भारतीय मानक

Indian Standard

IS 15938 : 2024
ISO 13496 : 2021

मांस और मांस उत्पाद — रंजन अभिकर्मक का संसुचन एवं निर्धारण

(पहला पुनरीक्षण)

Meat and Meat Products — Detection and Determination of Colouring Agents

(First Revision)

ICS 67.120.10

भारतीय मानक ब्यूरो
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November 2024 **Price Group 15**

Slaughter House and Meat Industry Sectional Committee, FAD 18

NATIONAL FOREWORD

This Indian Standard which is identical to ISO 13496 : 2021 ‘Meat and meat products — Detection and determination of colouring agents’ issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on recommendation of the Slaughter House and Meat Industry Sectional Committee and approval of the Food and Agriculture Division Council.

This standard was originally published in year 2011 and was an identical adoption of ISO 13496 : 2000 ‘Meat and meat products — Detection of colouring agents — Method using thin-layer chromatography’ under dual numbering. This first revision has been undertaken to harmonize it with the latest version of ISO 13496 and is an identical adoption of the ISO document under dual numbering. The main changes in this revision compared with previous version are as follows:

a) A new test method, high performance liquid chromatography (HPLC), has been added;

b) The order of the clauses has been rearranged;

c) The title of the document has been modified; and

d) The scope has been modified.

The text of ISO standard has been approved as suitable for publication as an Indian Standard without deviations. Certain terminologies and conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words ‘International Standard’ appear referring to this standard, they should be read as ‘Indian Standard’; and

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to the following International Standard for which Indian Standard also exists. The corresponding Indian Standard which is to be substituted in its place, it listed below along with its degree of equivalence for the edition indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 4793 Laboratory sintered (fritted) filters — Porosity grading, classification and designation	IS 12305 : 2018/ISO 4793 : 1980 Laboratory sintered (fritted) filters — Porosity grading, classification and designation (*first revision*)	Identical

The Committee has reviewed the provisions of the following International Standards referred in this adopted standard and has decided that they are acceptable for use in conjunction with these standards:

International Standard	*Title*
ISO 3696	Water for analytical laboratory use — Specification and test methods
AOAC 46.1.08	Official methods of analysis (AOAC International)

In reporting the result of a test made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 ‘Rules for rounding off numerical values (*second revision*)’.

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Indian Standard

MEAT AND MEAT PRODUCTS — DETECTION AND DETERMINATION OF COLOURING AGENTS

(First Revision)

1 Scope

This document specifies a detection method using thin-layer chromatography and a determination method using high performance liquid chromatography (HPLC) for synthetic colouring agents in meat and meat products.

This document specifies the HPLC method as the reference method.

This document is applicable to meat and meat products, including livestock and poultry products.

The method using thin-layer chromatography can detect the following colouring agents:

— Tartrazine
— Quinoline Yellow
— Sunset Yellow FCF
— Amaranth
— Ponceau 4R
— Erythrosine
— Patent Blue V
— Indigotine
— Brilliant Black PN
— Black 7984
— Fast Green FCF
— Blue VRS

Synonyms and identity numbers of these colouring agents are listed in Annex A. The plant colours and plant extracts which have been observed not to interfere with this method are listed in B.1. Natural colours which in some cases have been shown to interfere with this method are listed in B.2.

The method using HPLC can detect the following colouring agents:

— Tartrazine
— Amaranth
— Ponceau 4R
— Sunset Yellow FCF
— Erythrosine
— Allura Red AC
— Brilliant Blue FCF
— New Red
— Carmoisine
— Indigotine

Chromatograms of these standard reference colours are shown in Annex D.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 4793, *Laboratory sintered (fritted) filters — Porosity grading, classification and designation*

AOAC 46.1.08, *Official Methods of Analysis (AOAC International)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
detection of colouring agents
detection of the presence or absence of colouring agents in accordance with the method specified in this document

4 Principle

4.1 Thin-layer chromatography

The colouring agents are extracted from a test portion with hot water and adsorbed onto polyamide powder. The extracted colouring agents are purified by column chromatography and the colours are eluted from the column. The colouring agents are identified by thin-layer chromatography.

4.2 HPLC

The colouring agents are extracted from a test portion with hot water and adsorbed onto polyamide powder. The extracted colouring agents are injected into the column and chromatographed in HPLC in reverse phase (RP). The colouring agents are identified according to retention time and quantified with external standard method.

5 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Proceed from a representative sample of at least 200 g. Store the sample in such a way that deterioration and change in composition are prevented.

6 Preparation of test sample

Homogenize the laboratory sample with the appropriate equipment (7.2.1). Take care that the temperature of the sample material does not rise above 25 °C. If a mincer is used, pass the sample at least twice through the equipment.

Fill a suitable airtight container with the prepared sample. Close the container and store in such a way that deterioration and change in the composition of the sample are prevented. Analyse the sample as soon as practicable, but always within 24 h after homogenization.

7 Test method of thin-layer chromatography

7.1 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

7.1.1 **Water**, conforming to at least grade 3 in accordance with ISO 3696.

7.1.2 Petroleum ether, boiling range 40 °C to 60 °C.

7.1.3 Methanol.

7.1.4 Ammonia, 25 % aqueous solution, ρ_{20} = 0,910 g/ml.

7.1.5 Acetic acid, 100 % mass fraction, ρ_{20} = 1,050 g/ml.

7.1.6 Trisodium citrate dihydrate.

7.1.7 Propan-1-ol.

7.1.8 Ethyl acetate.

7.1.9 2-Methyl-2-propanol.

7.1.10 Propionic acid.

7.1.11 Eluent solution for column chromatography.

Mix 95 volumes of methanol (7.1.3) with five volumes of ammonia solution (7.1.4).

7.1.12 Acetic acid, 50 % solution in methanol.

Mix one volume of acetic acid (7.1.5) with one volume of methanol (7.1.3).

7.1.13 Polyamide powder, of particle size 0,05 mm to 0,16 mm.

7.1.14 Sand, fine granular, hydrochloric acid-washed, neutralized and calcinated.

7.1.15 Standard reference colours.

The purities of the standard colours can vary so it is necessary to know the purity of the colours to be used as standards. The purity shall be determined by the method given in AOAC 46.1.08.

NOTE Certified food colours can also be used as standards.

7.1.16 Standard reference solutions for thin-layer chromatography.

Separately make solutions in water of each of the standard reference colours (7.1.15) with a standard colour content of about 1 g/l.

Prepare solutions of Indigotine on the day of use. Other solutions will keep for at least three months (solutions of Erythrosine for one month) when stored in the dark.

7.1.17 Eluent for thin-layer chromatography: solution I.

Weigh, to the nearest 0,1 g, 25 g of trisodium citrate dihydrate (7.1.6) into a 1 000 ml one-mark volumetric flask. Dissolve in water, dilute to the mark with water and mix.

Mix 80 volumes of this citrate solution with 20 volumes of ammonia solution (7.1.4) and 12 volumes of methanol (7.1.3).

To avoid or reduce interference from safflor or saffran, it is advisable to use chromatography solution II (7.1.18).

7.1.18 Eluent for thin-layer chromatography: solution II.

Mix six volumes of propan-1-ol (7.1.7) with one volume of ethyl acetate (7.1.8) and three volumes of water.

7.1.19 Eluent for thin-layer chromatography: solution III.

Mix 50 volumes of 2-methyl-2-propanol (7.1.9) with 12 volumes of propionic acid (7.1.10) and 38 volumes of water.

7.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used.

7.2.1 Mechanical or **electrical homogenizing equipment,** capable of homogenizing the laboratory sample.

Use a high-speed rotational cutter, or a mincer fitted with a plate with apertures not exceeding 4,0 mm in diameter.

7.2.2 Centrifuge tubes.

7.2.3 Flat-bottomed flasks, of capacity 250 ml, with ground glass stoppers.

7.2.4 Round-bottomed flasks, of capacity 100 ml, with ground glass joint.

7.2.5 Centrifuge, operating at a radial acceleration of about 2 000*g*.

7.2.6 Rotary evaporator.

7.2.7 Chromatographic column, of glass, with fritted filter and tap, of length about 20 cm, diameter about 30 mm, filter pore size 40 µm to 100 µm (porosity grade P 100 in accordance with ISO 4793).

Put some glass wool in the column and add 1 g to 2 g of sand (7.1.14).

7.2.8 Plastics container, of volume about 10 ml, with lid.

7.2.9 Thin-layer plates, coated with a layer of cellulose powder of 0,10 mm thickness, or equivalent.

Ready-to-use plates are suitable.

7.2.10 Micropipettes, of capacity approximately 5 µl.

7.2.11 pH-meter, accurate to within 0,1 pH unit.

7.3 Procedure

WARNING — If the sample contains Indigotine, the temperature shall not at any time during the analysis exceed 35 °C. Indigotine partially decomposes in chromatography solution I, so chromatography solution II shall be used.

WARNING — Erythrosine is sensitive to light. When pausing in the course of the analysis, solutions and plates shall be stored in the dark. The same also holds for Indigotine.

7.3.1 Test portion

Weigh, to the nearest 0,1 g, 5 g of the prepared test sample (see Clause 6) into a centrifuge tube (7.2.2).

For fatty samples, proceed in accordance with 7.3.2.

For non-fatty samples, proceed in accordance with 7.3.3.

7.3.2 Fatty samples

Add about 20 ml of petroleum ether (7.1.2) to the centrifuge tube and mix with a glass rod. Decant the petroleum ether.

Repeat this procedure three times.

7.3.3 Non-fatty samples

Add 25 ml of boiling water (see warning above) and mix. Add 25 ml of the eluent solution (7.1.11).

Check that the pH is 9 ± 0,5 using the pH-meter (7.2.11). If not, adjust the pH with acetic acid (7.1.5) or ammonia solution (7.1.4).

Mix well. Chill the sample in a freezer for 15 min (to prevent turbidity).

Centrifuge (7.2.5) for 10 min at a radial acceleration of about 2 000*g*.

Decant the clear solution into a flat-bottomed flask (7.2.3). In the case of Indigotine, use a round-bottomed flask (7.2.4).

Add 5 ml of water to the centrifuge tube containing the residue. Mix and add 10 ml of the eluent solution (7.1.11). Mix and centrifuge as above.

Repeat the procedure until all colour has been extracted from the sample then combine all the extracts.

Evaporate the combined extracts in a water bath to about 25 ml in order to remove the methanol. In the case of Indigotine, use a round-bottomed flask (7.2.4) and the rotary evaporator (7.2.6) at 35 °C.

Add 25 ml of boiling water (see warnings) and mix.

7.3.4 Transfer of the colours to polyamide powder

Using acetic acid (7.1.5) or ammonia solution (7.1.4) adjust the pH to between 4 and 5.

Add 1 g of polyamide powder (7.1.13) to the warm solution (see warnings). Shake vigorously for 1 min.

Allow the powder to form a sediment.

Check that no colour remains in the solution. If the solution is coloured, add some more polyamide powder and shake vigorously.

NOTE Some natural colours (see Annex B) are not entirely adsorbed on the polyamide powder, leaving the solution coloured even if all synthetic colours have been completely adsorbed. It is usually possible to decide from the type of sample whether or not such natural colours are present.

Shake and transfer the warm suspension to the chromatographic column (7.2.7).

Rinse the flat-bottomed flask with three 10 ml portions of hot water (see warnings) and add the rinsings, portion by portion, to the column. Wash the column another three times with 10 ml portions of hot water (see warnings) and finally three times with 5 ml of methanol (7.1.3). If natural colours are eluted, continue washing the column with methanol until the eluted methanol is colourless.

7.3.5 Elution and concentration of isolated colours

Place a flask (7.2.4) under the column and elute the colours from the polyamide powder with 5 ml portions of the eluent solution (7.1.11), at an elution volume flow rate of 2 ml/min, until the polyamide is colourless.

Evaporate the eluate to dryness using the evaporator (7.2.6) at a temperature of at most 35 °C (see warnings).

Add 1,0 ml or 2,0 ml of eluent solution (7.1.11) depending on the amount and number of colours and dissolve the residue. Transfer the colour solution to a plastics container (7.2.8).

7.3.6 Thin-layer chromatographic separation

7.3.6.1 Standard reference plates

Prepare three standard reference thin-layer chromatographic plates. Using a micropipette (7.2.10), dispense a spot of about 5 µl (diameter, $d < 5$ mm) of each standard solution (7.1.16) separately on each plate (7.2.9). Develop these separately, one with each chromatography eluent (7.1.17, 7.1.18 and 7.1.19) in an unsaturated tank until the solvent front is about 10 cm to 12 cm from the starting line. Remove the plates from the tank and dry in air under a hood. Store the plates in the dark. The spots, except for that of Indigotine, are stable for several years.

7.3.6.2 Samples

Using a micropipette (7.2.10), apply to a thin-layer plate (7.2.9) a just-visible amount of sample solution (see 7.3.5). Dry using a hair dryer. In the case of Indigotine, dry in air.

Develop the plate in an unsaturated tank to a height of approximately 10 cm to 12 cm using a suitable chromatography solution (7.1.16, 7.1.17 or 7.1.18), i.e. the solution which gives the best separation of the colours detected in the sample (see Clause 1). Sometimes it will be necessary to prepare a second sample plate and develop this in one of the other two eluents to obtain the best separation.

Remove the plate from the tank and dry in air under a hood.

Compare the sample spots with the appropriate standard reference plate (see 7.3.6.1).

It is recommended that different amounts of sample solutions be applied in the case of mixtures of colorants, because colorants can be present in various concentrations in the concentrate.

Tailing is usually caused by inadequate purification. If this is the case, adsorb the colorant again with the adsorbent, wash with hot water and remove the adsorbent as previously described.

7.3.7 Confirmation

Confirm the identity of the colorants by chromatographing the concentrate (see 7.3.6.2) in a mixture of standards for the colorants identified in the first chromatogram.

In case of doubt, elute the colorant from the plate with a neutral solution (water or ethanol, or 0,2 g/l ammonium acetate solution), an acid (0,1 mol/l hydrochloric acid) and an alkali (0,1 mol/l sodium hydroxide solution), and compare the absorption spectrum of the colorant to that of the standard. See the absorbance spectra shown in Annex C.

