DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

DLA 09/2014

Food Dyes in Sweets

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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. Realisation

2.1 Test material

The test material was a mixture of a custard powder (Ingredients: corn starch, common salt, natural vanilla flavouring, colouring agents:

- E 101/ riboflavin,
- E 160b/ norbixin)

with the additional added colouring agents

- E 102 (tartrazine, appr. 300 mg/kg),
- E 104 (quinoline yellow, appr. 100 mg/kg),
- E 131 (patent blue V, appr. 300 mg/kg) and
- E 132 (indigotine, appr. 350 mg/kg)

and glucose (appr. 15%) added for the homogeneity test.

Approximately 0,6 kg of the material were homogenized and then packaged lightproof in portions to approximately 9 g. The portions were numbered chronologically. The material was checked for homogeneity.

2.1.1 Homogeneity

To verify the homogeneity of the test material glucose was added before homogenisation. The homogeneity was examined with glucose/ HPLC.

The homogeneity is considered verified with a standard deviation of 1,8 %, see in the documentation.

2.2 Test

Two portions of test material were sent to every participating laboratory in the 34th week of 2014. The testing method was optional. The tests should be finished at 6th October 2014 the latest.

2.3 Results

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

Only if at least 7 results were submitted, the statistical evaluation has been carried out: positive/ negative results for each parameter and the respective concentration (in mg/kg) of the identified dyes.

Queried and documented were single results, recovery and the used testing method.

From the 13 participants have 12 participants submitted at least one result.

3. Evaluation

The evaluation of this PT was performed qualitative and quantitative.

3.1 Evaluation of the qualitative results

The results of each dye are presented in a separate table in the result section. If a participant could not prove a dye of a sample, this result would be added to the negative results.

The dyes had been assessed qualitative by the percentage shares positive or negative results. If ≥ 75 % positive or negative results were available, for this sample a consensus result (positive or negative) was determined.

3.2 Evaluation of the quantitative results

The results of the dyes E 102 and E 131 were used for a statistical analysis. (Based on the wide variance of the results we only declared the mean, the median and the robust mean for the dye E 102).

There were only < 7 results for the dyes E 101, 104, E 132 and E 160b, so it was impossible to perform a statistical evaluation. These result are recorded in the documentation.

3.2.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X (6). The distribution of submitted results showed no hint for bimodal distribution or other reasons for a higher variability.

3.2.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (S_x) was calculated (6).

3.2.3 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic (significance level: 5%) (5). Detected outliers were stated for information only, when z-score simultaneously was < -2 or > 2.

3.2.4 Target standard deviation

The relative target standard deviation in % of the assigned value is calculated according to the following equation.

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

Out of this is calculated the target standard deviation in µg/kg

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

This PT was characterised by a limited number of participants (12 thereof 5 included only qualitative results) and due to the method a large standard deviation. A publication about the "Separation and Determination of dyes in Sweet with HPLC" (11) reports on relative repeatability standard deviation (RSD_r) of 6% to 8%. It can be assumed that an empiric factor will be 1,5 – 3 between the RSD_r and the relative reproducibility standard deviation (RSD_R).

For this PT a RSD_R of 15 % was set as target standard deviation.

3.2.5 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation ($\hat{\sigma}$) the result (x) of the participant is deviating from the assigned value (X)(6).

Participants' z-scores were derived as:

$$z = (x - X) / \hat{\sigma} \ ;$$

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

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

3.2.6 Quotient $S^x/\hat{\sigma}$

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.

For this PT the results of E 131 show a sufficient comparability.

3.7 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 (6).

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

If $u_X \leq 0{,}3 * \hat{\sigma}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT (6). The quotient $u_X / \hat{\sigma}$ is reported in the characteristics of the test.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

The qualitative results of die participants are shown in following format:

dye	E101	E102	E104	E131	E132	E160b	qualitative assessment*
Evaluation number	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	

* In the column "quantitative assessment" it is stated how much of the dyes are in agreement with the consensus result.

The quantitative results of die participants are shown in following format:

Number of results	
Number of outliers	
Mean	
Median	
Robust mean (X)	
Robust standard deviation (S^x)	
Target standard deviation($\hat{\sigma}$)	
Lower limit of target range (X - 2 $\hat{\sigma}$)	
Upper limit of target range (X + 2 $\hat{\sigma}$)	
Quotient $S^x/\hat{\sigma}$	
Standard uncertainty u_x	
Quotient $u_X/\hat{\sigma}$	
Number of results in the target range	

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

Evaluation number	Result	Deviation	z-Score	Remark

4.1 Qualitative results

To make it possible to classify the results of the participants, a qualitative assessment in relation to the consensus result was made.

