DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 48/2016

Food Supplement II:

Biotin, Niacin, Pantothenic Acid and Vitamin C

in Multi Vitamin Capsule Powder

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, Germany

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

Coordinator of this PT:
Dr. Matthias Besler

Inhalt / Content

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

2. Realisation

2.1 Test material

The test material is a mixture of two common in commerce food supplements "multi vitamin capsules" and lactose as bulking agent from European Suppliers. The first food supplement was crushed including the capsule shells, while the second food supplement was added without capsule shells. The materials were sieved, mixed and homogenized. Afterwards the samples were portioned to approximately 25 g into metallised PET film bags and chronologically numbered.

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 capsules
Ingredients including capsule shell (1. food supplement): Dicalcium phosphate, magnesium oxide, gelatin, vitamin C, potassium chloride, niacin, magnesium stearate, vitamin E acetate, calcium D-pantothenate, ferrous sulfate, zinc oxide, vitamin B6 hydrochloride, copper sulfate, vitamin B2, vitamin B1 mononitrate, vitamin A acetate, folic acid, biotin, potassium iodide, chromium-III-chloride, sodium molybdate, sodium selenite, vitamin K1, vitamin D3, vitamin B12. Ingredients without capsule shell (2. food supplement): Bulking agent lactose, vitamin C, nicotinamide, vitamin E acetate, calcium D-pantothenate, vitamin B6, vitamin B2, vitamin B1, separating agent: magnesium stearate, silica, beta-carotene, biotin, folic acid, vitamin B12. additional ingredient: Lactose

Table 2: Calculated amounts according to labelled values of vitamins

Vitamin	Content per 100 g
Biotin	10.000 µg
Niacin	1.500 mg
Pantothenic Acid	520 mg
Vitamin C	6.100 mg

2.1.1 Homogeneity

The **homogeneity of bottled numbered DLA-samples** was checked by 8-fold determination of niacin by HPLC-UV. The repeatability standard deviation of 0,8 % is below the range of the repeatability standard deviations of method EN 15652:2009 for determination of niacin, which are in the range of 1,1% to 5,6% [18]. The results of the homogeneity test are given in the documentation.

The calculation of the **repeatability standard deviation S_r of the participants** was also used as an indicator of homogeneity. It is < 2,5% (1,45% - 2,47%) for all analytes. Therefore these repeatability standard deviations are similar to precision data of the referring standardized methods (e.g. ASU §64 L 00.00-86, s. 3.6.2) (see Tab. 3) [16-19]. The repeatability standard deviations of the participants' results are given in the tables of statistic data (see 4.1 to 4.20).

Furthermore, the homogeneity was characterized by the **trend line function of participants' results for chronological bottled single samples**. The maximum deviations from the mean value of the trend lines for niacin and pantothenic acid were at approximately 30% and below 30% of the target standard deviations $\sigma_{pt'}$ and σ_{pt}, respectively (s. 5.2 homogeneity) and can therefore be regarded as low.

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 25th week of 2016. The testing method was optional. The tests should be finished at 19th August 2016 the latest.

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

The two identical samples are powdered multivitamin capsules including capsule shells (gelatin) containing biotin, niacin, pantothenic acid and vitamin C. The recommendation is to take 3 g per day. Each sample bag contains 50 g. The samples contain vitamins in the form of approved food supplements nutrient compounds. The material was tested for homogeneity and is intended for laboratory use only. The methods for determination are optional (e.g. HPLC, ELISA).
In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.

2.3 Submission of results

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

The finally calculated concentrations of the parameter as average of duplicate determinations of both numbered samples were 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 methods.

In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

All 9 participants submitted the result in time. A 10th registration was cancelled before sample shipment.

3. Evaluation

3.1 Consensus value from participants (assigned value)

The robust mean of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

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

The actual measurement results will be drafted. Individual results, which are outside the specified measurement range of the participating laboratory (for example with the result > 25 mg/kg or < 2,5 mg/kg) or the indicating "0" will not be considered for the statistic evaluation [3].

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S^x) was calculated. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

3.3 Repeatability standard deviation

The repeatability standard deviation S_r is based on the laboratory´s standard deviation of (outlier free) individual participant results, each under repeatability conditions, that means analyses was performed on the same sample by the same operator using the same equipment in the same laboratory within a short time. It characterizes the mean deviation of the results within the laboratories [3] and is used by DLA as an indication of the homogeneity of the sample material.

In case single results from participants are available the calculation of the repeatability standard deviation S_r, also known as standard deviation within laboratories S_w, is performed by: [3, 4].

The relative repeatability standard deviation as a percentage of the mean value is indicated as coefficient of variation CV_r in the table of statistical characteristics in the results section in case single results from participants are available.

3.4 Reproducibility standard deviation

The reproducibility standard deviation S_R represents a inter-laboratory estimate of the standard deviation for the determination of each parameter on the bases of (outlier free) individual participant results. It takes into account both the repeatability standard deviation S_r and the within-laboratory standard deviation S_S. Reproducibility standard deviations of PT´s may differ from reproducibility standard deviations of ring trials, because the participating laboratories of a PT generally use different internal conditions and methods for determining the measured values.

In the present evaluation, the specification of the reproducibility standard deviation, therefore, does not refer to a specific method, but characterizes approximately the comparability of results between the laboratories, assumed the effect of homogeneity and stability of the sample are negligible.

In case single results from participants are available the calculation of the reproducibility standard deviation S_R is performed by: [3, 4].

The relative reproducibility standard deviation as a percentage of the mean value is indicated as coefficient of variation CV_R in the table of statistical characteristics in the results section in case single results from participants are available. Its meaning is explained in more detail in 3.9.

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 for evaluation of interlaboratory studies, where different analytical methods are applied by the participants. On the other hand the target standard deviation from the evaluation of precision data of an precision experiment is derived from collaborative studies with specified analytical methods.

In cases where both above-mentioned models are not suitable, the target standard deviation is determined based on values by perception, see under 3.6.3.

For information the z-scores of both models are given in the evaluation, if available.

In the present PT for valuation of biotin, niacin, pantothenic acid and vitamin C the target standard deviation according to the general model of Horwitz was applied (see 3.6.1).
Additionally for vitamin C the standard uncertainty was considered by valuating with z'-scores (see 3.6.8).

Due to the low number of < 7 the results of biotin were not evaluated by means of z-scores.

3.6.1 General model (Horwitz)

Based on statistical characteristics obtained in numerous PTs for different parameters and methods Horwitz has derived a general model for estimating the reproducibility standard deviation σ_R [6]. Later the model was modified by Thompson for certain concentration ranges [10]. The reproducibility standard deviation σ_R can be applied as the relative target standard deviation σ_{pt} in % of the assigned values and calculated according to the following equations [3]. For this the assigned value X_{pt} is used for the concentration c.

