
**Milk and milk products —
Determination of the sugar contents
— High performance anion exchange
chromatography with pulsed
amperometric detection method
(HPAEC-PAD)**

*Lait et produits laitiers — Détermination de la teneur en sucre —
Chromatographie d'échange d'anions haute performance couplée à la
détection par ampérométrie pulsée (HPAEC-PAD)*



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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).

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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 5, *Milk and milk products*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 302, *Milk and milk products — Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement), and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

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.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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Milk and milk products — Determination of the sugar contents — High performance anion exchange chromatography with pulsed amperometric detection method (HPAEC-PAD)

1 Scope

This document specifies the quantitative liquid chromatographic determination of specific sugars (galactose, glucose, fructose, sucrose, lactose and maltose) in various milk and milk products, applying arabinose as an internal standard.

The method is applicable to the following dairy matrices: milk, sweetened condensed milk, milk powder, cheese, whey powder, infant formula, milk dessert and yoghurt.

The method does not apply to dairy products containing soy or to the determination of the lactose content in low-lactose milk products at levels below 1 mg/g.

A high performance anion exchange chromatography method in combination with pulsed amperometric detection (HPAEC-PAD) method is applied^{[5][3][4]}. With this method, thirteen different monosaccharides, disaccharides and trisaccharides can be separated: fucose, arabinose, galactose, glucose, fructose, sucrose, lactose, lactulose, maltose, melibiose, trehalose, isomaltulose and maltotriose.

The method is applicable to labelling for the six most important sugars that can be present by nature or by addition in milk and milk products. The method does not apply to sugar contents less than 0,1 %.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 3696, *Water for analytical laboratory use — Specification and test methods*

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

The sugars present in the sample are extracted with an aqueous ethanol buffer solution in order to inhibit potential probiotic activities. The obtained extract is deproteinized with a Carrez clarification. After clarification, the solution is diluted and the sugars present are separated and quantified by HPAEC. HPAE allows carbohydrates separation at high pH. In order to improve sensitivity and stability, post-column sodium hydroxide solution is added to the HPAEC-PAD. GOS (galacto-oligosaccharides) and fructans do not interfere with the analysis of the sugars^[5]. Arabinose is applied as an internal standard for the quantification of the sugars.

5 Reagents

Use only reagents of recognized analytical grade and water in accordance with ISO 3696, unless otherwise specified.

5.1 Water, conforming to ISO 3696, grade 3 and grade 1.

5.2 Sodium hydroxide (NaOH) pellets.

5.3 Aqueous sodium hydroxide solution, substance concentration $c = 1 \text{ mol/l}$.

Add to a 1 000 ml volumetric flask $40 \text{ g} \pm 1 \text{ g}$ NaOH pellets (5.2), dissolve in about 500 ml of water and, after cooling down, dilute with water to the mark and homogenize.

5.4 Sodium hydroxide solution, mass fraction $w(\text{NaOH}) = 33 \%$ in water.

5.5 Sodium hydroxide solution, mass fraction $w(\text{NaOH}) = 50 \%$ in water.

The amount of carbonate and mercury in the reagent should be minimized. Do not shake or stir the solution before use. A suitable commercially available carbonate sodium hydroxide solution may also be used.

5.6 Concentrated hydrochloric acid (HCl), mass fraction of 36 % to 38 % in water.

5.7 Aqueous hydrochloric acid solution, $c = 1 \text{ mol/l}$.

Add to a 1 000 ml volumetric flask (6.2) 500 ml of water followed by 83 ml of concentrated HCl (5.6) and, after cooling down, dilute with water to the mark and homogenize.

5.8 Acetonitrile (HPLC quality).

5.9 Acetonitrile in water, a volume fraction of 5 % in water.

Add to a 1 000 ml volumetric flask (6.2) 50 ml of acetonitrile (5.8), dilute with water grade 3 to the mark and homogenize.

5.10 Anhydrous sodium acetate (CH_3COONa) (HPLC quality).

5.11 Eluent 1 (E1), aqueous solution of sodium acetate (CH_3COONa), $c = 1,0 \text{ mol/l}$.

Add to a 1 000 ml volumetric flask (6.2) about 800 ml of degassed water grade 1 (eluent 3, 5.13) followed by 82,0 g sodium acetate (5.10). Then dilute the aqueous solution with degassed water (eluent 3, 5.13) to the mark and homogenize. Store the eluent under an inert atmosphere.

5.12 Eluent 2 (E2), aqueous solution of carbonate free sodium hydroxide (NaOH), $c = 0,2 \text{ mol/l}$.

Add to a 1 000 ml volumetric flask (6.2) about 800 ml of degassed water grade 1 (eluent 3, 5.13) and purge for 15 min with helium. Add 16,0 g of sodium hydroxide solution (5.5). Then quickly dilute the aqueous solution with degassed water grade 1 (eluent 3, 5.13) to the mark, immediately close the bottle and homogenize. Store the eluent under an inert atmosphere.

5.13 Eluent 3 (E3), degassed water grade 1, stored under an inert atmosphere.

5.14 Eluent 4 (E4), aqueous solution of sodium acetate (CH_3COONa), $c = 0,025 \text{ mol/l}$.

Add to a 1 000 ml volumetric flask (6.2) about 800 ml of degassed water grade 1 (eluent 3, 5.13) followed by 2,05 g of sodium acetate (5.10). Then dilute the aqueous solution with degassed water (eluent 3, 5.13) to the mark and homogenize. Store the eluent under an inert atmosphere.

