



BSI Standards Publication

**Determination of the
triacylglycerol composition
of fats and oils —
Determination by capillary gas
chromatography**

National foreword

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**Determination of the triacylglycerol
composition of fats and oils —
Determination by capillary gas
chromatography**

*Détermination de la composition des triacylglycérols des corps
gras — Détermination par chromatographie en phase gazeuse sur
colonne capillaire*



Reference number
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Contents

	Page
Foreword	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Reagents	1
6 Apparatus	2
7 Sampling	3
8 Preparation of test sample	3
9 Procedure	3
9.1 Sample purification (if necessary)	3
9.2 Separation of individual triglycerides by GC	3
9.3 Identification	3
9.4 Verification of the response factors	3
10 Calculation	4
11 Precision	4
11.1 Interlaboratory test	4
11.2 Repeatability limit (<i>r</i>)	4
11.3 Reproducibility limit (<i>R</i>)	5
12 Test report	5
Annex A (informative) Chromatograms	6
Annex B (informative) Results of interlaboratory test	10

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Determination of the triacylglycerol composition of fats and oils — Determination by capillary gas chromatography

1 Scope

This Technical Specification describes the procedure for the capillary gas chromatographic determination of the qualitative and semi-quantitative composition of individual triglycerides of fats, oils, and fat mixtures. The separation of the triglycerides is based on their retention depending on the carbon number of the fatty acids in the triglycerides and their degree of unsaturation.

This Technical Specification is applicable to animal and vegetable fats, as well as to mixtures of natural and synthetic triglycerides, as long as

- the oil fatty acid composition does not contain high content of linolenic acid such as linseed oil and
- the total chain length does not exceed a total carbon number of C60.

NOTE If quantitative results are expected, the response factors of several triglycerides have to be checked as the increase of the triglyceride unsaturation reduces the sensitivity.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

proportion of the triglyceride or triglyceride group

composition of the mixture of triglycerides is expressed as a percentage of area assuming the total of the triglyceride peaks is normalized to 100 %

4 Principle

Triglycerides of different polarities are separated by capillary gas chromatography on a highly polar stationary phase without any further sample preparation. After normalization of all peak areas, the content of the relevant triglycerides of the same retention time is expressed as a percentage proportion of the sum of all peak areas in percent.

5 Reagents

WARNING — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational, and personal safety measures shall be followed.

Unless otherwise stated, analytically pure reagents are to be used.

5.1 n-Hexane, analytical grade.

5.2 Diethyl ether, analytical grade.

5.3 Solvent mixture of hexane/diethyl ether, volume fraction hexane $\varphi = 87$ ml/100 ml, volume fraction diethyl ether $\varphi = 13$ ml/100 ml.

5.4 Isooctane, analytical grade.

5.5 Reference substances, triglycerides such as tripalmitin, tristearin, triolein, trilinolein, etc., as well as vegetable and animal fats of known composition.

5.6 Synthetic air, suitable for gas chromatography.

5.7 Hydrogen, for gas chromatography.

6 Apparatus

6.1 Injection vials for GC.

6.2 Gas chromatograph (GC), a chromatograph fitted with a cold on-column injection system and a flame ionization detector (FID).

NOTE Alternative injection systems, e.g. a split injector, a programmed-temperature vaporizer (PTV), or a moving-needle injector, can be used provided the same results are obtained as indicated in [Annex A](#).

6.3 The separation and detection has been proven to be satisfactory if the following experimental conditions are followed:

- High temperature capillary GC column: fused silica coated with thermo stable 50 % to 65 % phenylmethyl-polysiloxane, 25 m to 30 m \times 0,25 i.d., film thickness of 0,1 μ m to 0,15 μ m.
- Temperature program: 100 °C held 1 min (initial temperature), 100 °C to 325 °C at 30 °C/min, 325 °C to 345 °C at 1 °C/min, 345 °C to 370 °C at 5 °C/min, 370 °C held 5 min (final temperature).
- Carrier gas: hydrogen (purity $\geq 99,999$ %).

