DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 41/2016

Sugar Alcohols in Plant Product
E 420, E 421, E 953, E 966 and E 967

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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

The test material was a sauce powder (ingredients: corn starch, salt, flavour, colours: E 101 and E 160b), added were sorbitol (E 420/ 4,0%), mannitol (E 421/ 4,0%), isomalt (E 953/ 4,0%), lactitol (E 966/ 4,0%) and xylitol (E 967/ 4,0%). To the mixture were further added microtracer iron particles (FSS red lake) to homogeneity verification.

Approximately 650 g of the material were mixed, sieved, homogenized and then packaged in portions to approximately 10 g. The portions were numbered chronologically.

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 10-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [13].

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 showed probabilities of 68%. 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 a HorRat value of 0,78. The results of microtracer analysis are given in the documentation.

The calculation of the repeatability standard deviation of the participants for the sugar alcohols E 420, E 421, E 953, E 966 and E 967 were used as an indicator of homogeneity. The result is similar to the repeatability standard deviation of the official method ASU § 64 LFGB 48.03-2 [15]. The repeatability standard deviation of the participants is given in the documentation and in the statistic data (see 4.1 to 4.5).

In the documentation the portion numbers are graphically assigned to the results of xylitol. There is no trend recognizable in the results which could suggest inhomogeneity.

If the criteria for sufficient homogeneity of the test material are not fulfilled on a particular parameter, the impact on the target standard deviation is checked and optionally the evaluation of the results of the participants will be done using the z´-score considering the standard uncertainty of the assigned value (see 3.8 and 3.11) [3].

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 19th week of 2016. The testing method was optional. The tests should be finished at June 24th 2016 the latest.

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

The two portions contain identical samples of the same plant powder with a common in commerce content of several sugar alcohols such as Isomalt (E 953), Lactitol (E 966), Mannitol (E421), Sorbitol (E 420) or Xylitol (E 967) to perform a complete duplicate determination. In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.

The analytical methods are optional.

2.3 Results

The participants submitted their results in standard forms, which have been handed out with the samples (by email). The finally calculated concentrations as average of duplicate determinations of both numbered samples was used for the statistical evaluation. For the calculation of the Repeatability- and Reproducibility standard deviation the single values of the double determination were used.

Queried and documented were single results, recovery and the used testing method for E 420, E 421, E 953, E 966, E 967 and further sugar alcohols, Information on the limit of quantification, the date of the analysis and general points to the method.

From the 10 participants one participant has not delivered a result. All other participants submitted the result in time.

3. Evaluation

3.1 Consensus values from participants (Assigned value)

The robust mean of the submitted results was used as assigned value (X) („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.

The calculation of the repeatability standard deviation S_r is performed by: [3, 4].

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.

The calculation of the reproducibility standard deviation S_R is performed by: [3, 4].

3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, and results for a another proficiency test item can be removed from the data set [2]. 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. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

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.

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)

The target standard deviation according to Horwitz [6, 8, 9] was used for the sugar alcohols E 420, E 421, E 953, E 966 and E 967.

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 values given in Table 1 relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) were determined in collaborative trials using the specified methods.

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Table 1: Relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) for the sugar alcohols according to evaluations of experiments for precision [15, 16]

Parameter	**Matrix**	**Mean**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method/ Literature**
Xylitol (E967)*	cookies	3,03%	1,62%	3,76%	3,58%	HPLC-RI/15
Sorbitol (E420)*	cookies	3,76%	1,52%	3,91%	3,76%	HPLC-RI/15
Sorbitol (E420)	cookies	4,66%	1,65%	2,66%	2,66%	Enzymatic/16
Mannitol (E421)*	cookies	4,34%	1,24%	3,55%	3,44%	HPLC-RI/15
GPS[1] (E953)*	cookies	13,5%	0,52%	3,41%	3,41%	HPLC-RI/15
GPM[1] (E953)	cookies	12,6%	0,66%	4,47%	4,47%	HPLC-RI/15
Lactitol (E966)*	cookies	6,11%	1,82%	7,83%	7,73%	HPLC-RI/15

[1] Chemical isomalt (E953) is a mixture of 6-O-α-D-Gucopyranosyl-D-sorbitol (1,6-GPS) and 1-O-α-D-Glucopyranosyl-D-mannitol (1,1-GPM).

* The precision data are used to calculate the target standard deviation according ASU. This target standard deviation is given for information in the evaluation.

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 of results the target standard deviation according to Horwitz was applied.

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

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

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 (x) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation ($\hat{\sigma}$) 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

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

In this PT was present an increased HorRat ratio (> 2.0) for the parameters sorbitol (E420) and lactitol (E 966). For this reason these parameters were evaluated with the target standard deviation ***σ_{pt}'.***

3.8.1 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. For example a fault isolation or a root cause analysis through the examination of transmission error or an error in the calculation, in the trueness and precision must be performed and 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].

3.9 Reproducibility coefficient (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

The consensus value has a standard uncertainty $U(X_{pt})$ that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories (P) and perhaps on other factors. The standard uncertainty of the assigned value ($U_{(Xpt)}$) 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 consensus value needs not to be included in the interpretation of the results of the PT [3]. A clear exceeded the value of 0.3 is an indication that the target standard deviation was possibly set too low for the standard uncertainty of the assigned value. The quotient $U(X_{pt})/\sigma_{pt}$ is reported in the characteristics of the test.

4. Results

In the present PT the sugar alcohols E 420, E 421, E 953, E 966 and E 967 were mixed in a concentration of 4,0% to the sample to be examined. For comparison of the recoveries and precision of measurement, we have compiled the statistic data of the results in Table 2.

Table 2: Compilation of the characteristics of the sugar alcohols E 420, E 421, E 953, E 966 and E 967

Parameter	E 420	E 421	E 953	E 966	E 967
Rob. mean (X_{pt})	4,29%	4,19%	4,16%	4,31%	4,39%
Recovery	107,3%	104,8%	104,0%	107,8%	109,8%
Rob. Standard deviation (S*)	0,325	0,159	0,253	0,426	0,277
repeatability standard deviation (S_r)	0,138	0,096	0,276	0,270	0,110
reproducibility standard deviation (S_R)	0,343	0,205	0,530	0,569	0,287
Target standard deviation σ_{pt} or σ_{pt}'	0,193	0,134	0,134	0,244	0,140
Variation coefficient (CV_R)	8,0	4,9	12,7	13,2	6,5
Standard uncertainty $U_{(Xpt)}$	0,140	0,066	0,120	0,201	0,120
Quotient S*/σ_{pt} *or* S*/σ_{pt}'	1,7	1,2	1,9	1,7	2,0
Quotient $U_{(Xpt)}/\sigma_{pt}$ *or* $U_{(Xpt)}/\sigma_{pt}$'	0,70	0,49	0,84	0,82	0,82

The reproducibility coefficients of variation are in the range of 4,9 – 13,2 and are to be assessed with thus as low. The recovery is slightly above 100% (104% – 110%).

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^x)
repeatability standard deviation (S_r)
reproducibility standard deviation (S_R)
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 CV_R in %
Quotient S/σ_{pt} or S*/σ_{pt}'*
Standard uncertainty $U(X_{pt})$
Quotient $U(X_{pt})/\sigma_{pt}$ or $U(X_{pt})/\sigma_{pt}$'
Number of results in the target range
Percent in the target range

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

In the second table the individual results of the participating laboratories are listed:

Auswerte-nummer	**Parameter [Einheit/ Unit]**	**Abweichung**	**Z-Score** σ_{pt}	**z-Score** (Info)	**Hinweis**
Evaluation number		**Deviation**			**Remark**

4.1 Sorbitol (E420) in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	9
Number of outliers	1
Mean	4,52
Median	4,30
Robust Mean (X_{pt})	4,29
Robust standard deviation (S^x)	0,325
Repeatability standard deviation (S_r)	0,138
Reproducibility standard Deviation (S_R)	0,343
Target range:	
Target standard deviation Horwitz (σ_{pt}')	0,193
Target standard deviation ASU (for Information)	0,161
lower limit of target range	3,91
upper limit of target range	4,68
coefficient of variation (CV_R) in %	8,0
Quotient S^x/ σ_{pt}'	*1,7*
Standard uncertainty $u(X_{pt})$	*0,14*
Quotient $u(X_{pt})$ /σ_{pt}'	*0,70*
Results in the target range	*8*
Percent in the target range	*89*

Comments:

The target standard deviation was calculated by Horwitz as σ_{pt}' .

