Proficiency Tests
DLA
food
cosmetics
consumer goods
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Evaluation Report
proficiency test

DLA 18/2018

Lactose and Fructose

in “lactose free” Food - Cereal Pap Powder (Infant Food)

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

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EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (28. August 2018) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 7. November 2018
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
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 / Content

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 mixture of common in commerce infant food products "cereal pap" for children from 4th and 6th month (labeled as milk- and gluten-free). 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 A** 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 and B were portioned to approximately 25 g, the spiking level sample to approximately 15 g into metallised PET film bags.

Table 1: Composition of the DLA-Samples

Ingredients	**Sample A**	**Sample B**	**Spiking Level Sample**
Organic-Cereal-Pap, infant pap after 4th and 6th month Ingredients: Millet whole flour (68%), rice flour (25%), thiamine Nutrients per 100g: Fat 3,6 g, carbohydrates 79 g, sugar 0,7 g, fiber 3,5 g, protein 9,9 g, salt 0,01 g	99,2 g/100g	100 g/100g	-
Potato powder Ingredients: Potatoes, E471, E304, E223, E100	-	-	99,3 g/100g
Lactose*	298 mg/100g	-	288 mg/100g
Fructose*	536 mg/100g	-	458 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 sample A and the spiking level sample showed a probability of 97% and 94%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave HorRat values of 0,75 and 0,85 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 PT samples was approx. 0,13 (23,6°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

One portion of each test material (sample A, sample B and spiking level sample) was sent to every participating laboratory in the 15th week of 2018. The testing method was optional. The tests should be finished at 9st June 2017 the latest.

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 infant food (cereal pap powder). 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**.*

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 single results, recovery and the used testing methods. 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.

14 out of 15 registered participants submitted the results in time. One participant submitted no results.

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].

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 statistical evaluation is carried out for all the parameters for a minimum of 7 values are present.

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 were submitted.

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 PT´s 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 in percent of the mean is given as variation coefficient VK_R in the statistical data in the results, if single results of participants were submitted. The meaning is explained in section 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) and results for a another proficiency test item can be removed from the data set [2]. Even if a result clearly deviates from the robust mean (e.g. factor >10) and has an influence on the robust statistics, a result can be excluded from statistical evaluation [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 identified as 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 is classified as an outlier [3]. Due to the using of robust statistics, outliers are generally excluded from the evaluation, unless there are other reasons (see above) [3]. Determined 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.

Additionally for the evaluation of the spiking level sample the standard uncertainty was considered for both PT-parameters and the results were evaluated by z´-score (see 3.8).

Since no quantitative results were available for galactose, no statistical evaluation was performed.

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 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 deviation (RSD_R) given in Table 2 were determined in ring tests using 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, respectively.

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].
For the present evaluation the target standard deviation according to 3.6.1 and 3.6.2 considering the standard uncertainty (s. 3.8 and 3.11) was regarded suitable.

Table 3 shows selected data of the participant 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 2013 (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	Cookies	288	119	41,3	(9,0)	DLA 8/2013 (Sample B)
Fructose	Crispbread	657	30,7	4,7	1,1	DLA 8/2014 (Sample B)
Fructose	Cookies	1130	122	10,8	1,7*	DLA 9/2015 (Sample B)
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)
Lactose	Cookies	142	37,1	26,1	(4,9)	DLA 8/2013 (Sample A)
Lactose	Crispbread	269	56,6	21,1	2,5*	DLA 8/2014 (Sample B)
Lactose	Cookies	116	37,3	32,2	2,8*	DLA 9/2015 (Sample 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)

** 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 valid z-score is indicated as z-score (σ_{pt}) in the evaluation. The as z-score (info) designated value only obtains an informative character. The both z-scores were calculated with different target standard deviations described in 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 process, calibration of equipment and composition of reagents, transmission or calculation errors, trueness and precision, and use of reference material. If necessary, the problems must be addressed through appropriate corrective action [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. 3.8). The z'-score represents the relation of the deviation of the result (xi) of the participant from the respective consensus value 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) 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 gives the relative variability within a data region. While a low CV, e.g. <5-10% can be taken as evidence for a homogeneous set of results, a CV 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 (PT) 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 of the assigned value

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 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).
For lactose results of the spiking level sample and the spiked sample recovery rates were calculated with respect to the known added content of lactose. The recovery rates were given for information only. No statistical evaluation was done. The recovery rates should exclusively give an estimation of the matrix- and/or processing influences.

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 ($X_{pt} + 2\sigma_{pt}$´) *
Variation coefficient V_K in %
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 **	9
Number of outliers	0
Mean	536
Median	540
Robust Mean (X_{pt})	**544**
Robust standard deviation (S*)	**41,3**
Target range:	
Target standard deviation σ_{pt}	**23,9**
Target standard deviation (for Information)	12,7
lower limit of target range	**496**
upper limit of target range	**592**
Quotient S/σ_{pt}*	*1,7*
Standard uncertainty $U_{(Xpt)}$	*17,2*
Results in the target range	*8*
Percent in the target range	*89%*

** without result No. 12 (excluded in advance)*

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) 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.*

89% of results were in the target range.

The robust mean of the participants' results were at 101% of the spiking level of fructose to the spiking level sample (s. p. 5).