5.15 Post column reagent, aqueous solution of sodium hydroxide, $c = 0,3 \text{ mol/l}$.

Add to a 1 000 ml volumetric flask (6.2) about 800 ml of degassed water grade 1 (eluent 3, 5.13). Purge for 15 min with helium. Add 24,0 g of sodium hydroxide solution (5.5) and quickly fill up to the mark with the degassed water grade 1 (eluent 3, 5.13). Immediately close the flask and homogenize. Store the post column reagent under an inert atmosphere.

IMPORTANT — It is extremely important to remove dissolved carbon dioxide from the eluents and post column reagent prior to use and during use to avoid fast reduction in detector sensitivity. The eluents and post column reagent are maintained under an inert gas during use.

5.16 Mixture of a volume fraction of 95 % of ethanol (with a volume fraction of 96 % ethanol and 4 % of water) and a volume fraction of 5 % methanol.

5.17 Potassium hexacyanoferrate (II) trihydrate, $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$.

5.18 Zinc acetate dihydrate, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$.

5.19 Glacial acetic acid.

5.20 Carrez reagent I.

Weigh 106 g of $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ (5.17) in a 1 000 ml volumetric flask (6.2), dissolve in 800 ml of water (5.1) and dilute with water grade 3 to the mark. Store the Carrez reagent I in the refrigerator.

5.21 Carrez reagent II.

Weigh 220 g of $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ (5.18) in a 1 000 ml volumetric flask (6.2), dissolve in 800 ml water, add 30 ml of glacial acetic acid (5.19) and dilute with water grade 3 to the mark. Store the Carrez reagent II in the refrigerator.

IMPORTANT — Do not use Carrez reagent II with zinc sulfate.

5.22 Buffer solution of piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) ($c = 1,5 \text{ mol/l}$ and $\text{pH} = 6,9$).

Add 22,5 g of the PIPES buffer solution to a 100 ml conical flask and add 20 ml of sodium hydroxide solution (5.3). Adjust the pH to $\text{pH} = 6,9$ with $\text{NaOH} 33 \%$ (5.4) in water. Transfer the PIPES buffer solution quantitatively into a 50 ml calibrated tube and fill up with water grade 3 till 50 ml. The pH of the obtained buffer solution shall be within the range of 6,8 to 7,0.

5.23 Arabinose.

5.24 Galactose.

5.25 Glucose.

5.26 Fructose.

5.27 Sucrose.

5.28 Lactose.

5.29 Maltose.

5.30 Internal standard stock solution arabinose.

Weigh, to the nearest mg, approximately 7 g of arabinose (5.23) into a 50 ml volumetric flask (6.2). Add about 30 ml of water grade 3 and dissolve the arabinose. Add 2,5 ml of acetonitrile (5.8), fill up to the mark with water and homogenize the solution.

5.31 Sugar standard stock solution.

Weigh, to the nearest 0,1 mg, approximately 260 mg of the monosaccharides galactose (5.24), glucose (5.25) and fructose (5.26), and approximately 400 mg of the disaccharide sucrose (5.27), lactose (5.28) and maltose (5.29) into a 500 ml volumetric flask (6.2). Add about 200 ml of water grade 3 and dissolve the sugars. Add 25 ml of acetonitrile (5.8), fill up to the mark with water grade 3 and homogenize the solution.

5.32 Sugar standard solutions for calibration.

Prepare the different dilutions of the sugar calibration standards as specified in Table 1. Mix the specified volumes of the internal standard stock solution arabinose (5.30) and sugar standard stock solution (5.31) in a 200 ml volumetric flask, add about 50 ml of water grade 3 and homogenize. Add 10 ml of acetonitrile (5.8), fill up to the mark with water and homogenize.

Table 1 — Preparation of the sugar standard solutions for calibration

Sugar standard solution	Volume of sugar standard stock solution (5.31) ml	Volume of arabinose internal standard stock solution (5.30) ml
1	0,2	0,050
2	1,0	0,050
3	6,0	0,050
4	10,0	0,050
5	20,0	0,050
6	40,0	0,050
7	80,0	0,050
8	100,0	0,050

6 Apparatus

6.1 Analytical balance, capable of weighing to an accuracy of $\pm 0,1$ mg.

6.2 Volumetric flask, volume of 50 ml, 500 ml and 1 000 ml.

6.3 pH meter.

6.4 Black band filter paper.

6.5 Centrifugation tubes.

- 6.6 Homogenizer¹⁾.**
- 6.7 Vortex mixer.**
- 6.8 Screw cap closed graduated tubes, 50 ml volume.**
- 6.9 Positive displacement multipipette.**
- 6.10 Dispensers,** resistant to organic solvent and adjusted to 2,5 ml and 25 ml.
- 6.11 HPLC vials.**
- 6.12 Metal-free liquid chromatographic system,** e.g. Thermo Dionex ICS 3000²⁾, applicable for a (quaternary) gradient elution.
- 6.13 Column oven,** with a temperature stability of ± 1 °C with an operating temperature of 20 °C to 35 °C.
- 6.14 High performance anion exchange analytical column³⁾,** filled with pellicular polystyrene-divinylbenzene resin or filled with an anion exchanger resin enabling the required separation.
- 6.15 High performance anion exchange guard column⁴⁾,** filled with pellicular polystyrene-divinylbenzene resin or filled with an anion exchanger resin enabling the required separation.
- 6.16 Pulsed amperometric detector (PAD)⁵⁾** with a stability of ± 1 °C and an operating temperature range of 20 °C to 35 °C.
- Use pulsed amperometric detection and potential settings and waveforms recommended by the instrument supplier. Example detector potential settings (versus Ag/AgCl reference) are given in [Table 2](#).
- 6.17 Metal-free post column reagent pump⁶⁾.**