8 Test method of HPLC

8.1 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

8.1.1 Acetonitrile, HPLC quality.

8.1.2 Ammonium acetate.

8.1.3 Ammonium acetate solution (0,02 mol/l).

Weigh 1,54 g of ammonium acetate (8.1.2), add appropriate water to dissolve and dilute to 1 000 ml with water. Filter through 0,45 µm microporous membrane (8.2.2).

8.1.4 Methanol, 10 % solution in water.

Mix 10 volumes of methanol (7.1.3) with 90 volumes of water (7.1.1).

8.1.5 Stock solutions (1 mg/ml).

Separately make solutions in 10 % methanol (8.1.4) of each of the standard reference colours (7.1.15) with a standard colour content of about 1 mg/ml.

8.1.6 Working reference solutions (50 µg/ml).

Dilute the 1 mg/ml stock solutions (8.1.5) 20 times with 10 % methanol (8.1.4) and filter through 0,45 µm microporous membrane (8.2.2).

8.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used.

8.2.1 HPLC chromatographic system, with column thermostat and UV/visible or diode array detector.

8.2.2 Micro filters with membranes (diameter of the pores: 0,45 µm).

8.3 Procedure

WARNING — If the sample contains Indigotine, the temperature shall not at any time during the analysis exceed 35 °C.

WARNING — Erythrosine is sensitive to light. When pausing in the course of the analysis, the solutions shall be stored in the dark. The same also holds for Indigotine.

8.3.1 Test portion

Weigh, to the nearest 0,001 g, 5 g of the prepared test sample (see Clause 6) into a centrifuge tube (7.2.2).

For fatty samples, proceed in accordance with 8.3.2.

For non-fatty samples, proceed in accordance with 8.3.3.

8.3.2 Fatty samples

See 7.3.2.

8.3.3 Non-fatty samples

See 7.3.3.

8.3.4 Transfer of the colours to polyamide powder

See 7.3.4.

8.3.5 Elution and concentration of isolated colours

Place a flask (7.2.4) under the column and elute the colours from the polyamide powder with 5 ml portions of the eluent solution (7.1.11), at an elution volume flow rate of 2 ml/min, until the polyamide is colourless.

Evaporate the eluate to dryness using the evaporator (7.2.6) at a temperature of at most 35 °C (see warnings).

Add 1,0 ml or 2,0 ml of water (7.1.1) depending on the amount and number of colours and dissolve the residue. Filter the colour solution through 0,45 µm microporous membrane (8.2.2) for injection into the HPLC chromatographic system (8.2.1).

8.3.6 HPLC analysis

8.3.6.1 Operating conditions

The operating conditions are as follows:

a) Column: C18 (5 µm, 4,6 × 250 mm).

b) Mobile phase:

A: 0,02 mol/l ammonium acetate solution (8.1.3);

B: acetonitrile (8.1.1), elution gradient see Table 1.

c) Column temperature: 35 °C.

d) Flow rate: 1,0 ml/min.

e) Injection volume: 20 µl.

f) Wavelength range of diode array detector: 400 nm to 800 nm, or wavelength of UV detector detection: see Annex D.

Table 1 — Elution gradient

Time, min	**Phase A,** %	**Phase B,** %
0	95	5
3	65	35
7	0	100
10	0	100
10,1	95	5
21	95	5

8.3.6.2 Determination

Under above conditions, when the retention time for the peak of analyte in the unknown sample is the same as the retention time of the standard, the sample can be assumed to contain synthetical pigments. The chromatogram of synthetical pigments standard is given in Annex D. The method is quantified by the external standard curve. The responses of synthetical pigments in the sample solution should be in the linear range of the instrumental detection.

8.3.6.3 Parallel test

According to the above procedure, the same sample was tested in a parallel test.

8.3.6.4 Blank test

Except for weighing the sample, follow the procedure described above.

8.4 Calculation

The level of colorant is calculated as shown by Formula (1):

$$X = \frac{C \times V}{m} \quad (1)$$

where

X is the content of the colorant in the sample, in grams per kilogram (mg/kg);

C is the concentration of the colorant in the sample solution, in milligrams per litre (mg/l);

V is the final diluted volume of the sample solution, in millilitres (ml);

m is the sample mass, in grams (g).

The result is subtracted from the blank value.

Express the calculation result as the arithmetic average of two single test results obtained under repetitive conditions. Express the results to two significant figures. Interlaboratory testing results are shown in Annex E.

8.5 Precision

The absolute difference between two single test results obtained under repetitive conditions shall not exceed 10 % of the arithmetic mean.

8.6 Limit of detection (LOD) and limit of quantification (LOQ)

For Tartrazine, Amaranth, Ponceau 4R, Sunset Yellow FCF, Erythrosine, Allura Red AC, Brilliant Blue FCF, New Red, Carmoisine and Indigotine, the LOD is 0,15 mg/kg and the LOQ is 0,5 mg/kg.

9 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this document including its year of publication, i.e. ISO 13496:2021;

— all operating details not specified in this document, or regarded as optional, together with details of any incidents which can have influenced the test result;

— the test result obtained, including a reference to the clause which explains how the results were calculated;

— the date of the test.

Annex A
(informative)

Synonyms and identity numbers of synthetic, water-soluble colouring agents

Table A.1 — Synonyms and identity numbers of synthetic, water-soluble colouring agents

Name	Synonym 1	Synonym 2	C.I.[a]	E No.[b]	CAS No.[d]
Tartrazine	FD&C Yellow No. 5		19140	E 102	1934-21-0
Quinoline Yellow			47005	E 104	8004-92-0
Sunset Yellow FCF	FD&C Yellow No. 6		15985	E 110	2783-94-0
Amaranth	FD&C Red No. 2	Naphthol Red S	16185	E 123	915-67-3
Ponceau 4R	New coccine	Cochineal Red A	16255	E 124	2611-82-7
Erythrosine	FD&C Red No. 3		45430	E 127	16423-68-0
Patent Blue V			42051	E 131	3536-49-0
Indigotine	FD&C Blue No. 2		73015	E 132	860-22-0
Brilliant Black PN			28440	E 151	2519-30-4
Black 7984			27755	E 152	2118-39-0
Fast Green FCF	FD&C Green No. 3		42053	[c]	2353-45-9
Blue VRS			2045	[c]	[c]
Allura Red AC			16035	E129	25956-17-16
Brilliant Blue FCF			42090	E133	3844-45-9
New Red			[c]	[c]	220658-76-4
Carmoisine	Azorubine		14720	E122	3567-69-6

[a] C.I.: Identity number according to the Colour Index[4].

[b] E No.: Current number within the European Community (EC).

[c] E No. is not available.

[d] CAS No.: Chemical abstracts service number.

Annex B
(informative)

Possible interference by colours

B.1 Colours which do not interfere

The following plant colours or plant extracts have been observed not to interfere with this method:

- alfalfa
- annatto (bixin and norbixin)
- anthocyanins
- beetroot red
- *β*-apocarotenal
- *β*-apocarotenic acid ethyl ester
- *β*-carotene
- canthaxanthin
- chlorophyll
- chlorophyllin copper complex
- flower of tagetes
- marigold
- mustard
- paprika oleoresin
- riboflavin
- tea
- tomato

B.2 Colours which can interfere

In some cases, natural colours have been observed to interfere with this method. Their uses in foods and the synthetic colours of which the determination can be affected are given in Table B.1.

Table B.1 — Colours which can interfere

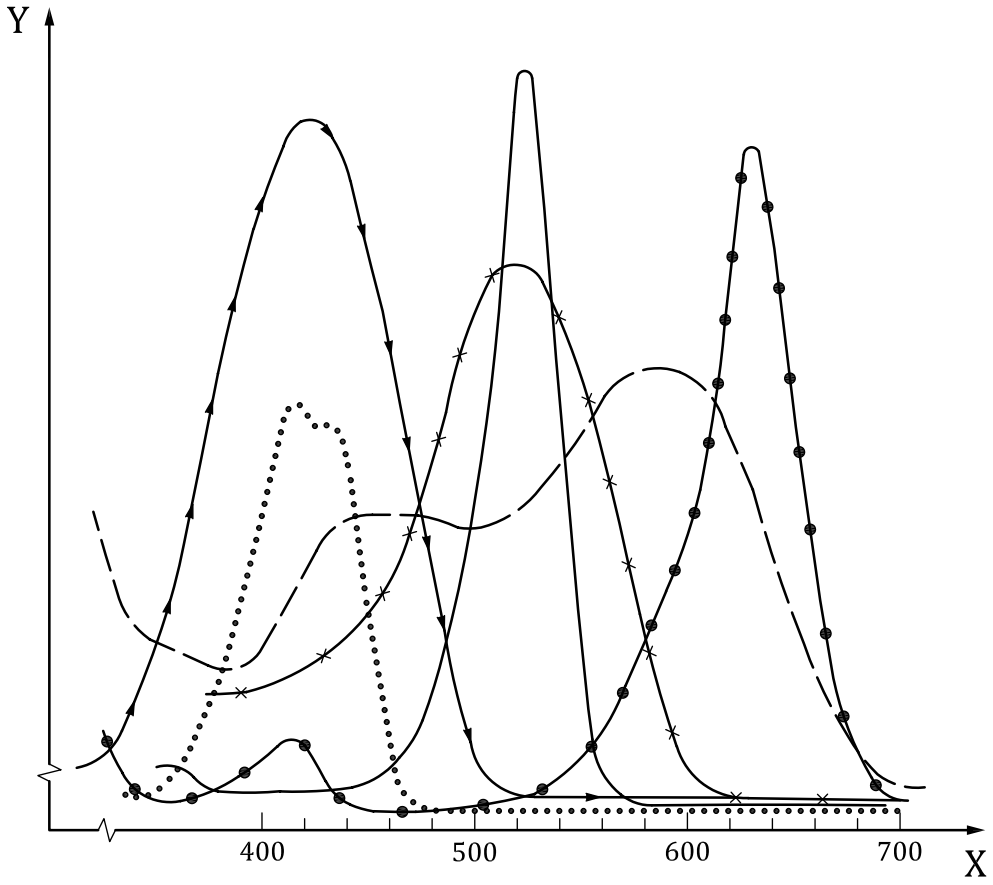
Substance	Use in foods	Interferes with analysis of
Curcumin	Spice, also used as yellow colour	Quinoline Yellow (E 104)[a] Brilliant Black PN (E 151)[a] Black 7984 (E 152)[a]
Saffran	Spice, too expensive for use as a colour	Erythrosine (E 127)[b] Quinoline Yellow (E 104)[a,b] Brilliant Black PN (E 151)[a,b] Black 7984 (E 152)[b]
Safflor	Substitute for saffran	Tartrazine (E 102)[b]

[a] The interferences are minor and may be considered negligible.

[b] To avoid or reduce interference from safflor or saffran, it is advisable to use chromatography solution II (7.1.18).

Annex C
(informative)

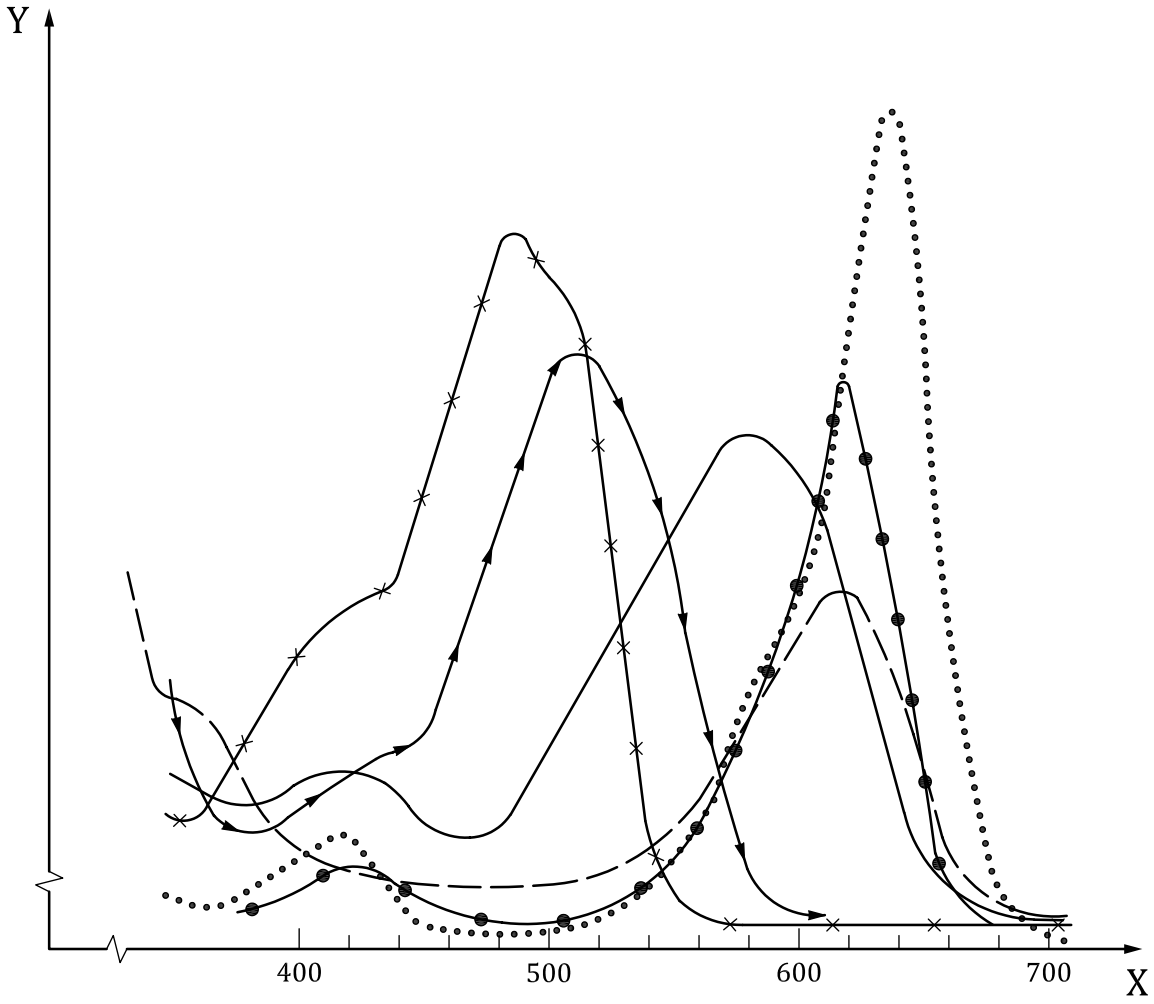
Absorbance spectra



Key

X	wavelength, nm
Y	absorbance
.....	Quinoline Yellow
———	Erythrosine
——→——	Tartrazine
— — —	Black 7984
——×——	Amaranth
——●——	Blue VRS