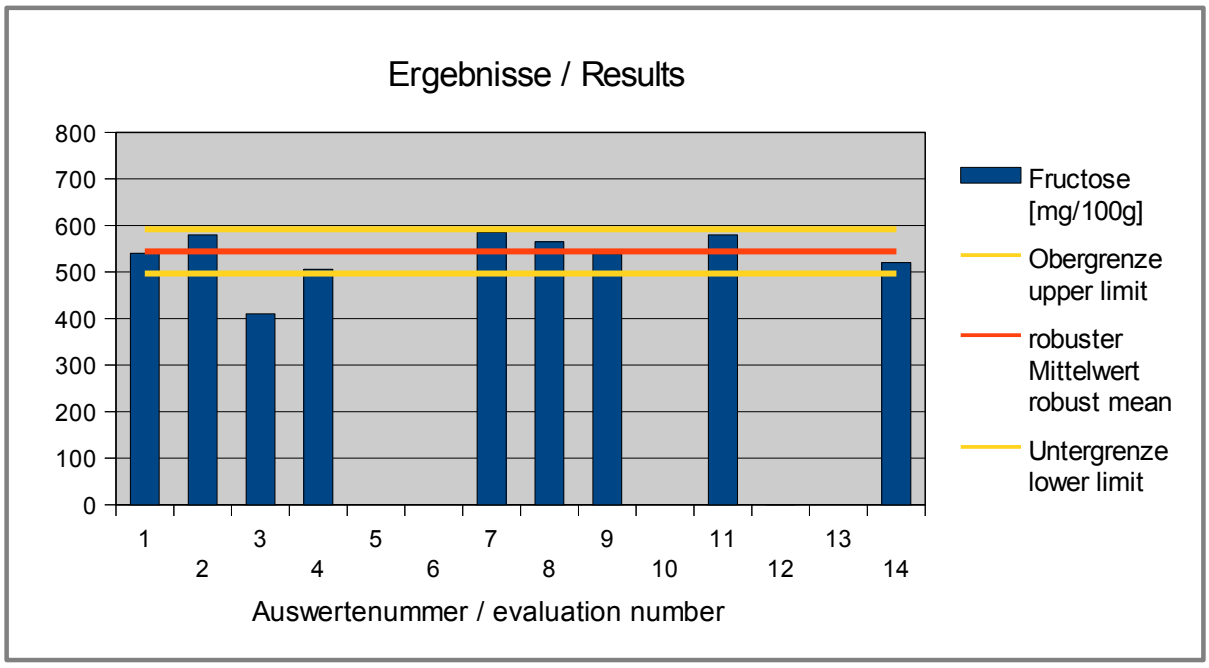

Abb. / Fig. 1: Ergebnisse Fructose / Results Fructose

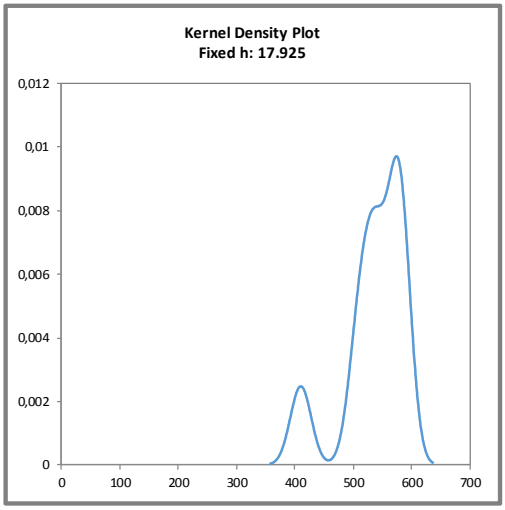

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse
(mit h = 0,75 x σ_{pt} von Xpt)

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

Comment:
The kernel density shows almost a symmetrical distribution of results with a shoulder and an additional peak at approx. 400 mg/100g, due to a single result 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	540	-4	-0,17	-0,33	
2	580	36	1,5	2,8	
3	410	-134	-5,6	-11	
4	506	-38	-1,6	-3,0	
5					
6					
7	585	41	1,7	3,2	
8	565	21	0,87	1,6	
9	539	-5	-0,22	-0,40	
10					
11	580	36	1,5	2,8	
12	0,540				Ergebnis ausgeschlossen / Result excluded
13					
14	520	-24	-1,0	-1,9	

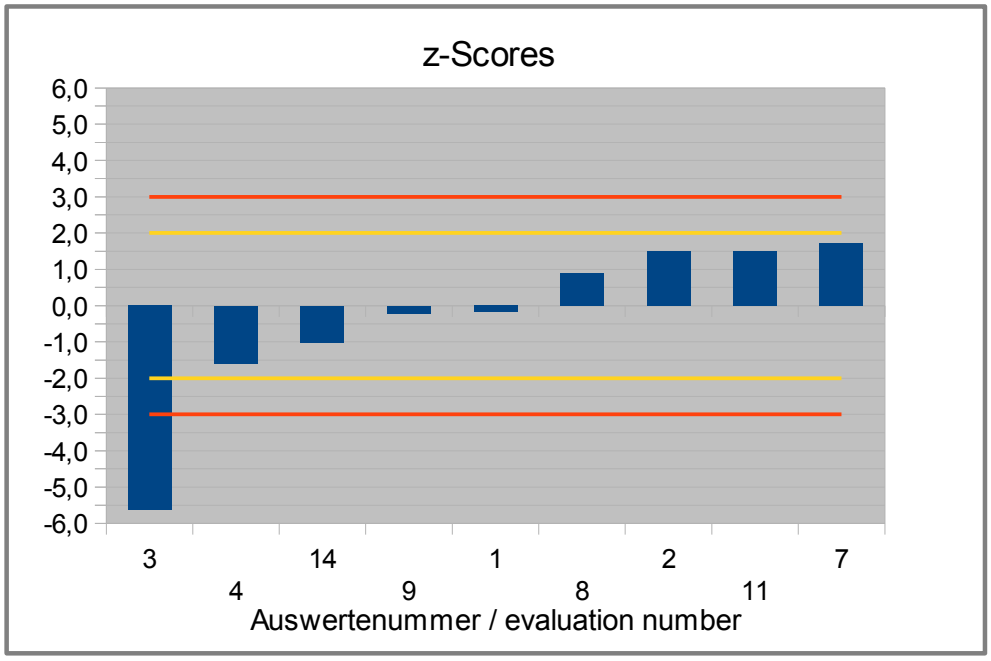

Abb. / Fig. 3: z-Scores Fructose (Probe A / Sample A)

4.1.2 Fructose Sample B (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Due to the small number of available results (<7), no statistical evaluation was made.

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	16,0				
2					
3	14,0				
4	<50				
5	-				
6					
7	<LOQ				
8	<50				
9	<20				
10					
11	n.d.				
12	<0,1				
13					
14	<100				

Comments:
Fructose was not added to sample B. Two participants detected an amount of about 15 mg/100g in sample B.

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

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	9
Number of outliers	0
Mean	451
Median	475
Robust Mean (x_{pt})	**452**
Robust standard deviation (S*)	**91,1**
Target range:	
Target standard deviation σ_{pt}'	**43,1**
Target standard deviation (for Information)	10,5
lower limit of target range	**366**
upper limit of target range	**538**
Quotient S/σ_{pt}'*	*2,1*
Standard uncertainty $U_{(Xpt)}$	*37,9*
Results in the target range	*7*
Percent in the target range	*78%*

** without result No. 12 (excluded in advance)*

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) is given for information.

The distribution of results showed an increased variability. Therefore the valuation was done by z'-scores considering the standard uncertainty. The quotient S/σ_{pt}' was then 2,1. The robust standard deviation of 20% was higher than values of established, standardized methods (see 3.6.2).*

78% of results were in the target range.

The robust mean of participant results was 98 % of the spiking level of fructose to the spiking level sample (s. p. 5).

