DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 28/2014

Sugar Alcohols in Sweets:
E 420, E 421, E 953, E 965, E 966, E 967, E 968

Dienstleistung Lebensmittel Analytik GbR
Pinnberg 5
22927 Großhansdorf

proficiency-testing@dla-lvu.de **www.dla-lvu.de**

Coodinator: Dr. G. Wichmann

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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. 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 validity of the particular testing method.
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.

2. Realisation

2.1 Test material

The test material was a sauce powder (ingredients: corn starch, salt, vanilla flavour, colours: E 101 and E 160b), added were sorbitol (E 420, app. 2%), mannitol (E 421, app. 6%), isomalt (E 953, app. 2%) and xylitol (E 967, app. 4%) as well as magnesium oxide (approx. 35 mg Mg/kg) for the homogeneity test.
Approximately 600 g of the material were homogenized and then packaged in portions to approximately 10 g. The portions were numbered chronologically.
The detectability of the sugar alcohols was assured.

2.1.1 Homogeneity

The calculation of the repeatability standard deviation of the participants for mannitol was used as an indicator of homogeneity. The result is similar to the repeatability standard deviation of the German official method ASU § 64 LFGB L00.00-59 (11). The repeatability standard deviation of the participants is given in the documentation.

To verify the homogeneity of the test material magnesium oxide was added before homogenisation additionally. The homogeneity was examined with ICP.

Probe/ sample	Magnesium		
1	33,2	mg/kg	
2	38,2	mg/kg	
3	35,3	mg/kg	
4	36,7	mg/kg	
5	36,5	mg/kg	
Mittelwert/ mean	36,0	mg/kg	
Standardabw./ standard deviation	1,86	5,2	%

The homogeneity is considered verified with a standard deviation of 5,2%.

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

2.2 Test

Two portions of test material were sent to every participating laboratory in the 21st week of 2014. The testing method was optional. The tests should be finished at 4th July 2014 the latest.

2.3 Results

The participants submitted their results in standard forms, which have been handed out with the samples.

From the requested sugar alcohols E 420, E 421, E 953, E 965, E 966, E 967 and E 968 in the samples only the sugar alcohols E 420, E 421, E 953 and E 967 were available. The finally calculated concentrations from these sugar alcohols as average of duplicate determinations of both numbered samples was used for the statistical evaluation.

Queried and documented were single results, recovery and the testing method used. One participant has not submitted any results. All other participants have submitted at least one result in time.

3. Evaluation

3.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X (6). The distribution of submitted results showed no hint for bimodal distribution or other reasons for a higher variability.

3.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (S_x) was calculated (6).

3.3 Outliers

Statistical outliers were determined by Mandel´s-h-Statistic (5). Detected outliers were stated for information only, when z-score was < -2 or > 2.

3.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

3.4.1 General model (Horwitz)

The relative target standard deviation in % of the assigned value is calculated according to the following equation.

$$\hat{\sigma} \ (\%) = 2^{(1-0,5\log X)}$$

Out of this is calculated the target standard deviation in mg/kg

$$\hat{\sigma} = X * \hat{\sigma} \ (\%) / 100.$$

The target standard deviation according to Horwitz is given for information in the evaluation.

3.4.2 Precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment the between-laboratories standard deviation (σ_L) can be calculated :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} \ .$$

And then, using the number of replicate measurements n, each participant is to perform, the standard deviation for proficiency assessment is calculated :

$$\hat{\sigma} = \sqrt{(\sigma_L^2 + (\sigma_r^2/n))} \quad .$$

From the precision data obtained from the relevant official methods for sugar alcohols the target standard deviation for the relevant parameter was calculated and used for evaluation (6,11).

3.5 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation ($\hat{\sigma}$) the result (x) of the participant is deviating from the assigned value (X)(6).

Participants' z-scores were derived as:

$$z = (x - X) / \hat{\sigma} \quad ;$$

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

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

3.6 Quotient $S^x/\hat{\sigma}$

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation and target standard deviation 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.

For this PT the results for E 420, E 421, E 953 and E 967 showed a suitable comparability.

3.7 Standard uncertainty

The assigned value X has a standard uncertainty u_X that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty (u_X) for this PT is calculated as follows (6).

$$u_X = 1{,}25 * S^x / \sqrt{(p)}$$

If $u_X \leq 0{,}3 * \hat{\sigma}$ the standard uncertainty of the assigned value needs not be included in the interpretation of the results of the PT (6). The Quotient $u_X/\hat{\sigma}$ is reported in the characteristics of the test.

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 upper table – test - the characteristics are listed:

