DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

29/2014

Food Supplement I:

Vitamins A, D3, E, K1 and beta-Carotene

in Multi Vitamin Powder

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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.

2. Realisation

2.1 Test material

The test material is a of common in commerce food supplement "multi vitamin powder plus minerals" with addition of drinking powder (appr. 8,8%), powdered sugar (appr. 11%) and potato starch powder (appr. 67%). The materials were sieved, mixed and homogenized. Afterwards the samples were packaged lightproof in portions to approximately 50 g. The portions were numbered chronologically.

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

Table 1: Composition of DLA-Samples

Multi vitamin powder with minerals / drink powder / sugar / starch
Ingredients: Starch, Sugar, Sorbit, Calcium carbonate, Magnesium oxide, Food dyes: beta-Carotene and Anthocyans, Ascorbic acid, L-Carnitine, Acidifying agent: Citric acid, Acid regulator: Sodium hydrogencarbonate, Maltodextrin, Orange fruit powder, Glucose syrup, Vegetable oils, Modified starch, D,L-Alpha-Tocopheryl acetate, Aroma, Separating agent: Magnesium salts of fatty acids, Calcium-D-Pantothenate, Zinc oxide, Sweetener: Aspartam, Pyridoxinhydrochloride, Riboflavin, Thiaminhydrochloride, Retinylacetate, Pteroylmonoglutamiic acid, Chrom-(III)-chloride, Potassium Iodide, Sodium molybdate, Phytomenadione, Cholecalciferol, Cyanocobalamine

Table 2: Calculated amounts according to labelled values of vitamins

Vitamin	**Content per 100 g**	
Vitamin A	800	µg
Vitamin D	33	µg
Vitamin E	80	mg
Vitamin K	250	µg
beta-Carotene	0,67	mg

2.1.1 Homogeneity

The calculation of the repeatability standard deviation of the participants was used as an indicator of homogeneity. For vitamin E it was 7,0% and in the range of common relative repeatability standard deviations of methods for fat-soluble vitamins (16). The repeatability standard deviation of the participants for vitamin E is given in the documentation.

Additionally in the documentation the portion numbers are graphically assigned to the results of vitamin E. 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 22nd week of 2014. The testing method was optional. The tests should be finished at 11th july 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.
With one exception all participants submitted the results in time. One participant submitted no results.

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.

For the proficiency assessment preferably the target standard deviation from the general model (Horwitz) is used, when an acceptable quotient $S^x/\hat{\sigma}$ was achieved. 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 the evaluation of vitamins in the present proficiency testing the model according to Horwitz was applied for vitamin A, D, K and beta-carotene (3.4.1.), for vitamin E the target standard deviation from a precision experiment was used (3.4.2).

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 (ASU)

Method	**Parameter**	**Matrix**	**relative Target standard deviation** $\hat{\sigma}$	**Literature**
HPLC	Vitamin A	Milk powder	2,9 %[1]	L 06.00-63/1
HPLC	Vitamin D3	2 Milk powders	3,2 % / 11,6 %[1]	L 00.00-61
HPLC	Vitamin E	2 Milch powders	6,1 % / 12,5 %[1]	L 00.00-62
HPLC	Vitamin K1	6 Infant food with milk and soy protein	4,4 %[1] (Mean)	L 00.00-86
HPLC	beta-Carotene	Vitamin drink Pudding powder	6,0 % 7,9 %[1]	L 06.00-63/2

[1] *values used for informational or evaluation purposes*

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 vitamin E the target standard deviation from a precision experiment was used (3.4.2). For the other parameters the target standard deviation of the general model (Horwitz) was used (3.4.1).

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 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 a higher than 2 for vitamin A and beta-carotene and for the other vitamins below 2.

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 to 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. In the present proficiency test the quotients ranged from 0,3 to 0,7 and for beta-carotene it was 0,9. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.

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 $\hat{\sigma}$
lower limit of target range (X - 2 $\hat{\sigma}$)
upper limit of target range (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

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	Remarks

4.1 Vitamin A (as Retinol in µg / 100 g)

Statistic Data	
Number of results	*18**
Number of outliers	*2*
Mean	710
Median	673
Robust mean (X)	690
Robust standard deviation (S^x)	180
Target range:	
Target standard deviation $\hat{\sigma}$ (Horwitz)	83
Target standard deviation for Information (ASU §64)	20
lower limit of target range (X - 2 $\hat{\sigma}$)	525
upper limit of target range (X + 2 $\hat{\sigma}$)	855
Quotient $S^x/\hat{\sigma}$	*2,2*
Standard uncertainty u_X	*53*
Quotient $u_X/\hat{\sigma}$	*0,64*
Number of results in the target range	*12 (67%)*

