Evaluation Report
proficiency test

DLA ptAI01 (2020)

Lactose and Fructose

in “lactose free” Food - Cookies “brown Cakes”

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Allgemeine Informationen zur Eignungsprüfung (EP)
General Information on the proficiency test (PT)

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EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 16 September 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Keine As part of the present proficency test the following services were subcontracted: none
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Inhalt / Contents

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

2. Realisation

2.1 Test material

Two PT-samples for the detection of lactose/galactose and fructose with contents in the range of mg/100g and one spiking level sample with a simple matrix were provided for analysis. To one of the PT-samples (spiked sample) and the spiking level sample the EP-paramaters lactose and fructose were added in similar concentrations. The results of the spiking level sample should give the possibility of a comparison with the spiked sample in respect to the detectability of the paramaters with and without the influence of matrix and / or food processing.

The test material is a common in commerce cookie without lactose. The basic composition of both samples A and B was the same (see table 1).
After crushing and sieving by means of an impact mill (mesh 1,5 mm) the basic mixture was homogenized. Afterwards the **spiked sample B** was produced as follows:
The spiking materials lactose and fructose were sieved by means of a centrifugal mill (mesh 250 µm), added to an aliquot of the basic mixture and the mixture was homogenized. Subsequently, the basic mixture was again added in 3 additional steps and homogenized in each case until the total quantity had been reached.
For the **spiking level sample**, the spiking materials above mentioned were added during a multi-stage addition of potato powder (mesh 500 µm) and homogenized at each stage.

Afterwards the samples A, B and the spiking level sample were portioned to approximately 25 g into metallised PET film bags.

The composition of the PT samples is shown in Table 1.

Table 1: Composition of DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Cookies "brown Cake" Ingredients: Wheat flour, invert sugar syrup, glucose syrup, drained sugar syrup, sugar, vegetable fat (palm), raising agents: potassium carbonate, ammonium hydrogen carbonate, spices (cinnamon, cloves), salt, lemon peel Nutrients per 100g: Fat 12 g, carbo-hydrates 74 g, protein 6,7 g	100 g/100 g	99,3 g/100g	-
Potato powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,4 g/100 g
Lactose*	-	224 mg/100g	213 mg/100g
Fructose*	-	440 mg/100g	417 mg/100g

*All contents according to gravimetric mixture

Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **micro-tracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples B and the spiking level sample showed a probability of 77% and 93%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17].
This gave a HorRat value of 1,1 and 0,71 respectively. The results of microtracer analysis are given in the documentation.

In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 3.8 and 3.11) [3].

2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].

The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the EP samples was approx. 0,27 (22°C) and 0,29 (23°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of test materials sample A, B and spiking level sample were sent to every participating laboratory in the 16th week of 2020. The testing method was optional. The tests should be finished at 26th June 2020 the latest (extended).

With the cover letter along with the sample shipment the following information was given to participants:

*There are **two different samples A and B** possibly containing the parameters lactose/galactose and fructose in the range relevant for labeling (of lactose) of mg/100g in the **matrix** of **cookie** (lactose-free). One of these samples and the "spiking level sample" were prepared adding lactose and fructose. The **"spiking level sample"** contains the parameters in a simple matrix in **similar amounts**. The spiking level sample should be analysed like a regular sample.*

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).
Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 23 participants submitted the results in time.

3. Evaluation

3.1 Consensus value from participants (assigned value)

The robust mean of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3]. If there are < 12 quantitative results and an increased difference between robust mean and median, the median may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [3].
The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].
In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.
The evaluation is usually carried out starting from 7 results, in justified cases a valuation is also allowed from 5 results.
The actual measurement results will be drafted. Individual results, which are outside the specified measurement range of the participating laboratory (for example with the result > 25 mg/kg or < 2,5 mg/kg) or the indicating "0" will not be considered for the statistic evaluation [3].

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S^x) was calculated. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

3.3 Repeatability standard deviation

The repeatability standard deviation S_r is based on the laboratory´s standard deviation of (outlier free) individual participant results, each under repeatability conditions, that means analyses was performed on the same sample by the same operator using the same equipment in the same laboratory within a short time. It characterizes the mean deviation of the results within the laboratories [3] and is used by DLA as an indication of the homogeneity of the sample material.

In case single results from participants are available the calculation of the repeatability standard deviation S_r, also known as standard deviation within laboratories S_w, is performed by: [3, 4].

The relative repeatability standard deviation as a percentage of the mean value is indicated as coefficient of variation CV_r in the table of statistical characteristics in the results section in case single results from participants are available.

3.4 Reproducibility standard deviation

The reproducibility standard deviation S_R represents a inter-laboratory estimate of the standard deviation for the determination of each parameter on the bases of (outlier free) individual participant results. It takes into account both the repeatability standard deviation S_r and the within-laboratory standard deviation S_S. Reproducibility standard deviations of PTs may differ from reproducibility standard deviations of ring trials, because the participating laboratories of a PT generally use different internal conditions and methods for determining the measured values.
In the present evaluation, the specification of the reproducibility standard deviation, therefore, does not refer to a specific method, but characterizes approximately the comparability of results between the laboratories, assumed the effect of homogeneity and stability of the sample are negligible.

In case single results from participants are available the calculation of the reproducibility standard deviation S_R is performed by: [3, 4].

The relative reproducibility standard deviation as a percentage of the mean value is given as the coefficient of variation CV_R in the statistical characteristics in the results section, provided that the individual results of the participants are available, and the meaning is explained in more detail under 3.9.

3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, too few significant digits (valid digits) or results for another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].
All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

Results obtained by different analytical methods causing an increased variability and/or a bi- or multimodal distribution of results, are treated separately or could be excluded in case of too few numbers of results. For this results are checked by kernel density estimation [3, 12].

Results are tested for outliers by the use of robust statistics (algorithm A): If a value deviates from the robust mean by more than 3 times the robust standard deviation, it can be classified as an outlier (see above) [3]. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3]. Detected outliers are only mentioned in the results section, if they have been excluded from the statistical evaluation.

3.6 Target standard deviation (for proficiency assessment)

The target standard deviation of the assigned value σ_{pt} (= standard deviation for proficiency assessment) can be determined according to the following methods.

If an acceptable quotient S^*/σ_{pt} is present, the target standard deviation of the general model by Horwitz is preferably used for the proficiency assessment. It is usually suitable for evaluation of interlaboratory studies, where different methods are applied by the participants. On the other hand the target standard deviation from the evaluation of precision data of an precision experiment is derived from collaborative studies with specified analytical methods.

In cases where both above-mentioned models are not suitable, the target standard deviation is determined based on values by perception, see under 3.6.3.

For information, the z-scores of both models are given in the evaluation, if available.

In the present PT for evaluation of the results of the parameter fructose the target standard deviation according to the general model of Horwitz was applied (see 3.6.1).
For the parameter lactose the target standard deviation from evaluation of a precision experiment (see 3.6.2) was used (ASU §64 Method: L 01.00-90, [19]).

Additionally for the evaluation of fructose (samples A, B and spiking level sample) and lactose (sample B) the standard uncertainty was considered and the results were evaluated by z´-score (see 3.8).

Due to the low number of < 7 the results of galactose were not evaluated by means of z-scores.

3.6.1 General model (Horwitz)

Based on statistical characteristics obtained in numerous PTs for different parameters and methods Horwitz has derived a general model for estimating the reproducibility standard deviation σ_R [6]. Later the model was modified by Thompson for certain concentration ranges [10]. The reproducibility standard deviation σ_R can be applied as the relative target standard deviation σ_{pt} in % of the assigned values and calculated according to the following equations [3]. For this the assigned value X_{pt} is used for the concentration c.

Equations	**Range of concentrations**	**corresponds to**
$\sigma_R = 0{,}22c$	$c < 1{,}2 \times 10^{-7}$	< 120 µg/kg
$\sigma_R = 0{,}02c^{0{,}8495}$	$1{,}2 \times 10^{-7} \leq c \leq 0{,}138$	≥ 120 µg/kg
$\sigma_R = 0{,}01c^{0{,}5}$	$c > 0{,}138$	> 13,8 g/100g

with c = mass content of analyte (as relative size, e.g. 1 mg/kg = 1 ppm = 10^{-6} kg/kg)

3.6.2 Value by precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment (collaborative trial or proficiency test) the target standard deviation σ_{pt} can be derived considering the number of replicate measurements m of participants in the present PT [3]:

$$\sigma_{pt} = \sqrt{\sigma_R^2 - \sigma_r^2 \left(m - 1 / m \right)}$$

The relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) given in table 2 were obtained in precision experiments by the indicated methods.
The resulting target standard deviations σ_{pt}, which were identified there, were used to evaluate the results and to provide additional information for the statistical data.

Table 2: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviation (RSD_R) according to selected evaluations of tests for precision and the resulting target standard deviation σ_{pt} [18-23]

Parameter	**Matrix**	**Mean** [g/100g]	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Fructose	Rusk	7,0%	1,59%	2,59%	2,33%[1]	ASU §64 L 48.02.07-1
Lactose	Baby food	28,7%	1,66%	3,33%	3,12%	ASU §64 L 48.02.07-1
Lactose	"lactose free" skimmed Milk	0,13%	20%	30%	26,5%	ASU §64 L 01.00-17
Lactose	"lactose free" Milk (3 samples)	0,0282% 0,0804% 0,1257%	6,74% 1,71% 6,25%	10,9% 3,95% 7,33%	9,76%[1] 3,76% 5,85%[1]	ASU §64 L 01.00-90
Lactose	Milk	4,55%	0,48%	1,01%	1,01%	ISO 22662
Lactose	Cream	3,04%	0,66%	4,41%	4,41%	ISO 22662
Lactose	Milk powder	44,5%	0,30%	2,36%	2,36%	ISO 22662

[1] *values used or given for information in the evaluation (s. section 4), for lactose calculated from means of the standard deviations (7,85%)*

3.6.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].

In the present PT, the target standard deviations of 3.6.1. and 3.6.2 were considered suitable.

Table 3 shows selected statistic data of participants results of the present PT compared to PT results of previous years.

Table 3: Characteristics of the present PT (on grey) in comparison to previous PTs since 2016 (SD = standard deviation, CV = coefficient of variation)

Parameter	**Matrix**	**robust Mean** [mg/100g]	**rob. SD (S*)** [mg/100g]	**rel. SD (VK_{S*}) [%]**	**Quotient S^*/σ_{pt}**	**DLA-report**
Fructose	Bread baking mixture	880 660	105 187	11,9 28,3	1,6* 2,1*	DLA 14/2016 (Sample B)**
Fructose	Bread baking mixture	999	287	28,7	2,3*	DLA 18/2017 (Sample B)
Fructose	Cereal pap powder	544	41,3	7,6	1,7	DLA 18/2018 (Sample A)
Fructose	Cake baking mixture	525	38,1	7,3	1,6	DLA 18/2019 (Sample B)
Fructose	Cookies	2390	506	21,2	2,5*	DLA ptAI01 2020 (Probe B)
Lactose	Bread baking mixture	154	26,7	17,3	1,6*	DLA 14/2016 (Sample B)
Lactose	Bread baking mixture	77,7	10,5	13,5	1,9*	DLA 18/2017 (Sample B)
Lactose	Cereal pap powder	289	29,2	10,1	1,3	DLA 18/2018 (Sample A)
Lactose	Cake baking mixture	104	13,1	12,6	1,6	DLA 18/2019 (Sample B)
Lactose	Cookies	209	35,2	16,8	1,9*	DLA ptAI01 2020 (Probe B)

** with target standard deviation σ_{pt}'*
*** enzyme methods (1st line) and other methods (2nd line)*

3.7 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}) [3].
Participants' z-scores are derived from:

$$z_i = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}}$$

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

The z-score valid for the proficiency test is called z-score (σ_{pt}) in the evaluation, while the value called z-score (info) is purely informative. The two z scores are calculated with the different target standard deviations according to 3.6.

3.7.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation.

An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision and use of reference material. If necessary appropriate corrective measures should be applied [3].

In the figures of z-scores DLA gives the limits of warning and action signals as yellow and red lines respectively. According to ISO 13528 the signals are valid only in case of a number of ≥ 10 results [3].

3.8 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.11). The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty (Ux_{pt}) [3].

The calculation is performed by:

$$z'_i = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u_{(x_{pt})}^2}}$$

If carried out an evaluation of the results by means of z 'score, we have defined below the expression in the denominator as a target standard deviation σ_{pt}'.

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 \ .$$

For warning and action signals see 3.7.1.

3.9 Reproducibility coefficient of variation (CV)

The variation coefficient (CV_R) of the reproducibility (= *relative reproducibility standard deviation)* is calculated from the standard deviation and the mean as follows [4, 13]:

$$CV_R = \frac{S_R * 100}{X}$$

In contrast to the standard deviation as a measure of the absolute variability the CV_R gives the relative variability within a data region. While a low CV_R, e.g. <5-10% can be taken as evidence for a homogeneous set of results, a CV_R of more than 50% indicates a "strong inhomogeneity of statistical mass", so that the suitability for certain applications such as the assessment of exceeded maximum levels or the performance evaluation of the participating laboratories possibly can not be done [3].

