (hold for 18 min).

Peak assignment and identification of individual components
Identify the main components using a reference sample of known composition. The table of relative retention times given below should

be used as a further guide. All other peaks should be identified as unknown.

Component	Relative retention time (-)
5α-cholestane (internal standard)	0.761
Cholesterol	0.929
Cholestanol	0.934
Brassicasterol	0.958
Cholestanone	0.967
24-methylcholesterol	0.989
Campesterol	1.000
Campestanol	1.007
Stigmasterol	1.021
Unidentified stanol	1.028
δ7-campesterol	1.044
Unidentified sterol 1	1.048
Clerosterol	1.053
Sitosterol	1.066
Sitostanol	1.073
δ5-avenasterol	1.080
Unidentified sterol 2	1.094
δ7-stigmastenol	1.103
δ7-avenasterol	1.115
Unidentified sterol 3	1.133

Calculation of result

Calculation of the concentration of the individual components (mg/kg)

$$C_I = \frac{C_{IS} \times V_{IS} \times A_{component} \times PURITY_{IS} \times 10^6}{A_{IS} \times W_s \times RF}$$

where:
C_I = component
C_{IS} = internal standard concentration (mg/ml)
V_{IS} = internal standard volume (ml)
$A_{component}$ = peak area of individual component
$PURITY_{IS}$ = purity internal standard (%)
A_{IS} = internal standard peak area
W_s = sample weight (mg)
RF = response factor of FID, RF = 1.05 for stanols and 1.00 for other components

Report all sterols/stanols individually. Report the sum of the unidentified sterols/stanols as “unknown sterols/stanols”. Report all other peaks in the chromatogram as unknowns (sum value).