1) An Ultra turrax with an appropriate probe is an example of a suitable homogenizer available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

2) Thermo Dionex ICS 3000 is an example of a suitable homogenizer available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

3) A Thermo-Dionex Carbowac PA1 analytical column (2 mm × 250 mm) is an example of a suitable high performance anion exchange column available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

4) A Dionex Carbowac PA1 guard column (2 mm × 50 mm) is an example of a suitable high performance anion exchange column available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

5) A Thermo-Dionex model PAD is an example of a suitable PAD available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

6) A single piston Thermo Dionex Axp pump is an example of a suitable metal-free post column reagent pump available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

6.18 Integrator chromatography data system⁷⁾.

Table 2 — Time programming of the detector potential settings of the gold working electrode

Step	Time s	Potential gold electrode ^a	Integration detector signal
1	0,00	0,10	
2	0,20	0,10	Start
3	0,40	0,10	End
4	0,41	-2,00	
5	0,42	-2,00	
6	0,43	0,60	
7	0,44	-0,10	
8	0,50	-0,10	

^a Potential versus Ag/AgCl reference electrode.
NOTE The integration window is 0,20 s to 0,40 s.

7 Sampling

It is important that the laboratory receives a sample that is truly representative and has not been damaged or changed during transport and/or storage. Sampling is not a part of the method specified in this document.

8 Preparation of the test sample

8.1 General

Prepare the sample in accordance with an appropriate sampling procedure. Sweetened condensed milk requires a dedicated sample preparation, see ISO 2911 | IDF 35 and [8.2](#).

8.2 Sample preparation of sweetened condensed milk

8.2.1 Samples of recently manufactured products in which no appreciable separation of components can be expected

Open the container, transfer all material adhering to the lid into the container and thoroughly mix by an up-and-down movement of a spoon, in such a way that the top layers and contents of the lower corners are moved and mixed. When the product is in a can, transfer the contents into a jar with a well-fitting lid. When the product is in a collapsible tube, transfer as much as possible of the content to a jar with a well-fitting lid, then cut open the tube, scrape out all material adhering to the interior and also transfer this to the jar. Mix the contents of the jar as described above.

8.2.2 Samples of older products and samples in which separation of components can be expected

Heat the sample in a water bath at about 40 °C until the sample has nearly reached this temperature. Open the container and proceed as described in [8.2.1](#). When the product is in a can or tube, transfer the contents to a jar, scrape out all material adhering to the walls (in the case of a collapsible tube, after cutting open the tube) and continue the mixing until the whole mass is homogeneous, reducing the size of any crystals by crushing with a glass rod. Close the jar with a well-fitting lid. Allow to cool.

⁷⁾ A Thermo Dionex Chromeleon software package is an example of a suitable chromatographic integration software package available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

9 Procedure

9.1 Sample extraction and clean up

9.1.1 General

In cases where the integrator chromatography software package has the option “variable internal standard” (e.g. in Chromeleon™⁸), apply the procedure given in 9.1.2. In these cases, just one sample extraction and a clean-up procedure with two different dilutions have to be done. Adjust the integrator settings in the variable internal standard option for the 50-fold dilution.

For low sugar contents, apply the procedure given in 9.1.2.2. For high sugar contents, apply the procedure given in 9.1.2.3. The limits for low and high sugar contents are given in Table 3.

Table 3 — Limits for low and high sugar content

Sugar	Low content g/100 g	High content g/100 g
Galactose	≤ 12	> 12
Glucose	≤ 12	> 12
Fructose	≤ 12	> 12
Sucrose	≤ 18	> 18
Lactose	≤ 18	> 18
Maltose	≤ 18	> 18

In cases where the chromatographic integration software has no option for “variable internal standard” and there is no prior knowledge about the sugar levels in the dairy sample to be investigated, it is recommended to apply the procedure given in 9.1.2.2 for the low sugar levels. In cases where a sugar peak is bigger than the corresponding sugar peak in the highest calibration standard, the procedure given in 9.1.2.3 shall be applied for a correct quantitation of the sugar content.

9.1.2 Sample extraction and clean-up

9.1.2.1 General

Weigh, to the nearest 0,000 1 g, 1,0 g ± 0,1 g in a 50 ml calibrated tube (6.8).

Add 4 ml of the denatured ethanol (5.16), 0,125 ml of the arabinose internal standard solution (5.30) and 0,5 ml of the PIPES buffer solution (5.22).

Mix well by vortexing (6.7) and let the sample suspension rest for 10 min at an ambient temperature.

Fill up the calibrated tube to the 25 ml mark with demineralized water grade 3 (5.1) with a temperature of 40 °C ± 2 °C, followed by homogenization with a homogenizer (6.6) for 1 min.

NOTE Already well-dissolved samples can be homogenized by shaking.

Check the pH of the homogenized sample solution. The pH shall be within the values pH = 5,0 to pH = 7,0. If necessary, adjust the pH with NaOH solution (5.3) or HCl solution (5.7).

