DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

30/2014

Food Supplement II:

Biotin, Niacin, Pantothenic Acid and Vitamin C

in Multi Vitamin Tablets

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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 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 (6).

2. Realisation

2.1 Test material

The test material is a mixture of two common in commerce food supplements "multi vitamin effervescent tablets" with food starch from EU suppliers. The material was crushed, sieved, mixed and homogenized. Afterwards the samples were packaged lightproof in portions to approximately 25 g. The portions were numbered chronologically.

The composition (list of ingredients) and the amounts of vitamins were calculated according to the labeled values as given in table 1 and table 2 respectively.

Table 1: Composition of DLA-Samples

Multi vitamin capsules with minerals
Ingredients: Acidifying agent: Citric acid, Sodium hydrogencarbonate, Maltodextrin, Starch, Vitamin C, Beetroot powder, Flavours, Sweeteners: Sodium Cyclamate, Sodium Saccharin, Vitamin E, Niacin, Calcium-D-Pantothenate, Riboflavin, Vitamin B6, Vitamin B2, Vitamin B1, Folic acid, Biotin, Vitamin B12

Table 2: Calculated amounts according to labeled values of vitamins

Vitamin	**Content per 100 g**
Biotin	0,54 mg
Niacin	155 mg
Pantothenic Acid	58 mg
Vitamin C	1060 mg

2.1.1 Homogeneity

Homogeneity of the test material was checked by 5fold determination of vitamin C by enzyme-UV-test. With a repeatability standard deviation of 4,1 % the homogeneity of the test material was considered acceptable. The results are given in the documentation.

The calculation of the repeatability standard deviation of the participants was also used as an indicator of homogeneity. For pantothenic acid it was 3,4 % and for vitamin C 1,7 % and in the range of common relative repeatability standard deviations of methods for water-soluble vitamins (13-16). The repeatability standard deviation of the participants for both vitamins is given in the documentation.

Additionally in the documentation the portion numbers are graphically assigned to the results of vitamin C. There is no 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 44th week of 2014. The testing method was optional. The tests should be finished at 12th December 2014.

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples.
The finally calculated concentration of each vitamin as an average of a duplicate determination of both numbered samples was used for each statistical evaluation.
Queried and documented were single results, recovery and the testing methods used.
All participants submitted their results 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 for 95% significance niveau (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.

In general the Horwitz target standard deviation is suitable for the statistical evaluation of interlaboratory tests where different analytical methods are applied. The standard deviation from precision experiments are derived from proficiency tests where a specific analytical method is mandatory.

For all analytes the target standard deviation according to the general model (Horwitz) was applied.

3.4.1 General model (Horwitz)

The relative target standard deviation in % of the assigned value is derived from following equation (Horwitz)

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

From the result the target standard deviation is calculated

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

3.4.2 Value by 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 can be calculated σ_L :

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

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

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

The target standard deviations given in table 3 were calculated from the precision data of the respective methods.

Table 3: Target standard deviations from precision experiments

Method	**Parameter** (per 100 g)	**Matrix**	**relative Target standard deviation** $\hat{\sigma}$	**Literature**
HPLC	Biotin (16 - 200 µg)	Cereal Powder Milk Powder Orange Juice Chicken Soup dry	17,0 % 28,1 % 19,7 % 14,2 %	EN 15607:2009
HPLC-MS/MS	Biotin	Feeding Stuffs	7,0 - 20,9 %	EURL (2011)
Protein Binding Assay	Biotin	Fortified Foods	5,6 - 18,6 %	AOAC Performance Test (in: Rychlik 2011)
HPLC	Niacin (17 - 21 mg)	Milk Powder Chocolate Muesli	3,6 % / 3,5 % 4,2 % / 4,4 % after acidic / enzym. Hydrolysis	EN 15652:2009
HPLC	Vitamin C (55 - 170 mg)	Cereal Powder Milk Powder Orange Juice Soup dry	17,3 % 10,0 % 19,4 % 13,5 %	EN 14130:2003
HPLC	Vitamin C	Fruit Juices and Infant Formulas	3,9 - 33,1 %	Brause et al. (2003)
HPLC (Multi-Method	Biotin, Vitamin C, Nicotinamide, Pantothenic Acid	Multivitamin Premixes	< 8,0 %	Heudi et al. (2005)

3.4.3 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 (6).

For the evaluation in the present proficiency testing the model according to Horwitz was applied.

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:

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

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

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

3.6 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 assigned value (X) to the square root of quadrat sum of the target standard deviation ($\hat{\sigma}$) and the standard uncertainty (Ux) (6).

Participants' z'-scores are derived as:

$$z' = (x - X) / \sqrt{\hat{\sigma}^2 + u_X^2}$$

In the following we define the denominator $\sqrt{\hat{\sigma}^2 + u_X^2}$ as the target standard deviation $\hat{\sigma}'$.

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

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

3.7 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 (11).

In the present proficiency tests the quotients $S^x/\hat{\sigma}$ were for biotin 1,7, for niacin 1,3, for pantothenic acid 1,5 and for vitamin C 1,3.

3.8 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 to be included in the interpretation of the results of the PT (6).

