Evaluation Report
proficiency test

DLA 53/2019

Cosmetic Products III:

Coenzyme Q10, Panthenol and Tocopherol

in Skin Cream

DLA - Proficiency Tests GmbH
Kalte Weide 21
24641 Sievershütten/Germany

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

Koordinator der LVU:
Dr. Matthias Besler-Scharf

Allgemeine Informationen zur Eignungsprüfung (EP)
General Information on the proficiency test (PT)

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Kalte Weide 21, 24641 Sievershütten, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
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Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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1. Introduction

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

2. Realisation

2.1 Test material

The test material is a mixture of a common in commerce hand cream and a body lotion from European Suppliers.

The materials were mixed and homogenized. The composition of the PT samples (list of ingredients) is shown in table 1.

Afterwards the samples were portioned to approximately 25 g into 28 ml plastic containers, sealed in metallised PET film bags and chronologically numbered.

Table 1: Composition of DLA-Samples

PT-Samples Skin Cream
Skin cream / Body lotion 1 Ingredients: Aqua, Glycerin, Glycine Soja Oil, Dicaprylyl Ether, Glyceryl Sterate SE, Cetearyl Alcohol, Phenoxyethanol, Simmondsia Chinesis Seed Oil, Panthenol, Tocophryl Acetate, Carbomer, Parfum, Sodium Hydroxide, Allantoin, Ubiquinone, Ethylhexylglycerin
Skin cream / Hand cream 2 Ingredients: Aqua, Glycerin, Ethylhexyl Salicylate, Butylmethoxydibenzoylmethane, Glyceryl Stearate SE, Caprylic/Capric Triglyceride, Cetyl Alcohol, Isopropyl Palmitate Glycine Soja Oil, Helianthus Annuus Seed Oil, Ubiquinone, Panthenol, Tocopherylacetate, Butylene Glycol, Carbomer, Phenoxyethanol, Sodium Cetearyl Sulfate, Parfum, Glyceryl Stearate Citrate, Ethylhexylglycerin, Tetrasodium Glutamate Diacetate, Sodium Hydroxide, Citric Acid

Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

2.1.1 Homogeneity

The calculation of the **repeatability standard deviations S_r of the participants' double determination** was used as an indicator of homogeneity for this PT. The repeatability standard deviation was in the range of 1,4% - 7,0%) (see Table 2) and thus in the normal to low range of comparable methods.
The repeatability standard deviations of the participants' results are given in the documentation in the statistic data (see 4.1 to 4.3).

Table 2: Repeatability standard deviation S_r of double determinations of the participants (coefficient of variation CV_r in %)

Parameter	**CV_r**
Coenzyme Q10	1,43 %
Panthenol	7,03 %
DL-alpha-Tocopheryl Acetate	4,05 %

Furthermore, the homogeneity was graphically characterized for information by the **trend line function of participants' results for chronological bottled single samples** (s. 5.2.1).

In case the criterion for sufficient homogeneity of the test items is not fulfilled the impact on the target standard deviation will be verified. If necessary the evaluation of results will be done considering the standard uncertainty of the assigned value by z'-scores (s. 3.8 and 3.11) [3].

2.1.2 Stability

Experience has shown that unopened preserved skin creams are stable for several years. For the products, the manufacturer gave a shelf life of 12 months after opening. The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 49th week of 2019. The testing method was optional. The tests should be finished at 24th January 2020 the latest.

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

The two portions contain identical samples of a mixture of common in commerce skin creams with the parameters Coenzyme Q10 (Ubiquinone), Panthenol and Tocopherol (Tocopheryl acetate) to be determined.

Note: Please store the samples at 2-10°C on arrival.

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

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.

14 of 15 participants submitted their results in time. One participant submitted no results.

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]. If there are < 12 quantitative results and an increased difference between robust mean and median, the median may be used as the assigned value (criterion: Δ median - rob. mean > 0,3 σ_{pt}) [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, in justified cases, an evaluation may also be carried out from 5 results onwards.
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*) 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 CV_R in percent of the mean is given as variation coefficient in the statistical data of participant for each parameter. The significance of CV_R is further explained in section 3.9.

3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, too few significant digits (valid digits) or results for another proficiency test item can be removed from the data set [2]. Even if a result e.g. with a factor >10 deviates significantly from the mean and has an influence on the robust statistics, a result of the statistical evaluation can be excluded [3].
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 tested for outliers by the use of robust statistics (algorithm A): If a value deviates from the robust mean by more than 3 times the robust standard deviation, it can be classified as an outlier (see above) [3]. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3]. Detected outliers are only mentioned in the results section, if they have been excluded from the statistical evaluation.

3.6 Target standard deviation (for proficiency assessment)

The target standard deviation of the assigned value σ_{pt} (= standard deviation for proficiency assessment) can be determined according to the following methods.
If an acceptable quotient S*/σ_{pt} is present, the target standard deviation of the general model by Horwitz is preferably used for the proficiency assessment. It is usually suitable for evaluation of interlaboratory studies, where different methods are applied by the participants. On the other hand the target standard deviation from the evaluation of precision data of an precision experiment is derived from collaborative studies with specified analytical methods.

For valuation of coenzyme Q10, panthenol and tocopherol (calculated as DL-alpha-tocopheryl acetate) in the present PT the target standard deviation according to the general model of Horwitz was applied (see 3.6.1). Additionally for DL-alpha-tocopheryl acetate the standard uncertainty was considered by evaluation using z'-scores (see 3.8).

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]:

$$\sigma_{pt} = \sqrt{\sigma_R^2 - \sigma_r^2\left(m - 1/m\right)}$$

For the determination of coenzyme Q10, panthenol and tocopherol in cosmetic products to our knowledge currently there are no sufficient data available on relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from interlaboratory comparisons or ring trials.

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 was regarded suitable.

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 valid z-Score for each parameter is indicated as z-Score (σ_{pt}).

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.
An error or cause analysis can be carried out by checking the analysis process including understanding and implementation of the measurement by the staff, details of the measurement procedure, calibration of equipment and composition of reagents, transmission error or an error in the calculation, in the trueness and precision and use of reference material. If necessary, the problems must be addressed through appropriate corrective action [3].

In the figures of z-scores DLA gives the limits of warning and action signals as yellow and red lines respectively. According to ISO 13528 the signals are valid only in case of a number of ≥ 10 results [3].

3.8 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. 3.11). The z'-score represents the relation of the deviation of the result (x_i) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation (σ_{pt}) and the standard uncertainty ($U_{(Xpt)}$) [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 cofficient of variation (CV_R)

The variation coefficient (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_R 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 levels or the performance evaluation of the participating laboratories possibly can not be done [3].