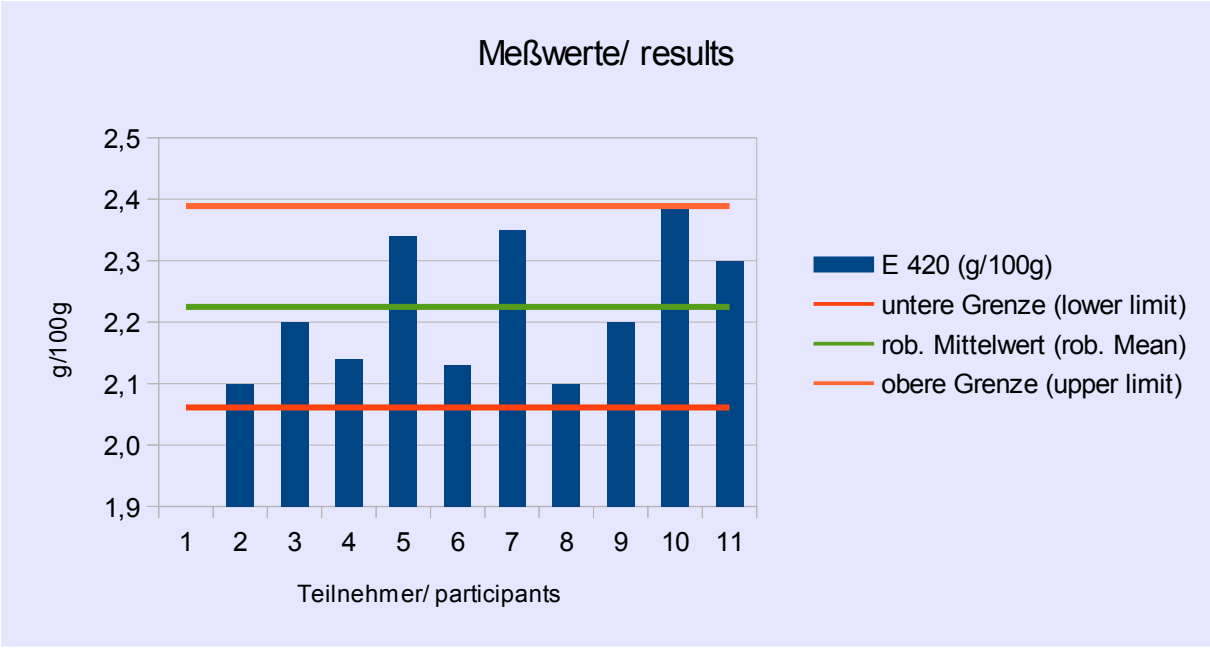
Number of results	
Number of outliers	
Mean	
Median	
Robust mean (X)	
Robust standard deviation (S^x)	
Target standard deviation($\hat{\sigma}$)(Horwitz) for Information	
Target standard deviation (ASU § 64 LFGB L00.00-59)	
Lower limit of target range	
Upper limit of target range	
Quotient $S^x/\hat{\sigma}$	
Standard uncertainty u_x	
Quotient $u_X/\hat{\sigma}$	
Number of results in the target range	

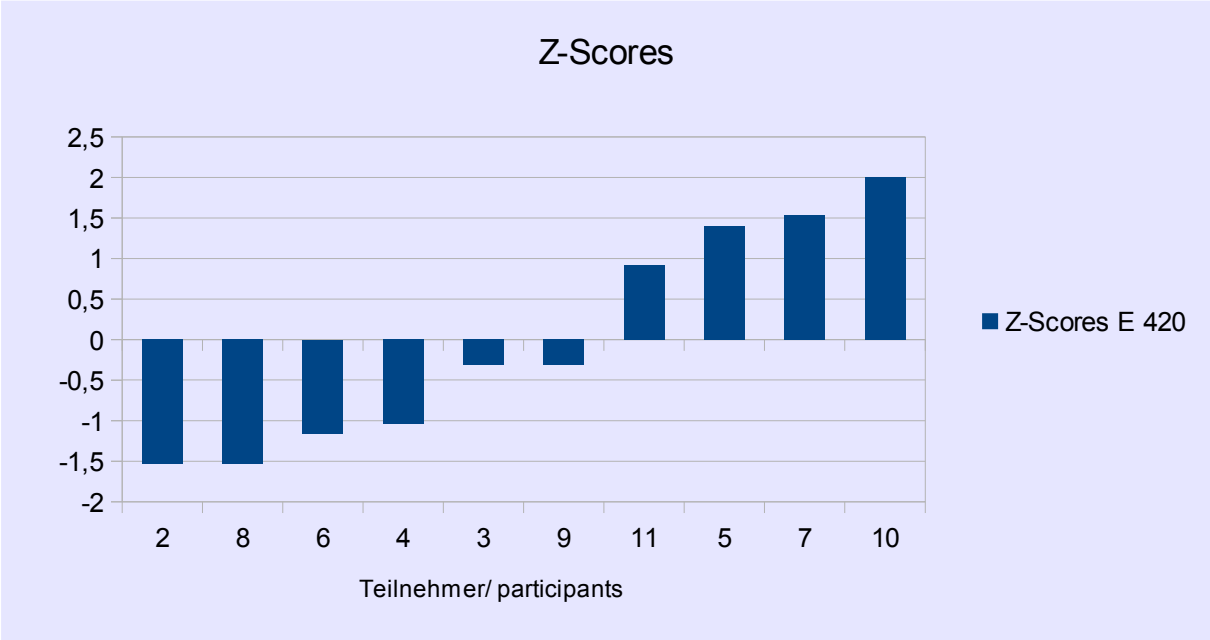
In the lower table - Laboratories - the individual results of the participating laboratories are listed:

Evaluation number	Result	Deviation	z-Score	Remarks

4.1 Sorbitol(E 420) in g/100g

Characteristics	
Number of results	10
Number of outliers	0
Mean	2,23
Median	2,20
Robust mean (X)	2,23
Robust standard deviation (S^x)	0,126
Target standard deviation($\hat{\sigma}$)(Horwitz) for Information	0,079
Target standard deviation (ASU § 64 LFGB L00.00-59)	0,082
Lower limit of target range (X - 2 $\hat{\sigma}$)	2,06
Upper limit of target range (X + 2 $\hat{\sigma}$)	2,39
Quotient $S^x/\hat{\sigma}$	1,5
standard uncertainty u_x	0,050
Quotient $u_X/\hat{\sigma}$	0,63
Number of results in the target range	10 (100%)