In the present proficiency tests the quotients $U_x/\hat{\sigma}$ were between 0,4 and 0,8. Because the quotients $S^x/\hat{\sigma}$ were all below 2,0 the target range was not extended according 3.6 z'-score.

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 the characteristics are listed:

Statistic Data
Number of results
Number of outliers
Mean
Median
Robust mean (X)
Robust standard deviation (S^x)
Target range:
Target standard deviation for information
Target standard deviation $\hat{\sigma}$ '
lower limit of target range (X - 2 $\hat{\sigma}$) or (X - 2 $\hat{\sigma}$ ') *
upper limit of target range (X + 2 $\hat{\sigma}$) or (X + 2 $\hat{\sigma}$ ') *
Quotient $S^x/\hat{\sigma}$ '
Standard uncertainty u_X
Quotient $u_X/\hat{\sigma}$ '
Number of results in the target range

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

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

evaluation number	test result	deviation from assigned value	Z-Score Horwitz	Z'-Score Horwitz	Remarks

4.1 Biotin (in mg/100 g)

Statistic Data	
Number of results	*8 **
Number of outliers	*1*
Mean	0,719
Median	0,603
Robust mean (X)	0,633
Robust standard deviation (S^x)	0,131
Target range:	
Target standard deviation $\hat{\sigma}$ (Horwitz)	0,077
lower limit of target range (X - 2 $\hat{\sigma}$)	0,479
upper limit of target range (X + 2 $\hat{\sigma}$)	0,786
Quotient $S^x/\hat{\sigma}$	*1,7*
Standard uncertainty u_X	*0,058*
Quotient $u_X/\hat{\sigma}$	*0,76*
Number of results in the target range	*6 (75%)*

** without results 5 and 9*

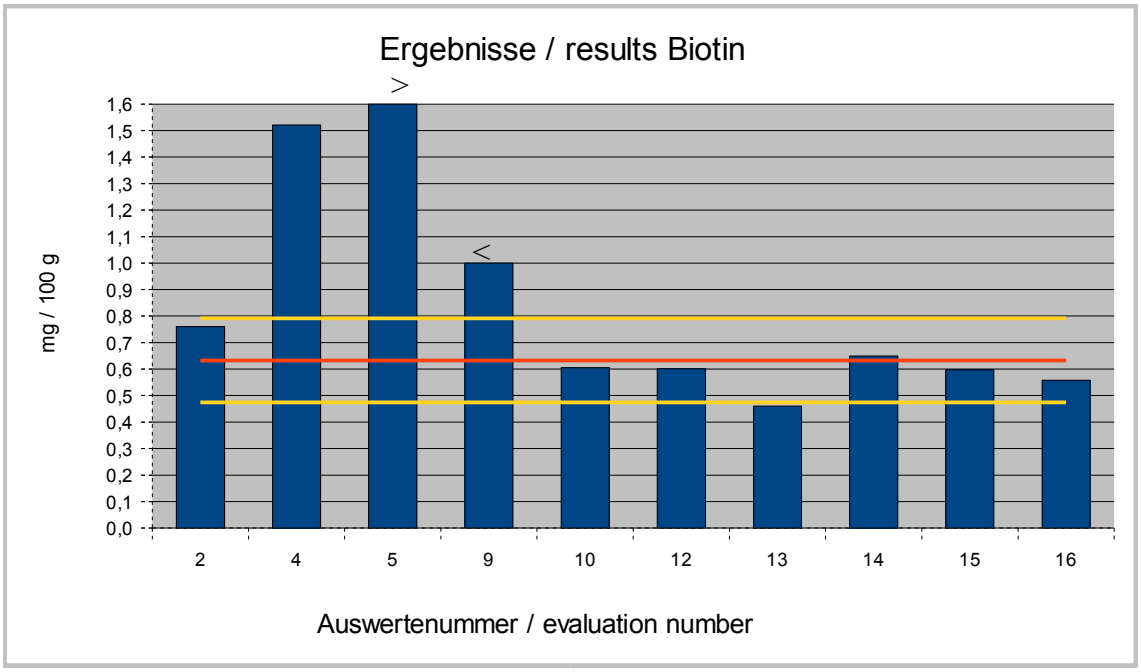


Fig. 1: Results Biotin
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score $\hat{\sigma}$	Remarks
	[g/100g]	X rob. Mean	Horwitz	
2	0,76	0,127	1,7	
4	1,5208	0,888	11,6	outlier
5	448	-	-	result excluded, unit error?
9	< 1	-	-	result not considered
10	0,605	-0,028	-0,4	
12	0,601	-0,032	-0,4	
13	0,46	-0,173	-2,3	
14	0,65	0,017	0,2	
15	0,597	-0,036	-0,5	
16	0,558	-0,075	-1,0	

