Evaluation Report

proficiency test

DLA ptAU05 (2021)

Steviosides

in Drink Powder

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General Information on the proficiency test (PT)

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1. Introduction

The participation in proficiency testing (PT) 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 drink powder with plant protein with an addition of steviosides. The mixture consists of commercially available ingredients for dietary supplements for athletes.
The raw 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 stevioside content calculated on the basis of the declaration are given in tables 1 and 2.

Table 1: Composition of DLA-Samples

Drink powder with plant protein
Ingredients: Soyprotein isolate, maltodextrin, acai powder, steviol glycosides (E960)

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

Table 2: Calculated amounts of the parameter according to the manufacturers specification (conversion to steviol equivalents not given)

Parameter	**Content per kg**
Steviol Glycosides	1370 mg

2.1.1 Homogeneity

The homogeneity of the mixture before the filling was examined in 8-fold determinations by means of microtracer analysis. It is a standardized method that is part of the international GMP certification system for feed [14]. Before mixing, iron particles coated with dye are added to the sample in µm size and the number of particles is determined in aliquots taken after homogenization. The evaluation of the homogeneity of the mixture is based on the Poisson distribution using the chi-square test. A probability of ≥ 5% is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15]. The microtracer analysis of the present PT sample showed a probability of 98%. The particle results were also converted into concentrations, statistically evaluated as normal distribution and compared with the standard deviation according to Horwitz. For the assessment, HorRat values between 0,3 and 1,3 under repeated conditions (measurements within the laboratory) are to be accepted [16, 17]. A HorRat value of 0,64 was obtained for the present PT sample. The results of the microtracer analysis are given in the documentation.

The calculation of the **repeatability standard deviations S_r of the participants** was also used as an indicator of homogeneity. It is 9,70% for stevioside, 4,23% for rebaudioside A and 3,88% for steviol glycosides in sum. Thus they were similar to corresponding repeatability standard deviations of precision data of the standardized methods (e.g. ASU §64 L 43.00-2, s. 3.6.2) (see Table 3) [20].
The repeatability standard deviations of the participants' results are given in the statistic data (see 4.1 to 4.3).

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 Homogeneity).

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

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA materials shows, with comparable matrix and water activity (a_W value <0.5), good durability of the PT samples and storage stability against microbial spoilage and with regard to the content of the PT parameters.
The a_W value of the PT samples was approx. 0,34 (21,3°C). 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 24th week of 2021. The testing method was optional. The tests should be finished at 13th August 2021 the latest.

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

*There are **two identical samples I and II** with the parameters stevioside and rebaudioside A to be determined as well as the sum of all steviol glycosides in the matrix of drink powder with plant protein. All contents should be given as **steviol equivalents**. The analysis method is optional.*

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 by means of transmission tables handed over to the participating laboratories (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 10 participants submitted at least one result.

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"). 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. The use of different examination methods is often an option. 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*) of the submitted results 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 in percent of the mean is given as variation coefficient CV_R in the statistical data of participant for each parameter if the single results from participants are available. 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.

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 the parameters stevioside, rebaudioside A and steviol glycosides in sum the target standard deviation according to data from a precision experiment was applied (see 3.6.2)(ASU §64 Methods: L 43.00-2). The parameter stevioside could not be evaluated due to the small number of results and their heterogeneity.

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 relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviation (RSD_R) given in Table 3 were determined in ring tests using the indicated methods.
The resulting target standard deviations σ_{pt}, which were identified there, were used to evaluate the results and to provide additional information for the statistical data.

Table 3: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) according to selected evaluations of tests for precision and the resulting target standard deviation σ_{pt} [20]

Parameter	**Matrix**	**Mean** (mg/kg)	**RSD_r** (%)	**RSD_R** (%)	**σ_{pt}** (%)	**Method / Literature**
Steviosside	Caffeinated drink	7,84	3,26%	28,3%	28,2%*	ASU §64 L 43.00-2 [20]
Rebaudioside A	Caffeinated drink A	85,1	1,25%	11,9%	11,9%*	ASU §64 L 43.00-2 [20]
Rebaudioside A	Caffeinated drink B	75,6	2,77%	14,2%	14,1%	ASU §64 L 43.00-2 [20]

The target standard deviations marked with "" were given in the evaluations.*

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 3.6.2 was regarded suitable.

Table 4 shows selected statistic data of participants' results of present PT compared to PT results of previous years.

Table 4: Characteristics of the present PT (on dark grey) in comparison to the previous PT from 2015 (SD = standard deviation, CV = coefficient of variation)

Parameter	Matrix (Powder)	robust Mean	rob. SD (S*)	rel. SD (CV_{S*}) [%]	Quotient S*/σpt	DLA-report
Stevioside	Drink powder	269	93,0	34,6	2,3*	DLA 28/2015
Stevioside	Drink powder	70,9	-	-	-	DLA ptAU05 (2021)
Rebaudioside A	Drink powder	180	160	88,9	2,3*	DLA 28/2015
Rebaudioside A	Drink powder	359	61,0	17,0	1,4	DLA ptAU05 (2021)
Steviol glycosides in sum	Drink powder	413	-	-	-	DLA 28/2015
Steviol glycosides in sum	Drink powder	443	122	27,5	1,0	DLA ptAU05 (2021)

