INTERNATIONAL STANDARD

ISO 23349

First edition
2020-01

Animal and vegetable fats and oils — Determination of sterols and stanols in foods and dietary supplements containing added phytosterols

Corps gras d'origines végétale et animale — Dosage des stérols et des stanols dans les aliments et les compléments alimentaires contenant des phytostérols ajoutés

Reference number
ISO 23349:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Plant sterols and plant stanols, collectively referred to as phytosterols, are increasingly recognized for their role in reducing the risk of coronary heart disease by lowering serum total and low-density lipoprotein cholesterol. Because of these potential health benefits, health claim regulations authorizing the addition of phytosterols and phytosterol esters to foods and dietary supplements now exist in countries throughout Europe, North and South America, Asia, New Zealand and Australia.

This document was developed in response to the worldwide demand for a reference method for quantifying total and individual phytosterols in foods and dietary supplements containing added phytosterols, and in the phytosterol food additive concentrates used to prepare such products. This reference method is based on the single-laboratory validated methods of Clement et al.[1] and Srigley and Haile[2] for the preparation and gas chromatographic separation of phytosterol trimethylsilyl ether derivatives, respectively.

In 2016 to 2017, an international collaborative study co-organized by the United States Food and Drug Administration (FDA), Cargill (USA) and the American Oil Chemists' Society (AOCS) was carried out to evaluate the performance of this method for the determination of total and individual phytosterols in foods, dietary supplements and phytosterol concentrates.[3] A total of 14 laboratories from 6 countries successfully completed the analysis of 18 test materials, upon which the method was approved as AOCS Official Method Ce 12-16[4].

This reference method is appropriate for the determination of the five major phytosterols (i.e. campesterol, campestanol, stigmasterol, β-sitosterol and sitostanol) that are the subject of FDA's health claim regulation for phytosterols and the reduced risk of coronary heart disease[5].

Animal and vegetable fats and oils — Determination of sterols and stanols in foods and dietary supplements containing added phytosterols

1 Scope

This document specifies a reference method for the determination of free sterols/stanols and steryl/stanol esters (0,1 % to 97 % mass fraction) in foods and dietary supplements containing added phytosterols and in phytosterol food additive concentrates.

Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this document. This method does not apply to matrices that contain rice bran oil at concentrations of more than 20 % due to possible interferences in the gas chromatogram.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Principle

Free sterols/stanols and saponified steryl/stanol esters are derivatized to phytosterol trimethylsilyl (TMS) ethers and separated on a capillary gas chromatography column.

5 Reagents

Use only reagents of recognized analytical grade.

WARNING — Attention is drawn to regulations that specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed. A chemical fume hood shall be used for the work.

5.1 Pyridine.

5.2 *N,O*-Bis(trimethylsilyl)trifluoroacetamide +1 % trimethylchlorosilane (BSTFA).

5.3 Internal standard dissolving solvent, toluene (recommended) or ethyl acetate.

5.4 Methanol.

5.5 Sodium hydroxide, pellets, of purity ≥ 97 %.

5.6 Sodium hydroxide solution, 2,3 N.

Weigh 92 g of sodium hydroxide (5.5) into a 1 000 ml volumetric flask (6.14). Add a stir bar (6.20) and 750 ml of methanol (5.4). Place the flask on a magnetic stirrer (6.21) to disperse. Dilute to the mark with methanol, and invert to mix. The sodium hydroxide solution shall be stirred each time before use because it will settle out with time.

5.7 Hydrochloric acid, 3,0 N.

5.8 Epicoprostanol (5β-cholestan-3α-ol), of purity > 95 %.

5.9 Epicoprostanol internal standard (IS) solution, 5 mg/ml.

Accurately weigh 5,0 g of epicoprostanol (5.8) into a glass weighing scoop (6.8). Transfer the epicoprostanol to a 1 000 ml volumetric flask (6.14). Rinse the scoop with internal standard dissolving solvent (5.3) into the flask. Dilute to the mark with toluene or ethyl acetate and invert to mix. The epicoprostanol IS solution of concentration 5 mg/ml is used for sterol/stanol concentrates, steryl/stanol ester concentrates and some dietary supplements.

5.10 Epicoprostanol internal standard (IS) solution, 2 mg/ml.

Accurately weigh 2,0 g of epicoprostanol (5.8) into a glass weighing scoop (6.8). Transfer the epicoprostanol to a 1 000 ml volumetric flask (6.14). Rinse the scoop with internal standard dissolving solvent (5.3) into the flask. Dilute to the mark with toluene or ethyl acetate and invert to mix. The epicoprostanol IS solution of concentration 2 mg/ml is used for analysis of foods and some dietary supplements.

5.11 Phytosterols, mixture of soya sterols, gas chromatography reference standard.

5.12 Sodium sulfate, anhydrous, granular, of purity ≥ 99 %.

5.13 Sodium chloride, crystalline, of purity ≥ 99 %.

5.14 Sodium chloride solution, saturated.

Weigh approximately 400 g of sodium chloride (5.13) into a storage bottle (6.15). Dilute with 1 000 ml of deionized water.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Gas-liquid chromatograph, equipped with flame ionization detector and capillary split injection system.

6.2 Carrier gas, hydrogen (recommended) or helium, of purity ≥ 99,99 % pure or better, gas chromatography quality, dried, oxygen removed by suitable filters (< 0,1 mg/kg), free from organic impurities.

6.3 Other gases, nitrogen, hydrogen and synthetic air, of gas chromatography quality and purity ≥ 99,99 %.

6.4 Capillary column, with a stationary phase that has been successfully employed to perform the separation of phytosterol TMS ethers.

The use of a bonded (5 %-phenyl)-methylpolysiloxane stationary phase column, such as the HP-5, of length 30 m, internal diameter 0,32 mm and film thickness 0,25 µm, is recommended. The use of a non-bonded (5 %-phenyl)(1 %-vinyl)-methylpolysiloxane stationary phase column, such as the SE-54, of length 30 m, internal diameter 0,32 mm and film thickness 0,25 µm, is also appropriate.

6.5 Injection port split liner, tapered, deactivated, of length 78,5 mm, internal diameter 4 mm and outer diameter 6,3 mm, with glass wool.

6.6 Microsyringe, of capacity 10 µl, with hardened needle for gas chromatography.

6.7 Sample preparation system, suitable for performing saponification and acid hydrolysis procedures, and equipped with the following.