** result of participant 5 excluded*

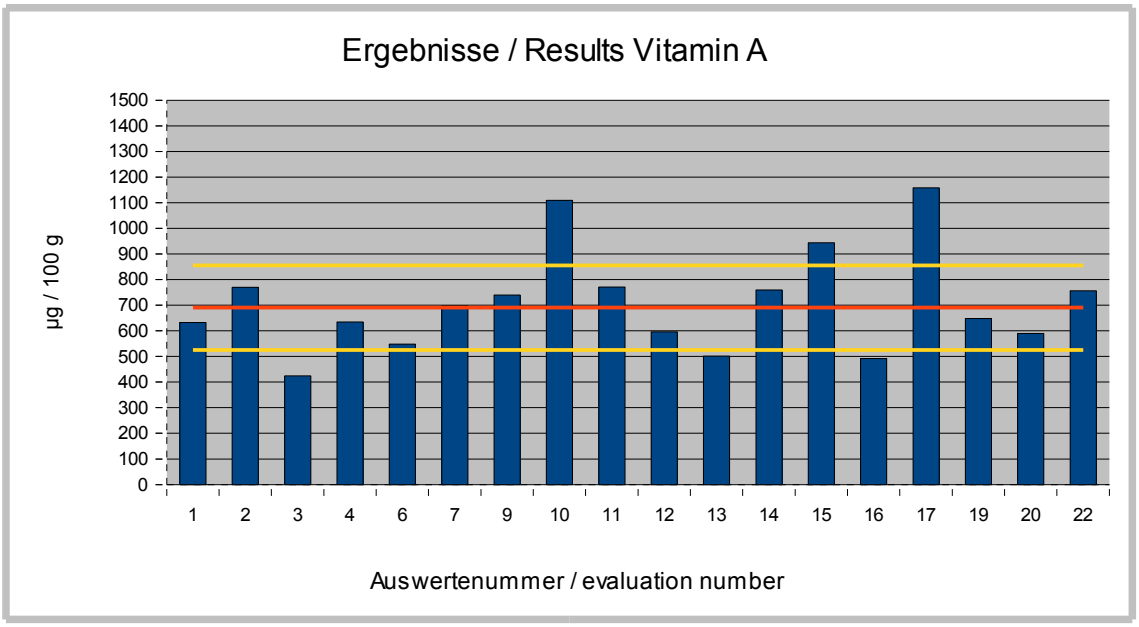


Fig. 1: Results Vitamin A
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score $\hat{\sigma}$	Z-Score´ (for Info)	Remarks
	[µg/100g]	X rob. Mean	Horwitz	ASU	
1	633	-57	-0,7	-2,9	
2	770	80	1,0	4,0	
3	424,2	-266	-3,2	-13,4	
4	634,4	-56	-0,7	-2,8	
5	< 33000				
6	548	-142	-1,7	-7,2	
7	698	8	0,1	0,4	
9	740	49	0,6	2,5	
10	1109,47	419	5,1	21,1	Outlier
11	771	81	1,0	4,1	
12	596	-94	-1,1	-4,8	
13	500,9	-189	-2,3	-9,5	
14	760	70	0,8	3,5	
15	944	254	3,1	12,8	
16	492	-198	-2,4	-10,0	
17	1159	469	5,7	23,6	Outlier
19	648	-42	-0,5	-2,1	
20	589,53	-101	-1,2	-5,1	
22	756	66	0,8	3,3	

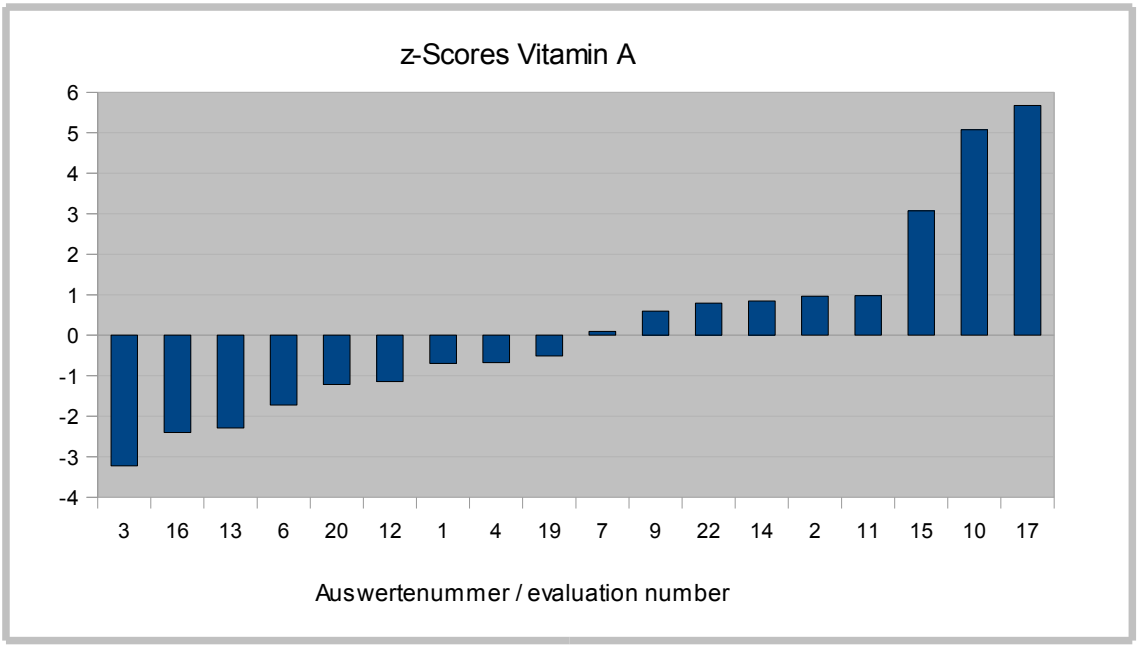


Fig. 2: Z-Scores Vitamin A

4.2 Vitamin D3 (as Cholecalciferol in µg / 100 g)

Statistic Data	
Number of results	*15**
Number of outliers	*0*
Mean	28,6
Median	31,0
Robust mean (X)	28,6
Robust standard deviation (S^x)	11,2
Target range:	
Target standard deviation $\hat{\sigma}$ (Horwitz)	5,5
Target standard deviation for Information (ASU §64)	3,3
lower limit of target range (X - 2 $\hat{\sigma}$)	17,6
upper limit of target range (X + 2 $\hat{\sigma}$)	39,6
Quotient $S^x/\hat{\sigma}$	*2,0*
Standard uncertainty u_X	*3,6*
Quotient $u_X/\hat{\sigma}$	*0,65*
Number of results in the target range	*10 (67%)*