Equations	**Range of concentrations**	**corresponds to**
$\sigma_R = 0{,}22c$	$c < 1{,}2 \times 10^{-7}$	< 120 µg/kg
$\sigma_R = 0{,}02c^{0{,}8495}$	$1{,}2 \times 10^{-7} \leq c \leq 0{,}138$	≥ 120 µg/kg
$\sigma_R = 0{,}01c^{0{,}5}$	$c > 0{,}138$	> 13,8 g/100g

with c = mass content of analyte (as relative size, e.g. 1 mg/kg = 1 ppm = 10^{-6} kg/kg)

3.6.2 Value by 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 3 relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) were determined in collaborative trials using the specified methods.
The in the table indicated resulting target standard deviations σ_{pt} were used for evaluation of the results.
For information the target standard deviations according to Horwitz are given additionally.

Table 3: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [16-19]

Parameter	**Matrix**	**Mean values**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Biotin	Cereal breakfast powder	197 µg/100 g	4,5%	17,4%	17,1%[1]	HPLC [17]
	Infant milk powder	18,0 µg/100 g	11,6%	29,8%	27,5%	HPLC [17]
	Feed	15-58 µg/100g	7,2-9,4%	9,4-22,4%*	-	HPLC-MS/MS [19]
Vitamin C	Breakfast cereals	102,6 mg/100g	9,9%	19,3%	18,0%	HPLC [16]
	Milk powder	100,3 mg/100 g	6,3%	11,4%	10,5%[1]	HPLC [16]
Niacin	Chocolate cereals	21,03 mg/100g	1,1%	4,3%	4,23%	HPLC [18]
	Milk powder	16,66 mg/100 g	2,8%	4,3%	3,82%[1]	HPLC [18]
	Wheat flour	0,72 mg/100 g	3,9%	29,2%	29,1%	HPLC [18]

[1] *used in evaluation (s. chapter 4)*
** intermediär precision*

3.6.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].

For the present evaluation the target standard deviation according to 3.6.1 were regarded suitable. Table 4 shows selected characteristics of participants results of the present PT in comparison to previous years.

3.7 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}) [3].
Participants' z-scores are derived from:

$$z_i = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}}$$

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

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

The z-score valid for the PT evaluation is designated z-score (σ_{pt}), while the value of z-score (Info) is for information only. The two z-scores are calculated using the different target standard deviations according to 3.6.

3.7.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation. 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].

Table 4: Characteristics of the present PT (on dark gray) in comparison to previous PTs since 2014 (SD = standard deviation, CV = coefficient of variation)

Parameter	**Matrix** (Powder)	**robust Mean**	**rob. SD (S*)**	**rel. SD (VK_{S*}) [%]**	**Quotient S^*/σ_{pt}**	**DLA-Report**
Biotin	Multivitamin Effervescent Tablets	633 µg/100g	131 µg/100g	20,7%	1,7	DLA 30/2014
Biotin	Multivit-amin-Capsule Powder	23100 µg/100g	5500 µg/100g	23,8%	1,6	DLA 33/2015
Biotin	Multivit-amin-Capsule Powder	11200 µg/100g	1190 µg/100g	10,6%	1,4	DLA 48/2016
Niacin	Multivitamin Effervescent Tablets	172 mg/100g	11,5 mg/100g	6,69%	1,3	DLA 30/2014
Niacin	Multivit-amin-Capsule Powder	3100 mg/100g	115 mg/100g	3,71%	1,1	DLA 33/2015
Niacin	Multivit-amin-Capsule Powder	1530 mg/100g	107 mg/100g	6,98%	1,9	DLA 48/2016
Pantothe-nic acid	Multivitamin Effervescent Tablets	61,0 mg/100g	5,67 mg/100g	9,30%	1,5	DLA 30/2014
Pantothe-nic acid	Multivit-amin-Capsule Powder	1060 mg/100g	99 mg/100g	9,34%	1,5	DLA 33/2015
Pantothe-nic acid	Multivit-amin-Capsule Powder	598 mg/100g	41,1 mg/100g	6,88%	1,6	DLA 48/2016
Vitamin C	Multivitamin Effervescent Tablets	1088 mg/100g	54,8 mg/100g	5,04%	1,3	DLA 30/2014
Vitamin C	Multivit-amin-Capsule Powder	11200 mg/100g	951 mg/100g	8,49%	1,9	DLA 48/2016
Vitamin C	Multivit-amin-Capsule Powder	6133 mg/100g	365 mg/100g	5,96%	1,4	DLA 48/2016

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

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

For warning and action signals see 3.7.1.

3.9 Reproducibility coefficient of variation (CV_R)

The coefficient of variation (CV_R) 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_K gives the relative variability within a data region. While a low CV_R, e.g. < 5-10% can be taken as evidence for a homogeneous set of results, a CV_R 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 values or the performance evaluation of the participants 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 ($U(X_{pt})$) for this PT is calculated as follows [3]:

$$u_{(x_{pt})} = 1{,}25 \times \frac{s^*}{\sqrt{p}}$$

If $U(X_{pt}) \leq 0{,}3\ \sigma_{pt}$ 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

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^*)
Number with m replicate measurements
Repeatability standard deviation (S_r)
Coefficient of Variation (CV_r) in %
Reproducibility standard deviation (S_R)
Coefficient of Variation (CV_R) in %
Target range:
Target standard deviation σ_{pt} or σ_{pt}'
Target standard deviation for information
lower limit of target range ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}$') *
upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}$') *
Variation coefficient V_K in %
Quotient S/σ_{pt} or S*/σ_{pt}'*
Standard uncertainty $U(X_{pt})$
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 **Evaluation number**	**Parameter [Einheit / Unit]**	**Abweichung** **Deviation**	**z-Score σpt**	**z-Score** (Info)	**Hinweis** **Remark**

4.1 Biotin in µg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	6
Number of outliers	0
Mean	11200
Median	10900
Robust Mean (X)	**11200**
Robust standard deviation (S*)	**1190**
Number with 2 replicates	5
Repeatability SD (S_r)	157
Repeatability (CV_r)	1,45%
Reproducibility SD (S_R)	763
Reproducibility (CV_R)	7,02%
Target range:	
Target standard deviation σ_{pt}	**881**
Target standard deviation (for Information)	1920
lower limit of target range	**-**
upper limit of target range	**-**
Quotient S/σ_{pt}*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*608*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,69*
Results in the target range	-
Percent in the target range	-

Comments to the statistic data:

A valuation of results using z-scores was not performed due to the small number of results of < 7. The following comments are for information only:

The target standard deviation was calculated according to the general model of Horwitz. All results were in the target range of 9440-13000 µg/100g.

The evaluation showed a normal variability of results. The quotient S*/σ_{pt} was below 2,0. The robust standard deviation is in the range of previous PTs (see 3.6.3). The repeatability and reproducibility standard deviations were in the range of established values for the applied methods (see 3.6.2). The quotient $U_{(Xpt)}/\sigma_{pt}$ was 0,69. Although it was not below 0,3 it is acceptable due to the other statistical data and the use of different analytical methods.