6.7.1 Round bottom flasks, of capacity 50 ml, with 24/40 joints.

6.7.2 Flask neck sleeves, polytetrafluoroethylene (PTFE), size 24/40.

6.7.3 Glass stoppers, full-length, size 24/40.

6.7.4 Hot plate, explosion-proof.

6.7.5 Condensers, water-cooled.

6.8 Glass weighing scoops, suitable for the test portion and insertion into volumetric flasks (6.14).

6.9 Analytical balance, of readability 0,000 1 g and weighing accuracy 0,001 g.

6.10 Spatulas, PFTE-coated.

6.11 Volumetric pipette, of capacities 5 ml, 40 ml and 50 ml, class A, with aspiration bulb.

6.12 Pipette or automatic pipette, of capacities 1 ml and 10 ml, with pipette tips.

6.13 Pasteur pipettes, of length 150 mm, with aspiration bulb.

6.14 Volumetric flasks, of capacities 100 ml and 1 000 ml, class A.

6.15 Storage bottle, of capacity 1 000 ml, glass or polymethylpentene, with screw cap.

6.16 Glass tubes, of length 100 mm and outer diameter 13 mm, with PTFE-lined screw caps.

6.17 Autosampler vials, of capacity 2 ml, amber, with PTFE-lined caps.

6.18 Vortex mixer.

6.19 Boiling chips.

6.20 Stir bar and stir bar remover tool.

6.21 Magnetic stirrer.

7 Sampling

Treat the sample as needed (e.g. cryogrind, homogenize or shake) to obtain a homogeneous composite. Refer to the appropriate International Standard for the matrix concerned.

8 Test portion

Use Table 1 to select the appropriate test portion size and concentration of IS solution based on the estimated content of total phytosterols in the sample.

Table 1 — Guidelines for determining the sample test portion size and concentration of IS solution

Sample type	Phytosterol concentration % (mass fraction)	Test portion mg	IS concentration mg/ml	IS added mg
Sterol/stanol concentrates	> 90	50	5	25
Steryl/stanol ester concentrates, dietary supplements	~50	100	5	25
Foods and dietary supplements	~20	250	5	25
Food and dietary supplements	~10	500	5	25
Food and dietary supplements	< 10	1 000	2	10

9 Procedure

9.1 Selection of analysis protocol

For new formulations (phytosterol type and matrix), determine the optimal protocol by triplicate analysis according to the following protocols: a) 120 min alkaline, b) 15 min alkaline and c) 45 min acid/15 min alkaline. Select the protocol that produces the highest phytosterol recovery and lowest variability. Further refine this protocol, as needed, by decreasing the saponification time from 120 min to 60 min to 30 min, or the acid hydrolysis time from 45 min to 30 min to 15 min, with triplicate analyses for each. The optimal protocol will show a repeatability coefficient of variation ($C_{V,r}$) of less than 5 % for triplicate analyses over a period of five days of testing.

The 120 min alkaline protocol is appropriate for the analysis of most foods and dietary supplements containing added phytosterols. The 45 min acid/15 min alkaline protocol can be required for complete liberation of phytosterol esters from certain matrices, such as some cereals.

9.2 Dilute and shoot protocol

Use this protocol for sterol/stanol concentrates and phytosterol reference standards.

a) Accurately weigh 50 mg of sample into a tared glass tube (6.16). Record the exact weight.

b) Add 5,00 ml of 5 mg/ml epicoprostanol IS solution (5.9). Cap tightly and vortex (6.18) to mix.

c) Add 0,5 ml of pyridine (5.1) and 1 ml of BSTFA (5.2). Cap tightly and vortex (6.18) to mix. Heating can be required because some concentrates are pellets that fail to dissolve at room temperature.

d) Transfer 300 µl of derivatized sample solution, 1 ml of toluene or ethyl acetate (5.3) and 25 mg of sodium sulfate (5.12) to an autosampler vial (6.17). Cap tightly and vortex (6.18) to mix. The sample is now ready for gas chromatographic separation (see Clause 10).

9.3 15 min and 120 min alkaline protocols

Use this protocol for most foods, dietary supplements and steryl/stanol ester concentrates.

a) Accurately weight the test portion into a tarred round bottom flask (6.7.1). Record the exact weight.

b) Add a few boiling chips (6.19) and place a PTFE sleeve (6.7.2) in the neck of the flask.

c) Add 5,00 ml of the appropriate epicoprostanol IS solution (5.9 or 5.10).

d) Add 5 ml of sodium hydroxide solution (5.6).

e) Boil the sample at 100 °C for 15 min or 120 min (see 9.1).

f) Remove the flask from the heat source, insert a stopper (6.7.3) and let cool to room temperature.

g) Add 7 ml of hydrochloric acid (5.7), insert a stopper (6.7.3) and shake to mix.

h) Add 40 ml of sodium chloride solution (5.14), insert a stopper (6.7.3) and shake to mix. Allow the two phases to separate. Wait until the cloudy organic phase is clear before proceeding.

i) Transfer 300 µl of organic phase and 25 mg of sodium sulfate (5.12) to an autosampler vial (6.17).

j) Add 0,5 ml of pyridine (5.1) and 1 ml of BSTFA (5.2). Cap tightly and vortex (6.18) to mix. The sample is now ready for gas chromatographic separation (see Clause 10).

9.4 45 min acid/15 min alkaline protocol

Use this protocol for some foods and dietary supplements containing added phytosterol esters. Acid hydrolysis prior to saponification is needed for the complete liberation of phytosterol esters from certain matrices, such as some cereals (see 9.1).

a) Accurately weight the test portion into a tarred round bottom flask (6.7.1). Record the exact weight.

b) Add a few boiling chips (6.19) and place a PTFE sleeve (6.7.2) in the neck of the flask.

c) Add 5 ml of hydrochloric acid (5.7).

d) Add 5,00 ml of the appropriate epicoprostanol IS solution (5.9 or 5.10).

e) Boil the sample at 100 °C for 45 min.

f) Remove the flask from the heat source, insert a stopper (6.7.3) and let cool to room temperature.

g) Add 40 ml of sodium chloride solution (5.14), insert a stopper (6.7.3) and shake to mix. Allow the two phases to separate.

h) Transfer the organic phase (as much as possible without disrupting the solids on the surface) to a new round bottom flask (6.7.1).

i) Add 5 ml of sodium hydroxide solution (5.6).

j) Boil the sample at 100 °C for 15 min.

k) Remove the flask from the heat source, insert a stopper (6.7.3) and let cool to room temperature.

l) Add 7 ml of hydrochloric acid (5.7), insert a stopper (6.7.3) and shake to mix.

m) Add 40 ml of sodium chloride solution (5.14), insert a stopper (6.7.3) and shake to mix. Allow the two phases to separate. Wait until the cloudy organic phase is clear before proceeding.

n) Transfer 300 µl of organic phase and 25 mg of sodium sulfate (5.12) to an autosampler vial (6.17).

o) Add 0,5 ml of pyridine (5.1) and 1 ml of BSTFA (5.2). Cap tightly and vortex (6.18) to mix. The sample is now ready for gas chromatographic separation (see Clause 10).