NOTE Operating conditions and type of GC column should be used provided the same results are obtained as indicated in [Annex A](#).

6.4 Microliter syringe for the GC, injection volume 1 μ l to 2 μ l.

6.5 Pipettes, 1 ml.

6.6 Analytical balance, readability 0,1 mg.

6.7 Volumetric flasks, 10 ml.

6.8 Silica gel cartridges for solid phase extraction, 1 g (6 ml).

6.9 Rotary evaporator.

6.10 Micropipette.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

Heat solid and semi-solid fats to temperatures slightly above their melting point to be completely clear, then mix well. Filter liquid or melted samples, or their solutions, which still contain visible contaminations.

9 Procedure

9.1 Sample purification (if necessary)

If a sample contains monoglycerides, diglycerides, free fatty acids, or polymerized fats in greater proportions, they should be separated in advance by preparative column chromatography according to the following procedure.

Wash an SPE silica gel cartridge (6.8) with 6 ml of hexane. Place a conical flask under the cartridge. Load a solution of the sample (0,12 g, approximately) in 0,5 ml of hexane (5.1) into the column and then elute the triglycerides fraction with 10 ml of the solvent mixture (5.3) of hexanediethyl ether (87:13 v/v). Evaporate to dryness the eluted solvent in a rotary evaporator (6.9) under reduced pressure at room temperature.

NOTE Oil purification can also be done using a silica gel column, as described in ISO 8420.

9.2 Separation of individual triglycerides by GC

Prepare a solution of the sample at approx. 0,50 mg/ml in isooctane (5.4). Weigh 50 mg of the sample in a 10 ml volumetric flask (6.7) and bring to volume with isooctane (5.4). Pipette 1 ml (6.5) of the resulting solution in another 10 ml volumetric flask (6.7) and bring to volume with the same solvent.

Inject 1,0 µl of the final test solution into the GC system using the cold on-column injection system.

Working (temperature) conditions shall be used to get a good separation C50-triglycerides (POP/PLP), C52-triglycerides (POS/POO/PLO), and C54-triglycerides (S00/O00/OLO) as shown in [Annex A](#).

9.3 Identification

For the identification of the peaks in the gas chromatogram, the relative retention times, e.g. relative to tripalmitin, shall be compared to those of the test substances.

In general, triglycerides appear in order of increasing number of C atoms and of increasing unsaturation for the same number of C atoms. The elution order of the triglycerides is given in the example chromatograms ([Annex A](#)).

9.4 Verification of the response factors

Prepare a solution of the reference substances (5.5) at approx. 0,1 mg/ml in isooctane (5.4). Weigh 50 mg of the sample in a 10 ml volumetric flask (6.7) and bring to volume with isooctane (5.4). Pipette 0,2 ml (6.10) of the resulting solution in another 10 ml volumetric flask (6.7) and bring to volume with the same solvent.

Inject 1,0 µl of the final test solution into the GC system using the cold on-column injection system.

Calculate the response factor as a ratio of the area of the tripalmitin, divided by the area of the triglyceride being checked:

$$F_{TGi} = \frac{A_{PPP}}{A_{TGi}} \quad (1)$$

where

F_{TGi} is the response factor of the triglyceride i ;

A_{TGi} is the peak area of the triglyceride i ;

A_{PPP} is the peak area of the tripalmitin.

10 Calculation

Calculate the composition of the mixture as a percentage of area by integrating all the peaks present in the chromatogram. The unknown peaks will be summed up in a category called "other TAGs". A prerequisite for the calculation according to the 100 % method is the assumption that all triglyceride groups contained in the sample are fully separated in the gas chromatogram with the same response.