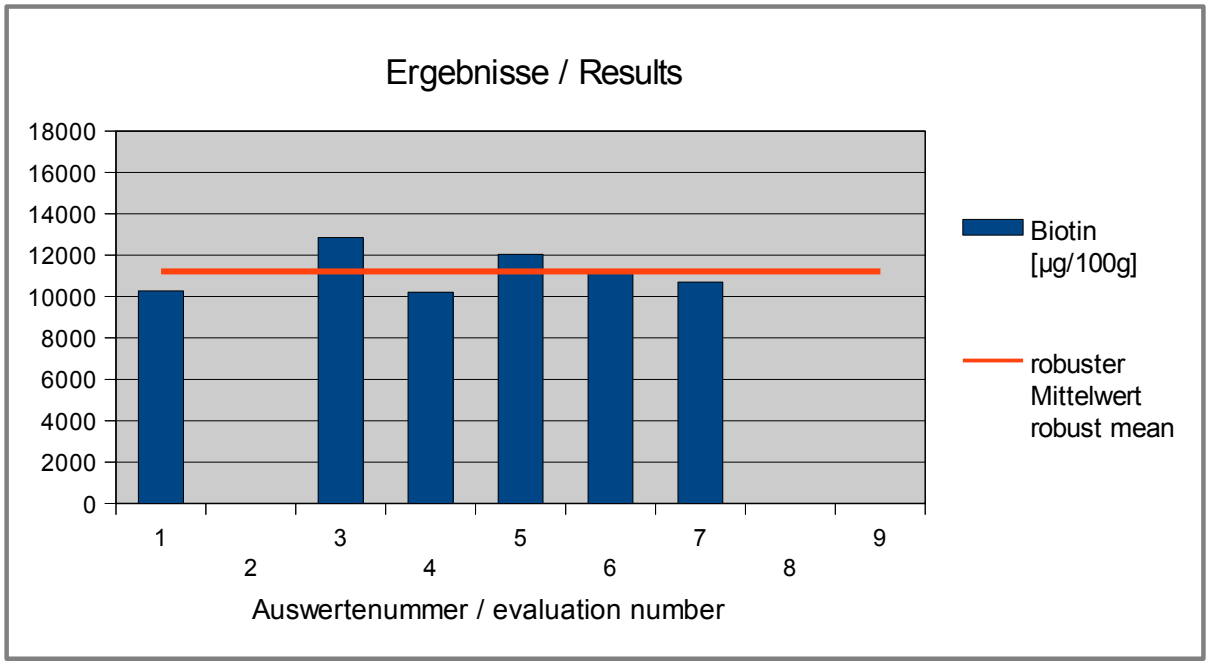

Abb. 1: Ergebnisse Biotin
Fig. 1: Results biotin

Comments:
The kernel density estimation was not calculated due to a number of < 8 results.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Biotin [µg/100g]	Abweichung [µg/100g] Deviation [µg/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	10278	-929			
2					
3	12853	1646			
4	10213	-993			
5	12040	834			
6	11154	-52			
7	10700	-506			
8					
9					

4.2 Niacin in mg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	8
Number of outliers	0
Mean	1520
Median	1520
Robust Mean (X)	**1530**
Robust standard deviation (S*)	**107**
Number with 2 replicates	8
Repeatability SD (S_r)	22,7
Repeatability (CV_r)	1,50%
Reproducibility SD (S_R)	126
Reproducibility (CV_R)	8,29%
Target range:	
Target standard deviation σ_{pt}	**57,4**
Target standard deviation (for Information)	58,3
lower limit of target range	**1410**
upper limit of target range	**1640**
Quotient S/σ_{pt}*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*47*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,82*
Results in the target range	*6*
Percent in the target range	*75%*

Comments to the statistic data:

The target standard deviation was calculated according to the general model of Horwitz.

The evaluation showed a normal variability of results. The quotient S^*/σ_{pt} was below 2,0. The robust standard deviation is in the range of previous PTs (see 3.6.3). The comparability of results is given.

The repeatability and reproducibility standard deviations were in the range of established values for the applied methods (see 3.6.2).

The quotient $U_{(Xpt)}/\sigma_{pt}$ was 0,82. Although it was not below 0,3 it is acceptable due to the other statistical data and the use of different analytical methods.

75% of results were in the target range.

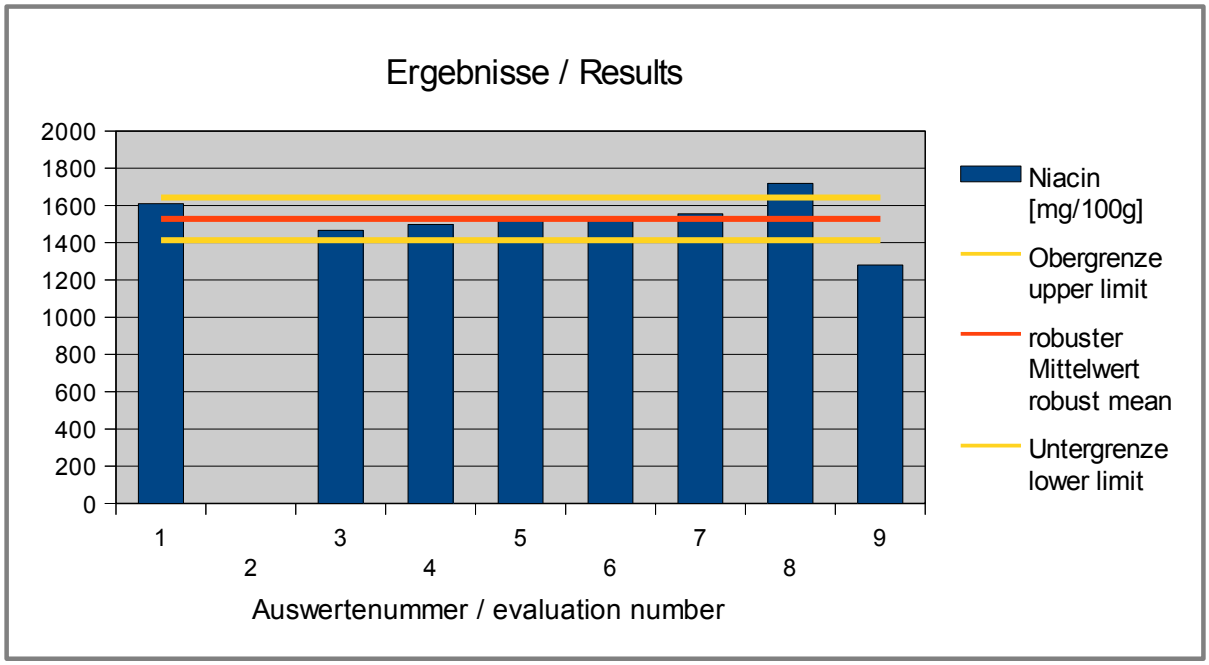

Abb. 2: Ergebnisse Niacin
Fig. 2: Results niacin

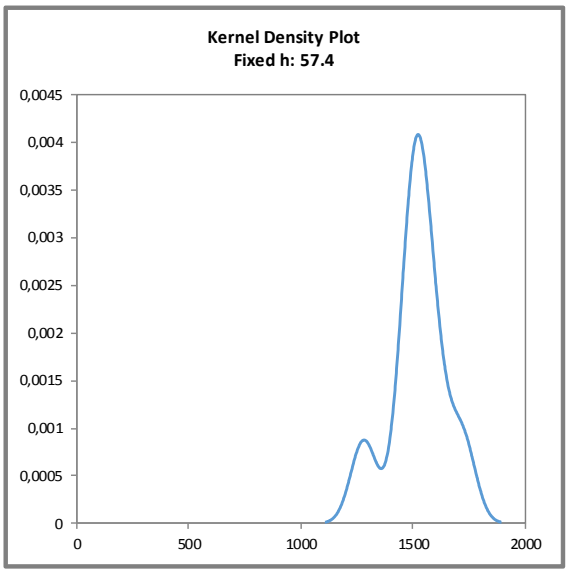

Abb. 3: Kerndichte-Schätzung der Ergebnisse für Niacin
(mit h = 1,0 x σ_{pt} von X_{pt})

Fig. 3: Kernel density plot of niacin results
(with h = 1,0 x σ_{pt} von X_{pt})

Comments:
The kernel density estimation shows a normal distribution of results with a minor peak and a shoulder, which are due to two participants' results outside the target range (s. fig. 3).