The evaluation of the results shows an acceptable variability of results, in particular because the tests using different methods (HPAEC-PAD,HPLC-RI, GC-FID)). The quotient $S^x/\sigma_{pt}'$was below 2,0. The quotient $U(X_{pt})/\sigma_p'$ is 0,70 above 0,3, but to accept because of the different methods.

The reproducibility coefficient of variation is low. The robust standard deviation is in the range of established values for the standard deviation of the methods used. The reproducibility of the results is given.

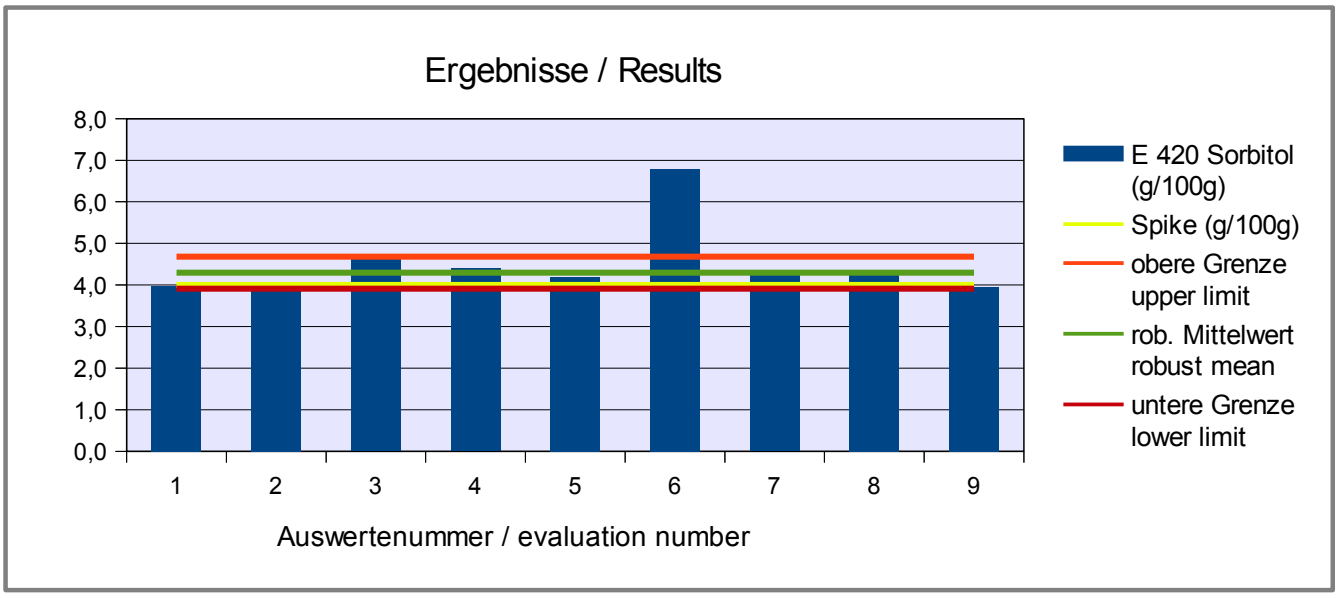

Abb. 1: Ergebnisse E 420
Fig. 1: Results E 420

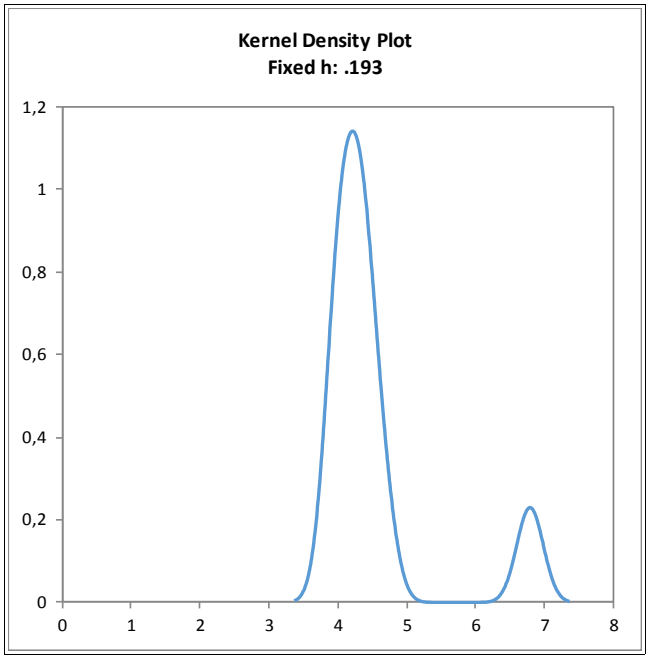

Abb. 2: Kern Dichte Plot der Ergebnisse E 420 mit h = Zielstandardabweichung (0,193 g/100g)
Fig. 2: Kernel density plot of the E 420 results with h = target standard deviation (0,193 g/100g)

Comments:
The kernel density plot shows a normal distribution of results. The second peak at 6,8 g/100g denotes the outlier (no. 6).

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	E 420 Sorbitol (g/100g)	Abweichung [g/100g] Deviation [g/100g]	Z-Score σ_{pt}	z-Score (Info)	Hinweis Remark
1	3,98	-0,315	-1,6	-1,9	
2	4,07	-0,225	-1,2	-1,4	
3	4,64	0,342	1,8	2,1	
4	4,40	0,105	0,5	0,7	
5	4,18	-0,112	-0,6	-0,7	
6	6,79	2,50	12,9	15,5	Ausreisser / Outlier
7	4,30	0,005	0,0	0,0	
8	4,36	0,065	0,3	0,4	
9	3,94	-0,355	-1,8	-2,2	

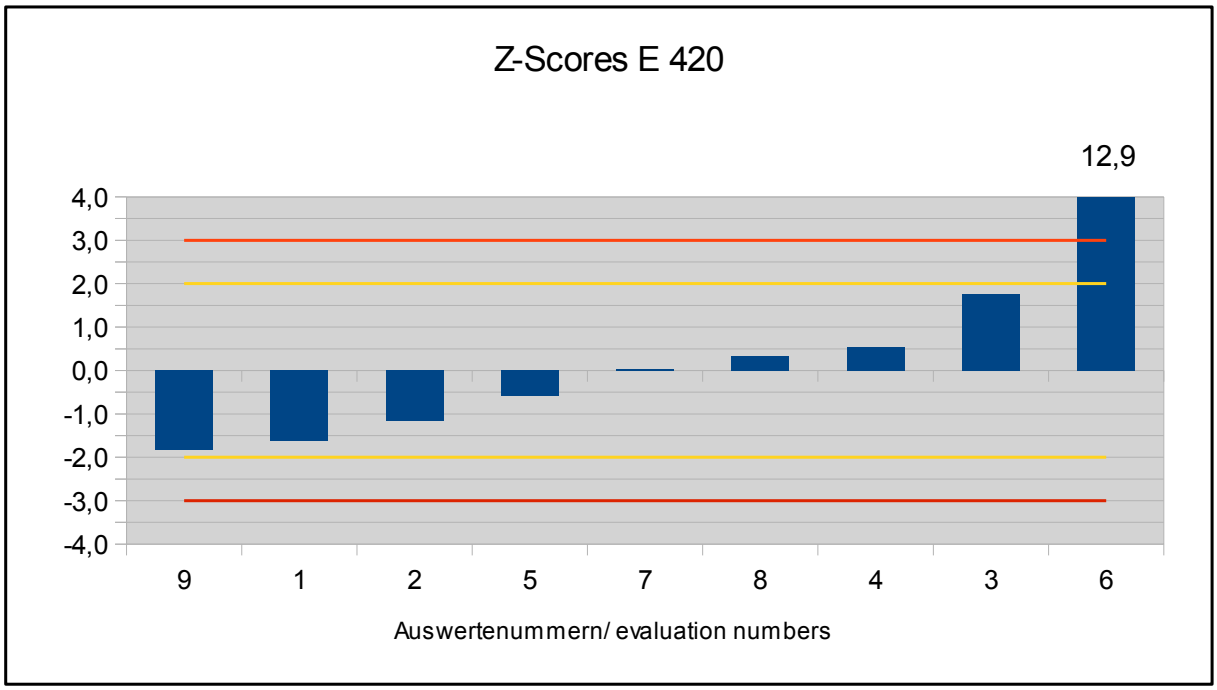

Abb. 3: Z-Scores E 420
Fig. 3: Z-Scores E 420

4.2 Mannitol (E421) in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	9
Number of outliers	1
Mean	4,10
Median	4,20
Robust Mean (X_{pt})	4,16
Robust standard deviation (S^x)	0,159
Repeatability standard deviation (S_r)	0,096
Reproducibility standard Deviation (S_R)	0,205
Target range:	
Target standard deviation Horwitz (σ_{pt})	0,134
Target standard deviation ASU (for Information)	0,143
lower limit of target range	3,89
upper limit of target range	4,43
coefficient of variation (CV_R) in %	4,9
Quotient S^x/σ_{pt}	*1,2*
Standard uncertainty $u(X_{pt})$	*0,066*
Quotient $u(X_{pt})/\sigma_{pt}$	*0,49*
Results in the target range	*8*
Percent in the target range	*89*

Comments:

The target standard deviation was calculated by Horwitz.