** result of participant 5 excluded*

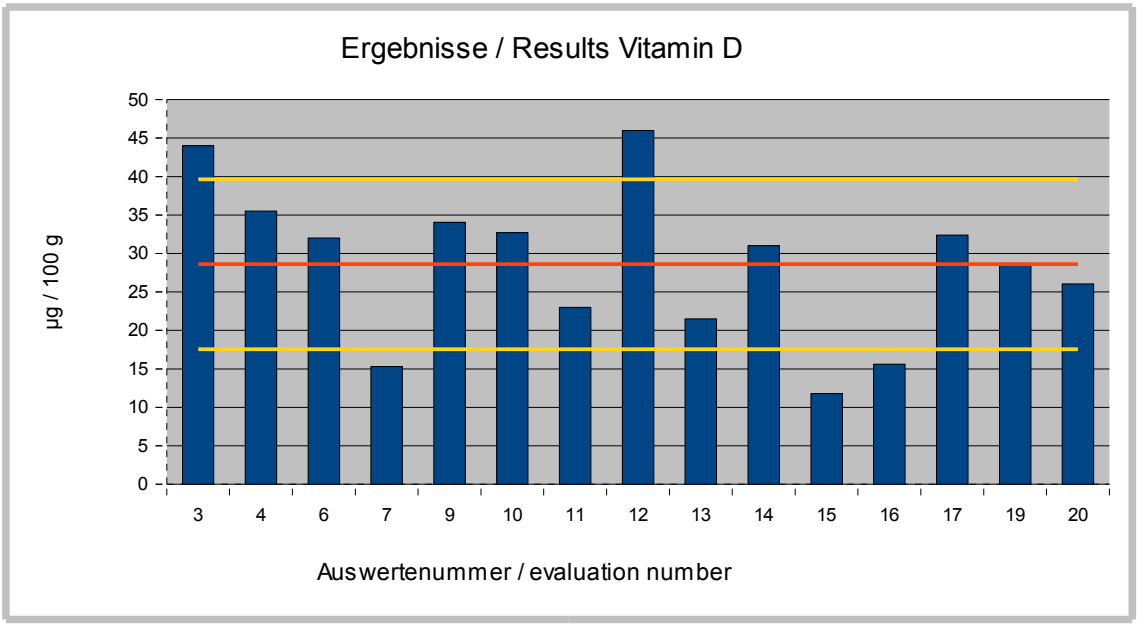


Fig. 3: Results Vitamin D
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score $\hat{\sigma}$	Z-Score´ (for Info)	Remarks
	[µg/100g]	X rob. Mean	Horwitz	ASU	
3	44	15	2,8	4,6	
4	35,5	7	1,2	2,1	
5	< 540				
6	32	3	0,6	1,0	
7	15,3	-13	-2,4	-4,0	
9	34,1	5	1,0	1,6	
10	32,7	4	0,7	1,2	
11	23	-6	-1,0	-1,7	
12	46	17	3,1	5,2	
13	21,5	-7	-1,3	-2,1	
14	31	2	0,4	0,7	
15	11,8	-17	-3,0	-5,1	
16	15,6	-13	-2,4	-3,9	
17	32,4	4	0,7	1,1	
19	28,7	0	0,0	0,0	
20	26,05	-3	-0,5	-0,8	

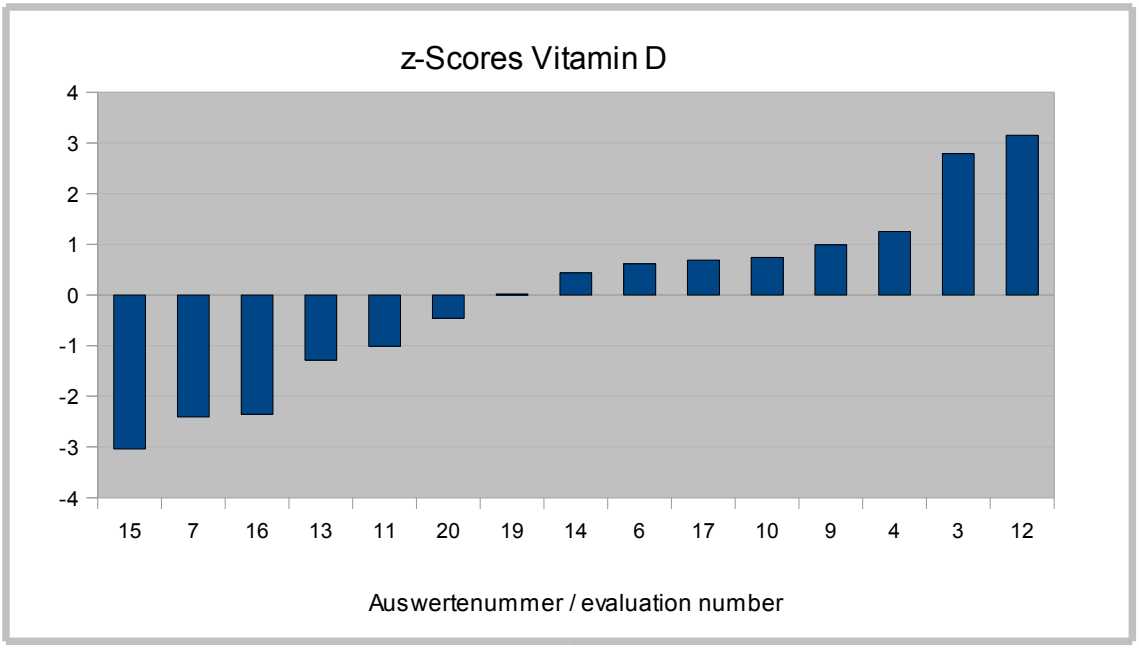


Fig. 4: Z-Scores Vitamin D3

4.3 Vitamin E (as D-alpha-Tocopherol in mg / 100 g)

Statistic Data	
Number of results	*22*
Number of outliers	*0*
Mean	91,5
Median	95,0
Robust mean (X)	92,7
Robust standard deviation (S^x)	16,3
Target range:	
Target standard deviation or Information (Horwitz)	5,3
Target standard deviation $\hat{\sigma}$ (ASU §64)	11,6
lower limit of target range (X - 2 $\hat{\sigma}$)	69,4
upper limit of target range (X + 2 $\hat{\sigma}$)	116
Quotient $S^x/\hat{\sigma}$	*1,4*
Standard uncertainty u_X	*4,4*
Quotient $u_X/\hat{\sigma}$	*0,37*
Number of results in the target range	*20 (91%)*