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Niacin [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	1609	81	1,4	1,4	
2					
3	1466	-62	-1,1	-1,1	
4	1498	-31	-0,5	-0,5	
5	1522	-6	-0,1	-0,1	
6	1520	-8	-0,1	-0,1	
7	1555	27	0,5	0,5	
8	1719	190	3,3	3,3	result without recovery 92,7% = 1594 (z-Score 1,1)
9	1280	-248	-4,3	-4,3	

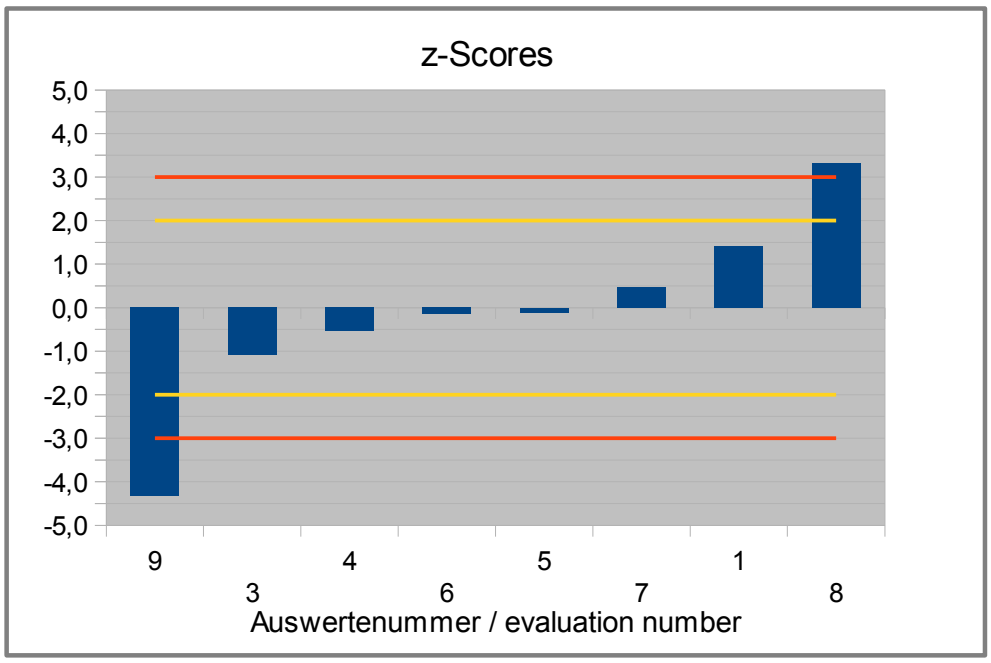

Abb. 4: Z-Scores Niacin
Fig. 4: Z-Scores niacin

4.3 Pantothenic Acid in mg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	8
Number of outliers	0
Mean	598
Median	589
Robust Mean (X)	**598**
Robust standard deviation (S*)	**41,1**
Number with 2 replicates	8
Repeatability SD (S_r)	11,0
Repeatability (CV_r)	1,84%
Reproducibility SD (S_R)	37,3
Reproducibility (CV_R)	6,23%
Target range:	
Target standard deviation σ_{pt}	**25,8**
lower limit of target range	**546**
upper limit of target range	**650**
Quotient S/σ_{pt}*	*1,6*
Standard uncertainty $U_{(Xpt)}$	*18,2*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,70*
Results in the target range	*7*
Percent in the target range	*88%*

Comments to the statistic data:

The target standard deviation was calculated according to the general model of Horwitz.

The evaluation showed a low variability of results. The quotient S^*/σ_{pt} was clearly below 2,0. The robust standard deviation is in the the range of previous PTs (see 3.6.3). The comparability of results is given.

The repeatability and reproducibility standard deviations were in the range of established values of methods for water-soluble vitamins (see 3.6.2).

The quotient $U_{(Xpt)}/\sigma_{pt}$ was 0,70. Although it was not below 0,3 it is acceptable due to the other statistical data and the use of different analytical methods.

88% of results were in the target range.