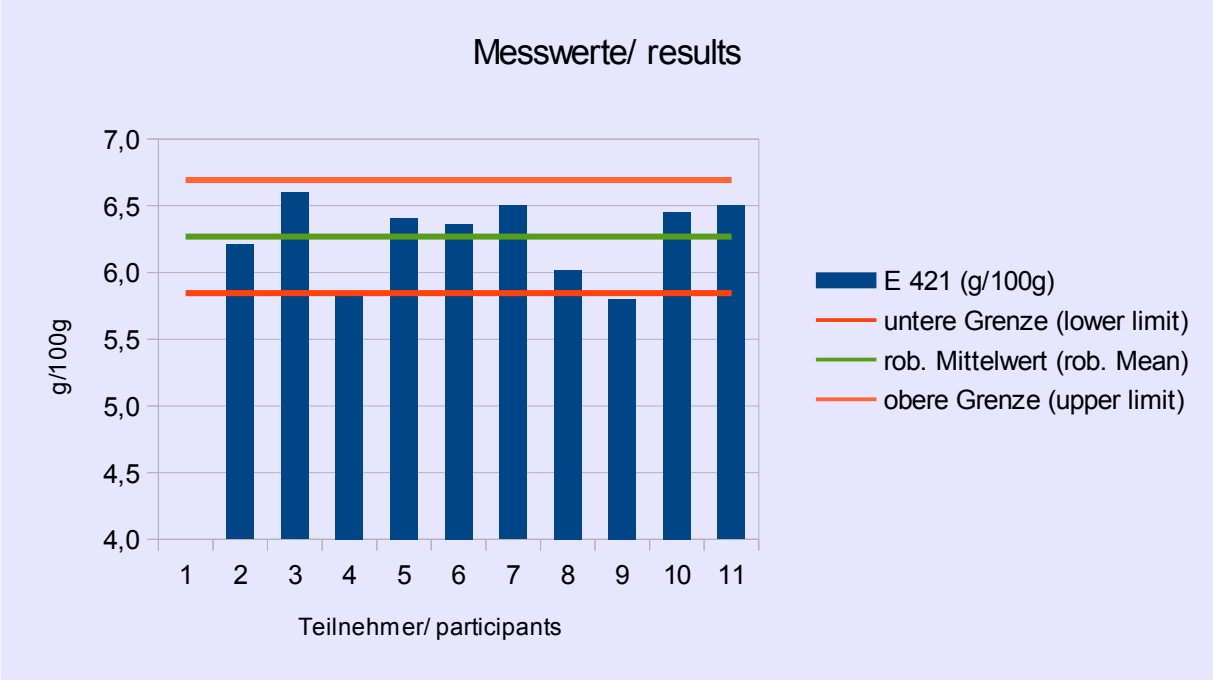


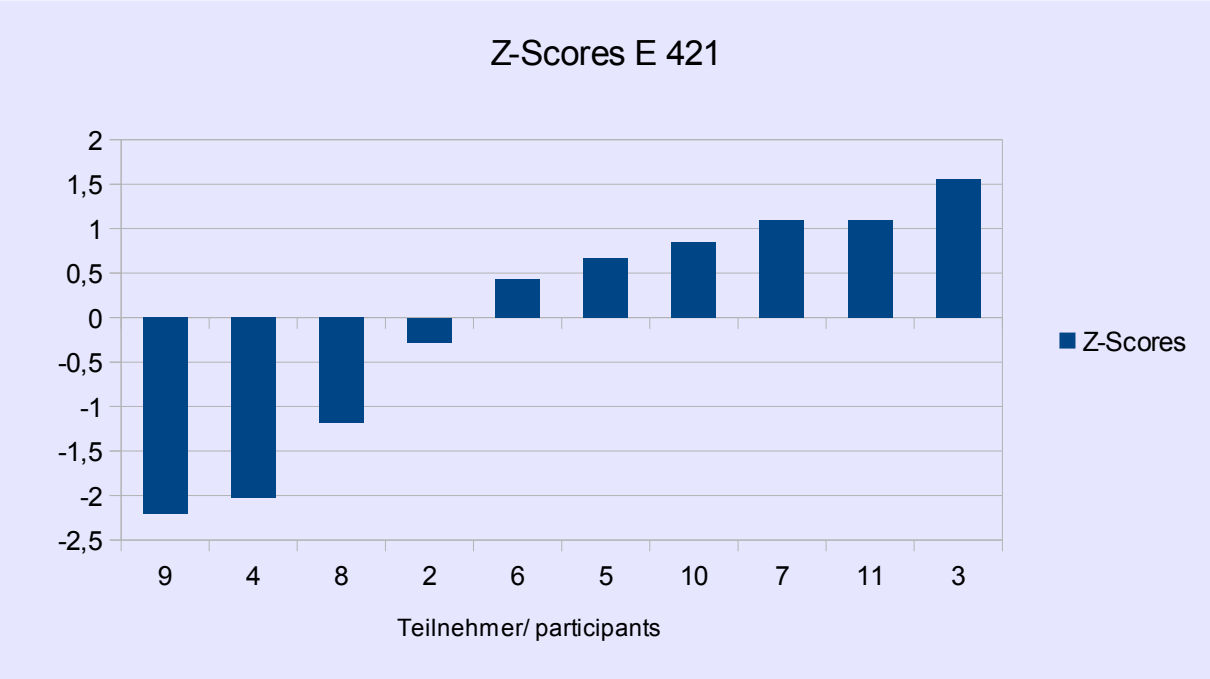
Laboratories

Teilnehmer/ participant	E 420 (g/100g)	Abweichung/ deviation	Z-Scores	Bemerkung/ remark
1				
2	2,1	-0,13	-1,5	
3	2,2	-0,02	-0,3	
4	2,14	-0,09	-1,0	
5	2,34	0,12	1,4	
6	2,13	-0,10	-1,2	
7	2,35	0,13	1,5	
8	2,1	-0,13	-1,5	
9	2,2	-0,02	-0,3	
10	2,39	0,17	2,0	
11	2,3	0,07	0,9	