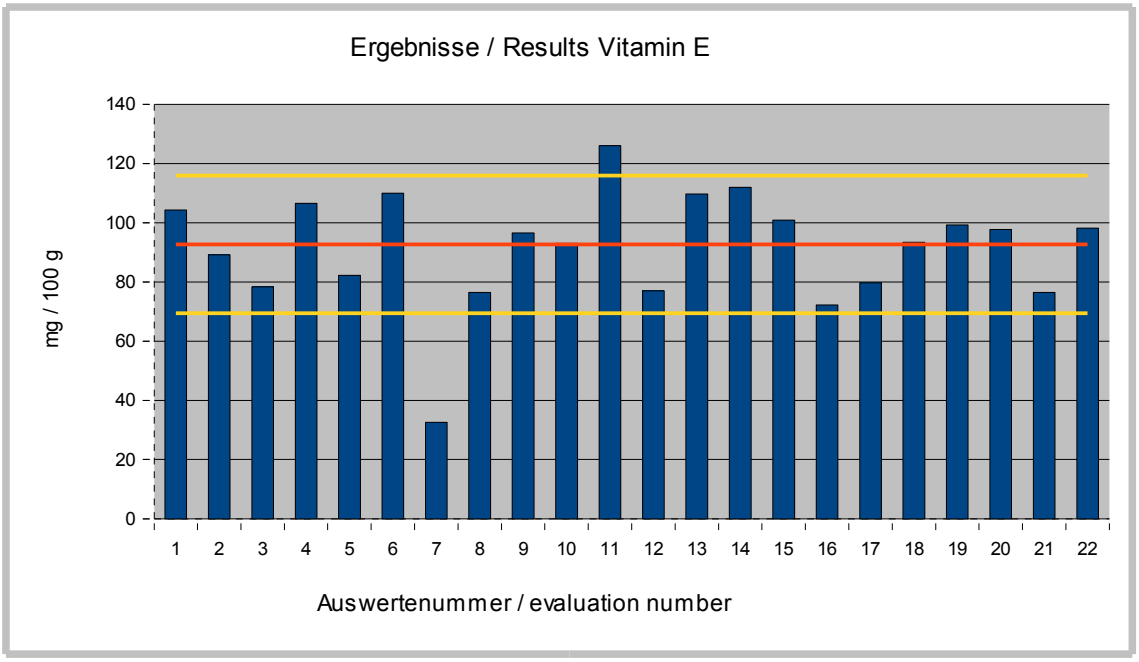


Fig. 5: Results Vitamin E
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score´ (for Info)	Z-Score $\hat{\sigma}$	Remarks
	[mg/100g]	X rob. Mean	Horwitz	ASU	
1	104,3	12	2,2	1,0	
2	89,2	-3	-0,7	-0,3	
3	78,4	-14	-2,7	-1,2	
4	106,6	14	2,6	1,2	
5	82,2	-10	-2,0	-0,9	
6	110	17	3,3	1,5	
7	32,6	-60	-11,3	-5,2	Outlier
8	76,5	-16	-3,1	-1,4	
9	96,6	4	0,7	0,3	
10	93,04	0	0,1	0,0	
11	126	33	6,3	2,9	
12	77	-16	-3,0	-1,3	
13	109,7	17	3,2	1,5	
14	112	19	3,6	1,7	
15	100,9	8	1,6	0,7	
16	72,2	-20	-3,9	-1,8	
17	79,7	-13	-2,4	-1,1	
18	93,41	1	0,1	0,1	
19	99,3	7	1,2	0,6	
20	97,76	5	1,0	0,4	
21	76,5	-16	-3,1	-1,4	
22	98,2	6	1,0	0,5	

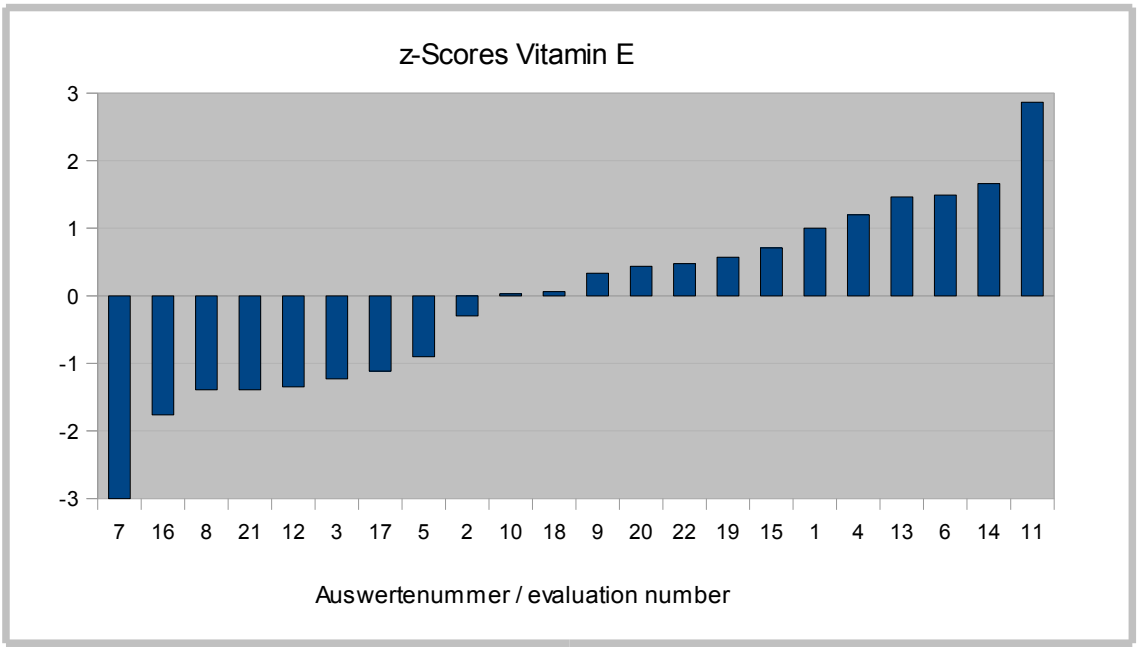


Fig. 6: Z-Scores Vitamin E

4.4 Vitamin K1 (as Phylloquinone in µg / 100 g)

Statistic Data	
Number of results	*5**
Number of outliers	*0*
Mean	233
Median	239
Robust mean (X)	233
Robust standard deviation (S^x)	21,3
Target range:	
Target standard deviation $\hat{\sigma}$ (Horwitz)	32,8
Target standard deviation for Information (ASU §64)	10,3
lower limit of target range (X - 2 $\hat{\sigma}$)	167
upper limit of target range (X + 2 $\hat{\sigma}$)	298
Quotient $S^x/\hat{\sigma}$	*0,7*
Standard uncertainty u_X	*12*
Quotient $u_X/\hat{\sigma}$	*0,36*
Number of results in the target range	*5 (100%)*