3.10 Quotient S*/σ_{pt}

Following the HorRat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation S* and target standard deviation σ_{pt} does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

3.11 Standard uncertainty and traceability

Every assigned value has a standard uncertainty that depends on the analytical method, differences between the analytical methods used, the test material, the number of participating laboratories (P) and on other factors. The standard uncertainty ($U_{(Xpt)}$) for this PT is calculated as follows [3]:

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

If $U_{(Xpt)} \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT [3]. Values exceeding 0,3 imply, that the target standard deviation could be too low with respect to the standard uncertainty of the assigned value.

The traceability of the assigned value is ensured on the basis of the consensus value as a robust mean of the participant results.

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}'
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 ($X_{pt} + 2\sigma_{pt}$´) *
Quotient S/σ_{pt} or S*/σ_{pt}'*
Standard uncertainty U(X_{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 table below, the results of the participating laboratories are formatted in 3 valid digits**:

Auswerte-nummer / **Evaluation number**	**Parameter [Einheit / Unit]**	**Abweichung** / **Deviation**	**z-Score σpt**	**Hinweis** / **Remark**

*** In the documentation part, the results are given as they were transmitted by the participants.*

4.1 Coenzyme Q10 (Ubiquinone) in mg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	11
Number of outliers	0
Mean	49,9
Median	49,0
Robust Mean (X_{pt})	**49,9**
Robust standard deviation (S*)	**4,85**
Number with 2 replicates	11
Repeatability SD (S_r)	0,713
Repeatability (CV_r)	1,43%
Reproducibility SD (S_R)	4,32
Reproducibility (CV_R)	8,66%
Target range:	
Target standard deviation σ_{pt}	**3,13**
lower limit of target range	**43,6**
upper limit of target range	**56,1**
Quotient S/σ_{pt}*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*1,83*
Results in the target range	*10*
Percent in the target range	*91%*

Comments to the statistic data:

The target standard deviation was calculated according to the general model of Horwitz (s. 3.6.1).
The evaluation of all methods showed a normal variability of results, with a quotient S/σ_{pt} below 2,0. The comparability of results is given.*

75% of results were in the target range.

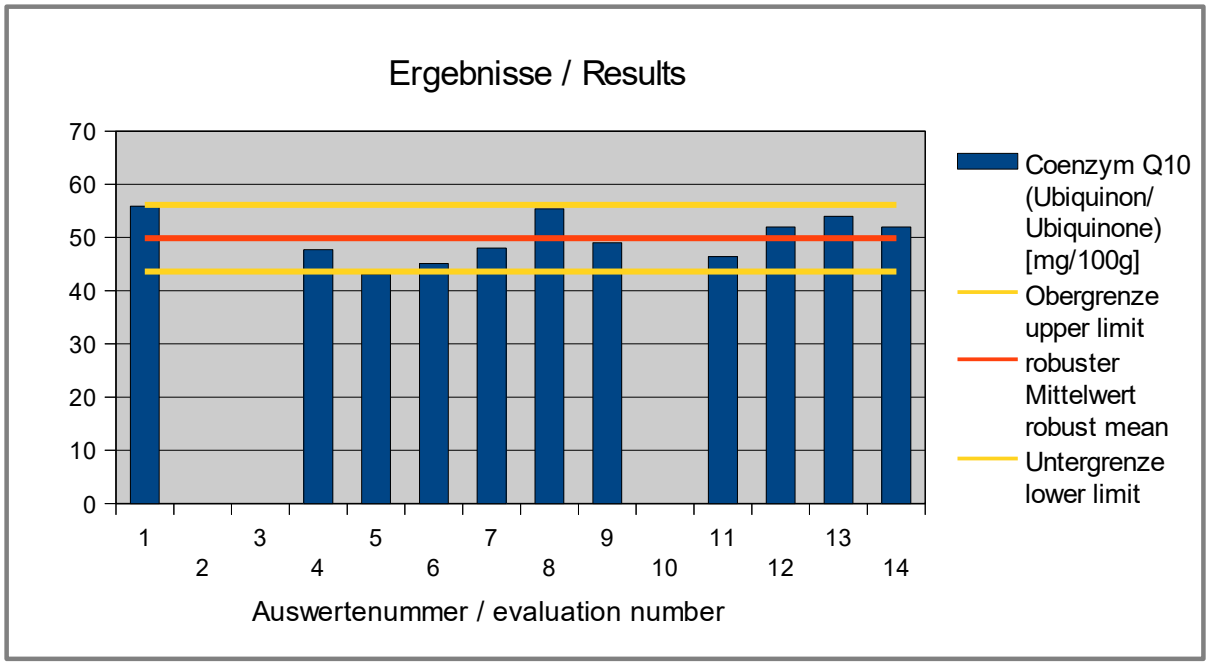

Abb. / Fig. 1: Ergebnisse Coenzyme Q10 (Ubiquinon)/ Results Coenzyme Q10 Ubiquinone)

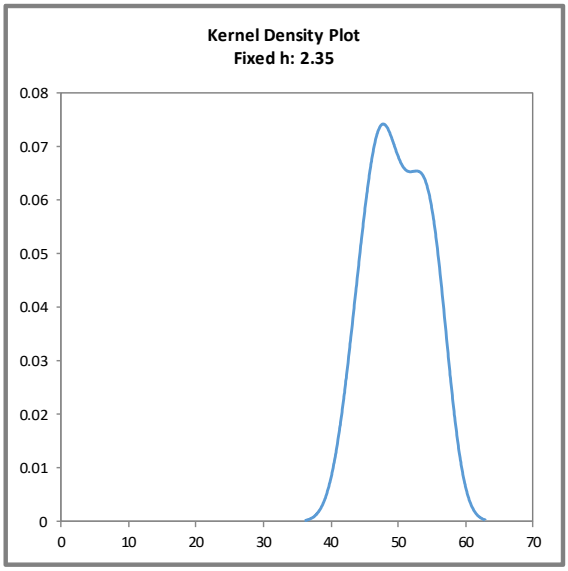

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

Kernel density plot of results (with h = 0,75 x σ_{pt} of X_{pt})

Comment:
The kernel density plot shows almost a symmetrical distribution of results with a clear shoulder at approx. 55 mg/100g.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Coenzyme Q10 (Ubiquinon/ Ubiquinone) [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z-Score (σ_{pt})	Hinweis Remark
1	55,9	6,03	1,9	
2				
3				
4	47,7	-2,18	-0,69	
5	43,1	-6,78	-2,2	
6	45,1	-4,76	-1,5	
7	48,0 *	-1,88	-0,60	
8	55,4 *	5,52	1,8	
9	49,0	-0,88	-0,28	
10				
11	46,4	-3,48	-1,1	
12	52,0	2,12	0,68	
13	54,0	4,12	1,3	
14	52,0	2,12	0,68	

