Proficiency Tests

DLA

food
cosmetics
consumer goods
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Evaluation Report
proficiency test

DLA 46/2018

Food Supplement II:

Taurine and Caffeine

in Athletes Product

Dienstleistung Lebensmittel Analytik GbR
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Allgemeine Informationen zur Eignungsprüfung (EP)
General Information on the proficiency test (PT)

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EP-Nummer *PT-Number*	DLA 46/2018
EP-Koordinator *PT-Coordinator*	Dr. Matthias Besler-Scharf
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (26. November 2018) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager) *- gezeichnet / signed G. Wichmann* Datum / Date: 26. November 2018
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
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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Inhalt / Content

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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 common in commerce food supplements "drink powders for athlets" and maltodextrin as bulking agent/carrier material from European suppliers.
The raw materials were crushed, sieved (mesh 600 µm), mixed and homogenized.

Afterwards the samples were portioned to approximately 25 g into metallised PET film bags and numbered chronologically.

The composition (list of ingredients) and the contents of taurine and caffeine were calculated according to the manufacturers specification and are given in table 1 and 2.

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Table 1: Composition of DLA-Samples

Food supplement - Drink Powder
Ingredients (1. food supplement): Glucose, acidulant: citric acid, taurine, flavor, acidity regulator: dipotassium phosphate, trimagnesium dicitrate, magnesium carbonate, trisodium citrate, caffeine, vitamin C, sweetener: acesulfame-K, aspartame, inositol, salt, palm oil, dye: azorubin, niacin, vitamin B12, vitamin B1 Ingredients (2. food supplement): Glucose, maltodextrin, fructose, isomaltulose, acidulants sodium citrate, citric acid and tartaric acid, flavor, taurine, magnesium citrate, L-carnitine, potassium chloride, L-leucine, guarana extract, caffeine, L-isoleucine, L-valine, dye: caramel (E150a), ascorbic acid , Nicotinic acid amide, DL-alpha tocopheryl acetate, calcium d-pantothenate, riboflavin, pyridoxine hydrochloride, thiamine hydrochloride, folic acid, chromium III chloride, biotin, cyanocobalamin Further Ingredient: Maltodextrin

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 parameters according to the manufacturers specification

Parameter	**Content per 100 g**
Caffeine	444 mg
Taurine	4990 mg

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2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity 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 56%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [16, 17]. This gave a HorRat value of 1,2. The results of microtracer analysis are given in the documentation.

The calculation of the **repeatability standard deviations S_r of the duplicate determination of the participants** was also used as an indicator of homogeneity. For caffeine it is 2,7% and for taurine 5,3%. Thus they were similar to corresponding repeatability standard deviations of precision data of the standardized methods (e.g. ASU- §64 L 46.00-3, s. 3.6.2) (see Table 3) [18].
The repeatability standard deviations of the participants' results are given in the documentation in the statistic data (see 4.1 to 4.2).

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].

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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 test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,29 (21,2°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 25th week of 2018. The testing method was optional. The tests should be finished at 17th August 2018 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 food supplement with the parameters taurine and caffeine in the matrix of drink powder for athletes. The recommended dosage for the food supplement is given with 20-40 g per 500 ml for the preparation of the drink powder (Note: The present LVU samples are to be used exclusively for laboratory tests and are not suitable for consumption).

The analysis methods are 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, 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 results in time.

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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^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 stat-

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istical 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.

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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.

The target standard deviation of the evaluation by precision experiment (s. 3.6.2) was considered for the parameter caffeine (ASU §64 method: L 46.00-3).

For evaluation of the parameter taurine in the present PT the target standard deviation according to the general model of Horwitz was applied (see 3.6.1).

Additionally for taurine the standard uncertainty was considered by evaluation using z'-scores (see 3.6.8).

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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/or to provide additional information for the statistical data.