The evaluation of the results shows a low variability of results, in particular because the tests using different methods (HPAEC-PAD,HPLC-RI, GC-FID)). The quotient S^x/σ_{pt} was below 2,0. The quotient $U(X_{pt})/\sigma_p$ is 0,49 above 0,3, but to accept because of the different methods.

The reproducibility coefficient of variation is low. The robust standard deviation is in the range of established values for the standard deviation of the methods used. The reproducibility of the results is given.

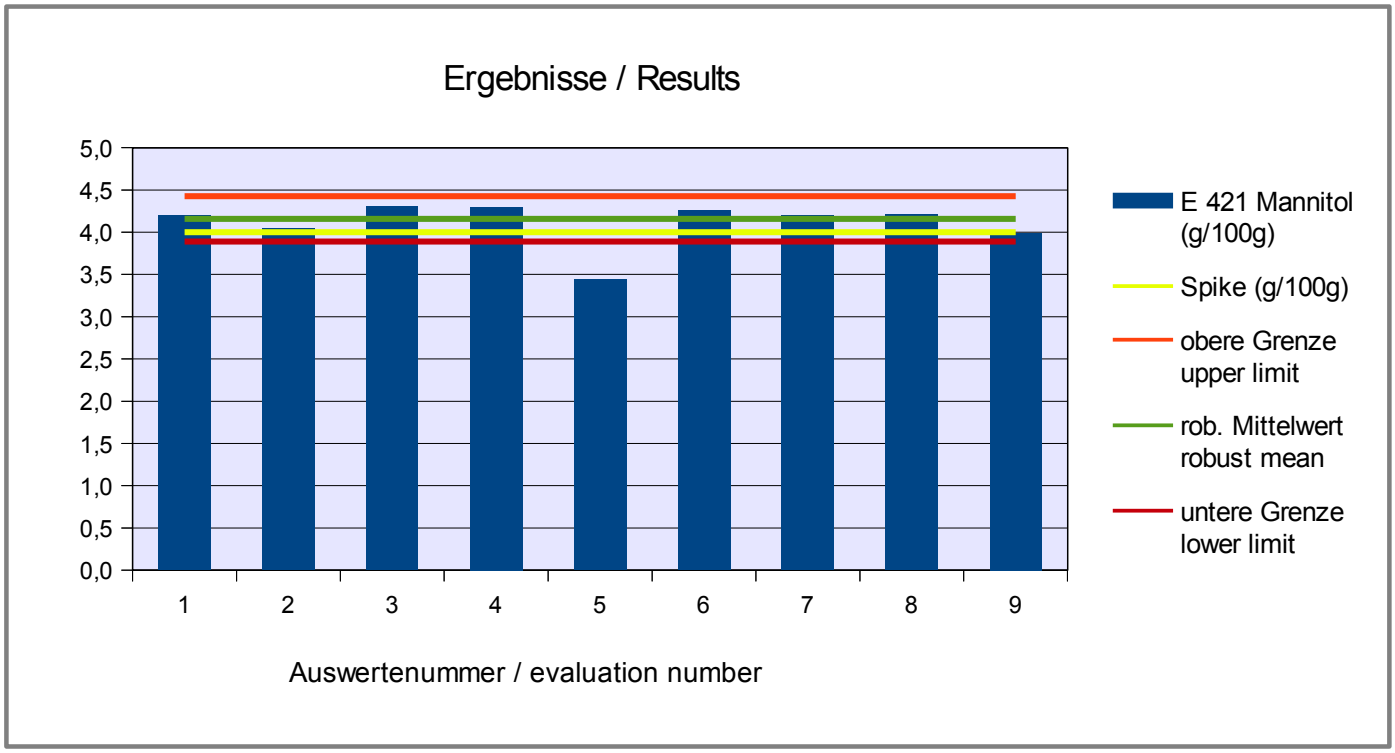

Abb. 4: Ergebnisse E 421
Fig. 4: Results E 421

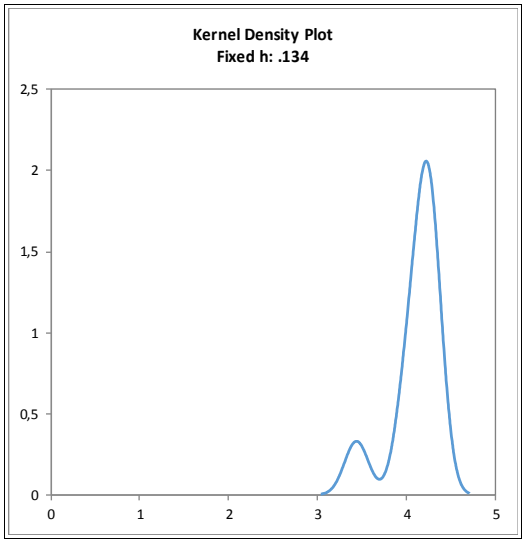

Abb. 5: Kern Dichte Plot der Ergebnisse E 421 mit h = Zielstandardabweichung (0,134 g/100g)
Fig. 5: Kernel density plot of the E 421 results with h = target standard deviation (0,134 g/100g)

Comments:
The kernel density plot shows a normal distribution of results. The second peak at 3,4 g/100g denotes the outlier (no. 5).

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	E 421 Mannitol (g/100g)	Abweichung [g/100g] Deviation [g/100g]	Z-Score σ_{pt}	z-Score (Info)	Hinweis Remark
1	4,20	0,042	0,3	0,3	
2	4,04	-0,118	-0,9	-0,8	
3	4,30	0,146	1,1	1,0	
4	4,30	0,142	1,1	1,0	
5	3,44	-0,718	-5,4	-5,0	Ausreisser / Outlier
6	4,26	0,102	0,8	0,7	
7	4,20	0,042	0,3	0,3	
8	4,21	0,052	0,4	0,4	
9	3,99	-0,168	-1,3	-1,2	