* Mean calculated by DLA

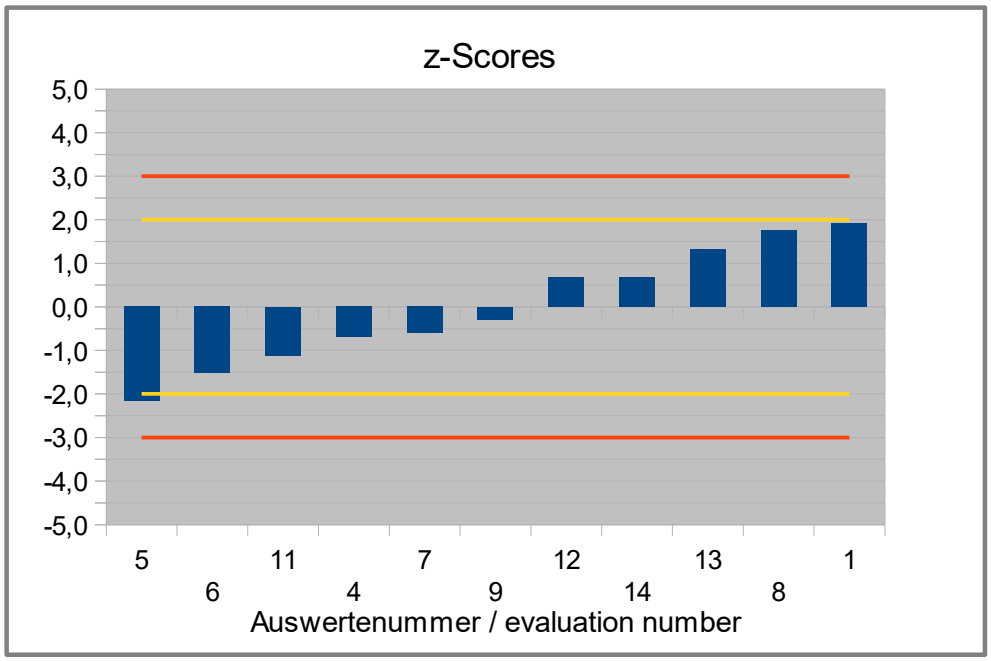

Abb. / Fig. 3: z-Scores Coenzyme Q10 (Ubiquinon/ Ubiquinone)

4.2 Panthenol in mg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results°	11
Number of outliers	2
Mean	428
Median	433
Robust Mean (X_{pt})	**429**
Robust standard deviation (S*)	**16,7**
Number with 2 replicates	11
Repeatability SD (S_r)	4,03
Repeatability (CV_r)	0,944%
Reproducibility SD (S_R)	15,9
Reproducibility (CV_R)	3,73%
Target range:	
Target standard deviation σ_{pt}	**19,5**
lower limit of target range	**390**
upper limit of target range	**468**
Quotient S/σ_{pt}*	*0,86*
Standard uncertainty $U_{(Xpt)}$	*6,30*
Results in the target range	*11*
Percent in the target range	*100%*

° *results without outliers (reults no. 2 and 10)*

Comments to the statistic data:

The target standard deviation was calculated according to the general model of Horwitz (s. 3.6.1).
The evaluation of all methods showed a low variability of results, with a quotient S/σ_{pt} below 1,0. The comparability of results is given.*

100% of results were in the target range.

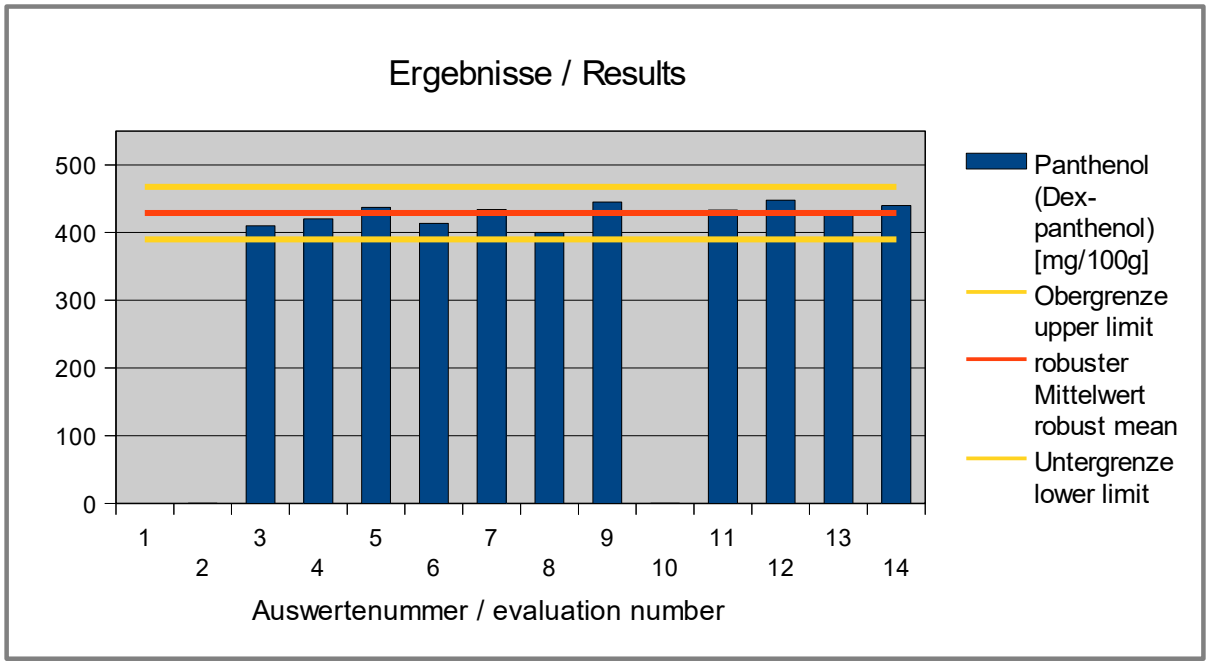

Abb. / Fig. 4: Ergebnisse / Results Panthenol

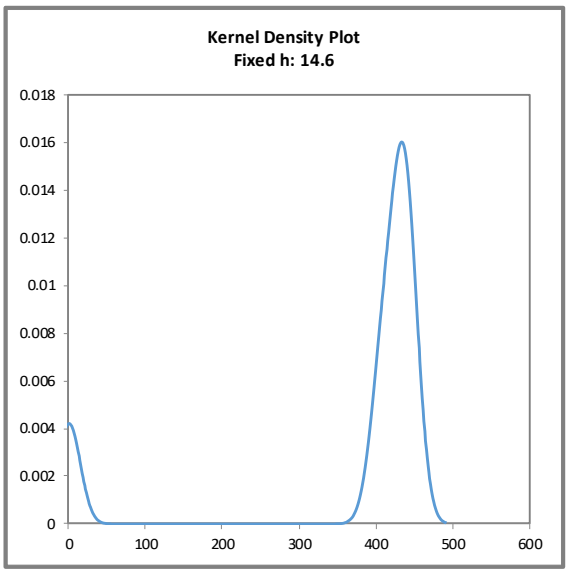

Abb. / Fig. 5:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von X_{pt})