** with target standard deviation σpt'*

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}). The value indicated as z-Score (Info) only obtains an informative character. The both z-Scores were calculated with the different target standard deviations in accordance with 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.
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 inter alia 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_{pt}) 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 coefficient of variation (CV_R)

The variation coefficient (CV_R) of the reproducibility (= *relative reproducibility standard deviation)* is calculated from the reproducibility standard deviation S_R 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 of the assigned value

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

According to EU Regulation 231/2012, the food additive E 960 (steviol glycosides) is made up of at least 95% of the substances listed below:

Trivial name	Formula	Conversion factor
Steviol	$C_{20}H_{30}O_3$	1,00
Steviolbioside	$C_{32}H_{50}O_{13}$	0,50
Rubusoside	$C_{32}H_{50}O_{13}$	0,50
Dulcoside A	$C_{38}H_{60}O_{17}$	0,40
Stevioside	$C_{38}H_{60}O_{18}$	0,40
Rebaudioside A	$C_{44}H_{70}O_{23}$	0,33
Rebaudioside B	$C_{38}H_{60}O_{18}$	0,40
Rebaudioside C	$C_{44}H_{70}O_{22}$	0,34
Rebaudioside D	$C_{50}H_{80}O_{28}$	0,29
Rebaudioside E	$C_{44}H_{70}O_{23}$	0,33
Rebaudioside F	$C_{43}H_{68}O_{22}$	0,34
Rebaudioside M	$C_{56}H_{90}O_{33}$	0,25

In this proficiency test, the results of the parameters stevioside, rebaudioside A and the sum of the steviol glycosides were to be given as steviol equivalents. The corresponding conversion factors are listed in EU Regulation 231/2012 or ASU §64 Method L 43.00-2.

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 ($X_{pt} + 2\sigma_{pt}$´) *
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	**Parameter [Einheit / Unit]**	**Abweichung**	**z-Score σpt**	**z-Score** (Info)	**Hinweis**
Evaluation number		**Deviation**			**Remark**

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

4.1 Sum of Steviol Glycosides (as steviol equivalents) in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	9
Number of outliers	-
Mean	530
Median	443
Robust Mean (X)	**443**
Robust standard deviation (S*)	**122**
Number with 2 replicates	9
Repeatability SD (S_r)	20,5
Repeatability (CV_r)	3,88%
Reproducibility SD (S_R)	339
Reproducibility (CV_R)	64,0%
Target range:	
Target standard deviation σ_{pt}	**125**
Target standard deviation (for Information)	28,3
lower limit of target range	**193**
upper limit of target range	**693**
Quotient S/σ_{pt}*	*1,0*
Standard uncertainty $U_{(Xpt)}$	*50,8*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,41*
Results in the target range	*8*
Percent in the target range	*89%*

Comments:

The target standard deviation was calculated according to 3.6.2 data from precision experiments (ASU §64 L 43.00-2). Additionally the target standard deviation using data from the general model of Horwitz is given for information (s. 3.6.1).

The distribution of results showed a low variability. The quotient S/σ_{pt} was 1,0. The robust standard deviation was in the range of previous PTs (see 3.6.3). The comparability of results is given.*

The repeatability and reproducibility standard deviation were in the range of of established values for the used determination methods (s. 3.6.2).

89% of results were in the target range.