3.10 Quotient S*/σ_{pt}

Following the HorRat-value the results of a proficiency-test can be considered convincing, if the quotient of robust standard deviation S* and target standard deviation σ_{pt} does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

3.11 Standard uncertainty and traceability

Every assigned value has a standard uncertainty that depends on the analytical method, differences between the analytical methods used, the test material, the number of participating laboratories (P) and on other factors. The standard uncertainty ($U_{(Xpt)}$) for this PT is calculated as follows [3]:

$$u_{(x_{pt})} = 1{,}25 \times \frac{s^*}{\sqrt{p}}$$

If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.

The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

3.12 Recovery rates: Spiking

For the lactose results of the spiking level sample and the spiked sample recovery rates were calculated by DLA with respect to the known content of added lactose. The related values of added lactose are given in 2.1 test material in table 1. As a range of acceptance RA for valuating participant's results the range of 85 - 115% for the recovery rates were deduced from published methods [18-23]. The calculation of the associated z-scores was carried out according to 3.5 with the target standard deviation of 7,5%

4. Results

All following tables are anonymized. With the delivering of the evaluation report the participants are informed about their individual evaluation number.

In the first table the characteristics are listed:

Statistic Data
Number of results
Number of outliers
Mean
Median
Robust mean(X_{pt})
Robust standard deviation (S^*)
Target range:
Target standard deviation σ_{pt} or σ_{pt}'
Target standard deviation for information
lower limit of target range ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}$') *
upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}$´) *
Quotient S^*/σ_{pt} *or* S^*/σ_{pt}'
Standard uncertainty $U(X_{pt})$
Number of results in the target range
Percent in the target range

* Target range is calculated with z-score or z'-score

In the table below, the results of the participating laboratories are formatted in 3 valid digits**:

Auswerte-nummer **Evaluation number**	**Parameter [Einheit / Unit]**	**Abweichung** **Deviation**	**z-Score σpt**	**z-Score** (Info)	**Hinweis** **Remark**

*** In the documentation part, the results are given as they were transmitted by the participants.*

4.1 Fructose

4.1.1 Fructose Sample A (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results°	11
Number of outliers	2
Mean	1940
Median	1820
Robust Mean (X_{pt})	**1940**
Robust standard deviation (S*)	**322**
Target range:	
Target standard deviation σ_{pt}'	**140**
Target standard deviation (for Information)	45,2
lower limit of target range	**1660**
upper limit of target range	**2220**
Quotient S/σ_{pt}'*	*2,3*
Standard uncertainty $U_{(Xpt)}$	*121*
Results in the target range	7
Percent in the target range	*64%*

° number without outliers (results no. 4 and no. 13)

Comments:

The target standard deviation was calculated according to the model of Horwitz (s. 3.6.1). Additionally the target standard deviation using data from precision experiments (ASU §64 L 48.02.07-1, [22]) is given for information (s. 3.6.2).

The distribution of results showed an increased variability. The quotient S^*/σ_{pt} was well above 2. Therefore the valuation was done by z'-scores considering the standard uncertainty. The quotient S^*/σ_{pt}' was then 2,3. The robust standard deviation was in the range of previous PTs (see 3.6.3). The comparability of results is given.

64% of results were in the target range.

Fructose was not added to sample A, the fructose content comes from the ingredients of the basic matrix (s. p. 5).

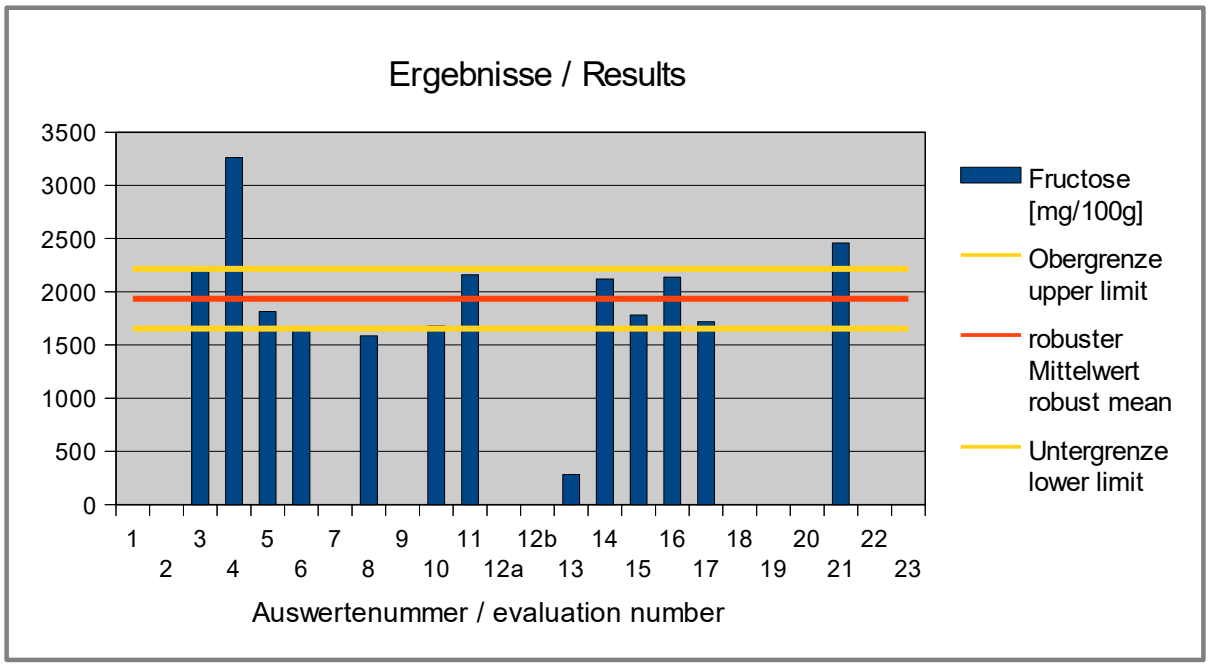

Abb. / Fig. 1: Ergebnisse Fructose Probe A/ Results fructose sample A

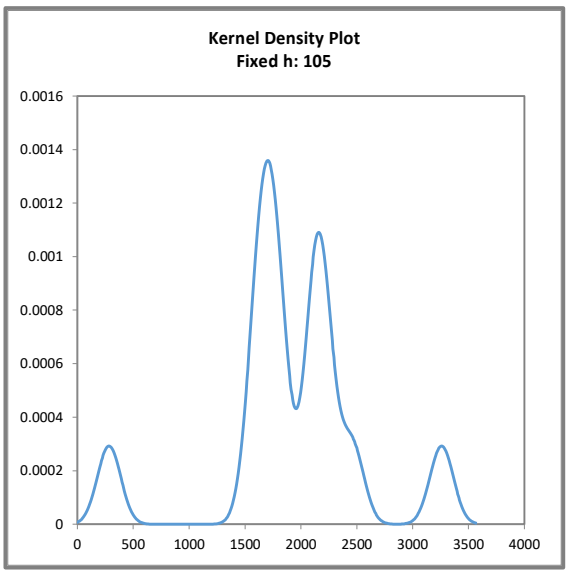

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

Kernel density plot of results (with h = 0,75 x σ_{pt} of X_{pt})

Comment:
The kernel density showed a distribution with two maxima and two smaller side peaks, due to two outliers. Since the differences in the results were not dependent on the applied methods, all results were evaluated together (see documentation).

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Fructose [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2					
3	2230	295	2,1	6,5	
4	3260				Outlier excluded
5	1820	-120	-0,86	-2,7	
6	1640	-296	-2,1	-6,6	
7					
8	1590	-350	-2,5	-7,8	
9					
10	1680	-255	-1,8	-5,7	
11	2160	225	1,6	5,0	
12a					
12b					
13	283				Outlier excluded
14	2120	185	1,3	4,1	
15	1780	-154	-1,1	-3,4	
16	2140	204	1,5	4,5	
17	1720	-215	-1,5	-4,8	
18					
19					
20					
21	2460	525	3,7	11,6	
22					
23					

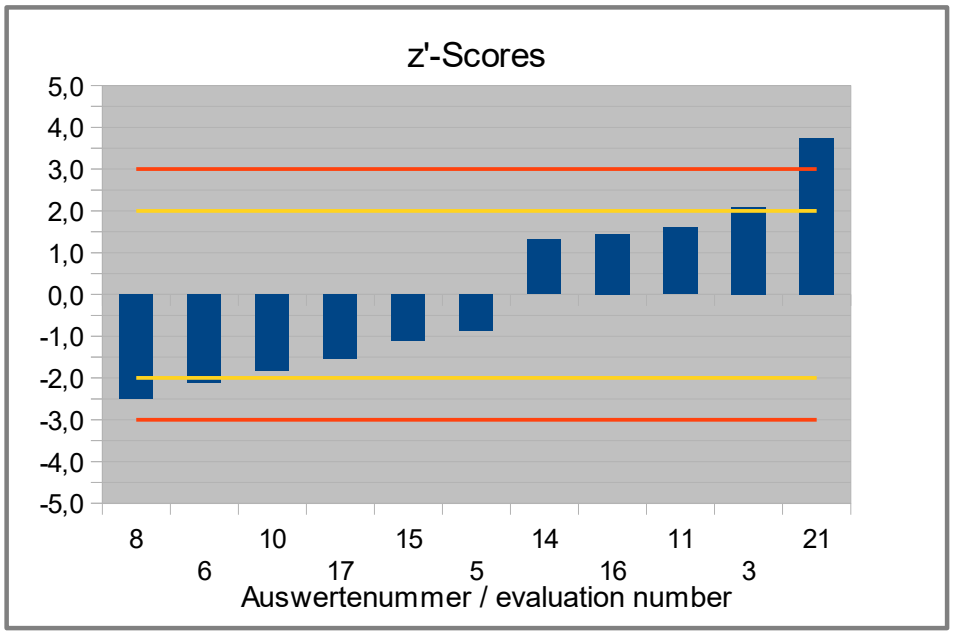

Abb. / Fig. 3: z'-Scores Fructose Probe A / fructose sample A

4.1.2 Fructose Sample B (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results°	12
Number of outliers	1
Mean	2420
Median	2370
Robust Mean (X_{pt})	**2390**
Robust standard deviation (S*)	**506**
Target range:	
Target standard deviation σpt'	**201**
Target standard deviation (for Information)	55,7
lower limit of target range	**1990**
upper limit of target range	**2790**
Quotient S/σ_{pt}'*	*2,5*
Standard uncertainty $U_{(Xpt)}$	*183*
Results in the target range	*7*
Percent in the target range	*58%*

° *number without outliers (result no. 13)*

Comments:

The target standard deviation was calculated according to the model of Horwitz (s. 3.6.1). Additionally the target standard deviation using data from precision experiments (ASU §64 L 48.02.07-1, [22]) is given for information (s. 3.6.2).

The distribution of results showed an increased variability. The quotient S/σ_{pt} was well above 2. Therefore the valuation was done by z'-scores considering the standard uncertainty. The quotient S*/σ_{pt}´ was then 2,5. The robust standard deviation was in the range of previous PTs (see 3.6.3). The comparability of results is given.*

58% of results were in the target range.

Fructose was not added to the basic matrix of sample A (s. p. 5).

The difference of the robust means of the participants' results for sample B and sample A (450 mg/100g) was at 103% of the spiking level of fructose to the sample B (s. p. 5).