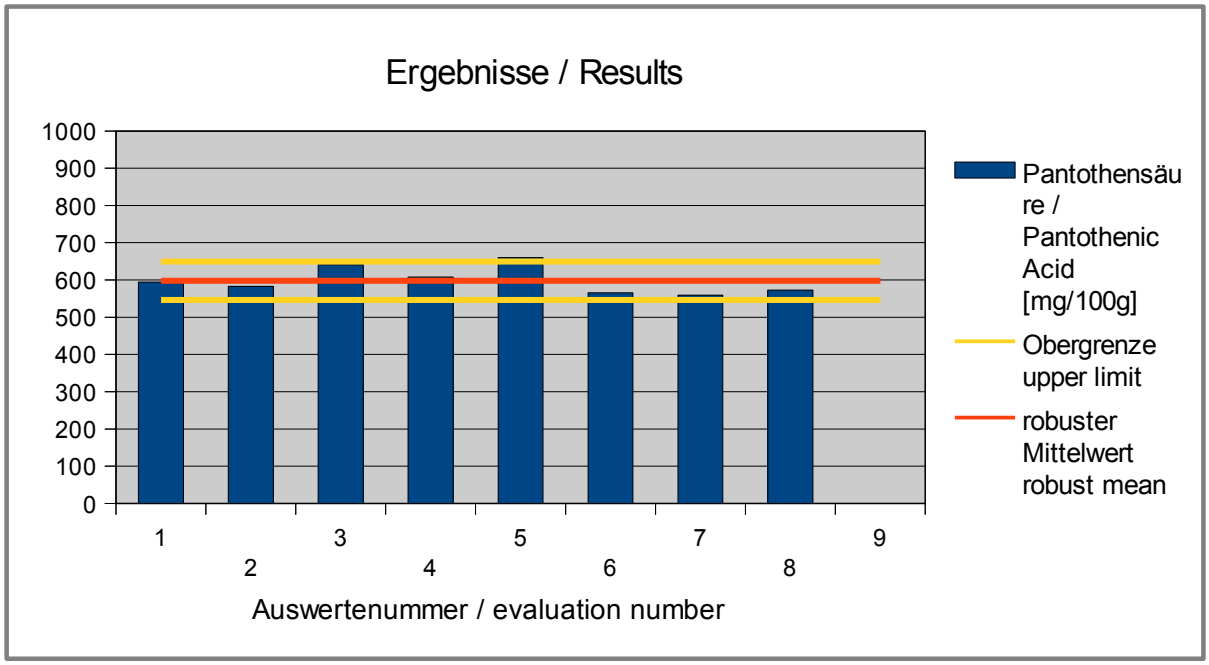

Abb. 5: Ergebnisse Pantothensäure
Fig. 5: Results pantothenic acid

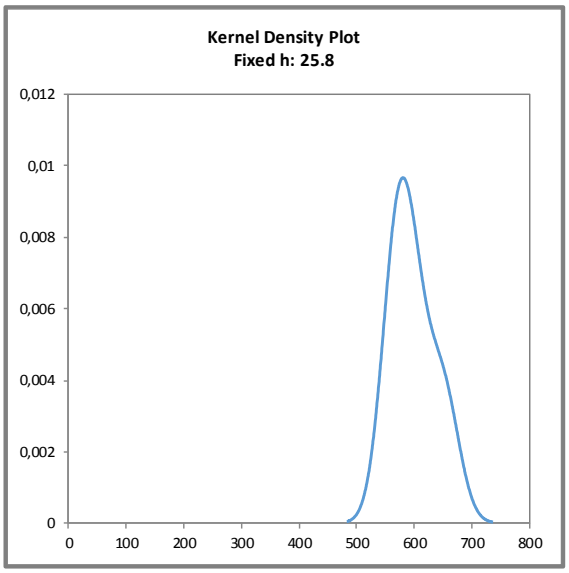

Abb. 6: Kerndichte-Schätzung der Ergebnisse für Pantothensäure
(mit h = 1,0 x σ_{pt} von X_{pt})

Fig. 6: Kernel density plot of pantothenic acid results
(with h = 1,0 x σ_{pt} von X_{pt})

Comments:
The kernel density estimation shows a normal distribution of results with a slight shoulder, which is due to a participants' result outside the target range (s. fig. 6).

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Pantothen-säure / Pantothenic Acid [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	594	-4	-0,1		
2	583	-15	-0,6		
3	641	43	1,7		
4	608	10	0,4		
5	660	62	2,4		
6	566	-32	-1,3		
7	559	-39	-1,5		
8	573	-25	-1,0		
9					

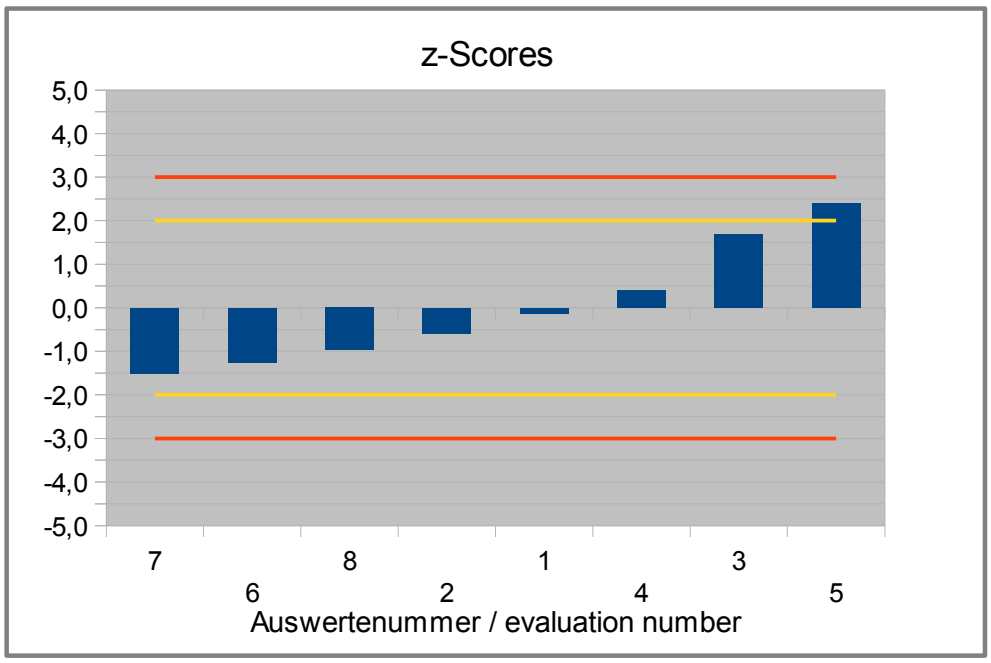

Abb. 7: Z-Scores Pantothensäure
Fig. 7: Z-Scores pantothenic acid

4.4 Vitamin C in mg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	7
Number of outliers	1*
Mean	6130
Median	6130
Robust Mean (X)	**6130**
Robust standard deviation (S*)	**365**
Number with 2 replicates	7
Repeatability SD (S_r)	152
Repeatability (CV_r)	2,47%
Reproducibility SD (S_R)	340
Reproducibility (CV_R)	5,54%
Target range:	
Target standard deviation σ_{pt}'	**254**
Target standard deviation (for Information)	644
lower limit of target range	**5620**
upper limit of target range	**6640**
Quotient S/σ_{pt}'*	*1,4*
Standard uncertainty $U_{(Xpt)}$	*173*
Quotient $U_{(Xpt)}/\sigma_{pt}$'	*0,68*
Results in the target range	*7*
Percent in the target range	*100%*

* The result of participant no. 3 was excluded, due to its influence on statistical valuation of the remaining results despite applying robust statistics.

Comments to the statistic data:

The target standard deviation was calculated according to the general model of Horwitz considering the standard uncertainty.

The distribution of results showed a normal variability in comparison to the target standard deviation of standardized methods, but it was increased in comparison to the target standard deviation according to Howitz. Therefore the target standard deviation σ_{pt}' and the z'-Score were used for evaluation. The quotient S*/σ_{pt}' was below 2,0. The robust standard deviation is in the the range of previous PTs (see 3.6.3). The comparability of results is given.

The repeatability and reproducibility standard deviations were in the range of established values for the applied methods (see 3.6.2).

The quotient $U_{(Xpt)}/\sigma_{pt}$ was 0,68. Although it was not below 0,3 it is acceptable due to the other statistical data and the use of different analytical methods.

All results were in the target range.