4.2 Mannitol (E 421) in g/100g

Characteristics	
Number of results	10
Number of outliers	0
Mean	6,27
Median	6,39
Robust mean (X)	6,27
Robust standard deviation (S^x)	0,327
Target standard deviation($\hat{\sigma}$)(Horwitz) for Information	0,19
Target standard deviation (ASU § 64 LFGB L00.00-59)	0,21
Lower limit of target range (X - 2 $\hat{\sigma}$)	5,85
Upper limit of target range (X + 2 $\hat{\sigma}$)	6,69
Quotient $S^x/\hat{\sigma}$	1,5
standard uncertainty u_x	0,13
Quotient $u_X/\hat{\sigma}$	0,68
Number of results in the target range	9 (90%)



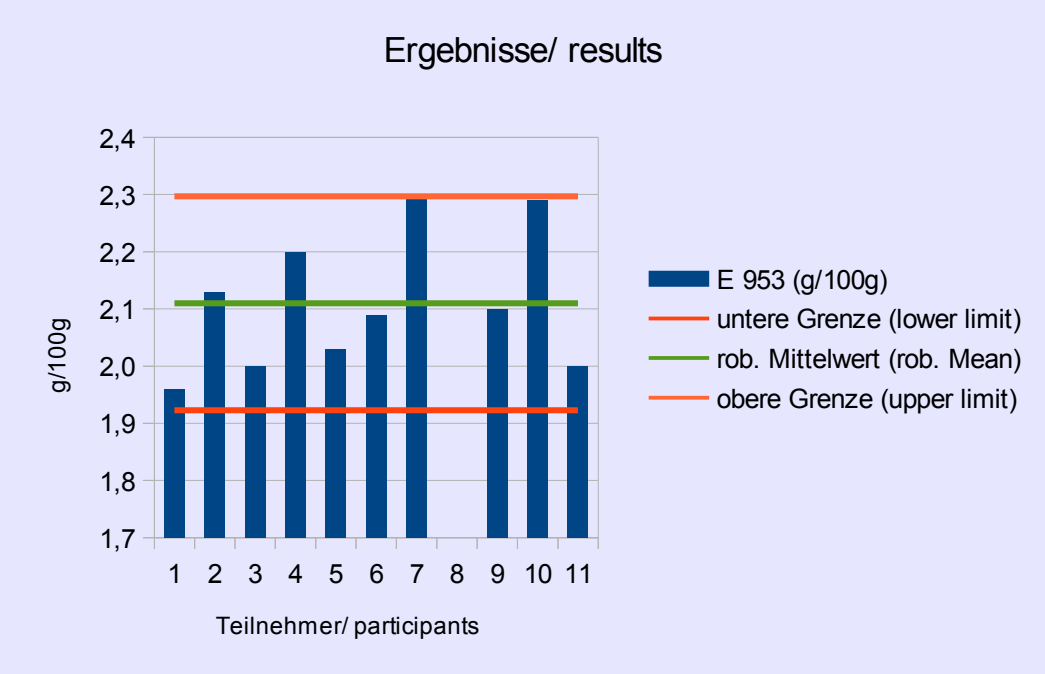


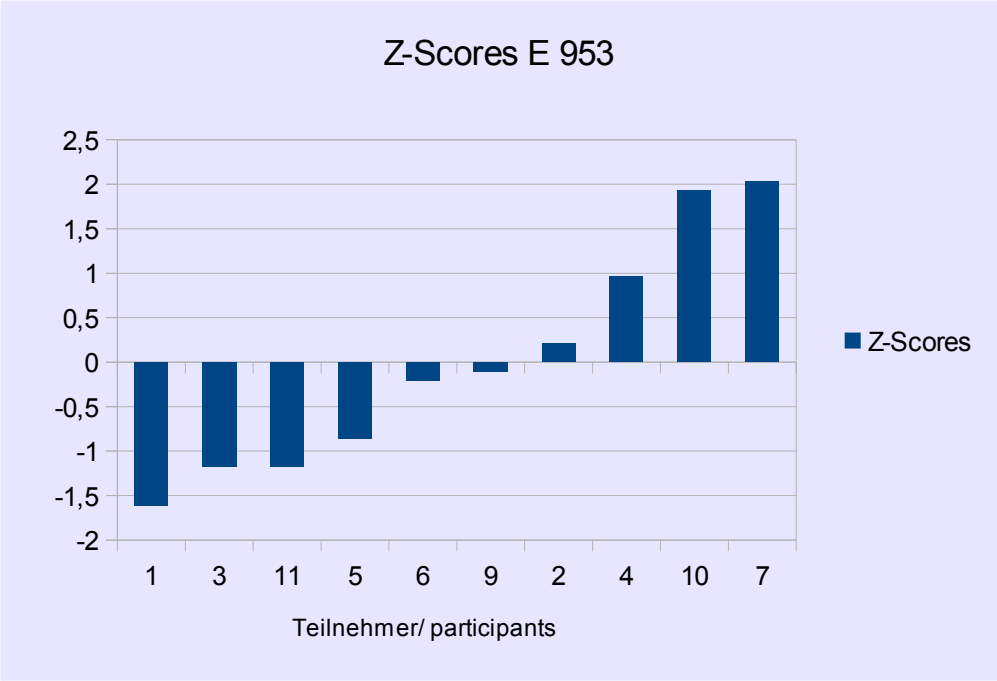
Laboratories

Teilnehmer/ participant	E 421 (g/100g)	Abweichung/ deviation	Z-Scores	Bemerkung/ remark
1				
2	6,21	-0,06	-0,3	
3	6,6	0,33	1,6	
4	5,84	-0,43	-2,0	
5	6,41	0,14	0,7	
6	6,36	0,09	0,4	
7	6,5	0,23	1,1	
8	6,02	-0,25	-1,2	
9	5,8	-0,47	**-2,2**	
10	6,45	0,18	0,9	
11	6,5	0,23	1,1	