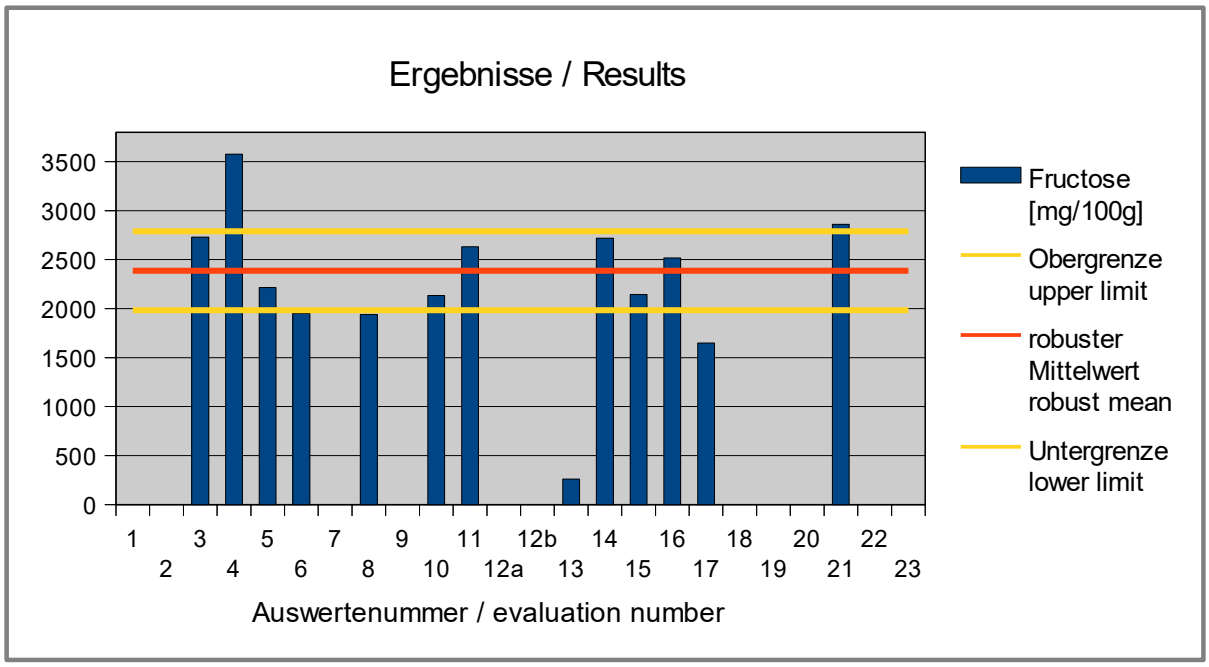

Abb. / Fig. 4: Ergebnisse Fructose Probe B/ Results fructose sample B

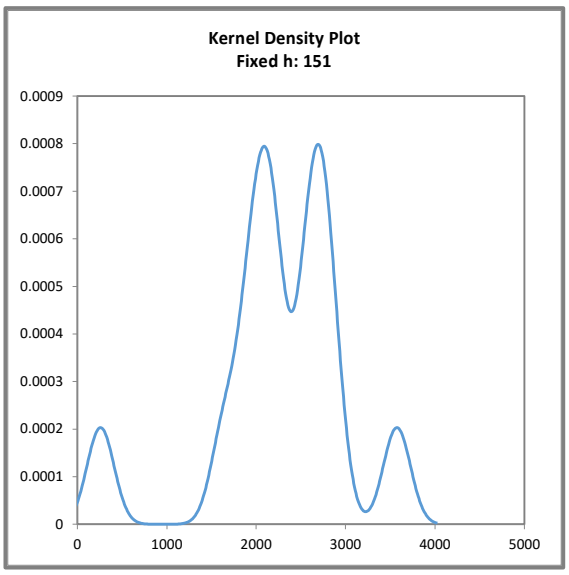

Abb. / Fig. 5:
Kerndichte-Schätzung der Ergebnisse
(mit h = 0,75 x σ_{pt} von X_{pt})

Kernel density plot of results
(with h = 0,75 x σ_{pt} of X_{pt})

Comment:
The kernel density showed a distribution with two maxima and two smaller side peaks, due to two outliers. Since the differences in the results were not dependent on the applied methods, all results were evaluated together (see documentation).

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Fructose [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2					
3	2730	344	1,7	6,2	
4	3580	1189	5,9	21,4	
5	2220	-171	-0,85	-3,1	
6	1950	-435	-2,2	-7,8	
7					
8	1940	-446	-2,2	-8,0	
9					
10	2130	-254	-1,3	-4,6	
11	2630	244	1,2	4,4	
12a					
12b					
13	260				Outlier excluded
14	2720	334	1,7	6,0	
15	2140	-242	-1,2	-4,4	
16	2520	131	0,65	2,3	
17	1650	-736	-3,7	-13,2	
18					
19					
20					
21	2860	474	2,4	8,5	
22					
23					

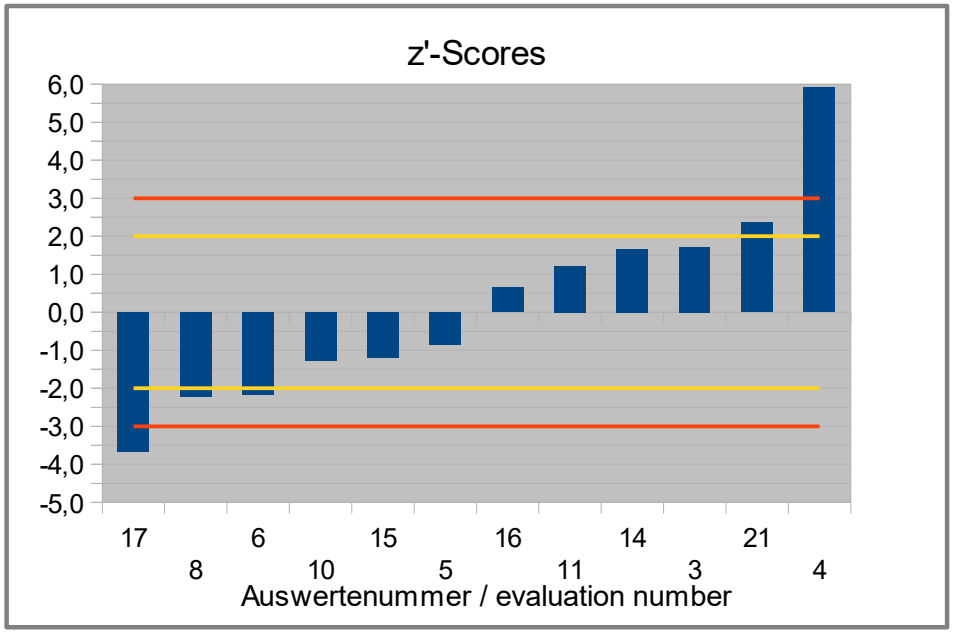

Abb. / Fig. 6: z-Scores Fructose Probe B / fructose sample B

4.1.3 Fructose Spiking Level Sample (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results°	12
Number of outliers	0
Mean	478
Median	480
Robust Mean (x_{pt})	**482**
Robust standard deviation (S*)	**55,9**
Target range:	
Target standard deviation $\sigma_{pt'}$	**29,5**
Target standard deviation (for Information)	11,2
lower limit of target range	**423**
upper limit of target range	**541**
Quotient S/$\sigma_{pt'}$*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*20,2*
Results in the target range	*9*
Percent in the target range	*75%*

° *number without result no. 14 (excluded)*

Comments:

The target standard deviation was calculated according to the model of Horwitz (s. 3.6.1). Additionally the target standard deviation using data from precision experiments (ASU §64 L 48.02.07-1, [22]) is given for information (s. 3.6.2).

The distribution of results showed a slightly increased variability with a quotient S/σ_{pt} of 2,6. Therefore the valuation was done by z'-scores considering the standard uncertainty. The quotient S*/σ_{pt}´ was then 1,9. The robust standard deviation was in the range of previous PTs (see 3.6.3). The comparability of results is given.*

75% of results were in the target range.

The robust mean of participant results was 116 % of the spiking level of fructose to the spiking level sample (s. p. 5).

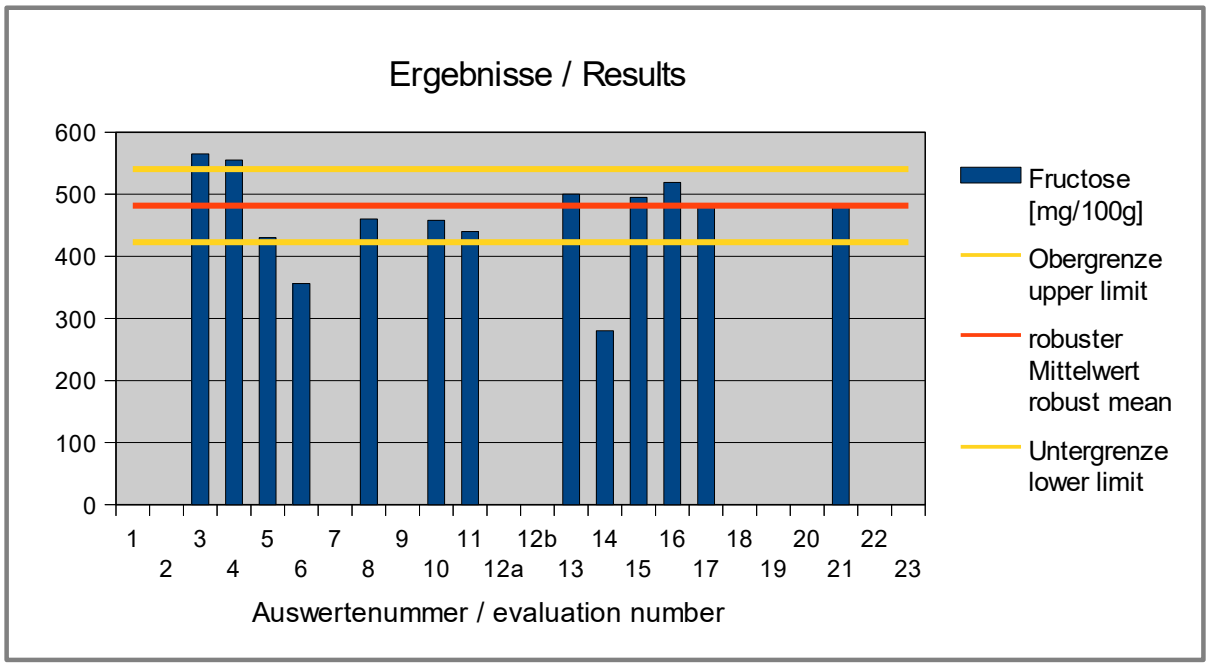

Abb. / Fig. 7: Ergebnisse Fructose Dotierungsniveauprobe / Results Fructose spiking level sample

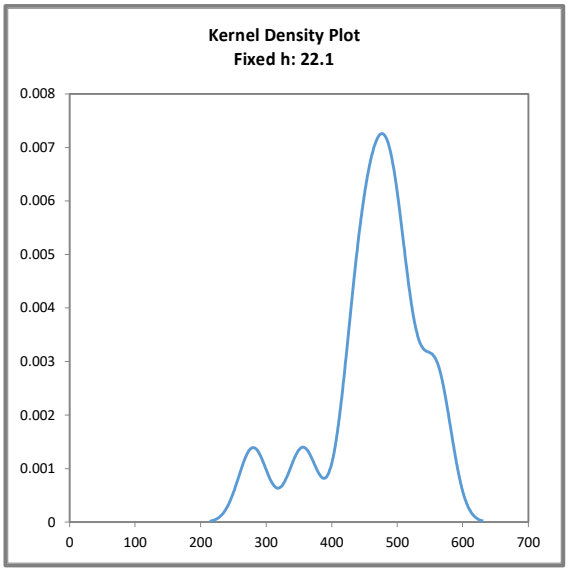

Abb. / Fig. 8:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

Kernel density plot of results (with h = 0,75 x σ_{pt} of X_{pt})

Comment:
The kernel density shows almost a symmetrical distribution of results with a small shoulder at approx. 550 mg/100g and two additional peaks at < 400 mg/100g due to two results outside the target range.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Fructose [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2					
3	565	83,4	2,8	7,4	
4	555	73,4	2,5	6,5	
5	430	-51,6	-1,8	-4,6	
6	356	-125,6	-4,3	-11	
7					
8	460	-21,6	-0,73	-1,9	
9					
10	458	-23,8	-0,81	-2,1	
11	440	-41,6	-1,4	-3,7	
12a					
12b					
13	500	18,4	0,62	1,6	
14	280				Result excluded
15	495	13,4	0,45	1,2	
16	519	37,4	1,3	3,3	
17	480	-1,6	-0,06	-0,15	
18					
19					
20					
21	480	-1,6	-0,06	-0,15	
22					
23					

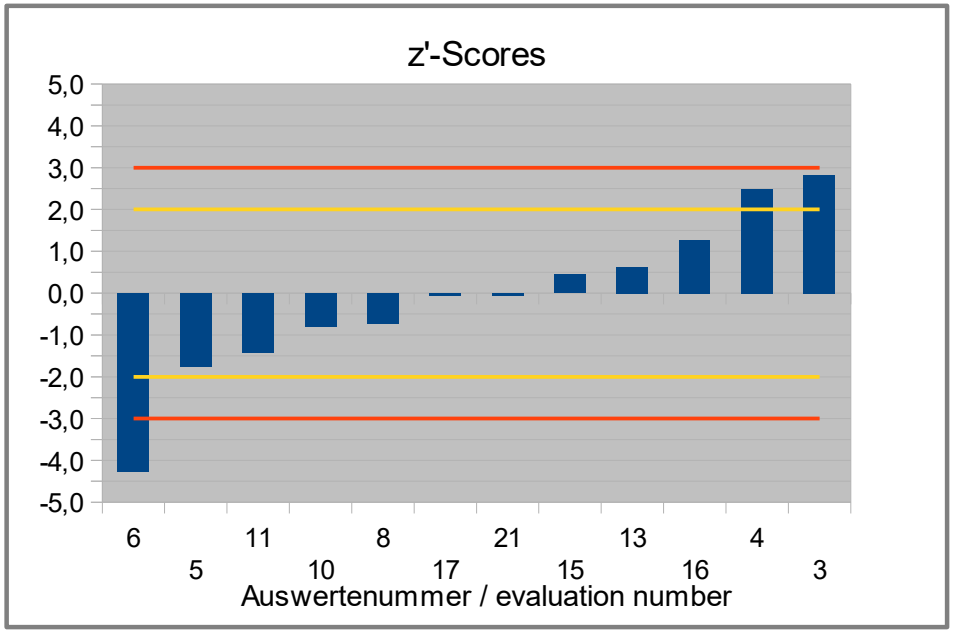

Abb. / Fig. 9: z'-Scores Fructose Dotierungsniveauprobe / fructose spiking level sample