Kernel density plot of results (with h = 0,75 x σ_{pt} of X_{pt})

Comment:
The kernel density plot shows a symmetrical distribution of results with smaller peaks at approx. 0,4 mg/100g, due to two results out of the target range.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Panthenol (Dex-panthenol) [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z-Score (σ_{pt})	Hinweis Remark
1				
2	0,420			Ausreißer ausgeschlossen / Outlier excluded
3	410	-18,8	-0,96	
4	420	-8,5	-0,44	
5	437	8,2	0,42	
6	413	-15,4	-0,79	
7	434 *	5,2	0,27	
8	400 *	-28,8	-1,5	
9	445	16,2	0,83	
10	0,445			Ausreißer ausgeschlossen / Outlier excluded
11	433	4,4	0,23	
12	448	19,2	0,99	
13	432	3,2	0,17	
14	440	11,2	0,58	

* Mean calculated by DLA

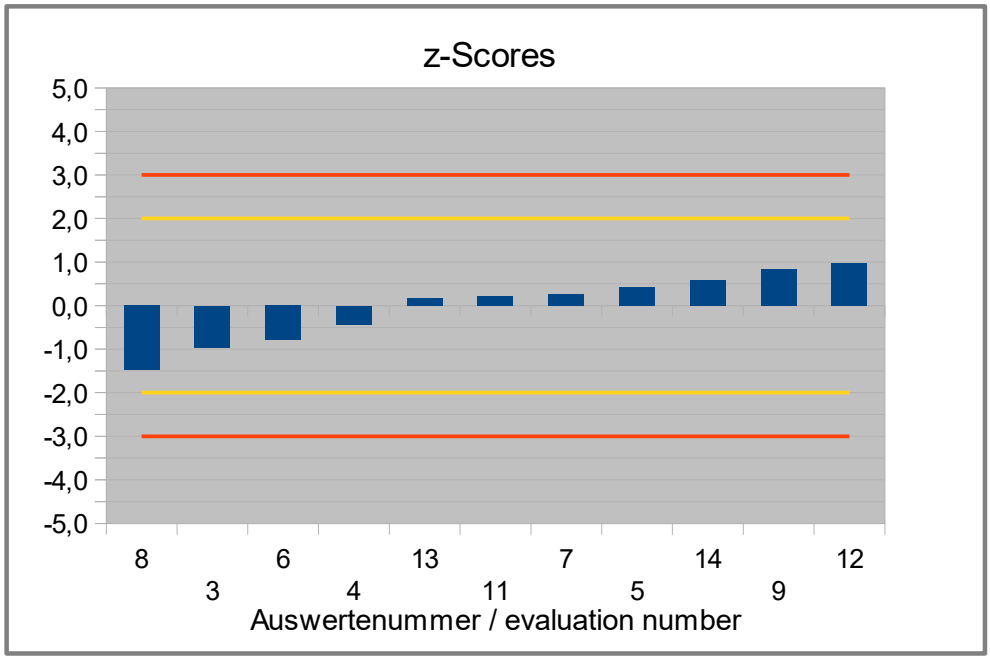

Abb. / Fig. 6: z-Scores Panthenol

4.3 DL-alpha-Tocopheryl Acetate in mg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results°	12
Number of outliers	1
Mean	273
Median	273
Robust Mean (x_{pt})	**271**
Robust standard deviation (S*)	**23,9**
Number with 2 replicates	10
Repeatability SD (S_r)	7,8
Repeatability (CV_r)	2,89%
Reproducibility SD (S_R)	16,9
Reproducibility (CV_R)	6,24%
Target range:	
Target standard deviation $\sigma_{pt'}$	**15,8**
lower limit of target range	**240**
upper limit of target range	**303**
Quotient S/$\sigma_{pt'}$*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*8,63*
Results in the target range	*10*
Percent in the target range	*83%*

° *results without outlier result no. 2*

Comments to the statistic data:

Preliminary remark: The results were given inconsistently by the participants. As far as DLA can judge this, the analytical determination was made by all laboratories as alpha-tocopheryl acetate without differentiation of the D and L forms. Only a few participants converted their results into tocopherol. Also different factors were used there. For this reason, if necessary, all results were converted by DLA and evaluated as DL-alpha-tocopheryl acetate.

The target standard deviation was calculated according to the general model of Horwitz (s. 3.6.1).
The evaluation showed a normal variability of results with a quotient S^*/σ_{pt} in the upper range of 1,8. Due to the recalculation of results the evaluation was done by z'-score considering the standard uncertainty. The comparability of results is given.

83% of results were in the target range.