** result of participant 5 excluded*

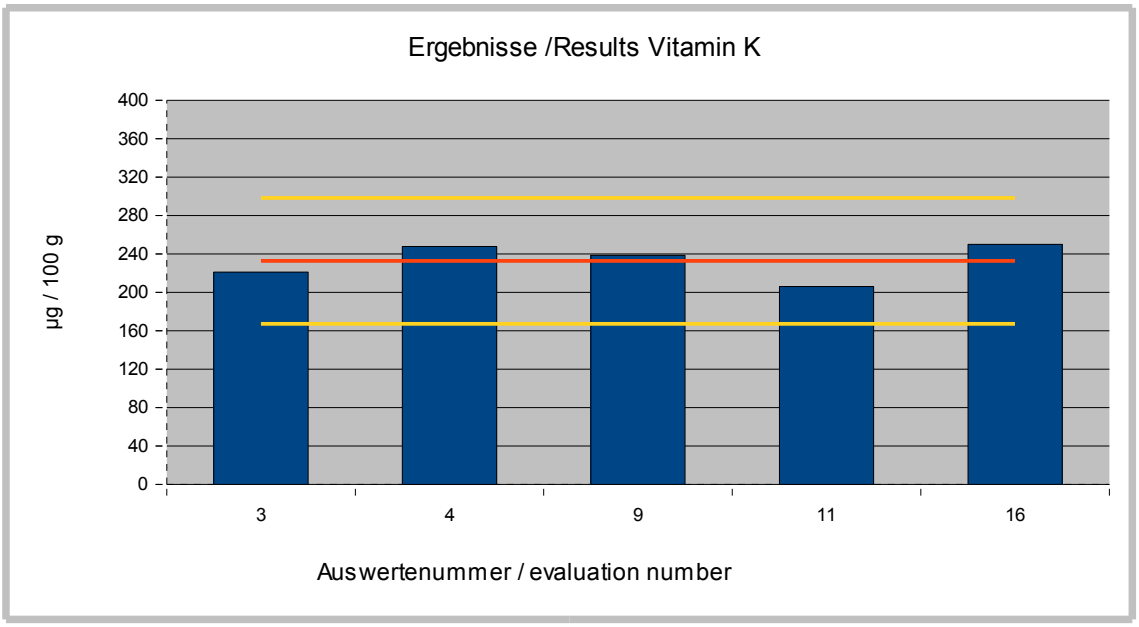


Fig. 7: Results Vitamin K1
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score $\hat{\sigma}$	Z-Score´ (for Info)	Remarks
	[µg/100g]	X rob. Mean	Horwitz	ASU	
3	220,9	-12	-0,4	-1,1	
4	247,8	15	0,5	1,5	
5	< 8000	-	-	-	
9	239	6	0,2	0,6	
11	206	-27	-0,8	-2,6	
16	250	17	0,5	1,7	

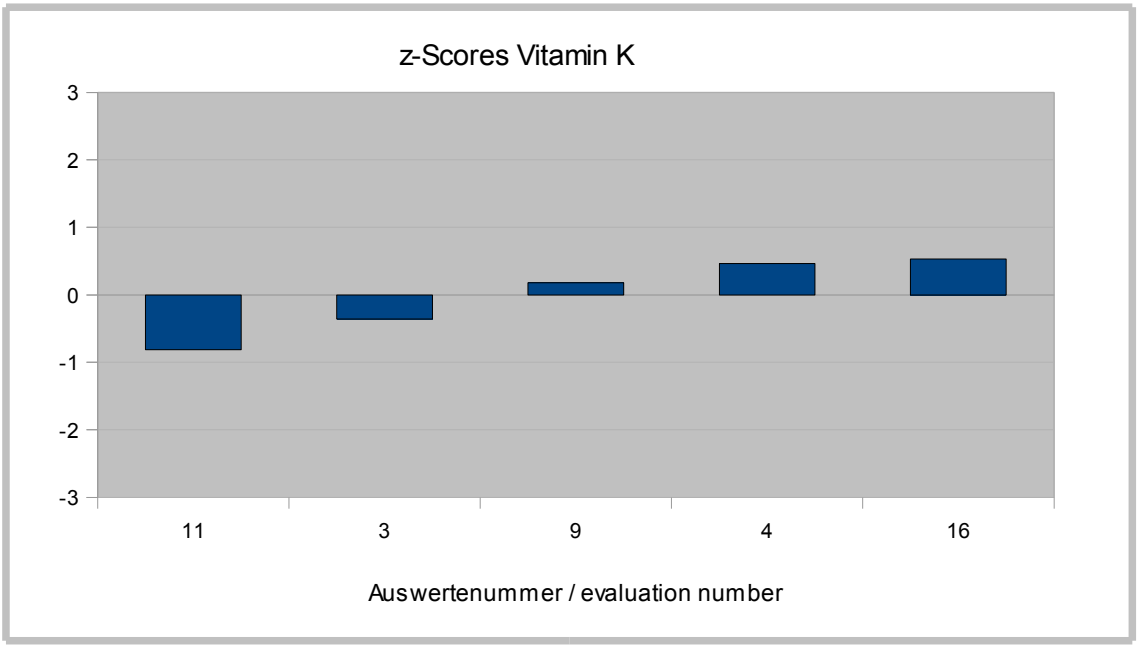


Fig. 8: Z-Scores Vitamin K1

4.5 beta-Carotene (in mg / 100 g)

Statistic Data	
Number of results	*13*
Number of outliers	*1*
Mean	0,515
Median	0,550
Robust mean (X)	0,509
Robust standard deviation (S^x)	0,160
Target range:	
Target standard deviation $\hat{\sigma}$ (Horwitz)	0,064
Target standard deviation for Information (ASU §64)	0,043
lower limit of target range (X - 2 $\hat{\sigma}$)	0,381
upper limit of target range (X + 2 $\hat{\sigma}$)	0,636
Quotient $S^x/\hat{\sigma}$	*2,5*
Standard uncertainty u_X	*0,055*
Quotient $u_X/\hat{\sigma}$	*0,87*
Number of results in the target range	*9 (69%)*