Dye	E 101	E 102	E 104	E 131	E 132	E 160b	Qualitative assessment
Teilnehmer/ participant	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	
1	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)
2	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)
3	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)
4	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)
5	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)
6	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)
7	pos.	pos.	pos.	pos.	pos.	neg.	5/6 (83%)
8	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)
9	neg.	pos.	pos.	pos.	neg.	neg.	5/6 (83%)
10	neg.	pos.	neg.	pos.	neg.	neg.	4/6 (66%)
11	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)
12	neg.	pos.	pos.	pos.	pos.	neg.	6/6 (100%)

	E 101	E 102	E 104	E 131	E 132	E 160b
Number positive	1	12	11	12	10	0
Number negative	11	0	1	0	2	12
Percentage positive	8%	100%	92%	100%	83%	100%
Percentage negative	92%	0%	8%	0%	17%	100%
Consensus result	**neg.**	**pos.**	**pos.**	**pos.**	**pos.**	**neg.**

The dyes E 101 and E 160b should be according to the declaration in the sample but the consensus result revealed a „negative" result. More than 75% of the participants could not detect these dyes. Participant no. 7 was the only one who could detect the labelled dye E 101. But the result must be assessed with "nonconforming", because no other participant could detect this dye.

4.2 Quantitative results

4.2.1 Overview

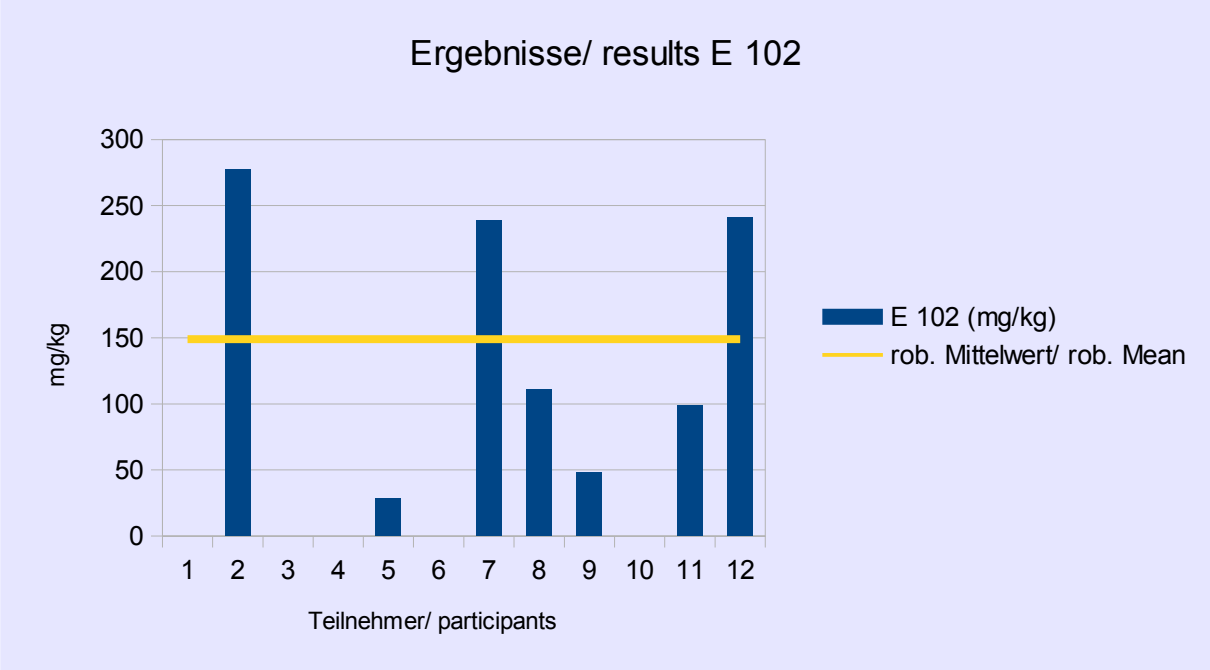
Overall results (in mg/kg):