4.2 Lactose

4.2.1 Qualitative Evaluation Sample A and Sample B

Vergleichsuntersuchung / Proficiency Test

Evaluation number	Sample A	Sample A	Sample B	Sample B	Qualitative Valuation	Remarks
	pos/neg	[mg/kg]	pos/neg	[mg/kg]	Agreement with consensus value	
1	negative	-	positive	210	1/1 (100%)	
2	negative	0	positive	>100	1/1 (100%)	
3	positive	<BG	positive	105	1/1 (100%)	
4	positive	145	positive	160	1/1 (100%)	
5	negative	0	positive	201	1/1 (100%)	
6	negative	<2	positive	235	1/1 (100%)	
7	negative	<2	positive	202	1/1 (100%)	
8	negative	<20	positive	219	1/1 (100%)	
9						
10	positive	119	positive	316	1/1 (100%)	
11	positive	160	positive	170	1/1 (100%)	
12a	negative	<10	positive	220	1/1 (100%)	
12b	negative	<5	positive	225	1/1 (100%)	
13	positive	2,85	positive	199	1/1 (100%)	
14	negative	<10	positive	200	1/1 (100%)	
15	negative		positive	213	1/1 (100%)	
16	positive	81,4	positive	260	1/1 (100%)	
17	negative	<LOD	positive	200	1/1 (100%)	
18	negative	<20	positive	>200	1/1 (100%)	
19	negative	<20	positive	181	1/1 (100%)	
20	positive	450	positive	164	1/1 (100%)	
21	negative	<5	positive	234	1/1 (100%)	
22	negative	<3,64	positive	220	1/1 (100%)	
23	positive	40,8	positive	255	1/1 (100%)	

	Sample A		Sample B	
Number positive	8		23	
Number negative	15		0	
Percent positive	35		100	
Percent negative	65		0	
Consensus value	none		positive	

Comments:
The consensus value for sample B is in qualitative agreement with the spiking of sample B. For sample A (no added lactose) no consensus value of ≥75% positive or negative results was obained.

4.2.2 Lactose Sample A (in mg/100g)

Due to the small number of available quantitative results (7) and their high variability, no statistical analysis was carried out.

4.2.3 Lactose Sample B (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	21
Number of outliers	-
Mean	209
Median	210
Robust Mean (x_{pt})	**209**
Robust standard deviation (S*)	**35,2**
Target range:	
Target standard deviation σ_{pt}'	**19,0**
Target standard deviation (for Information)	10,6
lower limit of target range	**171**
upper limit of target range	**247**
Quotient S/σ_{pt}'*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*9,59*
Results in the target range	*15*
Percent in the target range	*71%*

Comments:

The target standard deviation was calculated using data from a precision experiment (ASU §64 L 01.00-90, [19]*)(3.6.2). Additionally the target standard deviation according to the model of Horwitz (s. 3.6.1) is given for information.*

The distribution of results showed a slightly increased variability with a quotient S/σ_{pt} of 2,1. Therefore the valuation was done by z'-scores considering the standard uncertainty. The quotient S*/σ_{pt}´ was then 1,9. The robust standard deviation was in the range of previous PTs (see 3.6.3). The comparability of results is given.*

71% of results were in the target range.

The robust mean of participant results was 93 % of the spiking level of fructose to sample B (s. p. 5).

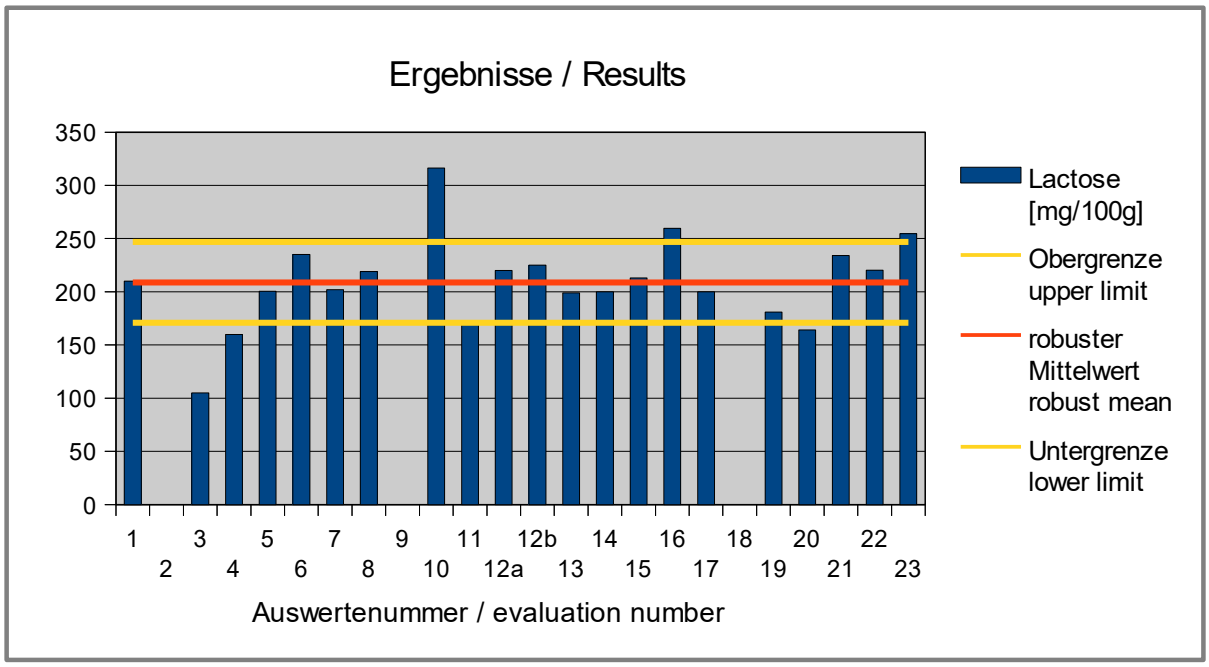

Abb. / Fig. 10: Ergebnisse Lactose Probe B / Results lactose sample B

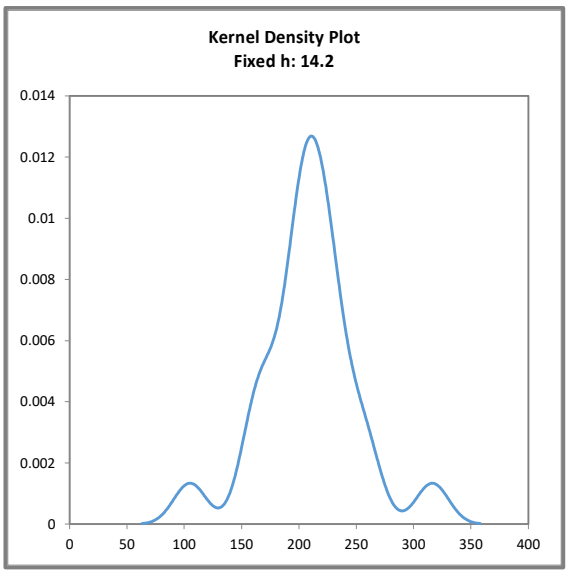

Abb. / Fig. 11:
Kerndichte-Schätzung der Ergebnisse
(mit h = 0,75 x σ_{pt} von X_{pt})

Kernel density plot of results
(with h = 0,75 x σ_{pt} of X_{pt})

Comment:
The kernel density shows almost a symmetrical distribution of results with a small shoulder at approx. 170 mg/100g and two additional peaks at 105 mg/100g und 316 mg/100g due to two results outside the target range.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Lactose [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	210	1,2	0,06	0,11	
2	>100				
3	105	-103,8	-5,5	-9,8	
4	160	-48,8	-2,6	-4,6	
5	201	-8,3	-0,44	-0,78	
6	235	26,2	1,4	2,5	
7	202	-6,8	-0,36	-0,64	
8	219	10,2	0,54	0,97	
9					
10	316	107,6	5,7	10	
11	170	-38,8	-2,0	-3,7	
12a	220	11,2	0,59	1,1	
12b	225	16,2	0,85	1,5	
13	199	-9,8	-0,52	-0,93	
14	200	-8,8	-0,46	-0,83	
15	213	4,2	0,22	0,40	
16	260	50,8	2,7	4,8	
17	200	-8,8	-0,46	-0,83	
18	>200				
19	181	-27,8	-1,5	-2,6	
20	164	-44,8	-2,4	-4,2	
21	234	25,2	1,3	2,4	
22	220	11,6	0,61	1,1	
23	255	45,7	2,4	4,3	

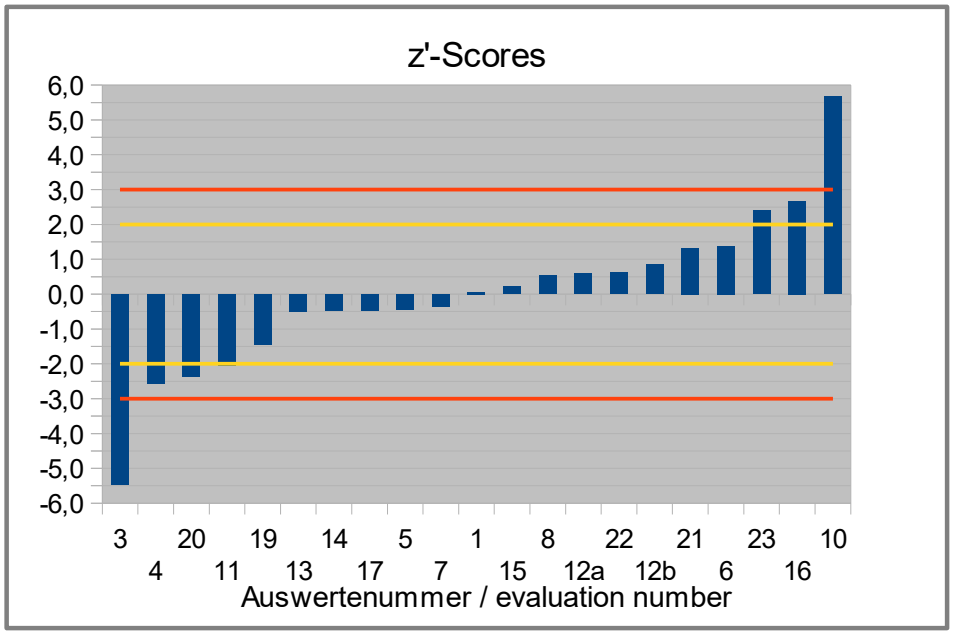

Abb. / Fig. 12: z'-Scores Lactose Probe B / lactose sample B

4.2.3 Lactose Spiking Level Sample (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results°	20
Number of outliers	1
Mean	190
Median	193
Robust Mean (x_{pt})	**190**
Robust standard deviation (S*)	**27,8**
Target range:	
Target standard deviation σ_{pt}	**14,9**
Target standard deviation (for Information)	9,76
lower limit of target range	**160**
upper limit of target range	**220**
Quotient S/σ_{pt}*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*7,77*
Results in the target range	*15*
Percent in the target range	*75%*

° *number without outliers (result no. 9)*

Comments:

The target standard deviation was calculated using data from a precision experiment (ASU §64 L 01.00-90, [19]*)(3.6.2). Additionally the target standard deviation according to the model of Horwitz (s. 3.6.1) is given for information.*

The distribution of results showed a normal variability. The quotient S/σ_{pt} was below 2,0. The robust standard deviation was in the range of previous PTs (see 3.6.3). The comparability of results is given.*

75% of results were in the target range.

The robust mean of participant results was 89 % of the spiking level of lactose to the spiking level sample (s. p. 5).