Calculation of the individual triglyceride groups according to the following Formula (2):

$$C_{TGi} = \frac{A_{TGi}}{A_T} \times 100 \quad (2)$$

where

C_{TGi} is the proportion of the triglyceride or triglyceride group i , in %;

A_{TGi} is the peak area of the triglyceride i ;

A_T is the sum of the peak area of all triglycerides (ΣA_{TGi}).

NOTE For a quantitative analysis, a correction of the peak areas by means of the response factors is not necessary as long as the verification of the response factor leads to values lower or equal to 1,2 for the triglycerides present in the sample. As an example, the response factor for triolein was estimated at 1,18.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in [Annex B](#). The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability limit (r)

The repeatability limit (r) is the value less than or equal to which the absolute difference between two test results obtained under repeatability conditions may be expected to be with a probability of 95 %.

Repeatability conditions are conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

11.3 Reproducibility limit (*R*)

The reproducibility limit (*R*) is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions may be expected to be with a probability of 95 %.

Reproducibility conditions are conditions where independent test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment within short intervals of time.

12 Test report

The test report shall include the following information:

- the method in accordance with which sampling was carried out (if known);
- the method used;
- the test result(s) obtained;
- if the repeatability has been checked, the final quoted result obtained.

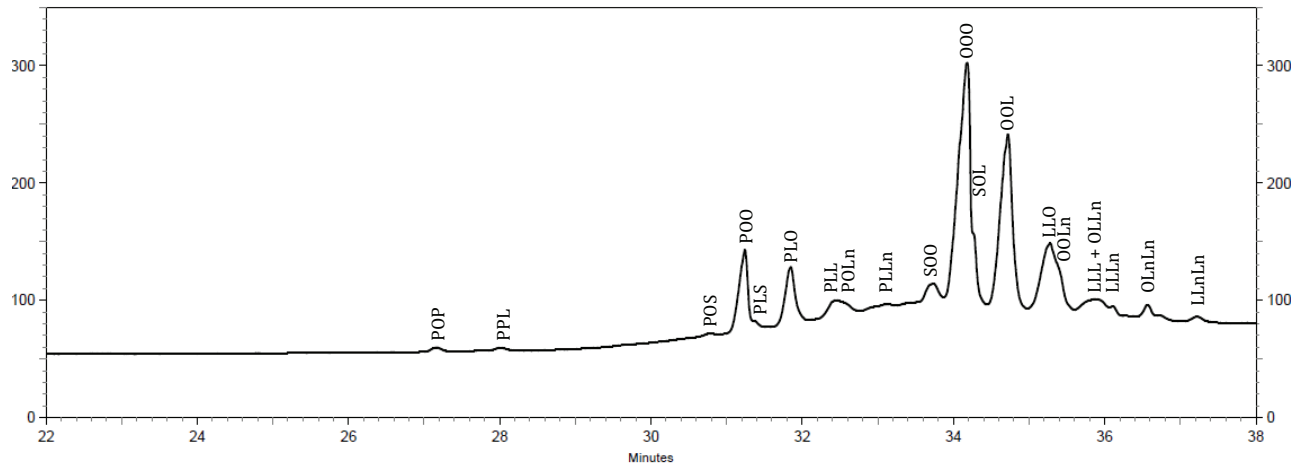
It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the result(s). The test report shall include all information necessary for the complete identification of the sample.

Annex A (informative)

Chromatograms

Experimental conditions:	GC column: 30 m × 0,25 mm fused silica capillary column coated with 0,1 µm Restek RTX65TG
Oven temperature:	100 °C held 1 min, 100 °C to 325 °C at 30 °C/min, 325 °C to 345 °C at 1 °C/min, 345 °C to 370 °C at 5 °C/min, 370 °C held 5 min.
Injector:	Cold on-column
Detector (FID):	370 °C
Carrier gas:	H ₂ at 1,1 ml/min
Injection volume:	1 µl of a 0,25 mg/ml solution