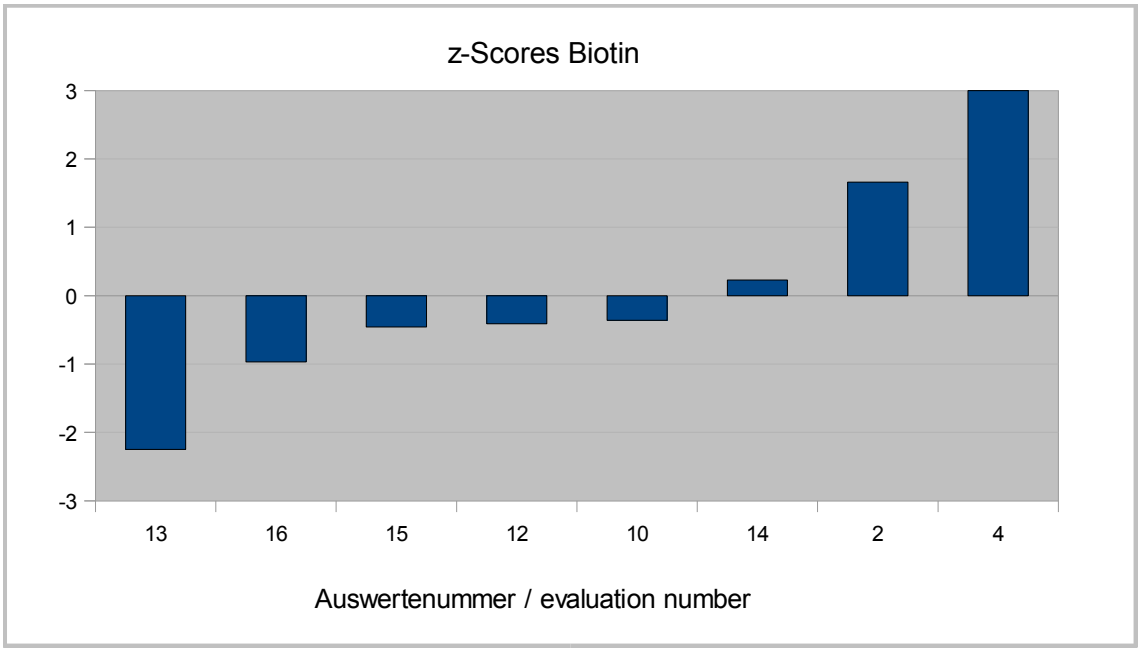


Fig. 2: Z-Scores Biotin

4.2 Nicotinamide (in mg/100 g)

Statistic Data	
Number of results	*15*
Number of outliers	*2*
Mean	173
Median	172
Robust mean (X)	172
Robust standard deviation (S^x)	11,5
Target range:	
Target standard deviation $\hat{\sigma}$	8,96
lower limit of target range	154
upper limit of target range	190
Quotient $S^x/\hat{\sigma}$'	*1,3*
Standard uncertainty u_X	*3,72*
Quotient $u_X/\hat{\sigma}$'	*0,41*
Number of results in the target range	*12 (80%)*

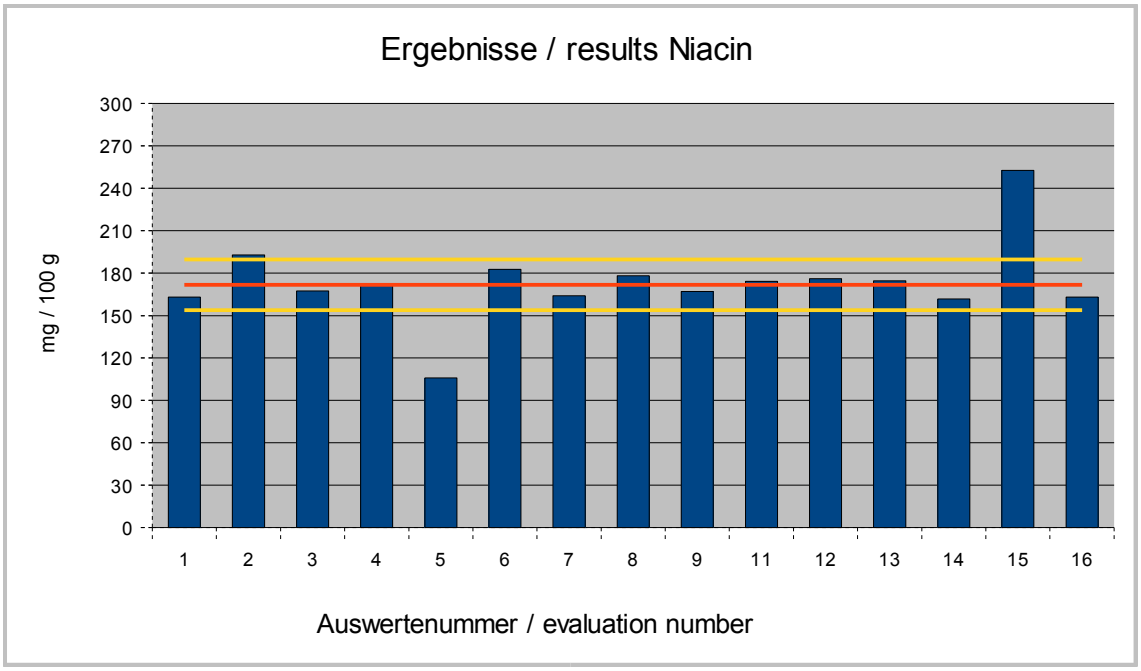


Fig. 3: Results Nicotinamide
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score	Remarks
	[mg/100g]	X rob. MW	Horwitz	
1	163	-8,7	-1,0	
2	193	21,3	2,4	
3	167,4	-4,3	-0,5	
4	171,5	-0,2	0,0	
5	106	-65,7	-7,3	outlier
6	182,7	11,0	1,2	
7	164	-7,7	-0,9	
8	178,1	6,4	0,7	
9	167	-4,7	-0,5	
11	174,32	2,6	0,3	
12	176	4,3	0,5	
13	174,7	3,0	0,3	
14	161,72	-10,0	-1,1	
15	252,8	81,1	9,1	outlier
16	163	-8,7	-1,0	