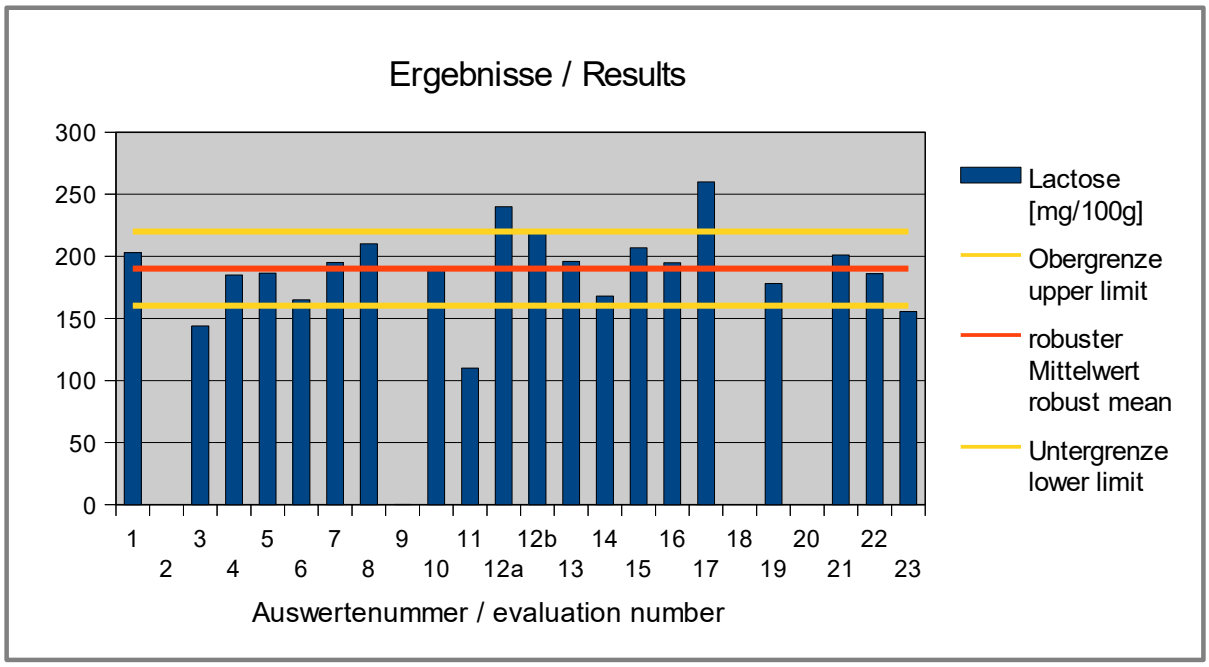

Abb. / Fig. 13: Ergebnisse Lactose Dotierungsniveauprobe / Results lactose spiking level sample

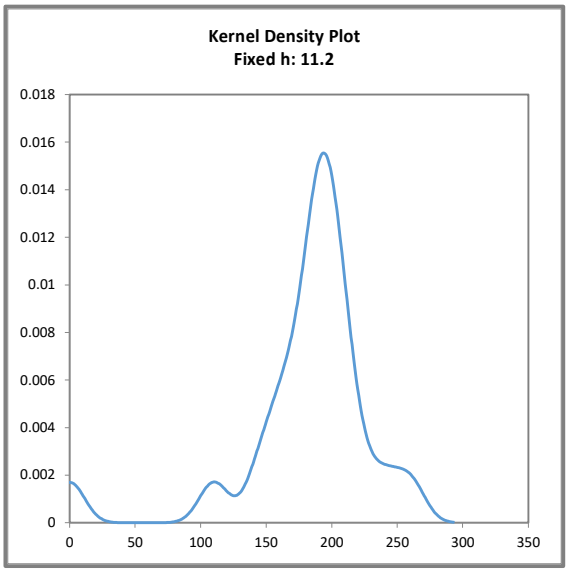

Abb. / Fig. 14:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

Kernel density plot of results (with h = 0,75 x σ_{pt} of X_{pt})

Comment:
The kernel density shows nearly a symmetrical distribution of results with three small side peaks < 130 mg/100g and > 240 mg/100g, due to three results outside the target range.

Ergebnisse der Teilnehmer / Results of Participants:

Auswerte-nummer Evaluation number	Lactose [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	203	12,9	0,87	1,3	
2	>100				
3	144	-46,1	-3,1	-4,7	
4	185	-5,1	-0,34	-0,52	
5	187	-3,6	-0,24	-0,36	
6	165	-25,1	-1,7	-2,6	
7	195	4,9	0,33	0,51	
8	210	19,9	1,3	2,0	
9	0,180				Outlier excluded
10	190	0,1	0,00	0,01	
11	110	-80,1	-5,4	-8,2	
12a	240	49,9	3,3	5,1	
12b	220	29,9	2,0	3,1	
13	196	5,9	0,40	0,61	
14	168	-22,1	-1,5	-2,3	
15	207	16,9	1,1	1,7	
16	195	4,8	0,32	0,50	
17	260	69,9	4,7	7,2	
18	>200				
19	178	-12,1	-0,81	-1,2	
20					
21	201	10,9	0,73	1,1	
22	186	-4,1	-0,27	-0,42	
23	156	-34,6	-2,3	-3,5	

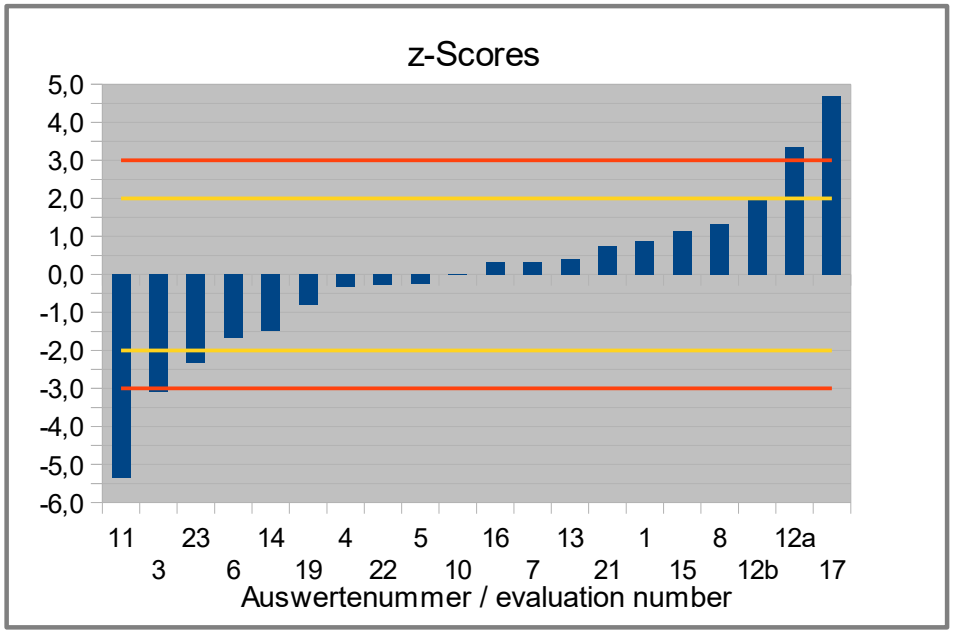

Abb. / Fig. 15: z-Scores Lactose Dotierungsniveauprobe / lactose spiking level sample

4.2.4 Recovery Rates for Lactose

Hereafter the recovery rates of the participants' results with respect to the level of addition (page 5, table 1) were calculated by DLA and given for information only. The related z-scores are based on the target standard deviation of 7,5%.

Spiking Level Sample and Sample B

Auswerte-nummer	Dotierungs-niveauprobe	Wiederfin-dungsrate*		Probe B	Wiederfin-dungsrate*		Hinweis
	[mg/kg]	[%]	[Z_{RR}]	[mg/kg]	[%]	[Z_{RR}]	
1	203	**95**	-0,63	210	**94**	-0,83	
2	>100			>100			
3	144	68	-4,3	105	47	-7,1	
4	185	**87**	-1,8	160	71	-3,8	
5	187	**88**	-1,7	201	**90**	-1,4	
6	165	77	-3,0	235	**105**	0,65	
7	195	**92**	-1,1	202	**90**	-1,3	
8	210	**99**	-0,19	219	**98**	-0,30	
9	0,180	0	-13				
10	190	**89**	-1,4	316	141	5,5	
11	110	52	-6,4	170	76	-3,2	
12a	240	**113**	1,7	220	**98**	-0,24	
12b	220	**103**	0,44	225	**100**	0,06	
13	196	**92**	-1,1	199	**89**	-1,5	
14	168	79	-2,8	200	**89**	-1,4	
15	207	**97**	-0,38	213	**95**	-0,65	
16	195	**92**	-1,1	260	116	2,1	
17	260	122	2,9	200	**89**	-1,4	
18	>200			>200			
19	178	84	-2,2	181	81	-2,6	
20				164	73	-3,6	
21	201	**94**	-0,75	234	**104**	0,60	
22	186	**87**	-1,7	220	**98**	-0,21	
23	156	73	-3,6	255	**114**	1,8	

AB**	85-115 %	AB**	85-115 %
Anzahl im AB	**13**	Anzahl im AB	**14**
Prozent im AB	**62**	Prozent im AB	**67**

* Wiederfindungsrate 100% Bezugsgröße: Lactose, s. Seite 5

** Akzeptanzbereich Kapitel 3.12 (S. 14)

Comments:

For the spiking level sample 62% (13) of the participants obtained a recovery rate within the range of 85-115%. For the spiked food matrix sample B 67% (14) of the recovery rates were in this range.

4.3 Galactose

4.3.1 Galactose Sample A (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Due to the low number of results <7 and variability of results no statistical evaluation was done.

Statistic Data	
Number of results	5
Number of outliers	0
Mean	69,8
Median	28,0
Robust Mean (x_{pt})	**69,8**
Robust standard deviation (S*)	
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Results in the target range	
Percent in the target range	

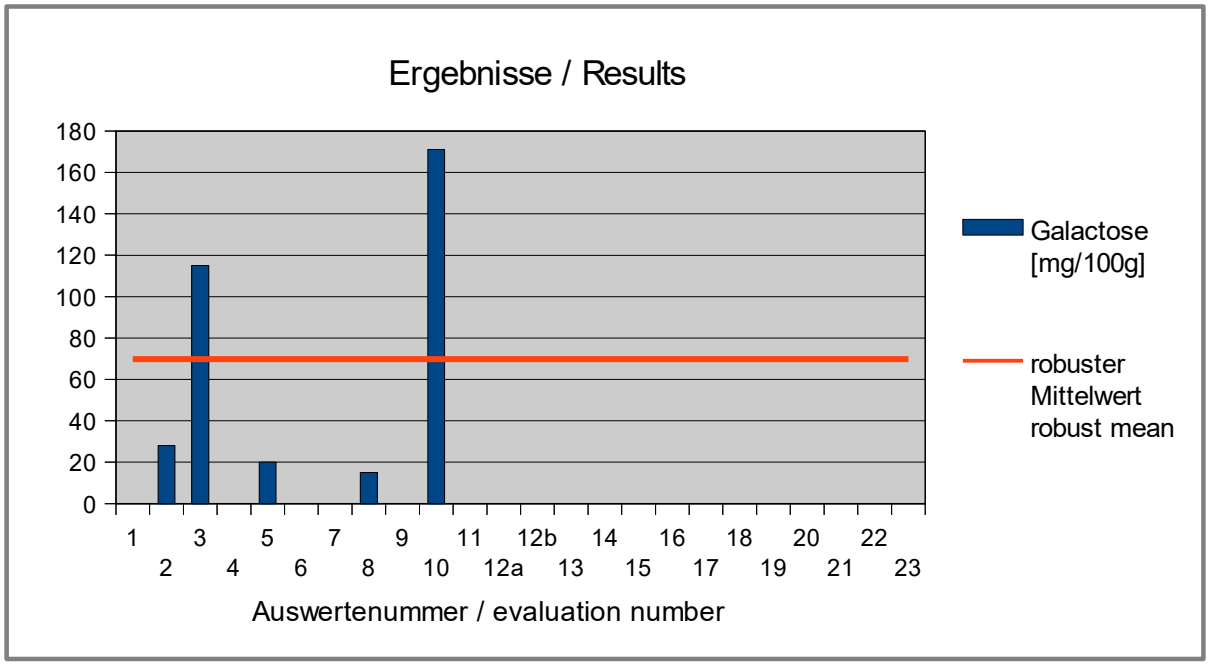

Abb. / Fig. 16: Ergebnisse Galactose Probe A / Results galactose sample A

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Galactose [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2	28,0				
3	115				
4					
5	20,0				
6	<50				
7					
8	15,0				
9					
10	171				
11					
12a					
12b					
13					
14	<200				
15					
16	<100				
17					
18					
19	<10				
20					
21	<100				
22					
23					

4.3.2 Galactose Sample B (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Due to the low number of results <7 no statistical evaluation was done.