Add 0,5 ml of the Carrez reagent I solution (5.20), mix and then add 0,5 ml of the Carrez reagent II solution (5.21). Add 2,5 ml acetonitrile (5.8) and fill up with water (5.1) to the 50 ml mark. Close the calibrated tube with the screw cap and mix well.

Filter the suspension over black band filter paper (6.4) and collect the filtrate.

8) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

Prepare from each filtrate two different dilutions directly in the HPLC vials (6.11):

- 10-fold dilution: mix 100 µl of filtrate with 900 µl of water grade 3 (5.1);
- 50-fold dilution: mix 20 µl of filtrate with 980 µl of water grade 3 (5.1) and adjust the integrator settings for these 50-fold diluted filtrates in the variable internal standard procedure.

Inject 5 µl of the diluted filtrates in the metal free liquid chromatographic system (6.12).

In cases where the measured sugar peak is outside the calibration graph in the 10-fold diluted sample, use the results measured in the 50-fold diluted samples after properly adjusting the settings for the variable internal standard option.

9.1.2.2 Sample extraction and clean-up for the low sugar contents (10-fold dilution)

Weigh, to the nearest 0,000 1 g, 1,0 g ± 0,1 g in a 50 ml calibrated tube (6.8).

Add 4 ml of the mixture of denatured ethanol (5.16), 0,125 ml of the arabinose internal standard solution (5.30) and 0,5 ml of the PIPES buffer solution (5.22).

Mix well by vortexing (6.7) and let the sample suspension rest for 10 min at an ambient temperature.

Fill up the calibrated tube to the 25 ml mark with demineralized water grade 3 (5.1) with a temperature of 40 °C ± 2 °C, followed by homogenization with a homogenizer (6.6) for 1 min.

NOTE Already well-dissolved samples can be homogenized by shaking.

Check the pH of the homogenized sample solution. The pH shall be within the values pH = 5,0 to pH = 7,0. If necessary, adjust the pH with NaOH solution (5.3) or HCl solution (5.7).

Add 0,5 ml of the Carrez reagent I solution (5.20), mix and then add 0,5 ml of the Carrez reagent II solution (5.21). Add 2,5 ml acetonitrile (5.8) and fill up with water (5.1) to the 50 ml mark. Close the calibrated tube with the screw cap and mix well.

Mix well by vortexing (6.7) and let the sample suspension rest for 10 min at an ambient temperature.

Fill up the calibrated tube to the 25 ml mark with demineralized water grade 3 (5.1) with a temperature of 40 °C ± 2 °C, followed by homogenization with a homogenizer (6.6) for 1 min.

NOTE Already well-dissolved samples can be homogenized by shaking.

Check the pH of the homogenized sample solution. The pH shall be within the values pH = 5,0 to pH = 7,0. If necessary, adjust the pH with NaOH solution (5.3) or HCl solution (5.7).

Add 0,5 ml of the Carrez reagent I solution (5.20), mix and then add 0,5 ml of the Carrez reagent II solution (5.21). Add 2,5 ml acetonitrile (5.8) and fill up with water (5.1) to the 50 ml mark. Close the calibrated tube with the screw cap and mix well.

Filter the suspension over black band filter paper (6.4), collect the filtrate and mix 100 µl of filtrate with 900 µl of water grade 3 (5.1) in an HPLC vial (6.11).

Inject 5 µl of the diluted filtrates in the metal-free liquid chromatographic system (6.12).

9.1.2.3 Sample extraction and clean-up for the high sugar contents (50-fold dilution)

Weigh, to the nearest 0,000 1 g, 1,0 g ± 0,1 g in a 50 ml calibrated tube (6.8).

Add 4 ml of the mixture of denatured ethanol (5.16), 0,625 ml of the arabinose internal standard solution (5.30) and 0,5 ml of the PIPES buffer solution (5.22).

Filter the suspension over black band filter paper (6.4), collect the filtrate and mix 20 µl of filtrate with 980 µl of water grade 3 (5.1) in an HPLC vial (6.11).

Inject 5 µl of the diluted filtrates in the metal-free liquid chromatographic system (6.12).

9.2 Chromatographic analysis

Set up the chromatographic system (6.12) with the column oven (6.13), the high-performance anion exchange guard (6.15) and the analytical (6.14) column, the post column reagent pump (6.17), the detector (6.16) and the integrator (6.18). Programme the linear gradient elution profile as presented in Table 4.

Allow the chromatographic system to equilibrate for at least 15 min, followed by a threefold injection of a test standard in order to stabilize the chromatographic system under the conditions specified in Tables 2, 3 and 4.

Calibrate the chromatographic system by successive 5 µl injections of the 8 sugar calibration samples (5.32) under the conditions specified in Tables 2, 3 and 4.

Analyse the prepared filtrates by injecting 5 µl of filtrate samples (9.1) into the chromatographic system under the conditions specified in Tables 2, 3 and 4 (the gradient elution is given as an example for a specific instrument setting).

Inject a series of 4 sugar calibration samples (5.32) after every series of 8 filtrate samples (9.1) in order to account for minor changes in retention times and/or detector sensitivity. Use, alternately, the 4 calibration samples: numbers 1, 3, 5 and 7, and the 4 calibration samples: numbers 2, 4, 6 and 8.