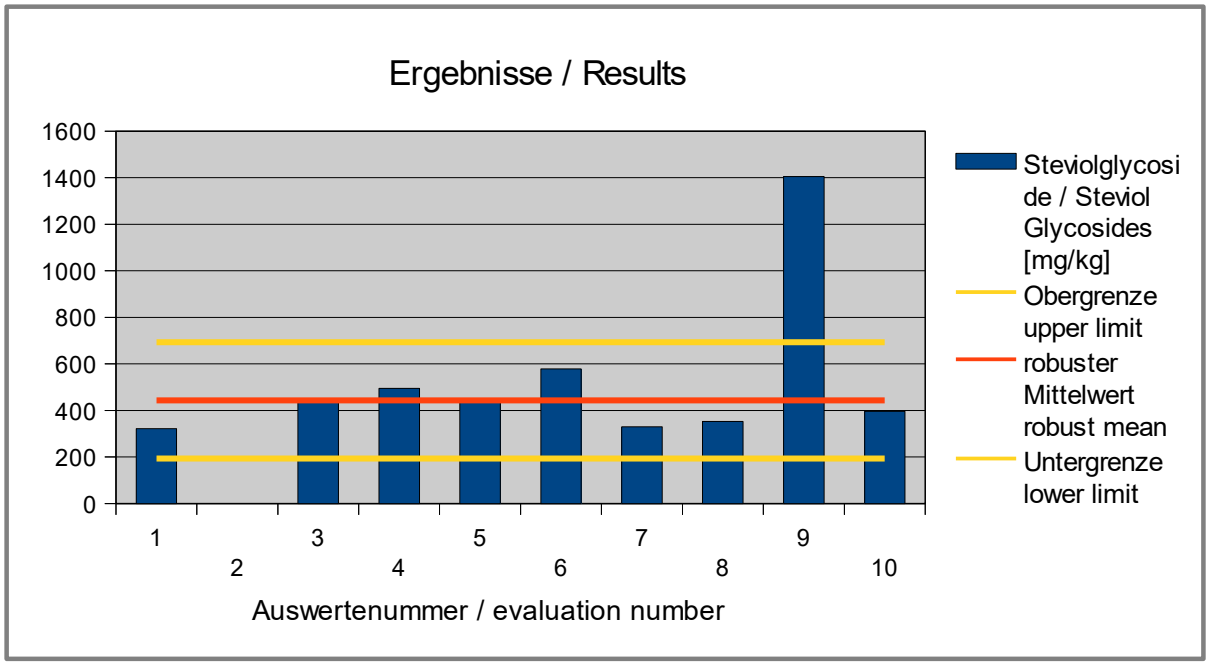

Abb. / Fig. 1: Ergebnisse Steviolglycoside / Results Steviol Glycosides

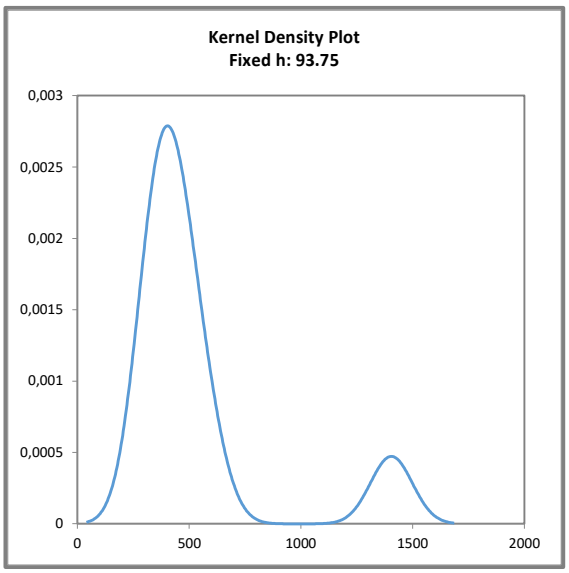

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse
(mit h = 0,75 x σ_{pt} von X_{pt})

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

Comment:
The kernel density shows almost a symmetrical distribution with a side-peak at approx. 1400 mg/kg, which is based on a participant result outside the target range.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Steviolglyco-side / Steviol Glycosides [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	322	-121	-1,0	-4,3	
2					
3	444	1,0	0,01	0,03	
4	495	51,9	0,42	1,8	
5	443	0,0	0,00	0,00	
6	579	136	1,1	4,8	
7	330 *	-114	-0,91	-4,0	
8	353	-90,0	-0,72	-3,2	
9	1405 *	961	7,7	34	not given as steviol equivalent?
10	396	-47,0	-0,38	-1,7	

* Mean calculated by DLA, if difference of single samples ≤ 2 σpt

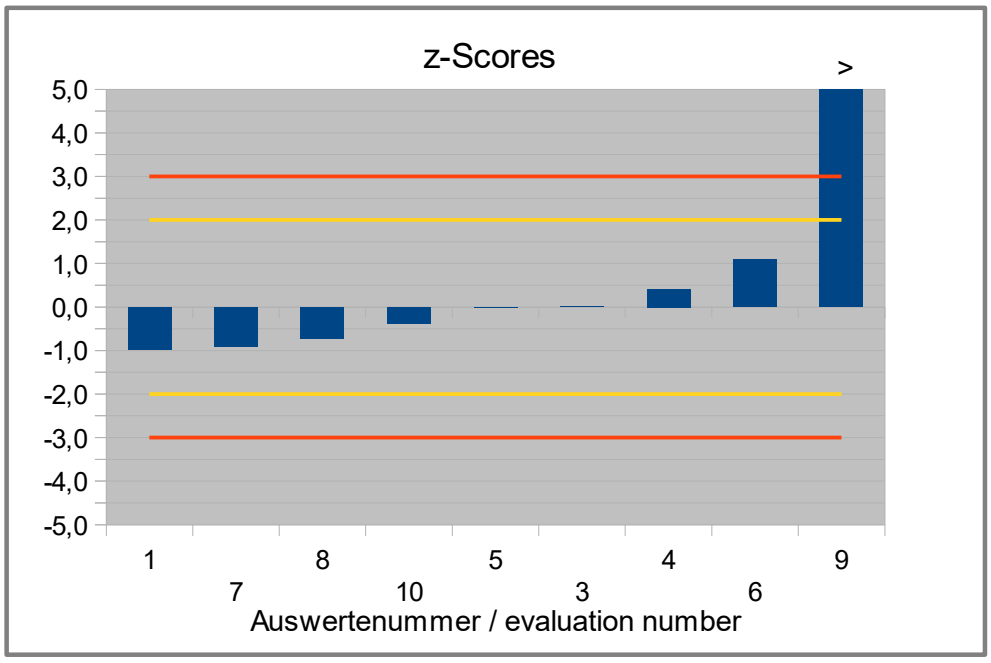

Abb. / Fig. 3: z-Scores Steviolglycoside / Steviol Glycosides

4.2 Stevioside (as steviol equivalents) in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	5
Number of outliers	-
Mean	70,9
Median	46,1
Robust Mean (X)	**70,9**
Robust standard deviation (S*)	**54,7**
Number with 2 replicates	5
Repeatability SD (S_r)	6,88
Repeatability (CV_r)	9,70%
Reproducibility SD (S_R)	48,5
Reproducibility (CV_R)	68,4%

Comments:

The above characteristics are for information purposes only.