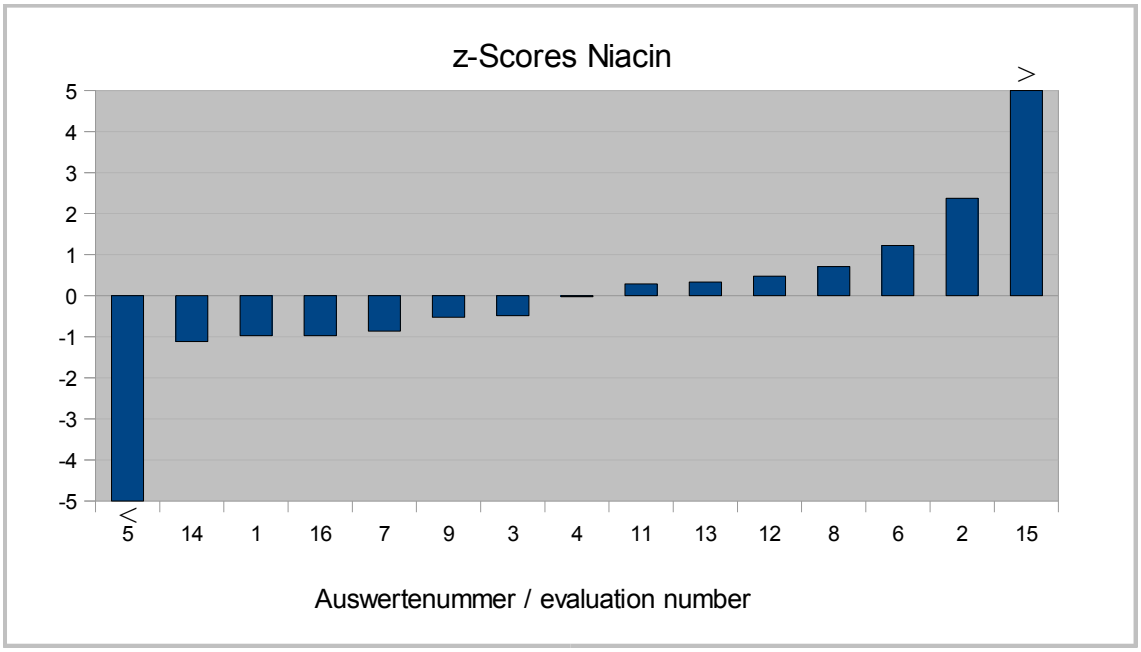


Fig. 4: Z'-Scores Nicotinamide

4.3 Pantothenic Acid (in mg/100 g)

Statistic Data	
Number of results	*14*
Number of outliers	*1*
Mean	62,5
Median	61,3
Robust mean (X)	61,0
Robust standard deviation (S^x)	5,67
Target range:	
Target standard deviation $\hat{\sigma}$ (Horwitz)	3,72
lower limit of target range (X - 2 $\hat{\sigma}$)	53,6
upper limit of target range (X + 2 $\hat{\sigma}$)	68,5
Quotient $S^x/\hat{\sigma}$	*1,5*
Standard uncertainty u_X	*1,89*
Quotient $u_X/\hat{\sigma}$	*0,51*
Number of results in the target range	*12 (86%)*