Figure C.1 — Absorbance spectra of Amaranth, Black 7984, Blue VRS, Erythrosine, Tartrazine and Quinoline Yellow



Key

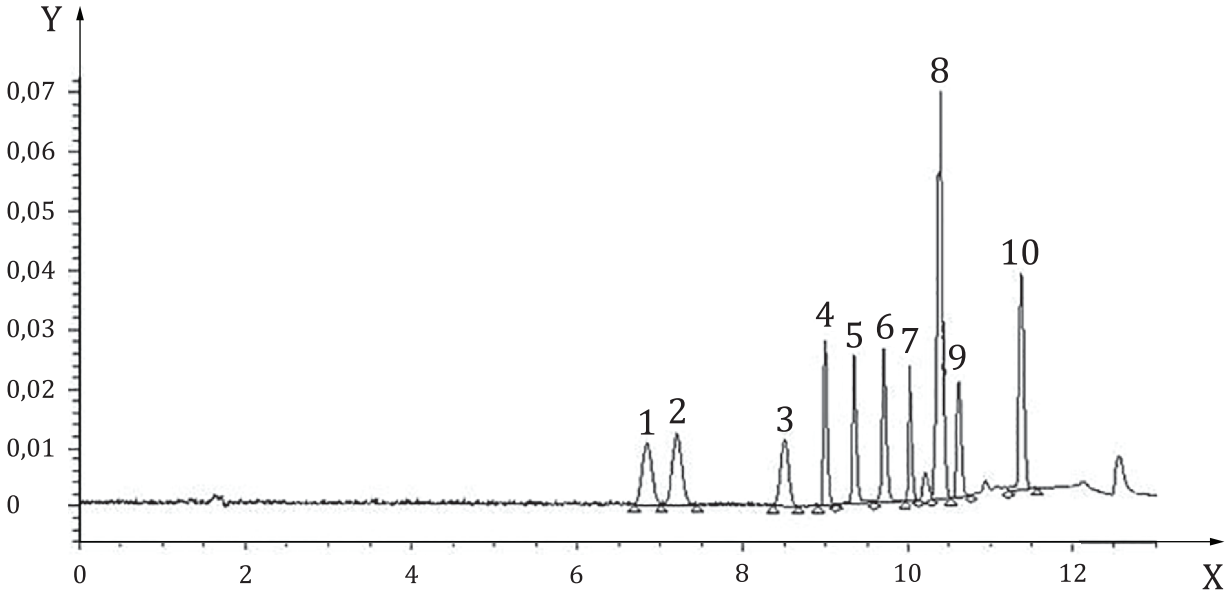
X	wavelength, nm
Y	absorbance
—×—	Sunset Yellow FCF
———	Brilliant Black PN
—●—	Fast Green FCF
• • • • •	Patent Blue V
——→	Ponceau 4R
— —	Indigotine

Figure C.2 — Absorbance spectra of Brilliant Black PN, Fast Green FCF, Indigotine, Patent Blue V, Ponceau 4R and Sunset Yellow FCF

Annex D
(informative)

Chromatogram and wavelength

D.1 Chromatogram of standard



Key

X time, min

Y absorbance

1. Tartrazine	2. New Red	3. Amaranth	4. Indigotine	5. Ponceau 4R
6. Sunset Yellow FCF	7. Allura Red AC	8. Brilliant Blue FCF	9. Carmoisine	10. Erythrosine

Figure D.1 — Chromatogram (λ: 400 nm to 800 nm) of Tartrazine, New Red, Amaranth, Indigotine, Ponceau 4R, Sunset Yellow FCF, Allura Red AC, Brilliant Blue FCF, Carmoisine and Erythrosine

D.2 Recommended wavelength of UV detector detection

The recommended detection wavelength for different colouring agents using UV detector detection is:

Tartrazine:	428 nm	Allura Red AC:	508 nm
Amaranth:	525 nm	Brilliant Blue FCF:	628 nm
Ponceau 4R:	508 nm	New Red:	508 nm
Sunset Yellow FCF:	480 nm	Carmoisine:	518 nm
Erythrosine:	531 nm	Indigotine:	610 nm

Annex E
(informative)

Interlaboratory testing

E.1 Overview

The international laboratory ring test of this document was conducted from 27th June 2020 to 26th July 2020. Eleven laboratories participated in two parallel tests of five samples each.

The test was conducted by the Shanghai Institute of Quality Inspection and Technical Research, China, which also prepared the statistical analysis and final report.

The test method described in this document is adopted here for the determination of colouring agents in meat product samples.

Five different kinds of meat samples (A: chicken, B: mutton, C: pork, D: beef; and E: duck) were used during the ring test, and each with several mean levels.

E.2 Statistical analysis of the test results of colouring agents

E.2.1 Tartrazine

E.2.1.1 Original test results

Eleven laboratories participated in the determination of Tartrazine content in meat samples. The results are shown in Table E.1.

Table E.1 — Original test results — Tartrazine content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,492	0,443	9,07	9,75	2,09	2,17	5,11	3,73	0,876	0,900
2	0,512	0,516	10,60	10,13	2,25	2,34	4,92	4,91	0,935	0,962
3	0,510	0,503	10,50	10,50	2,17	2,20	4,81	4,86	0,929	0,976
4	0,480	0,496	9,62	9,81	2,32	2,29	4,63	4,51	0,813	0,931
5	0,481	0,502	9,23	9,34	2,48	2,50	4,65	4,65	0,947	0,996
6	0,479	0,501	9,83	10,01	2,25	2,64	4,41	4,70	0,866	0,885
7	0,544	0,528	10,06	10,52	2,27	2,31	4,57	4,76	0,854	0,886
8	0,492	0,521	10,32	10,01	2,11	2,52	4,57	4,80	0,951	0,908
9	0,479	0,459	9,81	10,10	2,07	2,45	4,60	4,46	0,889	0,842
10	0,500	0,532	8,38	8,56	2,67	2,99	4,39	4,78	1,279	1,181
11	0,549	0,573	10,68	10,87	2,28	2,51	4,67	5,04	0,906	0,925

E.2.1.2 Cell means

The cell means of the determination of Tartrazine content are shown in Table E.2.

Table E.2 — Cell means — Tartrazine content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,467 5	9,410	2,130	4,420	0,888 0
2	0,514 0	10,365	2,295	4,915	0,948 5
3	0,506 5	10,500	2,185	4,835	0,952 5
4	0,488 0	9,715	2,305	4,570	0,872 0
5	0,491 5	9,285	2,490	4,650	0,971 5
6	0,490 0	9,920	2,445	4,555	0,875 5
7	0,536 0	10,290	2,290	4,665	0,870 0
8	0,506 5	10,165	2,315	4,685	0,929 5
9	0,469 0	9,955	2,260	4,530	0,865 5
10	0,516 0	8,470	2,830	4,585	1,230 0
11	0,561 0	10,775	2,395	4,855	0,915 5

E.2.1.3 Cell absolute differences

The cell absolute differences of the determination of Tartrazine content are shown in Table E.3.

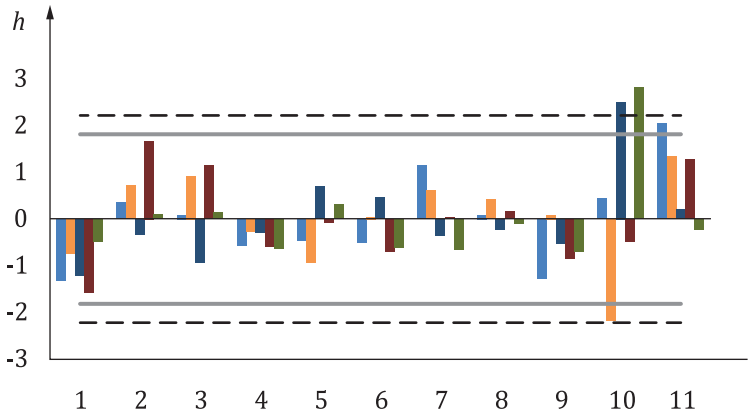
Table E.3 — Cell absolute differences — Tartrazine content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,049	0,68	0,08	1,38	0,024
2	0,004	0,47	0,09	0,01	0,027
3	0,007	0,00	0,03	0,05	0,047
4	0,016	0,19	0,03	0,12	0,118
5	0,021	0,11	0,02	0,00	0,049
6	0,022	0,18	0,39	0,29	0,019
7	0,016	0,46	0,04	0,19	0,032
8	0,029	0,31	0,41	0,23	0,043
9	0,020	0,29	0,38	0,14	0,047
10	0,032	0,18	0,32	0,39	0,098
11	0,024	0,19	0,23	0,37	0,019

E.2.1.4 Scrutiny of results for consistency and outliers

E.2.1.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.1 and E.2.

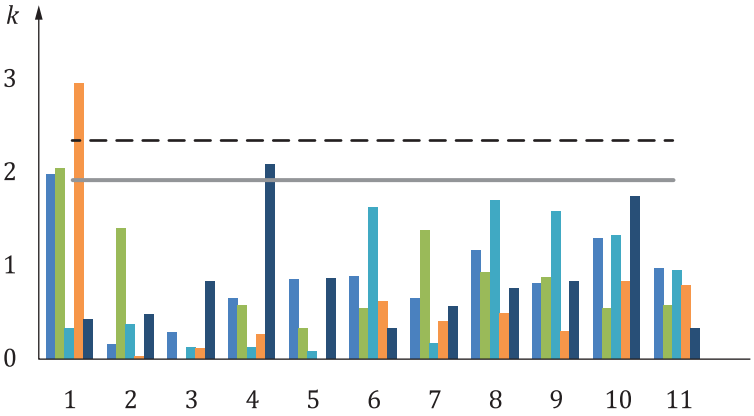


Key

1 % critical values

5 % critical values

Figure E.1 — Tartrazine content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure E.2 — Tartrazine content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 10 had a straggler at level B, as did laboratory 11 at level A, while laboratory 10 had outliers at levels C and E. As further validation, laboratory 10 obtained much higher test results than all other laboratories at levels C and E, and therefore these two results were rejected.

The *k* graph shows that laboratory 1 had stragglers at levels A and B, as did laboratory 4 on level E, while laboratory 1 had an outlier at level D. As further validation, the absolute difference between the data measured by laboratory 1 at level D is the largest, and therefore the result was rejected.

E.2.1.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C shown in Table E.4.

Table E.4 — Tartrazine content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,356 0	0,377 7	0,313 7	0,305 0	0,543 0
Stragglers (*n* = 2)	0,570	0,570	0,602	0,602	0,602
Outliers (*n* = 2)	0,684	0,684	0,718	0,718	0,718
Number of laboratories, *p*	11	11	10	10	10

If the test statistic is greater than its 5 % critical value and less than or equal to its 1 % critical value, the item tested is regarded as a straggler. If the test statistic is greater than its 1 % critical value, the item tested is regarded as an outlier.

The application of the Cochran's test confirms no stragglers and outliers.

E.2.1.4.3 Grubbs' test

The application of the Grubbs' test to the cell means gives the values of the test statistic G shown in Table E.5.

Table E.5 — Tartrazine content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, *p*	Single low	Single high	Double low	Double high
A	11	1,322	2,048	0,589 7	0,335 4
B	11	2,183	1,347	0,328 2	0,675 5
C	10	1,644	1,626	0,445 9	0,429 2
D	10	1,124	1,677	0,701 8	0,398 5
E	10	1,093	1,579	0,701 2	0,488 7
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

For the Grubbs' test for one outlying observation, outliers and stragglers give rise to values which are larger than its 1 % and 5 % critical values, respectively.

For the Grubbs' test for two outlying observations, outliers and stragglers give rise to values which are smaller than its 1 % and 5 % critical values, respectively.

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

E.2.1.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Tartrazine content in each sample gives the values shown in Table E.6.

Table E.6 — Tartrazine content — Computed values of m, s_r, s_R

Parameter	Level j mg/kg				
	A	B	C	D	E
Number of laboratories, p	11	11	10	10	10
General mean, m	0,504	9,90	2,31	4,68	0,909
Repeatability standard deviation, s_r	0,017 5	0,235 9	0,163 7	0,157 9	0,035 8
Reproducibility standard deviation, s_R	0,030 4	0,674 0	0,159 7	0,177 1	0,047 1

E.2.1.6 Dependence of precision on general mean, m

Table E.6 shows that s_r and s_R have a strong positive correlation with m. The actual calculation demonstrates a linear correlation between $\log s_r$ (or $\log s_R$) with $\log m$, as follows:

$\log s_r = 0{,}885\ 9 \quad \log m - 1{,}383\ 1$, $R^2 = 0{,}891\ 4$

$\log s_R = 0{,}994\ 7 \quad \log m - 1{,}249\ 3$, $R^2 = 0{,}958\ 4$

E.2.1.7 Final values of precision

The precision of the Tartrazine content measurement method should be given as follows:

— $s_r = 0{,}041 + m^{0{,}89}$

— $s_R = 0{,}056 + m^{0{,}99}$

E.2.2 New Red

E.2.2.1 Original test results

Eleven laboratories participated in the determination of New Red content in meat samples. The test results are shown in Table E.7.

Table E.7 — Original test results — New Red content

Laboratory i	Level j mg/kg									
	A		B		C		D		E	
1	0,476	0,522	10,13	10,36	2,05	2,24	4,60	3,91	0,975	0,938
2	0,486	0,510	9,87	9,77	2,13	2,19	4,82	4,77	0,995	1,016
3	0,487	0,494	9,75	9,78	2,04	2,08	4,62	4,67	0,952	0,922
4	0,484	0,543	9,74	9,85	2,17	2,12	4,68	4,72	0,963	0,941
5	0,495	0,510	9,71	9,58	2,26	2,46	4,61	4,60	0,941	0,920
6	0,551	0,496	10,27	10,64	2,36	2,75	4,49	4,57	0,969	0,948
7	0,502	0,461	10,78	10,18	2,17	2,76	4,62	4,41	0,882	0,906
8	0,523	0,503	10,18	10,21	2,48	2,45	4,84	4,23	0,963	0,902
9	0,507	0,448	9,71	10,19	2,54	2,09	4,26	4,59	0,846	0,889
10	0,478	0,534	10,44	10,73	2,93	2,78	4,48	4,37	1,121	1,072
11	0,525	0,493	11,99	9,96	2,60	2,58	4,72	4,87	0,908	0,967

E.2.2.2 Cell means

The cell means of the determination of New Red content are shown in Table E.8.