Due to the small number of results and the heterogeneous distribution into 3 lower values (mean 37 mg/kg) and 2 higher values (mean 122 mg/kg), no statistical analysis was carried out.

It is possible that the higher results of participants 2 and 10 were not converted into steviol equivalents using the conversion factor of 0,40.

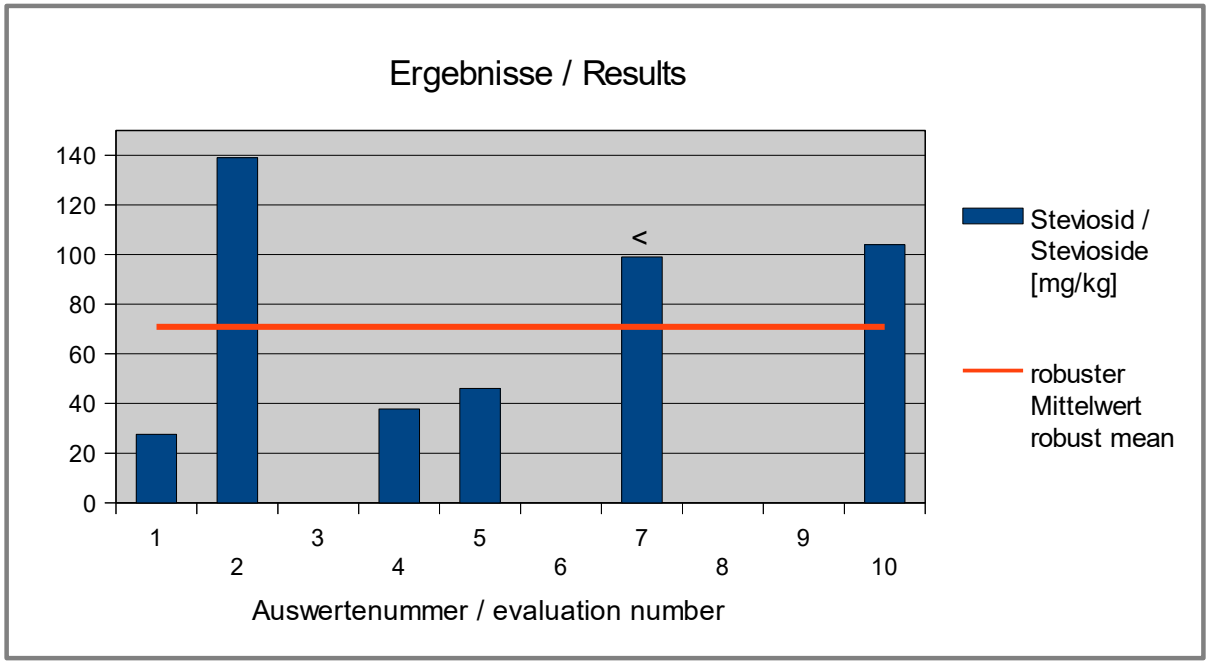

Abb. / Fig. 4: Ergebnisse Steviosid / Results Stevioside

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Steviosid / Stevioside [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	27,6				
2	139				not given as steviol equivalent?
3					
4	37,8				
5	46,1				
6					
7	<99				
8	<BG				
9					single values: 161 mg/kg und 38 mg/kg
10	104				not given as steviol equivalent?

* Mean calculated by DLA, if difference of single samples ≤ 2 σpt

4.3 Rebaudioside A (as steviol equivalents) in mg/kg

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	8
Number of outliers	-
Mean	665
Median	408
Robust Mean (X)	**665**
Robust standard deviation (S*)	**484**
Number with 2 replicates	8
Repeatability SD (S_r)	26,8
Repeatability (CV_r)	4,04%
Reproducibility SD (S_R)	427
Reproducibility (CV_R)	64,2%

Comments:

The above characteristics are for information purposes only.
Due to the small number of results and the heterogeneous distribution into 5 lower values (mean value 359 mg/kg) and 3 higher values (mean value 1175 mg/kg), no common statistical evaluation was carried out.
It is possible that the higher results of participants 2, 9 and 10 were not converted into steviol equivalents using the conversion factor of 0,33.
A separate evaluation is carried out for the lower values in the following section.