4.3 Isomalt (E 953) in g/100g

Characteristics	
Number of results	10
Number of outliers	0
Mean	2,11
Median	2,10
Robust mean (X)	2,11
Robust standard deviation (S^x)	0,14
Target standard deviation($\hat{\sigma}$)(Horwitz) for Information	0,075
Target standard deviation (ASU § 64 LFGB L00.00-59)	0,094
Lower limit of target range (X - 2 $\hat{\sigma}$)	1,92
Upper limit of target range (X + 2 $\hat{\sigma}$)	2,30
Quotient $S^x/\hat{\sigma}$	1,5
standard uncertainty u_x	0,054
Quotient $u_X/\hat{\sigma}$	0,72
Number of results in the target range	10 (100%)





Laboratories

Teilnehmer/ participant	E 953 (g/100g)	Abweichung/ deviation	Z-Scores	Bemerkung/ remark
1	1,96	-0,15	-1,6	
2	2,13	0,02	0,2	
3	2,0	-0,11	-1,2	
4	2,2	0,09	1,0	
5	2,03	-0,08	-0,9	
6	2,09	-0,02	-0,2	
7	2,3	0,19	**2,0**	
8				
9	2,1	-0,01	-0,1	
10	2,29	0,18	1,9	
11	2	-0,11	-1,2	

4.4 Maltitol (E 965) in g/100g

To the test material was added no maltitol (E 965).

All participants have submitted for E 965 a negative result. The limit of detection ranged from < 0,01 to < 0,5 g/100g.

Teilnehmer/ participant	E 965 (g/100g)
1	
2	< 0,01
3	<0,1
4	n.d.
5	n.d.
6	<0,2
7	n.d.
8	<0,04
9	<0,1
10	< 0,04
11	<0,5

n.d. = not detectable

4.5 Lactitol (E 966) in g/100g

To the test material was added no lactitol (E 966).

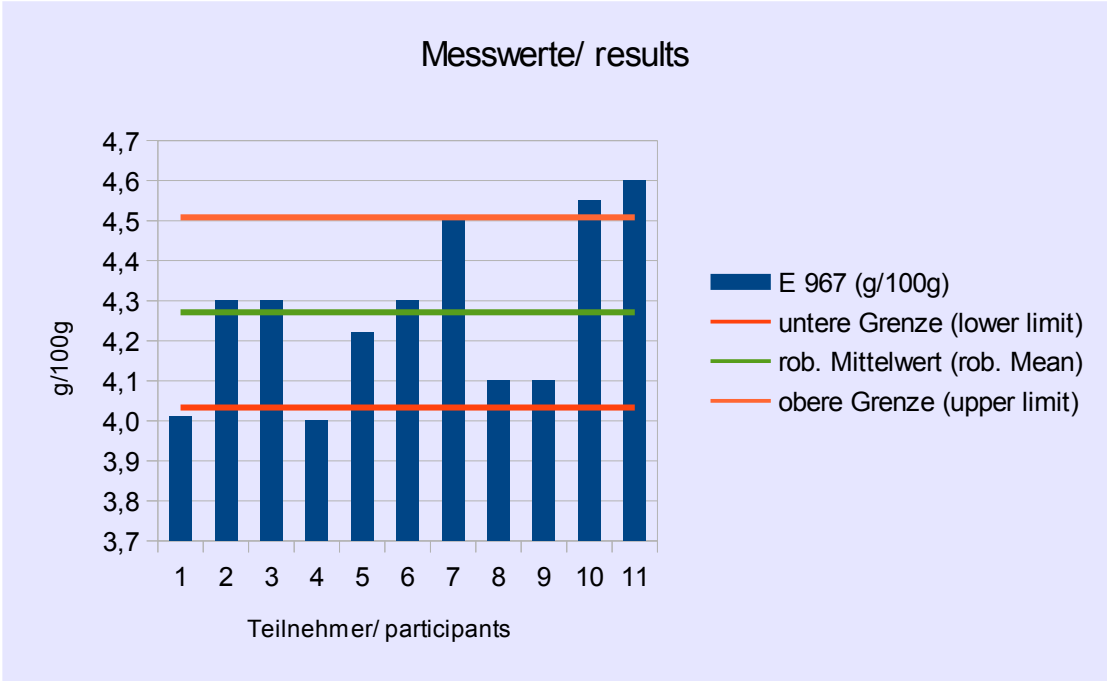
All participants have submitted for E 966 a negative result. The limit of detection ranged from < 0,01 to < 0,5 g/100g.