Table 4 — Typical example of a suitable gradient elution profile for the chromatographic separation of the sugars

Time min	Percentage E1 CH ₃ COONa, c = 1 mol/l	Percentage E2 NaOH, c = 0,2 mol/l	Percentage E3 H ₂ O	Percentage E4 CH ₃ COONa, c = 0,025 mol/l	
initial	0	5	88	7	
0	0	5	88	7	Sample injection and start data acquisition
10	0	5	88	7	
15	0	17	76	7	
25	0	93	0	7	
28,1	20	73	0	7	Stop data acquisition and start of column wash
32,0	20	73	0	7	End of column wash
32,1	0	5	88	7	Start column equilibrium
52	0	5	88	7	End column equilibrium and end of cycle

The gradient profile is specific for the applied chromatographic system. It is strongly advised to check the gradient profile. The required chromatographic resolutions are listed in Table 5. In case the resolutions between glucose and sucrose and between sucrose and fructose are less than the required 1,5, it is advised to increase the percentage of eluent E2 (NaOH, 0,2 mol/l) a little bit (from 5 % to 5,5 %) and to decrease the percentage of eluent E3 (degassed water) with the same amount.

Table 5 — Demands chromatographic resolution

Sugars	Minimal required resolution
Arabinose-galactose	> 1,5
Galactose-glucose	> 1,5
Glucose-sucrose	> 1,5
Sucrose-fructose	> 1,5
Fructose-melibiose	> 1,5

10 Calculation and expression of the results

Identify the sugars in the sample solutions by comparing them with the retention times of the corresponding peaks in the sugar calibration samples. Calibrate the chromatographic system by using both series of sugar calibration samples (5.32), which are run before and after a series of 8 filtrate samples (see 9.1).

Calculate the mass concentration of sugar in the stock solution (5.31), ρ_{st} , in mg/ml, using Formula (1):

$$\rho_{st} = \frac{(m \times P / 100)}{500} \quad (1)$$

where

m is the mass of the sugar standard, in mg;

P is the purity of the sugar standard, in %;

100 is a conversion factor;

500 is the volume of the stock solution, in ml.

Calculate the mass concentration of sugar in the calibration solution (5.32), ρ_{cal} , in mg/ml, using Formula (2):

$$\rho_{cal} = \frac{(V_{st} \times \rho_{st})}{200} \quad (2)$$

where

V_{st} is the volume of the sugar stock solution, in ml;

ρ_{st} is the mass concentration of sugar in the stock solution (5.31), in mg/ml;

200 is the volume of the sugar calibration solution, in ml.

For the calibration graphs, use the instrument software for each sugar analyte to plot an eight-point standard curve of the ratio of the instrument response for the analyte and the instrument response for the internal standard arabinose against the concentration of the analyte. Fit a quadratic curve ($y = ax^2 + bx + c$) to the data without forcing through zero, where y is the sugar concentration in mg/ml in the injected 5 μ l calibration samples and x is the relative peak area defined by the ratio of sugar concentration and internal standard concentration of arabinose.

Calculate the mass concentration of sugar in the injected 5 µl of the prepared sample solution, ρ_{inj} , in mg/ml, using [Formula \(3\)](#):

$$\rho_{inj} = ax^2 + bx + c \quad (3)$$

where

a, b, c are values obtained from the quadratic fit;

x is the relative peak area (ratio of sugar concentration and internal standard concentration of arabinose).

Calculate the mass fraction of sugar in the test sample, w_s , in mg/kg, using [Formula \(4\)](#):

$$w_s = \frac{\rho_{inj}}{m} \times D \times 50 \times 1\,000 \quad (4)$$

where

ρ_{inj} is the mass concentration of sugar in the injected 5 µl of the prepared sample solution, in mg/ml;

m is the mass of the test sample, in g;

D is the dilution factor for the filtrated sample; $D = 10$ applying a 10-fold dilution of the filtrated injected sample and $D = 50$ applying a 50-fold dilution of the filtrated injected sample;

50 is a conversion factor, ml;

1 000 is the conversion factor for g to kg.

Quantify the carbohydrates in the samples by applying the calibrations.

11 Precision

11.1 General

Details of the interlaboratory test of the precision of the method are summarized in [Annexes A](#) and [B](#). The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and/or matrices other than those given in [Annexes A](#) and [B](#).

11.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus with the shortest feasible time interval will exceed the repeatability limit r in not more than 5 % of the cases.

The values for galactose, glucose, fructose, sucrose, lactose and maltose are shown in [Tables 6](#) to [11](#), respectively.

Table 6 — Values for galactose

Mean value	Repeatability limit	Test material
$\bar{x} = 0,70 \text{ g/100 g}$	$r = 0,03 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = 0,10 \text{ g/100 g}$	$r = 0,02 \text{ g/100 g}$	commercial infant formula
$\bar{x} = 2,14 \text{ g/100 g}$	$r = 0,07 \text{ g/100 g}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	infant formula (MUVA reference sample)
$\bar{x} = 0,40 \text{ g/100 g}$	$r = 0,03 \text{ g/100 g}$	cream cheese (MUVA reference sample)
$\bar{x} = 0,60 \text{ g/100 g}$	$r = 0,02 \text{ g/100 g}$	whey powder (MUVA reference sample)
$\bar{x} = 0,24 \text{ g/100 g}$	$r = 0,02 \text{ g/100 g}$	processed cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	sweetened condensed milk
$\bar{x} = \text{nd}$	$r = \text{nd}$	infant formula (NIST certified reference material)
Key nd = not detectable		