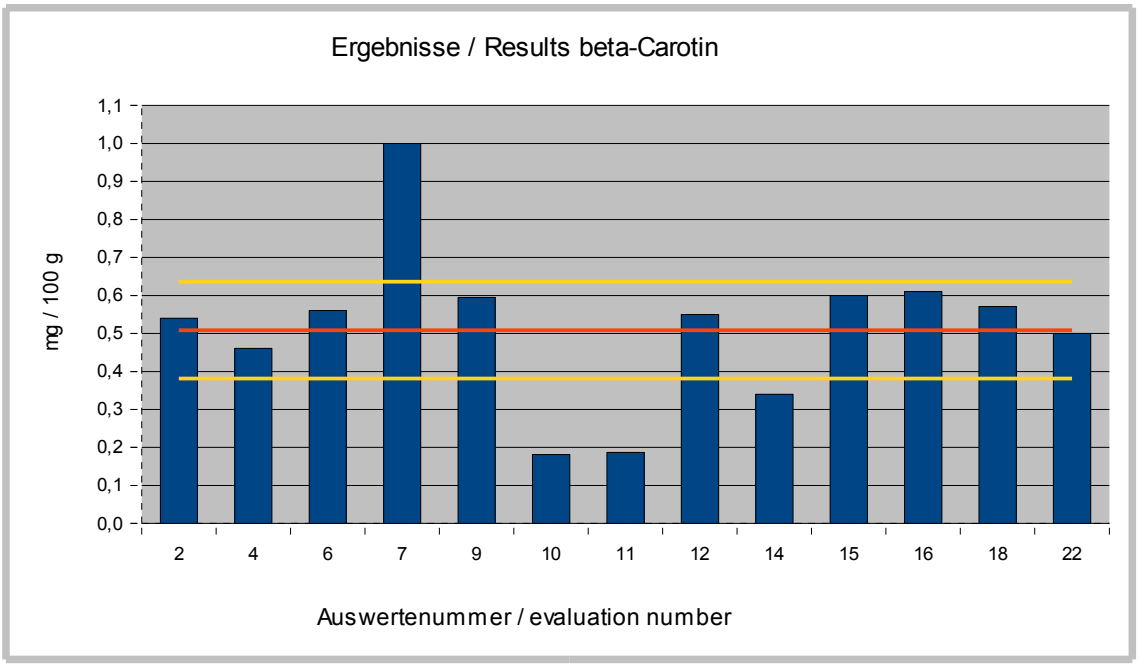


Fig. 9: Results beta-Carotene
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score $\hat{\sigma}$	Z-Score´ (zur Info)	Remarks
	[mg/100g]	X rob. Mean	Horwitz	ASU	
2	0,54	0,03	0,5	0,8	
4	0,461	-0,05	-0,7	-1,2	
6	0,56	0,05	0,8	1,3	
7	1,0	0,49	7,7	12,2	Outlier
9	0,60	0,09	1,4	2,1	
10	0,181	-0,33	-5,1	-8,1	
11	0,187	-0,32	-5,0	-8,0	
12	0,55	0,04	0,6	1,0	
14	0,34	-0,17	-2,6	-4,2	
15	0,6	0,09	1,4	2,3	
16	0,61	0,10	1,6	2,5	
18	0,571	0,06	1,0	1,5	
22	0,5	-0,01	-0,1	-0,2	

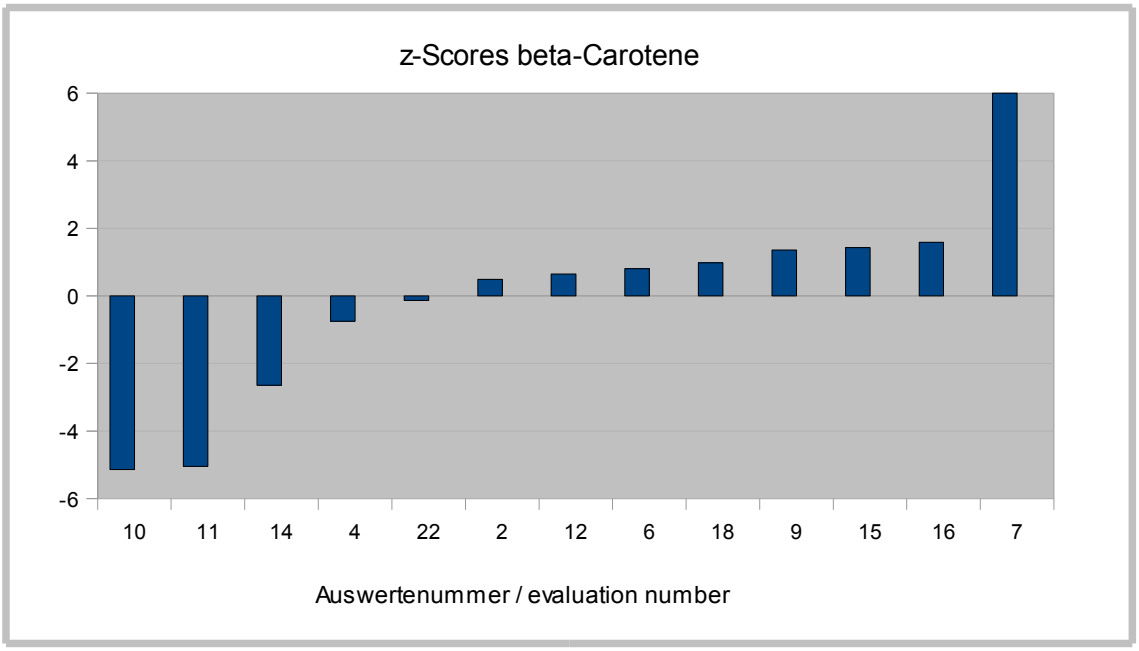


Fig. 10: Z-Scores beta-Carotene

5. Documentation

5.1 Primary data

5.1.1 Vitamin A

Evaluation number	Result	DLA-No Sample 1	DLA-No Sample 2	Result 1	Result 2	Recovery rate
	[µg/100g]			[µg/100g]	[µg/100g]	in %
1	633	6	39	622	644	
2	770	21	37			not done
3	424,2	20	41	364,8	483,6	
4	634,4	24	53	663,9	605,0	93,5
5	< 33000					
6	548	1	26	580	515	N/A
7	698	7	54	676	720	-
9	740	2	64	771	708	
10	1109,47	58	15	942,43	1276,51	no internal standard
11	771	33	52	785	756	100
12	596	11	50	606	585	
13	500,9	22	45			
14	760	17	42	729	788	89%
15	944	35	62	842	1045	77
16	492	19	43	448	537	97,0
17	1159	10	28	1156	1162	88
19	648	38	60	654	642	-
20	589,53	12	56	616,06	562,99	110
22	756	9	44	732	780	113