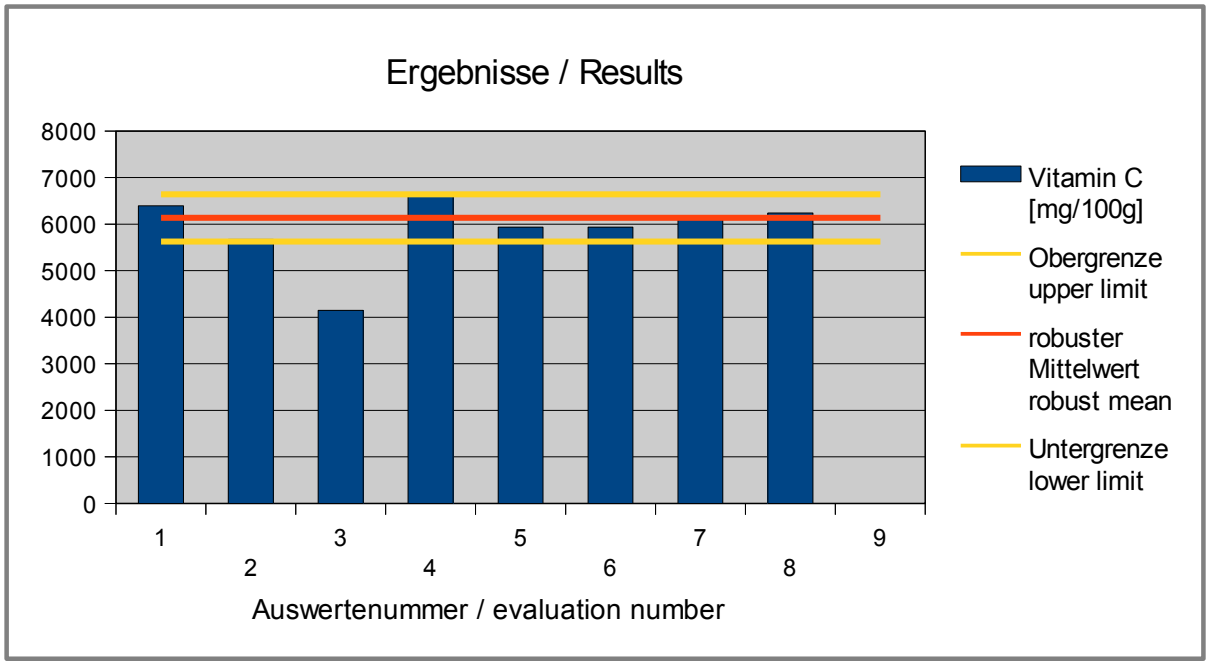

Abb. 8: Ergebnisse Vitamin C
Fig. 8: Results vitamin C

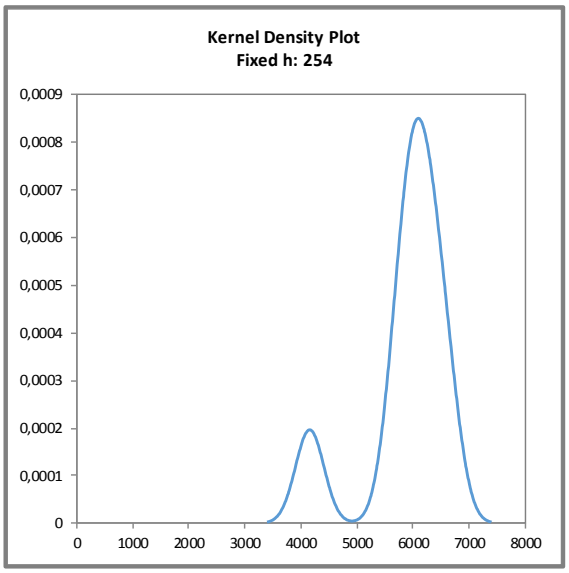

Abb. 9: Kerndichte-Schätzung der Ergebnisse für Vitamin C
(mit h = 1,0 x σ_{pt} von X_{pt})

Fig. 9: Kernel density plot of vitamin C results
(with h = 1,0 x σ_{pt} von X_{pt})

Comments:
The kernel density estimation shows a normal distribution of results with an additional peak, which is due to the excluded outlier (s. fig. 9).

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Vitamin C [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	6393	260	1,0	0,4	
2	5671	-462	-1,8	-0,7	
3	4149				Ausreißer ausgeschlossen / Outlier excluded
4	6635	501	2,0	0,8	
5	5935	-198	-0,8	-0,3	
6	5934	-200	-0,8	-0,3	
7	6128	-5	0,0	0,0	
8	6239	105	0,4	0,2	
9					

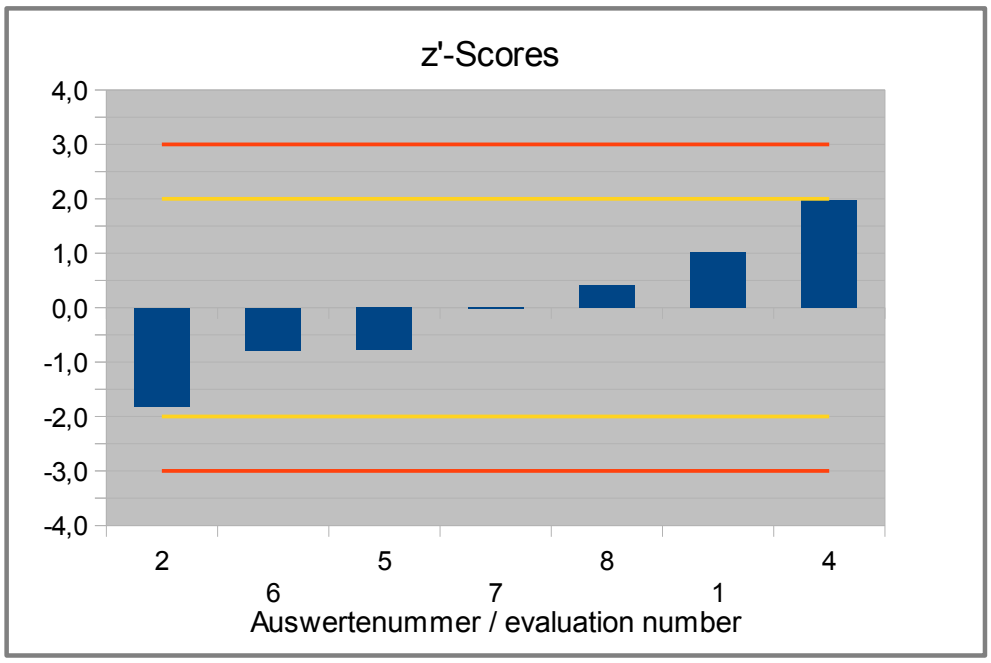

Abb. 10: Z-Scores Vitamin C
Fig. 10: Z-Scores vitamin C

5. Documentation

5.1 Primary data

Parameter	Teilnehmer	Einheit	Proben-Nr. A	Proben-Nr. B	Datum d. Analyse	Ergebnis (Mittel)	Ergebnis A	Ergebnis B	Bestim-mungsgren-ze	Inkl. WF	Wiederfin-dungsrate [%]
Analyte	**Participant**	**Unit**	**Sample No. A**	**Sample No. B**	**Date of analysis**	**Result (Mean)**	**Result A**	**Result B**	**Limit of de-termination**	**Incl. RR**	**Recovery rate [%]**
Biotin	1	µg/100g	26	48	08.07.16	10277,52	10356,64	10198,4	n/a	no	n/a
	2	µg/100g	06	39							
	3	µg/100g	13	51	13.07.16	12852,69	12852,69			yes	119
	4	µg/100g	32	54	22.06.16	10213,43	10341,32	10085,54	70µg/100g	no	103
	5	µg/100g	10	24	26.07.16	12040	12101	11979		no	
	6	µg/100g	2	47		11154,28	11268,45	11040,1		no	
	7	µg/100g	17	60	13.07.16	10700	10800	10500	10,4	n.a.	n.a.
	8	µg/100g	19	35							
	9	µg/100g	30	43							