Table 7 — Values for glucose

Mean value	Repeatability limit	Test material
$\bar{x} = 0,45 \text{ g/100 g}$	$r = 0,02 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = 1,17 \text{ g/100 g}$	$r = 0,06 \text{ g/100 g}$	commercial infant formula
$\bar{x} = 2,23 \text{ g/100 g}$	$r = 0,06 \text{ g/100 g}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 1,08 \text{ g/100 g}$	$r = 0,09 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = 0,38 \text{ g/100 g}$	$r = 0,02 \text{ g/100 g}$	cream cheese (MUVA reference sample)
$\bar{x} = 0,48 \text{ g/100 g}$	$r = 0,06 \text{ g/100 g}$	whey powder (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	processed cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	sweetened condensed milk
$\bar{x} = 2,00 \text{ g/100 g}$	$r = 0,09 \text{ g/100 g}$	infant formula (NIST certified reference material)
Key nd = not detectable		

Table 8 — Values for fructose

Mean value	Repeatability limit	Test material
$\bar{x} = 2,16 \text{ g/100 g}$	$r = 0,10 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = \text{nd}$	$r = \text{nd}$	commercial infant formula
$\bar{x} = \text{nd}$	$r = \text{nd}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 5,53 \text{ g/100 g}$	$r = 0,98 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	cream cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	whey powder (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	processed cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	sweetened condensed milk
$\bar{x} = \text{nd}$	$r = \text{nd}$	infant formula (NIST certified reference material)
Key nd = not detectable		

Table 9 — Values for sucrose

Mean value	Repeatability limit	Test material
$\bar{x} = 2,29 \text{ g/100 g}$	$r = 0,10 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = 7,80 \text{ g/100 g}$	$r = 0,72 \text{ g/100 g}$	commercial infant formula
$\bar{x} = \text{nd}$	$r = \text{nd}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 1,08 \text{ g/100 g}$	$r = 0,11 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	cream cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	whey powder (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	processed cheese (MUVA reference sample)
$\bar{x} = 45,1 \text{ g/100 g}$	$r = 1,7 \text{ g/100 g}$	sweetened condensed milk
$\bar{x} = 27,6 \text{ g/100 g}$	$r = 0,8 \text{ g/100 g}$	infant formula (NIST certified reference material)
Key nd = not detectable		

Table 10 — Values for lactose

Mean value	Repeatability limit	Test material
$\bar{x} = 2,67 \text{ g/100 g}$	$r = 0,19 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = 29,5 \text{ g/100 g}$	$r = 0,4 \text{ g/100 g}$	commercial infant formula
$\bar{x} = 0,22 \text{ g/100 g}$	$r = 0,07 \text{ g/100 g}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 21,6 \text{ g/100 g}$	$r = 4,2 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = 2,52 \text{ g/100 g}$	$r = 0,12 \text{ g/100 g}$	cream cheese (MUVA reference sample)
$\bar{x} = 68,6 \text{ g/100 g}$	$r = 3,2 \text{ g/100 g}$	whey powder (MUVA reference sample)
$\bar{x} = 1,07 \text{ g/100 g}$	$r = 0,08 \text{ g/100 g}$	processed cheese (MUVA reference sample)
$\bar{x} = 10,5 \text{ g/100 g}$	$r = 0,7 \text{ g/100 g}$	sweetened condensed milk
$\bar{x} = 0,52 \text{ g/100 g}$	$r = 0,03 \text{ g/100 g}$	infant formula (NIST certified reference material)
Key nd = not detectable		

Table 11 — Values for maltose

Mean value	Repeatability limit	Test material
$\bar{x} = \text{nd}$	$r = \text{nd}$	sweetened drinking yoghurt
$\bar{x} = 1,77 \text{ g/100 g}$	$r = 0,11 \text{ g/100 g}$	commercial infant formula
$\bar{x} = \text{nd}$	$r = \text{nd}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 1,28 \text{ g/100 g}$	$r = 0,08 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	cream cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	whey powder (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	processed cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$r = \text{nd}$	sweetened condensed milk
$\bar{x} = 2,43 \text{ g/100 g}$	$r = 0,22 \text{ g/100 g}$	infant formula (NIST certified reference material)
Key nd = not detectable		

11.3 Reproducibility

The absolute difference between two single test results found on identical test material reported by two laboratories will exceed the reproducibility limit R in not more than 5 % of the cases.

The values for galactose, glucose, fructose, sucrose, lactose and maltose are shown in [Tables 12 to 17](#), respectively.

Table 12 — Values for galactose

Mean value	Reproducibility limit	Test material
$\bar{x} = 0,70 \text{ g/100 g}$	$R = 0,11 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = 0,10 \text{ g/100 g}$	$R = 0,05 \text{ g/100 g}$	commercial infant formula
$\bar{x} = 2,14 \text{ g/100 g}$	$R = 0,27 \text{ g/100 g}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	infant formula (MUVA reference sample)
$\bar{x} = 0,40 \text{ g/100 g}$	$R = 0,08 \text{ g/100 g}$	cream cheese (MUVA reference sample)
$\bar{x} = 0,60 \text{ g/100 g}$	$R = 0,09 \text{ g/100 g}$	whey powder (MUVA reference sample)
$\bar{x} = 0,24 \text{ g/100 g}$	$R = 0,06 \text{ g/100 g}$	processed cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	sweetened condensed milk
$\bar{x} = \text{nd}$	$R = \text{nd}$	infant formula (NIST certified reference material)
Key		
nd = not detectable		