Table E.8 — Cell means — New Red content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,499 0	10,245	2,145	4,255	0,956 5
2	0,498 0	9,820	2,160	4,795	1,005 5
3	0,490 5	9,765	2,060	4,645	0,937 0
4	0,513 5	9,795	2,145	4,700	0,952 0
5	0,502 5	9,645	2,360	4,605	0,930 5
6	0,523 5	10,455	2,555	4,530	0,958 5
7	0,481 5	10,480	2,465	4,515	0,894 0
8	0,513 0	10,195	2,465	4,535	0,932 5
9	0,477 5	9,950	2,315	4,425	0,867 5
10	0,506 0	10,585	2,855	4,425	1,096 5
11	0,509 0	10,975	2,590	4,795	0,937 5

E.2.2.3 Cell absolute differences

The cell absolute differences of the determination of New Red content are shown in Table E.9.

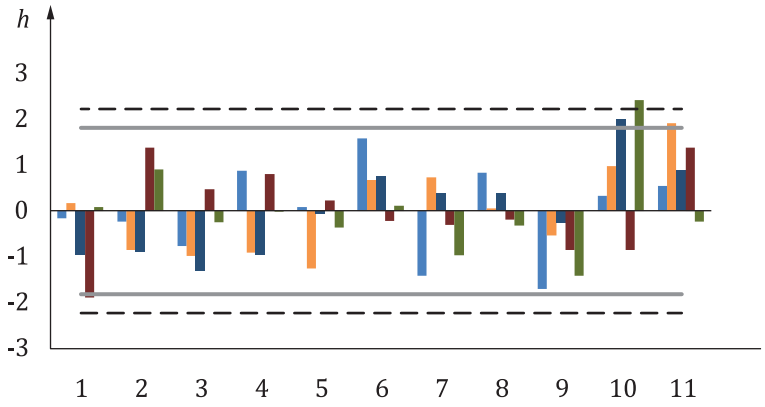
Table E.9 — Cell absolute differences — New Red content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,046	0,23	0,19	0,69	0,037
2	0,024	0,10	0,06	0,05	0,021
3	0,007	0,03	0,04	0,05	0,030
4	0,059	0,11	0,05	0,04	0,022
5	0,015	0,13	0,20	0,01	0,021
6	0,055	0,37	0,39	0,08	0,021
7	0,041	0,60	0,59	0,21	0,024
8	0,020	0,03	0,03	0,61	0,061
9	0,059	0,48	0,45	0,33	0,043
10	0,056	0,29	0,15	0,11	0,049
11	0,032	2,03	0,02	0,15	0,059

E.2.2.4 Scrutiny of results for consistency and outliers

E.2.2.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.3 and E.4.

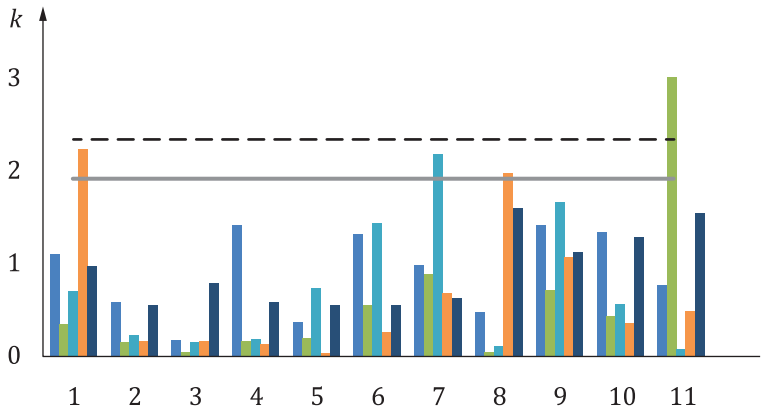


Key

1 % critical values

5 % critical values

Figure E.3 — New Red content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure E.4 — New Red content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 1 had a straggler at level D, laboratory 10 at level C and laboratory 11 at level B, while laboratory 10 had an outlier at level E. As further validation, laboratory 10 obtained higher test results than the other laboratories at level E, and therefore the results were rejected.

The *k* graph shows that laboratory 1 had a straggler at level D, laboratory 7 at level C and laboratory 8 at level D, while laboratory 11 had an outlier at level B. As further validation, the absolute difference between the data measured by laboratory 11 at level B is the largest, and therefore the result was rejected.

E.2.2.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.10.

Table E.10 — New Red content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,181 4	0,397 7	0,429 6	0,453 9	0,271 5
Stragglers (n = 2)	0,570	0,602	0,570	0,570	0,602
Outliers (n = 2)	0,684	0,718	0,684	0,684	0,718
Number of laboratories, p	11	10	11	11	10

The application of the Cochran's test confirms no stragglers and outliers.

E.2.2.4.3 Grubbs' test

The application of the Grubbs' test to the cell means gives the values of the test statistic G shown in Table E.11.

Table E.11 — New Red content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, p	Single low	Single high	Double low	Double high
A	11	1,701	1,591	0,402 5	0,602 9
B	10	1,314	1,440	0,633 2	0,535 2
C	11	1,304	1,996	0,683 1	0,428 1
D	11	1,885	1,389	0,488 1	0,528 2
E	10	1,869	1,834	0,335 8	0,509 4
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

E.2.2.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of New Red content in each sample gives the values shown in Table E.12.

Table E.12 — New Red content — Computed values of m, s_r, s_R

Parameter	Level *j* mg/kg				
	A	B	C	D	E
Number of laboratories, p	11	10	11	11	10
General mean, m	0,501	10,09	2,37	4,57	0,937
Repeatability standard deviation, s_r	0,029 5	0,212 7	0,191 9	0,218 4	0,026 2
Reproducibility standard deviation, s_R	0,025 1	0,373 0	0,276 5	0,225 9	0,041 6

E.2.2.6 Dependence of precision on general mean, *m*

Table E.12 shows that there is no obvious dependence between s_r, s_R and m. Therefore, m can be considered as the final mean, the average s_r of five matrices as the final repeatability standard deviation, and the average s_R of five matrices as final reproducibility standard deviation.

E.2.2.7 Final values of precision

The precision of the New Red content measurement method should be given as follows:

— s_r = 0,135 7

— s_R = 0,188 4

E.2.3 Amaranth

E.2.3.1 Original test results

Eleven laboratories participated in the determination of Amaranth content in meat samples. The test results are shown in Table E.13.

Table E.13 — Original test results — Amaranth content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,421	0,445	8,02	9,02	2,01	2,40	4,77	4,26	0,988	0,970
2	0,479	0,494	9,57	9,54	2,02	2,22	4,78	4,75	0,997	0,989
3	0,489	0,507	9,68	9,73	2,07	2,12	4,58	4,56	0,888	0,857
4	0,459	0,489	10,32	10,35	2,27	2,00	4,61	4,70	0,836	0,884
5	0,493	0,502	9,81	9,70	2,46	2,50	4,45	4,56	0,905	0,884
6	0,439	0,448	8,50	10,08	1,96	2,55	3,94	4,34	0,875	0,848
7	0,448	0,474	9,03	9,62	2,47	2,46	4,58	4,45	0,853	0,897
8	0,451	0,437	9,52	9,44	2,35	2,31	5,00	4,71	0,960	0,980
9	0,470	0,423	8,41	8,77	1,98	2,03	4,03	4,35	0,756	0,936
10	0,514	0,443	7,90	7,95	2,08	2,59	4,21	4,42	0,932	0,917
11	0,480	0,485	9,58	9,99	2,24	2,28	4,65	4,57	0,904	0,926

E.2.3.2 Cell means

The cell means of the determination of Amaranth content are shown in Table E.14.

Table E.14 — Cell means — Amaranth content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,433 0	8,520	2,205	4,515	0,979 0
2	0,486 5	9,555	2,120	4,765	0,993 0
3	0,498 0	9,705	2,095	4,570	0,872 5
4	0,474 0	10,335	2,135	4,655	0,860 0
5	0,497 5	9,755	2,480	4,505	0,894 5
6	0,443 5	9,290	2,255	4,140	0,861 5
7	0,461 0	9,325	2,465	4,515	0,875 0
8	0,444 0	9,480	2,330	4,855	0,970 0
9	0,446 5	8,590	2,005	4,190	0,846 0
10	0,478 5	7,925	2,335	4,315	0,924 5
11	0,482 5	9,785	2,260	4,610	0,915 0

E.2.3.3 Cell absolute differences

The cell absolute differences of the determination of Amaranth content are shown in Table E.15.

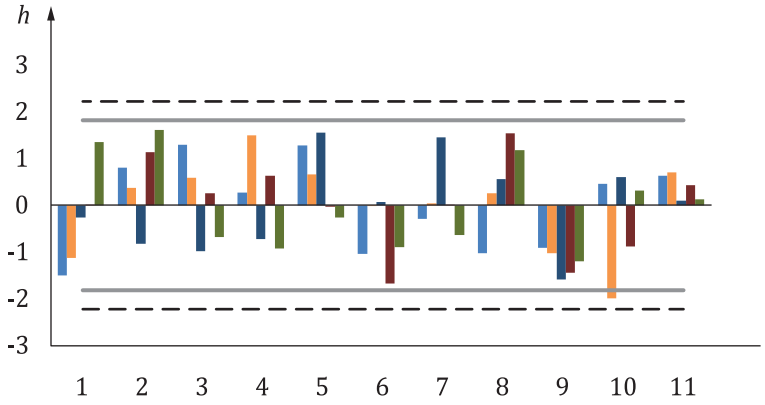
Table E.15 — Cell absolute differences — Amaranth content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,024	1,00	0,39	0,51	0,018
2	0,015	0,03	0,20	0,03	0,008
3	0,018	0,05	0,05	0,02	0,031
4	0,030	0,03	0,27	0,09	0,048
5	0,009	0,11	0,04	0,11	0,021
6	0,009	1,58	0,59	0,40	0,027
7	0,026	0,59	0,01	0,13	0,044
8	0,014	0,08	0,04	0,29	0,020
9	0,047	0,36	0,05	0,32	0,180
10	0,071	0,05	0,51	0,21	0,015
11	0,005	0,41	0,04	0,08	0,022

E.2.3.4 Scrutiny of results for consistency and outliers

E.2.3.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.5 and E.6.

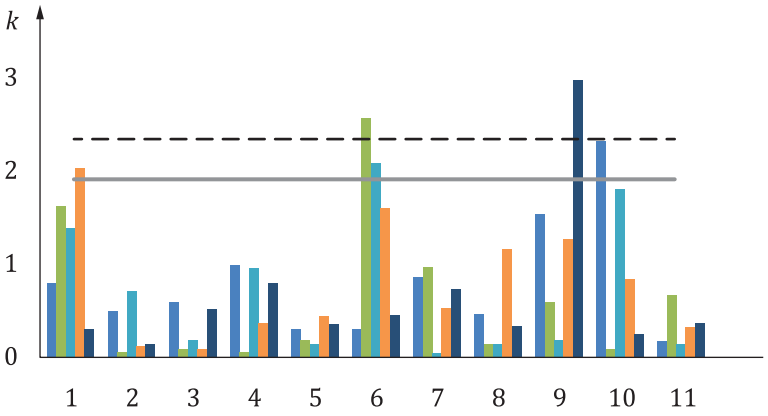


Key

1 % critical values

5 % critical values

Figure E.5 — Amaranth content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure E.6 — Amaranth content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 10 had a straggler at level B, while no outlier has been found herein.

The *k* graph shows that laboratory 1 had a straggler at level D, laboratory 6 at level C and laboratory 10 at level A, while laboratory 6 had an outlier at level B, as did laboratory 9 at level E. As further validation, the absolute difference between the data measured by laboratory 6 at level B and laboratory 9 at level E is the largest, and therefore the results were rejected.

E.2.3.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.16.

Table E.16 — Amaranth content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,487 8	0,598 4	0,394 2	0,374 0	0,292 8
Stragglers (n = 2)	0,570	0,602	0,570	0,570	0,602
Outliers (n = 2)	0,684	0,718	0,684	0,684	0,718
Number of laboratories, p	11	10	11	11	10

The application of the Cochran's test confirms no stragglers and outliers.

E.2.3.4.3 Grubbs' test

The application of the Grubbs' test to the cell means gives the values of the test statistic G shown in Table E.17.

Table E.17 — Amaranth content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, p	Single low	Single high	Double low	Double high
A	11	1,497	1,305	0,595 0	0,590 5
B	10	1,884	1,424	0,358 4	0,664 2
C	11	1,581	1,560	0,579 6	0,441 9
D	11	1,672	1,539	0,403 2	0,555 0
E	10	1,078	1,553	0,686 0	0,440 2
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

E.2.3.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Amaranth content in each sample gives the values shown in Table E.18.

Table E.18 — Amaranth content — Computed values of m, s_r, s_R

Parameter	Level *j* mg/kg				
	A	B	C	D	E
Number of laboratories, p	11	10	11	11	10
General mean, m	0,468	9,30	2,24	4,51	0,915
Repeatability standard deviation, s_r	0,021 7	0,289 1	0,200 4	0,177 8	0,019 8
Reproducibility standard deviation, s_R	0,027 8	0,756 8	0,207 2	0,255 8	0,052 5

E.2.3.6 Dependence of precision on general mean, *m*

Table E.18 shows that there is no obvious dependence between s_r and *m*. Therefore, *m* can be considered as the final mean, and the average s_r of five matrices as the final repeatability standard deviation. The actual calculation demonstrates a linear correlation between logs_R with log*m*, as follows:

$\log s_R = 1{,}085\ 6\ \ \log m - 1{,}195\ 0,\ R^2 = 0{,}976\ 4$

E.2.3.7 Final values of precision

The precision of the Amaranth content's measurement method should be given as follows:

— $s_r = 0{,}141\ 7$

— $s_R = 0{,}064\ m^{1{,}09}$

E.2.4 Indigotine

E.2.4.1 Original test results

Eleven laboratories participated in the determination of Indigotine content in meat samples. The test results are shown in Table E.19.