10 Gas chromatography

Operating conditions are as follows.

a) Injection port temperature: 290 °C.

b) Detector temperature: 290 °C.

c) Oven temperature: 250 °C for 60 min, ramp at 15 °C/min to 265 °C, hold for 7 min.

d) Post-run temperature: 250 °C for 3 min.

e) Carrier gas (6.2): hydrogen or helium, 1,0 ml/min, constant flow rate.

f) Detector gases (6.3): air, 400 ml/min, hydrogen, 30 ml/min.

g) Makeup gas (6.3): nitrogen, 30 ml/min.

h) Split ratio: 25:1.

i) Injection volume: 1,0 µl.

j) Phytosterol concentration: 10 mg/ml.

11 Peak identification

Identify phytosterol TMS ethers by retention time (see Table 2) and by comparison with reference chromatograms (see Figures A.1 to A.4). Peaks of unknown identity should be omitted from the summation of peak areas, unless they are confirmed to be phytosterols.

Samples containing added plant stanols and stanol esters require special attention during peak identification and integration. Refer to Figure A.4 when analysing samples known to contain high concentrations of plant stanols.

Table 2 — Elution order and theoretical correction factors (TCFs) for sterols and stanols commonly found in foods and dietary supplements containing added phytosterols

Peak #[a]	Compound	Retention time min	Relative retention time min	TCF[b]
1	Epicoprostanol	25,3	1,00	1,000 0
2	Cholesterol	30,5	1,21	0,995 6
3	Brassicasterol	33,7	1,33	0,988 7
4	Ergosterol	37,8	1,49	0,984 5
5	24-Methylene cholesterol	38,3	1,51	0,988 7

[a] Peak numbers are used in Figures A.1 to A.4. Peaks corresponding to cholesterol and ergosterol are not included in the quantitation of total phytosterols.

[b] TCFs were calculated relative to epicoprostanol TMS ether, which had a factor of 1,000 0. Atomic weights were as follows: carbon, 12,011; hydrogen, 1,007 9; oxygen, 15,999 4; silicon, 28,085 5. TCFs account for the proportion of active carbon atoms contributing to the response of the flame ionization detector[6].

Table 2 *(continued)*

Peak #[a]	Compound	Retention time min	Relative retention time min	TCF[b]
6	Campesterol	39,1	1,55	0,993 0
7	Campestanol	39,9	1,58	0,997 2
8	Stigmasterol	42,0	1,66	0,986 4
9	Δ22-Stigmastenol	43,0	1,70	0,990 5
10	Δ7-Campesterol	44,4	1,75	0,993 0
11	Clerosterol + Δ5,23-Stigmastadienol	46,3	1,83	0,986 4
12	β-Sitosterol	48,1	1,90	0,990 5
13	Sitostanol	49,0	1,94	0,994 6
14	Δ5-Avenasterol	50,0	1,98	0,986 4
15	Fucosterol + unidentified	53,0	2,09	0,986 4
16	Δ7-Stigmastenol	54,8	2,17	0,990 5
17	Δ7-Avenasterol	57,0	2,25	0,986 4

[a] Peak numbers are used in Figures A.1 to A.4. Peaks corresponding to cholesterol and ergosterol are not included in the quantitation of total phytosterols.

[b] TCFs were calculated relative to epicoprostanol TMS ether, which had a factor of 1,000 0. Atomic weights were as follows: carbon, 12,011; hydrogen, 1,007 9; oxygen, 15,999 4; silicon, 28,085 5. TCFs account for the proportion of active carbon atoms contributing to the response of the flame ionization detector[6].

12 Calculations

12.1 Calculate the mass of individual phytosterols (in mg) in a test sample by using Formula (1):

$$m_{PSx} = \left(A_{PSx} \times m_{IS} \times F_{TC}\right) \div A_{IS} \quad (1)$$

where

m_{PSx} is the mass of phytosterol x;

A_{PSx} is the area count for phytosterol x;

m_{IS} is the mass of epicoprostanol IS (in mg, corrected for purity) added to the test sample;

F_{TC} is the theoretical correction factor for phytosterol x (see Table 2);

A_{IS} is the area count for epicoprostanol IS.

12.2 Calculate the concentration of individual phytosterols (in percentage) in a test sample by using Formula (2):

$$C_{PSx} = \left(m_{PSx} \div m_{TS}\right) \times 100 \quad (2)$$

where

C_{PSx} is the concentration of phytosterol *x*;

m_{PSx} is the mass (in mg) of phytosterol *x*;

m_{TS} is the mass (in mg) of the test sample.

12.3 Calculate the concentration of total phytosterols in a test sample (% mass fraction) by using Formula (3):

$$C_{PSt} = \left(\sum m_{PSx} \div m_{TS}\right) \times 100 \quad (3)$$

where

C_{PSt} is the concentration of total phytosterols *t*;

Σm_{PSx} is the sum of the mass (in mg) of all phytosterols identified in a test sample (cholesterol and ergosterol are not included in the quantitation of total phytosterols);

m_{TS} is the mass (in mg) of the test sample.

13 Quality control/quality assurance

13.1 Verify acceptable chromatographic separations by analysing the phytosterol reference standard. Ensure that a baseline resolution is achieved between peaks corresponding to campesterol and campestanol, and β-sitosterol and sitostanol. Decrease the flow rate of the H_2 carrier gas (e.g. by 0,05 ml/min to 0,1 ml/min) if a greater resolution is needed.

13.2 Perform a spike-recovery experiment to verify the accuracy of the method for determining phytosterols in the subject sample matrix. Prepare the spiked sample by adding a known amount of stigmasterol to the subject sample and analyse according to the appropriate sample preparation protocol. Calculate the recovery of stigmasterol by determining the analysed content, taken as a proportion of the spiked content and corrected for any incurred amount in the sample.

13.3 Analyse a blank matrix sample to verify the absence of interferences in the analysis due to matrix, reagents or equipment used. Confirm that the chromatogram of the blank matrix sample is devoid of peaks eluting in the region in which phytosterols elute (i.e. 23 min to 60 min).

13.4 A standard reference material has yet to be created for food and dietary supplement samples containing added phytosterols.