Dye	E 101	E 102	E 104	E 131	E 132	E 160b	Bemerkung/ remark
Teilnehmer/ participant							
1	-	positive	positive	positive	positive	-	
2	-	277,2	74,1	182,6	256,4	-	
3	-	positive	positive	positive	positive	-	
4	-	positive	positive	positive	positive	-	
5	-	28,74	15,59	129,98	44,64	-	
6	-	positive	positive	positive	positive	-	
7	positive	238,785	positive	153,165	266,275	-	
8	-	110,7	12,4	83,8*	214,15	-	E 131 Ausreißer/ outlier
9	-	47,8	15,3	163,2	-	-	
10	-	positive	-	positive	-	-	
11	negative	99	23,65	139,7	78,55	negative	
12	-	241*[1]	54*[1]	180*[1]	218*[1]	-	

*[1] *The final result was calculated by DLA*

4.2.2 Results dye E 102 (mg/kg)

Characteristics	
Number of results (without no. 14)	7
Number of outliers	0
Mean	149
Median	112
Robust mean (X)	149
Robust standard deviation (S^x)	not evaluated
Target standard deviation($\hat{\sigma}$)	not evaluated
Lower limit of target range (X - 2 $\hat{\sigma}$)	not evaluated
Upper limit of target range (X + 2 $\hat{\sigma}$)	not evaluated
Quotient $S^x/\hat{\sigma}$	not evaluated
Standard uncertainty u_x	not evaluated
Quotient $u_X/\hat{\sigma}$	not evaluated
Number of results in the target range	not evaluated



Because of the limited number of results and high standard deviation of the methods we do not report the key figures of this PT.