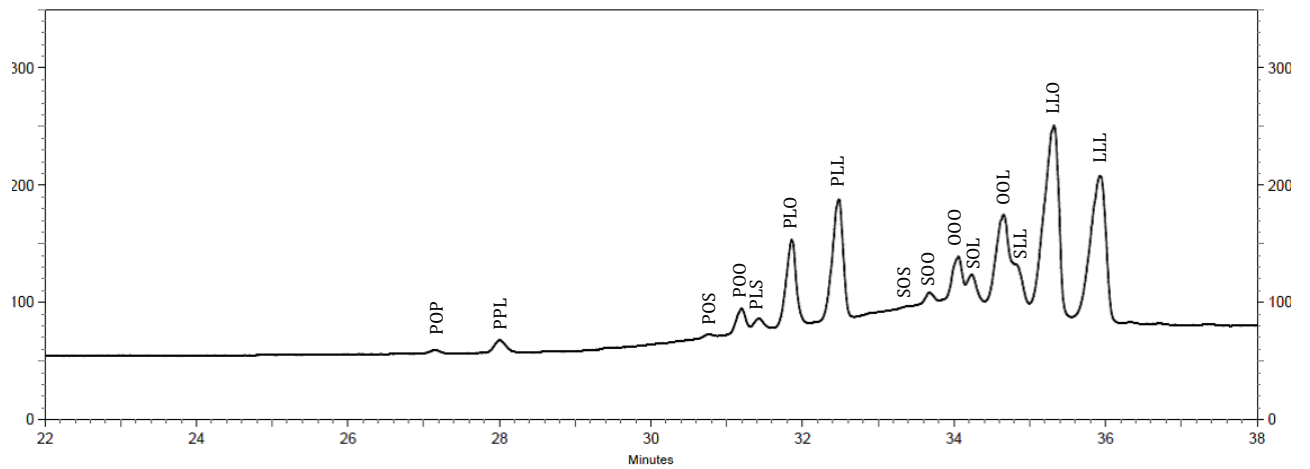
See [Figures A.1](#) to [A.5](#) for the example chromatograms.



Key

- P palmitic
- S stearic
- O oleic
- L linoleic
- Ln Linolenic

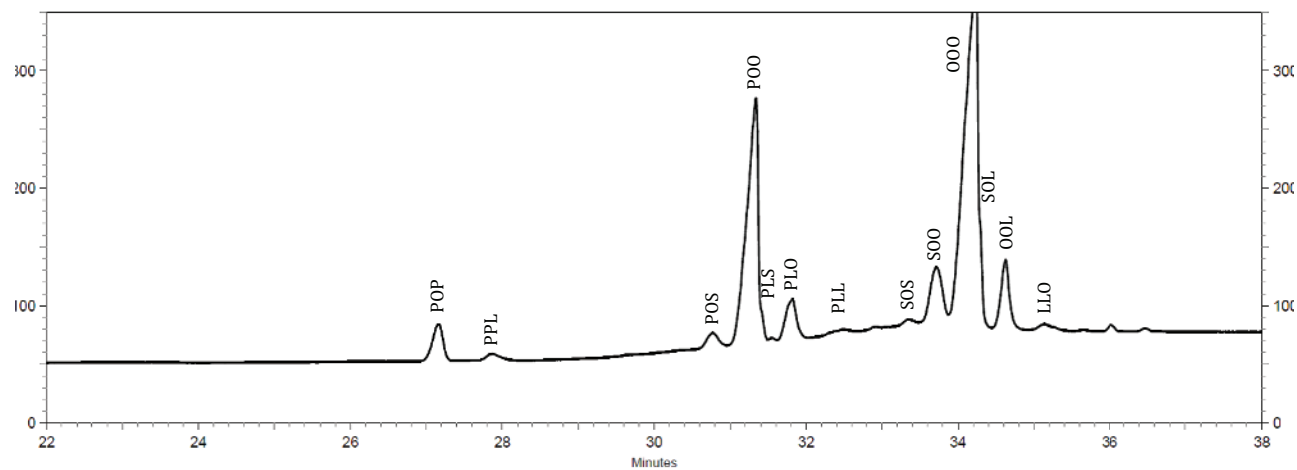
Figure A.1 — Triacylglycerol profile of rapeseed oil



