DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 20/2014

Sudan-Dyes in spices

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Contents

1 Introduction

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

2 Evaluation

2.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X.

2.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (S^x) was calculated.

2.3 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic (95% significance). Detected outliers were stated for information only, when z-score was < -2 or > 2.

2.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

2.4.1 General model (Horwitz / Thompson)

The relative target standard deviation in % of the assigned value was derived from following equation (Horwitz)

$$\sigma_{(\%)} = 2^{(1-0,5\log X)} .$$

From the result the target standard deviation is calculated

$$\sigma = X * \sigma_{(\%)} / 100.$$

For analytes with a content below 120 µg/kg after the evaluation of a lot of mycotoxin- proficiency testing schemes after 1997 it was suggested for the target standard deviation a steady value of 22 % (Thompson), analogical:

$$\sigma = 0,22\ C / mr;$$

with σ = Target standard deviation for contents < 120 µg/kg
C = assigned content, expressed as a dimensionless mass ratio
mr = dimensionless mass ratio.

2.4.2 Precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment the between-laboratories standard deviation (σ_L) can be calculated :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} .$$

And then, using the number of replicate measurements n, each participant is to perform, the standard deviation for proficiency assessment is calculated :

$$\sigma = \sqrt{(\sigma_L^2 + (\sigma_r^2 / n))} .$$

If available, the precision data from official methods for each parameter are used to calculate the target standard deviation.

2.5 Z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ) the result (x) of the participant is deviating from the assigned value (X).

Participants' z-scores are derived as:

$$z = (x - X) / \sigma \quad ;$$

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

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

2.6 Z'-Score

The z'-Score can be used to assess the results of the participants in case the standard uncertainty must be considered (s. 2.8).

The calculation is carried out as follows (3)

$$z' = (x - X) / \sqrt{\hat{\sigma}^2 + u_X^2}$$

For the following evaluation $\sqrt{\hat{\sigma}^2 + u_X^2}$ is defined as $\hat{\sigma}'$, the target standard deviation considering the standard uncertainty of the results.

The requirements for the analytical performance are considered as fulfilled then, if

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

2.7 Quotient S^x/σ

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

2.8 Standard uncertainty

The assigned value X has a standard uncertainty u_X that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty u_X for this PT is calculated as follows

$$u_X = 1{,}25 * S^x / \sqrt{(p)} \quad .$$

If $U_x \leq 0{,}3 * \sigma$ the standard uncertainty of the assigned value has not to be included in the interpretation of the results of the PT. The Quotient U_x/σ is given in the evaluation.

3 Realisation

3.1 Test material

The test material was a mixture of different spices like paprica, chilli and curry, to which glucose for the test of homogeneity was added. The spices contained Sudan I, III and IV, known from former PT. Auramine was detected in one spice by single analysis with a content of app. 3500 µg/kg.
App. 1000 g of the material were mixed, homogenized and then packaged in portions to approximately 10 g. The portions were numbered chronologically.
The expected minimum contents of the known, detectable dyes are given in the following table.

Dye	**Minimum expected content**
Sudan I	2.920 µg/kg
Sudan III	410 µg/kg
Sudan IV	600 µg/kg
Auramine	1.980 µg/kg

Due to the small amount of participants and an obvious bimodal distribution of the results, the minimum expected contents of the dyes were considered for the calculation of the assigned values.

3.1.1 Homogeneity

The material was checked for homogeneity. The content of the added glucose was determined in 5 samples after extraction and cleanup using HPLC/RI. The within-samples standard deviation was relative 3,9% and the between-samples standard deviation was relative 4,5%, with which the homogeneity was considered as given.
The repeatability standard deviation of the participants for Sudan I was also calculated. It is relative 4,8 %, which is in the range of the achievable precision. The repeatability standard deviation of the participants for Sudan I is given in the documentation.
Additionally in the documentation the portion numbers are graphically assigned to the results of Sudan I. There is no institute independent trend recognizable in the results which could suggest inhomogeneity.