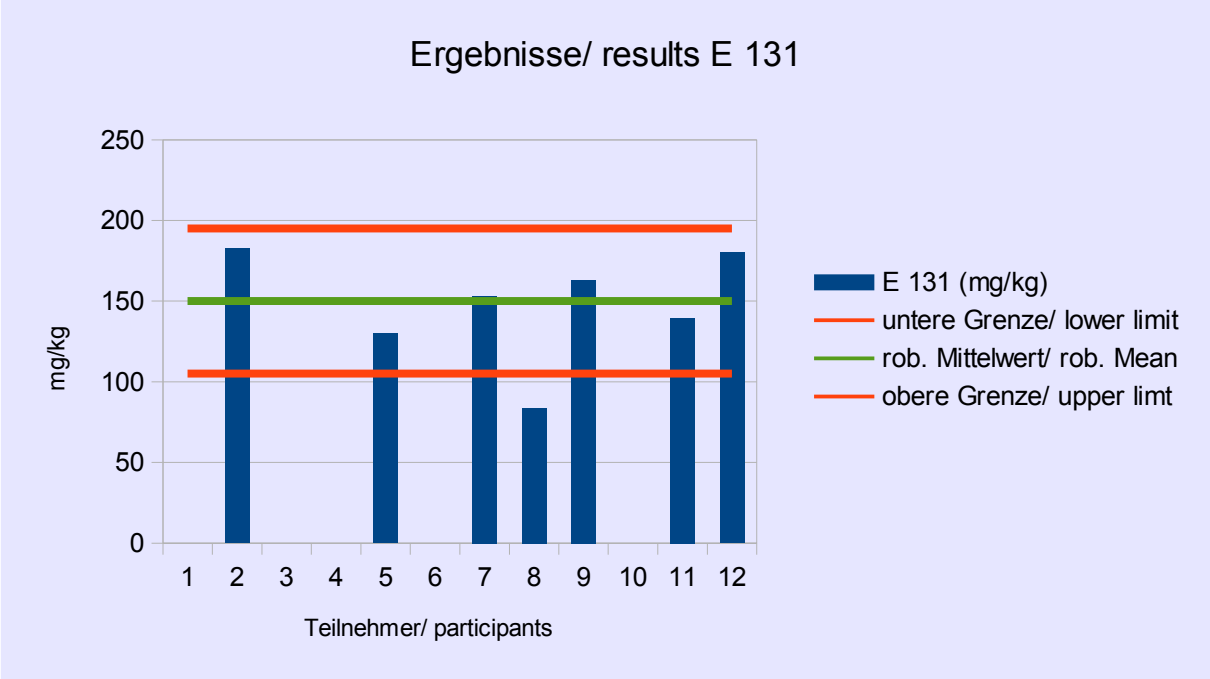
Laboratories

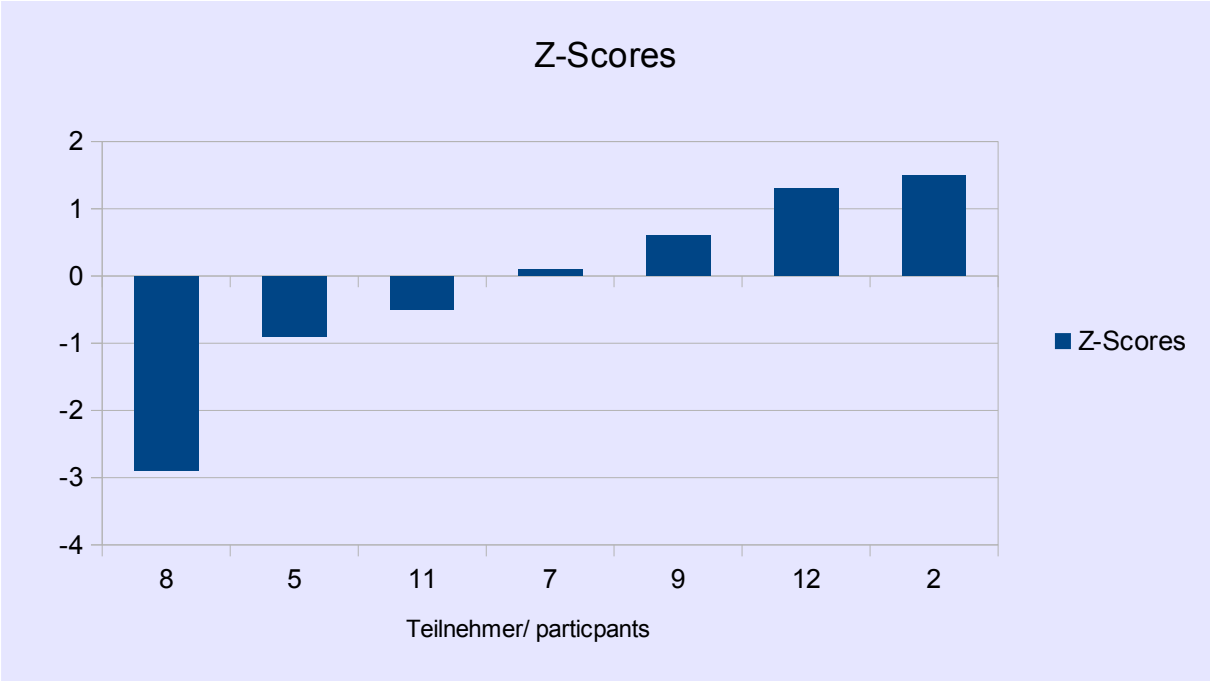
Teilnehmer/ participant	E 102
	mg/kg
1	
2	277,2
3	
4	
5	28,74
6	
7	238,785
8	110,7
9	47,8
10	
11	99
12	241*

** The final result was calculated by DLA*

4.2.3 Results dye E 131 (mg/kg)

Characteristics	
Number of results (without no. 14)	7
Number of outliers	1
Mean	147
Median	153
Robust mean (X)	150
Robust standard deviation (S^x)	33,1
Target standard deviation($\hat{\sigma}$)	22,5
Lower limit of target range (X - 2 $\hat{\sigma}$)	104,9
Upper limit of target range (X + 2 $\hat{\sigma}$)	194,8
Quotient $S^x/\hat{\sigma}$	1,5
Standard uncertainty u_x	15,7
Quotient $u_X/\hat{\sigma}$	0,7
Number of results in the target range	6 (86%)





Laboratories

Teilnehmer/ participant	E 131	Abweichung/ deviation	Z-Scores	Bemerkungen/ remarks
	mg/kg	mg/kg		
1				
2	182,6	32,75	1,5	
3				
4				
5	129,98	-19,87	-0,9	
6				
7	153,165	3,32	0,1	
8	83,8	-66,05	**-2,9**	Ausreißer/ outlier
9	163,2	13,35	0,6	
10				
11	139,7	-10,15	-0,5	
12	180*	30,15	1,3	