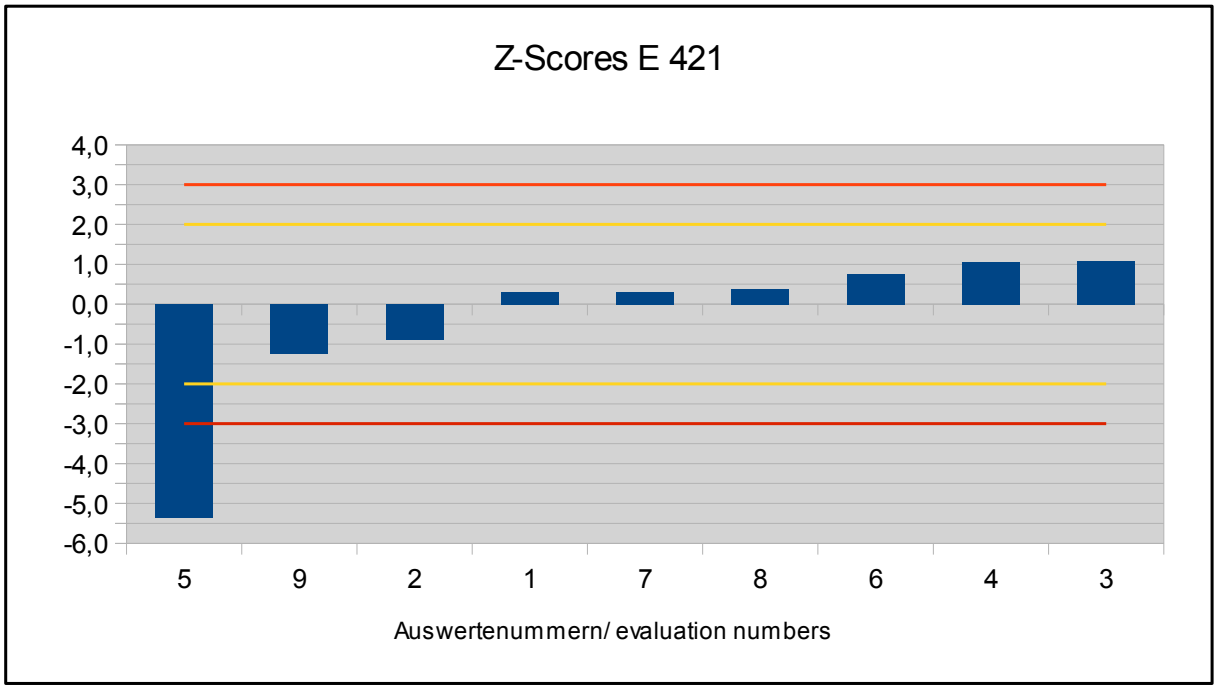

Abb. 6: Z-Scores E 421
Fig. 6: Z-Scores E 421

4.3 Isomalt (E953) in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	7
Number of outliers	0
Mean	4,16
Median	4,14
Robust Mean (X_{pt})	4,16
Robust standard deviation (S^x)	0,253
Repeatability standard deviation (S_r)	0,276
Reproducibility standard Deviation (S_R)	0,530
Target range:	
Target standard deviation Horwitz (σ_{pt})	0,134
Target standard deviation ASU (for Information)	0,141
lower limit of target range	3,90
upper limit of target range	4,43
coefficient of variation (CV_R) in %	12,7
Quotient S^x/σ_{pt}	*1,9*
Standard uncertainty $u(X_{pt})$	*0,120*
Quotient $u(X_{pt})/\sigma_{pt}$	*0,89*
Results in the target range	*6*
Percent in the target range	*86*

Comments:

The target standard deviation was calculated by Horwitz.

The evaluation of the results shows a acceptable variability of results, in particular because the tests using different methods (HPAEC-PAD,HPLC-RI, GC-FID)). The quotient S^x/σ_{pt} was below 2,0. The quotient $U(X_{pt})/\sigma_p$ is 0,89 above 0,3, but to accept because of the different methods.

The reproducibility coefficient of variation is low. The robust standard deviation is in the range of established values for the standard deviation of the methods used. The reproducibility of the results is given.

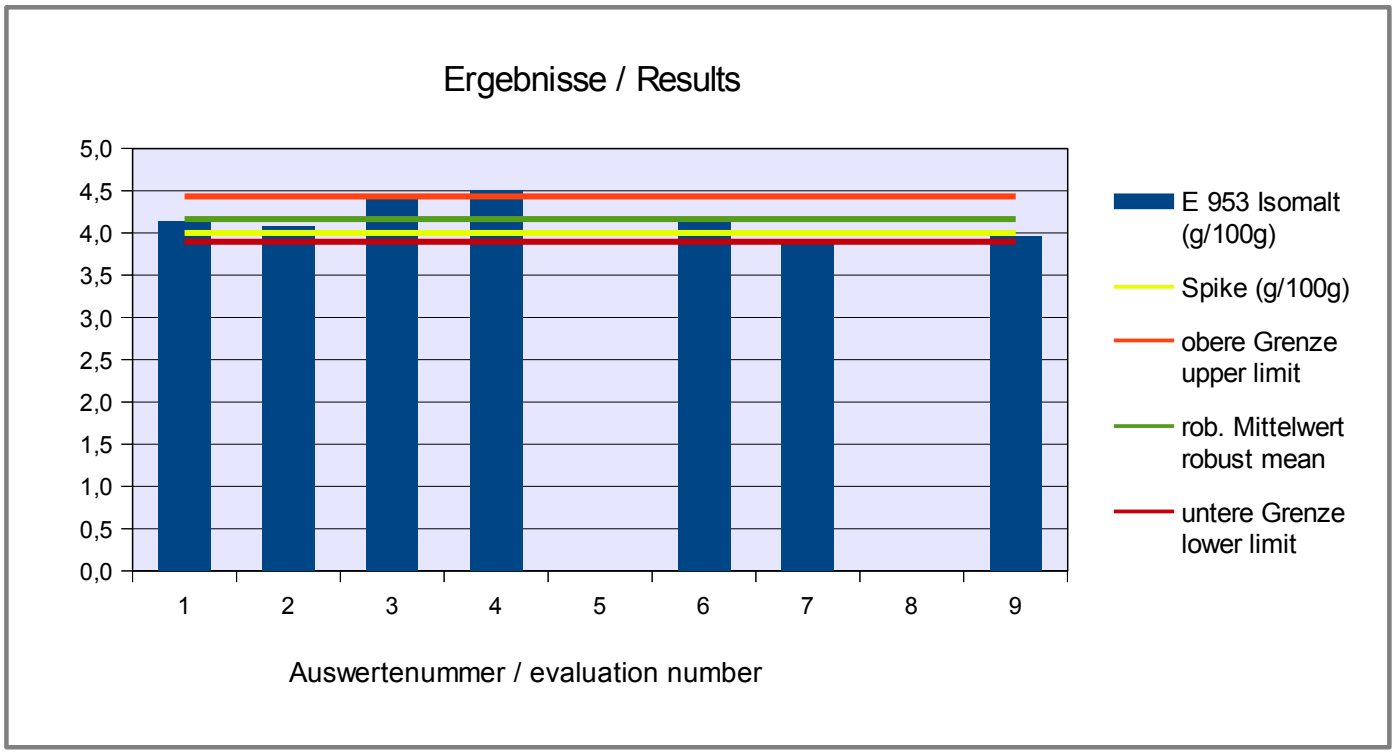

Abb. 7: Ergebnisse E 953
Fig. 7: Results E 953

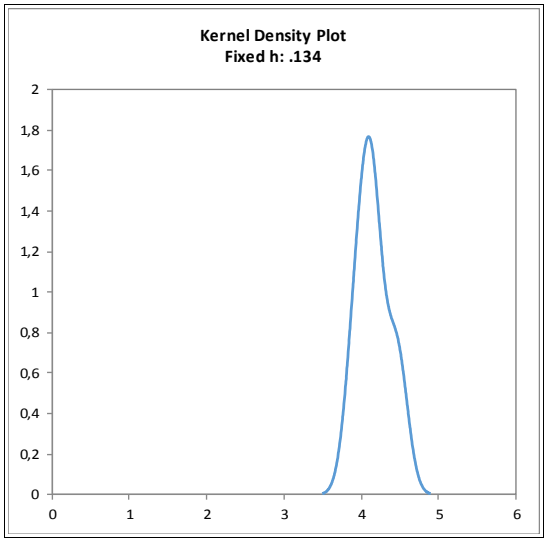

Abb. 8: Kern Dichte Plot der Ergebnisse E 953 mit h = Zielstandardabweichung (0,134 g/100g)
Fig. 8: Kernel density plot of the E 953 results with h = target standard deviation (0,134 g/100g)

Comments:
The kernel density plot shows nearly a normal distribution of results (with a slight shoulder at 4,5 g/100g), the reason is the increased results of participants 3 and 4.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	E 953 Isomalt (g/100g)	Abweichung [g/100g] Deviation [g/100g]	Z-Score σ_{pt}	z-Score (Info)	Hinweis Remark
1	4,14	-0,025	-0,2	-0,2	
2	4,08	-0,085	-0,6	-0,6	
3	4,42	0,259	1,9	1,8	
4	4,50	0,335	2,5	2,4	
5					
6	4,15	-0,015	-0,1	-0,1	
7	3,90	-0,265	-2,0	-1,9	
8					
9	3,96	-0,205	-1,5	-1,5	