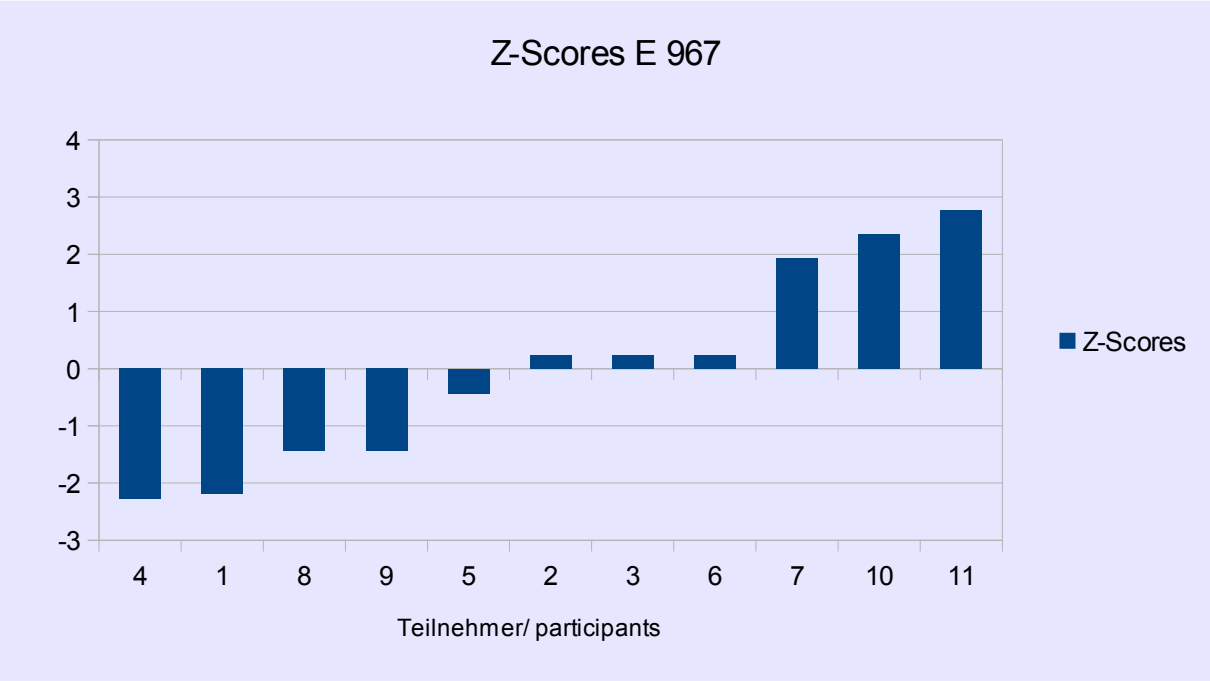
Teilnehmer/ participant	E 966 (g/100g)
1	
2	< 0,01
3	<0,1
4	
5	n.d.
6	<0,2
7	n.d.
8	
9	<0,1
10	< 0,04
11	<0,5

n.d. = not detectable

4.6 Xylitol (E 967) in g/100g

Characteristics	
Number of results	11
Number of outliers	0
Mean	4,27
Median	4,30
Robust mean (X)	4,27
Robust standard deviation (S^x)	0,239
Target standard deviation($\hat{\sigma}$)(Horwitz) for Information	0,137
Target standard deviation (ASU § 64 LFGB L00.00-59)	0,119
Lower limit of target range (X - 2 $\hat{\sigma}$)	4,03
Upper limit of target range (X + 2 $\hat{\sigma}$)	4,51
Quotient $S^x/\hat{\sigma}$	2,0
standard uncertainty u_x	0,090
Quotient $u_X/\hat{\sigma}$	0,66
Number of results in the target range	7 (64%)





Laboratories

Teilnehmer/ participant	E 967 (g/100g)	Abweichung/ deviation	Z-Scores	Bemerkung/ remark
1	4,01	-0,26	**-2,2**	
2	4,3	0,03	0,2	
3	4,3	0,03	0,2	
4	4	-0,27	**-2,3**	
5	4,22	-0,05	-0,4	
6	4,3	0,03	0,2	
7	4,5	0,23	1,9	
8	4,1	-0,17	-1,4	
9	4,1	-0,17	-1,4	
10	4,55	0,28	**2,3**	
11	4,6	0,33	**2,8**	

4.7 Erythritol (E 968) in g/100g

To the test material was added no erythritol (E 968).

All participants have submitted for E 968 a negative result. The limit of detection ranged from < 0,01 to < 0,5 g/100g.

Teilnehmer/ participant	E 968 (g/100g)
1	
2	< 0,01
3	<0,1
4	
5	n.d.
6	
7	
8	
9	<0,1
10	< 0,04
11	<0,5

n.d. = not detectable

5. Documentation

5.1 Primary data in g/100g

5.1.1 Sorbitol (E 420)

Teilnehmer/ participant	Ergebnis/ result	DLA-No Probe I/ sample I	DLA-No Probe II/ sample II	Ergebnis I/ result I	Ergebnis II/ result II
	g/100g			g/100g	g/100g
1		9	16		
2	2,1	10	33	2,04	2,15
3	2,2	5	36	2,19	2,22
4	2,14	32	7	2,22	2,05
5	2,34	13	34	2,29	2,39
6	2,13	15	26	2,16	2,09
7	2,35	11	23	2,3	2,4
8	2,1	2	25	2,05	2,1
9	2,2	6	27	2,2	2,1
10	2,39	3	29	2,42	2,35
11	2,3	12	18	2,3	2,3

5.1.2 Mannitol (E 421)

Teilnehmer/ participant	**Ergebnis/ result**	**DLA-No Probe I/ sample I**	**DLA-No Probe II/ sample II**	**Ergebnis I/ result I**	**Ergebnis II/ result II**
	g/100g			g/100g	g/100g
1					
2	6,21	10	33	6,28	6,13
3	6,6	5	36	6,63	6,47
4	5,84	32	7	5,71	5,96
5	6,41	13	34	6,49	6,32
6	6,36	15	26	6,37	6,34
7	6,5	11	23	6,5	6,5
8	6,02	2	25	**60,5**	6
9	5,8	6	27	5,9	5,7
10	6,45	3	29	6,4	6,49
11	6,5	12	18	6,5	6,4