Parameter	Teilnehmer	Einheit	Proben-Nr. A	Proben-Nr. B	Datum d. Analyse	Ergebnis (Mittel)	Ergebnis A	Ergebnis B	Bestim-mungsgren-ze	Inkl. WF	Wiederfin-dungsrate [%]
Analyte	**Participant**	**Unit**	**Sample No. A**	**Sample No. B**	**Date of analysis**	**Result (Mean)**	**Result A**	**Result B**	**Limit of de-termination**	**Incl. RR**	**Recovery rate** [%]
Niacin	1	mg/100g	26	48	08.07.16	1609,39	1600,97	1617,81	n/a	no	n/a
	2	mg/100g	06	39							
	3	mg/100g	13	51	5.07. + 25.07.16	1465,98	1440	1491,95		yes	100 and 95,91
	4	mg/100g	32	54	22.06.16	1497,75	1523,09	1472,41	3mg/100g	no	103
	5	mg/100g	10	24	09.08.16	1522	1514	1530		no	
	6	mg/100g	2	47		1520,32	1524,15	1516,49		o	
	7	mg/100g	17	60	13.07.16	1555	1569	1542	0.00024	n.a.	n.a.
	8	mg/100g	19	35	04.-05.07.	1718,78	1732,34	1705,22	1	yes	92,33/93,11
	9	mg/100g	30	43	18.07.	1280,3	1295,7	1264,9	0,01	no	

Parameter	Teilnehmer	Einheit	Proben-Nr. A	Proben-Nr. B	Datum d. Analyse	Ergebnis (Mittel)	Ergebnis A	Ergebnis B	Bestim-mungsgren-ze	Inkl. WF	Wiederfin-dungsrate [%]
Analyte	**Participant**	**Unit**	**Sample No. A**	**Sample No. B**	**Date of analysis**	**Result (Mean)**	**Result A**	**Result B**	**Limit of de-termination**	**Incl. RR**	**Recovery rate** [%]
Pantothen-säure / pantothenic acid	1	mg/100g	26	48	08.07.16	594,4	595,05	593,74	n/a	no	n/a
	2	mg/100g	06	39	19.07.16	582,62	592,76	572,48		no	
	3	mg/100g	13	51	5.07. + 25.07.16	641,22	631	651,44		yes	100 and
	4	mg/100g	32	54	22.06.2016	608,01	613,87	602,14	3mg/100g	no	103
	5	mg/100g	10	24	28.07.16	660	670	650		no	
	6	mg/100g	2	47		565,55	567,88	563,22		no	
	7	mg/100g	17	60	13.07.16	559	570	547	0	n.a.	n.a.
	8	mg/100g	19	35	04.-05.07.	572,95	571,11	574,79	1	yes	97,73/97,41
	9	mg/100g	30	43							

Parameter	Teilnehmer	Einheit	Proben-Nr. A	Proben-Nr. B	Datum d. Analyse	Ergebnis (Mittel)	Ergebnis A	Ergebnis B	Bestim-mungsgren-ze	Inkl. WF	Wiederfin-dungsrate [%]
Analyte	**Participant**	**Unit**	**Sample No. A**	**Sample No. B**	**Date of analysis**	**Result (Mean)**	**Result A**	**Result B**	**Limit of de-termination**	**Incl. RR**	**Recovery rate** [%]
Vitamin C	1	mg/100g	26	48	17.08.16	6392,99	6402,62	6383,36	n/a	no	n/a
	2	mg/100g	06	39	26.07.16	5671	5710	5631		no	
	3	mg/100g	13	51	02.08. + 08.08.16	4149,14	4340,28	3958		yes	91,09 and 91,99
	4	mg/100g	32	54	22.06.16	6634,79	6676,69	6592,9	3mg/100g	no	101
	5	mg/100g	10	24	03.08.16	5935	5716	6154		no	
	6	mg/100g	2	47		5933,52	5874,55	5992,5		no	
	7	mg/100g	17	60	01.08.16	6128	6072	6185	0.6	n.a.	n.a.
	8	mg/100g	19	35	28.-29.06	6238,9	6388,77	6089,02	5	yes	102,10/104,63
	9	mg/100g	30	43							

5.2 Homogeneity

5.2.1 Homogeneity of bottled PT-samples

Homogeneity test of niacin by HPLC-UV:

Independant samples	mg/100g
1	16,2
2	16,3
3	16,3
4	16,1
5	16,3
6	16,0
7	16,3
8	16,4

Mean	16,2	
Repeatability Standard Deviation	0,130	0,80%

5.2.2 Comparison of sample numbers / test results and trend line

By comparison of the increasing sample numbers and the measurement results of participants, the homogeneity of the chronological bottled PT item can be characterized with the help of the trend line function:

Niacin				
Target standard deviation σ_{pt}	57			mg/100g
Sample numbers	2 - 60			
Total numbers of samples	16			
Slope	-2,29			
Trend line range	15370	-	15407	mg/100g
Deviation trend line	15389	±	18,5	mg/100g
Percent of σpt	32,2	%		

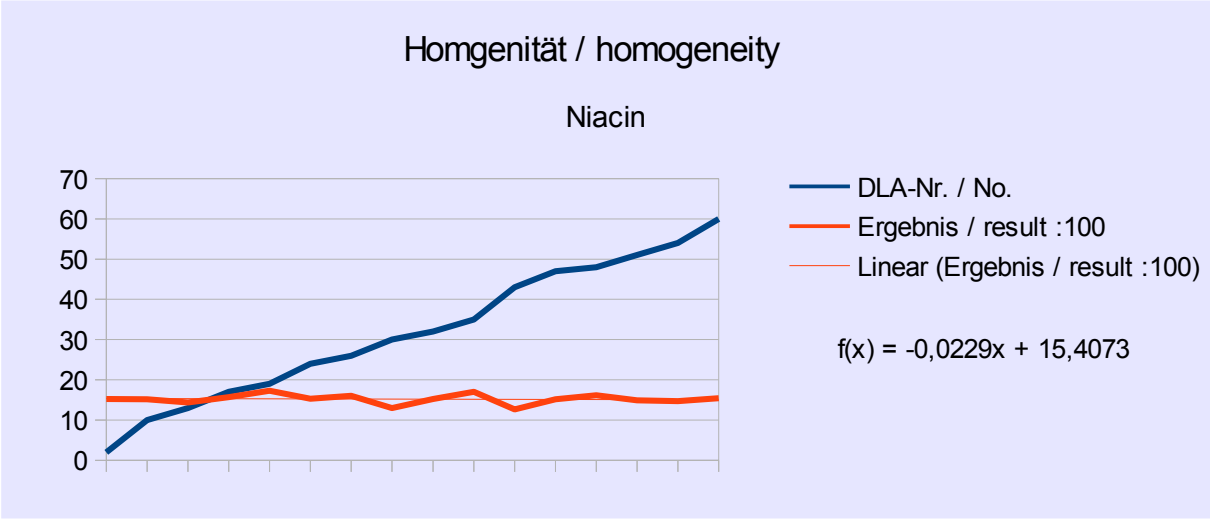

Abb. 11: Trendfunktion Probennummern / Niacin Ergebnisse (1/100 dargestellt)
Fig. 11: trend line function sample number / niacin results (1/100 shown)