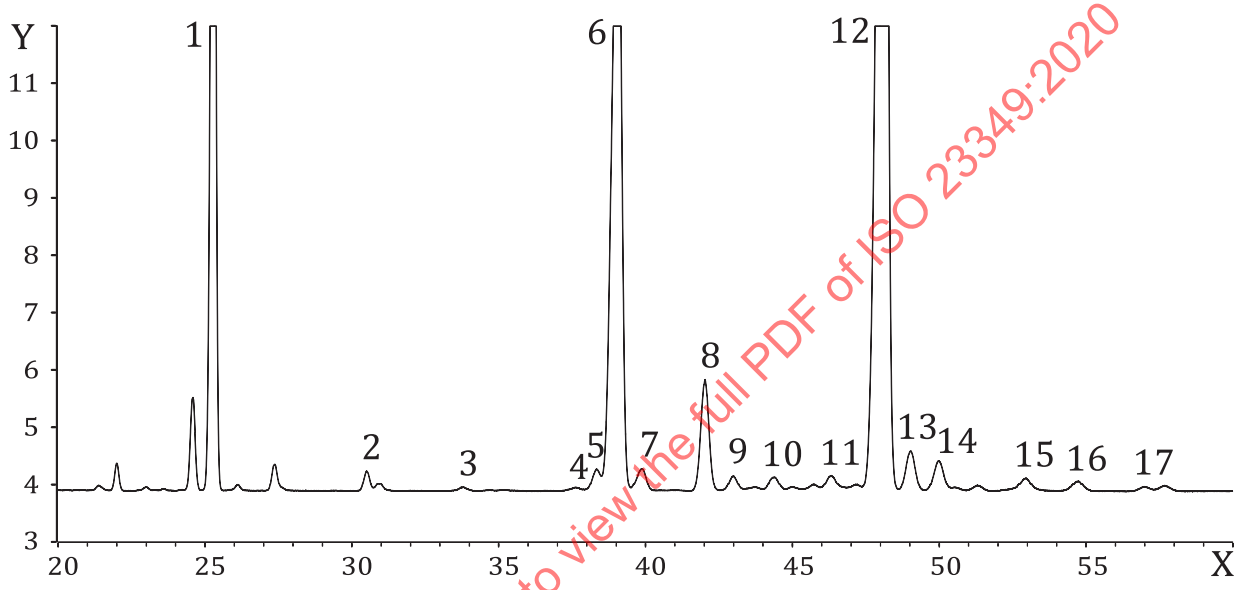
14 Precision

Results of the international collaborative study carried out to evaluate the performance of this reference method are presented in Annex B.

Annex A
(normative)

Reference gas chromatograms

Figure A.1 gives an example of a typical partial gas chromatogram of phytosterol TMS ethers derived from a phytosterol reference standard (5.11).

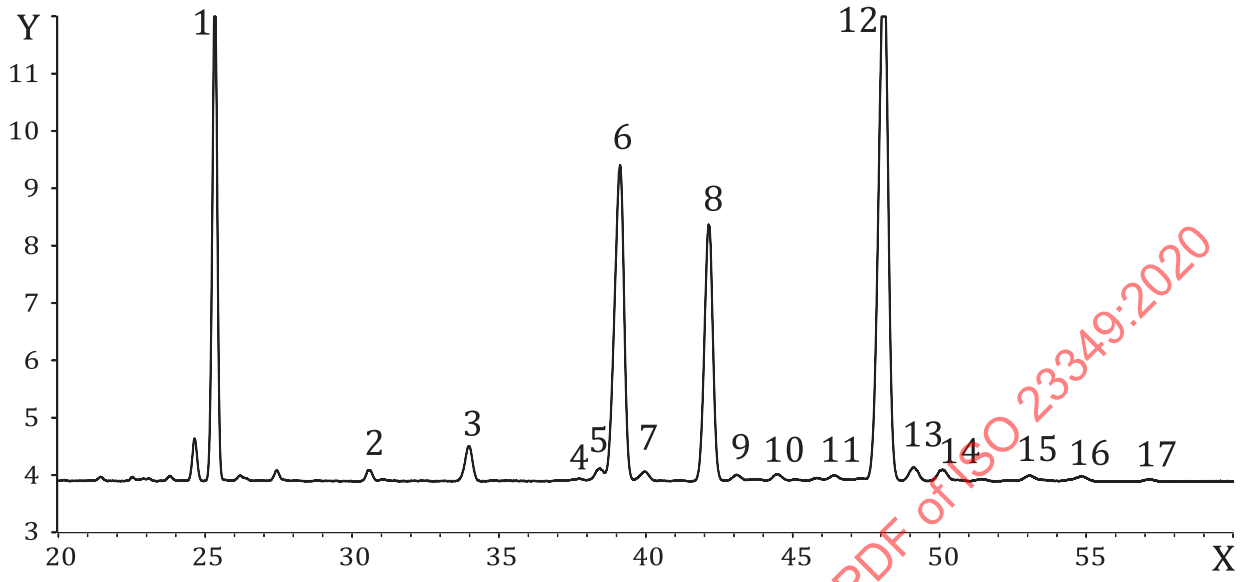


Key

X	min	9	Δ22-stigmastenol
Y	pA	10	Δ7-campesterol
1	epicoprostanol (IS)	11	clerosterol + Δ5,23-stigmastadienol
2	cholesterol	12	β-sitosterol
3	brassicasterol	13	sitostanol
4	ergosterol	14	Δ5-avenasterol
5	24-methylene cholesterol	15	fucosterol + unidentified
6	campesterol	16	Δ7-stigmastenol
7	campestanol	17	Δ7-avenasterol
8	stigmasterol		

Figure A.1 — Typical partial gas chromatogram from a phytosterol reference standard

Figure A.2 gives an example of a typical partial gas chromatogram of phytosterol TMS ethers derived from a vegetable spread containing added plant sterol esters.