Table 13 — Values for glucose

Mean value	Reproducibility limit	Test material
$\bar{x} = 0,45 \text{ g/100 g}$	$R = 0,07 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = 1,17 \text{ g/100 g}$	$R = 0,24 \text{ g/100 g}$	commercial infant formula
$\bar{x} = 2,23 \text{ g/100 g}$	$R = 0,25 \text{ g/100 g}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 1,08 \text{ g/100 g}$	$R = 0,29 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = 0,38 \text{ g/100 g}$	$R = 0,09 \text{ g/100 g}$	cream cheese (MUVA reference sample)
$\bar{x} = 0,48 \text{ g/100 g}$	$R = 0,12 \text{ g/100 g}$	whey powder (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	processed cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	sweetened condensed milk
$\bar{x} = 2,00 \text{ g/100 g}$	$R = 0,28 \text{ g/100 g}$	infant formula (NIST certified reference material)
Key		
nd = not detectable		

Table 14 — Values for fructose

Mean value	Reproducibility limit	Test material
$\bar{x} = 2,16 \text{ g/100 g}$	$R = 0,22 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = \text{nd}$	$R = \text{nd}$	commercial infant formula
$\bar{x} = \text{nd}$	$R = \text{nd}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 5,53 \text{ g/100 g}$	$R = 1,5 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	cream cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	whey powder (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	processed cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	sweetened condensed milk
$\bar{x} = \text{nd}$	$R = \text{nd}$	infant formula (NIST certified reference material)
Key		
nd = not detectable		

Table 15 — Values for sucrose

Mean value	Reproducibility limit	Test material
$\bar{x} = 2,29 \text{ g/100 g}$	$R = 0,23 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = 7,80 \text{ g/100 g}$	$R = 0,98 \text{ g/100 g}$	commercial infant formula
$\bar{x} = \text{nd}$	$R = \text{nd}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 1,08 \text{ g/100 g}$	$R = 0,36 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	cream cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	whey powder (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	processed cheese (MUVA reference sample)
$\bar{x} = 45,1 \text{ g/100 g}$	$R = 15,6 \text{ g/100 g}$	sweetened condensed milk
$\bar{x} = 27,6 \text{ g/100 g}$	$R = 3,0 \text{ g/100 g}$	infant formula (NIST certified reference material)
Key nd = not detectable		

Table 16 — Values for lactose

Mean value	Reproducibility limit	Test material
$\bar{x} = 2,67 \text{ g/100 g}$	$R = 0,44 \text{ g/100 g}$	sweetened drinking yoghurt
$\bar{x} = 29,5 \text{ g/100 g}$	$R = 8,1 \text{ g/100 g}$	commercial infant formula
$\bar{x} = 0,22 \text{ g/100 g}$	$R = 0,14 \text{ g/100 g}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 21,6 \text{ g/100 g}$	$R = 6,4 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = 2,52 \text{ g/100 g}$	$R = 0,48 \text{ g/100 g}$	cream cheese (MUVA reference sample)
$\bar{x} = 68,6 \text{ g/100 g}$	$R = 20,5 \text{ g/100 g}$	whey powder (MUVA reference sample)
$\bar{x} = 1,07 \text{ g/100 g}$	$R = 0,35 \text{ g/100 g}$	processed cheese (MUVA reference sample)
$\bar{x} = 10,5 \text{ g/100 g}$	$R = 2,5 \text{ g/100 g}$	sweetened condensed milk
$\bar{x} = 0,52 \text{ g/100 g}$	$R = 0,17 \text{ g/100 g}$	infant formula (NIST certified reference material)
Key nd = not detectable		

Table 17 — Values for maltose

Mean value	Reproducibility limit	Test material
$\bar{x} = \text{nd}$	$R = \text{nd}$	sweetened drinking yoghurt
$\bar{x} = 1,77 \text{ g/100 g}$	$R = 1,4 \text{ g/100 g}$	commercial infant formula
$\bar{x} = \text{nd}$	$R = \text{nd}$	low lactose UHT milk (MUVA reference sample)
$\bar{x} = 1,28 \text{ g/100 g}$	$R = 0,42 \text{ g/100 g}$	infant formula (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	cream cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	whey powder (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	processed cheese (MUVA reference sample)
$\bar{x} = \text{nd}$	$R = \text{nd}$	sweetened condensed milk
$\bar{x} = 2,43 \text{ g/100 g}$	$R = 0,64 \text{ g/100 g}$	infant formula (NIST certified reference material)
Key nd = not detectable		

12 Test report

The test report shall contain the following data:

- a) all information necessary for the identification of the sample (type of sample, origin and designation of the sample);
- b) a reference to this document, i.e. ISO 22184 | IDF 244:2021;
- c) the date and type of sampling procedure (if known);
- d) the date of receipt;
- e) the date of test;
- f) the test results and the units in which they have been expressed;
- g) any operations not specified in the method or regarded as optional, which could have affected the results.

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Annex A (informative)

Precision data

The data given in Tables A.1 to A.6 were obtained in an interlaboratory study, organized by Rotating Disc b.v. and Eurofins Carbohydrate Competence Centre, in accordance with ISO 5725-2 for collaborative study procedures to validate the characteristics of a method of analysis. The report on the interlaboratory study gives further information on chromatographic systems and settings, see <https://standards.iso.org/iso/22184/ed-1/en/> and Reference [12].