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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} [18]

Parameter	**Matrix**	**Mean**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Caffeine	Coffee beverage powder cappuccino	403 mg/100 g	2,1%	6,1%	5,9%[1]	ASU §64 L 46.00-3
Caffeine	Roasted coffee mixture not decaffeinated	642 mg/100g	3,0%	5,1%	4,6	ASU §64 L 46.00-3
Caffeine	Roasted coffee not decaffeinated	1220 mg/100 g	1,6%	5,2%	5,1	ASU §64 L 46.00-3
Caffeine	Soluble coffee not decaffeinated freeze-dried	2510 mg/100 g	0,7%	3,3%	3,3	ASU §64 L 46.00-3

[1] *used for evaluation or given for information (s. chapter 4)*

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 partly using the z'-scores.

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

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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 a 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 process, calibration of equipment and composition of reagents, transmission or calculation errors, 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].

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Table 4: Characteristics of the present PT (on dark grey) 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
Caffeine	Drink powder	783 mg/100g	20,7 mg/100g	2,64%	0,64	DLA 35/2015
Caffeine	Drink powder	420 mg/100g	14,5 mg/100g	3,46%	0,54	DLA 46/2018
Taurine	Drink powder	9745 mg/100g	657 mg/100g	6,74%	1,6	DLA 35/2015
Taurine	Drink powder	5289 mg/100g	719 mg/100g	13,6%	1,9	DLA 46/2018

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 (xi) of the participant from the respective consensus value to the square root of quadrat sum of the target standard deviation (σ_{pt}) 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.

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3.9 Reproducibility coefficient of variation (CV_R)

The variation coefficient (CV) of the reproducibility (= *relative reproducibility standard deviation)* is calculated from the 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 gives the relative variability within a data region. While a low CV, e.g. <5-10% can be taken as evidence for a homogeneous set of results, a CV 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.

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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 ($X_{pt} + 2\sigma_{pt}$´) *
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**	**z-Score** (Info)	**Hinweis** / **Remark**

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

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4.1 Caffeine in mg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	8
Number of outliers	–
Mean	424
Median	418
Robust Mean (X_{pt})	**420**
Robust standard deviation (S*)	**14,5**
Number with 2 replicates	8
Repeatability SD (S_r)	11,4
Repeatability (CV_r)	2,69%
Reproducibility SD (S_R)	24,0
Reproducibility (CV_R)	5,67%
Target range:	
Target standard deviation σ_{pt}	**24,9**
Target standard deviation (for Information)	19,2
lower limit of target range	**370**
upper limit of target range	**470**
Quotient S/σ_{pt}*	*0,58*
Standard uncertainty $U_{(Xpt)}$	*6,42*
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 evaluation of precision experiments (s. 3.6.2) (German official ASU §64 method: L 46.00-3). Additionally the target standard deviation calculated according to the general model of Horwitz was given for information (s. 3.6.1).

The distribution of results showed a low variability. The quotient S*/σ_{pt} was well below 1,0. The robust standard deviation is in the range of prior PTs (s. 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).

88% of results were in the target range.