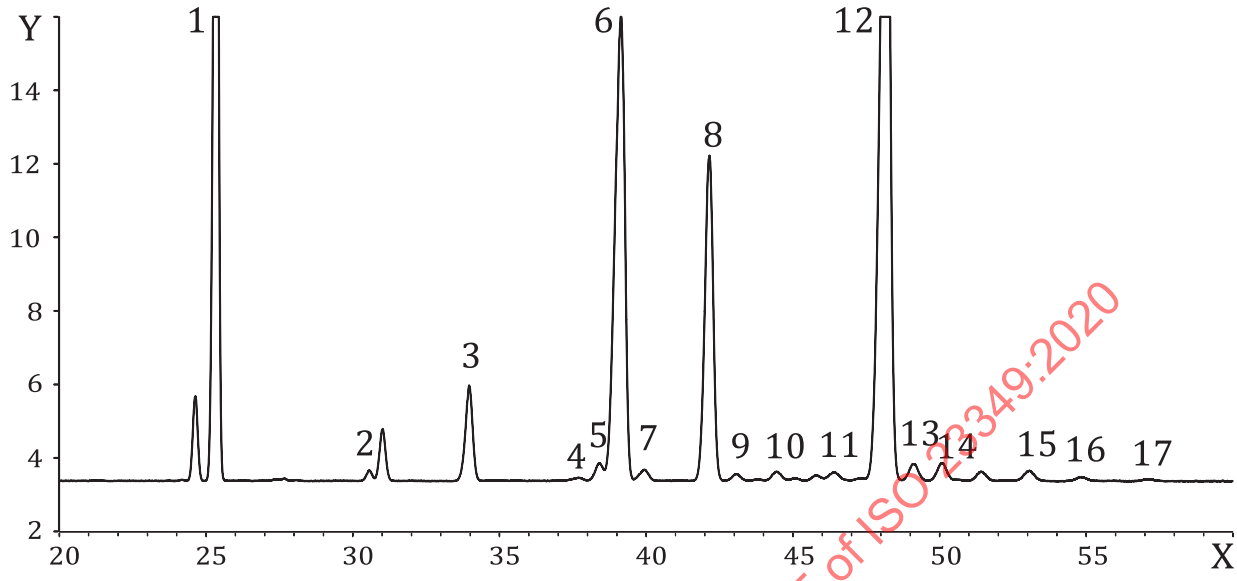


Key

X	min	9	Δ22-stigmastenol
Y	pA	10	Δ7-campesterol
1	epicoprostanol (IS)	11	clerosterol + Δ5,23-stigmastadienol
2	cholesterol	12	β-sitosterol
3	brassicasterol	13	sitostanol
4	ergosterol	14	Δ5-avenasterol
5	24-methylene cholesterol	15	fucosterol + unidentified
6	campesterol	16	Δ7-stigmastenol
7	campestanol	17	Δ7-avenasterol
8	stigmasterol		

Figure A.2 — Typical partial gas chromatogram from a margarine with added plant sterol esters

Figure A.3 gives an example of a typical partial gas chromatogram of phytosterol TMS ethers derived from a dietary supplement containing added phytosterols.

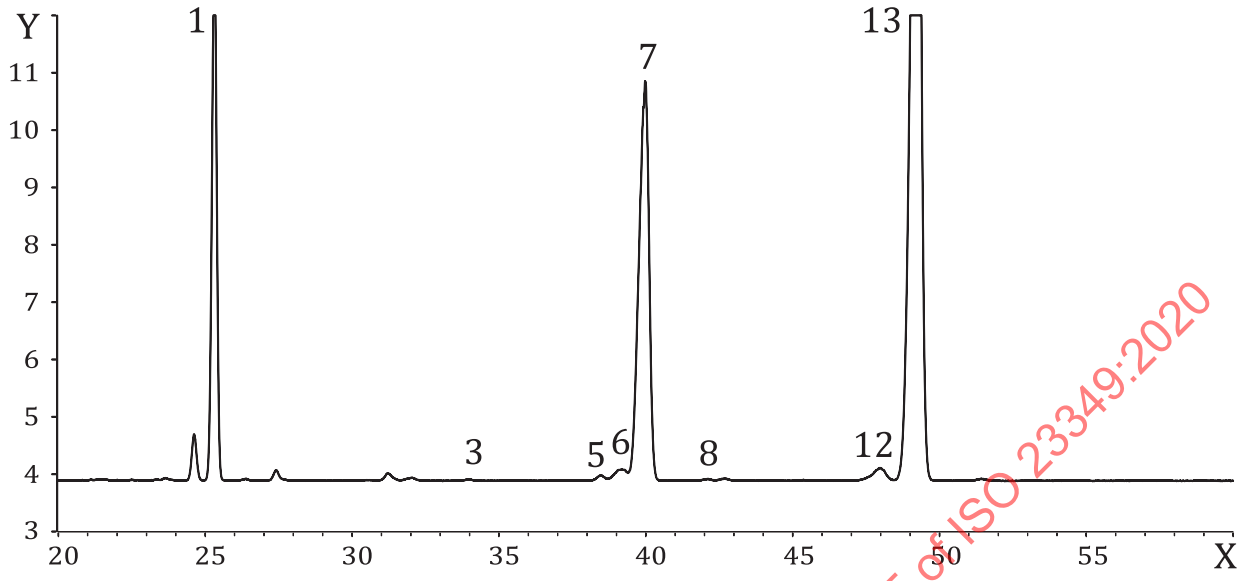


Key

X	min	9	Δ22-stigmastenol
Y	pA	10	Δ7-campesterol
1	epicoprostanol (IS)	11	clerosterol + Δ5,23-stigmastadienol
2	cholesterol	12	β-sitosterol
3	brassicasterol	13	sitostanol
4	ergosterol	14	Δ5-avenasterol
5	24-methylene cholesterol	15	fucosterol + unidentified
6	campesterol	16	Δ7-stigmastenol
7	campestanol	17	Δ7-avenasterol
8	stigmasterol		

Figure A.3 — Typical partial gas chromatogram from a dietary supplement with added phytosterols

Figure A.4 gives an example of a typical partial gas chromatogram of phytosterol TMS ethers derived from a dietary supplement containing added plant stanol esters.



Key

X	min	6	campesterol
Y	pA	7	campestanol
1	epicoprostanol (IS)	8	stigmasterol
3	brassicasterol	12	β-sitosterol
5	24-methylene cholesterol	13	sitostanol

Figure A.4 — Typical partial gas chromatogram from a dietary supplement with added plant stanol esters

Annex B
(informative)

Results of the interlaboratory test

An international collaborative study, co-organized by the United States Food and Drug Administration (FDA), Cargill (USA) and the American Oil Chemists' Society (AOCS, USA), was carried out in 2016 to 2017 to verify the performance of this reference method for quantifying total and individual plant sterols and stanols in foods and dietary supplements containing added phytosterols and in phytosterol food additive concentrates. A total of 14 laboratories from 6 countries completed the analysis of foods (n = 9), dietary supplements (n = 5) and phytosterol concentrates (n = 4). The statistical results are presented in Tables B.1 to B.17. Study results for the contents of total phytosterols (mass fraction) were 0,19 % to 8,4 % for foods, 8,7 % to 49 % for dietary supplements and 57 % to 97 % for concentrates. This method showed acceptable performance for total and individual phytosterols, indicating its suitability for a wide range of marketed products containing added phytosterols.

Table B.1 — Sample 1: Phytosterol concentrate

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,335	0,011	3,277	0,015	4,340	0,031	0,041
Brassicasterol	1,455	0,068	4,689	0,068	4,689	0,191	0,191
Ergosterol	0,128	0,014	11,332	0,028	21,751	0,041	0,078
24-Methylene cholesterol	0,687	0,023	3,313	0,031	4,544	0,064	0,087
Campesterol	25,003	0,146	0,584	0,440	1,759	0,409	1,231
Campestanol	0,729	0,020	2,779	0,024	3,342	0,057	0,068
Stigmasterol	20,337	0,134	0,660	0,415	2,040	0,376	1,162
Δ22-Stigmastenol	0,493	0,012	2,343	0,033	6,653	0,032	0,092
Δ7-Campesterol	0,471	0,024	5,029	0,048	10,279	0,066	0,135
Clerosterol + Δ5,23-Stigmastadienol	0,395	0,010	2,546	0,077	19,614	0,028	0,217
β-Sitosterol	40,123	0,218	0,542	0,854	2,127	0,609	2,390
Sitostanol	1,094	0,010	0,884	0,065	5,966	0,027	0,183
Δ5-Avenasterol	0,888	0,014	1,545	0,132	14,837	0,038	0,369
Fucosterol + Unidentified	0,497	0,014	2,819	0,116	23,295	0,039	0,324
Δ7-Stigmastenol	0,465	0,019	4,062	0,049	10,489	0,053	0,137
Δ7-Avenasterol	0,173	0,011	6,226	0,028	16,395	0,030	0,079
Total Phytosterols	92,662	0,441	0,476	1,698	1,833	1,234	4,755