3.2 Tests

Two test samples were sent to every participating laboratory in the 4th week of 2014. The test method was optional. The tests should be finished at 10th march 2014.

3.3 Results and statistic evaluation

The participants submitted their results in standard forms, which have been handed out with the samples.
The statistical evaluation was carried out according to 2.4.1 (Horwitz) with the final results calculated as mean of both samples, if at least 7 results were submitted:
Sudan I, Sudan III, Sudan IV.
For all 3 dyes the assigned value was not calculated as robust mean of all the results. Due to a bimodal distribution of the results, the assigned value was calculated only with results of the participants, which were in the same magnitude as the expected minimum contents (see 3.1).
Because Sudan I results showed high variation the target range was extended according to 2.6. Both, Z-Score and Z´-Score of Sudan I are given in 4.1. The number of participants inbetween the limits of the target range does not change with the extension.
Queried and documented were further results and the testing method applied.
The submitted results showed a bimodal distribution. This distribution does not depend on inhomogeneity of the samples (see 5.2.2/5.1).
9 out of 11 participants submitted 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 upper table the characteristics are listed:

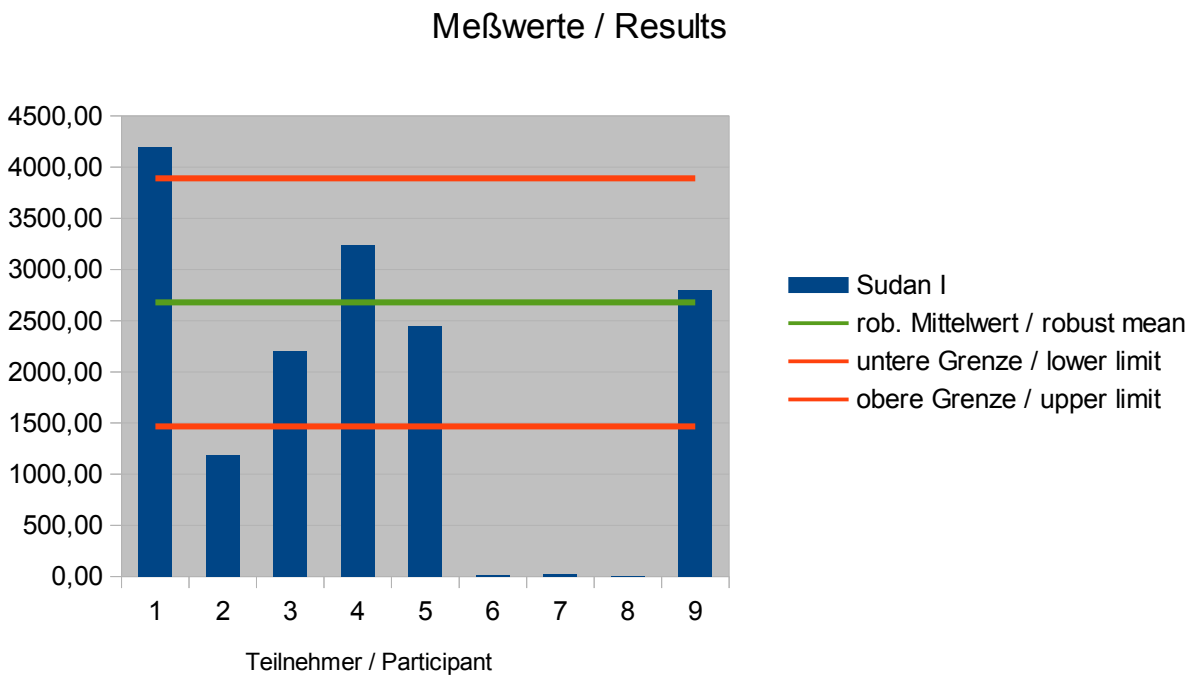
Number of results
Number of outliers
Mean
Median
Robust mean (X)
Robust standard deviation(S^x)
Target standard deviation (σ)
Lower limit of target range (X – 2σ) or (X – 2σ´)
Upper limit of target range (X + 2σ) or (X + 2σ´)
Quotient S^x/σ
Standard uncertainty U_x
Quotient U_x/σ
Results in target range.