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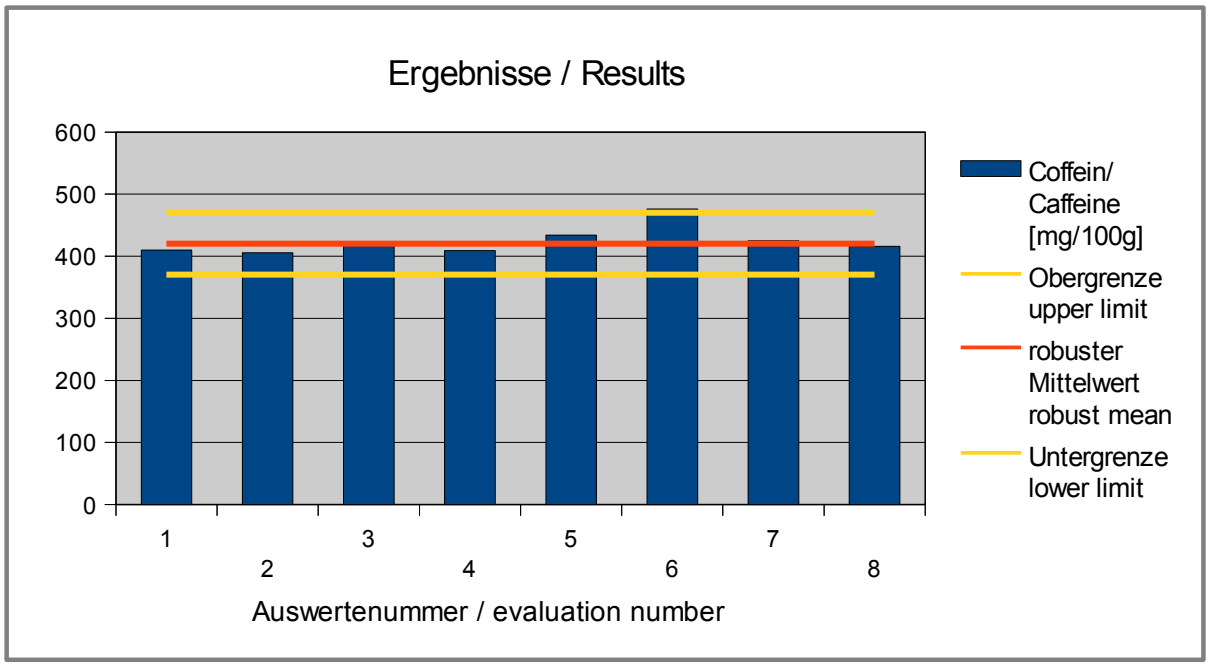

Abb. / Fig. 1: Ergebnisse Coffein / Results caffeine

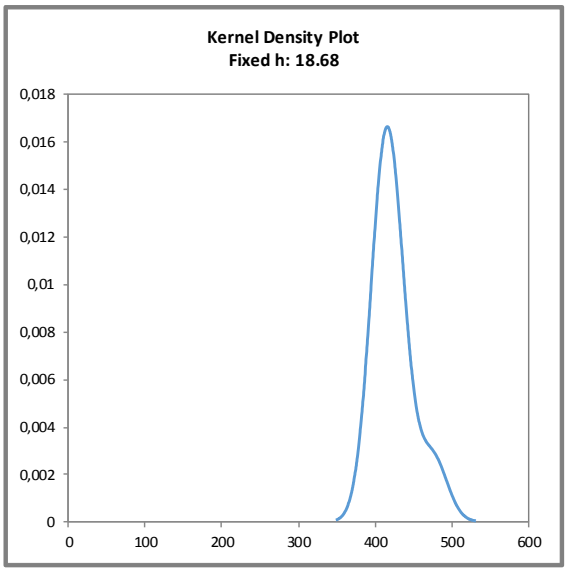

Abb. / Fig. 2:
Kerndichte-Schätzung der Ergebnisse
(mit h = 0,75 x σ_{pt} von X_{pt})

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

Comment:
The kernel density plot shows a symmetrical distribution of results with a slight shoulder, caused by one result above the target range.

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Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Coffein/ Caffeine [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	410	-10,2	-0,41	-0,53	
2	406	-14,6	-0,59	-0,76	
3	420	-0,2	-0,01	-0,01	
4	409	-11,2	-0,45	-0,58	
5	434	13,8	0,56	0,72	
6	476	55,8	2,2	2,9	
7	425	4,8	0,19	0,25	
8	416	-4,2	-0,17	-0,22	
9					