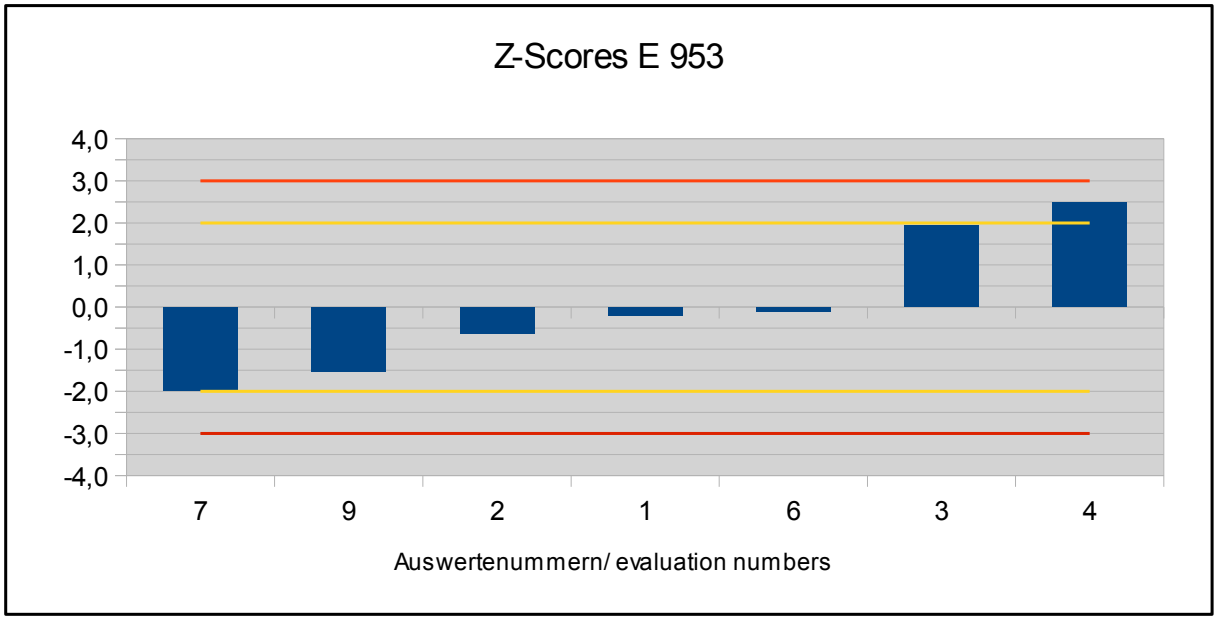

Abb. 9: Z-Scores E 953
Fig. 9: Z-Scores E 953

4.4 Lactitol (E966) in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	7
Number of outliers	0
Mean	4,31
Median	4,10
Robust Mean (X_{pt})	4,31
Robust standard deviation (S^x)	0,426
Repeatability standard deviation (S_r)	0,270
Reproducibility standard Deviation (S_R)	0,569
Target range:	
Target standard deviation Horwitz (σ_{pt}')	0,244
Target standard deviation ASU (for Information)	0,333
lower limit of target range	3,82
upper limit of target range	4,80
coefficient of variation (CV_R) in %	13,2
Quotient S^x/σ_{pt}'	*1,7*
Standard uncertainty $u(X_{pt})$	*0,201*
Quotient $u(X_{pt})/\sigma_{pt}'$	*0,82*
Results in the target range	7
Percent in the target range	*100*

Comments:

The target standard deviation was calculated by Horwitz as σ_{pt}' .

The evaluation of the results shows an acceptable variability of results, in particular because the tests using different methods (HPAEC-PAD,HPLC-RI, GC-FID)). The quotient $S^x/\sigma_{pt}'$was below 2,0. The quotient $U(X_{pt})/\sigma_p'$ is 0,82 above 0,3, but to accept because of the different methods.

The reproducibility coefficient of variation is low. The robust standard deviation is in the range of established values for the standard deviation of the methods used. The reproducibility of the results is given.

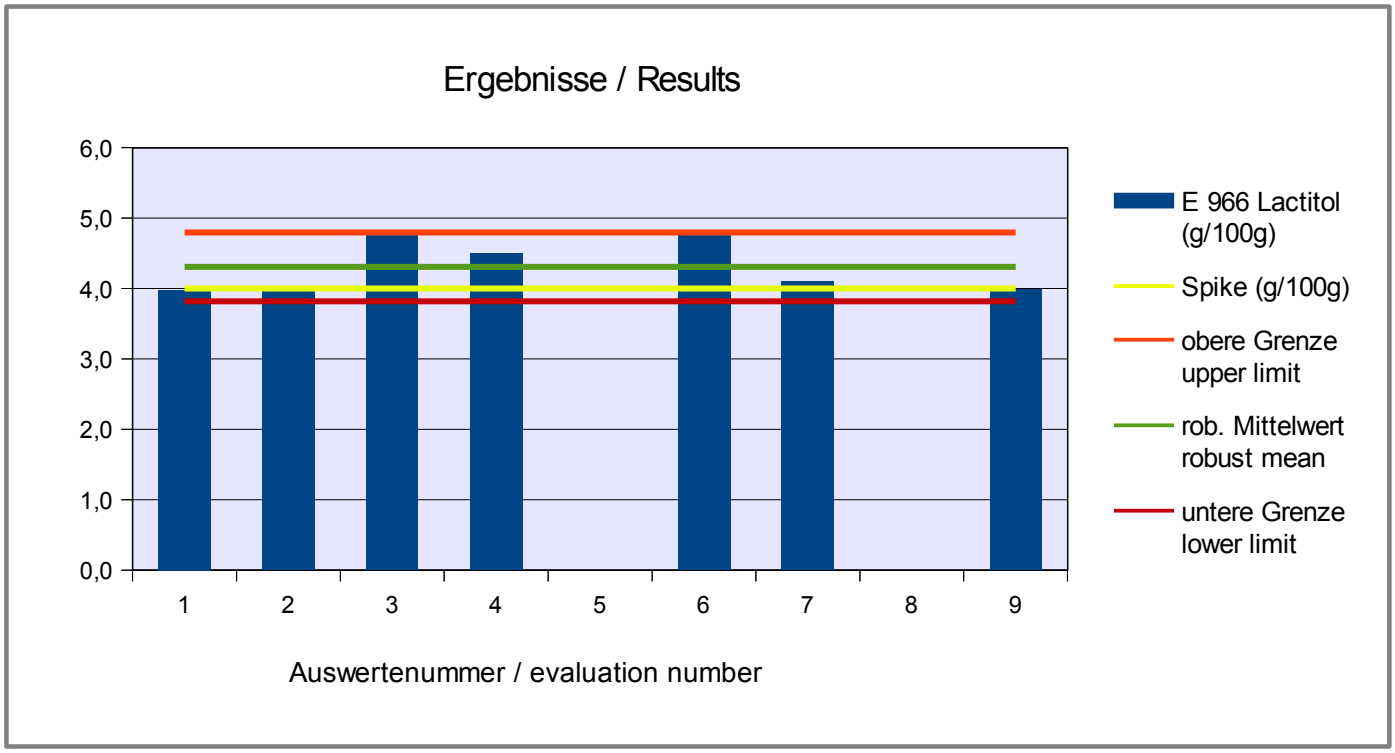

Abb. 10: Ergebnisse E 966
Fig. 10: Results E 966

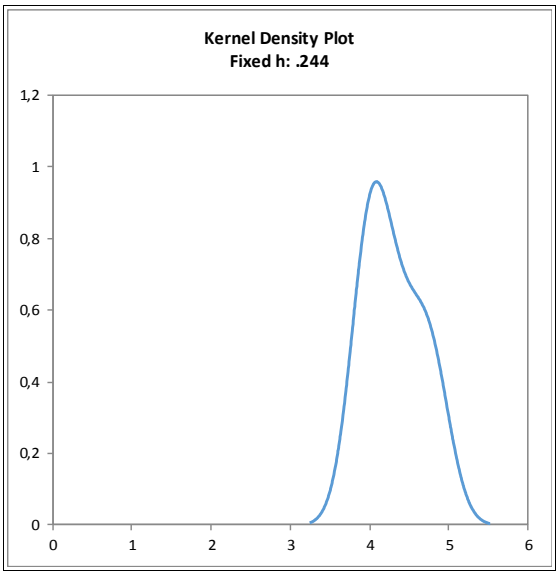

Abb. 11: Kern Dichte Plot der Ergebnisse E 966 mit h = Zielstandardabweichung (0,244 g/100g)
Fig. 11: Kernel density plot of the E 966 results with h = target standard deviation (0,244 g/100g)

Comments:
The kernel density plot shows nearly a normal distribution of results (with a slight shoulder at 4,8 g/100g), the reason is the increased results of participants 3 and 6.