Table E.19 — Original test results — Indigotine content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,388	0,338	7,84	8,26	1,99	2,19	4,36	3,60	0,850	0,831
2	0,419	0,431	8,36	8,15	1,94	2,07	4,41	4,08	0,877	0,933
3	0,434	0,404	8,61	8,94	2,07	2,12	4,35	3,94	0,836	0,851
4	0,426	0,406	8,57	8,46	2,07	2,00	4,03	4,27	0,853	0,806
5	0,413	0,375	9,01	8,40	1,92	2,15	4,37	4,15	0,801	0,844
6	0,375	0,421	7,81	8,31	1,87	2,26	3,47	3,55	0,813	0,898
7	0,380	0,367	7,94	8,42	2,04	1,81	4,17	3,72	0,827	0,817
8	0,394	0,426	8,33	8,08	1,97	2,34	4,31	3,88	0,846	0,829
9	0,429	0,390	7,61	8,16	1,71	1,50	3,99	4,37	0,729	0,796
10	0,399	0,347	8,03	8,32	1,85	2,16	3,77	4,32	0,807	0,878
11	0,412	0,371	8,36	8,99	1,82	2,37	4,37	4,08	0,819	0,917

E.2.4.2 Cell means

The cell means of the determination of Indigotine content are shown in Table E.20.

Table E.20 — Cell means — Indigotine content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,363 0	8,050	2,090	3,980	0,840 5
2	0,425 0	8,255	2,005	4,245	0,905 0
3	0,419 0	8,775	2,095	4,145	0,843 5
4	0,416 0	8,515	2,035	4,150	0,829 5
5	0,394 0	8,705	2,035	4,260	0,822 5
6	0,398 0	8,060	2,065	3,510	0,855 5
7	0,373 5	8,180	1,925	3,945	0,822 0
8	0,410 0	8,205	2,155	4,095	0,837 5
9	0,409 5	7,885	1,605	4,180	0,762 5
10	0,373 0	8,175	2,005	4,045	0,842 5
11	0,391 5	8,675	2,097	4,225	0,868 0

E.2.4.3 Cell absolute differences

The cell absolute differences of the determination of Indigotine content are shown in Table E.21.

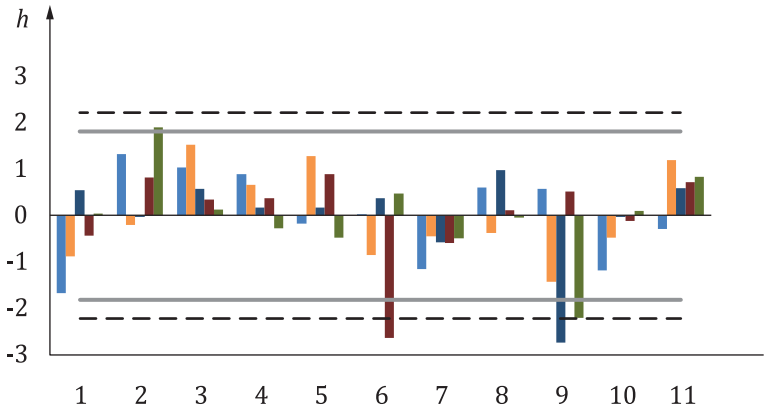
Table E.21 — Cell absolute differences — Indigotine content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,050	0,42	0,20	0,76	0,019
2	0,012	0,21	0,13	0,33	0,056
3	0,030	0,33	0,05	0,41	0,015
4	0,020	0,11	0,07	0,24	0,047
5	0,038	0,61	0,23	0,22	0,043
6	0,046	0,50	0,39	0,08	0,085
7	0,013	0,48	0,23	0,45	0,010
8	0,032	0,25	0,37	0,43	0,017
9	0,039	0,55	0,21	0,38	0,067
10	0,052	0,29	0,31	0,55	0,071
11	0,041	0,63	0,55	0,29	0,098

E.2.4.4 Scrutiny of results for consistency and outliers

E.2.4.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.7 and E.8.

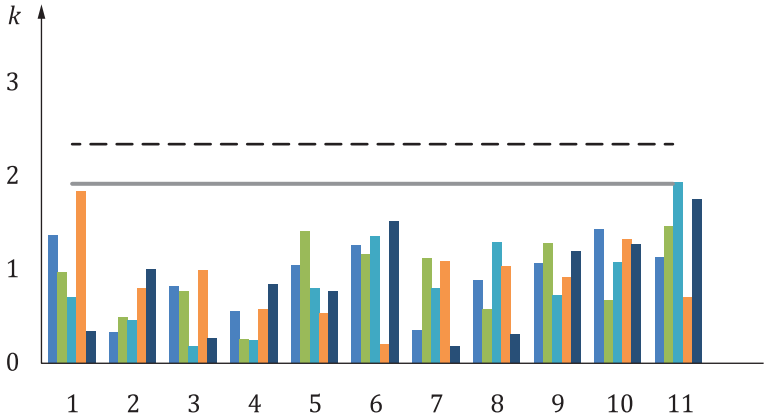


Key

1 % critical values

5 % critical values

Figure E.7 — Indigotine content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure E.8 — Indigotine content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 2 and laboratory 9 had two stragglers at level E, while laboratory 6 had an outlier at level D, as did laboratory 9 at level C. As further validation, laboratory 6 and laboratory 9 obtained lower test results than the other laboratories at level D and C, respectively, and therefore the results were rejected.

The *k* graph shows that laboratory 11 had a straggler at level C, while no outlier has been found herein.

E.2.4.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.22.

Table E.22 — Indigotine content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,185 2	0,194 6	0,356 0	0,307 4	0,278 2
Stragglers (n = 2)	0,570	0,570	0,602	0,602	0,570
Outliers (n = 2)	0,684	0,684	0,718	0,718	0,684
Number of laboratories, *p*	11	11	10	10	11

The application of the Cochran's test confirms no stragglers and outliers.

E.2.4.4.3 Grubbs' test

The application of the Grubbs' test to the cell means gives the values of the test statistic G shown in Table E.23.

Table E.23 — Indigotine content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, *p*	Single low	Single high	Double low	Double high
A	11	1,668	1,330	0,490 8	0,652 6
B	10	1,432	1,523	0,657 0	0,513 6
C	11	1,955	1,622	0,420 5	0,573 8
D	11	1,666	1,218	0,364 2	0,632 2
E	10	2,207	1,904	0,407 8	0,483 8
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

E.2.4.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Indigotine content in each sample gives the values shown in Table E.24.

Table E.24 — Indigotine content — Computed values of m, s_r, s_R

Parameter	Level *j* mg/kg				
	A	B	C	D	E
Number of laboratories, *p*	11	11	10	10	11
General mean, m	0,398	8,32	2,05	4,13	0,839
Repeatability standard deviation, s_r	0,025 8	0,304 5	0,207 6	0,306 5	0,039 6
Reproducibility standard deviation, s_R	0,027 6	0,370 2	0,160 3	0,242 7	0,044 6

E.2.4.6 Dependence of precision on general mean, *m*

Table E.24 shows that s_r and s_R have a strong positive correlation with m. The actual calculation demonstrates a linear correlation between logs_r (or logs_R) with log$m$, as follows:

$\log s_r = 0{,}924\ 1 \quad \log m - 1{,}194\ 1,\ R^2 = 0{,}895\ 0$

$\log s_R = 0{,}904\ 6 \quad \log m - 1{,}198\ 5,\ R^2 = 0{,}971\ 6$

E.2.4.7 Final values of precision

The precision of the Indigotine content measurement method should be given as follows:

— $s_r = 0{,}064\ m^{0{,}92}$

— $s_R = 0{,}063\ m^{0{,}90}$

E.2.5 Ponceau 4R

E.2.5.1 Original test results

Eleven laboratories participated in the determination of Ponceau 4R content in meat samples. The test results are shown in Table E.25.

Table E.25 — Original test results — Ponceau 4R content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,428	0,453	8,44	8,07	2,16	2,38	4,75	3,95	0,975	0,899
2	0,479	0,495	8,03	7,98	2,29	2,28	4,69	4,64	0,966	0,994
3	0,474	0,436	8,52	8,74	2,31	2,47	4,66	4,71	0,896	0,914
4	0,455	0,440	8,39	8,50	2,30	2,07	4,67	4,74	0,952	0,923
5	0,443	0,446	8,77	8,65	2,30	2,28	4,91	4,79	0,983	1,014
6	0,414	0,436	8,07	8,49	2,28	2,27	4,23	4,34	0,871	0,893
7	0,470	0,467	8,27	8,50	2,20	2,22	4,56	4,45	0,877	0,934
8	0,462	0,429	8,45	8,35	2,35	2,36	4,87	4,27	0,948	0,966
9	0,437	0,421	7,90	8,30	2,16	2,13	3,92	5,00	0,781	0,816
10	0,427	0,474	8,18	8,35	2,20	2,22	4,37	4,55	0,890	0,872
11	0,471	0,518	8,77	8,78	2,30	2,32	4,97	4,52	0,805	1,037

E.2.5.2 Cell means

The cell means of the determination of Ponceau 4R content are shown in Table E.26.

Table E.26 — Cell means — Ponceau 4R content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,440 5	8,255	2,270	4,350	0,937 0
2	0,487 0	8,005	2,285	4,665	0,980 0
3	0,455 0	8,630	2,390	4,685	0,905 0
4	0,447 5	8,445	2,185	4,705	0,937 5
5	0,444 5	8,710	2,290	4,850	0,998 5
6	0,425 0	8,280	2,275	4,285	0,882 0
7	0,468 5	8,385	2,210	4,505	0,905 5
8	0,445 5	8,400	2,355	4,570	0,957 2
9	0,429 0	8,100	2,145	4,460	0,798 5
10	0,450 5	8,265	2,210	4,460	0,881 0
11	0,494 5	8,775	2,310	4,745	0,921 0

E.2.5.3 Cell absolute differences

The cell absolute differences of the determination of Ponceau 4R content are shown in Table E.27.

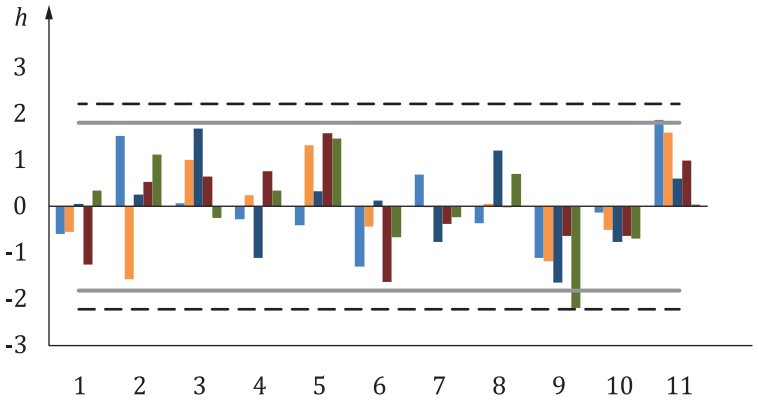
Table E.27 — Cell absolute differences — Ponceau 4R content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,025	0,37	0,22	0,80	0,076
2	0,016	0,05	0,01	0,05	0,028
3	0,038	0,22	0,16	0,05	0,018
4	0,015	0,11	0,23	0,07	0,029
5	0,003	0,12	0,02	0,12	0,031
6	0,022	0,42	0,01	0,11	0,022
7	0,003	0,23	0,02	0,11	0,057
8	0,033	0,10	0,01	0,60	0,018
9	0,016	0,40	0,03	1,08	0,035
10	0,047	0,17	0,02	0,18	0,018
11	0,047	0,01	0,02	0,45	0,232

E.2.5.4 Scrutiny of results for consistency and outliers

E.2.5.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.9 and E.10.

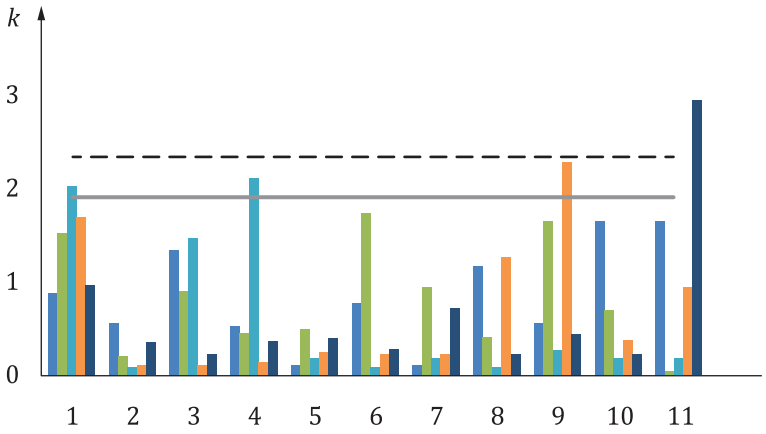


Key

1 % critical values

5 % critical values

Figure E.9 — Ponceau 4R content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure E.10 — Ponceau 4R content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 9 had a straggler at level E, as did laboratory 11 at level A, while no outlier has been found herein.

The *k* graph shows that laboratory 1 and laboratory 4 had two stragglers at level C, as did laboratory 9 at level D, while laboratory 11 had an outlier at level E. As further validation, the absolute difference between the data measured by laboratory 11 at level E is the largest, and therefore the result was rejected.

E.2.5.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.28.

The application of the Cochran's test confirms no stragglers and outliers.

Table E.28 — Ponceau 4R content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,250 6	0,274 5	0,407 9	0,476 1	0,403 8
Stragglers (n = 2)	0,570	0,570	0,570	0,570	0,602
Outliers (n = 2)	0,684	0,684	0,684	0,684	0,718
Number of laboratories, *p*	11	11	11	11	10

E.2.5.4.3 Grubbs' test

The application of the Grubbs' test to the cell means gives the values of the test statistic G shown in Table E.29.