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.2 — Sample 2: Phytosterol ester concentrate

Phytosterol[a]	Mean % (mass fraction)[b]	Repeatability standard deviation s_r	Coefficient of variation of repeatability $C_{V,r}$	Reproducibility standard deviation s_R	Coefficient of variation of reproducibility $C_{V,R}$	Repeatability limit r (= 2,8 s_r)	Reproducibility limit R (= 2,8 s_R)
Cholesterol	0,275	0,010	3,542	0,013	4,842	0,027	0,037
Brassicasterol	1,863	0,010	0,510	0,040	2,149	0,027	0,112
Ergosterol	0,127	0,017	13,784	0,033	26,076	0,049	0,092
24-Methylene cholesterol	0,604	0,008	1,287	0,016	2,684	0,022	0,045
Campesterol	16,093	0,108	0,674	0,438	2,720	0,303	1,226
Campestanol	0,439	0,013	3,022	0,018	4,017	0,037	0,049
Stigmasterol	10,147	0,054	0,532	0,247	2,431	0,151	0,691
Δ22-Stigmastenol	0,244	0,009	3,565	0,034	13,833	0,024	0,094
Δ7-Campesterol	0,329	0,003	0,825	0,038	11,608	0,008	0,107
Clerosterol + Δ5,23-Stigmastadienol	0,225	0,005	2,219	0,121	53,671	0,014	0,338
β-Sitosterol	25,126	0,148	0,589	1,240	4,935	0,415	3,472
Sitostanol	0,657	0,019	2,837	0,051	7,814	0,052	0,144
Δ5-Avenasterol	0,603	0,017	2,736	0,056	9,346	0,046	0,158
Fucosterol + Unidentified	0,331	0,027	8,255	0,133	40,255	0,076	0,373
Δ7-Stigmastenol	0,226	0,017	7,659	0,037	16,505	0,048	0,104
Δ7-Avenasterol	0,083	0,006	6,813	0,014	17,518	0,016	0,040
Total Phytosterols	57,375	0,403	0,703	2,000	3,486	1,129	5,601

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.3 — Sample 3: Phytosterol concentrate

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,364	0,008	2,262	0,023	6,345	0,023	0,065
Brassicasterol	0,646	0,013	2,005	0,024	3,789	0,036	0,069
Ergosterol	0,159	0,026	16,058	0,037	23,172	0,072	0,103
24-Methylene cholesterol	0,669	0,013	1,897	0,028	4,172	0,036	0,078
Campesterol	21,658	0,093	0,427	0,499	2,302	0,259	1,396
Campestanol	0,494	0,016	3,279	0,023	4,606	0,045	0,064
Stigmasterol	19,402	0,102	0,526	0,751	3,870	0,286	2,103
Δ22-Stigmastenol	0,433	0,019	4,396	0,037	8,534	0,053	0,103
Δ7-Campesterol	0,475	0,044	9,265	0,049	10,250	0,123	0,136
Clerosterol + Δ5,23-Stigmastadienol	0,547	0,054	9,868	0,197	36,038	0,151	0,552
β-Sitosterol	49,123	0,544	1,107	2,381	4,847	1,522	6,667
Sitostanol	0,863	0,008	0,933	0,047	5,449	0,023	0,132
Δ5-Avenasterol	1,022	0,008	0,760	0,075	7,369	0,022	0,211
Fucosterol + Unidentified	0,504	0,045	8,957	0,048	9,603	0,126	0,136
Δ7-Stigmastenol	0,258	0,007	2,713	0,035	13,658	0,020	0,099
Δ7-Avenasterol	0,148	0,015	10,448	0,039	26,542	0,043	0,110
Total Phytosterols	97,074	0,533	0,549	3,127	3,222	1,492	8,756

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.4 — Sample 4: Phytosterol concentrate

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,479	0,007	1,394	0,036	7,529	0,019	0,101
Brassicasterol	0,373	0,014	3,868	0,033	8,801	0,040	0,092
Ergosterol	0,178	0,025	14,132	0,036	20,055	0,070	0,100
24-Methylene cholesterol	0,929	0,015	1,567	0,040	4,304	0,041	0,112
Campesterol	23,693	0,209	0,881	1,160	4,896	0,585	3,248
Campestanol	0,754	0,020	2,716	0,055	7,253	0,057	0,153
Stigmasterol	12,741	0,204	1,605	1,368	10,740	0,572	3,832
Δ22-Stigmastenol	0,355	0,012	3,519	0,064	18,020	0,035	0,179
Δ7-Campesterol	0,618	0,016	2,650	0,123	19,897	0,046	0,344
Clerosterol + Δ5,23-Stigmastadienol	0,668	0,015	2,292	0,260	38,970	0,043	0,729
β-Sitosterol	41,296	0,442	1,071	0,843	2,040	1,238	2,359
Sitostanol	1,055	0,047	4,417	0,207	19,633	0,131	0,580
Δ5-Avenasterol	1,256	0,013	1,016	0,119	9,458	0,036	0,333
Fucosterol + Unidentified	0,699	0,025	3,586	0,078	11,094	0,070	0,217
Δ7-Stigmastenol	0,447	0,014	3,219	0,081	18,040	0,040	0,226
Δ7-Avenasterol	0,214	0,020	9,219	0,038	17,841	0,055	0,107
Total Phytosterols	86,073	0,744	0,864	5,643	6,556	2,083	15,800

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.5 — Sample 5: Pita bread with plant sterols

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,004	0,000	7,305	0,001	18,522	0,001	0,002
Brassicasterol	0,020	0,000	2,177	0,001	6,054	0,001	0,003
Ergosterol	0,005	0,001	10,714	0,003	65,763	0,002	0,010
24-Methylene cholesterol	0,010	0,000	1,457	0,000	4,573	0,000	0,001
Campesterol	0,212	0,003	1,264	0,023	10,961	0,007	0,065
Campestanol	0,008	0,000	3,586	0,001	6,368	0,001	0,001
Stigmasterol	0,147	0,001	0,689	0,001	0,689	0,003	0,003
Δ22-Stigmastenol	0,004	0,000	7,662	0,000	10,679	0,001	0,001
Δ7-Campesterol	0,004	0,000	7,372	0,001	21,340	0,001	0,002
Clerosterol + Δ5,23-Stigmastadienol	0,006	0,000	7,328	0,002	29,976	0,001	0,005
β-Sitosterol	0,437	0,003	0,618	0,004	0,855	0,008	0,010
Sitostanol	0,013	0,000	2,830	0,001	9,267	0,001	0,003
Δ5-Avenasterol	0,013	0,000	3,332	0,001	10,317	0,001	0,004
Fucosterol + Unidentified	0,004	0,000	6,844	0,001	25,599	0,001	0,003
Δ7-Stigmastenol	0,004	0,000	10,212	0,002	44,729	0,001	0,005
Δ7-Avenasterol	0,002	0,000	16,908	0,001	49,665	0,001	0,003
Total Phytosterols	0,892	0,005	0,549	0,007	0,773	0,014	0,019