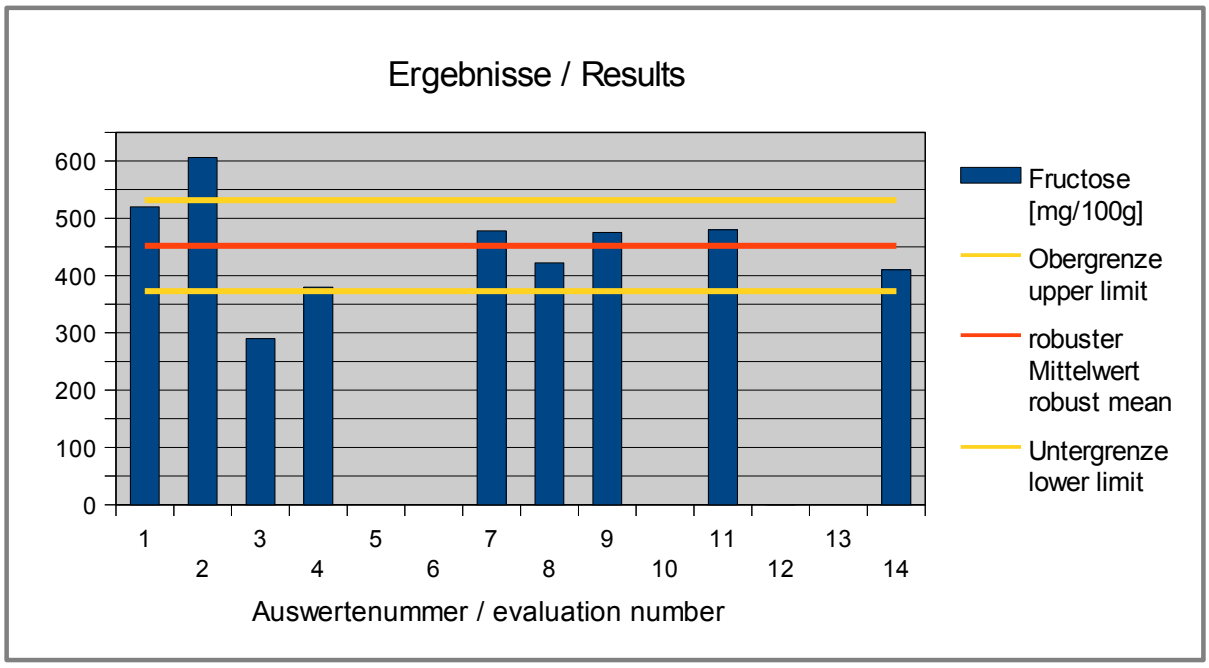

Abb. / Fig. 4: Ergebnisse Fructose / Results Fructose

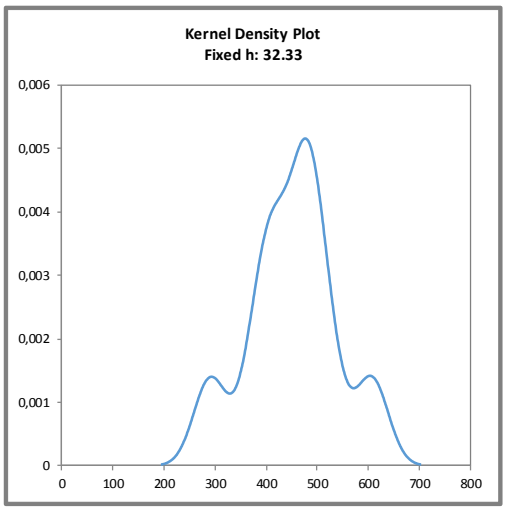

Abb. / Fig. 5:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

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

Comment:
The kernel density shows almost a symmetrical distribution of results with a shoulder and two additional peaks, due to a single result below and above the target range, respectively.

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	520	68	1,6	6,4	
2	606	154	3,6	15	
3	290	-162	-3,8	-15	
4	380	-72	-1,7	-6,8	
5					
6					
7	478	26	0,60	2,5	
8	422	-30	-0,70	-2,9	
9	475	23	0,53	2,2	
10					
11	480	28	0,65	2,6	
12	0,42				Ergebnis ausgeschlossen / Result excluded
13					
14	410	-42	-1,0	-4,0	

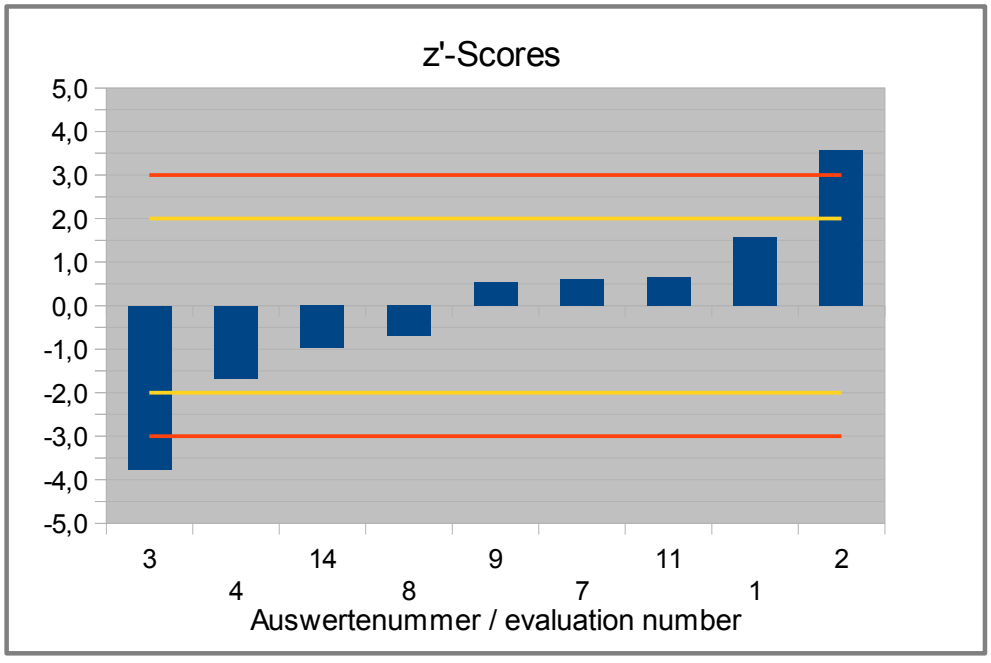

Abb. / Fig. 6: z´-Scores Fructose (Dotierungsniveauprobe/ Spiking Level Sample)

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	positive	270	positive	39	1/2 (50%)	
2	positive	287	negative		2/2 (100%)	
3	positive	230	negative	< LOD	2/2 (100%)	
4	positive	265	negative	<50	2/2 (100%)	
5	positive	291	positive	15	1/2 (50%)	
6	positive	300	positive	< 10	1/2 (50%)	
7	positive	303	negative	<BG	2/2 (100%)	
8	positive	283	negative	<50	2/2 (100%)	
9	positive	558	negative	<20	2/2 (100%)	
10	positive	455	negative	<14.5	2/2 (100%)	
11	positive	285	negative	n.n.	2/2 (100%)	
12	positive	0,300	negative	<0,01	2/2 (100%)	
13	positive	274	negative	0	2/2 (100%)	
14	positive	370	negative	<5	2/2 (100%)	

	Sample A		Sample B	
Number positive	14		3	
Number negative	0		11	
Percent positive	100		21	
Percent negative	0		79	
Consensus value	positive		negative	

Comments:
The consensus values are in qualitative agreement with the spiking of sample A. 3 of the 14 participants obtained a positive result for sample B, partly below the limit of quantification.

4.2.2 Lactose Sample A (in mg/100g)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	12
Number of outliers	0
Mean	301
Median	286
Robust Mean (X_{pt})	**289**
Robust standard deviation (S*)	**29,2**
Target range:	
Target standard deviation σ_{pt}	**22,7**
Target standard deviation (for Information)	13,9
lower limit of target range	**244**
upper limit of target range	**334**
Quotient S/σ_{pt}*	*1,3*
Standard uncertainty $U_{(Xpt)}$	*10,5*
Results in the target range	*9*
Percent in the target range	*75%*

** without result No. 9 and 12 (excluded in advance)*

Comments:

The target standard deviation was calculated using data from a precision experiment (ASU §64 L 01.00-90)(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 97 % of the spiking level of lactose to sample A (s. p. 5).