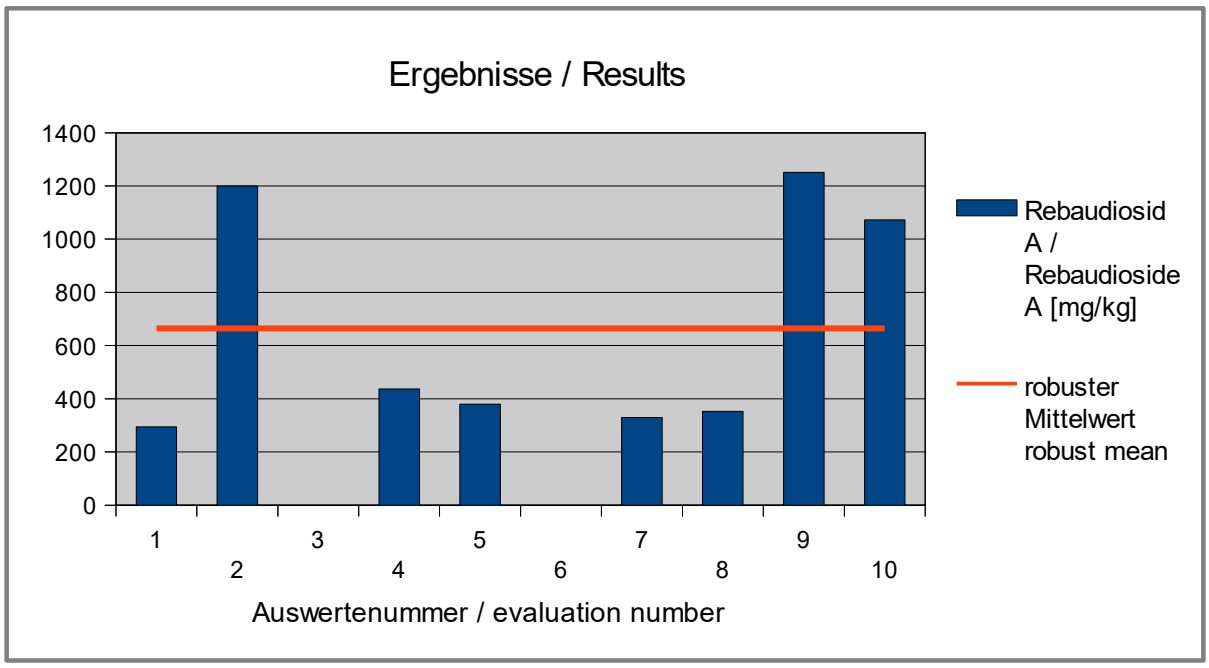

Abb. / Fig. 5: Ergebnisse Rebaudiosid **A** / Results Rebaudioside A

4.3.1 Rebaudioside A: Evaluation without higher values (in mg/kg)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	5°
Number of outliers	0
Mean	359
Median	353
Robust Mean (X)	**359**
Robust standard deviation (S*)	**61,0**
Number with 2 replicates	5
Repeatability SD (S_r)	15,2
Repeatability (CV_r)	4,23%
Reproducibility SD (S_R)	54,9
Reproducibility (CV_R)	15,3%
Target range:	
Target standard deviation σ_{pt}	**42,6**
Target standard deviation (for Information)	23,7
lower limit of target range	**274**
upper limit of target range	**444**
Quotient S^*/σ_{pt}	*1,4*
Standard uncertainty $U_{(Xpt)}$	*34,1*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,80*
Results in the target range	*5*
Percent in the target range	*100%*

° without results of participants 2, 9 and 10

Comments:

The target standard deviation was calculated according to 3.6.2 data from precision experiments (ASU §64 L 43.00-2). Additionally the target standard deviation using data from the general model of Horwitz is given for information (s. 3.6.1).

The distribution of results showed a normal variability. The quotient S^*/σ_{pt} was below 2,0. The comparability of results is given.

The repeatability and reproducibility standard deviation were in the range of of established values for the used determination methods (s. 3.6.2).

100% of results were in the target range.