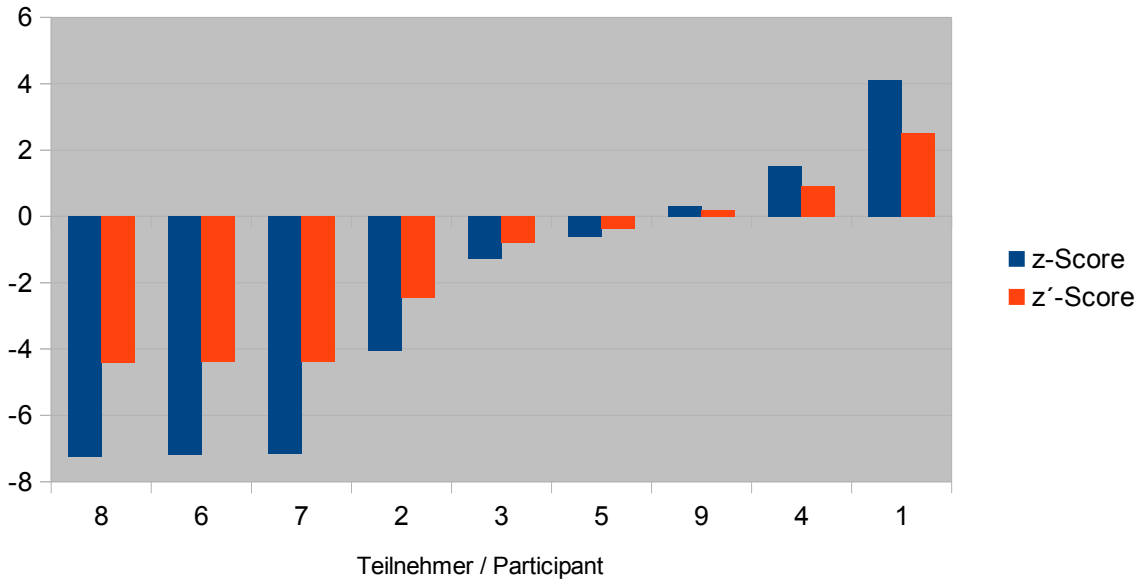
In the lower table -laboratories- the individual results of the participating laboratory are listed:

Evaluation number	Test result	Deviation from assigned value	Z-Score (σ)	Remarks

4.1 Sudan I in µg/kg

Statistic data	
Number of results	9
Number of outliers	0
Mean	1791
Median	2200
Robust mean (X)	2679
Robust standard deviation (Sx)	1151
Target standard deviation (sigma)	370
Lower limit of target range	1468
Upper limit of target range	3890
Quotient Sx/sigma	3,1
Standard uncertainty Ux	480
Quotient Ux/sigma	1,3
Results in target range	4
Percent in target range	44