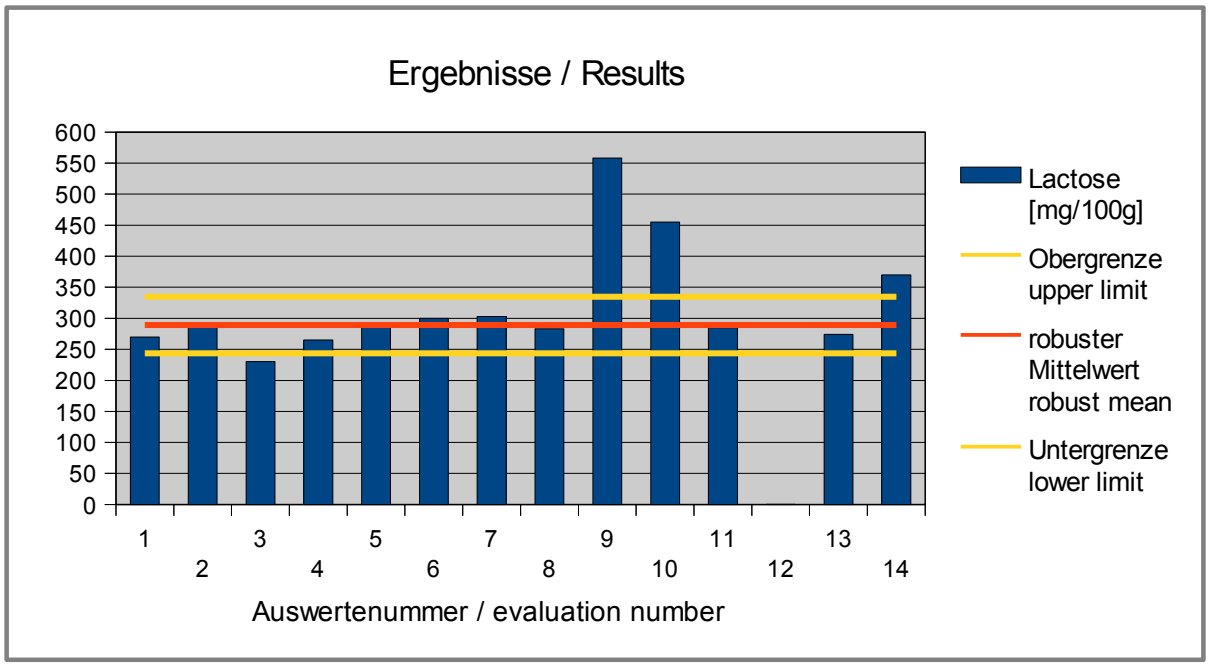

Abb. / Fig. 7: Ergebnisse Lactose / Results Lactose

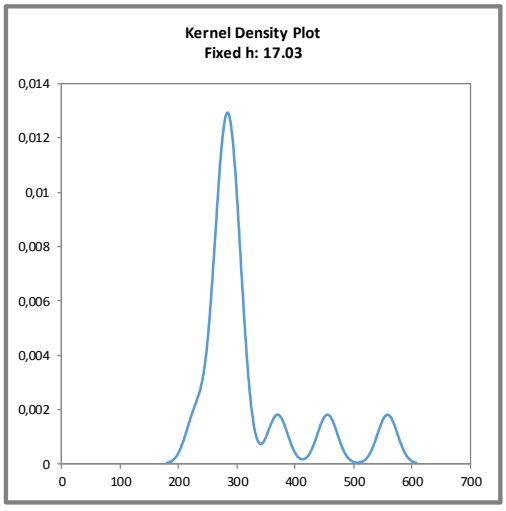

Abb. / Fig. 8:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

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

Comment:
The kernel density shows nearly a symmetrical distribution of results with three small side peaks, due to three single results above 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	270	-19	-0,84	-1,4	
2	287	-2	-0,09	-0,15	
3	230	-59	-2,6	-4,2	
4	265	-24	-1,1	-1,7	
5	291	2	0,09	0,14	
6	300	11	0,48	0,78	
7	303	14	0,61	1,0	
8	283	-6	-0,27	-0,44	
9	558				Ausreisser ausgeschlossen/ Outlier excluded
10	455	166	7,3	12	Ausreisser / Outlier
11	285	-4	-0,18	-0,29	
12	0,300				Ergebnis ausgeschlossen / Result excluded
13	274	-15	-0,67	-1,1	
14	370	81	3,6	5,8	

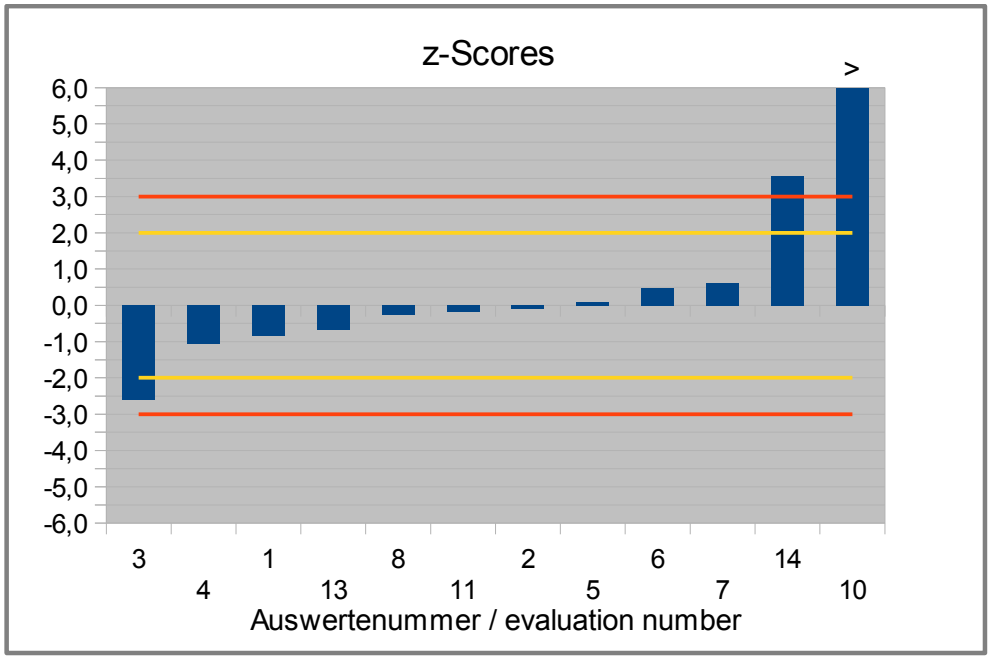

Abb. / Fig. 9: z-Scores Lactose (Probe A / Sample A)

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

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	13
Number of outliers	0
Mean	281
Median	270
Robust Mean (x_{pt})	**279**
Robust standard deviation (S*)	**62,1**
Target range:	
Target standard deviation σ_{pt}'	**30,7**
Target standard deviation (for Information)	13,5
lower limit of target range	**218**
upper limit of target range	**340**
Quotient S/σ_{pt}'*	*2,0*
Standard uncertainty $U_{(xpt)}$	*21,5*
Results in the target range	*8*
Percent in the target range	*62%*

** without result No. 12 (excluded in advance)*

Comments:

The target standard deviation was calculated using data from a precision experiment (ASU §64 L 01.00-90)(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 an increased variability. Therefore the valuation was done by z'-scores considering the standard uncertainty. The quotient S/σ_{pt}' was then 2,0. The robust standard deviation was 22% and higher than values of established, standardized methods (see 3.6.2).*

62% of results were in the target range.