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

Table E.29 — Ponceau 4R content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, *p*	Single low	Single high	Double low	Double high
A	11	1,296	1,875	0,643 3	0,284 3
B	11	1,571	1,601	0,530 1	0,470 5
C	11	1,649	1,693	0,521 9	0,471 8
D	11	1,630	1,591	0,482 7	0,574 0
E	10	2,078	1,394	0,370 7	0,571 9
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

E.2.5.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Ponceau 4R content in each sample gives the values shown in Table E.30.

Table E.30 — Ponceau 4R content — Computed values of m, s_r, s_R

Parameter	Level *j* mg/kg				
	A	B	C	D	E
Number of laboratories, *p*	11	11	11	11	10
General mean, *m*	0,453	8,39	2,27	4,57	0,918
Repeatability standard deviation, s_r	0,020 0	0,170 9	0,076 8	0,333 7	0,026 7
Reproducibility standard deviation, s_R	0,026 1	0,271 2	0,091 2	0,294 0	0,060 6

E.2.5.6 Dependence of precision on general mean, *m*

Table E.30 shows that there is no obvious dependence between s_r and m. Therefore, m can be considered as the final mean, and the average s_r of five matrices as the final repeatability standard deviation. The actual calculation demonstrates a linear correlation between $\log s_R$ with $\log m$, as follows:

$$\log s_R = 0{,}843\ 7\ \ \log m - 1{,}250\ 8,\ R^2 = 0{,}938\ 0$$

E.2.5.7 Final values of precision

The precision of the Ponceau 4R content measurement method should be given as follows:

— $s_r = 0\ 125\ 6$

— $s_R = 0{,}056\ m^{0{,}84}$

E.2.6 Sunset Yellow FCF

E.2.6.1 Original test results

Eleven laboratories participated in the determination of Sunset Yellow FCF content in meat samples. The test results are shown in Table E.31.

Table E.31 — Original test results — Sunset Yellow FCF content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,448	0,469	9,35	9,50	2,30	2,48	4,75	4,04	1,010	0,951
2	0,461	0,466	9,23	8,95	2,44	2,48	4,90	4,85	0,933	1,085
3	0,459	0,465	8,37	8,90	2,22	2,50	4,75	4,77	0,944	0,996
4	0,459	0,471	8,43	8,57	2,44	2,41	4,77	4,74	0,912	0,975
5	0,491	0,500	8,93	8,95	2,22	2,24	4,86	4,89	0,925	0,884
6	0,508	0,537	9,73	9,91	3,07	2,28	4,25	4,93	1,030	0,942
7	0,503	0,480	9,76	10,19	2,48	2,53	4,47	4,98	0,972	0,957
8	0,507	0,489	10,14	9,43	2,96	2,06	5,04	4,65	0,957	1,040
9	0,501	0,467	9,23	9,94	2,42	2,38	4,63	4,87	0,883	0,967
10	0,485	0,500	10,04	9,54	2,38	2,71	4,67	4,99	0,987	0,942
11	0,535	0,473	10,04	10,84	2,60	2,64	4,82	5,10	1,015	1,025

E.2.6.2 Cell means

The cell means of the determination of Sunset Yellow FCF content are shown in Table E.32.

Table E.32 — Cell means — Sunset Yellow FCF content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,458 5	9,425	2,390	4,395	0,980 5
2	0,463 5	9,090	2,460	4,875	1,009 0
3	0,462 0	8,635	2,360	4,760	0,970 0
4	0,465 0	8,500	2,425	4,755	0,943 5
5	0,495 5	8,940	2,230	4,875	0,904 5
6	0,522 5	9,820	2,675	4,590	0,986 0
7	0,491 5	9,975	2,505	4,725	0,964 5
8	0,498 0	9,785	2,510	4,845	0,998 5
9	0,484 0	9,585	2,400	4,750	0,925 0
10	0,492 5	9,790	2,545	4,830	0,964 5
11	0,504 0	10,440	2,620	4,960	1,020 0

E.2.6.3 Cell absolute differences

The cell absolute differences of the determination of Sunset Yellow FCF content are shown in Table E.33.

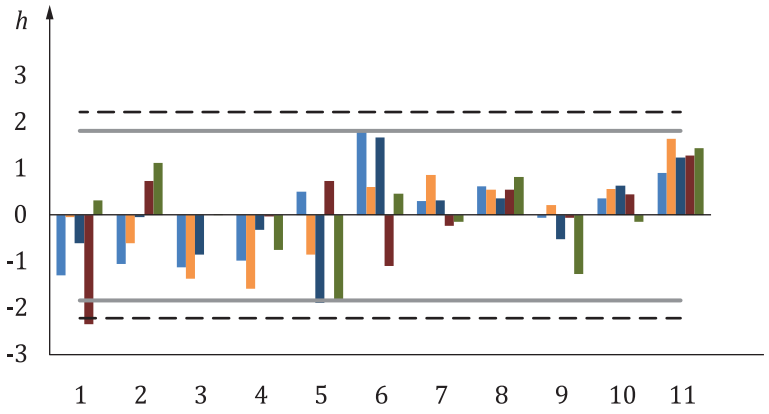
Table E.33 — Cell absolute differences — Sunset Yellow FCF content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,021	0,15	0,18	0,71	0,059
2	0,005	0,28	0,04	0,05	0,152
3	0,006	0,53	0,28	0,02	0,052
4	0,012	0,14	0,03	0,03	0,063
5	0,009	0,02	0,02	0,03	0,041
6	0,029	0,18	0,79	0,68	0,088
7	0,023	0,43	0,05	0,51	0,015
8	0,018	0,71	0,90	0,39	0,083
9	0,034	0,71	0,04	0,24	0,084
10	0,015	0,50	0,33	0,32	0,045
11	0,062	0,80	0,04	0,28	0,010

E.2.6.4 Scrutiny of results for consistency and outliers

E.2.6.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.11 and E.12.

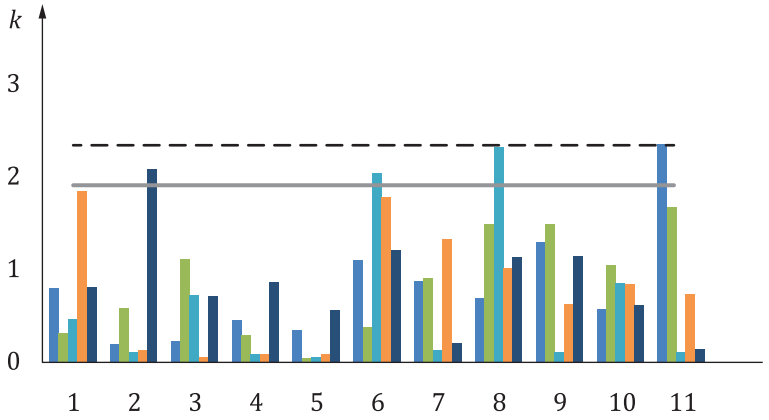


Key

1 % critical values

5 % critical values

Figure E.11 — Sunset Yellow FCF content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure E.12 — Sunset Yellow FCF content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 5 had two stragglers at levels C and E, while laboratory 1 had an outlier at level D. As further validation, laboratory 1 obtained a lower test result than the other laboratories at level D, and therefore the result was rejected.

The *k* graph shows that laboratory 2 had a straggler at level E, as did laboratory 6, and laboratory 8 had two stragglers at level C, while laboratory 11 had an outlier at level A. As further validation, the absolute difference between the data measured by laboratory 11 at level A is the largest, and therefore the result was rejected.

E.2.6.4.2 Cochran's test

E.2.6.4.2.1 General

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.34.

Table E.34 — Sunset Yellow FCF content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,304 1	0,254 2	0,487 2	0,413 7	0,391 7
Stragglers (n = 2)	0,602	0,570	0,570	0,602	0,570
Outliers (n = 2)	0,718	0,684	0,684	0,718	0,684
Number of laboratories, *p*	10	11	11	10	11

The application of the Cochran's test confirms no stragglers and outliers.

E.2.6.4.2.2 Grubbs' test

Application of Grubbs' test to cell means led to the values of the test statistic G shown in Table E.35.

Table E.35 — Sunset Yellow FCF content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, *p*	Single low	Single high	Double low	Double high
A	10	1,199	1,895	0,653 4	0,450 4
B	11	1,590	1,646	0,464 2	0,583 1
C	11	1,886	1,678	0,490 1	0,470 6
D	10	2,005	1,587	0,398 8	0,578 9
E	11	1,859	1,437	0,383 0	0,594 3
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

E.2.6.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Sunset Yellow FCF content in each sample gives the values shown in Table E.36.

Table E.36 — Sunset Yellow FCF content — Computed values of m, s_r, s_R

Parameter	Level *j* mg/kg				
	A	B	C	D	E
Number of laboratories, *p*	10	11	11	10	11
General mean, *m*	0,483	9,45	2,47	4,80	0,970
Repeatability standard deviation, s_r	0,013 8	0,338 3	0,274 9	0,236 4	0,051 8
Reproducibility standard deviation, s_R	0,022 9	0,645 6	0,231 0	0,196 4	0,050 7

E.2.6.6 Dependence of precision on general mean, *m*

Table E.36 shows that there is no obvious dependence between s_r and m. Therefore, m can be considered as the final mean, and the average s_r of five matrices as the final repeatability standard deviation. The actual calculation demonstrates a linear correlation between $\log s_R$ with $\log m$, as follows:

$$\log s_R = 1{,}072\ 7 \quad \log m - 1{,}262\ 7,\ R^2 = 0{,}942\ 6$$

E.2.6.7 Final values of precision

The precision of the Sunset Yellow FCF content measurement method should be given as follows:

— $s_r = 0{,}183\ 0$

— $s_R = 0{,}055\ m^{1{,}07}$

E.2.7 Allura Red AC

E.2.7.1 Original test results

Eleven laboratories participated in the determination of Allura Red AC content in meat samples. The test results are shown in Table E.37.

Table E.37 — Original test results — Allura Red AC content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,469	0,451	9,15	9,91	2,26	2,44	4,86	3,93	1,017	0,977
2	0,496	0,479	8,61	8,57	2,31	2,08	4,97	5,10	0,978	0,989
3	0,481	0,509	9,20	9,87	2,38	2,13	4,74	5,14	0,990	0,953
4	0,493	0,534	9,32	9,01	2,38	2,33	4,97	4,92	0,950	0,918
5	0,467	0,486	9,51	9,28	2,51	2,57	4,87	4,77	0,937	0,954
6	0,453	0,496	9,83	9,82	3,05	3,44	4,65	4,75	0,940	0,977
7	0,487	0,508	10,16	10,14	2,65	2,41	4,99	4,57	0,902	0,967
8	0,499	0,486	9,90	10,03	2,46	2,88	4,74	4,92	0,954	0,971
9	0,495	0,470	9,34	9,94	2,47	3,32	4,54	4,87	0,852	0,888
10	0,531	0,518	10,07	10,02	2,53	2,89	4,71	4,79	0,948	0,919
11	0,533	0,511	10,65	10,09	2,60	2,79	4,89	5,13	0,952	1,240

E.2.7.2 Cell means

The cell means of the determination of Allura Red AC content are shown in Table E.38.

Table E.38 — Cell means — Allura Red AC content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,460 0	9,530	2,350	4,395	0,997 0
2	0,487 5	8,590	2,195	5,035	0,983 5
3	0,495 0	9,535	2,255	4,940	0,971 5
4	0,513 5	9,165	2,355	4,945	0,934 0
5	0,476 5	9,395	2,540	4,820	0,945 5
6	0,474 5	9,825	3,245	4,700	0,958 5
7	0,497 5	10,150	2,530	4,780	0,934 5
8	0,492 5	9,965	2,670	4,830	0,962 5
9	0,482 5	9,640	2,895	4,705	0,870 0
10	0,524 5	10,045	2,710	4,750	0,933 5
11	0,522 0	10,370	2,695	5,010	1,096 0

E.2.7.3 Cell absolute differences

The cell absolute differences of the determination of Allura Red AC content are shown in Table E.39.

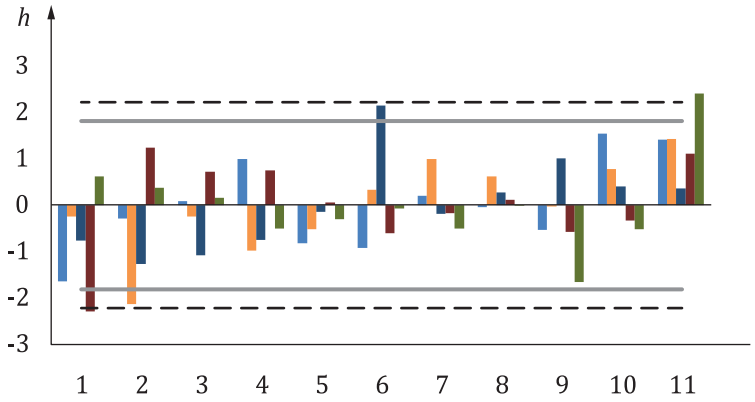
Table E.39 — Cell absolute differences — Allura Red AC content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,018	0,76	0,18	0,93	0,040
2	0,017	0,04	0,23	0,13	0,011
3	0,028	0,67	0,25	0,40	0,037
4	0,041	0,31	0,05	0,05	0,032
5	0,019	0,23	0,06	0,10	0,017
6	0,043	0,01	0,39	0,10	0,037
7	0,021	0,02	0,24	0,42	0,065
8	0,013	0,13	0,42	0,18	0,017
9	0,025	0,60	0,85	0,33	0,036
10	0,013	0,05	0,36	0,08	0,029
11	0,022	0,56	0,19	0,24	0,288

E.2.7.4 Scrutiny of results for consistency and outliers

E.2.7.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.13 and E.14.