Statistic Data	
Number of results	4
Number of outliers	0
Mean	80,9
Median	74,5
Robust Mean (x_{pt})	**80,9**
Robust standard deviation (S*)	
Target range:	
Target standard deviation σ_{pt}	
Target standard deviation (for Information)	
lower limit of target range	
upper limit of target range	
Quotient S/σ_{pt}*	
Standard uncertainty $U_{(Xpt)}$	
Results in the target range	
Percent in the target range	

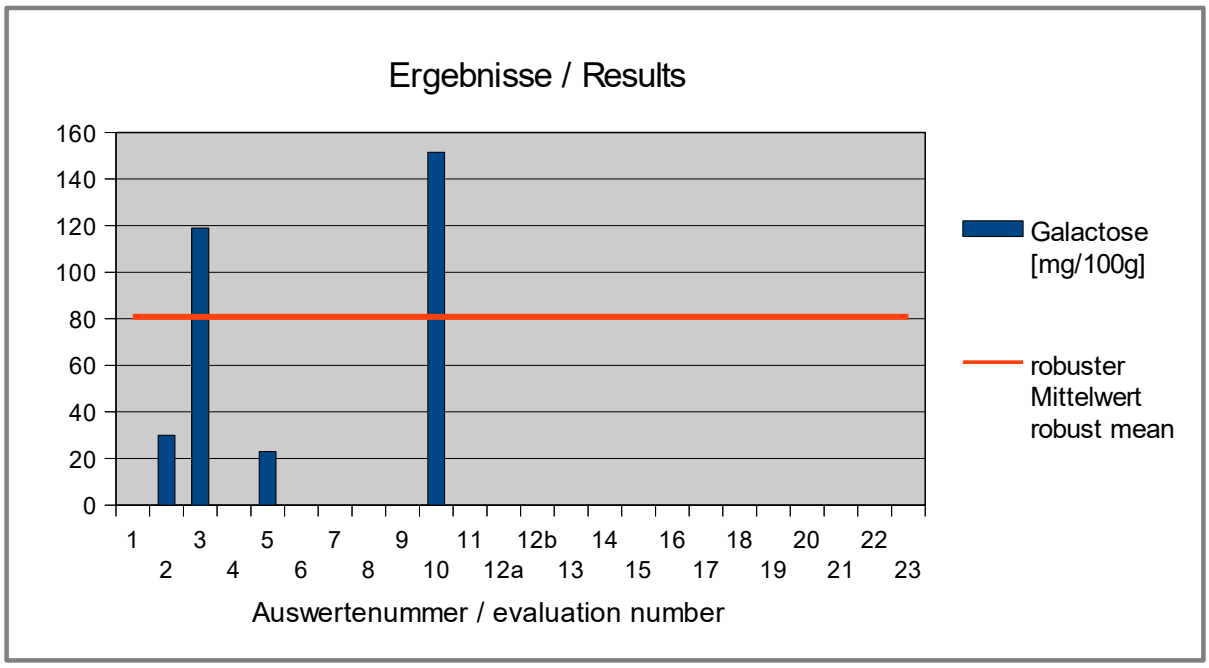

Abb. / Fig. 17: Ergebnisse Galactose Probe B / Results galactose sample B

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Galactose [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2	30				
3	119				
4					
5	23				
6	<50				
7					
8	<20				
9					
10	151				
11					
12a					
12b					
13					
14	<200				
15					
16	<100				
17					
18					
19	<10				
20					
21	<100				
22					
23					

4.3.3 Galactose Spiking Level Sample (in mg/100g)

For galactose, no results were given above the limit of detection or limit of quantitation for the spiking level sample (see documentation).

4.4 Participant z-Scores: overview table

Z-Scores for the assigned values from participants results (consensus values)

Auswerte-nummer	Fructose			Lactose	
	Sample A°	Sample B°	Spiking Le-vel Sample°	Sample B°	Spiking Le-vel Sample°
1				0,06	0,87
2					
3	2,1	1,7	2,8	-5,5	-3,1
4		5,9	2,5	-2,6	-0,34
5	-0,86	-0,85	-1,8	-0,44	-0,24
6	-2,1	-2,2	-4,3	1,4	-1,7
7				-0,36	0,33
8	-2,5	-2,2	-0,73	0,54	1,3
9					
10	-1,8	-1,3	-0,81	5,7	0,00
11	1,6	1,2	-1,4	-2,0	-5,4
12a				0,59	3,3
12b				0,85	2,0
13			0,62	-0,52	0,40
14	1,3	1,7		-0,46	-1,5
15	-1,1	-1,2	0,45	0,22	1,1
16	1,5	0,65	1,3	2,7	0,32
17	-1,5	-3,7	-0,06	-0,46	4,7
18					
19				-1,5	-0,81
20				-2,4	
21	3,7	2,4	-0,06	1,3	0,73
22				0,61	-0,27
23				2,4	-2,3

° z'-Score

Z-Scores for the assigned values from spiking level (recovery rates)

Evaluation number	Lactose	
	Sample B°	Spiking Level Sample
1	-0,83	-0,63
2		
3	-7,1	-4,3
4	-3,8	-1,8
5	-1,4	-1,7
6	0,65	-3,0
7	-1,3	-1,1
8	-0,30	-0,19
9		-13
10	5,5	-1,4
11	-3,2	-6,4
12a	-0,24	1,7
12b	0,06	0,44
13	-1,50	-1,1
14	-1,40	-2,8
15	-0,65	-0,4
16	2,1	-1,1
17	-1,40	2,9
18		
19	-2,6	-2,2
20	-3,6	
21	0,6	-0,75
22	-0,21	-1,7
23	1,8	-3,6

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal" / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal" / action signal (in red)

5. Documentation

Note: Information given in German were translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1 Details by the participants

5.1.1 Primary Data

Fructose Sample A

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Sample A			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Fructose	1	mg/100g							
	2	mg/100g							
	3	mg/100g	06.05.20	2230	yes	500	1600	no	98
	4	mg/100g	07.05.20	3260	yes			no	
	5	mg/100g	20.05.20	1815	yes			no	
	6	mg/100g	15.05.20	1639	YES	-	50	NO	-
	7	mg/100g							
	8	mg/100g	11.05.20	1585	yes		100	no	
	9	mg/100g							
	10	mg/100g	10/06	1679,99	yes	2	5	no	>90%
	11	mg/100g		2160	yes				
	12a	mg/100g							
	12b	mg/100g							
	13	mg/100g	19.06.20	283	yes		2	no	
	14	mg/100g	12.05.20	2120	yes	100	200	no	
	15	mg/100g	05.05.20	1781	yes	30	100	no	
	16	mg/100g	04.05.20	2139	yes	20	100	no	
	17	mg/100g	18.05.20	1720	YES	100	300	NO	
	18	mg/100g	-	-	-	-	-	-	-
	19	mg/100g							
	20	mg/100g							
	21	mg/100g	11.05.20	2460	yes		100	no	
	22	mg/100g							
	23	mg/100g							

Fructose Sample B

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Sample B			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Fructose	1	mg/100g							
	2	mg/100g							
	3	mg/100g	06.05.20	2730	yes	500	1600	no	98
	4	mg/100g	07.05.20	3575	yes			no	
	5	mg/100g	20.05.20	2215	yes			no	
	6	mg/100g	15.05.20	1951	YES	-	50	NO	-
	7	mg/100g							
	8	mg/100g	11.05.20	1940	yes		100	no	
	9	mg/100g							
	10	mg/100g	10/06	2132,41	yes	2	5	no	>90%
	11	mg/100g		2630	yes				
	12a	mg/100g							
	12b	mg/100g							
	13	mg/100g	19.06.20	260	yes		2	no	
	14	mg/100g	12.05.20	2720	yes	100	200	no	
	15	mg/100g	05.05.20	2144	yes	30	100	no	
	16	mg/100g	04.05.20	2517	yes	20	100	no	
	17	mg/100g	18.05.20	1650	YES	100	300	NO	
	18	mg/100g	-	-	-	-	-	-	-
	19	mg/100g							
	20	mg/100g							
	21	mg/100g	11.05.20	2860	yes		100	no	
	22	mg/100g							
	23	mg/100g							

Fructose Spiking Level Sample

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Spiking Level Sample			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Fructose	1	mg/100g							
	2	mg/100g							
	3	mg/100g	06.05.20	565	yes	500	1600	no	98
	4	mg/100g	07.05.20	555	yes			no	
	5	mg/100g	20.05.20	430	yes			no	
	6	mg/100g	15.05.20	356	YES	-	50	NO	-
	7	mg/100g							
	8	mg/100g	11.05.20	460	yes		100	no	
	9	mg/100g							
	10	mg/100g	28/05	457,87	yes	2	5	no	>90%
	11	mg/100g		440	yes				
	12a	mg/100g							
	12b	mg/100g							
	13	mg/100g	19.06.20	500	yes		9	no	
	14	mg/100g	12.05.20	280	yes	100	200	no	
	15	mg/100g	05.05.20	495	yes	30	100	no	
	16	mg/100g	04.05.20	519	yes	20	100	no	
	17	mg/100g	18.05.20	480	YES	100	300	NO	
	18	mg/100g	-	-	-	-	-	-	-
	19	mg/100g							
	20	mg/100g							
	21	mg/100g	11.05.20	480	yes		100	no	
	22	mg/100g							
	23	mg/100g							

Lactose Sample A

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Sample A			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Lactose	1	mg/100g	21.-28.04.20	negativ	no	40 (not verified, therefore LOQ taken as LOD)	40	no	104
	2	mg/100g	29.04.20	0	no	7			
	3	mg/100g	06.05.20	< BG	yes	14	46	no	98
	4	mg/100g	07.05.20	145	yes			no	
	5	mg/100g	25.05.20	0	no	4	14	no	
	6	mg/100g	18.05.20	<2	NO	-	2	NO	-
	7	mg/100g	26.05.20	< 2	no		2	yes	100
	8	mg/100g	05.05.20	<20	no		20	no	
	9	mg/100g							
	10	mg/100g	10/06	118,58	yes	2	5	no	>90%
	11	mg/100g		160	yes				
	12a	mg/100g	28.05.20	<10	no	10	50	no	
	12b	mg/100g	19.06.20	<5	no	5	15	no	
	13	mg/100g	19.06.20	2,85	yes		17	no	
	14	mg/100g	22.06.20	<10	no	5	10	no	
	15	mg/100g	05.05.20		no	30	100	no	
	16	mg/100g	15.06.20	81,4	yes	1	2,5	no	
	17	mg/100g	18.05.20	<LOD	NO	100	300	NO	
	18	mg/100g	24.06.20	<20	Not Detected	20			
	19	mg/100g	26.05.2020, 27.05.2020	<20	no	20	60	no	95
	20	mg/100g	24.06.20	450	yes		2,5	no	
	21	mg/100g	11.05.20	< 5	no	2	5	no	
	22	mg/100g	08.05.20	<3.64	No	3,64	0,0036	No	N/A
	23	mg/100g	16.04.20	40,8	yes	0.6 mg/100g	1.8 mg/100g	no	0,9925

Lactose Sample B

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Sample B			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Lactose	1	mg/100g	21.-28.04.20	210	yes	40 (not verified, therefore LOQ taken as LOD)	40	no	104
	2	mg/100g	29.04.20	> 100	yes	7			
	3	mg/100g	06.05.20	105	yes	14	46	no	98
	4	mg/100g	07.05.20	160	yes			no	
	5	mg/100g	25.05.20	200,5	yes	4	14	no	
	6	mg/100g	15.05.20	235	YES	-	50	NO	-
	7	mg/100g	26.05.20	202	yes		2	yes	100
	8	mg/100g	05.05.20	219	yes		20	no	
	9	mg/100g							
	10	mg/100g	10/06	316,38	yes	2	5	no	>90%
	11	mg/100g		170	yes				
	12a	mg/100g	28.05.20	220	yes	10	50	no	
	12b	mg/100g	19.06.20	225	yes	5	15	no	
	13	mg/100g	19.06.20	199	yes		17	no	
	14	mg/100g	22.06.20	200	yes	5	10	no	
	15	mg/100g	05.05.20	213	yes	30	100	no	
	16	mg/100g	15.06.20	259,6	yes	1	2,5	no	
	17	mg/100g	18.05.20	200	YES	100	300	NO	
	18	mg/100g	24.06.20	>200	Detected	20			
	19	mg/100g	26.05.2020, 27.05.2020	181	yes	20	60	no	95
	20	mg/100g	24.06.20	164	yes		2,5	no	
	21	mg/100g	11.05.20	234	yes	2	5	no	
	22	mg/100g	08.05.20	220,4	Yes	3,64	0,0036	No	N/A
	23	mg/100g	16.04.20	254,5	yes	0.6 mg/100g	1.8 mg/100g	no	0,9925

Lactose Spiking Level Sample

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Spiking Level Sample			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Lactose	1	mg/100g	21.-28.04.20	203	yes	100 (not verified, therefore LOQ taken as LOD)	100	no	95
	2	mg/100g	29.04.20	> 100	yes	7			
	3	mg/100g	06.05.20	144	yes	14	46	no	98
	4	mg/100g	07.05.20	185	yes			no	
	5	mg/100g	25.05.20	186,5	yes	4	14	no	
	6	mg/100g	15.05.20	165	YES	-	50	NO	-
	7	mg/100g	26.05.20	195	yes		2	yes	100
	8	mg/100g	05.05.20	210	yes		20	no	
	9	mg/100g	16th June 2020	0,18	yes	0,01	0,01	NO	
	10	mg/100g	28/05	190,13	yes	2	5	no	>90%
	11	mg/100g		110	yes				
	12a	mg/100g	28.05.20	240	yes	10	50	no	
	12b	mg/100g	19.06.20	220	yes	5	15	no	
	13	mg/100g	19.06.20	196	yes		73	no	
	14	mg/100g	22.06.20	168	yes	5	10	no	
	15	mg/100g	05.05.20	207	yes	30	100	no	
	16	mg/100g	15.06.20	194,9	yes	1	2,5	no	
	17	mg/100g	18.05.20	260	YES	100	300	NO	
	18	mg/100g	24.06.20	>200	Detected	20			
	19	mg/100g	26.05.2020, 27.05.2020	178	yes	20	60	no	95
	20	mg/100g							
	21	mg/100g	11.05.20	201	yes	2	5	no	
	22	mg/100g	08.05.20	185,96	Yes	3,64	0,0036	No	N/A
	23	mg/100g	16.04.20	155,5	yes	0.6 mg/100g	1.8 mg/100g	no	0,9925