The robust mean of participant results was 97 % of the spiking level of lactose to the spiking level sample (s. p. 5).

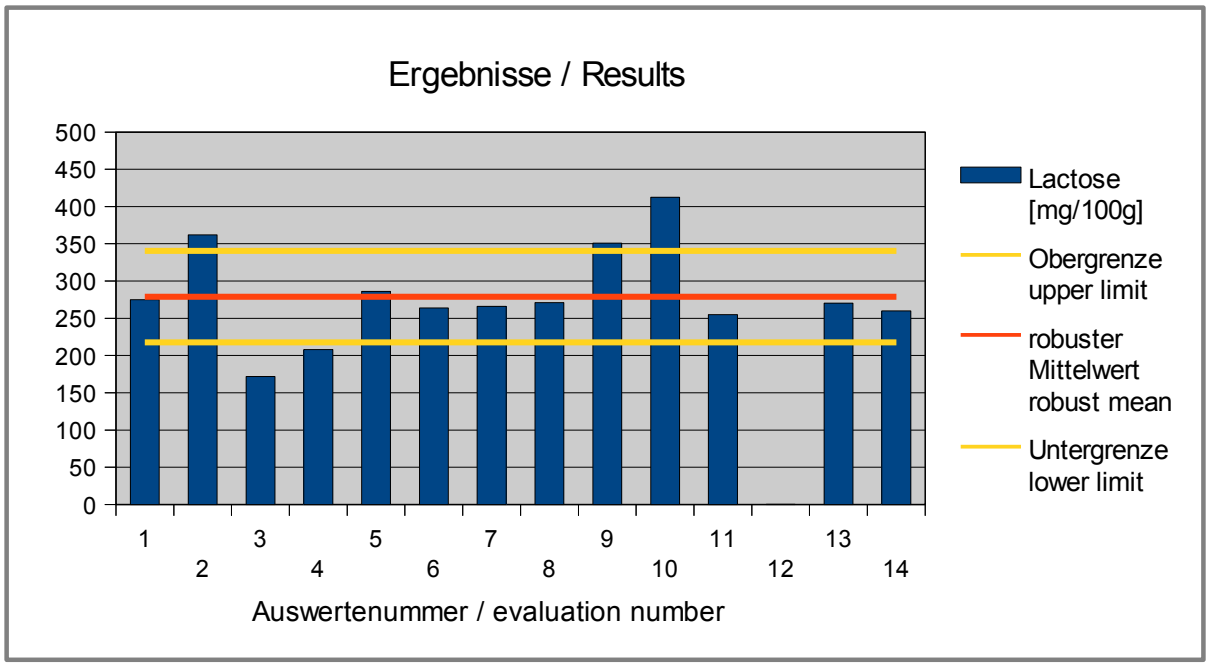

Abb. / Fig. 10: Ergebnisse Lactose / Results Lactose

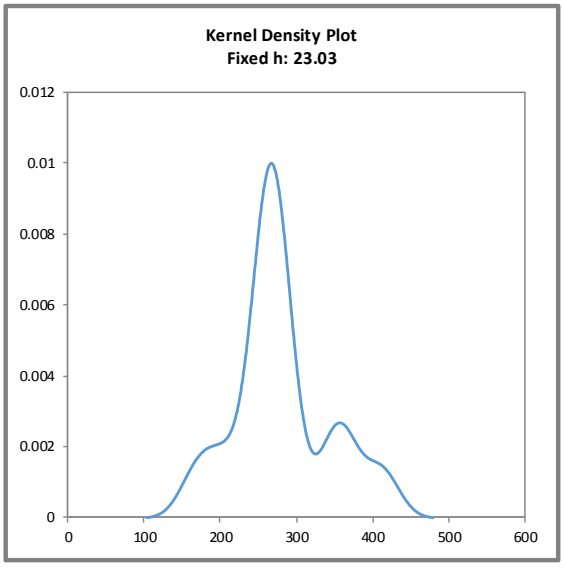

Abb. / Fig. 11:
Kerndichte-Schätzung der Ergebnisse
(mit h = 0,75 x σ_{pt} von X_{pt})

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

Comment:
The kernel density shows nearly a symmetrical distribution of results with a broad side peak, due to three results above the target range and a shoulder, due to two results below the target range.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Lactose [mg/100g]	Abweichung [mg/kg] Deviation [mg/kg]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	275	-4	-0,13	-0,29	
2	362	83	2,7	6,1	
3	172	-107	-3,5	-7,9	
4	208	-71	-2,3	-5,2	
5	286	7	0,23	0,52	
6	264	-15	-0,49	-1,1	
7	266	-13	-0,42	-1,0	
8	271	-8	-0,26	-0,59	
9	351	72	2,3	5,3	
10	412	133	4,3	9,9	
11	255	-24	-0,78	-1,8	
12	0,275				Ergebnis ausgeschlossen / Result excluded
13	270	-9	-0,28	-0,63	
14	260	-19	-0,62	-1,4	

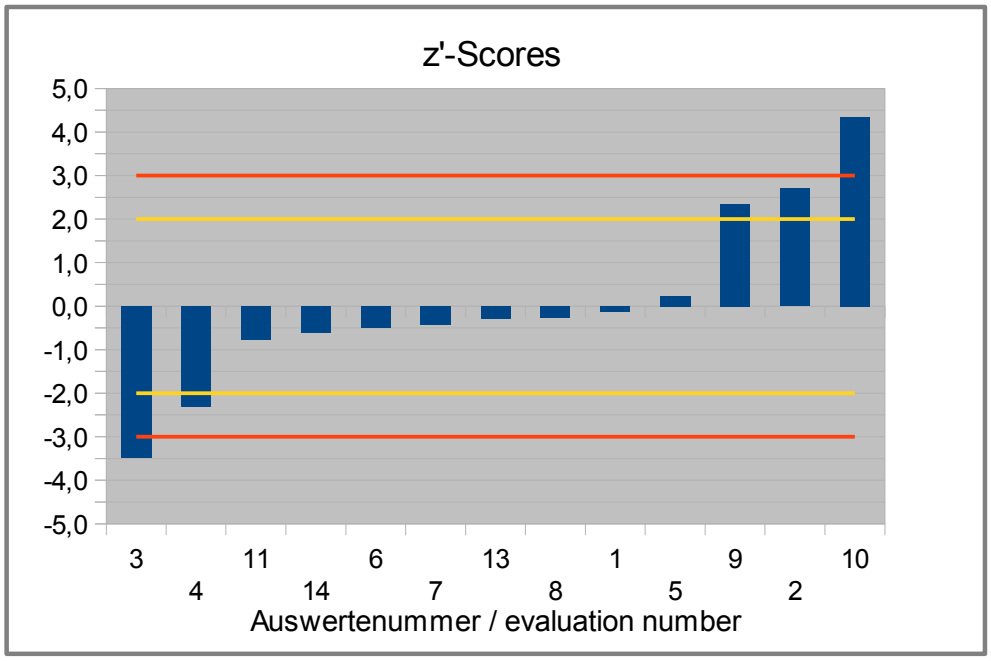

Abb. / Fig. 12: z´-Scores Lactose

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.