5.1.3 Isomalt (E 953)

Teilnehmer/ participant	Ergebnis/ result	DLA-No Probe I/ sample I	DLA-No Probe II/ sample II	Ergebnis I/ result I	Ergebnis II/ result II
	g/100g			g/100g	g/100g
1	1,96	9	16	1,97	1,95
2	2,13	10	33	1,91	2,35
3	2,0	5	36	1,90	2,11
4	2,2	32	7	2,5	1,9
5	2,03	13	34	1,99	2,06
6	2,09	15	26	2,13	2,04
7	2,3	11	23	2,4	2,2
8	-			-	-
9	2,1	6	27	2,3	1,9
10	2,29	3	29	2,37	2,2
11	2	12	18	2	2

5.1.4 Maltitol (E 965)

Teilnehmer/ participant	Ergebnis/ result	DLA-No Probe I/ sample I	DLA-No Probe II/ sample II	Ergebnis I/ result I	Ergebnis II/ result II
	g/100g			g/100g	g/100g
1		9	16		
2	n.d., <0,01	10	33	n.d., <0,01	n.d., <0,01
3	<0,1	5	36	<0,1	<0,1
4	n.d.	32	7	n.d.	n.d.
5	n.d.	13	34	n.d.	n.d.
6	<0,2	15	26	<0,2	<0,2
7	n.d.	11	23	n.d.	n.d.
8	<0,04	2	25	<0,04	<0,04
9	<0,1	6	27	<0,1	<0,1
10	< 0,04	3	29	< 0,04	< 0,04
11	<0,5	12	18	<0,5	<0,5

n.d. = not detectable

5.1.5 Lactitol (E 966)

Teilnehmer/ participant	Ergebnis/ result	DLA-No Probe I/ sample I	DLA-No Probe II/ sample II	Ergebnis I/ result I	Ergebnis II/ result II
	g/100g			g/100g	g/100g
1		9	16		
2	n.d., <0,01	10	33	n.d., <0,01	n.d., <0,01
3	<0,1	5	36	<0,1	<0,1
4		32	7		
5	n.d.	13	34	n.d.	n.d.
6	<0,2	15	26	<0,2	<0,2
7	n.d.	11	23	n.d.	n.d.
8	-	2	25	-	-
9	<0.1	6	27	<0.1	<0.1
10	< 0,04	3	29	< 0,04	<0,04
11	<0,5	12	18	<0,5	<0,5

n.d. = not detectable

5.1.6 Xylitol (E 967)

Teilnehmer/ participant	Ergebnis/ result	DLA-No Probe I/ sample I	DLA-No Probe II/ sample II	Ergebnis I/ result I	Ergebnis II/ result II
	g/100g			g/100g	g/100g
1	4,01	9	16	4,04	3,97
2	4,3	10	33	4,39	4,2
3	4,3	5	36	4,36	4,26
4	4	32	7	3,95	4,05
5	4,22	13	34	4,3	4,14
6	4,3	15	26	4,33	4,27
7	4,5	11	23	4,6	4,4
8	4,1	2	25	4,15	4,05
9	4,1	6	27	4,2	4,1
10	4,55	3	29	4,58	4,52
11	4,6	12	18	4,6	4,6

5.1.7 Erythritol (E 968)

Teilnehmer/ participant	Ergebnis/ result	DLA-No Probe I/ sample I	DLA-No Probe II/ sample II	Ergebnis I/ result I	Ergebnis II/ result II
	g/100g			g/100g	g/100g
1		9	16		
2	n.d., <0,01	10	33	n.d., <0,01	n.d., <0,01
3	<0,1	5	36	<0,1	<0,1
4		32	7		
5	n.d.	13	34	n.d.	n.d.
6		15	26		
7	not examined	11	23		
8	-	2	25	-	-
9	<0.1	6	27	<0.1	<0.1
10	< 0,04	3	29	< 0,04	< 0,04
11	<0,5	12	18	<0,5	<0,5

n.d. = not detectable

5.2 DLA-portion-numbers and homogeneity

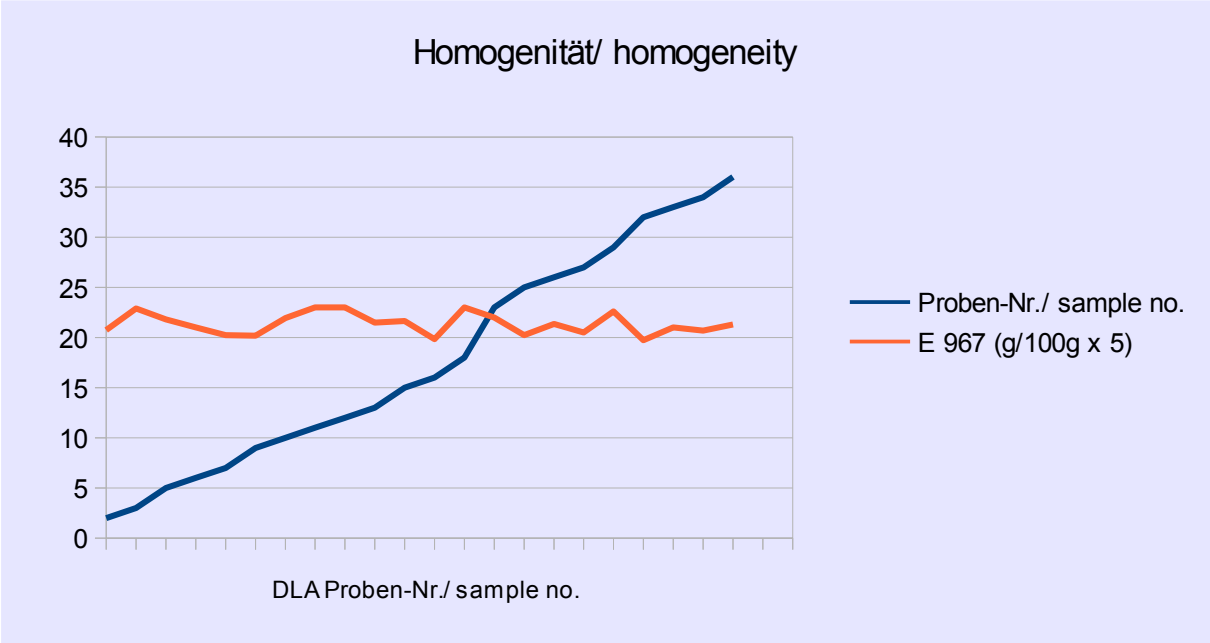
5.2.1 Repeatability standard deviation of participants