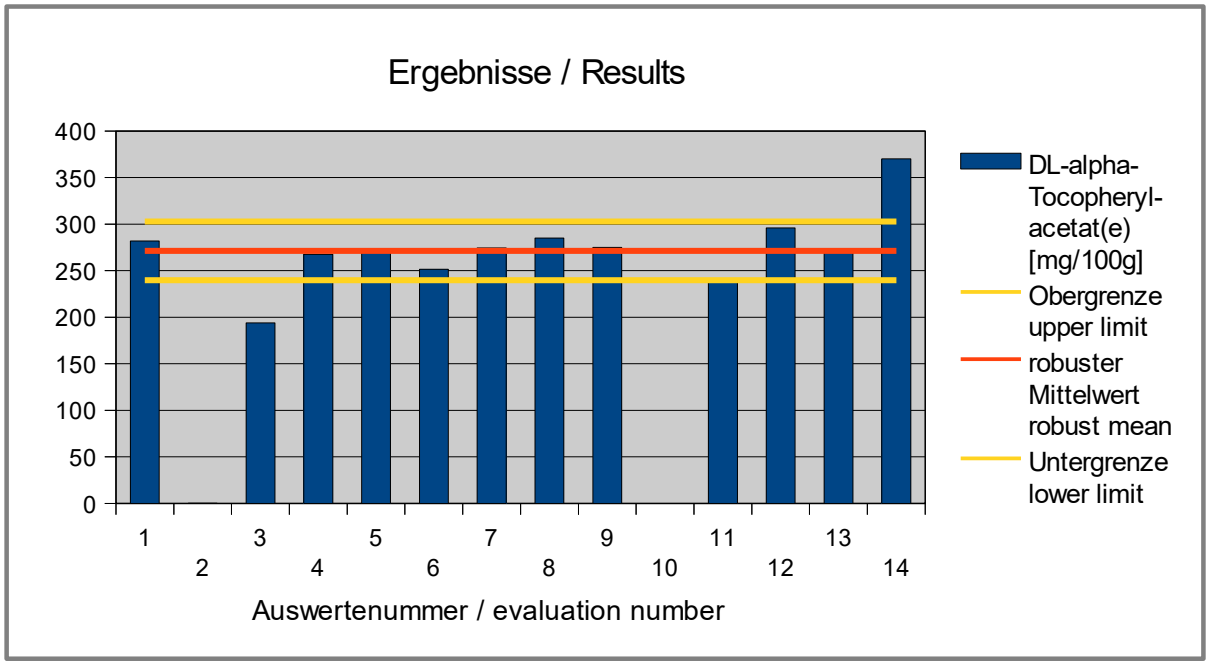

Abb. / Fig. 7: Ergebnisse DL-alpha-Tocopherylacetat/ Results DL-alpha-tocopheryl acetate

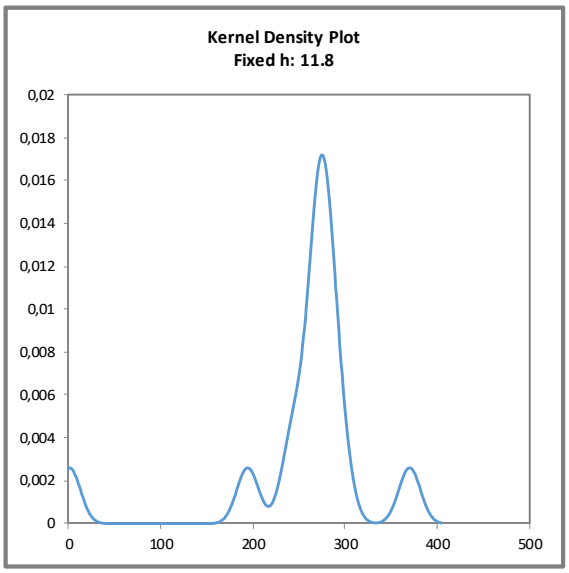

Abb. / Fig. 8:
Kerndichte-Schätzung der Ergebnisse (mit h = 0,75 x σ_{pt} von Xpt)

Kernel density plot of results (with h = 0,75 x σ_{pt} von Xpt)

Comment:
The kernel density plot shows almost a symmetrical distribution of results with a slight shoulder at approx. 250 mg/100g and three smaller peaks at 0,4 mg/100g, 194 mg/100g and 370 mg/100g, due to single results out of the target range.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	DL-alpha-Tocopheryl acetate [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z'-Score (σ_{pt})	Hinweis Remark
1	282	10,5	0,66	
2	0,370			Ausreißer ausgeschlossen / Outlier excluded
3	194	-77,3	-4,9	
4	268	-3,7	-0,24	
5	270	-1,3	-0,08	
6	251	-19,9	-1,3	
7	275 *	3,2	0,20	
8	285 *	13,7	0,87	
9	275	3,7	0,23	
10				
11	240	-31,2	-2,0	
12	296	24,7	1,6	
13	272	0,7	0,04	
14	370	98,7	6,3	

* Mean calculated by DLA

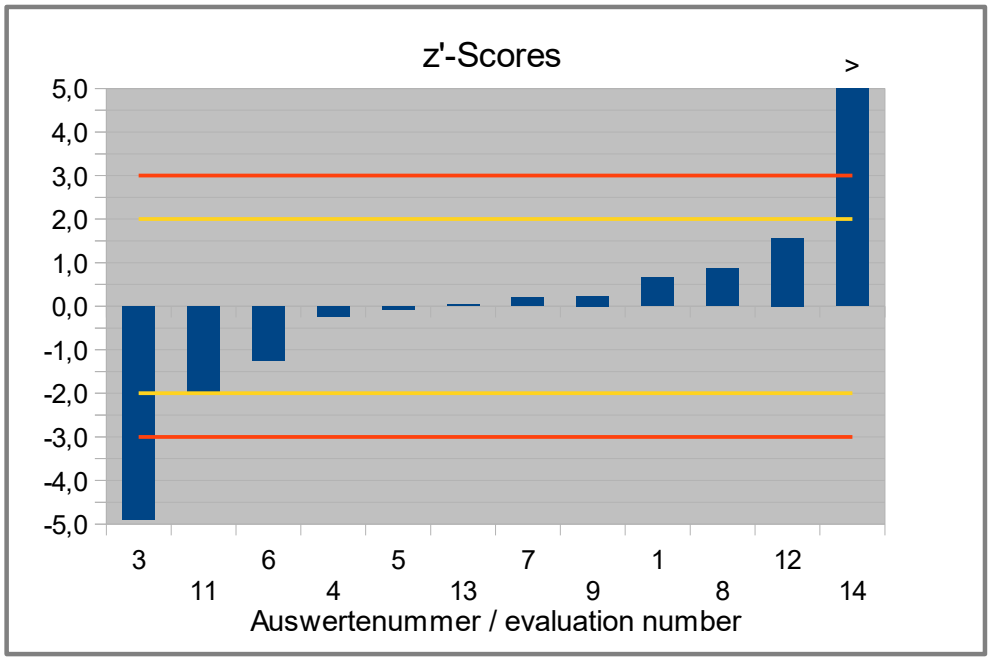

Abb. / Fig. 9: z'-Scores DL-alpha-Tocopherylacetat / DL-alpha-tocopheryl acetate

4.4 Participant z-Scores: overview table

Evaluation number	Coenzyme Q10	Panthenol	D,L-alpha-Tocopheryl Acetate
	z-Score	z-Score	z'-Score
1	1,9	-	0,66
2	-	-	-
3	-	-0,96	-4,9
4	-0,69	-0,44	-0,24
5	-2,2	0,42	-0,08
6	-1,5	-0,79	-1,3
7	-0,60	0,27	0,20
8	1,8	-1,5	0,87
9	-0,28	0,83	0,23
10	-	-	-
11	-1,1	0,23	-2,0
12	0,68	0,99	1,6
13	1,3	0,17	0,04
14	0,68	0,58	6,3