Spiking Level Sample and Sample A

Evaluation number	Spiking Level Sample	Recovery rate*	Sample A	Recovery rate*	Remarks
	[mg/kg]	[%]	[mg/kg]	[%]	
1	275	**95**	270	**91**	
2	362	126	287	**96**	
3	172	60	230	77	
4	208	72	265	**89**	
5	286	**99**	291	**98**	
6	264	**92**	300	**101**	
7	266	**92**	303	**102**	
8	271	**94**	283	**95**	
9	351	122	558	187	
10	412	143	455	153	
11	255	**89**	285	**96**	
12	0,275	0	0,300	0	
13	270	**94**	274	**92**	
14	260	**90**	370	124	

RA**	85-115 %	RA**	85-115 %
Anzahl im AB	**8**	Anzahl im AB	**9**
Prozent im AB	**57**	Prozent im AB	**64**

* Recovery rate 100% relative size: lactose, s. page 5

** Range of acceptance 3.12 (s. page 15)

Comments:

For the spiking level sample 57% (8) of the participants obtained a recovery rate within the range of 85-115%. For the spiked food matrix sample A 64% (9) of the recovery rates were in this range.

4.3 Galactose

For galactose no results above the detection or quantification limits were reported (see documentation).

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				
Fructose	1	mg/100g	30.05.18	540	yes	5	10	no	-
	2	mg/100g	25.05.18	580	YES		20	NO	
	3	mg/100g	24.05.18	410	yes	10	30	no	89,3
	4	mg/100g	22.05.	506	yes	50	200	no	
	5	mg/100g	-	-					
	6	mg/100g							
	7	mg/100g	03.05.	585	yes	10	30	no	
	8	mg/100g	02.05.18	565	yes	-	50	no	-
	9	mg/100g	18.05.18	539	yes	20	100	yes	not determined
	10	mg/100g							
	11	mg/100g	03.05.18	580			80	no	102
	12	mg/100g	16.04.18	0,54	yes		0,1		
	13	mg/100g							
	14	mg/100g	16.04.18	520	yes	100		no	

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				
Fructose	1	mg/100g	30.05.18	16	yes	5	10	no	-
	2	mg/100g	25.05.18	<LC	NO		20		
	3	mg/100g	24.05.18	14	yes	10	30	no	89,3
	4	mg/100g	22.05.	<50	no	50	200	no	
	5	mg/100g	-	-					
	6	mg/100g							
	7	mg/100g	03.05.	<LOQ	no	10	30	no	
	8	mg/100g	02.05.18	<50	no	-	50	no	-
	9	mg/100g	18.05.18	<20	no	20	100	yes	not determined
	10	mg/100g							
	11	mg/100g	03.05.18	n.d.	no		80	no	107
	12	mg/100g	16.04.18	<0,1	no		0,1		
	13	mg/100g							
	14	mg/100g	16.04.18	<100	no	100		no	

Fructose Spiking Level Sample

Analyte	Participant	Unit	Date of analysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
			Day /Month	mg/100g	yes / no				
Fructose	1	mg/100g	30.05.18	520	yes	5	10	no	-
	2	mg/100g	25.05.18	606	YES		20	NO	
	3	mg/100g	24.05.18	290	yes	10	30	no	89,3
	4	mg/100g	22.05.	380	yes	50	200	no	
	5	mg/100g	-	-					
	6	mg/100g							
	7	mg/100g	03.05.	478	yes	10	30	no	
	8	mg/100g	02.05.18	422	yes	-	50	no	-
	9	mg/100g	18.05.18	475	yes	20	100	yes	not determined
	10	mg/100g							
	11	mg/100g	03.05.18	480			80	no	99
	12	mg/100g	19.04.18	0,42	yes		0,1		
	13	mg/100g							
	14	mg/100g	16.04.18	410	yes	100		no	

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				
Lactose	1	mg/100g	30.05.18	270	yes	5	10	no	-
	2	mg/100g	25.05.18	287	YES		1	NO	
	3	mg/100g	24.05.18	230	yes	1,4	5	no	96,1
	4	mg/100g	22.05.	265	yes	50	200	no	
	5	mg/100g	02.05.18	291	yes	0,6 mg/100g	1,8 mg/100g	no	101,7
	6	mg/100g	22/05	300	yes	5	25	no	
	7	mg/100g	03.05.	303	yes	10	30	no	
	8	mg/100g	02.05.18	283	yes	-	50	no	-
	9	mg/100g	18.05.18	558	yes	20	100	yes	not determined
	10	mg/100g	18.04.18	455,2	Yes	14,5	14,5	No	
	11	mg/100g	03.05.18	285			22	no	112
	12	mg/100g	16.04.18	0,3	yes		0,01		
	13	mg/100g	26.04.18	273,9	yes		2	yes	99
	14	mg/100g	16.04.18	370	yes	5		no	

Lactose Sample B

Analyte	Participant	Unit	Date of ana-lysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Sample B			Day /Month	mg/100g	yes / no				
Lactose	1	mg/100g	30.05.18	39	yes	5	10	no	-
	2	mg/100g	25.05.18	<LC	NO		1		
	3	mg/100g	24.05.18	< lod	no	1,4	5	no	
	4	mg/100g	22.05.	<50	no	50	200	no	
	5	mg/100g	02.05.18	15	yes	0,6 mg/100g	1,8 mg/100g	no	101,24
	6	mg/100g	17/05	< 10	yes	2	10	no	
	7	mg/100g	03.05.	<LOQ	no	10	30	no	
	8	mg/100g	02.05.18	<50	no	-	50	no	-
	9	mg/100g	18.05.18	<20	no	20	100	yes	not determined
	10	mg/100g	18.04.18	<14.5	No	14,5	14,5	No	
	11	mg/100g	03.05.18	n.d.	no		22	no	106
	12	mg/100g	16.04.18	<0,01	no		0,01		
	13	mg/100g	26.04.18	0	no		2	yes	99
	14	mg/100g	16.04.18	<5	no	5		no	

Lactose Spiking Level Sample

Analyte	Participant	Unit	Date of ana-lysis	Final result	Detectable	LOD	LOQ	Incl. RR	Recovery rate [%]
Spiking Level Sample			Day /Month	mg/100g	yes / no				
Lactose	1	mg/100g	30.05.18	275	yes	5	10	no	-
	2	mg/100g	25.05.18	362	YES		1	NO	
	3	mg/100g	24.05.18	172	yes	1,4	5	no	96,1
	4	mg/100g	22.05.	208	yes	50	200	no	
	5	mg/100g	02.05.18	286	yes	0,6 mg/100g	1,8 mg/100g	no	101,24
	6	mg/100g	22/05	264	yes	5	25	no	
	7	mg/100g	03.05.	266	yes	10	30	no	
	8	mg/100g	02.05.18	271	yes	-	50	no	-
	9	mg/100g	18.05.18	351	yes	20	100	yes	not determined
	10	mg/100g	18.04.18	412,4	Yes	14,5	14,5	No	
	11	mg/100g	03.05.18	255			22	no	112
	12	mg/100g	11.05.18	0,275	yes		0,01		
	13	mg/100g	26.04.18	270,4	yes		2	yes	99
	14	mg/100g	16.04.18	260	yes	5		no	