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.6 — Sample 6: Nutritional shake with plant sterol esters

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,004	0,000	5,313	0,003	63,085	0,001	0,007
Brassicasterol	0,005	0,000	3,614	0,001	10,922	0,001	0,002
Ergosterol	0,000	0,000	63,830	0,000	63,830	0,000	0,000
24-Methylene cholesterol	0,003	0,000	15,869	0,001	21,045	0,001	0,002
Campesterol	0,053	0,000	0,595	0,002	3,322	0,001	0,005
Campestanol	0,001	0,000	6,665	0,001	42,361	0,000	0,002
Stigmasterol	0,037	0,001	1,769	0,002	4,244	0,002	0,004
Δ22-Stigmastenol	0,001	0,000	56,474	0,001	126,161	0,001	0,002
Δ7-Campesterol	0,001	0,000	62,392	0,001	118,944	0,001	0,002
Clerosterol + Δ5,23-Stigmastadienol	0,000	0,000	20,254	0,000	69,723	0,000	0,000
β-Sitosterol	0,085	0,001	0,824	0,003	3,719	0,002	0,009
Sitostanol	0,003	0,000	6,592	0,001	22,790	0,000	0,002
Δ5-Avenasterol	0,003	0,000	13,110	0,000	14,817	0,001	0,001
Fucosterol + Unidentified	0,001	0,000	16,626	0,001	101,814	0,000	0,003
Δ7-Stigmastenol	0,001	0,001	53,230	0,001	78,028	0,002	0,002
Δ7-Avenasterol	0,000	0,000	47,246	0,000	60,179	0,000	0,000
Total Phytosterols	0,194	0,003	1,313	0,007	3,841	0,007	0,021

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.7 — Sample 7: Nutritional shake with plant sterol esters

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,002	0,000	3,861	0,000	12,178	0,000	0,001
Brassicasterol	0,004	0,000	9,211	0,001	22,663	0,001	0,003
Ergosterol	0,000	0,000	76,819	0,000	76,819	0,000	0,000
24-Methylene cholesterol	0,002	0,000	9,755	0,001	24,949	0,001	0,001
Campesterol	0,043	0,000	1,044	0,002	4,396	0,001	0,005
Campestanol	0,001	0,000	20,746	0,001	79,252	0,001	0,002
Stigmasterol	0,030	0,001	1,748	0,002	5,276	0,001	0,004
Δ22-Stigmastenol	0,000	0,000	77,521	0,001	157,593	0,001	0,002
Δ7-Campesterol	0,000	0,000	49,407	0,000	57,309	0,000	0,000
Clerosterol + Δ5,23-Stigmastadienol	0,001	0,000	49,523	0,001	94,523	0,001	0,002
β-Sitosterol	0,082	0,000	0,554	0,004	4,830	0,001	0,011
Sitostanol	0,002	0,000	12,556	0,001	42,583	0,001	0,002
Δ5-Avenasterol	0,002	0,000	8,992	0,001	40,377	0,001	0,003
Fucosterol + Unidentified	0,001	0,000	8,421	0,001	130,206	0,000	0,003
Δ7-Stigmastenol	0,000	0,000	37,060	0,000	47,375	0,000	0,000
Δ7-Avenasterol	0,000	0,000	67,965	0,000	67,965	0,000	0,000
Total Phytosterols	0,167	0,002	1,140	0,017	9,896	0,005	0,046

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.8 — Sample 8: Oatmeal square with plant sterols

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,001	0,000	13,091	0,001	50,031	0,000	0,002
Brassicasterol	0,000	0,000	50,180	0,000	50,180	0,000	0,000
Ergosterol	0,001	0,000	16,759	0,002	129,800	0,001	0,005
24-Methylene cholesterol	0,000	0,000	47,191	0,000	50,608	0,000	0,000
Campesterol	0,115	0,004	3,155	0,007	5,773	0,010	0,019
Campestanol	0,020	0,001	6,818	0,002	7,952	0,004	0,004
Stigmasterol	0,011	0,003	32,094	0,004	34,591	0,010	0,010
Δ22-Stigmastenol	0,001	0,000	17,065	0,001	134,195	0,000	0,002
Δ7-Campesterol	0,001	0,000	26,154	0,001	114,146	0,001	0,004
Clerosterol + Δ5,23-Stigmastadienol	0,008	0,001	8,481	0,004	52,988	0,002	0,011
β-Sitosterol	1,217	0,015	1,260	0,101	8,330	0,043	0,284
Sitostanol	0,168	0,002	1,288	0,010	5,904	0,006	0,028
Δ5-Avenasterol	0,016	0,001	5,198	0,001	9,034	0,002	0,004
Fucosterol + Unidentified	0,004	0,000	9,328	0,003	72,333	0,001	0,009
Δ7-Stigmastenol	0,015	0,001	8,923	0,002	11,902	0,004	0,005
Δ7-Avenasterol	0,009	0,000	5,727	0,004	42,413	0,001	0,010
Total Phytosterols	1,589	0,022	1,366	0,072	4,561	0,061	0,203

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.9 — Sample 9: Chocolate chip cookie with plant sterol esters

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,037	0,003	8,714	0,008	22,227	0,009	0,023
Brassicasterol	0,160	0,001	0,680	0,005	2,954	0,003	0,013
Ergosterol	0,006	0,001	24,263	0,003	56,683	0,004	0,009
24-Methylene cholesterol	0,030	0,002	7,938	0,003	8,610	0,007	0,007
Campesterol	1,178	0,004	0,348	0,036	3,084	0,011	0,102
Campestanol	0,022	0,000	0,892	0,001	4,442	0,001	0,003
Stigmasterol	0,898	0,002	0,238	0,028	3,124	0,006	0,079
Δ22-Stigmastenol	0,018	0,001	5,390	0,001	5,886	0,003	0,003
Δ7-Campesterol	0,021	0,004	18,375	0,008	39,567	0,011	0,023
Clerosterol + Δ5,23-Stigmastadienol	0,020	0,002	11,883	0,007	36,881	0,007	0,020
β-Sitosterol	1,934	0,007	0,375	0,104	5,372	0,020	0,291
Sitostanol	0,050	0,000	0,974	0,002	3,937	0,001	0,006
Δ5-Avenasterol	0,040	0,001	2,692	0,002	4,137	0,003	0,005
Fucosterol + Unidentified	0,024	0,003	11,781	0,004	17,883	0,008	0,012
Δ7-Stigmastenol	0,016	0,002	12,668	0,005	32,096	0,006	0,015
Δ7-Avenasterol	0,008	0,001	16,367	0,004	54,847	0,004	0,012
Total Phytosterols	4,444	0,017	0,387	0,140	3,144	0,048	0,391