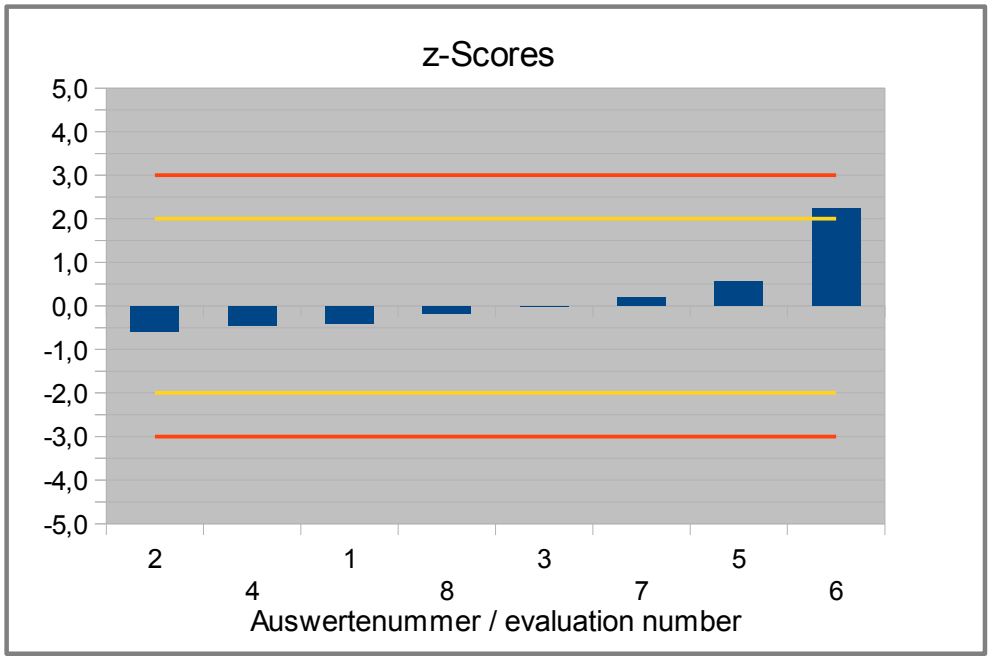

Abb. / Fig. 3: z-Scores Coffein/ Caffeine

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4.2 Taurine in mg/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	7
Number of outliers	-
Mean	5830
Robust Mean	5290
Median (x_{pt})	**5060**
Robust standard deviation (S*)	**719**
Number with 2 replicates	6
Repeatability SD (S_r)	269
Repeatability (CV_r)	5,28%
Reproducibility SD (S_R)	498
Reproducibility (CV_R)	9,74%
Target range:	
Target standard deviation σ_{pt}	**375**
lower limit of target range	**4310**
upper limit of target range	**5810**
Quotient S/σ_{pt}*	*1,9*
Standard uncertainty $U_{(Xpt)}$	*340*
Results in the target range	*5*
Percent in the target range	*71%*

Comments to the statistic data:

As assigned value the median of the participant results was used (see 3.1 Consensus of participants).

The target standard deviation was calculated according to the general model of Horwitz (s. 3.6.1).

The distribution of results showed a slightly increased variability. The quotient S*/σ_{pt} clearly above 2,0. Therefore the valuation was done considering the standard uncertainty by z'-score. The quotient S*/σ_{pt}' was below 2,0 then. The robust standard deviation is in the range of prior PTs (s. 3.6.3). The comparability of results is given.

71% of results were in the target range.