5. Documentation

5.1 Details by the participants

Note: Information given in German were translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1.1 Primary Data

Parameter	Parti-cipant	Unit	Sample I DLA No.	Sample II DLA No.	Date of ana-lysis	Result (Mean)	Result Sample I	Result Sample II	Limit of quanti-fication	Incl. RR	Recovery rate
					Day/Month					yes / no	in %
Coenzyme Q10 (Ubiquinon/ Ubiquinone)	1	mg/100g	27	39	09.12.19	55,91	56,31	55,55	11		
	2	mg/100g									
	3	mg/100g									
	4	mg/100g	14	52	09.12.19	47,7	47,7	47,8		no	
	5	mg/100g	15	51	14.01.20	43,1	44,1	42,1		no	
	6	mg/100g	1	65	10.12.19/ 08.01.20	45,12	45,1	45,14	2,5	no	101
	7	mg/100g	20	46	10.12.19	ja	49	47	1	no	
	8	mg/100g	56	10	18.12.19	19.12.19	55,6	55,2		no	
	9	mg/100g	53/2019	53/2019	03.01.	49	49	48	5	no	
	10	mg/100g									
	11	mg/100g	9	57	08.01.20	46,4	45,8	47	4,8	no	95,4
	12	mg/100g	26	40	17.01.20	52	52	52	0,01	yes	
	13	mg/100g	6	60	20.01.20	54	54	54	20	no	-
	14	mg/100g	35	36	21.01.2020	52	52	52	1	no	102

Parameter	Parti-cipant	Unit	Sample I DLA No.	Sample II DLA No.	Date of ana-lysis	Result (Mean)	Result Sample I	Result Sample II	Limit of quanti-fication	Incl. RR	Recovery rate
					Day/Month					yes / no	in %
Panthenol (Dexpanthenol)	1	mg/100g	27	39	-	-	-	-			
	2	mg/100g	25	41	17.12.19	0,42	0,42	0,41		no	
	3	mg/100g	21	45	06.01.20	410	406	413	50	no	
	4	mg/100g	14	52	12.12.19	420,3	420,5	420		no	
	5	mg/100g	15	51	09.01.20	437	440	434		no	
	6	mg/100g	1	65	03.01.20	413,4	413,6	413,3	3	no	101
	7	mg/100g	20	46	19.12.19	ja	431	437	28	no	
	8	mg/100g	56	10	18.12.19	19.12.19	405	395		no	
	9	mg/100g	53/2019	53/2019	03.01.	445	450	440	20	no	
	10	mg/100g	12	54	08.01.20	0,445	0,446	0,445		no	102
	11	mg/100g	9	57	15.01.20	433,2	432,5	433,8	60	no	102,5
	12	mg/100g	26	40	17.01.20	448	447	448	0,5	yes	
	13	mg/100g	6	60	23.01.20	432	432	431	8	no	-
	14	mg/100g	35	36	22.01.20	440	370	510	1	no	

Parameter	Parti-cipant	Unit	Sample I DLA No.	Sample II DLA No.	Date of ana-lysis	Result (Mean)	Result Sample I	Result Sample II	Limit of quanti-fication	Incl. RR	Recovery rate
					Day/Month					yes / no	in %
Tocopherol-Verbindungen (Original Ergebnisse der Teilnehmer)/ Tocopherol compounds (original results of participants)	1	mg/100g	27	39	12.12.	256,7	256,6	256,7	18,2		
	2	mg/100g	25	41	20.12.19	0,25	0,25	0,25		no	
	3	mg/100g	21	45	06.01.20	130	128	132	40	no	
	4	mg/100g	14	52	12.12.19	243,8	244,8	242,8		no	
	5	mg/100g	15	51	06.01.20	181	179	182		no	
	6	mg/100g	1	65	18.12.19	251,4	251,7	251,1	7,5	no	100
	7	mg/100g	20	46	09.01.20	ja	274	275	8	no	
	8	mg/100g	56	10	18.12.19	19.12.19	260	260		no	
	9	mg/100g	53/2019	53/2019	16.01.	336	336	335	10	no	
	10	mg/100g									
	11	mg/100g	9	57	16.01.20	161,1	172,2	150	5,2	no	91,8
	12	mg/100g	26	40	17.01.20	296	295	296	0,01	yes	
	13	mg/100g	6	60	21.01.20	248	249	246	5	no	-
	14	mg/100g	35	36	23.01.20	370	390	350	1	no	

Parameter	Parti-cipant	Unit	Sample I DLA No.	Sample II DLA No.	Date of ana-lysis	Result (Mean)	Result Sample I	Result Sample II
					Day/Month			
Ergebnisse als **DL-alpha-Tocopherylacetat** (von DLA vereinheitlicht*) / Results as **DL-alpha-tocopheryl acetate** (harmonized by DLA*)	1	mg/100g	27	39	12.12.	281,8	281,7	282,1
	2	mg/100g	25	41	20.12.19	0,37	0,37	0,37
	3	mg/100g	21	45	06.01.20	194	191	197
	4	mg/100g	14	52	12.12.19	267,6	268,7	266,5
	5	mg/100g	15	51	06.01.20	270	267	271
	6	mg/100g	1	65	18.12.19	251,4	251,7	251,1
	7	mg/100g	20	46	09.01.20	274,5	274	275
	8	mg/100g	56	10	18.12.19	285	290	280
	9	mg/100g	53/2019	53/2019	16.01.	275	276	274
	10	mg/100g						
	11	mg/100g	9	57	16.01.20	240,1	256,6	223,5
	12	mg/100g	26	40	17.01.20	296	295	296
	13	mg/100g	6	60	21.01.20	272	273	270
	14	mg/100g	35	36	23.01.20	370	390	350

* auf Basisder Teilnehmerangaben von S. 29 / based on the participants notes from p. 29