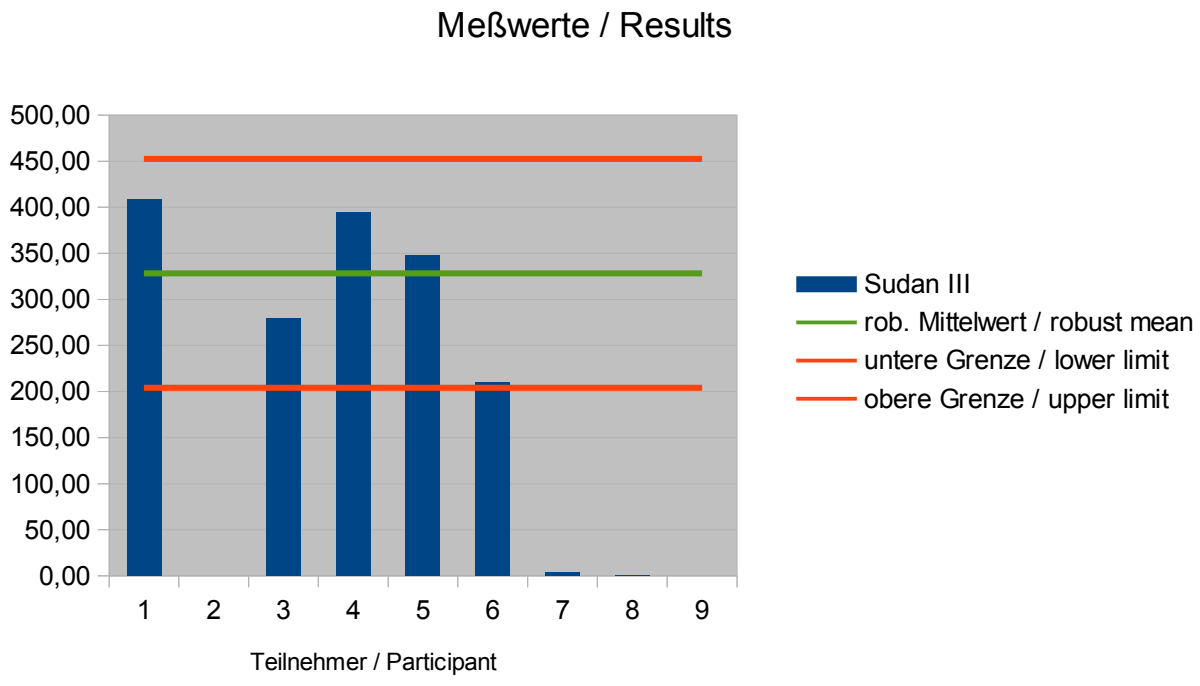


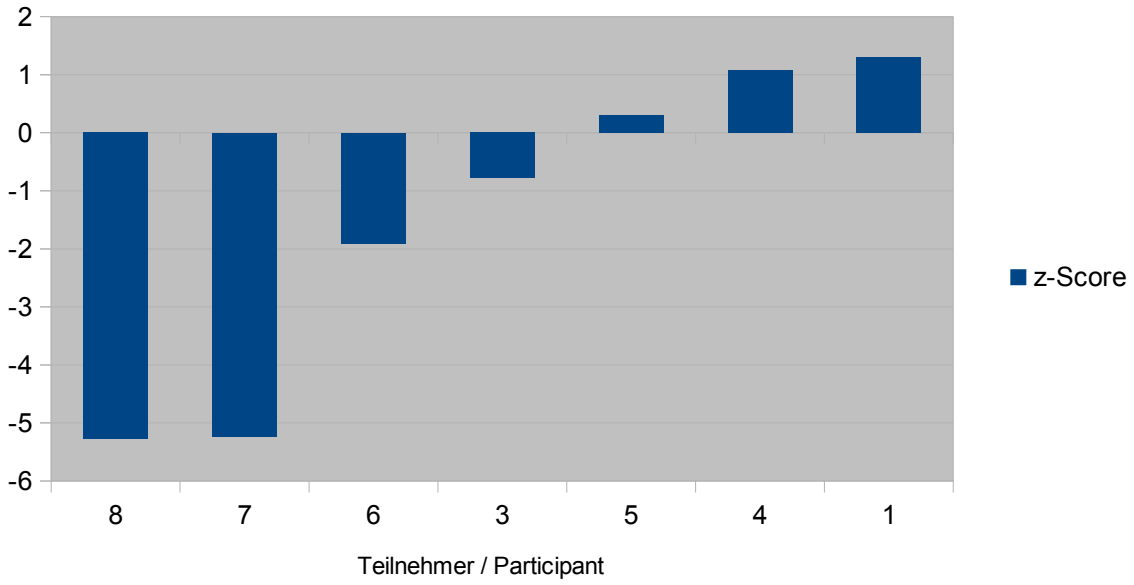


Auswerte nummer / Evaluation number	***Sudan I***	***Abweichung / Deviation***	***z-Score***	***z´-Score***	***Hinweis / Remark***
1	4199	1520	4,1	2,5	
2	1186	-1493	-4,0	-2,5	
3	2200	-479	-1,3	-0,8	
4	3240	561	1,5	0,9	
5	2450	-229	-0,6	-0,4	
6	< 10		< -7,2	< -4,4	To calculate (X) not included
7	28,62	-2650,38	-7,2	-4,4	To calculate (X) not included
8	2	-2677	-7,2	-4,4	To calculate (X) not included; Unit?
9	2799	120	0,3	0,2	

4.2 Sudan III in µg/kg

Statistic data	
Number of results	7
Number of outliers	0
Mean	235
Median	280
Robust mean (X)	328
Robust standard deviation (Sx)	94,3
Target standard deviation (sigma)	62,1
Lower limit of target range	204
Upper limit of target range	453
Quotient Sx/sigma	1,5
Standard uncertainty Ux	44,6
Quotient Ux/sigma	0,7
Results in target range	5
Percent in target range	71