Key

- P palmitic
- S stearic
- O oleic
- L linoleic
- Ln linolenic

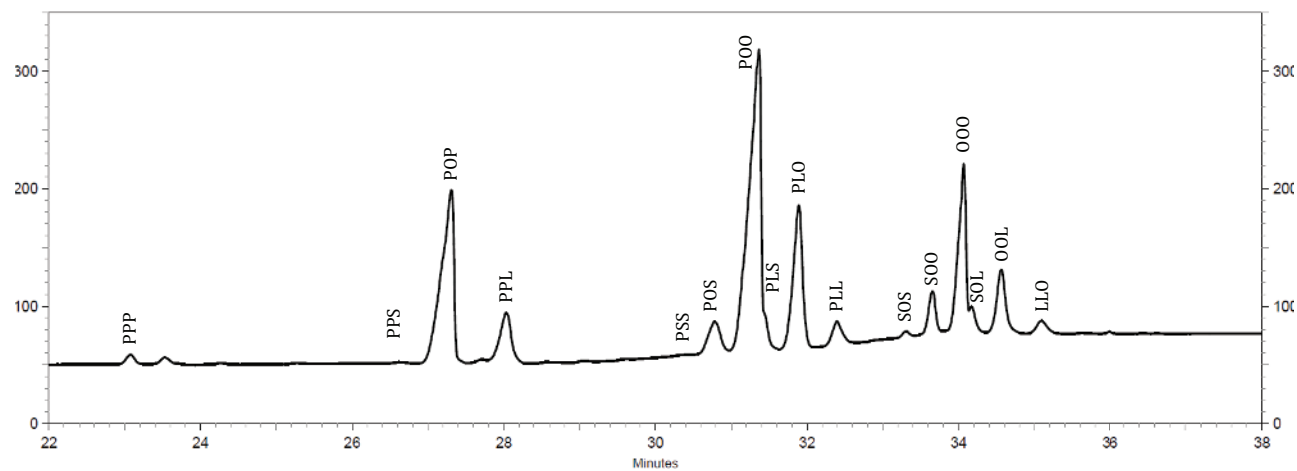
Figure A.2 — Triacylglycerol profile of the sunflower oil



Key

- P palmitic
- S stearic
- O oleic
- L linoleic
- Ln linolenic

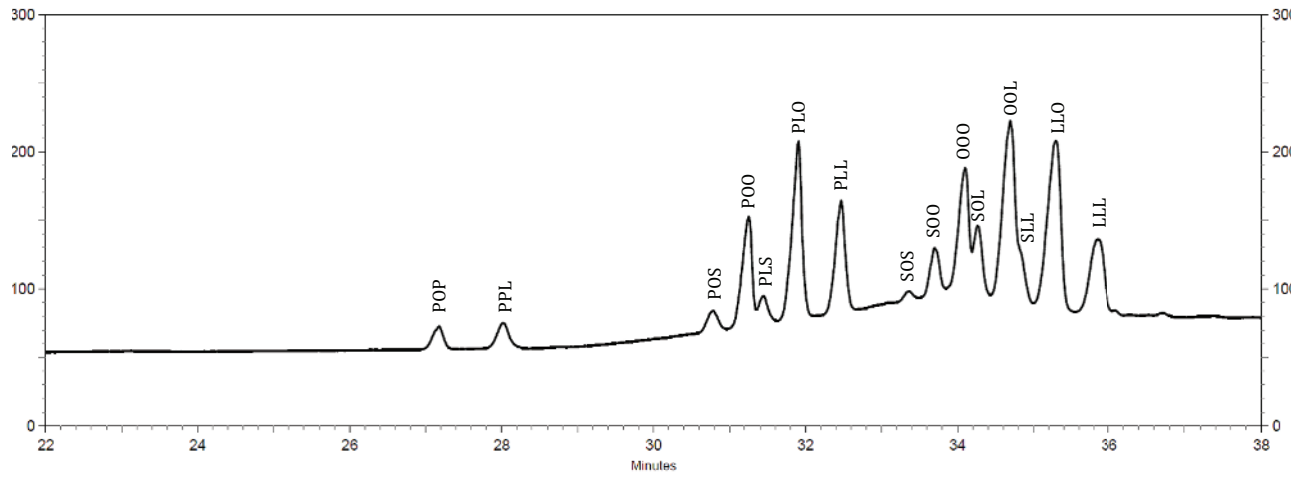
Figure A.3 — Triacylglycerol profile of olive oil