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	E 966 Lactitol (g/100g)	Abweichung [g/100g] Deviation [g/100g]	Z-Score σ_{pt}	z-Score (Info)	Hinweis Remark
1	3,98	-0,327	-1,3	-1,0	
2	4*	-0,307	-1,3	-0,9	
3	4,80	0,492	2,0	1,5	
4	4,50	0,193	0,8	0,6	
5					
6	4,78	0,473	1,9	1,4	
7	4,10	-0,207	-0,8	-0,6	
8					
9	3,99	-0,317	-1,3	-1,0	

*** Only for information: It is indicated by participant no. 2 at "further remarks": Lactitol is not part of our standard scope and is therefore not already calibrated.** Assuming that lactitol has a similar response as the other sugar alcohols for l**actitol a value of the order of 4g / 100 g was obtained also.**

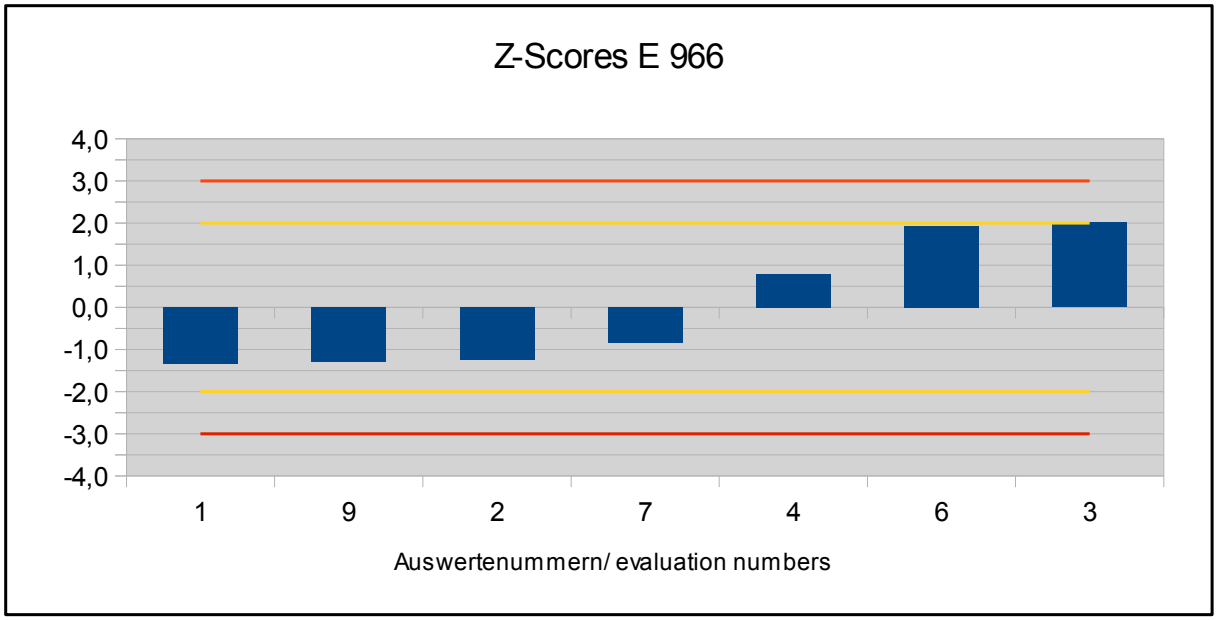

Abb. 12: Z-Scores E 966
Fig. 12: Z-Scores E 966

4.5 Xylitol (E967) in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	9
Number of outliers	1
Mean	4,57
Median	4,40
Robust Mean (X_{pt})	4,39
Robust standard deviation (S^x)	0,277
Repeatability standard deviation (S_r)	0,110
Reproducibility standard Deviation (S_R)	0,287
Target range:	
Target standard deviation Horwitz (σ_{pt})	0,140
Target standard deviation ASU (for Information)	0,157
lower limit of target range	4,11
upper limit of target range	4,67
coefficient of variation (CV_R) in %	6,5
Quotient S^x/σ_{pt}	*2,0*
Standard uncertainty $u(X_{pt})$	*0,12*
Quotient $u(X_{pt})/\sigma_{pt}$	*0,82*
Results in the target range	7
Percent in the target range	*78*

Comments:

The target standard deviation was calculated by Horwitz.

The evaluation of the results shows a acceptable variability of results, in particular because the tests using different methods (HPAEC-PAD,HPLC-RI, GC-FID)). The quotient S^x/σ_{pt} was below 2,0. The quotient $U(X_{pt})/\sigma_p$ is 0,82 above 0,3, but to accept because of the different methods.

The reproducibility coefficient of variation is low. The robust standard deviation is in the range of established values for the standard deviation of the methods used. The reproducibility of the results is given.

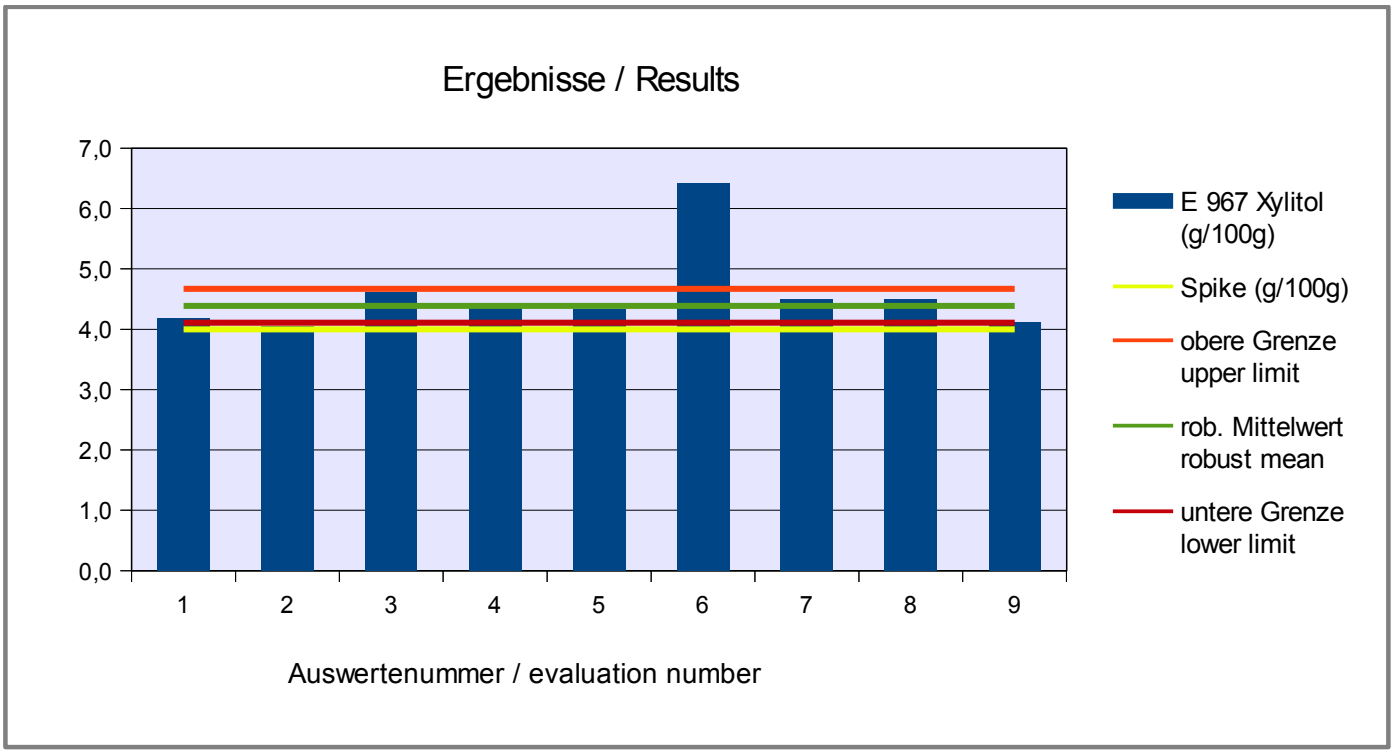

Abb. 13: Ergebnisse E 967
Fig. 13: Results E 967

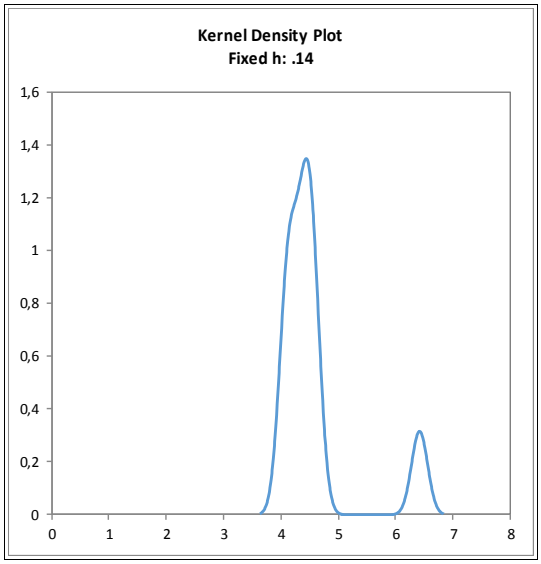

Abb. 14: Kern Dichte Plot der Ergebnisse E 967 mit h = Zielstandardabweichung (0,140 g/100g)
Fig. 14: Kernel density plot of the E 967 results with h = target standard deviation (0,140 g/100g)

Comments:
The kernel density plot shows a normal distribution of results. The second peak at 6,4 g/100g denotes the outlier (no. 6).