Table A.1 — Precision data for galactose in different dairy samples

Parameter	Sweetened drinking yoghurt	Commercial infant formula	UHT milk MUVA	Infant formula MUVA	Cream cheese MUVA	Whey powder MUVA	Processed cheese MUVA	Sweetened condensed milk	Infant formula NIST
Year of interlaboratory test	2018	2018	2018	2018	2018	2018	2018	2018	2018
n = number of laboratories	13	11	13	13	13	13	13		
Not detected or missing data laboratory no.	—	2	—	2	—	—	—		
Cochran outlier(s) (laboratory no.)	—	—	—	—	—	2	—		
Grubb outlier(s) (laboratory no.)	—	—	—	3	—	—	—		
n (excl. outliers)	13	11	13	10	13	11	13		
Mean value, $\bar{x}$, g/100 g	0,70	0,10	2,14	0,08	0,40	0,60	0,24	nd	nd
s_r (% mass fraction)	0,01	< 0,01	0,03	< 0,01	< 0,01	< 0,01	< 0,01		
$C_{V,r}$ in %	1,4	5,6	1,2	6,5	2,3	1,4	2,8		
$r = [2,8 \times s_r]$, g/100 g	0,03	0,02	0,07	0,02	0,03	0,02	0,02		
s_R (% mass fraction)	0,04	0,02	0,10	< 0,01	0,03	0,03	0,02		
$C_{V,R}$ in %	5,4	18,7	4,6	9,8	6,8	5,6	9,0		
$R = [2,8 \times s_R]$, g/100 g	0,11	0,05	0,27	0,02	0,08	0,09	0,06		
$\text{HoRSD}_R = 2 \times \text{mean}^{-0,15}$	4,2	5,6	3,6	5,8	4,6	4,3	4,9		
$\text{HorRat} = C_{V,R}/\text{HoRSD}_R$	1,27	3,31	1,29	1,70	1,47	1,30	1,82		
Key nd = not detectable									

Table A.2 — Precision data for glucose in different dairy samples

Parameter	Sweet-ened drink-ing yoghurt	Com-mercial infant formu-la	UHT milk MUVA	Infant for-mula MUVA	Cream cheese MUVA	Whey powder MUVA	Pro-cessed cheese MUVA	Sweet-ened con-densed milk	Infant formu-la NIST
Year of interlaboratory test	2018	2018	2018	2018	2018	2018	2018	2018	2018
n = number of laboratories	13	13	13	13	13	13			13
Not detected or missing data laboratory no.	1		—	—	—	1			—
Cochran outlier(s) (laboratory no.)	—	—	—	—	—	—			—
Grubb outlier(s) (laboratory no.)	—	—	—	—	—	—			—
n (excl. outliers)	12	13	13	13	13	12			13
Mean value, $\bar{x}$, g/100 g	0,45	1,17	2,23	1,08	0,38	0,48	nd	nd	2,00
s_r (% mass fraction)	0,01	0,02	0,02	0,03	< 0,01	0,02			0,03
$C_{V,R}$ in %	1,3	2,0	1,0	2,9	2,2	4,3			1,5
$r = [2,8 \times s_r]$, g/100 g	0,02	0,06	0,06	0,09	0,02	0,06			0,09
s_R (% mass fraction)	0,02	0,08	0,09	0,10	0,03	0,04			0,10
$C_{V,R}$ in %	5,3	7,4	4,0	9,7	8,1	8,5			5,0
$R = [2,8 \times s_R]$, g/100 g	0,07	0,24	0,25	0,29	0,09	0,12			0,28
$\text{HoRSD}_R = 2 \times \text{mean}^{-0,15}$	4,5	3,7	3,5	3,9	4,6	4,5			3,6
$\text{HorRat} = C_{V,R}/\text{HoRSD}_R$	1,18	1,90	1,14	2,44	1,75	1,91			1,39
Key nd = not detectable									

Table A.3 — Precision data for fructose in different dairy samples

Parameter	Sweet-ened drink-ing yoghurt	Com-mercial infant formu-la	UHT milk MUVA	Infant for-mula MUVA	Cream cheese MUVA	Whey powder MUVA	Pro-cessed cheese MUVA	Sweet-ened con-densed milk	Infant formu-la NIST
Year of interlaboratory test	2018	2018	2018	2018	2018	2018	2018	2018	2018
n = number of laboratories	13			13					
not detected or missing data laboratory no.	1			—					
Cochran outlier(s) (laboratory no.)	—			—					
Grubb outlier(s) (laboratory no.)	—			2					
n (excl. outliers)	12			11					
Mean value, $\bar{x}$, g/100 g	2,16	nd	nd	5,53	nd	nd	nd	nd	nd
s_r (% mass fraction)	0,04			0,35					
$C_{V,r}$ in %	1,7			6,3					
$r = [2,8 \times s_r]$, g/100 g	0,10			0,98					
s_R (% mass fraction)	0,08			0,54					
$C_{V,R}$ in %	3,6			9,8					
$R = [2,8 \times s_R]$, g/100 g	0,22			1,5					
$\text{HoRSD}_R = 2 \times \text{mean}^{-0,15}$	3,6			3,1					
$\text{HorRat} = C_{V,R}/\text{HoRSD}_R$	1,02			3,2					
Key nd = not detectable									