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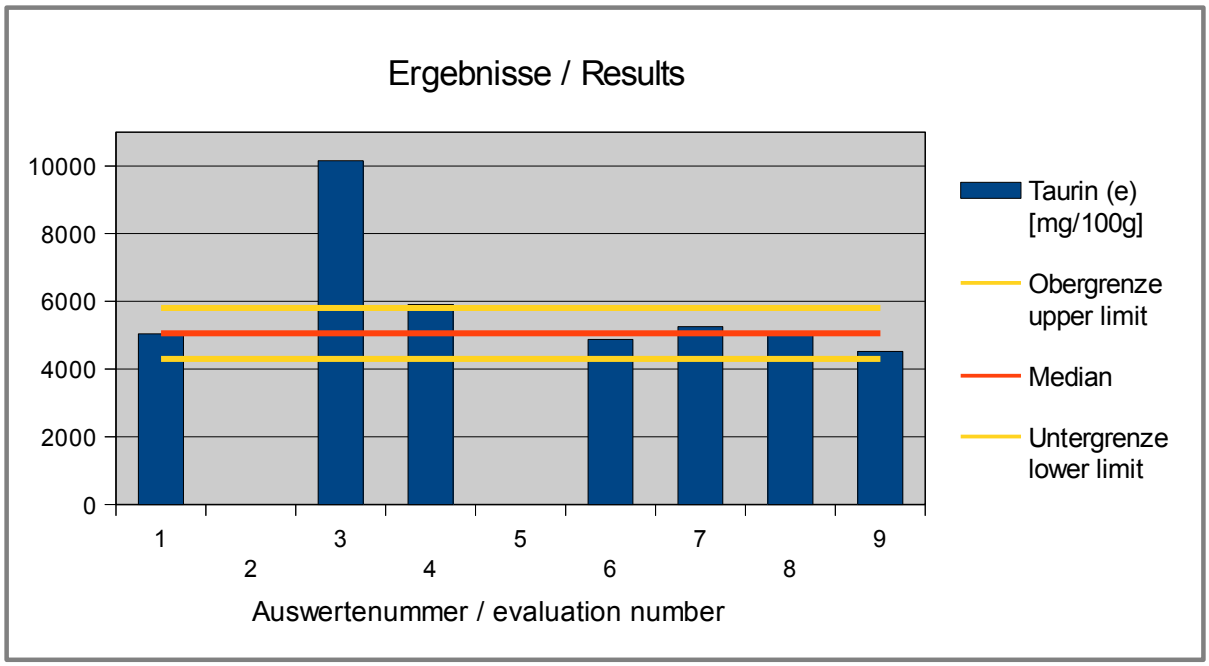

Abb. / Fig. 4: Ergebnisse Taurin / Results Taurine

Comment:
The kernel density estimation was not made, because there were less than 8 of results.

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Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Taurin (e) [mg/100g]	Abweichung [mg/100g] Deviation [mg/100g]	z'-Score (σ_{pt})	Hinweis Remark
1	5041	-14	-0,04	
2				
3	10157	5102	14	
4	5905	850	2,3	
5				
6	4878	-177	-0,47	
7	5250	195	0,52	
8	5055	0	0,00	
9	4524	-531	-1,4	