5.1.2 Analytical Methods

Parameter	Participant	Method specification, as in test report / standard / literature	Remarks about sample preparation and processing	Method description	Calibration and reference material	Recovery with same matrix	Method accredited to ISO / IEC 17025	Further remarks
						yes / no	yes / no	
Coenzyme Q10 (Ubiquinon/ Ubiquinone)	1	HPLC-DAD, house method					no	
	2							
	3							
	4	house method		HPLC-DAD		no	yes	
	5	SOP M 849, HPLC/UV		HPLC-UV house method	available		no	
	6	house method	Extraction with acetone	HPLC/DAD	yes	no	yes	
	7	DBQ10		House method, HPLC-DAD	external calibration		yes	
	8	HPLC-DAD	in EtOH				no	
	9	internal method	dissolve sample in a suitable solvent	LC-DAD	external standard		yes	
	10							
	11	House method B44.025.02	Extraction with ACN/THF/H2O and oxidation reduktion with Fe3Cl solution	HPLC/DAD		yes	yes	
	12	PV-SA-376		HPLC-UV		yes	yes	
	13	LAV 35-0030-01 (house method)	Extraction with acetone/ Isopropanol	HPLC-DAD (275 nm)	external calibration	no	yes	
	14	The sample is extracted with solvent and FeCl3 solution and then the content is determined using HPLC-DAD and an external calibration.		HPLC-DAD		yes	no	

Parameter	Participant	Method specification, as in test report / standard / literature	Remarks about sample preparation and processing	Method description	Calibration and reference material	Recovery with same matrix	Method accredited to ISO / IEC 17025	Further remarks
						yes / no	yes / no	
Panthenol (Dexpanthenol)	1							
	2	HPLC-UV internal method				no	no	
	3	House method HPLC-DAD	Extraction with acetate buffer				yes	
	4	house method		HPLC-DAD		no	yes	
	5	SOP M 855, HPLC/UV		HPLC-UV house method	available		yes	
	6	house method	extraction with ethanol in 0,01 M phosphoric acid (pH ca. 2,4)	HPLC/DAD	yes	no	yes	
	7	EBVITB		house method, HPLC-DAD	external calibration		yes	
	8	HPLC-DAD	in EtOH				no	
	9	internal method	Dissolve the sample in a suitable solvent	LC-DAD	external standard		yes	
	10	house method CU-3.P.K006 (HPLC-DAD)				no	yes	
	11	house method PM-228-008-01	extraction in buffer/ACN	HPLC/DAD		yes	yes	
	12	PV-SA-344		HPLC-UV		yes	yes	
	13	LAV 35-0010-06 (house method)	extraction with H2O/MeOH	HPLC-DAD (205 nm)	external calibration	no	yes	
	14	Determination after extraction and derivatization		GC/MS		no	no	

Parameter	Participant	Method specification, as in test report / standard / literature	Remarks about sample preparation and processing	Method description	Calibration and reference material	Recovery with same matrix	Method accredited to ISO / IEC 17025	Further remarks and messages from participants via email
						yes / no	yes / no	
Tocopherol-Verbindungen (als DL-alpha-Tocopherylacetat)/ Tocopherol compounds (as DL-alpha-tocopheryl acetate)	1	HPLC-DAD, house method					no	D/L-alpha-Tocopherol acetate (M=472,74 g/mol) determined analytically with 0.282 mg/100 g, then converted into D-alpha-tocopherol (M = 430.71 g/mol) with a conversion factor of 0.911
	2	HPLC-UV internal method				no	no	result was given as D-alpha-Tocopherol
	3	house method HPLC-DAD	extraction with isopropanol				yes	Determination as DL-alpha-tocopherol acetate, conversion factor: 0.671
	4	house method		HPLC-DAD		no	yes	in sample 1 an amount of tocopherol acetate of 268,7 mg/100 g and in sample 2 of 266,5 mg/100g were determined.This results in an mean of 267.6 mg/100g. Conversion factor is 0.911 (molar mass of tocopherol 430.71 g/mol and molar mass of tocopherol acetate 472.76 g/mol).
	5	SOP M 659, HPLC/UV		HPLC-UV house method	available		yes	*No information on the conversion made (factor 0.671 taken from DLA)*
	6	house method	extraction with isopropanol	HPLC/DAD	yes	no	yes	values converted into tocopherol: 168.7 mg/100g (individual values: 168.9 and 168.5 mg/100g)
	7	EBVITA		house method, HPLC-DAD	external calibration		yes	analyzed: alpha-tocopheryl acetate
	8	HPLC-DAD	in EtOH				no	to convert the DL-alpha-tocopheryl acetate into the D-alpha-tocopherol, the conversion factor 0.911 is used, which results from the two molar masses. Originally measured values for the DL-alpha tocopherol acetate (without any conversion): Sample I 290 mg/100g, Sample II 280 mg/100g
	9	internal method	Dissolve in THF	GC-FID	external standard with correction via internal standard		yes	Results separated for both substances: tocopherol (336/336/335 mg/100g) and tocopherol acetate (275/276/274 mg/100g)
	10							
	11	house method PM-228-002-01	mix with Na sulfate, extraction in 2-propanol	HPLC/DAD		yes	yes	Only tocopherol acetate was found in the samples. The results were then calculated as D-alpha tocopherol
	12	PV-SA-376		HPLC-UV		yes	yes	Tocopherol acetate measured
	13	LAV 35-0013-03 (house method)	extraction with isopropanol	HPLC-DAD (285 nm)	external calibration	no	yes	Tocopheryl acetate determined and converted into tocopherol (calculated with 472.76 and 430.71, approx.0.911)
	14	Determination after extraction		GC/MS		no	no	the DL-alpha-tocopheryl acetate was specified

5.2 Homogeneity

5.2.1 Trend line function of the participants results

By comparison of the increasing sample numbers and the measurement results of participants, the homogeneity of the chronological bottled PT items can be shown by the trend line for information:

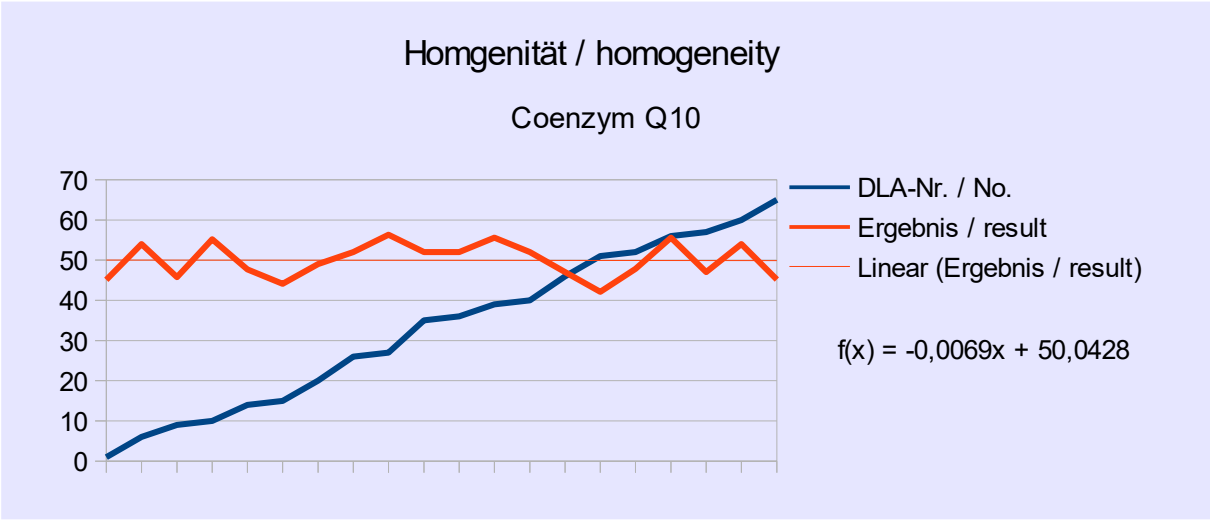

Abb./Fig. 10:
Trendfunktion Probennummern vs. Ergebnisse Coenzym Q10
trend line function sample number vs. results coenzyme Q10