Ergebnisse der teilnehmenden Institute:
Results of Participants:

Auswerte-nummer Evaluation number	E 967 Xylitol (g/100g)	Abweichung [g/100g] Deviation [g/100g]	Z-Score σ_{pt}	z-Score (Info)	Hinweis Remark
1	4,18	-0,207	-1,5	-1,3	
2	4,05	-0,337	-2,4	-2,1	
3	4,61	0,219	1,6	1,4	
4	4,40	0,013	0,1	0,1	
5	4,33	-0,062	-0,4	-0,4	
6	6,41	2,02	14,4	12,9	Ausreisser / Outlier
7	4,50	0,113	0,8	0,7	
8	4,50	0,113	0,8	0,7	
9	4,12	-0,267	-1,9	-1,7	

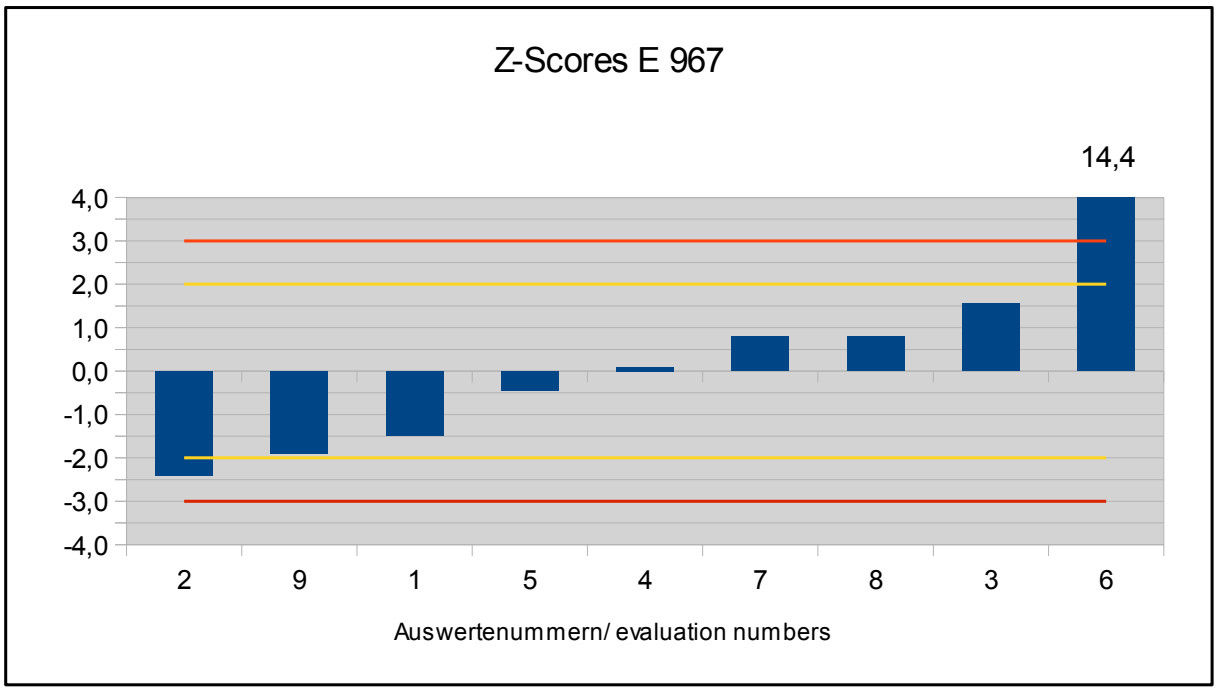

Abb. 15: Z-Scores E 967
Fig. 15: Z-Scores E 967

5. Documentation

5.1 Primary data

5.1.1 Sorbitol E 420

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe I/ sample I	DLA-Nr Probe II/ sample II	Datum der Analyse/ Date of analysis	Ergebnis I/ result I	Ergebnis II/ result II	Bestimmungs-grenze/ Limit of quantifica-tion
	g/100g			Tag/Monat	g/100g	g/100g	g/100g
1	3,98	11	30	22/06	4,1	3,86	0.05
2	4,07	18	44	13/06	4,1	4,03	0,1
3	4,637	6	39	06/06	4,726	4,548	0,1
4	4,4	24	47	09/06	4,43	4,38	0,1
5	4,1825	15	37		4,125	4,24	0,04
6	6,79	2	50	13/06			0,1
7	4,3	27	51	25/05	4,4	4,2	0,5
8	4,36		33	28/05	4,38	4,34	1
9	3,94	22	31	11/07	3,94	3,94	0,025

5.1.2 Mannitol E 421

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe I/ sample I	DLA-Nr Probe II/ sample II	Datum der Analyse/ Date of analysis	Ergebnis I/ result I	Ergebnis II/ result II	Bestimmungs-grenze/ Limit of quantifica-tion
	g/100g			Tag/Monat	g/100g	g/100g	g/100g
1	4,2	11	30	22/06	4,31	4,08	0.05
2	4,04	18	44	13/06	4,06	4,02	0,1
3	4,304	6	39	06/06	4,331	4,277	0,1
4	4,3	24	47	09/06	4,26	4,29	0,1
5	3,44	15	37		3,36	3,54	0,04
6	4,26	2	50	13/06			0,1
7	4,2	27	51	25/05	4,2	4,2	0,5
8	4,21		33	28/05	4,25	4,17	1
9	3,99	22	31	11/07	3,95	4,03	0,025

5.1.3 Isomalt E 953

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe I/ sample I	DLA-Nr Probe II/ sample II	Datum der Analyse/ Date of analysis	Ergebnis I/ result I	Ergebnis II/ result II	Bestimmungs-grenze/ Limit of quantifica-tion
	g/100g			Tag/Monat	g/100g	g/100g	g/100g
1	4,14	11	30	22/06	4,18	4,09	0.1
2	4,08	18	44	13/06	3,97	4,18	0,1
3	4,424	6	39	06/06	4,6	4,248	0,1
4	4,5	24	47	09/06	4,71	4,27	0,1
5							
6	4,15	2	50	13/06			0,1
7	3,9	27	51	25/05	4,0	3,8	0,5
8							
9	3,96	22	31	11/07	4,07	3,85	0,025

5.1.4 Lactit E 966

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe I/ sample I	DLA-Nr Probe II/ sample II	Datum der Analyse/ Date of analysis	Ergebnis I/ result I	Ergebnis II/ result II	Bestimmungs-grenze/ Limit of quantifica-tion
	g/100g			Tag/Monat	g/100g	g/100g	g/100g
1	3,98	11	30	22/06	3,76	4,19	0.1
2	(4)*	18	44	13/06			
3	4,799	6	39	06/06	4,92	4,679	0,1
4	4,5	24	47	09/06	4,73	4,38	0,1
5							
6	4,78	2	50	13/06			0,1
7	4,1	27	51	25/05	4,4	4,2	0,5
8							
9	3,99	22	31	11/07	3,9	4,08	0,025

*** Furtherremarks: Lactitol is not part of our standard scope and is therefore not already calibrated.** Assuming that lactitol has a similar response as the other sugar alcohols **lactitol also obtained for a value of the order of 4g / 100 g.**

5.1.5 Xylit E 967

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe I/ sample I	DLA-Nr Probe II/ sample II	Datum der Analyse/ Date of analysis	Ergebnis I/ result I	Ergebnis II/ result II	Bestimmungs-grenze/ Limit of quantifica-tion
	g/100g			Tag/Monat	g/100g	g/100g	g/100g
1	4,18	11	30	22/06	4,24	4,12	0.05
2	4,05	18	44	13/06	4,11	3,99	0,1
3	4,606	6	39	06/06	4,695	4,517	0,1
4	4,4	24	47	09/06	4,38	4,41	0,1
5	4,325	15	37		4,255	4,395	0,04
6	6,41	2	50	13/06			0,1
7	4,5	27	51	25/05	4,5	4,4	0,5
8	4,5		33	28/05	4,52	4,48	1
9	4,12	22	31	11/07	4,09	4,16	0,025

5.1.6 Further results

Parameter	Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe I/ sample I	DLA-Nr Probe II/ sample II	Datum der Analyse/ Date of analysis	Ergebnis I/ result I	Ergebnis II/ result II	Bestimmungs-grenze/ Limit of quantifica-tion
		g/100g			Tag/Monat	g/100g	g/100g	g/100g
E 968 Erythrit	7	<0,5	27	51	25/05	<0,5	<0,5	0,5
E 965 Maltit	7	<0,5	27	51	25/05	<0,5	<0,5	0,5