* *The final result was calculated by DLA*

4.2.4 Further submitted results

Dye	E 133	E 142	E 151
Teilnehmer/ participant			
	mg/kg	mg/kg	mg/kg
1	–	–	–
2	–	–	–
3	–	–	–
4	–	–	–
5	–	–	–
6	–	negative	negative
7	–	–	–
8	2,8	–	–
9	–	–	–
10	–	–	–
11	negative	negative	negative
12	–	–	–

5. Documentation

5.1 Primary data

5.1.1 Dye E 101

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe 1/ sample 1	DLA-Nr Probe 2/ sample 2	Ergebnis 1/ result 1	Ergebnis 2/ result 2	Wiederfindungs -rate/ recovery
	mg/kg			mg/kg	mg/kg	in %
7	positive	3	24	positive	positive	
11	< LOD	36	18	< LOD	< LOD	

5.1.2 Dye E 102

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe 1/ sample 1	DLA-Nr Probe 2/ sample 2	Ergebnis 1/ result 1	Ergebnis 2/ result 2	Wiederfindungs -rate/ recovery
				mg/kg	mg/kg	in %
1	positive	11	30			
2	277,2	4	42	278,1	276,4	90,1
3	positive	12	41	positive	positive	
4	positive	21	34			
5	28,74	38	25	28,74	28,73	100
6	positive	22	39	positive	positive	
7	238,785	3	24	236,61	240,96	
8	110,7	35	47	110,9	110,5	100
9	47,8	13		positive	positive	-
10	positive	10	32	positive		
11	99	36	18	93,3	104,7	
12		14	26	240,58	241,92	

5.1.3 Dye E 104

Teilnehmer/ participant	Ergebnis / result	DLA-Nr Probe 1/ sample 1	DLA-Nr Probe 2/ sample 2	Ergebnis 1/ result 1	Ergebnis 2/ result 2	Wiederfindungs-rate/ recovery
	mg/kg			mg/kg	mg/kg	in %
1	positive	11	30			
2	74,1	4	42	71,1	77,0	60,6
3						
4	positive	21	34			
5	15,59	38	25	15,35	15,84	100
6	negative	22	39	negative	negative	
7	positive	3	24	positive	positive	
8	12,4	35	47	12,3	12,5	100
9	15,3	13		positive		-
11	23,65	36	18	22,7	24,6	
12		14	26	65,07	43,4	

5.1.4 Dye E 131

Teilnehmer/ participant	Ergebniss/ result	DLA-Nr Probe 1/ sample 1	DLA-Nr Probe 2/ sample 2	Ergebnis 1/ result 1	Ergebnis 2/ result 2	Wiederfindungs-rate/ recovery
	mg/kg			mg/kg	mg/kg	in %
1	positive	11	30			
2	182,6	4	42	192,2	173,0	89,6
3	positive	12	41	positive	positive	
4	positive	21	34	positive	positive	
5	129,98	38	25	131,64	128,32	100
6	positive	22	39	positive	positive	
7	153,165	3	24	155,83	150,5	
8	83,8	35	47	84,1	83,5	99,8
9	163,2	13		positive		-
10	positive	10	32	positive	positive	
11	139,7	36	18	142,7	136,7	
12		14	26	180,92	179,71	

5.1.5 Dye E 132

Teilnehmer/ participants	Ergebnis/ resut	DLA-Nr Probe 1/ sample 1	DLA-Nr Probe 2/ sample 2	Ergebnis 1/ result 1	Ergebnis 2/ result 2	Wiederfindungs- rate/ recovery
	mg/kg			mg/kg	mg/kg	in %
1	positive	11	30			
2	256,4	4	42	256,4	positive	54,0
3	positive	12	41	positive	positive	
4	positive	21	34			
5	44,64	38	25	42,94	46,33	100
6	negative	22	39	negative	negative	
7	266,275	3	24	267,83	264,72	
8	214,15	35	47	210,7	217,6	86,3
11	78,55	36	18	74,5	82,6	
12		14	26	228,33	207,29	

5.1.6 Dye E 133

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe 1/ sample 1	DLA-Nr Probe 2/ sample 2	Ergebnis 1/ result 1	Ergebnis 2/ result 2	Wiederfindungs- rate/ recovery
	mg/kg			mg/kg	mg/kg	in %
8	2,8	35	47	2,6	3,0	98,9
11	negative	36	18	< LOD	< LOD	