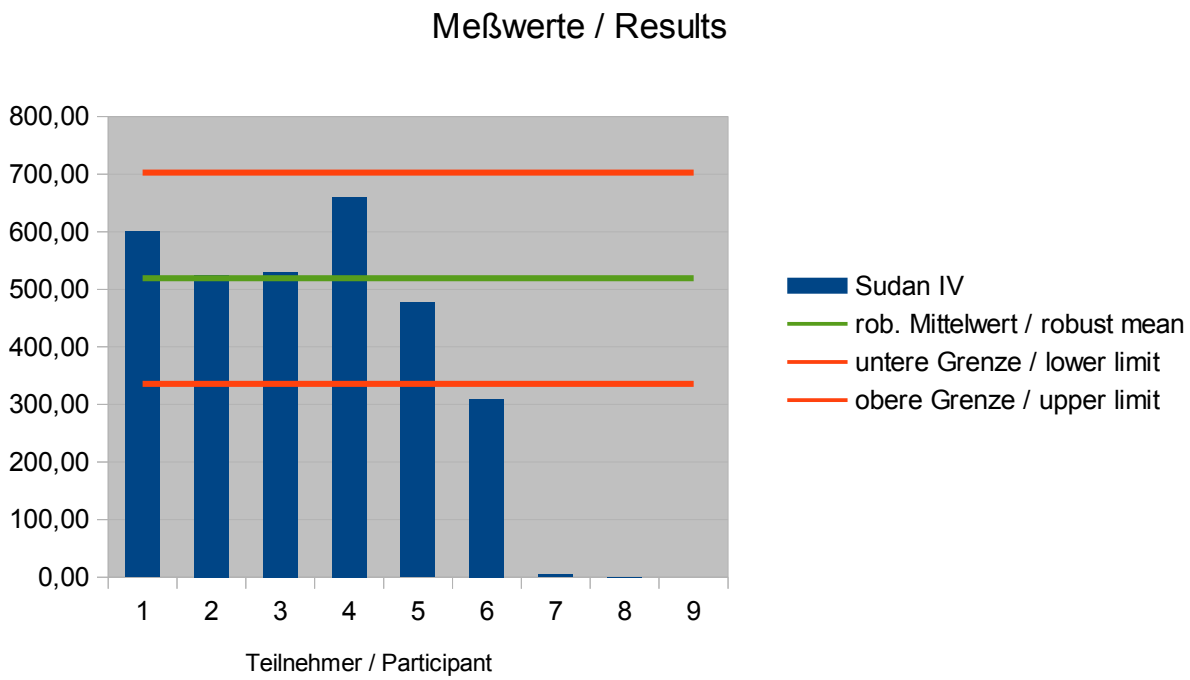


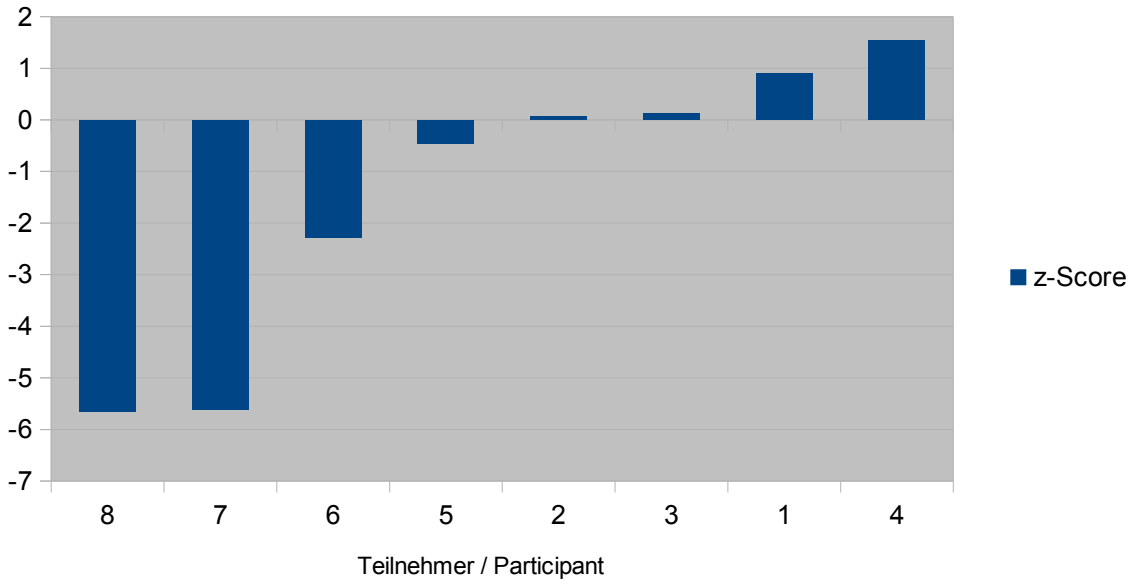


Auswerte nummer / Evaluation number	***Sudan III***	***Abweichung / Deviation***	***z-Score***	***Hinweis / Remark***
1	409	80,7	1,3	
2				
3	280	-48,3	-0,8	
4	395	66,7	1,1	
5	347,5	19,2	0,3	
6	210	-118,3	-1,9	
7	3,31		-5,2	To calculate (X) not included
8	0,27		-5,3	To calculate (X) not included; unit?
9				

4.3 Sudan IV in µg/kg

Statistic data	
Number of results	8
Number of outliers	0
Mean	389
Median	501
Robust mean (X)	519
Robust standard deviation (Sx)	132
Target standard deviation (sigma)	91,7
Lower limit of target range	336
Upper limit of target range	702
Quotient Sx/sigma	1,4
Standard uncertainty Ux	58,2
Quotient Ux/sigma	0,6
Results in target range	5
Percent in target range	63





Auswerte nummer / Evaluation number	***Sudan IV***	***Abweichung / Deviation***	***z-Score***	***Hinweis / Remark***
1	601	81,88	0,9	
2	525	5,88	0,1	
3	530	10,88	0,1	
4	660	140,88	1,5	
5	477	-42,12	-0,5	
6	310	-209,12	-2,3	
7	5,02	-514,1	-5,6	To calculate (X) not included
8	0,43	-518,69	-5,7	To calculate (X) not included; unit?
9				