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				
Galactose	1	mg/100g	30.05.18	<10	yes	5	10	no	-
	2	mg/100g							
	3	mg/100g							
	4	mg/100g							
	5	mg/100g	-	-					101,24
	6	mg/100g	22/05	< 25	yes	5	25	no	
	7	mg/100g							
	8	mg/100g	02.05.18	<50	no	-	50	no	-
	9	mg/100g							
	10	mg/100g							
	11	mg/100g	03.05.18	n.d.	no		40	no	
	12	mg/100g	16.04.18	<0,01	no		0,01		
	13	mg/100g							
	14	mg/100g	16.04.18	<100	no	100		yes	100

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				
Galactose	1	mg/100g	30.05.18	<10	yes	5	10	no	-
	2	mg/100g							
	3	mg/100g							
	4	mg/100g							
	5	mg/100g	-	-					
	6	mg/100g	17/05	< 10	yes	2	10	no	
	7	mg/100g							
	8	mg/100g	02.05.18	<50	no	-	50	no	-
	9	mg/100g							
	10	mg/100g							
	11	mg/100g	03.05.18	n.d.	no		40	no	
	12	mg/100g	16.04.18	<0,01	no		0,01		
	13	mg/100g							
	14	mg/100g	16.04.18	<100	no	100		no	

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				
Galactose	1	mg/100g	30.05.18	<10	yes	5	10	no	-
	2	mg/100g							
	3	mg/100g							
	4	mg/100g							
	5	mg/100g	-	-					
	6	mg/100g	22/05	< 25	yes	5	25	no	
	7	mg/100g							
	8	mg/100g	02.05.18	<50	no	-	50	no	-
	9	mg/100g							
	10	mg/100g							
	11	mg/100g	03.05.18	n.d.	no		40	no	
	12	mg/100g	16.04.18	<0,01	no		0,01		
	13	mg/100g							
	14	mg/100g	16.04.18	<100	no	100		no	

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								
Fructose	1	HPIEC-PAD	Water extraction				yes	
	2	HPLC/MS-MS				NO	NO	
	3	MP.0002.R1.2018	Water extraction	IC-PAD	D-(−)-Fructose Sigma	no	yes	
	4	HPLC/ELSD	Water extraction					
	5							
	6							
	7	Enzymatic	homogenize, aqueous extraction, Carrez clarification, filtration		standards from enzyme-kit r-biopharm	no	yes	HPAEC-PAD: 525 mg/100g
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and internal RM	no	no	
	9	ISO 22662		ELSD Detection		no	yes	recovery correction with internal standard
	10							
	11	ASU § 64 LFGB L31.00-12, modified, 1997-01	see Lactose	enzymatic, testkit r-biopharm	Fructose	yes	yes	
	12	Thermo Fisher Scientificv 984302					yes	
	13							
	14	HPAEC-PAD					no	

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								
Fructose	1	HPIEC-PAD	Water extraction				yes	
	2	HPLC/MS-MS				NO	NO	
	3	MP.0002.R1.2018	Water extraction	IC-PAD	D-(−)-Fructose Sigma	no	yes	
	4	HPLC/ELSD	Water extraction					
	5							
	6							
	7	Enzymatic	homogenize, aqueous extraction, Carrez clarification, filtration		standards from enzyme-kit r-biopharm	no	yes	HPAEC-PAD: n.d.
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and internal RM	no	no	
	9	ISO 22662		ELSD Detection		no	yes	
	10							
	11	ASU § 64 LFGB L31.00-12, modified, 1997-01	see lactose	enzymatic, testkit r-biopharm	Fructose	yes	yes	
	12	Thermo Fisher Scientificv 984302					yes	
	13							
	14	HPAEC-PAD					no	

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								
Fructose	1	HPIEC-PAD	Water extraction				yes	
	2	HPLC/MS-MS				NO	NO	
	3	MP.0002.R1.2018	Water extraction	IC-PAD	D-(−)-Fructose Sigma	no	yes	
	4	HPLC/ELSD	Water extraction					
	5							
	6							
	7	Enzymatic	homogenize, aqueous extraction, Carrez clarification, filtration		standards from enzyme-kit r-biopharm	no	yes	HPAEC-PAD: 439 mg/100g
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and internal RM	no	no	
	9	ISO 22662		ELSD Detection		no	yes	recovery correction with internal standard
	10							
	11	ASU § 64 LFGB L31.00-12, modified, 1997-01	see lactose	enzymatic, testkit r-biopharm	Fructose	yes	yes	
	12	Thermo Fisher Scientificv 984302					yes	
	13							
	14	HPAEC-PAD					no	

Lactose Sample A

Analyte	Partici-pant	Method description as in test report / norm / litera-ture	Sample preparation	Measuring me-thod	Calibration / Reference material	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Sample A								
Lactose	1	HPIEC-PAD	Water extraction				yes	
	2	HPLC/MS-MS				NO	NO	
	3	MP.0002.R1.2018	Water extraction	IC-PAD	Lactose mo-nohydrate Merck	no	yes	
	4	HPLC/ELSD	Extraktion mit Wasser					
	5	Enzymatic method using Boehringer/R-Biopharm Test-Combination kit for the quantitative determination of lac-tose 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 (o-riginal container) to room temperature and homogenise it by mixing. Take the test portion for analysis from the homogeneous test sample.	-	DS81 REF012 internal refe-rence sample	no	no	-
	6	Enzymatic	2,0 g / 100 ml; 70 °C 15 min., Carrez-clarification				no	
	7	Enzymatic	homogenize, aqueous extraction, Car-rez clarification, filtration		standards from enzyme-kit r-biopharm	no	yes	HPAEC-PAD: 310 mg/100g
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and in-ternal RM	no	no	
	9	ISO 22662		ELSD Detektion		no	yes	recovery cor-rection with in-ternal standard
	10	Enzymatic	Water Extraction	Megazyme Low-lac Kit	In House		No	
	11	ASU § 64 LFGB L01.00-17, modified, 2010-09	5 g sample in 100 ml flask with dist. Water extracted at 70 ° C in an ultra-sonic bath, after cooling Carrez clarifi-cation	enzymatic, test-kit, r-biopharm	Lactosemono-hydrat	yes	yes	
	12	r Biopharm Test-Combination 10176303035					yes	
	13	HPLC-MS		recovery calcula-ted by C13-Lactose internal standard	Anhydrous lac-tose (Sigma)	yes	yes	
	14	HPAEC-PAD					no	

Lactose Sample B

Analyte	Partici-pant	Method description as in test report / norm / litera-ture	Sample preparation	Measuring me-thod	Calibration / Reference ma-terial	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Sample B								
Lactose	1	HPIEC-PAD	Water extraction				yes	
	2	HPLC/MS-MS				NO	NO	
	3	MP.0002.R1.2018	Water extraction	IC-PAD	Lactose mono-hydrate Merck	no	yes	
	4	HPLC/ELSD	Water extraction					
	5	Enzymatic method using Boehringer/R-Biopharm Test-Combination kit for the quantitative determination of lac-tose 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 (o-riginal container) to room temperature and homogenise it by mixing. Take the test portion for analysis from the homogeneous test sample.	-	DS81 REF012 internal refe-rence sample	no	no	-
	6	Enzymatic	5,0 g / 100 ml; 70 °C 15 Min., Carrez clarification				no	
	7	Enzymatic	homogenize, aqueous extraction, Car-rez clarification, filtration		standards from enzyme-kit r-biopharm	no	yes	HPAEC-PAD: n.d.
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and inter-nal RM	no	no	
	9	ISO 22662		ELSD Detection		no	yes	
	10	Enzymatic	Water Extraction	Megazyme Low-lac Kit	In House		No	
	11	ASU § 64 LFGB L01.00-17, modified, 2010-09	5 g sample in 100 ml flask with dist. Water extracted at 70 ° C in an ultra-sonic bath, after cooling Carrez clarifi-cation	enzymatic, test-kit, r-biopharm	Lactosemono-hydrat	yes	yes	
	12	r Biopharm Test-Combination 10176303035					yes	
	13	HPLC-MS		recovery calcula-ted by C13-Lactose internal standard	Anhydrous lac-tose (Sigma)	yes	yes	
	14	HPAEC-PAD					no	