5.1.2 Vitamin D

Evaluation number	Result	DLA-No Sample 1	DLA-No Sample 2	Result 1	Result 2	Recovery rate
	[µg/100g]			[µg/100g]	[µg/100g]	in %
3	44	20	41	45,1	42,9	99
4	35,5	24	53	36,9	34,1	87,0
5	< 540					
6	32	1	26	28,3	35,6	N/A
7	15.3	23	61	15.0	15.6	-
9	34,1	2	64	33,4	34,7	
10	32,7	58	15	41,65	23,75	no internal standard
11	23	33	52	22	24	100
12	46	11	50	49	43	
13	21,5	22	45			
14	31	17	42	31,04	31,84	87%
15	11,8	35	62	12	11,5	no internal standard
16	15,6	19	43	15,6	15,7	-
17	32,4	10	28	32,8	32	79
19	28,7	38	60	29,8	27,6	-
20	26,05	12	56	25,2	26,9	119

5.1.3 Vitamin E

Evaluation number	Result	DLA-No Sample 1	DLA-No Sample 2	Result 1	Result 2	Recovery rate
	[µg/100g]			[µg/100g]	[µg/100g]	in %
1	104,3	6	39	105	102,9	
2	89,2	21	37	100,1	78,3	106
3	78,4	20	41	78,7	78	
4	106,6	24	53	104,3	108,8	96,6
5	82,2					
6	110	1	26	109	110	N/A
7	32.6	23	61	33.3	31.8	-
8	76,5	46	13	76,4	76,5	
9	96,6	2	64	95,5	97,6	
10	93,04	58	15	91,58	94,49	no internal standard
11	126	33	52	124	128	100
12	77	11	50	78	75	
13	109,7	22	45			
14	112	17	42	113,5	110,7	97%
15	100,9	35	62	99,9	102,1	95,1
16	72,2	19	43	70,8	73,5	-
17	79,7	10	28	79,7	81,7	93
18	93,41	25	47	93,49	93,33	89
19	99,3	38	60	98,6	100	-
20	97,76	12	56	95,49	100,03	99
21	76,5	8	40	77,2	75,8	
22	98,2	9	44	99,1	97,3	96

5.1.4 Vitamin K

Evaluation number	Result	DLA-No Sample 1	DLA-No Sample 2	Result 1	Result 2	Recovery rate
	[µg/100g]			[µg/100g]	[µg/100g]	in %
3	220,9	20	41	235	206,7	
4	247,8	24	53	253,1	242,4	98,3
5	< 8000					
9	239	2	64	231	246	
11	206	33	52	203	208	100
16	250	19	43	244	257	-

5.1.5 beta-Carotene

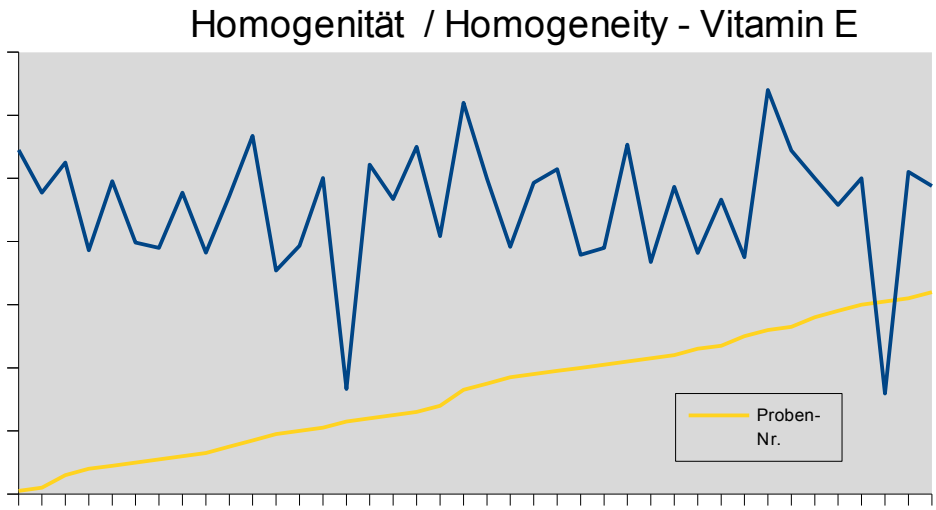
Evaluation number	Result	DLA-No Sample 1	DLA-No Sample 2	Result 1	Result 2	Recovery rate
	[µg/100g]			[µg/100g]	[µg/100g]	in %
2	0,54	21	37	0,54	0,53	85
4	0,461	24	53	0,493	0,429	102,9
6	0,56	1	26	0,57	0,55	N/A
7	1,0	61	54	1.0	1.0	-
9	0,60	2	64	0,59	0,6	
10	0,181	58	15	0,18	0,182	no internal standard
11	0,187	33	52	0,194	0,179	100
12	0,55	11	50	0,56	0,54	
14	0,34	17	42	0,331	0,347	---
15	0,6	35	62	0,58	0,61	80
16	0,61	19	43	0,61	0,61	-
18	0,571	25	47	0,572	0,57	106
22	0,5	9	44	0,501	0,498	96

5.2 Homogeneity

5.2.1 Repeatability standard deviation of duplicate tests of the participants

The repeatability standard deviation was calculated with the data documented in 5.1.3 for vitamin E. It is 6,5 mg/100g = 7,0 % of X.
The relative repeatability standard deviation from the German official method ASU L 00.00-62 is 12,8% for the determination of vitamin E in milk powder.