5 Documentation

5.1 Primary data

Teilnehmer / Participant	Probennummer / Sample number A	Probennummer / Sample number B	Einheit / Unit	Sudan I	Ergebnis / Result A	Ergebnis / Result B	Wiederfindung / Recovery
1	10	40	µg/kg	4.199	4.290	4.108	73,4
2	9	31	µg/kg	1186	1111	1157	105
3	28	29	µg/kg	2200	2100	2300	see remark
4	20	48	µg/kg	3240	3220	3260	81,5
5	4	37	µg/kg	2450	2340	2560	90
6	17	42	µg/kg	<10	<10	<10	80-120
7	15	34	µg/kg	28,62	29,16	28,07	
8	12	36	µg/kg	2	2,1	2	50
9	2	26	µg/kg	2799	2753	2845	100

Teilnehmer / Participant	Sudan II	Ergebnis / Result A	Ergebnis / Result B	Wiederfindung / Recovery	Sudan III	Ergebnis / Result A	Ergebnis / Result B	Wiederfindung / Recovery
1					409	432	385	135,7
2								
3	<10	<10	<10	see remark	280	240	310	see remark
4	n.d.				395	400	390	81,8
5	0	0	0	90	347,5	243	452	90
6	<10	<10	<10	80-120	210	190	200	80-120
7	<1	<1	<1		3,31	3,36	3,26	
8	nd	nd	nd	25	0,27	0,26	0,29	27
9	n.d.			100	n.d.			100

Teilnehmer / Participant	Sudan IV	Ergebnis / Result A	Ergebnis / Result B	Wiederfindung / Recovery	Sudan 7B	Ergebnis / Result A	Ergebnis / Result B	Wiederfindung / Recovery
1	601	648	555	88,8				
2	525	635	623	83,4				
3	530	550	510	see remark	<10	<10	<10	see remark
4	660	660	660	82	not tested			
5	477	505	449	90	0	0	0	90
6	310	380	350	80-120	<10	<10	<10	80-120
7	5,02	5,07	4,98		<1	<1	<1	
8	0,43	0,43	0,43	21	nd			
9	n.d.			100	n.d.			100

Teilnehmer / Participant	Auramin	Ergebnis / Result A	Ergebnis / Result B	Wiederfindung / Recovery
1	Not tested			
2				
3	1600	1500	1600	see remark
4	Not tested			
5	9170	7940	10400	90
6	170	130	150	80-120
7				
8	n.a.			
9	Not tested			100

5.2 Homogeneity

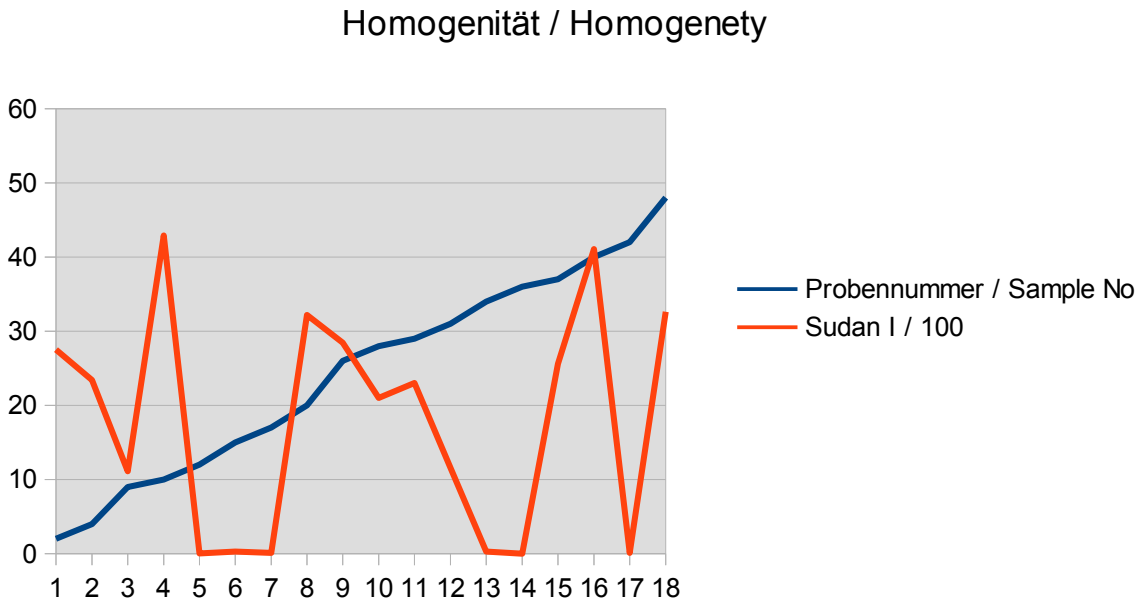
5.2.1 Test for homogeneity with additional glucose