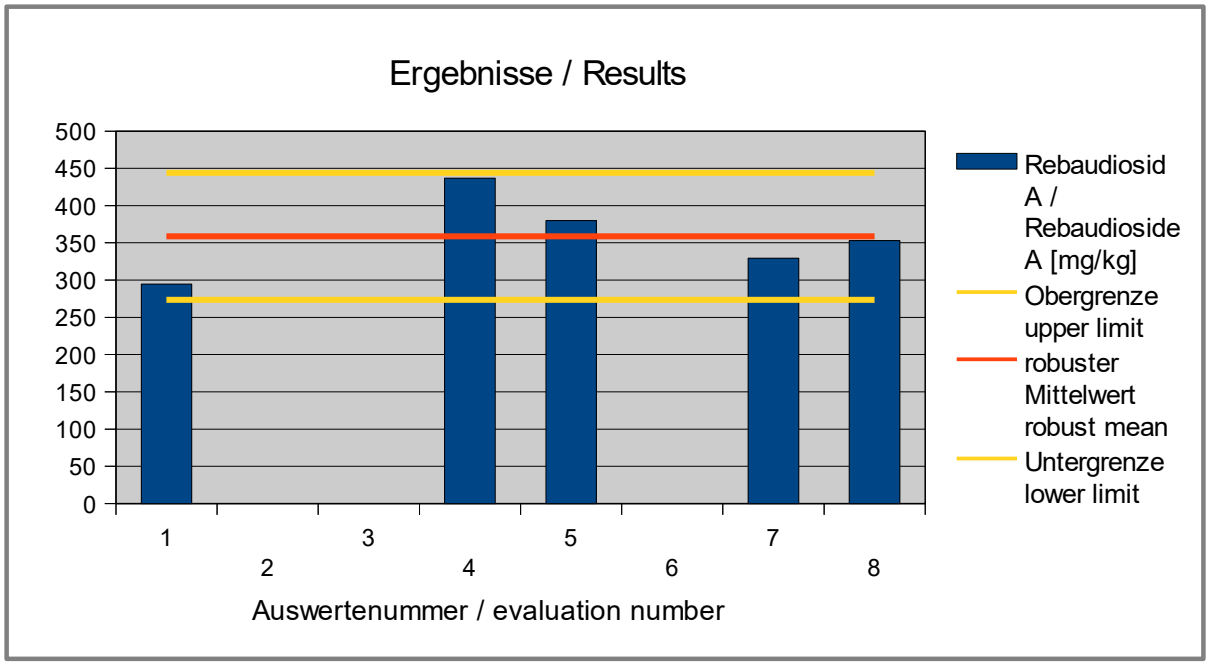

Abb. / Fig. 6: Ergebnisse Rebaudiosid **A** / Results Rebaudioside A

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Rebaudiosid A / Rebaudioside A [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	295	-64,3	-1,5	-2,7	
2					
3					
4	437	78,1	1,8	3,3	
5	380	21,2	0,50	0,90	
6					
7	330 *	-29,3	-0,69	-1,2	
8	353	-5,8	-0,14	-0,24	

* Mean calculated by DLA, if difference single samples ≤ 2 σpt

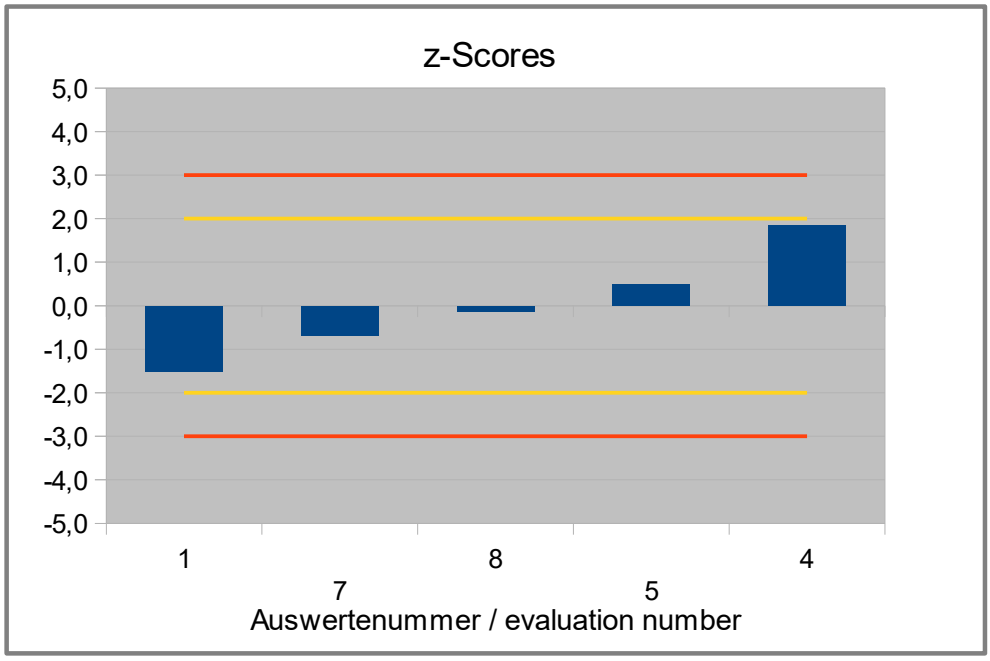

Abb. / Fig. 7: z-Scores Rebaudiosid A / Rebaudioside A

4.3.2 Rebaudioside A: higher values for information (in mg/kg)

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	3
Number of outliers	0
Mean	1175
Median	1200
Robust Mean (X)	**1175**
Robust standard deviation (S*)	**104**
Number with 2 replicates	3
Repeatability SD (S_r)	39,2
Repeatability (CV_r)	3,34%
Reproducibility SD (S_R)	95,6
Reproducibility (CV_R)	8,15%

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Rebaudiosid A / Rebaudioside A [mg/kg]	Abweichung [mg/kg] Deviation [mg/kg]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2	1200	25,2			not given as steviol equivalent?
3					
4					
5					
6					
7					
8					
9	1252 *	76,7			not given as steviol equivalent?
10	1073	-102			not given as steviol equivalent?

* Mean calculated by DLA

4.4 Partcipants' z-Scores: Overview table

Evaluation number	Stevioside	Rebaudioside A	Sum of Steviol Glycosides
1		-1,5	-1,0
2			
3			0,01
4		1,8	0,42
5		0,50	0,00
6			1,1
7		-0,69	-0,91
8		-0,14	-0,72
9			7,7
10			-0,38

Bewertung des z-Scores / valuation of z-score (DIN ISO 13528:2009-01):
-2 ≤ z-score ≤ 2 erfolgreich / successful (in green)
-2 > z-score > 2 „Warnsignal“ / warning signal (in yellow)
-3 > z-score > 3 „Eingriffssignal“ / action signal (in red)