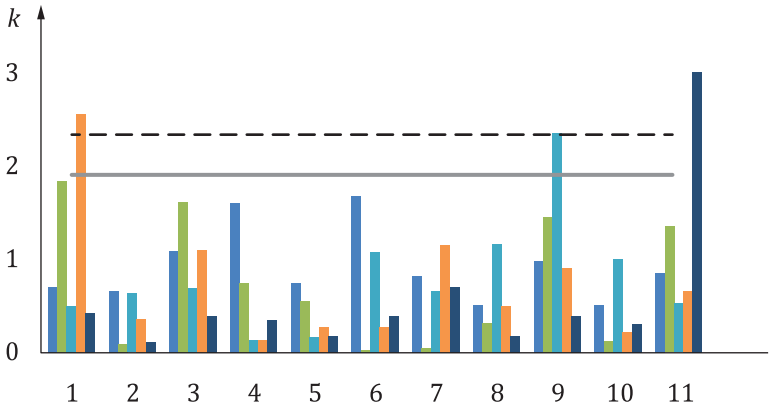


Key

- - - - 1 % critical values

——— 5 % critical values

Figure E.13 — Allura Red AC content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

- - - - 1 % critical values

——— 5 % critical values

Figure E.14 — Allura Red AC content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 2 had a straggler at level B, as did laboratory 6 at level C, while laboratory 1 and laboratory 11 had two outliers at level D and E, respectively. As further validation, laboratory 1 obtained a lower test result than the other laboratories at level D, and laboratory 11 obtained the highest test result at level E, and therefore the results were rejected.

The *k* graph shows that there was no straggler, while laboratory 1 had an outlier at level D, laboratory 9 at level C and laboratory 11 at level E. As further validation, the absolute difference between the data measured by laboratory 1 at level D, laboratory 9 at level C and laboratory 11 at level E is the largest, and therefore the results were rejected.

E.2.7.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.40.

Table E.40 — Allura Red AC content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,257 7	0,308 8	0,250 0	0,303 6	0,340 1
Stragglers (n = 2)	0,570	0,570	0,602	0,602	0,602
Outliers (n = 2)	0,684	0,684	0,718	0,718	0,718
Number of laboratories, *p*	11	11	10	10	10

The application of the Cochran's test confirms no stragglers and outliers.

E.2.7.4.3 Grubbs' test

Application of Grubbs' test to cell means led to the values of the test statistic G shown in Table E.41.

Table E.41 — Allura Red AC content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, *p*	Single low	Single high	Double low	Double high
A	11	1,639	1,538	0,572 8	0,466 2
B	11	2,129	1,428	0,343 6	0,633 8
C	10	1,180	2,266	0,672 9	0,293 2
D	10	1,229	1,489	0,593 8	0,462 8
E	10	2,241	1,359	0,320 7	0,613 0
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

E.2.7.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Allura Red AC content in each sample gives the values shown in Table E.42.

Table E.42 — Allura Red AC content — Computed values of m, s_r, s_R

Parameter	Level *j* mg/kg				
	A	B	C	D	E
Number of laboratories, *p*	11	11	10	10	10
General mean, m	0,493	9,66	2,55	4,85	0,949
Repeatability standard deviation, s_r	0,018 1	0,291 6	0,187 8	0,170 5	0,024 9
Reproducibility standard deviation, s_R	0,024 0	0,541 4	0,332 4	0,172 4	0,039 4

E.2.7.6 Dependence of precision on general mean, *m*

Table E.42 shows that there is no obvious dependence between s_r and m. Therefore, m can be considered as the final mean, and the average s_r of five matrices as the final repeatability standard deviation. The actual calculation demonstrates a linear correlation between $\log s_R$ with $\log m$, as follows:

$$\log s_r = 1{,}011\ 1 \quad \log m - 1{,}428\ 8,\ R^2 = 0{,}903\ 4$$

E.2.7.7 Final values of precision

The precision of the Allura Red AC content measurement method should be given as follows:

— $s_r = 0{,}037\ m^{1{,}01}$

— $s_R = 0{,}221\ 9$

E.2.8 Brilliant Blue FCF

E.2.8.1 Original test results

Eleven laboratories participated in the determination of Brilliant Blue FCF content in meat samples. The test results are shown in Table E.43.

Table E.43 — Original test results — Brilliant Blue FCF content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,384	0,367	7,78	7,99	2,13	2,01	5,28	4,62	0,868	0,833
2	0,395	0,404	8,47	8,40	2,06	2,25	4,18	3,79	0,886	0,841
3	0,405	0,418	7,78	7,83	2,21	2,28	4,20	4,25	0,844	0,843
4	0,403	0,407	7,74	7,47	2,01	1,94	4,07	4,11	0,815	0,828
5	0,383	0,392	8,01	8,16	1,98	2,09	3,84	4,00	0,793	0,811
6	0,427	0,395	8,19	7,98	1,99	2,42	3,87	4,21	0,768	0,826
7	0,401	0,372	8,09	8,40	2,34	1,88	3,88	4,04	0,797	0,762
8	0,387	0,418	8,08	8,33	1,79	2,20	3,87	4,19	0,839	0,879
9	0,407	0,361	7,61	8,03	2,37	1,84	3,78	4,01	0,770	0,809
10	0,385	0,403	8,31	8,09	1,94	2,21	3,97	4,03	0,858	0,787
11	0,420	0,397	8,48	8,75	2,03	2,22	4,22	4,20	0,803	0,844

E.2.8.2 Cell means

The cell means of the determination of Brilliant Blue FCF content are shown in Table E.44.

Table E.44 — Cell means —Brilliant Blue FCF content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,375 5	7,885	2,070	4,950	0,850 5
2	0,399 5	8,435	2,155	3,985	0,863 5
3	0,411 5	7,805	2,245	4,225	0,843 5
4	0,405 0	7,605	1,975	4,090	0,821 5
5	0,387 5	8,085	2,035	3,920	0,802 0
6	0,411 0	8,085	2,205	4,040	0,797 0
7	0,386 5	8,245	2,110	3,960	0,779 5
8	0,402 5	8,205	1,995	4,030	0,859 0
9	0,384 0	7,820	2,105	3,895	0,789 5
10	0,394 0	8,200	2,075	4,000	0,822 5
11	0,408 5	8,615	2,125	4,210	0,823 5

E.2.8.3 Cell absolute differences

The cell absolute differences of the determination of Brilliant Blue FCF content are shown in Table E.45.

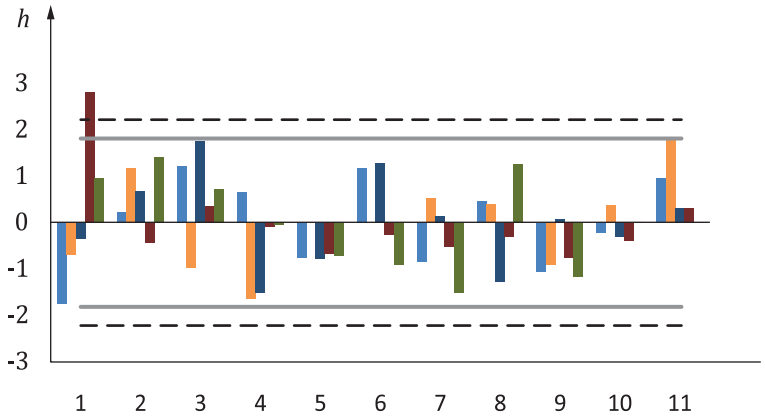
Table E.45 — Cell absolute differences — Brilliant Blue FCF content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,017	0,21	0,12	0,66	0,035
2	0,009	0,07	0,19	0,39	0,045
3	0,013	0,05	0,07	0,05	0,001
4	0,004	0,27	0,07	0,04	0,013
5	0,009	0,15	0,11	0,16	0,018
6	0,032	0,21	0,43	0,34	0,058
7	0,029	0,31	0,46	0,16	0,035
8	0,031	0,25	0,41	0,32	0,040
9	0,046	0,42	0,53	0,23	0,039
10	0,018	0,22	0,27	0,06	0,071
11	0,023	0,27	0,19	0,02	0,041

E.2.8.4 Scrutiny of results for consistency and outliers

E.2.8.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.15 and E.16.

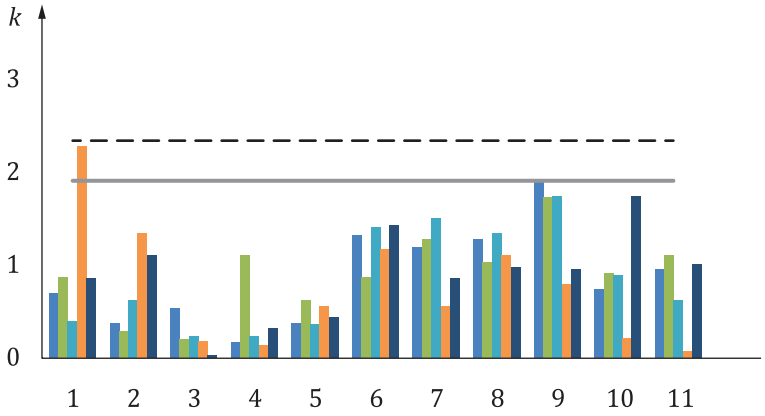


Key

1 % critical values

5 % critical values

Figure E.15 — Brilliant Blue FCF content —Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure E.16 — Brilliant Blue FCF content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that no straggler has been found, while laboratory 1 had an outlier at level D. As further validation, laboratory 1 obtained the highest test result at level D, and therefore the result was rejected.

The *k* graph shows that laboratory 1 had a straggler at level D, while no outlier has been found herein.

E.2.8.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.46.

Table E.46 — Brilliant Blue FCF content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,329 0	0,272 5	0,273 5	0,315 4	0,277 3
Stragglers (n = 2)	0,570	0,570	0,570	0,602	0,570
Outliers (n = 2)	0,684	0,684	0,684	0,718	0,684
Number of laboratories, *p*	11	11	11	10	11

The application of the Cochran's test confirms no stragglers and outliers.

E.2.8.4.3 Grubbs' test

The application of the Grubbs' test to the cell means gives the values of the test statistic G shown in Table E.47.

Table E.47 — Brilliant Blue FCF content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, *p*	Single low	Single high	Double low	Double high
A	11	1,756	1,203	0,491 7	0,658 0
B	11	1,638	1,776	0,564 2	0,452 0
C	11	1,508	1,761	0,527 0	0,424 3
D	10	1,260	1,699	0,631 4	0,259 4
E	11	1,512	1,413	0,556 7	0,563 0
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

E.2.8.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Brilliant Blue FCF content in each sample gives the values shown in Table E.48.

Table E.48 — Brilliant Blue FCF content — Computed values of m, s_r, s_R

Parameter	Level *j* mg/kg				
	A	B	C	D	E
Number of laboratories, *p*	11	11	11	10	11
General mean, *m*	0,397	8,09	2,10	4,04	0,823
Repeatability standard deviation, s_r	0,017 1	0,171 5	0,216 0	0,155 3	0,028 7
Reproducibility standard deviation, s_R	0,017 1	0,319 8	0,173 7	0,156 5	0,035 2

E.2.8.6 Dependence of precision on general mean, *m*

Table E.48 shows that there is no obvious dependence between s_r and m. Therefore, m can be considered as the final mean, and the average s_r of five matrices as the final repeatability standard deviation. The actual calculation demonstrates a linear correlation between logs_R with log$m$, as follows:

$$\log s_R = 0{,}978\ 1 \quad \log m - 1{,}320\ 1,\ R^2 = 0{,}933\ 1$$

E.2.8.7 Final values of precision

The precision of the Brilliant Blue FCF content measurement method should be given as follows:

— $s_r = 0{,}117\ 7$

— $s_R = 0{,}048\ m^{0{,}98}$

E.2.9 Carmoisine

E.2.9.1 Original test results

Eleven laboratories participated in the determination of Carmoisine content in meat samples. The test results are shown in Table E.49.

Table E.49 — Original test results — Carmoisine content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,394	0,390	8,20	8,45	2,53	2,21	4,56	3,54	0,892	0,877
2	0,386	0,411	8,57	8,40	2,44	2,52	4,39	4,17	0,875	0,813
3	0,394	0,401	8,16	8,17	2,29	2,44	4,39	4,66	0,890	0,854
4	0,399	0,441	8,24	8,23	2,50	2,27	4,59	4,50	0,867	0,860
5	0,421	0,434	7,93	8,11	2,28	2,18	4,23	4,25	0,805	0,815
6	0,429	0,470	7,58	9,56	2,42	2,39	4,15	4,14	0,831	0,800
7	0,439	0,498	8,86	8,87	2,46	2,27	4,29	4,29	0,809	0,781
8	0,478	0,405	8,76	8,65	2,30	2,27	4,01	4,61	0,855	0,877
9	0,458	0,419	8,32	8,73	1,82	2,64	3,99	4,61	0,750	0,794
10	0,501	0,438	8,56	8,93	2,36	2,41	4,20	4,37	0,836	0,770
11	0,431	0,539	9,57	8,96	2,57	2,33	4,48	4,53	0,836	0,813

E.2.9.2 Cell means

The cell means of the determination of Carmoisine content are shown in Table E.50.

Table E.50 — Cell means — Carmoisine content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,392 0	8,323	2,370	4,050	0,884 5
2	0,398 5	8,485	2,480	4,280	0,844 0
3	0,397 5	8,165	2,365	4,525	0,872 0
4	0,420 0	8,235	2,385	4,545	0,863 5
5	0,427 5	8,020	2,230	4,240	0,810 0
6	0,449 5	8,570	2,405	4,145	0,815 5
7	0,468 5	8,865	2,365	4,290	0,795 0
8	0,441 5	8,705	2,285	4,310	0,866 0
9	0,438 5	8,525	2,230	4,300	0,772 0
10	0,469 5	8,745	2,385	4,285	0,803 0
11	0,485 0	9,265	2,450	4,505	0,824 5

E.2.9.3 Cell absolute differences

The cell absolute differences of the determination of Carmoisine content are shown in Table E.51.

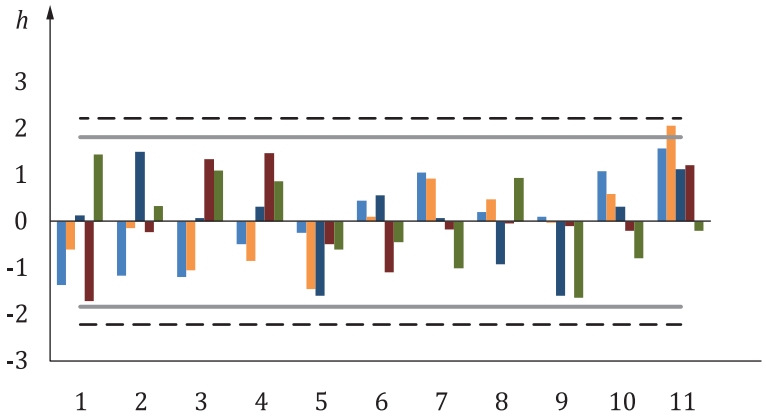
Table E.51 — Cell absolute differences — Carmoisine content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,004	0,25	0,32	1,02	0,015
2	0,025	0,17	0,08	0,22	0,062
3	0,007	0,01	0,15	0,27	0,036
4	0,042	0,01	0,23	0,09	0,007
5	0,013	0,18	0,10	0,02	0,010
6	0,041	1,98	0,03	0,01	0,031
7	0,059	0,01	0,19	0,00	0,028
8	0,073	0,11	0,03	0,60	0,022
9	0,039	0,41	0,82	0,62	0,044
10	0,063	0,37	0,05	0,17	0,066
11	0,108	0,61	0,24	0,05	0,023

E.2.9.4 Scrutiny of results for consistency and outliers

E.2.9.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

See Figures E.17 and E.18.