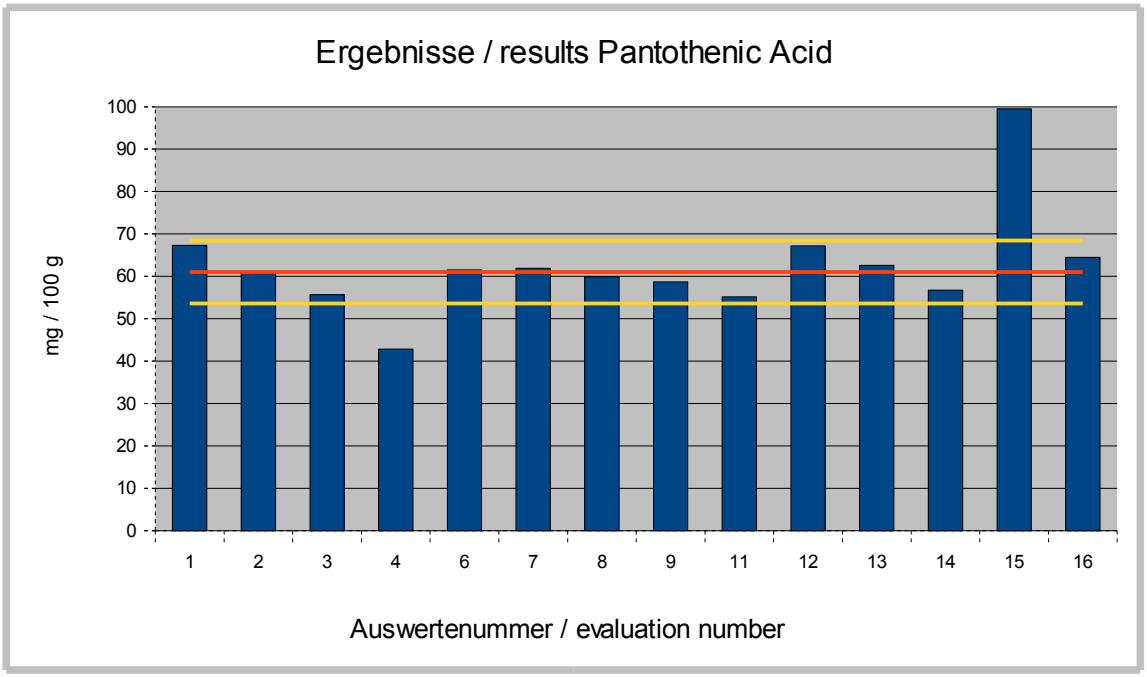


Fig. 5: Results Pantothenic Acid
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score	Remarks
	[mg/100g]	X rob. Mean	Horwitz	
1	67,3	6,3	1,7	
2	61	0,0	0,0	
3	55,7	-5,3	-1,4	
4	42,85	-18,2	-4,9	
6	61,5	0,5	0,1	
7	61,9	0,9	0,2	
8	59,9	-1,1	-0,3	
9	58,7	-2,3	-0,6	
11	55,2	-5,8	-1,6	
12	67,2	6,2	1,7	
13	62,6	1,6	0,4	
14	56,75	-4,3	-1,1	
15	99,52	38,5	10,4	outlier
16	64,5	3,5	0,9	

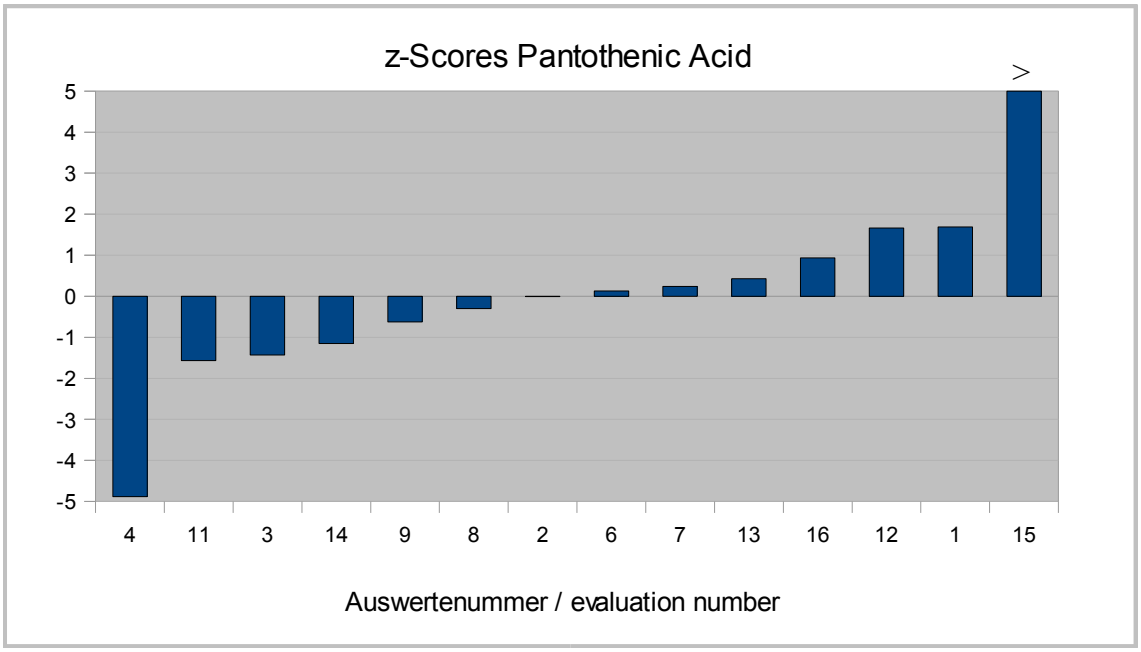


Fig. 6: Z-Scores Pantothenic Acid

4.4 Vitamin C (as Ascorbic Acid in mg/100 g)

Statistic Data	
Number of results	*15*
Number of outliers	*2*
Mean	1091
Median	1087
Robust mean (X)	1088
Robust standard deviation (S^x)	54,8
Target range:	
Target standard deviation $\hat{\sigma}$ (Horwitz)	42,9
lower limit of target range (X - 2 $\hat{\sigma}$)	1002
upper limit of target range (X + 2 $\hat{\sigma}$)	1174
Quotient $S^x/\hat{\sigma}$	*1,3*
Standard uncertainty u_X	*17,7*
Quotient $u_X/\hat{\sigma}$	*0,41*
Number of results in the target range	*13 (87%)*