5. Documentation

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

5.1 Details by the participants

5.1.1 Primary data

Parameter	Participant	Unit	Sample I DLA No.	Sample II DLA No.	Date of analysis	Result (Mean)	Result I	Result II	Limit of quantification	Incl. RR	Recovery rate [%]
Steviosid / Stevioside	1	mg/kg	12	50	17.06.21	27,6	28,6	26,6	1	no	96
	2	mg/kg	22	40	22.06.21	139	129	149	100	no	109
	3	mg/kg									
	4	mg/kg	3	59	30.06.21	37,77	38,76	36,77	20	yes	72,13
	5	mg/kg	20	42		46,1	46,6	45,7	10	no	-
	6	mg/kg	56	6							
	7	mg/kg	21	41	21.06.21		<99	<99	99	no	
	8	mg/kg	17	45	21.08.21		< LOD	< LOD	4	no	96
	9	mg/kg	4	58	04.08.21		161	38	35	no	
	10	mg/kg	7	55	11.08.21	104	108	100	19	no	

Parameter	Participant	Unit	Sample I DLA No.	Sample II DLA No.	Date of analysis	Result (Mean)	Result I	Result II	Limit of quantification	Incl. RR	Recovery rate [%]
Rebaudiosid A / Rebaudioside A	1	mg/kg	12	50	17.06.21	294,5	294,5	294,5	1	no	104
	2	mg/kg	22	40	22.06.21	1200	1220	1170	100	no	113
	3	mg/kg									
	4	mg/kg	3	59	30.06.21	436,93	458,4	415,46	20	yes	72,13
	5	mg/kg	20	42		380	385	376	10	no	-
	6	mg/kg									
	7	mg/kg	21	41	21.06.21		320	339		no	
	8	mg/kg	17	45	21.08.21	353	355	351	6	no	99
	9	mg/kg	4	58	04.08.21		1223	1280	50	no	
	10	mg/kg	7	55	11.08.21	1073	1043	1102	23	no	

Parameter	Participant	Unit	Sample I DLA No.	Sample II DLA No.	Date of analysis	Result (Mean)	Result I	Result II	Limit of quantification	Incl. RR	Recovery rate [%]
Steviolglycoside / Steviol Glycosides	1	mg/kg	12	50	17.06.21	322	323	321	1	no	
	2	mg/kg									
	3	mg/kg	2	60	12.07.21	444	460	427	10	no	
	4	mg/kg	3	59	30.06.21	494,98	517,85	472,1	20	yes	72,13
	5	mg/kg	20	42		443	448	439	10	no	-
	6	mg/kg			24.06.21	579	572	585	8	no	
	7	mg/kg	21	41	21.06.21		320	339		no	
	8	mg/kg	17	45	21.08.21	353	355	351		no	
	9	mg/kg	4	58	04.08.21		1434	1375		no	
	10	mg/kg	7	55	11.08.21	396	387	404			

5.1.2 Analytical Methods

Parameter	Participant	Method description	Sample preparation and processing	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited	Further Remarks
Steviosid / Stevioside	1	P4-02-01-12-7501	Extraction ACN / water 30:70; centrifugation; microfiltration; dilution;	HPLC-Orbitrap-MS	external calibration	no	yes	
	2	in house method					yes	
	3							
	4	in house method	SPE	HPLC DAD		jyes	yes	
	5	HPLC/ ASU L 43.00-2; 06/2018						
	6							
	7	LC/UV/DAD					no	
	8	§ 64 LFGB ASU L 43.00-2, modified			RM DLA 25/2013	yes	yes	
	9	in house method		HPLC			yes	
	10	ASU L43.00-2	5g/50 ml + Carrez		calibration with		no	

Parameter	Participant	Method description	Sample preparation and processing	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited	Further Remarks
Rebaudiosid A / Rebaudioside A	1	P4-02-01-12-7501	Extraction ACN/water 30:70; centrifugation; microfiltration; dilution;	HPLC-Orbitrap-MS	external calibration	no	yes	
	2	in house method					yes	
	3							
	4	in house method	SPE	HPLC DAD		yes	yes	
	5	HPLC/ ASU L 43.00-2; 06/2018						
	6							
	7	LC/UV/DAD					no	
	8	§ 64 LFGB ASU L 43.00-2, modified			RM DLA 25/2013	yes	yes	
	9	in house method		HPLC			yes	
	10				RebA, reference material: magnesium tablets, gummy bears			

Parameter	Participant	Method description	Sample preparation and processing	Measuring method	Calibration / Reference material	Recovery rate with same matrix	Method accredited	Further Remarks
Steviolglycoside / Steviol Glycosides	1	P4-02-01-12-7501	Extraction ACN/water 30:70; centrifugation; microfiltration; dilution;	HPLC-Orbitrap-MS	external calibration	no	yes	calculated
	2							
	3	in house method	Purification by means of SPE	HPLC-DAD	external calibration		yes	
	4	in house method	SPE	HPLC DAD		yes	yes	
	5	HPLC/ ASU L 43.00-2; 06/2018						Rebaudioside C detected (sample 1 17,2 mg/kg; sample 2 16,9 mg/kg (as equivalent))
	6	in house method, LC-MS/MS	Extraction with water/Hydrochloric acid, subsequent hydrolysis	LC-MS/MS	Isosteviol, CAS 27975-19-5		yes	
	7	LC/UV/DAD					no	
	8	§ 64 LFGB ASU L 43.00-2, modified					yes	
	9	in house method		HPLC			yes	
	10							