Lactose Spiking Level Sample

Analyte	Partici-pant	Method description as in test report / norm / litera-ture	Sample preparation	Measuring me-thod	Calibration / Reference ma-terial	Recovery rate with same matrix	Method accredited ISO/IEC 17025	Further Remarks
Lactose	1	HPIEC-PAD	Water extraction				yes	
	2	HPLC/MS-MS				NO	NO	
	3	MP.0002.R1.2018	Water extraction	IC-PAD	Lactose mono-hydrate Merck	no	yes	
	4	HPLC/ELSD	Water extraction					
	5	Enzymatic method using Boehringer/R-Biopharm Test-Combination kit for the quantitative determination of lac-tose 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 (o-riginal container) to room temperature and homogenise it by mixing. Take the test portion for analysis from the homogeneous test sample.	-	DS81 REF012 internal refe-rence sample	no	no	-
	6	Enzymatic	2,0 g / 100 ml; 70 °C 15 Min., Carrez-clarification				no	
	7	Enzymatic	homogenize, aqueous extraction, Car-rez clarification, filtration		standards from enzyme-kit r-biopharm	no	yes	HPAEC-PAD: 291 mg/100g
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and inter-nal RM	no	no	
	9	ISO 22662		ELSD Detection		no	yes	Recovery cor-rection with in-ternal standard
	10	Enzymatic	Water Extraction	Megazyme Low-lac Kit	In House		No	
	11	ASU § 64 LFGB L01.00-17, modified, 2010-09	5 g sample in 100 ml flask with dist. Water extracted at 70 ° C in an ultra-sonic bath, after cooling Carrez clarifi-cation	enzymatic, testkit, r-bio-pharm	Lactosemono-hydrat	yes	yes	
	12	r Biopharm Test-Combination 10176303035					yes	
	13	HPLC-MS		recovery calcu-lated by C13-Lactose internal stan-dard	Anhydrous lac-tose (Sigma)	yes	yes	
	14	HPAEC-PAD					no	

Galactose 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								
Galactose	1	HPIEC-PAD	extraction with water				yes	
	2							
	3							
	4							
	5	recovery rate lactose						
	6	Enzymatic	2,0 g / 100 ml; 70 °C 15 min., Carrez clarification				no	
	7							HPAEC-PAD: n.d.
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and internal RM	no	no	
	9							
	10							
	11	ASU § 64 LFGB L01.00-17, modified, 2010-09	like above	enzymatic, testkit r-biopharm	Galactose		yes	
	12	r Biopharm Test-Combination 10176303035					yes	
	13							
	14	HPAEC-PAD					no	

Galactose 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								
Galactose	1	HPIEC-PAD	Extraction with water				yes	
	2							
	3							
	4							
	5							
	6	Enzymatic	5,0 g / 100 ml; 70 °C 15 min., Carrez clarification				no	
	7							HPAEC-PAD: n.d.
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and internal RM	no	no	
	9							
	10							
	11	ASU § 64 LFGB L01.00-17, modified, 2010-09	like above	enzymatic, testkit r-biopharm	Galactose		yes	
	12	r Biopharm Test-Combination 10176303035					yes	
	13							
	14	HPAEC-PAD					no	

Galactose 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
Galactose	1	HPIEC-PAD	Extraction with water				yes	
	2							
	3							
	4							
	5							
	6	Enzymatic	2,0 g / 100 ml; 70 °C 15 min., Carrez clarification				no	
	7							HPAEC-PAD: n.d.
	8	HPAE-PAD - internal method PNTA0179			external calib. curve and internal RM	no	no	
	9							
	10							
	11	ASU § 64 LFGB L01.00-17, modified, 2010-09	like above	enzymatic, testkit, r-biopharm	Galactose		yes	
	12	r Biopharm Test-Combination 10176303035					yes	
	13							
	14	HPAEC-PAD					no	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogenitätstest
DLA 18-2018 Sample A

Weight whole sample	2,83	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	14,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	35	13,9
2	5,03	39	15,5
3	5,08	44	17,3
4	4,99	42	16,8
5	5,05	42	16,6
6	4,98	42	16,9
7	5,04	46	18,3
8	4,97	40	16,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	41,2	Particle
Standard deviation	3,24	Particle
χ^2 (CHI-Quadrat)	1,78	
Probability	**97**	%
Recovery rate	110	%

Normal distribution		
Number of samples	8	
Mean	16,4	mg/kg
Standard deviation	1,29	mg/kg
rel. Standard deviaton	7,85	%
Horwitz standard deviation	10,5	%
HorRat-value	**0,75**	
Recovery rate	110	%

Microtracer Homogenitätstest
DLA 18-2018 Spiking Level Sample

Weight whole sample	1,80	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	14,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,99	41	16,4
2	5,04	47	18,7
3	5,03	38	15,1
4	5,05	46	18,2
5	4,97	38	15,3
6	4,96	45	18,1
7	5,05	41	16,2
8	5,02	38	15,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	41,7	Particle
Standard deviation	3,72	Particle
χ^2 (CHI-Quadrat)	2,32	
Probability	**94**	%
Recovery rate	113	%

Normal distribution		
Number of samples	8	
Mean	16,7	mg/kg
Standard deviation	1,48	mg/kg
rel. Standard deviaton	8,91	%
Horwitz standard deviation	10,5	%
HorRat-value	**0,85**	
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	***DLA 18-2018***
PT name	***Lactose + Fructose in "lactose-free" infant-food with "Spiking Level Sample"***
*Sample matrix**	***Samples A + B:*** *"Lactose-free" infant food (cereal pap powder)/ingredients: sorghum whole meal, rice flour, thiamine and, potato powder, 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: 25 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.*
Result sheet	*One result each should be determined for Samples A and B and the Spiking Level Sample for submission.* *The results should be filled in the result submission file. In case of several determinations the mean.*
Units	*mg/100g*
Number of significant digits	*at least 2*
Further information	*For information please specify:* – *Date of analysis* – *DLA-sample-numbers (for sample A and B)* – *Limit of detection* – *Assignment incl. Recovery* – *Recovery with the same matrix* – *Method is accredited*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest 25th May 2018***
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	*Dr. Matthias Besler-Scharf*

** 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
		ITALY
		ITALY
		Germany
		Germany
		NETHERLANDS
		Germany
		SPAIN
		Germany
		ITALY
		GREAT BRITAIN
		NETHERLANDS
		Germany
		AUSTRIA
		Germany
		SPAIN

[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
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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)