5.2.2 Comparison of sample number / test result



Proben: DLA-Portionsnummer / sample: DLA portion numbers

5.3 Analytical Methods

Details by the participants

5.3.1 Vitamin A

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
1	alkaline hydrolysis; HPLC		yes	
2	HPLC - FLD		yes	
3	Vitamin A (USP)		yes	
4	PV 2.020/002-02	yes	yes	---
5				
6	HPLC/UV	N/A	No	None
7	HPLC-UV	no	no	None
9	VO (EG) 152/2009, IV, A		yes	recovery not determined
10	Determination of Vitamin A (Retinol) by HPLC-Fluorescence	no	no	
11	after alkaline hydrolysis by LC-DAD	no	yes	
12	HPLC-UV		yes	
13	EN 12823 (HPLC)	no	yes	
14	Hydrolysis with methanolic KOH, liquid-liquid extraction, HPLC with UV-Detektion; for feed according to VO EG 152/2009 An. IV/A; for food: Basic norm EN 12823-1	yes	yes	MU +- 25% relative
15	Vitamin A and E; Determination in food and food supplements by HPLC on basis of §64-Methods with modified hydrolysis temperatur	yes	yes	
16	In-house method STM No.03-021 based on Journal of Liquid Chromatography Analysis of Food and Beverage,Vol.2,1979,p.477-495	No	Yes	
17	L 00.00-63/1	yes	yes	badly soluble
19	§64 LFGB L 00.00-63/1	-	yes	
20	VD LUFA Method band III 13.1.2	no	yes	
22	HPLC-DAD after alkaline hydrolysis	yes	yes	

5.3.2 Vitamin D

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
3	Vitamin D3 (USP)	no	yes	
4	PV 2.020/004-02	yes	yes	---
5				
6	HPLC/UV	N/A	Yes	None
7	HPLC-UV	no	yes	None
9	VDLUFA III, 13.8.1		yes	recovery not determined
10	Determination of Vitamin D3 (Cholecalciferol) by HPLC-UV	no	no	
11	after alkaline hydrolysis by LC-MS	no	yes	
12	HPLC-UV		yes	
13	EN 12821 (HPLC)	no	yes	
14	alkaline hydrolysis, liquid-liquid extraction, Clean Up with HPLC and Fraction collector, HPLC with PDA-Detection: food: Basic norm EN 12821, feed: VDLUFA Method 13.8.1	yes	yes	MU +- 25% relativ
15	Determination of Vitamin D by LC-MS/MS		no	
16	Based on AOAC (2012), 995.05	Yes	No	
17	L 00.00-61	yes	yes	badly soluble
19	§64 LFGB L 00.00-61	-	yes	
20	VD LUFA Method band III 13.8.1	no	yes	

5.2.3 Vitamin E

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
1	alkaline hydrolysis; HPLC		yes	
2	HPLC -FLD	yes	yes	
3	Vitamin E (USP)		yes	
4	PV 2.020/003-02	yes	yes	---
5				
6	HPLC FLD	N/A	Yes	None
7	HPLC-UV	no	yes	None
8	UPLC-DAD	no	yes	
9	VO (EG) 152/2009, IV, B		yes	recovery not determined
10	Determination of Vitamin E (Tocopherols, Tocotrienols) by HPLC-FLD	no	yes	
11	after alkaline hydrolysis by LC-DAD	no	yes	
12	HPLC-UV		yes	
13	EN 12822 (HPLC)	no	yes	
14	Hydrolysis with methanolic KOH, liquid-liquid extraction, HPLC with Fluorescence detection for feed according to VO EG 152/2009 An. IV/B; for food: Basic norm EN 12822	yes	yes	MU +- 10% relativ
15	Vitamin A and E; Determination in food and food supplements by HPLC on basis of §64-Methods with modified hydrolysis temperatur	yes	yes	
16	In-house method STM No.03-021 based on Journal of Liquid Chromatography Analysis of Food and Beverage,Vol.2,1979,p.477-495	Yes	Yes	
17	L 00.00-62	yes	yes	badly soluble
18	HPLC-DAD	yes	yes	
19	§64 LFGB L 00.00-62	-	yes	
20	VD LUFA Method band III 13.5.4	no	yes	
21	HPLC-DAD		yes	Tocopherolacetat content of 114 mg/100g was determined and with Factor 0,671 multiplied
22	HPLC-DAD after alkaline hydrolysis	yes	yes	

5.2.4 Vitamin K

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
3	Vitamin K1 (USP)		yes	
4	in-house method based on ASU L 00.00-86 and USP 37	yes	yes	---
5				
9	in-house method		yes	recovery not determined
11	after extraction by LC-FLD with post-column derivatisation	no	yes	
16	Based on AOAC (2012), 999.15	Yes	No	

5.2.5 beta-Carotene

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
2	UV	yes	yes	
4	PV 2.019/012-02	yes	yes	---
6	HPLC/UV	N/A	Yes	None
7	Spectrophotometric	no	yes	None
9	in-house-method		yes	recovery not determined
10	Determination of beta-Carotene, HPLC	no	no	
11	after alkaline hydrolysis by LC-DAD	no	yes	
12	Spectrophotometry		no	
14	Hydrolysis with methanolic KOH, liquid-liquid extraction, HPLC with UV-Detection: Basic norm: EN 12823-2		yes	MU +- 20% relative
15	Photometric determination of total carotinoids and Beta-carotene in food and food supplements	yes	yes	
16	Based on JAOAC (1997)	Yes	No	
18	HPLC-DAD	yes	yes	
22	HPLC-DAD after alkaline hydrolysis	yes	yes	

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		AUSTRIA
		FRANCE
		THAILAND
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		DENMARK
		ENGLAND
		BELGIUM
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		SWEDEN
		GERMANY
		ESTLAND

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

7. Index of references

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2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
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4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
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15. ASU § 64 LFGB L 00.00-63/1 Bestimmung von Vitamin A in Lebensmitteln mittels HPLC, Teil 1: Bestimmung von all-trans-Retinol und 13-cis-Retinol
16. ASU § 64 LFGB L 00.00-63/2 Bestimmung von Vitamin A in Lebensmitteln mittels HPLC, Teil 2: Bestimmung von β-Carotin
17. ASU § 64 LFGB L 00.00-86 Bestimmung von Vitamin K_1 mit HPLC
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19. Blake CJ (2007), Analytical procedures for water-soluble vitamins in foods and dietary supplements: a review. Anal Bioanal Chem 389(1):63-76
20. Blake CJ (2007) Status of methodology for the determination of fat-soluble vitamins in foods, dietary supplements, and vitamin premixes. J AOAC Int 90(4):897-910

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