5.1.7 Dye E 142

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe 1/ sample 1	DLA-Nr Probe 2/ sample 2	Ergebnis 1/ result 1	Ergebnis 2/ result 2	Wiederfindungs- rate/ recovery
						in %
6	negative	22	39	negative	negative	
11	negative	36	18	negative	negative	

5.1.8 Dye E 151

Teilnehmer/ participant	Ergebnis/ result	DLA-Nr Probe 1/ sample 1	DLA-Nr Probe 2/ sample 2	Ergebnis 1/ result 1	Ergebnis 2/ result 2	Wiederfindungs- rate/ recovery
						in %
6	negative	22	39	negative	negative	
11	negative	36	18	negative	negative	

5.2 Homogeneity

5.2.1 Homogeneity testing before PT

To verify the homogeneity of the test material glucose was added before homogenisation. The homogeneity was examined with glucose/ HPLC.

Sample no.	**Glucose**		
52	15,5	g/100g	
49	15,7	g/100g	
32	15,3	g/100g	
20	15,1	g/100g	
5	15,8	g/100g	
mean	**15,5**		
standard deviation	**0,29**	**1,8**	**%**

5.3 Analytical methods

Teil-nehmer/ partici-pant	Methode/ method	Wiederfindung mit gleicher Matrix/ Recovery with same matrix	Akkreditiert/ Accredited	Hinweise/ remarks
1	In house method PC, according to Thaler-Sommer (E102, E 104); in house method, PC according to Serini (E 131, E 132)	-	yes	
2	SLMB no. 1576.2 (HPLC-DAD)	yes	no	The method is up to now only accredited for the qualitative determination of dyes. We could detect for E 132 (sample 2) no recovery.
3	HPLC		yes	
4	Qual. DC nach § 64, L 26.11.03-14 mod.		yes	Only qualitative determination.
5	Sample dissolved in water, filtered and detection with HPLC (DAD).	yes	yes	
6	Water-soluble dyes, qualitative, with thin-layer chromatography.		yes	
7	Extraction with water and analysis by HPLC with UV/VIS detector	no	no	
8	Ethanol/water (1/1) extraction and HPLC-PDA determination	yes	no	
9	HPLC method	no	-	
10	*Yoshioka & Ichihashi (2008) Determination of 40 synthetic food colours in drinks and candies by high-performance liquid chromatography using a short column with photo-diode array detection. Talanta 74:1408-1413*	no	no	
11	P3L99448 thin-layer chromatography and liquid chromatography.	no	yes	Qualitative DC and quantitative additional with HPLC. Verified E102, E104, E131 and E132; all the others unverifiable/ < LOD.
12	Water-soluble synthetic dyes - HPLC-DAD	no	no	

6. Index of participant laboratories

Teilnehmer/ participant	Ort/ location
	Spain
	Austria
	Italy
	Deutschland
	Deutschland
	Deutschland
	Deutschland
	Deutschland
	Deutschland
	Deutschland
	Australia
	Belgium
	Deutschland

[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)
9. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
10. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
11. Trennung und Bestimmung von Farbstoffen in Süßwaren mittels HPLC, S.Neier u. R.Matissek, Deutsche Lebensmittel-Rundschau, 94/11 (1998) 374-380
12. Schweizerisches Lebensmittelbuch SLMB, Künstliche, wasserlösliche Lebensmittelfarbstoffe, Nr. 1576/2006
13. Rapid planar chromatographic analysis of 25 water-soluble dyes used as food additives, G.Morlock, C.Oellig, J AOAC 2009 May-Jun,92(3), 745-756
14. Simultaneous determination of water-soluble and fat-soluble synthetic colorants in foodstuff by high-performance liquid chromatography-diode array detection-electrospray mass spectrometry, Ming Ma et al., J. Chromatogr. A 1103 (2006), 170-176
15. ASU §64 LFGB L08.00-51: Nachweis von Angkak, Rotsandelholz und Karminsäure (E 120) in Wurstwaren.
16. ASU §64 LFGB L08.00-50: Nachweis von färbenden Zusätzen in Wurstwaren (Screening-Verfahren)
17. Determination of 40 synthetic food colors in drinks and candies by high-performance liquid chromatography using a short column with photodiode array detection; Yoshioka et al., Talanta 2008 Feb. 15; 74(5): 1408-13

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