Key

- P palmitic
- S stearic
- O oleic
- L linoleic
- Ln linolenic

Figure A.4 — Triacylglycerol profile of palm oil



Key

- P Palmitic
- S Stearic
- O Oleic
- L Linoleic
- Ln Linolenic

Figure A.5 — Triacylglycerol profile of sesame oil

Annex B (informative)

Results of interlaboratory test

An interlaboratory test carried out at the International level in 2012 by France (ITERG), in which eight laboratories participated, each performing two determinations on each sample, gave the statistical results (determined in accordance with ISO 5725) given in [Tables B.1](#) to [B.11](#).

Sample identification:

Sample 1: refined rapeseed oil *Sample 4: virgin olive oil*
Sample 2: refined sunflower oil *Sample 5: crude palm oil*
Sample 3: roasted sesame oil

Table B.1 — Statistical results for POP (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	not treated mean value ≤1 %	not treated mean value ≤1 %	8	8	8
Number of laboratories retained after eliminating outliers (p)			8	8	8
Number of test results in all labs (n)			16	16	16
Mean (m)			1,58	3,30	18,25
Repeatability standard deviation (s r)			0,07	0,04	0,10
Repeatability relative standard deviation (CV r)			4,4 %	1,2 %	0,6 %
Repeatability limit, r (s r × 2,8)			0,19	0,11	0,29
Reproducibility standard deviation (s R)			0,33	0,53	2,11
Reproducibility relative standard deviation (CV R)			21,2 %	15,9 %	11,5 %
Reproducibility limit, R (s R × 2,8)			0,94	1,47	5,89
Horrat value			5,7	4,8	4,5

Table B.2 — Statistical results for PPL (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	not treated mean value ≤1 %	8	8	not treated mean value ≤1 %	8
Number of laboratories retained after eliminating outliers (p)		8	8		8
Number of test results in all labs (n)		16	16		16
Mean (m)		1,25	1,95		4,97
Repeatability standard deviation (s r)		0,04	0,06		0,11
Repeatability relative standard deviation (CV r)		3,0 %	3,2 %		2,2 %
Repeatability limit, r (s r × 2,8)		0,10	0,18		0,31
Reproducibility standard deviation (s R)		0,26	0,27		0,53
Reproducibility relative standard deviation (CV R)		20,5 %	13,7 %		10,7 %
Reproducibility limit, R (s R × 2,8)		0,72	0,75		1,49
Horrat value		5,3	3,8		3,4

Table B.3 — Statistical results for POS (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	not treated mean value ≤1 %	not treated mean value ≤1 %	8	8	8
Number of laboratories retained after eliminating outliers (p)			8	8	8
Number of test results in all labs (n)			16	16	16
Mean (m)			1,48	1,53	3,31
Repeatability standard deviation (s r)			0,08	0,06	0,18
Repeatability relative standard deviation (CV r)			5,1 %	3,9 %	5,4 %
Repeatability limit, r (s r × 2,8)			0,21	0,17	0,50
Reproducibility standard deviation (s R)			0,17	0,26	0,28
Reproducibility relative standard deviation (CV R)			11,7 %	17,1 %	8,3 %
Reproducibility limit, R (s R × 2,8)			0,48	0,74	0,77
Horrat value			3,8	3,7	3,3