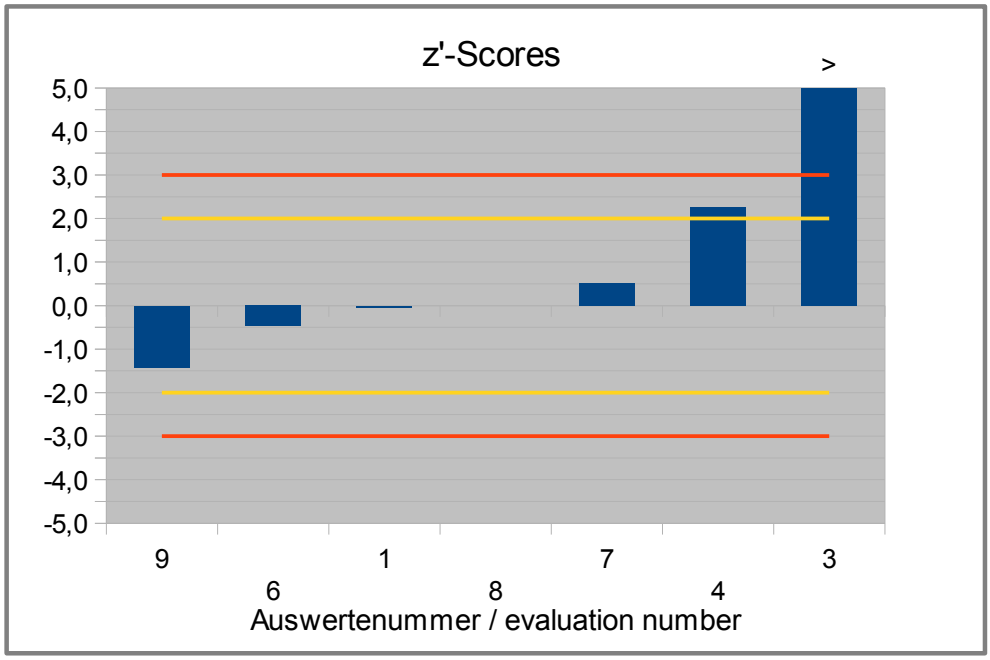

Abb. / Fig. 5: z'-Scores Taurin / parameter Taurine

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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	Evaluation number	Unit	Sample A DLA-No.	Sample B DLA-No.	Date of analysis	Final Result	Result Sample A	Result Sample B	LOQ (Limit of quantification)	Recovery included	Recovery rate
					Day/Month					yes/no	in %
Coffein/ Caffeine	1	mg/100g	57	11	18.07.18	410	408	412	0,5	no	101
	2	mg/100g	52	16	16.07.18	405,553	408,495	402,61	<0,2	no	
	3	mg/100g	25	43	09.08.18	420	427	413	5	no	
	4	mg/100g	30	38	02.07.18	409	407	411	0,2	no	
	5	mg/100g	26	42	13.07.18	434	454	413		no	
	6	mg/100g	31	37	05.07.18	476	471	480		no	
	7	mg/100g	22	46	19.07.18	425	422	428		No	
	8	mg/100g	2	66	29.06.18	416	418	413		no	
	9	mg/100g	63	5							
Taurin/ Taurine	1	mg/100g	57	11	31.07.18	5041	5023	5059	10	no	96,2
	2	mg/100g	52	16							
	3	mg/100g	25	43	04.08.18	10157	10373	9940	8	no	
	4	mg/100g	30	38	07.07.18	5905	5950	5860	0,3	no	
	5	mg/100g	26	42							
	6	mg/100g	31	37	04.07.18	4878	4861	4894		no	
	7	mg/100g	22	46	19.07.18	5250	5650	4850		No	
	8	mg/100g	2	66	29.06.18	5055	5060	5050	0,5	no	
	9	mg/100g	63	5	29.06.18	4524	4759	4289	0,05		

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5.1.2 Analytical Methods

Parameter	Evaluation number	Method description, like in a analysis report/ norm/ literature	Notes to sample preparation	Notes to analytical method	Calibration and reference material	Recovery with same matrix	Method accredited ISO/IEC 17025	Further Remarks
						yes/ no	yes/ no	
Coffein/ Caffeine	1	in-house method	hot water extraction	HPLC-DAD (detection 280 nm)	Sigma	yes	yes	
	2	ASU L 18.00-16 (1999-11)					yes	
	3	ASU L 00.00-28, extended regarding analyte caffeine	Extraction with Phosphate buffer-Acetonitrile mixture		Calibration with external standard		yes	
	4						yes	
	5	In house method 86.04	dilution	HPLC and UV	Calibration with 6 points Reference material = NIST	yes	yes	
	6	L 47.05-1: 1997-09 according to DIN 10810 (solid tea extract and food preparations with tea extract)		HPLC-UV			yes	
	7	Internal Method	Dilute, sonication prior to analysis		External calibration		Yes	
	8	HPLC with UV detection					no	
	9							
Taurin/ Taurine	1	in-house method	pre column derivatization	HPLC_FLD	Sigma	yes	yes	
	2							
	3	HPAEC-IPAD	aqueous extraction		Calibration with external standard		yes	
	4						yes	
	5							
	6	ASU § 64 LFGB L 49.07-1 and -2		HPLC-UV			no	
	7	Waters AcQTag Ultra Derivitization Method	Pre-Column derivitization		External calibration curve with internal standard		No	
	8	HPLC with fluorescence detection					no	
	9	In-house method, HPLC-UV			external Calibration; reference material: Energy Drink / Milk Powder	no	yes	

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5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 46-2018

Weight whole sample	1,81	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	21,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,06	55	21,7
2	4,97	60	24,1
3	5,05	64	25,3
4	4,99	58	23,2
5	5,07	74	29,2
6	4,98	53	21,3
7	5,07	62	24,5
8	5,02	51	20,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	59,6	Particles
Standard deviation	7,04	Particles
χ^2 (CHI-Quadrat)	5,82	
Probability	**56**	%
Recovery rate	111	%