The repeatability standard deviation was calculated like under 5.1 documented.
It is 0,142 g/100g = 2,3 % of X (= 6,3 g/100g) for Mannitol.

For comparison ASU § 64 LFGB L00.00-59 (11):
For mannitol (caramel): 0,135 g/100g = 3,4 % of X (= 4,0 g/100g)

5.2.2 Comparison of sample number/test result

From the comparison of the rising sample-numbers and measured Xylitol (E 967)-concentrations (x5) homogeneity can be seen.



5.3 Analytical methods

Inst.	Method	Details	Accredited
1	E 953, E 967: HPLC/RI	1. Mixed with a spatula 2. With MeOH 3. 2,5 g 4. yes 5. External, 5 point calibration	yes
2	E 420, E 421, E 953, E 965, E 966, E 967, E 968: HPAEC-PAD	1. Mixed manually 2. aqueous extraction 3. 1g/200 ml 4. D-Sorbitol, Lot 424214/165103034, Sigma Aldrich; D-Mannitol, Lot 61030, Sigma Aldrich; Palatinit, Palatinit GmbH; Maltit, Lot 412213/151804121,Sigma Aldrich; D-Lactitol Monohydrat, Lot 111K37931V, Sigma Aldrich; Xylit, Lot 447027/151104066, Sigma Aldrich; meso-Erythritol, Lot SLBF6576V, Sigma Aldrich 5. External standard	yes (E 968: no)
3	E 420, E 421, E 953, E 965, E 966, E 967, E 968: HRGC	1. - 2. aqueous extraction 3. 5 g 4. internal 5. Internal standard 6. GC after silylation	yes
4	E 420, E 421, E 953, E 965, E 967, E 968: HPLC	1. - 2. - 3. - 4. - 5. -	yes
5	E 420, E 421, E 953, E 965, E 966, E 967, E 968: Detection of the sugar alcohols with HPLC according to LFGB §64 ASU L 00.00-59 2008-12	1. - 2. - 3. - 4. - 5. external calibration, analysis with peak areas	no
6		1. - 2. - 3. - 4. - 5. -	yes
7	E 420: Enzymatically	1. - 2. - 3. - 4. - 5. -	yes
	E 421, E 953, E 965, E 966, E 967: HPLC	1. - 2. - 3. - 4. - 5. -	yes

Inst.	Method	Details	Accredited
8	E 420, E 421, E 953, E 965, E 966, E 967, E 968: HPAEC	1. Not needed 2. dissolved in water (80°C), shaken for 15 min. in waterbath with same temp. 3. 1 g 4. Control sample. Starch spike with std. 5. Std. curve	no
9	E 420, E 421, E 953, E 965, E 966, E 967, E 968: HPLC/ ELSD	1. no 2. aqueous, ultrasonic bath 3. 1 g 4. no 5. external, 5 points	yes
10	E 420, E 421, E 953, E 965, E 966, E 967, E 968: In house method, HPLC, RI	1. no 2. dissolve at 60°C in water, Carrenz clarification 3. 5 g/ 100 ml + dilute 1:10 4. no 5. external	yes
11	E 420, E 421, E 953, E 965, E 966, E 967, E 968: HPLC/RI – internal method PNTQ 1039	1. - 2. - 3. 2 g 4. internal reference material. 5. external calib. curve	no

1. = Homogenization
2. = Hydrolisation/ digestion
3. = Sample weight
4. = Reference material
5. = Calibration

6. Index of participant laboratories

Teilnehmer/ participant	Ort/ Location
	Germany
	Germany
	Germany
	Sweden
	Germany
	Germany
	Germany
	Germany
	Germany
	Australia
	Spain
	Germany

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

6. Index of literature

1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
3. 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
4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
5. ASU §64 LFGB : Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung
6. ISO 13528:2005; Statistische Verfahren für Eignungsprüfungen durch Ringversuche
7. The International Harmonised Protocol for the Proficiency Testing of Analytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories ; Pure Appl Chem, 78, 145 – 196 (2006)
9. Evaluation of analytical methods used for regulation of food and drugs;W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
10. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
11. ASU §64 LFGB: L 00.00-59; Bestimmung von Isomalt, Lactit, Maltit, Mannit, Sorbit und Xylit in Lebensmitteln; HPLC-Verfahren (2008)
12. ASU §64 LFGB: L 18.00-14; Bestimmung von D-Sorbit in Feinen Backwaren; Enzymatische Verfahren (1994)

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