Table B.4 — Statistical results for POO (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	8	8	8	8	8
Number of laboratories retained after eliminating outliers (p)	8	8	8	8	8
Number of test results in all labs (n)	16	16	16	16	16
Mean (m)	7,44	3,05	9,04	26,00	33,45
Repeatability standard deviation (s r)	0,12	0,24	0,25	0,13	0,36
Repeatability relative standard deviation (CV r)	1,6 %	7,7 %	2,8 %	0,5 %	1,1 %
Repeatability limit, r (s r × 2,8)	0,34	0,66	0,71	0,37	1,01
Reproducibility standard deviation (s R)	1,11	0,39	1,15	1,93	1,10
Reproducibility relative standard deviation (CV R)	15,0 %	12,9 %	12,7 %	7,4 %	3,3 %
Reproducibility limit, R (s R × 2,8)	3,12	1,10	3,22	5,39	3,08
Horrat value	5,1	4,1	4,4	3,0	1,4

Table B.5 — Statistical results for PLO (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	8	8	8	8	8
Number of laboratories retained after eliminating outliers (p)	8	8	8	8	8
Number of test results in all labs (n)	16	16	16	16	16
Mean (m)	5,43	7,48	11,98	4,11	11,91
Repeatability standard deviation (s r)	0,19	0,25	0,25	0,10	0,10
Repeatability relative standard deviation (CV r)	3,5 %	3,3 %	2,1 %	2,5 %	0,8 %
Repeatability limit, r (s r × 2,8)	0,53	0,69	0,71	0,28	0,28
Reproducibility standard deviation (s R)	0,76	1,30	1,35	0,30	0,41
Reproducibility relative standard deviation (CV R)	14,0 %	17,4 %	11,3 %	7,2 %	3,4 %
Reproducibility limit, R (s R × 2,8)	2,13	3,64	3,79	0,83	1,14
Horrat value	4,5	5,9	4,1	2,2	1,1

Table B.6 — Statistical results for PLL (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	8	8	8	not treated mean value ≤1 %	8
Number of laboratories retained after eliminating outliers (p)	8	8	8		8
Number of test results in all labs (n)	16	16	16		16
Mean (m)	2,82	11,26	7,52		2,11
Repeatability standard deviation (s r)	0,10	0,14	0,13		0,08
Repeatability relative standard deviation (CV r)	3,6 %	1,3 %	1,7 %		4,0 %
Repeatability limit, r (s r × 2,8)	0,28	0,40	0,36		0,24
Reproducibility standard deviation (s R)	0,59	1,63	0,61		0,24
Reproducibility relative standard deviation (CV R)	20,9 %	14,5 %	8,1 %		11,2 %
Reproducibility limit, R (s R × 2,8)	1,65	4,56	1,70		0,66
Horrat value	6,1	5,2	2,7		3,1

Table B.7 — Statistical results for S00 (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	8	not treated mean value ≤1 %	8	8	8
Number of laboratories retained after eliminating outliers (p)	8		8	8	8
Number of test results in all labs (n)	16		16	16	16
Mean (m)	2,13		3,42	6,47	2,83
Repeatability standard deviation (s r)	0,12		0,19	0,23	0,14
Repeatability relative standard deviation (CV r)	5,6 %		5,6 %	3,6 %	5,1 %
Repeatability limit, r (s r × 2,8)	0,34		0,53	0,65	0,40
Reproducibility standard deviation (s R)	0,35		0,71	0,64	0,61
Reproducibility relative standard deviation (CV R)	16,5 %		20,8 %	9,9 %	21,5 %
Reproducibility limit, R (s R × 2,8)	0,99		1,99	1,79	1,70
Horrat value	4,6		6,3	3,3	6,3