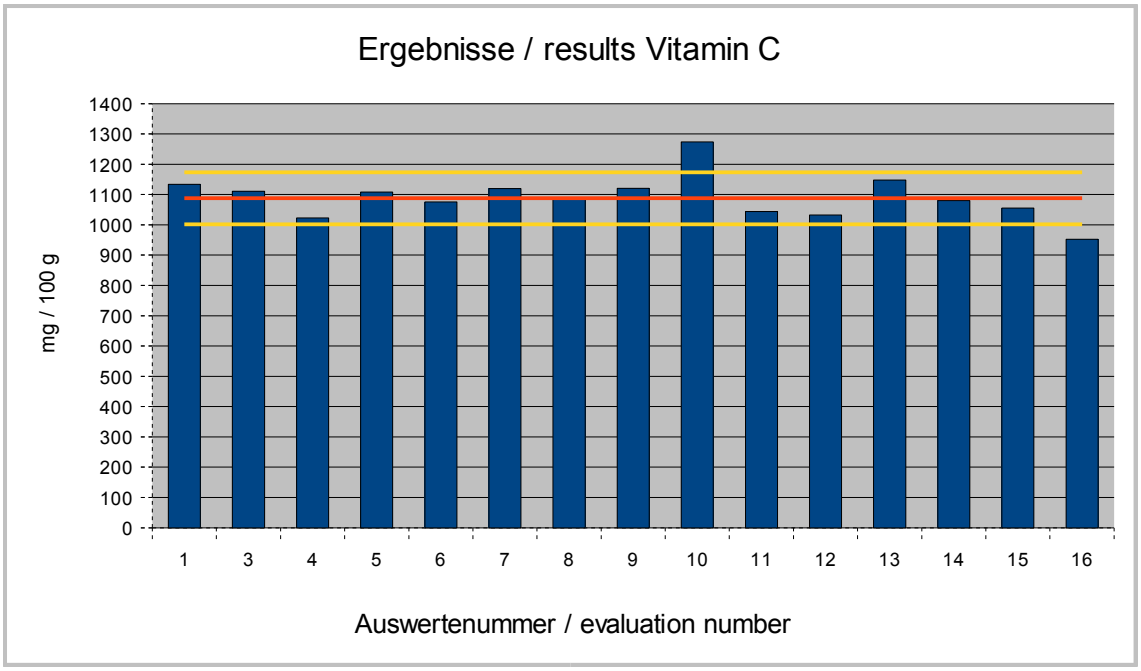


Fig. 7: Results Vitamin C
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score	Remarks
	[mg/100g]	X rob. Mean	Horwitz	
1	1134	46,2	1,1	
3	1111,4	23,6	0,5	
4	1023	-64,8	-1,5	
5	1108	20,2	0,5	
6	1076	-11,8	-0,3	
7	1120	32,2	0,7	
8	1087,5	-0,3	0,0	
9	1121	33,2	0,8	
10	1274	186,2	4,3	outlier
11	1044,5	-43,3	-1,0	
12	1033	-54,8	-1,3	
13	1148	60,2	1,4	
14	1.080,09	-7,7	-0,2	
15	1055	-32,8	-0,8	
16	952	-135,8	-3,2	outlier