Pantothenic Acid	ohne Teilnehmer 5*			
Target standard deviation σ_{pt}	25,8			mg/100g
Sample numbers	2 - 60			
Total numbers of samples	14			
Slope	0,0780			
Trend line range	588	-	589	mg/100g
Deviation trend line	589	±	0,5	mg/100g
Percent of σpt	1,9	%		

* höchste Werte außerhalb des Zielbereichs

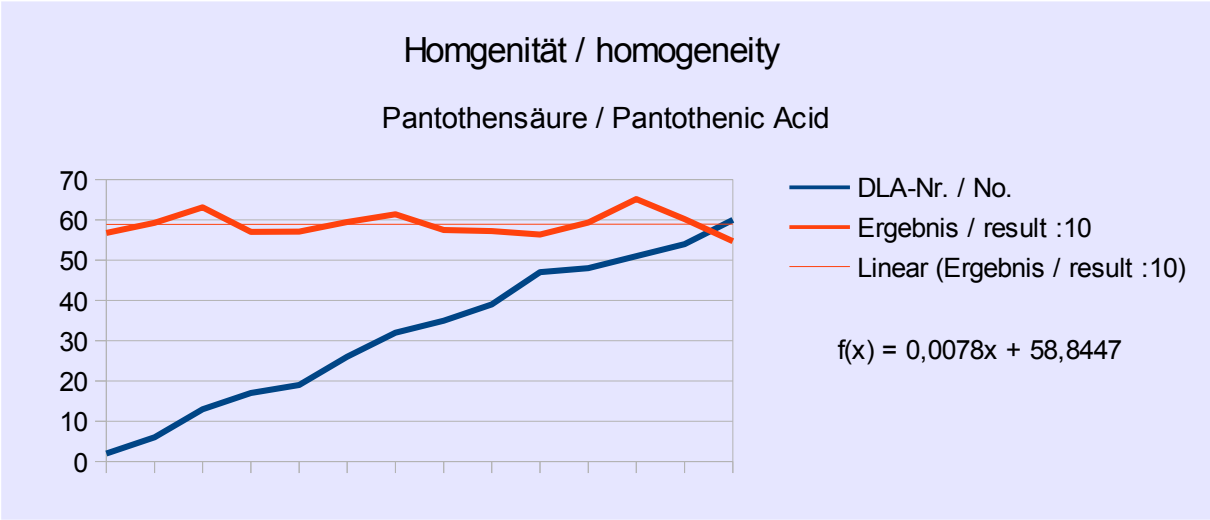

Abb. 12: Trendfunktion Probennummern / Pantothensäure Ergebnisse (:10 dargestellt)
Fig. 12: trend line function sample number / pantothenic acid results (:10 shown)

5.3 Analytical Methods

Details by the participants

Parameter	Teilnehmer	Methodenbeschreibung	Wiederfindung mit gleicher Matrix	Methode akkre-ditiert	Sonstige Hinweise
Analyte	**Participant**	**Method description**	**Recovery with same matrix**	**Method accredi-ted**	**Further remarks**
Biotin	1	HPLC	no	yes	n/a
	2				
	3	by ELISA	no	no	
	4	HPLC-DAD; in-house method	yes	yes	
	5	Microbiological (Vita fast Biotin) ratiopharm Nr.P1003		yes	
	6		no	yes	
	7	Extraction with formiate buffert. Determination with UHPLC-QQQ.	na	yes	
	8				
	9				

Parameter	Teilnehmer	Methodenbeschreibung	Wiederfindung mit gleicher Matrix	Methode akkreditiert	Sonstige Hinweise
Analyte	**Participant**	**Method description**	**Recovery with same matrix**	**Method accredited**	**Further remarks**
Niacin	1	HPLC	no	yes	n/a
	2				
	3	by HPLC/UV	yes	no	
	4	HPLC-DAD; in-house method	yes	yes	
	5	HPLC		yes	
	6		no	yes	
	7	Extraction with formiate buffert. Determination with UHPLC-QQQ.	na	yes	
	8	LAV 21.0017-02, HPLC-DAD	yes	yes	
	9	HPLC-DAD			

Parameter	Teilnehmer	Methodenbeschreibung	Wiederfindung mit gleicher Matrix	Methode akkreditiert	Sonstige Hinweise
Analyte	**Participant**	**Method description**	**Recovery with same matrix**	**Method accredited**	**Further remarks**
Pantothensäure / pantothenic acid	1	HPLC	no	yes	n/a
	2	HPLC-UV	yes	no	
	3	by HPLC/UV	yes	no	
	4	HPLC-DAD; in-house method	yes	yes	
	5	Microbiological (Vita fast Pantothenic acid) ratiopharm Nr.P1005		yes	
	6		no	yes	
	7	Extraction with formiate buffert. Determination with UHPLC-QQQ.	na	yes	
	8	LAV 21.0017-02, HPLC-DAD	yes	yes	
	9				

Parameter	Teilnehmer	Methodenbeschreibung	Wiederfindung mit gleicher Matrix	Methode akkreditiert	Sonstige Hinweise
Analyte	**Participant**	**Method description**	**Recovery with same matrix**	**Method accredited**	**Further remarks**
Vitamin C	1	Titration	no	yes	n/a
	2	HPLC-UV	yes	yes	
	3	enzymatically by UV/VIS-Test according to in-house method	yes	yes	
	4	HPLC-DAD; in-house method	yes	yes	
	5	UPLC		yes	
	6		no	yes	
	7	Extraction in water with DTT. Determination with UHPLC-DAD, wavelength: 245 nm	na	yes	
	8	LAV 21.0052-01, HPLC-DAD	yes	yes	
	9				

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		FRANCE
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		USA
		SWEDEN

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]

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

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
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 Anan-lytical 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. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. 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
16. EN 14130:2003; Untersuchung von Lebensmitteln: Bestimmung von Vitamin C mit HPLC (zurückgezogen) / Foodstuffs. Determination of vitamin C by HPLC (withdrawn)
17. EN 15607:2009; Untersuchung von Lebensmitteln: Bestimmung von D-Biotin mit HPLC / Foodstuffs. Determination of d-biotin by HPLC
18. EN 15652:2009; Untersuchung von Lebensmitteln: Bestimmung von Niacin mit HPLC / Foodstuffs. Determination of niacin by HPLC
19. EURL Evaluation Report on Analytical Methods D(+)Biotin, European Reference Laboratory Feed Additives, 2011
20. Rychlik M, Fortified Foods with Vitamins: Analytical Concepts to Assure Better and Safer Products, John Wiley & Sons, 2011
21. Brause et al., Determination of Total Vitamin C in Fruit Juices and Related Products by Liquid Chromatography: Interlaboratory Study, J AOAC Int 86(3): 367-374, 2003
22. Heudi et al., Separation of water-soluble vitamins by reversed-phase high

performance liquid chromatography with ultra-violet detection: application to polyvitaminated premixes, J Chromatogr A. 1070(1-2):49-56 (2005)
23. Ministry of Health and Welfare, JSM, Japan 2006
24. Blake CJ (2007), Analytical procedures for water-soluble vitamins in foods and dietary supplements: a review. Anal Bioanal Chem 389(1):63-76
25. 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