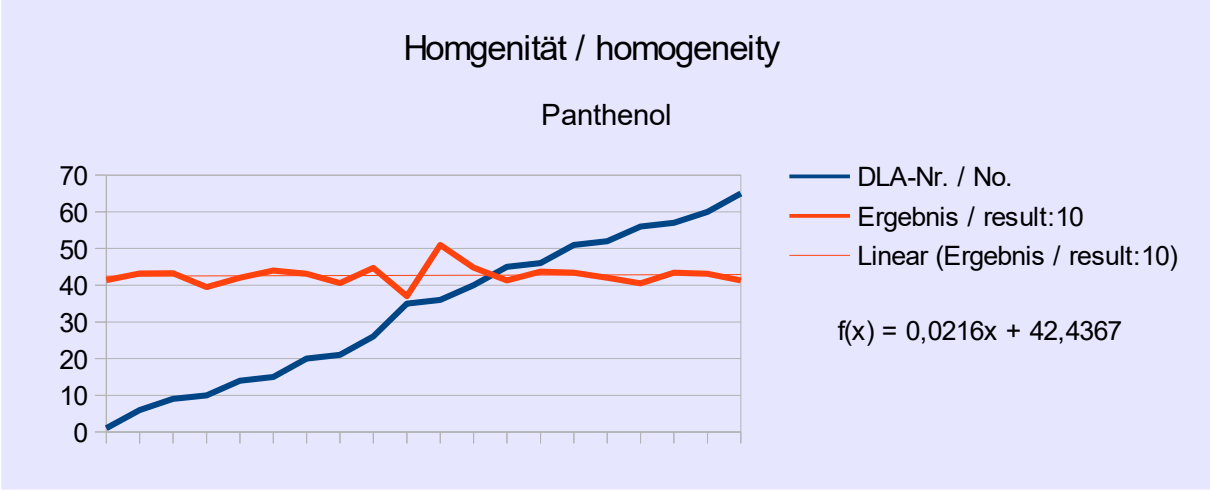

Abb./Fig. 11:
Trendfunktion Probennummern vs. Ergebnisse Panthenol (1:10 dargestellt)
trend line function sample number vs. results panthenol (1:10 shown)

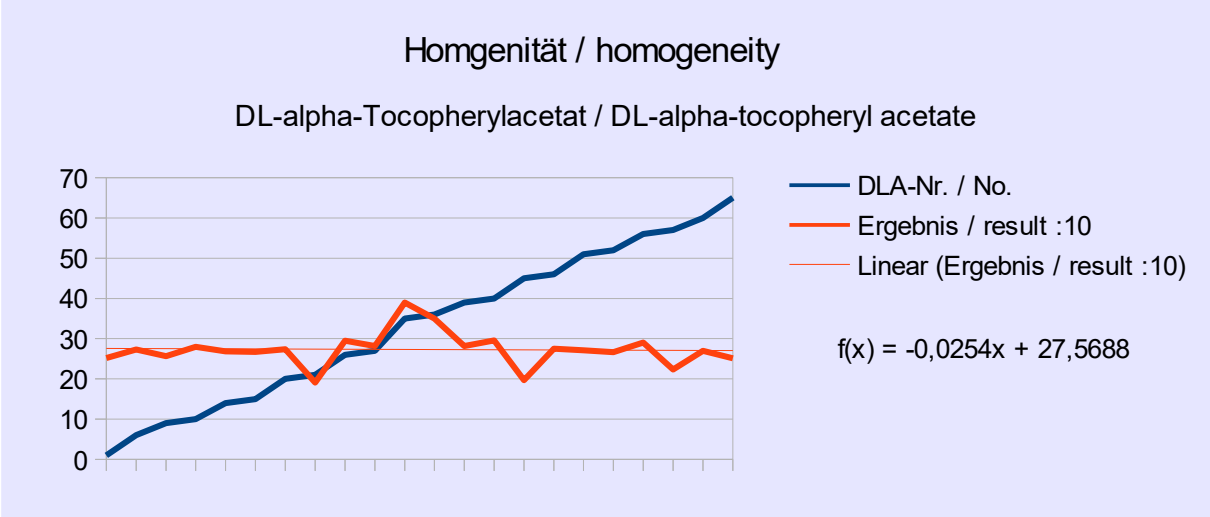

Abb./Fig. 12:
Trendfunktion Probennummern vs. Ergebnisse DL-alpha-Tocopherylacetat (1:10 dargestellt)
trend line function sample number vs. results DL-alpha-tocopheryl acetate (1:10 shown)

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

PT number	***DLA 53-2019***
PT name	***Cosmetic Products III: Coenzyme Q10, Panthenol and Tocopherol in Skin Cream***
*Sample matrix**	*Samples I + II: Skin Cream, common in commerce ingredients*
Number of samples and sample amount	*2 identical samples I + II, 25 g each.*
Storage	*Samples I + II: cooled 2 - 10°C*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative: Coenzyme Q10 (Ubiquinone), Panthenol and Tocopherol (Tocopheryl acetate)*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*The results for sample I and II as well as the final results calculated as mean of the double determination (samples I and II) should be filled in the result submission file. The recovery rates, if carried out, has to be included in the calculation.*
Units	*mg/100g*
Number of significant digits	*at least 2*
Further information	*For information please specify:* – *Date of analysis* – *DLA-sample-numbers (for sample I and II)* – *Limit of detection* – *Assignment incl. Recovery* – *Recovery with the same matrix* – *Method is accredited*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest 24th January 2019***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler-Scharf PhD*

** Control of mixture homogeneity and qualitative testings are carried out by DLA. Any testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.*

6. Index of participant laboratories in alphabetical order

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

[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 Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. 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. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)