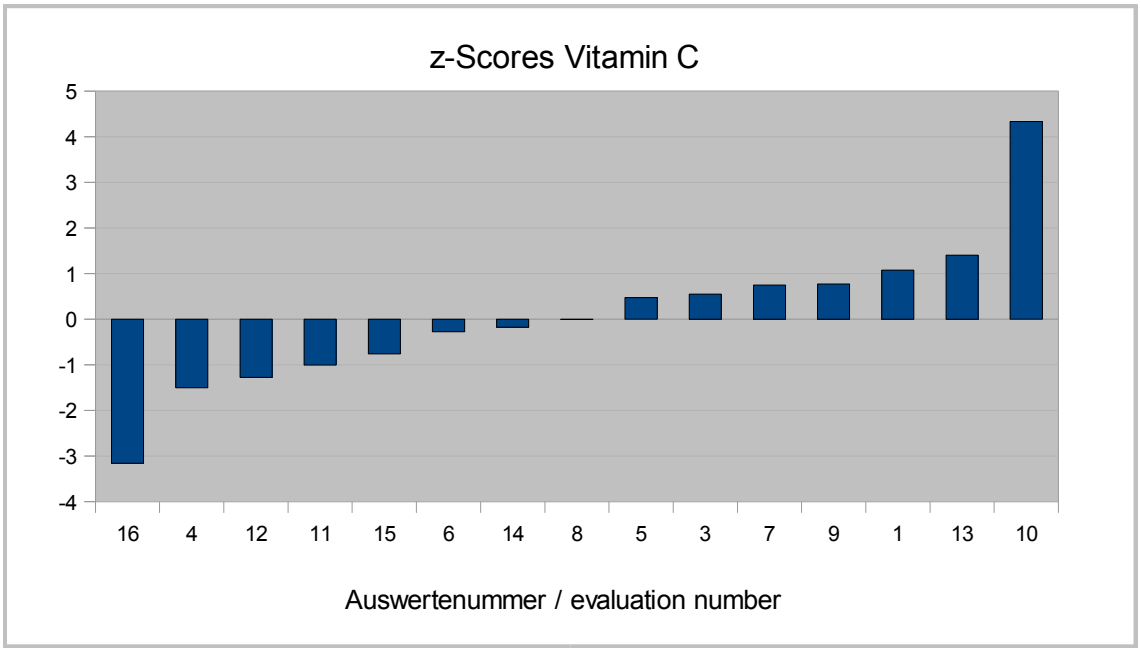


Fig. 8: Z-Scores Vitamin C

5. Documentation

5.1 Primary data

5.1.1 Biotin

Evaluation number	Result	Sample-No. A	Sample-No. B	Result A	Result B	Recovery rate
	[mg/100g]			[mg/100g]	[mg/100g]	%
1		7	15	/	/	/
2	0,76	29	48	0,7	0,82	not determined
3		49	70			
4	1,5208					
5	448	22	51	490	405	N/A
6		33	60			
7	-	25	55	-	-	-
8						
9	< 1	11	50	---	---	---
10	0,605	32	69	0,604	0,605	not applicable
11		17	46			
12	0,601	31	58	0,589	0,612	
13	0,46	9	39	0,42	0,51	-
14	0,65	41	59	0,64	0,66	
15	0,597	21	44			
16	0,558	8	66	0,558	0,557	

5.1.2 Nicotinamide

Evaluation number	Result	Sample-No. A	Sample-No. B	Result A	Result B	Recovery rate
	[mg/100g]			[mg/100g]	[mg/100g]	%
1	163	7	15	161	165	101
2	193	29	48	178	208	not determined
3	167,4	49	70	169,6	165,2	100,6 / 102,5
4	171,5					
5	106	22	51	113	99	N/A
6	182,7	33	60			100
7	164	25	55	162	167	-
8	178,1	16	53	174,6	181,6	
9	167	11	50	---	---	---
10		32	69			
11	174,32	17	46	173,85	174,78	
12	176	31	58	169	183	
13	174,7	9	39	174,9	174,4	-
14	161,72	41	59	160,1	163,33	
15	252,8	21	44			
16	163	8	66	160,8	166,0	

5.1.3 Pantothenic Acid

Evaluation number	Result	Sample-No. A	Sample-No. B	Result A	Result B	Recovery rate
	[mg/100g]			[mg/100g]	[mg/100g]	%
1	67,3	7	15	67,9	66,6	100
2	61	29	48	63,7	58,2	not determined
3	55,7	49	70	54,8	56,6	115,7 / 113,7
4	42,85					
5	N/A	22	51	N/A	N/A	N/A
6	61,5	33	60			100
7	61.9	25	55	61.6	62.3	-
8	59,9	16	53	58,7	61,2	
9	58,7	11	50	---	---	---
10		32	69			
11	55,2	17	46	55,04	55,36	
12	67,2	31	58	66,9	67,5	
13	62,6	9	39	62,6	62,6	-
14	56,75	41	59	56,92	56,58	
15	99,52	21	44			
16	64,5	8	66	64,0	64,9	

5.1.4 Vitamin C

Evaluation number	Result	Sample-No. A	Sample-No. B	Result A	Result B	Recovery rate
	[mg/100g]			[mg/100g]	[mg/100g]	%
1	1134	7	15	1137	1130	99
2		29	48			
3	1111,4	49	70	1104,5	1118,3	102,0 / 101,7
4	1023					
5	1108	22	51	1110	1105	N/A
6	1076	33	60			100
7	1120	25	55	1113	1127	-
8	1087,5	16	53	1091,4	1083,5	
9	1121	11	50	---	---	---
10	1274	32	69	1254	1294	not applicable
11	1044,5	17	46	1063,7	1025,3	
12	1033	31	58	1030	1035	
13	1148	9	39	1110	1120	103
14	1.080,09	41	59	1.078,48	1.081,70	
15	1055	21	44			
16	952	8	66	951,6	951,8	

5.2 Homogeneity

5.2.1 Homogeneity test befor the PT

Homogeneity test for vitamin C by enzymatic UV-test.