Results within one sample	2,24	Results between the samples	2,15
	2,02		2,25
	2,20		2,07
	2,18		2,00
	2,20		2,08
Mean	2,168		2,110
Standard deviation	0,086		0,095
In %	3,9		4,5

5.2.2 Repeatability standard deviation of duplicate tests of the participants

The repeatability standard deviation was calculated with the data documented in 5.1. It is 129 µg/kg = 4,9% of X for Sudan I.

5.2.3 Comparison of sample number / test result



5.3 Analytical methods

Parti-cipant	Method	Extraction	Clean up	Chromato-graphy	Detection	Standard material	Concentration and age of standard-solutions	Reference material	Recovery with same matrix	Method is accredited	Remarks
									Yes / no	Yes / no	
1	Direct	MeOH/Acetone	none	C18	ESI-MS/MS	Dr Ehrensdorfer	5-600 ppb		yes	no	
2		Acetone	H2O/Fil-tration	LC	MS/MS	Ehrensdorfer	0,1 - 2,5 µg/L/ January 2014	no	yes	no	
3	Fat soluble azo-dyes (Sudan)	Liquid extraction (Dichloromethane, Isopropanole and formic acid)	SPE (SRATA-X 33u)	HPLC	MSMS ESI+	Sigma-Aldrich		no	no	yes	Quantified using standard added sample (before clean up)
4	Sudan I to IV in spices	Cyclohexane / Ethylacetate	GPC	Cyclohexane/ Ethylacetate	DAD	Dr Ehrenstorfer	v.2012 90,5%	none	yes	yes	
5	In house method	Liquid extraction		LC	MSMS	External standards	3920	Solution	no	yes	
6	LC-MS/MS	Acetone	Zenrti-fuge	HPLC	MS/MS	various	1mg/mL, fresh	FAPAS	yes	yes	
7	Sudan dyes	Acetonitrile	none	HPLC	MSMS		Freshly prepared	none	no	no	
8	LC-MS	1g sample with 10 ml 1% formic acid in acetonitrile	none	UPLC	MS/MS	Sigma-Aldrich	5 ng/ml, max. 6 month	no	yes	no	LOD 0.05
9	Sudan dyes in powdered food are determined after extraction with acetonitrile and dilution with water using LC-MS/MS.	ACN; dilution with water	none	HPLC, RP-Phase	MS/MS	Dr Ehrensdorfer	app. 10mg/50ml; freshly prepared	-	yes	yes	-

6 Index of participant laboratories

Teilnehmer / Participant	Ort / Location	Land/Country
		Germany
		Germany
		Germany
		Germany
		Pakistan
		The Netherlands
		Germany
		Switzerland
		The Netherlands
		Southafrica
		Germany

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

7 Index of literature

1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
3. 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
4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
5. ASU §64 LFGB : Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung
6. DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Chemistry Laboratories ; Pure Appl Chem, 78, 145 – 196 (2006)
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10. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
11. 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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