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer homogeneity test
DLA ptAU05 (2021)

Weight of total sample	1,62	kg
Microtracer	FSS-red lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Tracer addition	24,2	mg/kg

Analysis results:

Sample	Weighted sample [g]	Number of particles	Particle [mg/kg]
1	4,99	59	23,6
2	4,97	61	24,5
3	4,99	57	22,8
4	4,99	66	26,5
5	5,05	65	25,7
6	5,00	57	22,8
7	4,97	55	22,1
8	4,95	58	23,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	59,7	Particle
Standard deviation	3,78	Particle
χ^2 (CHI-square)	1,67	
Probability	**98**	%
Recovery rate	99	%

Normal distribution		
Number of samples	8	
Mean	24,0	mg/kg
Standard deviation	1,51	mg/kg
rel. Standard deviation	6,32	%
Horwitz standard deviation	9,92	%
HorRat-value	**0,64**	
Recovery rate	99	%

5.2.2 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:

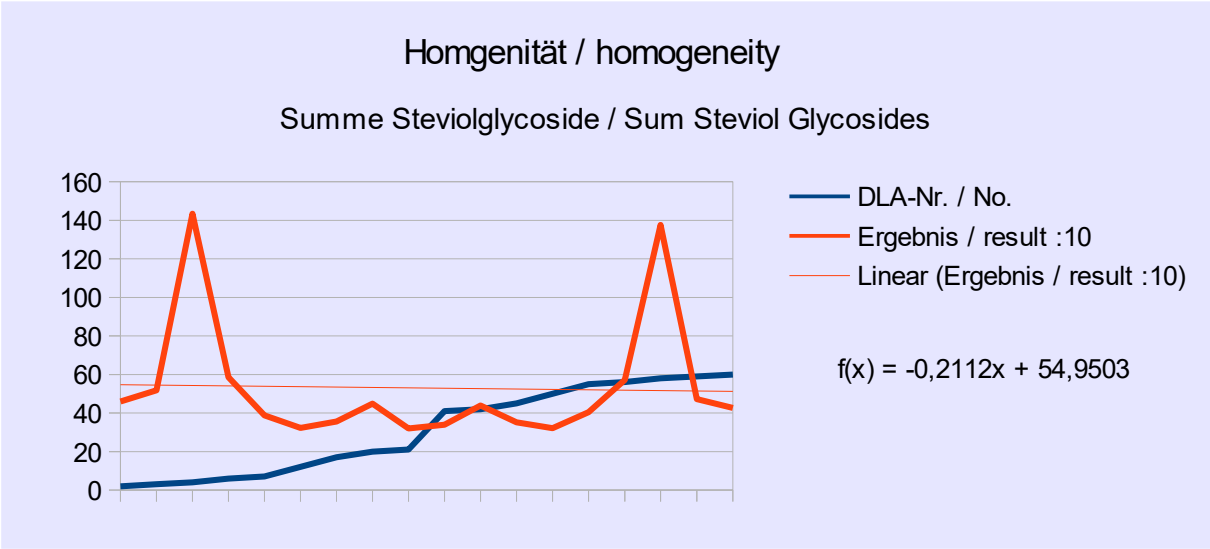

Abb./Fig. 8:
Trendfunktion Probennummern vs. Ergebnisse (1/10 dargestellt)
trend line function sample number vs. results (1/10 shown)

5.3 Sample cover letter: Information on the Proficiency Test (PT)

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

PT number	***DLA ptAU05 - 2021***
PT name	***Steviosides in Drink Powder***
*Sample matrix**	*Samples I + II: Drink powder with plant protein / ingredients: Soyprotein isolate, maltodextrin, acai powder, steviol glycosides (E960)*
Number of samples and sample amount	*2 identical samples I + II, 25 g each.*
Storage	*Samples I + II:* *room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative: Stevioside, rebaudioside A and sum of all steviol glycosides (as steviol equivalents)*
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.* *All contents should be given as steviol equivalents.*
Units	*mg/kg*
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***
Last Deadline	***the latest August 13th 2021***
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
		Germany
		Germany
		FRANCE
		BELGIUM
		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 17025:2005/2017; 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)
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13. EN ISO/IEC 17034:2016; Konformitätsbewertung - Allgemeine Anforderungen an die Kompetenz von Referenzmaterialherstellern / General requirements for the competence of reference material producers
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15. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
16. 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.
17. 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
18. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
19. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
20. ASU §64 LFGB L 43.00-2: Bestimmung von Steviol-Glycosiden in Süßwaren, Schokolade, koffeinhaltigen Brausen und Lebensmitteln für eine besondere Ernährungsform; HPLC-Verfahren (2018) [Determination of steviol glycosides in confectionery, chocolate, caffeinated drinks and foods for a special form of nutrition; HPLC method]
21. FAO JECFA Monographs 10, Compendium of food additive specifications, Joint FAO/WHO Expert Commitee on Food Additives, 73rd Meeting 2010
22. EU-VO 1131/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council with regard to steviol glycosides (11 November 2011)
23. EU-VO 231/2012 of laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council Text with EEA relevance (9 March 2012)