Normal distribution		
Number of samples	8	
Mean	23,7	mg/kg
Standard deviation	2,80	mg/kg
rel. Standard deviaton	11,8	%
Horwitz standard deviation	9,93	%
HorRat-value	**1,2**	
Recovery rate	111	%

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5.2.2 Trend line function of 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:

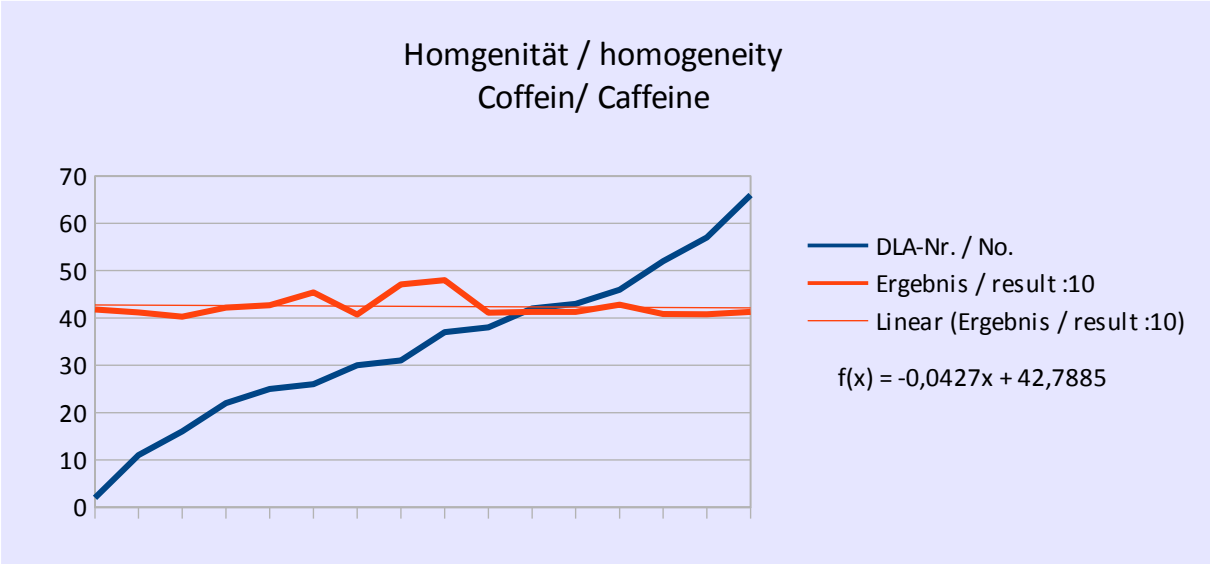

Abb./Fig. 6:
Trendfunktion Probennummern vs. Ergebnisse (1/10 dargestellt)
trend line function sample number vs. results (1/10 shown)

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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 46-2018***
PT name	***Food Supplement II: Athletes Product with Taurine + Caffeine***
*Sample matrix**	***2 Samples:*** *Drink powder for athletes / Ingredients: glucose, maltodextrin, fructose, isomaltulose, acidifier: citric acid, taurine, caffeine and other food additives*
Number of samples and sample amount	*2 identical samples, 25 g each.*
Storage	*room temperature*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*quantitative:* ***Taurine + Caffeine***
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 A and B as well as the final results calculated as mean of the double determination (samples A and B) 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 A and B)* – *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 August, 17th 2018***
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.

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6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		FRANCE
		UNITED KINGDOM
		CZECH REPUBLIC
		USA
		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.]

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7. Index of references

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17. AOAC Guidelines for Standard Method Performance Requirements (2016)
18. ASU §64 LFGB L 46.00-3 (2013), Untersuchung von Kaffee und Kaffee-Erzeugnissen; Bestimmung des Coffeingehaltes mittels HPLC; Referenzverfahren / ISO 20481 (2008): Coffee and coffee products - Determination of the caffeine content using high performance liquid chromatography (HPLC) - Reference method

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