Galactose Sample A

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Sample A			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Galactose	1	mg/100g							
	2	mg/100g	29.04.20	28	yes	4			
	3	mg/100g	06.05.20	115	yes	15	50	no	98
	4	mg/100g	n.a.	n.a.					
	5	mg/100g	25.05.20	20	yes			no	
	6	mg/100g	15.05.20	<50	NO	-	50	NO	-
	7	mg/100g							
	8	mg/100g	05.05.20	15	yes		10	no	
	9	mg/100g							
	10	mg/100g	10/06	171,13	yes	2	5	no	>90%
	11	mg/100g							
	12a	mg/100g							
	12b	mg/100g							
	13	mg/100g							
	14	mg/100g	12.05.20	<200	no	100	200	no	
	15	mg/100g	05.05.20		no	30	100	no	
	16	mg/100g	04.05.20	<100	no	20	100	no	
	17	mg/100g							
	18	mg/100g	-	-	-	-	-	-	-
	19	mg/100g	26.05.2020, 27.05.2020	<10	no	10	30	no	95
	20	mg/100g							
	21	mg/100g	11.05.20	< 100	no		100	no	
	22	mg/100g							
	23	mg/100g							

Galactose Sample B

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Sample B			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Galactose	1	mg/100g							
	2	mg/100g	29.04.20	30	yes	4			
	3	mg/100g	06.05.20	119	yes	15	50	no	98
	4	mg/100g	n.a.	n.a.					
	5	mg/100g	25.05.20	23	yes			no	
	6	mg/100g	15.05.20	<50	NO	-	50	NO	-
	7	mg/100g							
	8	mg/100g	05.05.20	<20	no		20	no	
	9	mg/100g							
	10	mg/100g	10/06	151,48	yes	2	5	no	>90%
	11	mg/100g							
	12a	mg/100g							
	12b	mg/100g							
	13	mg/100g							
	14	mg/100g	12.05.20	<200	no	100	200	no	
	15	mg/100g	05.05.20		no	30	100	no	
	16	mg/100g	04.05.20	<100	no	20	100	no	
	17	mg/100g							
	18	mg/100g	-	-	-	-	-	-	-
	19	mg/100g	26.05.2020, 27.05.2020	<10	no	10	30	no	95
	20	mg/100g							
	21	mg/100g	11.05.20	< 100	no		100	no	
	22	mg/100g							
	23	mg/100g							

Galactose Spiking Level Sample

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Spiking Level Sample			Day /Month	mg/100g	yes / no	mg/100g	mg/100g		
Galactose	1	mg/100g							
	2	mg/100g	29.04.20	0	no	4			
	3	mg/100g	06.05.20	< NG	no	15	50	no	98
	4	mg/100g	n.a.	n.a.					
	5	mg/100g	25.05.20	0	yes			no	
	6	mg/100g	15.05.20	<50	NO	-	50	NO	-
	7	mg/100g							
	8	mg/100g	05.05.20	<20	no		20	no	
	9	mg/100g							
	10	mg/100g	28/05	<5	no	2	5	no	>90%
	11	mg/100g							
	12a	mg/100g							
	12b	mg/100g							
	13	mg/100g							
	14	mg/100g	12.05.20	<200	no	100	200	no	
	15	mg/100g	05.05.20		no	30	100	no	
	16	mg/100g	04.05.20	<100	no	20	100	no	
	17	mg/100g							
	18	mg/100g	-	-	-	-	-	-	-
	19	mg/100g	26.05.2020, 27.05.2020	<10	no	10	30	no	95
	20	mg/100g							
	21	mg/100g	11.05.20	< 100	no		100	no	
	22	mg/100g							
	23	mg/100g							

5.1.2 Analytical Methods

Fructose Sample A

Analyte	Participant	Method description as in test report / norm / literature	Sample preparation	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Sample A						yes/no	yes/no	
Fructose	1							
	2							
	3	in-house method GC-FID	dissolved in 70 % MeOH, derivatization with Hxdroxylamine HCl and BSTFA	GC-FID	in solvent (70% MeOH)	no	yes	none
	4	Ion chromatography	in H20 dissolved, filtrated and diluted		Merck: D-Fructose, Art. No. 1.04007	no	no	Method development
	5	ASU L 48.01-3				no	yes	
	6	HPLC/PAD - internal method PNTA0179			external calib. curve and internal RM	no	yes	
	7							
	8	r-biopharm Test-Combination 10 139 106 035:2011-05					yes	
	9							
	10	Based in AOAC 2000.17	Dilution with hot water	Ion Chromatography		no	no	
	11	in-house method PV DE02.365 2019-03		HPLC-ELSD			yes	
	12a							
	12b							
	13	ASU § 64 LFGB L31.00-12, modified, 1997-01	5 g sample in 100 ml flask with dest. water ati 70°C in ultrasonic bath extracted, after cooling Carrez precipitation	Enzymatic, r-biopharm	Fructose	yes	yes	
	14	in house method GCFID					yes	
	15	Enzymatic	homogenized, aqueous extraction, Carrez precipitation, filtration		Standards from Enzyme-Kit r-biopharm	no	yes	HPAEC-PAD: 2056 mg/100g
	16						yes	
	17	HPLC-RID			STANDARD CALIBRATION		NO	
	18	-	-	-	-	-	-	
	19							
	20							
	21	HPAEC-PAD						
	22							
	23							

Fructose Sample B

Analyte	Participant	Method description as in test report / norm / literature	Sample preparation	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Sample B						yes/no	yes/no	
Fructose	1							
	2							
	3	in-house method GC-FID	dissolved in 70 % MeOH, derivatization with Hxdroxylamine HCl and BSTFA	GC-FID	in solvent (70% MeOH)	no	yes	none
	4	Ion chromatography	in H20 dissolved, filtrated and diluted		Merck: D-Fructose, Art. No. 1.04007	no	no	Method development
	5	ASU L 48.01-3				no	yes	
	6	HPLC/PAD - internal method PNTA0179			external calib. curve and internal RM	no	yes	
	7							
	8	r-biopharm Test-Combination 10 139 106 035:2011-05					yes	
	9							
	10	Based in AOAC 2000.17	Dilution with hot water	Ion Chromatography		no	no	
	11	in-house method PV DE02.365 2019-03		HPLC-ELSD			yes	
	12a							
	12b							
	13	ASU § 64 LFGB L31.00-12, modified, 1997-01	5 g sample in 100 ml flask with dest. water ati 70°C in ultrasonic bath extracted, after cooling Carrez precipitation	Enzymatic, r-biopharm	Fructose	yes	yes	
	14	in house method GCFID					yes	
	15	Enzymatic	homogenized, aqueous extraction, Carrez precipitation, filtration		Standards from Enzyme-Kit r-biopharm	no	yes	HPAEC-PAD: 2315 mg/100g
	16						yes	
	17	HPLC-RID			STANDARD CALIBRATION		NO	
	18	-	-	-	-	-	-	
	19							
	20							
	21	HPAEC-PAD						
	22							
	23							

Fructose Spiking Level Sample

Analyte	Participant	Method description as in test report / norm / literature	Sample preparation	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Spiking Level Sample						yes/no	yes/no	
Fructose	1							
	2							
	3	in-house method GC-FID	dissolved in 70 % MeOH, derivatization with Hxdroxylamine HCl and BSTFA	GC-FID	in solvent (70% MeOH)	no	yes	none
	4	Ion chromatography	in H20 dissolved, filtrated and diluted		Merck: D-Fructose, Art. No. 1.04007	no	no	Method development
	5	ASU L 48.01-3				no	yes	.2016
	6	HPLC/PAD - internal method PNTA0179			external calib. curve and internal RM	no	yes	
	7							
	8	r-biopharm Test-Combination 10 139 106 035:2011-05					yes	
	9							
	10	Based in AOAC 2000.17	Dilution with hot water	Ion Chromatography		no	no	
	11	in-house method PV DE02.365 2019-03		HPLC-ELSD			yes	
	12a							
	12b							
	13	ASU § 64 LFGB L31.00-12, modified, 1997-01	5 g sample in 100 ml flask with dest. water ati 70°C in ultrasonic bath extracted, after cooling Carrez precipitation	Enzymatic, r-biopharm	Fructose	yes	yes	
	14	in house method GCFID					yes	
	15	Enzymatic	homogenized, aqueous extraction, Carrez precipitation, filtration		Standards from Enzyme-Kit r-biopharm	no	yes	HPAEC-PAD: 515 mg/100g
	16						yes	
	17	HPLC-RID			STANDARD CALIBRATION		NO	
	18	-	-	-	-	-	-	
	19							
	20							
	21	HPAEC-PAD						
	22							
	23							

Lactose Sample A

Analyte	Participant	Method description as in test report / norm / literature	Sample preparation	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Sample A						yes/no	yes/no	
Lactose	1	ASU L 01.00-90, 2014-02 - Dermination of lactose in lactose-reduced milk products in the presence of glucose - Enzymatic method: , deviation: extension to other matrices, application of Testkits R-Biopharm AG, Glucose Remover, E3400, 2017-10	2,5 g sample weight; water extraction at 70 °C; Carrez precipitation; Glucose digestion with Glucose oxidase/ H2O2, R-Biopharm E3400	enzymatically	calculation with extinction differences, extinction coefficients / recovery rates with Lactose Monohydrate, Merck 1.07660	yes	yes	Lactose determination by Boehringer Mannheim/R-Biopharm Lactose/D-Glucose-Enzymtestkit 10 986 119 035, results as anhydrous free lactose
	2	UV-Detection, Difference of Lactose and D-Galactose	plus decoloration	measuring range 0-1.000 mg/kg Lactose (100 mg/100g)			yes	Lactose/D-Galactose BioAnalysis (Art. No. 10176303035)
	3	in-house method GC-FID	dissolved in 70 % MeOH, derivatization with Hxdroxylamine HCl and BSTFA	GC-FID	in solvent (70% MeOH)	no	yes	none
	4	Ion chromatography	in H20 dissolved, filtrated and diluted		Merck: Lactose, Art. No. 1.07660	no	no	Method development
	5	L01.00-17 § 64 LFGB				no	yes	
	6	LC/MS/MS - internal method PNTA0189			external calib. curve and internal RM	no	yes	
	7	HPLC-MS		recovery calculated by C13-Lactose internal standard	Anhydrous lactose (Sigma)	yes	yes	
	8	r-biopharm Test-Combination 10 176 303 035:2011-06					yes	
	9							
	10	Based in AOAC 2000.17	Dilution with hot water	Ion Chromatography		no	yes	
	11	in-house method PV DE02.365 2019-03		HPLC-ELSD			yes	
	12a	ISO 22662		HPLC-ELSD		no	yes	
	12b	in-house method		LC-MS		no	no	
	13	ASU § 64 LFGB L01.00-17, modified, 2010-09	5 g sample in 100 ml flask with dest. water ati 70°C in ultrasonic bath extracted, after cooling Carrez precipitation	Enzymatic, r-biopharm	Lactose monohydrate	yes	yes	
	14	in house method GCFID					yes	
	15	Enzymatic	homogenized, aqueous extraction, Carrez precipitation, filtration		Standards from Enzyme-Kit r-biopharm	no	yes	HPAEC-PAD: <25 mg/100g
	16						yes	
	17	HPLC-RID			STANDARD CALIBRATION		NO	
	18		-	-	-	-	-	
	19	enzymatically				no		
	20	colorimetrico			CMR MUVA-MP-0218 spray dried whole milk powder		no	
	21	LC-MS/MS						
	22	Megazyme K-LOLAC, Enzymatic	As per Kit Instruction	As per Kit Instruction	N/A	No	Yes	
	23	Enzymatic method using Boehringer/R-Biopharm Test-Combination kit for the quantitative determination of lactose in any foodstuff. The method has been validated at NRC on powdered beverages for aroma (PBA), and has been adapted and validated to enable the quantification of lactose in lactose-free infant formulae	Bring the whole laboratory sample (original container) to room temperature and homogenise it by mixing. Take the test portion for analysis from the homogeneous test sample.	-	DS81 REF012 internal reference sample	no	no	-