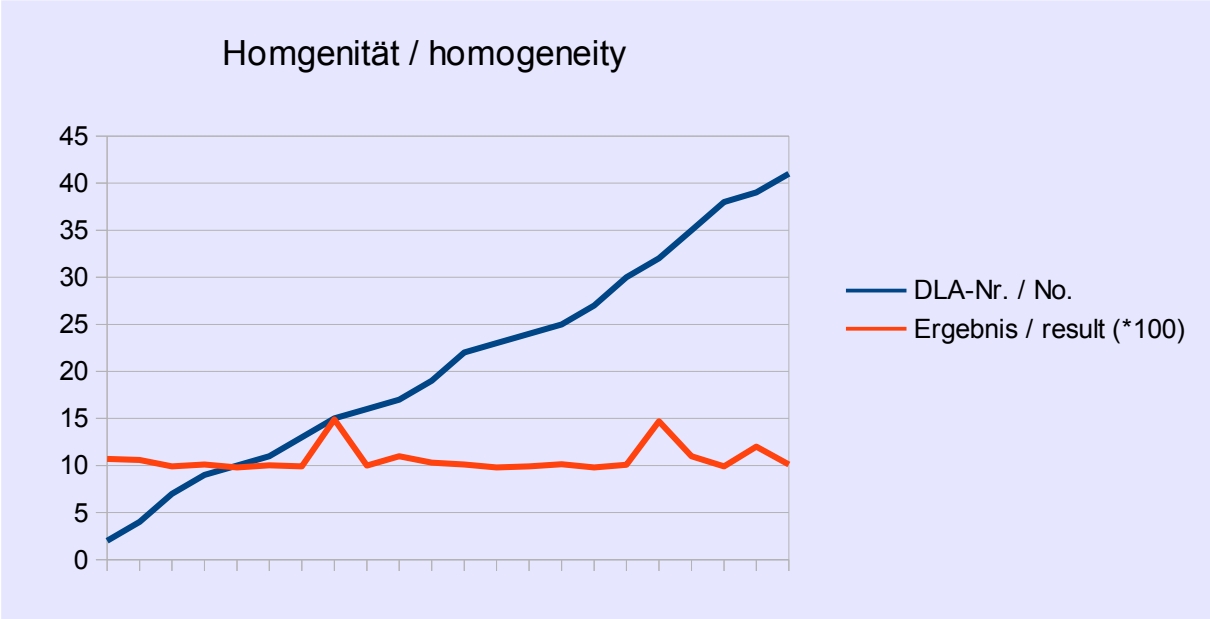
Independant samples	mg/100g
1	970
2	960
3	920
4	930
5	1020

Mean	960	
Repeatability Standard Deviation	39	4,1%

5.2.2 Repeatability standard deviation of duplicate tests of the participants

The repeatability standard deviation was calculated with the data documented in 5.1.3 for pantothenic acid. It is 2,08 mg/100g = 3,4 % of X.
The repeatability standard deviation was calculated with the data documented in 5.1.3 for vitamin C. It is 18,2 mg/100g = 1,7 % of X.

5.2.3 Comparison of sample number / test result (Vitamin C)



5.3 Analytical Methods

Details by the participants

5.3.1 Biotin

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
1	/	/	/	/
2	USP 21, 3. suppl., method 88, mikrob. Akt.		yes	
3				
4	LC-MS/MS	no	no	
5	Biotin	no	yes	none
6				
7	HPLC-UV	no	yes	-
8				not tested
9	HPLC-DAD		yes	
10	DIN EN 15607			
11				
12	Extraction with formiate buffert. Determination with UHPLC-QQQ.		yes	
13	Vita Fast Biotin (RBiopharm)	-	yes	
14	USP		yes	
15	microbiological, according to SLMB 1550.1:2008		yes	Vitafast from r-biopharm
16	in-house method LC-MSMS		yes	

5.3.2 Nicotinamide

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
1	HPLC-DAD	yes	yes	/
2	USP 34, method 441 (mod.), microb. Akt.		yes	
3	HPLC-DAD	yes	yes	
4	microbiological	no	no	
5	Niacinamide	no	yes	None
6		yes	yes	
7	HPLC-UV	no	yes	-
8			no	
9	HPLC-DAD		yes	
10				
11	internal method (HPLC-UV)		no	
12	Extraction with formiate buffert. Determination with UHPLC-QQQ.		yes	
13	HPLC according to VDLUFA Method book III No. 13.9.1	-	yes	
14	USP		yes	
15	microbiological, according to SLMB 1550.1:2007		yes	Vitafast from r-biopharm
16	in-house method LC-MSMS		yes	

5.2.3 Pantothenic Acid

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
1	HPLC-DAD	yes	yes	/
2	USP 34, method 91, microb. Akt.		yes	
3	HPLC-DAD	yes	yes	
4	microbiological	no	no	
5	N/A	N/A	N/A	N/A
6		yes	yes	
7	HPLC-UV	no	yes	-
8			yes	
9	HPLC-DAD		yes	
10				
11	internal method (HPLC-UV)		no	
12	Extraction with formiate buffert. Determination with UHPLC-QQQ.		yes	
13	Vita Fast Pantothenic Acid (RBiopharm)	-	yes	
14	USP		yes	
15	microbiological		yes	Vitafast from r-biopharm
16	in-house method LC-MSMS		yes	

5.2.4 Vitamin C

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
1	HPLC-FLD	yes	yes	/
2				
3	HPLC-DAD	yes	yes	
4	HPLC	no	yes	
5	Vitamin C as Ascorbic Acid	no	yes	None
6		yes	yes	
7	HPLC-UV	no	yes	-
8			yes	
9	HPLC-DAD		yes	
10	in-house method HPLC-DAD	yes / no		
11	NF V03-135 (HPLC-UV)		yes	
12	Extraction in water with DTT. Determination with UHPLC-DAD, wavelength: 245 nm		yes	
13	in-house method HPLC (DAD)	yes	yes	
14	USP		yes	
15	ASU § 64 LFGB L 00.00-85 mod.		yes	
16	in-house method HPLC-UV		yes	

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		FRANCE
		United Kingdom
		Germany
		DENMARK
		Germany
		Germany
		SWITZERLAND
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		SWEDEN
		ESTLAND

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

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