5.2 Homogeneity

5.2.1 Homogeneity testing before PT

The **mixture homogeneity before bottling** was examined 10-fold by **microtracer analysis.**

Microtracer Homogeneity Test		
Weight whole Sample	0,65	kg
Microtracer	FSS-rot lake	
Particle size	75-300	µm
Weight per particle	2,0	µg
Addition of tracer	23,2	mg/kg

Result of analysis:

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	9,95	124	24,9
2	10,54	138	26,2
3	11,52	149	25,9
4	10,07	126	25,0
5	10,22	126	24,7
6	10,48	127	24,2
7	9,41	116	24,7
8	9,8	103	21,0
9	9,97	105	21,1
10	9,83	129	26,2

Poisson distribution		
number of sample	10	
Degree of freedom	9	
Mean	121,9	Partikel
Standard deviation	9,45	Partikel
χ^2 (CHI-Quadrat)	6,59	
Probability	**68**	%
Recovery rate	105	%

Normal distribution		
Numbeer of samples	10	
Mean	24,39	mg/kg
Standard deviaton	1,89	mg/kg
rel. Standard deviation	7,7	%
Horwitz Standard deviation	9,9	%
HorRat-Value	**0,8**	
recovery rate	105	%

5.2.2 Repeatability standard deviation of participants

The repeatability standard deviations were calculated with the data documented in chapter 5.1, see also statistic data 4.1 to 4.5.
It is 0,14 g/100g = 3,2 % of X (E 420),
it is 0,096 g/100g = 2,3 % of X (E 421),
it is 0,28 g/100g = 6,6 % of X (E 963),
it is 0,27 g/100g = 6,3 % of X (E 966) and
it is 0,11 g/100g = 2,5 % of X (E 967).

In the ASU L00.00-59 the relative repeatability standard deviations were determined in a comparable range for cookies.

5.2.3 Comparison of sample number/test result

The comparison of the increasing sample-numbers and measured xylitol results shows a sufficient homogeneity. (Gradient of the trend line = 0)

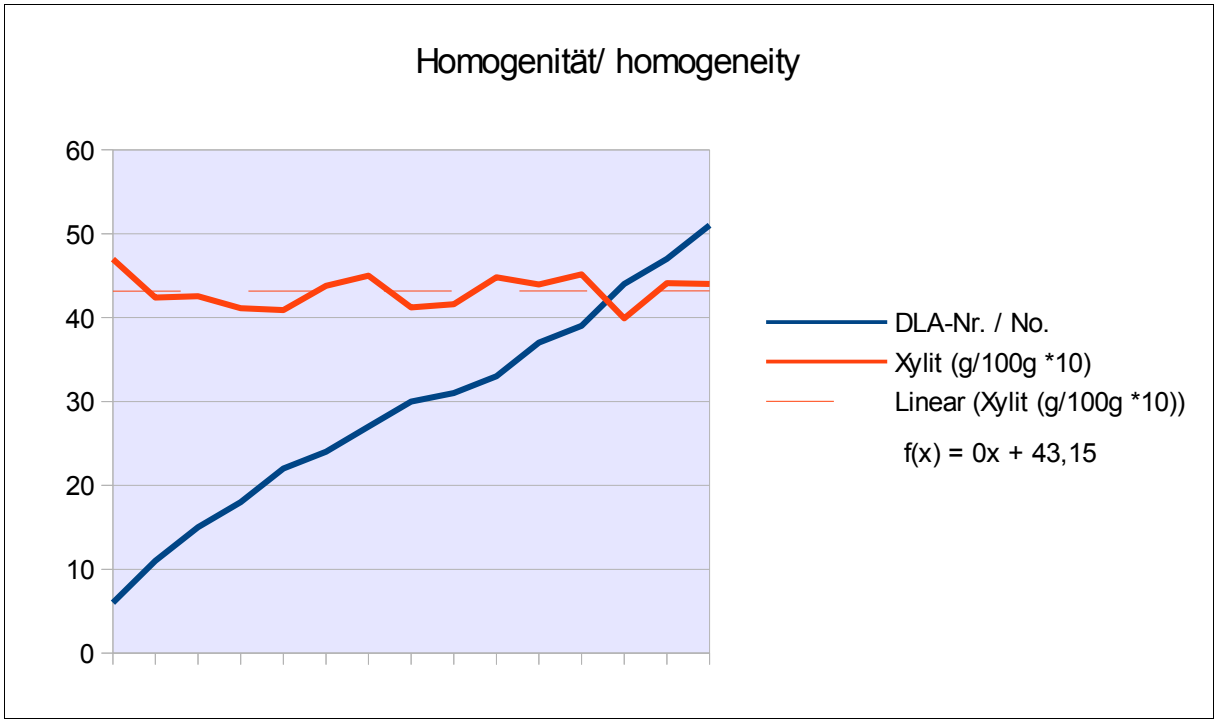

Abb. 16: Gegenüberstellung Probennummern/ Xylit-Ergebnisse (*10)
Fig. 16: Comparison of sample number/ Xylitol results

5.3 Analytical methods

To the participants:

5.3.1 Sugar alcohols E420, E421, E953, E966 a. E967

Teilnehmer/ participant	**Methode/ method**	**Angaben zur Methode/ details about the method***	**Hinweise/ further remarks**
1	LAM-MGC.M.0007HPAEC-PAD	1. yes 2. Water 3. 1g 4. - 5. ISTD 6. For any of the results: no	
2	HPAEC-PAD	1. yes 2. Water extraction, ultrasonic 60°C 3. 1g to 100 ml 4. - 5. external 6. For E450, E421, E953 a. E967: yes	
3	LG3Z8 Sugar alcohols: internal Method, SOP: 00.15610.L, GC-FID	1. yes, with a laboratory mill 2. warm Water + ultrasonic + Methanol 3. 5g 4. no 5. internal standard, Measurement of Sugar model mixture for correction factor 6. For any of the results: yes	
4	Hausmethode GC-FID	1. mixing 2. Carrez precipitation 3. 5g 4. spiked starch 5. internal standard 6. For any of the results: yes	
5	HPLC	1. - 2. Water ultrasonic bath 3. 1g 4. Sugar alcohol in cellulose 5. - 6. For any of the results: no	

6	Hausmethode PM DE01.277	1. Ultraturrax 2. aqueous extraction 3. 1g 4. - 5. external, 6-point 6. For any of the results: yes	
7	HPLC/RI - internal method PNTQ1039	1. - 2. n/a 3. 2g 4. internal RM 5. external calib. curve 6. For any of the results: no	
8	LC-RID	1. yes, water 2. no 3. 5g 4. - 5. 0,05 – 1 g/100 ml 6. For any of the results: no	
9	HPAEC-PAD	1. - 2. aqueous extraction 3. 0,4g/100 ml 4. : 1. D-Sorbitol for Sorbitol 2. D-Mannitol for Mannitol 3. Isomalt for Isomalt 4. Lactitol-Monohydrat for Lactitol 5. Xylitol for Xylitol 5. external standard 6. For any of the results: yes	

*
1. Homogenisation
2. digestion method/ Extraction
3. Weight
4. Reference material
5. Calibration method
6. Accredited

5.3.2 Further remarks of the participants

Participant 2:

Parameter	akkreditiert/ accredited	weitere Hinweise/ further remarks
E 953 Isomalt	yes	In our method is no separation between lactitol and GPS. Therefore is our calculation of the isomalt value based on the assumption, that GPM and GPS are in similar parts available.
E 966 Lactit		Lactitol is not part of our standard scope and is therefore not already calibrated. Assuming that lactitol has a similar response as the other sugar alcohols and taking into account the above-mentioned assumption lactitol is also obtained in a value of the order of 4g / 100 g.

6. Index of participant laboratories

Teilnehmer/ Participant	Ort/ Town	Land/ Country
		Deutschland
		Sweden
		Deutschland
		Deutschland
		Spain
		Deutschland
		Deutschland
		Great Britain
		Deutschland
		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 literature

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 interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) 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. Horwitz; 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; Analyst, 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 estimates, 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 Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. 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
15. ASU §64 LFGB: L 00.00-59; Bestimmung von Isomalt, Lactit, Maltit, Mannit, Sorbit und Xylit in Lebensmitteln; HPLC-Verfahren (2008)
16. ASU §64 LFGB: L 18.00-14; Bestimmung von D-Sorbit in Feinen Backwaren; Enzymatische Verfahren (1994)