Lactose Sample B

Analyte	Participant	Method description as in test report / norm / literature	Sample preparation	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Sample B						yes/no	yes/no	
Lactose	1	ASU L 01.00-90, 2014-02 - Dermination of lactose in lactose-reduced milk products in the presence of glucose - Enzymatic method: , deviation: extension to other matrices, application of Testkits R-Biopharm AG, Glucose Remover, E3400, 2017-10	2,5 g sample weight; water extraction at 70 °C; Carrez precipitation; Glucose digestion with Glucose oxidase/ H2O2, R-Biopharm E3400	enzymatically	calculation with extinction differences, extinction coefficients / recovery rates with Lactose Monohydrate, Merck 1.07660	yes	yes	Lactose determination by Boehringer Mannheim/R-Biopharm Lactose/D-Glucose-Enzymtestkit 10 986 119 035, results as anhydrous free lactose
	2	UV-Detection, Difference of Lactose and D-Galactose	plus decoloration	measuring range 0-1.000 mg/kg Lactose (100 mg/100g)			yes	Lactose/D-Galactose BioAnalysis (Art. No. 10176303035)
	3	in-house method GC-FID	dissolved in 70 % MeOH, derivatization with Hxdroxylamine HCl and BSTFA	GC-FID	in solvent (70% MeOH)	no	yes	none
	4	Ion chromatography	in H20 dissolved, filtrated and diluted		Merck: Lactose, Art. No. 1.07660	no	no	Method development
	5	L01.00-17 § 64 LFGB				no	yes	
	6	LC/MS/MS - internal method PNTA0189			external calib. curve and internal RM	no	yes	
	7	HPLC-MS		recovery calculated by C13-Lactose internal standard	Anhydrous lactose (Sigma)	yes	yes	
	8	r-biopharm Test-Combination 10 176 303 035:2011-06					yes	
	9							
	10	Based in AOAC 2000.17	Dilution with hot water	Ion Chromatography		no	yes	
	11	in-house method PV DE02.365 2019-03		HPLC-ELSD			yes	
	12a	ISO 22662		HPLC-ELSD		no	yes	
	12b	in-house method		LC-MS		no	no	
	13	ASU § 64 LFGB L01.00-17, modified, 2010-09	5 g sample in 100 ml flask with dest. wa-ter ati 70°C in ultrasonic bath extracted, after cooling Carrez precipitation	Enzymatic, r-biopharm	Lactose monohydrate	yes	yes	
	14	in house method GCFID					yes	
	15	Enzymatic	homogenized, aqueous extraction, Carrez precipitation, filtration		Standards from Enzyme-Kit r-biopharm	no	yes	HPAEC-PAD: 215 mg/100g
	16						yes	
	17	HPLC-RID			STANDARD CALIBRATION		NO	
	18		-	-	-	-	-	
	19	enzymatically				no		
	20	colorimetrico			CMR MUVA-MP-0218 spray dried whole milk powder		no	
	21	LC-MS/MS						
	22	Megazyme K-LOLAC, Enzymatic	As per Kit Instruction	As per Kit Instruction	N/A	No	Yes	
	23	Enzymatic method using Boehringer/R-Biopharm Test-Combination kit for the quantitative determination of lactose in any foodstuff. The method has been validated at NRC on powdered beverages for aroma (PBA), and has been adapted and validated to enable the quantification of lactose in lactose-free infant formulae	Bring the whole laboratory sample (original container) to room temperature and homogenise it by mixing. Take the test portion for analysis from the homogeneous test sample.	-	DS81 REF012 internal reference sample	no	no	-

Lactose Spiking Level Sample

Analyte	Participant	Method description as in test report / norm / literature	Sample preparation	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Spiking Level Sample						yes/no	yes/no	
Lactose	1	ASU L 01.00-90, 2014-02 - Dermination of lactose in lactose-reduced milk products in the presence of glucose - Enzymatic method: , deviation: extension to other matrices, application of Testkits R-Biopharm AG, Glucose Remover, E3400, 2017-10	1 g sample weight; water extraction at 70 °C; Carrez precipitation; Glucose digestion with Glucose oxidase/ H2O2, R-Biopharm E3400	enzymatically	calculation with extinction differences, extinction coefficients / recovery rates with Lactose Monohydrate, Merck 1.07660	yes	yes	Lactose determination by Boehringer Mannheim/R-Biopharm Lactose/D-Glucose-Enzymtestkit 10 986 119 035, results as anhydrous free lactose
	2	UV-Detection, Difference of Lactose and D-Galactose	plus decoloration	measuring range 0-1.000 mg/kg Lactose (100 mg/100g)			yes	Lactose/D-Galactose BioAnalysis (Art. No. 10176303035)
	3	in-house method GC-FID	dissolved in 70 % MeOH, derivatization with Hxdroxylamine HCl and BSTFA	GC-FID	in solvent (70% MeOH)	no	yes	none
	4	Ion chromatography	in H20 dissolved, filtrated and diluted		Merck: Lactose, Art. No. 1.07660	no	no	Method development
	5	L01.00-17 § 64 LFGB				no	yes	
	6	LC/MS/MS - internal method PNTA0189			external calib. curve and internal RM	no	yes	
	7	HPLC-MS		recovery calculated by C13-Lactose internal standard	Anhydrous lactose (Sigma)	yes	yes	
	8	r-biopharm Test-Combination 10 176 303 035:2011-06					yes	
	9							
	10	Based in AOAC 2000.17	Dilution with hot water	Ion Chromatography		no	yes	
	11	in-house method PV DE02.365 2019-03		HPLC-ELSD			yes	
	12a	ISO 22662		HPLC-ELSD		no	yes	
	12b	in-house method		LC-MS		no	no	
	13	ASU § 64 LFGB L01.00-17, modified, 2010-09	5 g sample in 100 ml flask with dest. wa-ter ati 70°C in ultrasonic bath extracted, after cooling Carrez precipitation	Enzymatic, r-biopharm	Lactose monohydrate	yes	yes	
	14	in house method GCFID					yes	
	15	Enzymatic	homogenized, aqueous extraction, Carrez precipitation, filtration		Standards from Enzyme-Kit r-biopharm	no	yes	HPAEC-PAD: 221 mg/100g
	16						yes	
	17	HPLC-RID			STANDARD CALIBRATION		NO	
	18		-	-	-	-	-	Information Only - test not Live
	19	enzymatically				no		
	20	colorimetrico			CMR MUVA-MP-0218 spray dried whole milk powder		no	
	21	LC-MS/MS						
	22	Megazyme K-LOLAC, Enzymatic	As per Kit Instruction	As per Kit Instruction	N/A	No	Yes	
	23	Enzymatic method using Boehringer/R-Biopharm Test-Combination kit for the quantitative determination of lactose in any foodstuff. The method has been validated at NRC on powdered beverages for aroma (PBA), and has been adapted and validated to enable the quantification of lactose in lactose-free infant formulae	Bring the whole laboratory sample (original container) to room temperature and homogenise it by mixing. Take the test portion for analysis from the homogeneous test sample.	-	DS81 REF012 internal reference sample	no	no	-

Galactose Sample A / Sample B / Spiking Level Sample

Analyte	Participant	Method description as in test report / norm / literature	Sample preparation	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
						yes/no	yes/no	
Galactose	1							
	2	UV-Detection, Differentiation between Lactose and D-Galactose	plus decoloration	measuring range 0-1.000 mg/kg Lactose (100 mg/100g)			yes	Lactose/D-Galactose BioAnalysis (Art. Nr. 10176303035)
	3	in-house method GC-FID	dissolved in 70 % MeOH, derivatization with Hxdroxylamine HCl and BSTFA	GC-FID	in solvent (70% MeOH)	no	yes	none
	4							n.a. not analyzed
	5	L01.00-17 § 64 LFGB				no	yes	
	6	HPLC/PAD - internal method PNTA0179			external calib. curve and internal RM	no	yes	
	7							
	8	r-biopharm Test-Combination 10 176 303 035:2011-06					yes	
	9							
	10	Based in AOAC 2000.17	Dilution with hot water	Ion Chromatography		no	no	
	11							
	12a							
	12b							
	13							
	14	in house method GCFID					yes	
	15	Enzymatically	homogenized, aqueous extraction, Carrez precipitation, filtration		Standards from Enzyme-Kit r-biopharm	no	yes	
	16						yes	
	17							
	18	-	-	-	-	-	-	
	19	enzymatically				no		
	20							
	21	HPAEC-PAD						
	22							
	23							

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA -ptAI01 Sample B

Weight whole sample	2,19	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	24,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	50	19,9
2	5,03	53	21,1
3	5,01	45	18,0
4	5,02	39	15,5
5	4,98	47	18,9
6	5,06	44	17,4
7	5,05	38	15,0
8	4,98	44	17,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	45,0	Particle
Standard deviation	5,12	Particle
χ^2 (CHI-Quadrat)	4,07	
Probability	**77**	%
Recovery rate	74	%

Normal distribution		
Number of samples	8	
Mean	17,9	mg/kg
Standard deviation	2,04	mg/kg
rel. Standard deviaton	11,4	%
Horwitz standard deviation	10,4	%
HorRat-value	**1,1**	
Recovery rate	74	%

Microtracer Homogeneity Test
DLA -ptAI01 Spiking Level Sample

Weight whole sample	1,73	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	27,6	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,96	73	29,4
2	5,01	78	31,1
3	5,03	73	29,0
4	5,01	84	33,5
5	4,97	80	32,2
6	5,02	74	29,5
7	4,97	75	30,2
8	4,95	86	34,7

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	77,9	Particle
Standard deviation	5,24	Particle
χ^2 (CHI-Quadrat)	2,47	
Probability	**93**	%
Recovery rate	113	%

Normal distribution		
Number of samples	8	
Mean	31,2	mg/kg
Standard deviation	2,10	mg/kg
rel. Standard deviaton	6,7	%
Horwitz standard deviation	9,5	%
HorRat-value	**0,71**	
Recovery rate	113	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

PT number	***ptAI01 – 2020***
PT name	***Lactose + Fructose in "lactose-free" Infant Food***
Sample matrix (processing)	***Samples A + B:*** *Cookies / ingredients: wheat flour, syrup (invert sugar syrup, glucose syrup, refined sugar syrup), sugar, vegetable oil (palm), powder, raising agent: potassium carbonate,ammonium bicarbonate, spices (cinnamon, cloves), salt, lemon zest paste and lactose and fructose (one of both samples)* ***Spiking Level Sample:*** *potato powder, lactose and fructose*
Number of samples and sample amount	*2 different Samples A + B: 25 g each* *+ 1 Spiking Level Sample: 15 g*
Storage	*Samples A + B: room temperature (long term 2 - 10°C)* *Spiking Level Sample: room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Lactose (optional: Galactose) + Fructose* *Samples A + B: Lactose < 500 mg/100g* *Spiking Level Sample: Lactose < 500 mg/100g*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. From* ***Samples A + B*** *the* ***total sample amount*** *should be* ***homogenized*** *each.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample.* *The results should be filled in the result submission file.*
Units	*mg/100g*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest June 26th 2020 (extended)***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Alexandra Scharf MSc.*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Any testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		GREAT BRITAIN
		SWITZERLAND
		SPAIN
		ITALY
		Germany
		Germany
		Germany
		Germany
		ITALY
		GREAT BRITAIN
		ITALY
		Germany
		SWITZERLAND
		BELGIUM
		Germany
		Germany
		SPAIN
		GREAT BRITAIN
		NETHERLANDS
		Germany
		AUSTRIA
		SPAIN
		GREECE

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]

[The address data of the participants were deleted for publication of the evaluation report.]

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methoden-validierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and preci-sion) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Hor-witz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Ana-lyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estim-ates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messun-gen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Check-ing procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Re-quirements, Appendix F, p. 2, AOAC Int (2016)
18. ASU §64 LFGB L 01.00-17 (2010) / DIN 10344 : Bestimmung des Lactose- und Galactosegehaltes von Milch und Milchprodukten; Enzymatisches Verfahren / Milk and milk products - Determination of lactose and D-galactose content - Enzymatic method
19. ASU §64 LFGB L 01.00-90 Bestimmung des Lactosegehaltes in lactosereduzierter Milch und lactosereduzierten Milchprodukten in Gegenwart von Glucose; Enzymatisches Verfahren (2014) [Milk and milk products - Determination of lactose in lactose-reduced milk products in the presence of glucose - Enzymatic method]
20. ASU §64 LFGB L 17.00-7 Bestimmung von Lactose in Brot einschließlich Kleingebäck aus Brotteigen (1983) [Determination of lactose in bread including small pastries from bread doughs]
21. ASU §64 LFGB L 48.01-4 Bestimmung von Lactose in teiladaptierter Säuglingsnahrung auf Milchbasis (1985) [Determination of lactose in partially-adapted infant milk-based food]
22. ASU §64 LFGB L 48.02.07-1 Bestimmung von Glucose und Fructose in Kinder-Zwieback und Zwiebackmehl (1985) [Determination of glucose and fructose in children's rusk and rusk flour]

23. ISO 22662:2012; Milch und Milchprodukte - Bestimmung des Lactosegehalts mit Hochleistungs-Flüssigchromatographie (Referenzverfahren) / Milk and milk products – Determination of lactose content by high-performance liquid chromatography (Reference method)