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.10 — Sample 10: Margarine with plant stanol esters

Phytosterol[a]	Mean % (mass fraction)[b]	Repeatability standard deviation s_r	Coefficient of variation of repeatability $C_{V,r}$	Reproducibility standard deviation s_R	Coefficient of variation of reproducibility $C_{V,R}$	Repeatability limit r (= 2,8 s_r)	Reproducibility limit R (= 2,8 s_R)
Cholesterol	0,000	0,000	50,925	0,000	60,176	0,000	0,000
Brassicasterol	0,018	0,001	4,228	0,001	6,195	0,002	0,003
Ergosterol	0,000	0,000	67,220	0,000	69,634	0,000	0,000
24-Methylene cholesterol	0,018	0,003	17,325	0,003	18,714	0,009	0,010
Campesterol	0,131	0,003	2,622	0,016	12,333	0,010	0,045
Campestanol	0,294	0,006	1,970	0,041	13,803	0,016	0,114
Stigmasterol	0,030	0,001	2,765	0,004	12,107	0,002	0,010
Δ22-Stigmastenol	0,001	0,001	43,504	0,001	114,398	0,001	0,004
Δ7-Campesterol	0,002	0,001	30,425	0,002	89,997	0,002	0,005
Clerosterol + Δ5,23-Stigmastadienol	0,000	0,000	89,848	0,000	89,848	0,000	0,000
β-Sitosterol	0,197	0,004	2,025	0,009	4,734	0,011	0,026
Sitostanol	3,220	0,058	1,787	0,126	3,918	0,161	0,353
Δ5-Avenasterol	0,005	0,000	10,167	0,004	71,938	0,001	0,010
Fucosterol + Unidentified	0,002	0,000	27,482	0,002	93,320	0,001	0,005
Δ7-Stigmastenol	0,007	0,001	13,016	0,005	75,581	0,002	0,014
Δ7-Avenasterol	0,001	0,000	25,926	0,001	115,128	0,001	0,004
Total Phytosterols	3,960	0,086	2,164	0,477	12,049	0,240	1,336

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.11 — Sample 11: Margarine with plant sterol esters

Phytosterol[a]	**Mean** % (mass fraction)[b]	**Repeatability standard deviation** s_r	**Coefficient of variation of repeatability** $C_{V,r}$	**Reproducibility standard deviation** s_R	**Coefficient of variation of reproducibility** $C_{V,R}$	**Repeatability limit** r (= 2,8 s_r)	**Reproducibility limit** R (= 2,8 s_R)
Cholesterol	0,038	0,001	2,520	0,009	22,933	0,003	0,025
Brassicasterol	0,211	0,004	1,998	0,006	2,646	0,012	0,016
Ergosterol	0,011	0,003	25,090	0,008	70,042	0,008	0,021
24-Methylene cholesterol	0,095	0,003	3,069	0,005	5,781	0,008	0,015
Campesterol	2,244	0,024	1,081	0,051	2,255	0,068	0,142
Campestanol	0,052	0,003	5,722	0,004	7,533	0,008	0,011
Stigmasterol	1,502	0,017	1,135	0,041	2,722	0,048	0,115
Δ22-Stigmastenol	0,034	0,002	6,974	0,005	15,091	0,007	0,015
Δ7-Campesterol	0,036	0,001	2,046	0,008	21,869	0,002	0,022
Clerosterol + Δ5,23-Stigmastadienol	0,047	0,005	11,370	0,016	35,073	0,015	0,046
β-Sitosterol	3,955	0,041	1,044	0,119	2,999	0,116	0,332
Sitostanol	0,086	0,002	1,792	0,006	6,435	0,004	0,015
Δ5-Avenasterol	0,116	0,006	5,215	0,011	9,876	0,017	0,032
Fucosterol + Unidentified	0,040	0,002	3,924	0,011	26,242	0,004	0,030
Δ7-Stigmastenol	0,024	0,002	7,666	0,004	17,046	0,005	0,011
Δ7-Avenasterol	0,013	0,003	19,240	0,003	19,240	0,007	0,007
Total Phytosterols	8,427	0,211	2,507	0,283	3,356	0,592	0,792

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.

Table B.12 — Sample 12: Orange juice with plant sterols

Phytosterol[a]	Mean % (mass fraction)[b]	Repeatability standard deviation s_r	Coefficient of variation of repeatability $C_{V,r}$	Reproducibility standard deviation s_R	Coefficient of variation of reproducibility $C_{V,R}$	Repeatability limit r (= 2,8 s_r)	Reproducibility limit R (= 2,8 s_R)
Cholesterol	0,002	0,000	3,976	0,001	65,721	0,000	0,004
Brassicasterol	0,007	0,000	2,981	0,001	7,174	0,001	0,001
Ergosterol	0,000	0,000	77,061	0,000	77,061	0,000	0,000
24-Methylene cholesterol	0,003	0,000	8,209	0,000	8,688	0,001	0,001
Campesterol	0,103	0,001	0,499	0,003	2,597	0,001	0,008
Campestanol	0,003	0,000	4,496	0,000	8,460	0,000	0,001
Stigmasterol	0,080	0,001	0,885	0,002	3,027	0,002	0,007
Δ22-Stigmastenol	0,002	0,000	11,095	0,000	14,925	0,001	0,001
Δ7-Campesterol	0,002	0,000	4,591	0,000	13,602	0,000	0,001
Clerosterol + Δ5,23-Stigmastadienol	0,002	0,000	12,581	0,001	62,593	0,001	0,003
β-Sitosterol	0,176	0,001	0,759	0,006	3,300	0,004	0,016
Sitostanol	0,005	0,000	3,051	0,001	10,840	0,000	0,001
Δ5-Avenasterol	0,004	0,000	5,926	0,001	15,518	0,001	0,002
Fucosterol + Unidentified	0,002	0,000	8,149	0,001	46,180	0,000	0,003
Δ7-Stigmastenol	0,003	0,001	21,420	0,001	21,420	0,002	0,002
Δ7-Avenasterol	0,001	0,000	8,468	0,001	144,651	0,000	0,002
Total Phytosterols	0,398	0,003	0,832	0,017	4,285	0,009	0,048

[a] Cholesterol and ergosterol were not included in the quantitation of total phytosterols.

[b] Samples analysed in duplicate by 14 laboratories.