Table B.8 — Statistical results for O00 (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	8	8	8	8	8
Number of laboratories retained after eliminating outliers (p)	8	8	8	8	8
Number of test results in all labs (n)	16	16	16	16	16
Mean (m)	34,04	6,79	14,68	49,05	14,02
Repeatability standard deviation (s r)	0,26	0,21	0,47	0,32	0,24
Repeatability relative standard deviation (CV r)	0,8 %	3,1 %	3,2 %	0,6 %	1,7 %
Repeatability limit, r (s r × 2,8)	0,72	0,59	1,33	0,89	0,68
Reproducibility standard deviation (s R)	2,16	1,15	2,07	2,46	1,99
Reproducibility relative standard deviation (CV R)	6,3 %	16,9 %	14,1 %	5,0 %	14,2 %
Reproducibility limit, R (s R × 2,8)	6,05	3,21	5,80	6,88	5,57
Horrat value	2,7	5,4	5,3	2,3	5,3

Table B.9 — Statistical results for OOL (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	8	8	8	8	8
Number of laboratories retained after eliminating outliers (p)	8	8	8	8	8
Number of test results in all labs (n)	16	16	16	16	16
Mean (m)	23,45	15,85	20,24	5,80	5,53
Repeatability standard deviation (s r)	0,31	0,14	0,24	0,06	0,12
Repeatability relative standard deviation (CV r)	1,3 %	0,9 %	1,2 %	1,0 %	2,1 %
Repeatability limit, r (s r × 2,8)	0,87	0,38	0,68	0,17	0,32
Reproducibility standard deviation (s R)	1,07	1,16	0,65	0,57	0,79
Reproducibility relative standard deviation (CV R)	4,5 %	7,3 %	3,2 %	9,9 %	14,2 %
Reproducibility limit, R (s R × 2,8)	2,99	3,26	1,83	1,60	2,21
Horrat value	1,8	2,8	1,3	3,2	4,6

Table B.10 — Statistical results for LLO (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	8	8	8	not treated mean value ≤1 %	8
Number of laboratories retained after eliminating outliers (p)	8	8	8		8
Number of test results in all labs (n)	16	16	16		16
Mean (m)	15,10	28,26	18,08		1,35
Repeatability standard deviation (s r)	0,16	0,14	0,20		0,05
Repeatability relative standard deviation (CV r)	1,1 %	0,5 %	1,1 %		3,9 %
Repeatability limit, r (s r × 2,8)	0,46	0,39	0,56		0,15
Reproducibility standard deviation (s R)	0,98	0,64	1,04		0,35
Reproducibility relative standard deviation (CV R)	6,5 %	2,2 %	5,8 %		25,9 %
Reproducibility limit, R (s R × 2,8)	2,75	1,78	2,91		0,98
Horrat value	2,5	0,9	2,2	6,8	

Table B.11 — Statistical results for LLL (area %)

Sample	1	2	3	4	5
Number of participating laboratories (P)	8	8	8	not treated mean value ≤1 %	not treated mean value ≤1 %
Number of laboratories retained after eliminating outliers (p)	8	8	8		
Number of test results in all labs (n)	16	16	16		
Mean (m)	4,88	23,55	8,84		
Repeatability standard deviation (s r)	0,14	0,17	0,19		
Repeatability relative standard deviation (CV r)	2,8 %	0,7 %	2,1 %		
Repeatability limit, r (s r × 2,8)	0,38	0,46	0,53		
Reproducibility standard deviation (s R)	1,23	0,84	1,08		
Reproducibility relative standard deviation (CV R)	25,3 %	3,6 %	12,2 %		
Reproducibility limit, R (s R × 2,8)	3,45	2,35	3,03		
Horrat value	8,0	1,4	4,2		

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