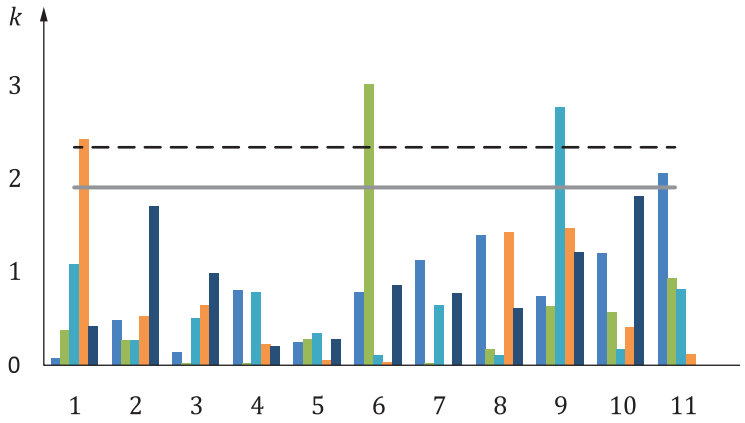


Key

1 % critical values

5 % critical values

Figure E.17 — Carmoisine content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

1 % critical values

5 % critical values

Figure E.18 — Carmoisine content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The *h* graph shows that laboratory 11 had a straggler at level B, while no outlier has been found herein.

The *k* graph shows that laboratory 11 had a straggler at level A, while laboratory 1 had an outlier at level D, laboratory 6 at level B and laboratory 9 at level C. As further validation, the absolute difference between the data measured by laboratory 1 at level D, laboratory 6 at level B and laboratory 9 at level C is the largest, and therefore the results were rejected.

E.2.9.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.52.

Table E.52 — Carmoisine content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,385 4	0,458 6	0,350 4	0,424 4	0,299 1
Stragglers (n = 2)	0,570	0,602	0,602	0,602	0,570
Outliers (n = 2)	0,684	0,718	0,718	0,718	0,684
Number of laboratories, *p*	11	10	10	10	11

The application of the Cochran's test confirms no stragglers and outliers.

E.2.9.4.3 Grubbs' test

The application of the Grubbs' test to the cell means gives the values of the test statistic G shown in Table E.53.

Table E.53 — Carmoisine content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, *p*	Single low	Single high	Double low	Double high
A	11	1,372	1,577	0,594 9	0,554 8
B	10	1,375	1,960	0,604 4	0,372 8
C	10	1,965	1,494	0,270 6	0,530 5
D	10	1,467	1,504	0,627 4	0,430 6
E	11	1,642	1,446	0,550 0	0,597 0
Stragglers (5 %)					
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

The application of the Grubbs' test to the cell means confirms no stragglers and outliers.

E.2.9.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Carmoisine content in each sample gives the values shown in Table E.54.

Table E.54 — Carmoisine content— Computed values of m, s_r, s_R

Parameter	Level *j* mg/kg				
	A	B	C	D	E
Number of laboratories, *p*	11	10	10	10	11
General mean, *m*	0,435	8,53	2,37	4,34	0,832
Repeatability standard deviation, s_r	0,037 1	0,201 4	0,120 9	0,212 8	0,025 7
Reproducibility standard deviation, s_R	0,041 0	0,399 6	0,111 9	0,201 9	0,040 7

E.2.9.6 Dependence of precision on general mean, *m*

Table E.54 shows that there is no obvious dependence between s_r and m. Therefore, m can be considered as the final mean, and the average s_r of five matrices as the final repeatability standard deviation. The actual calculation demonstrates a linear correlation between s_R with m, as follows:

$$s_R = 0{,}045\ 3\ m + 0{,}009\ 3,\ R^2 = 0{,}997\ 4$$

E.2.9.7 Final values of precision

The precision of the Carmoisine content measurement method should be given as follows:

— $s_r = 0{,}119\ 6$

— $s_R = 0{,}045\ 3\ m + 0{,}009\ 3$

E.2.10 Erythrosine

E.2.10.1 Original test results

Eleven laboratories participated in the determination of Erythrosine content in meat samples. The test results are shown in Table E.55. Due to the instability, Erythrosine with low level were easily to decompose. Therefore, the results of laboratory 6, 10 and 11 at level A which were obviously unreliable were not used.

Table E.55 — Original test results — Erythrosine content

Laboratory *i*	**Level** *j* mg/kg									
	A		**B**		**C**		**D**		**E**	
1	0,349	0,372	7,61	7,32	1,82	1,99	4,15	3,41	0,776	0,750
2	0,366	0,388	7,37	7,21	2,00	1,79	3,99	3,76	0,746	0,770
3	0,379	0,398	8,10	8,19	1,98	2,04	3,85	3,77	0,789	0,771
4	0,357	0,344	7,26	7,01	1,96	1,87	3,65	3,4	0,738	0,748
5	0,349	0,372	7,21	7,15	1,88	1,99	3,78	3,54	0,743	0,759
6	—	—	8,26	7,95	1,86	1,80	4,02	3,81	—	—
7	0,370	0,356	7,43	7,46	1,86	1,98	3,76	3,59	0,719	0,742
8	0,390	0,394	7,96	7,84	1,92	1,79	4,03	3,75	0,797	0,772
9	0,396	0,375	6,64	6,99	1,71	1,81	3,41	3,61	0,790	0,762
10	—	—	6,20	6,54	1,42	1,42	3,27	3,34	—	—
11	—	—	7,19	7,29	1,55	1,56	3,53	3,79	—	—

E.2.10.2 Cell means

The cell means of the determination of Erythrosine content are shown in Table E.56.

Table E.56 — Cell means — Erythrosine content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,360 5	7,465	1,905	3,780	0,763 0
2	0,377 0	7,290	1,895	3,875	0,758 0
3	0,388 5	8,145	2,010	3,810	0,780 0
4	0,350 5	7,135	1,915	3,525	0,743 0
5	0,360 5	7,180	1,935	3,660	0,751 0
6	—	8,105	1,830	3,915	—
7	0,363 0	7,445	1,920	3,675	0,730 5
8	0,392 0	7,900	1,855	3,890	0,784 5
9	0,385 5	6,815	1,760	3,510	0,776 0
10	—	6,370	1,420	3,305	—
11	—	7,240	1,555	3,660	—

E.2.10.3 Cell absolute differences

The cell absolute differences of the determination of Erythrosine content are shown in Table E.57.

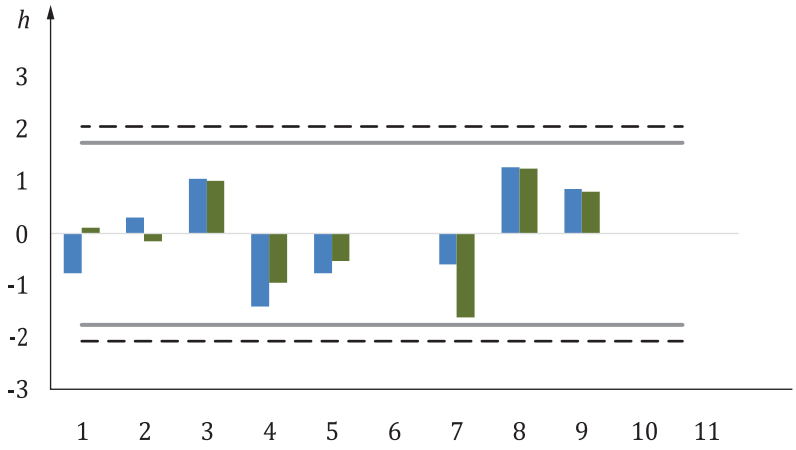
Table E.57 — Cell absolute differences — Erythrosine content

Laboratory *i*	Level *j* mg/kg				
	A	B	C	D	E
1	0,023	0,29	0,17	0,74	0,026
2	0,022	0,16	0,21	0,23	0,024
3	0,019	0,09	0,06	0,08	0,018
4	0,013	0,25	0,09	0,25	0,010
5	0,023	0,06	0,11	0,24	0,016
6	—	0,31	0,06	0,21	—
7	0,014	0,03	0,12	0,17	0,023
8	0,004	0,12	0,13	0,28	0,025
9	0,021	0,35	0,10	0,20	0,028
10	—	0,34	0,00	0,07	—
11	—	0,10	0,01	0,26	—

E.2.10.4 Scrutiny of results for consistency and outliers

E.2.10.4.1 Graphical consistency technique by Mandel's *h* and *k* statistics

Levels A and E had results from 8 valid laboratories, while other levels had results from 11 valid laboratories. Indicators for Mandel's *h* and *k* statistics at 1 % and 5 % significance level are related to the number of valid laboratories. Therefore, Mandel's *h* and *k* graphs for levels A and E are shown in Figures E.19 and E.20. The *h* and *k* graphs for levels B, C and D are shown in Figures E.21 and E.22.

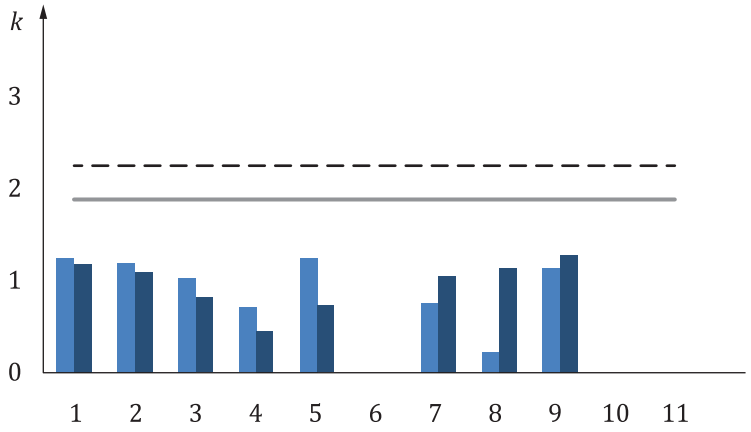


Key

1 % critical values

5 % critical values

Figure E.19 — Erythrosine content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories (levels A and E)

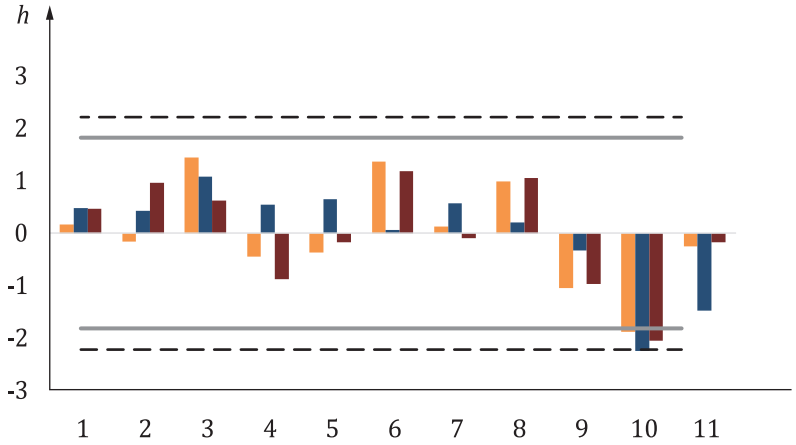


Key

1 % critical values

5 % critical values

Figure E.20 — Erythrosine content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories (levels A and E)

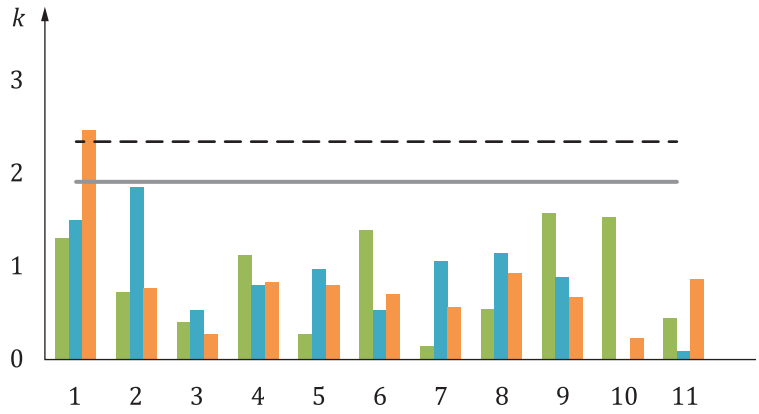


Key

1 % critical values

5 % critical values

Figure E.21 — Erythrosine content — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories (levels B, C and D)



Key

1 % critical values

5 % critical values

Figure E.22 — Erythrosine content — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories (levels B, C and D)

The *h* graphs show that laboratory 10 obtained much lower test results than all the other laboratories at all levels, and had an outlier at level C. As further validation, laboratory 10 obtained a lower test result than the other laboratories at level C, and therefore the result was rejected.

The *k* graphs show that there was no straggler, while laboratory 1 had an outlier at level D. As further validation, the absolute difference between the data measured by laboratory 1 at level D is the largest, and therefore the result was rejected.

E.2.10.4.2 Cochran's test

The remaining data are used to do a further Cochran's test which gives the values of the test statistic C given in Table E.58.

Table E.58 — Erythrosine content — Value of Cochran's test statistic C

Parameter	Level *j*				
	A	B	C	D	E
Cochran's test, *C*	0,194 1	0,225 4	0,311 0	0,176 9	0,202 6
Stragglers (*n* = 2)	0,680	0,570	0,602	0,602	0,680
Outliers (*n* = 2)	0,794	0,684	0,718	0,718	0,794
Number of laboratories, *p*	8	11	10	10	8

The application of the Cochran's test confirms no stragglers and outliers.

E.2.10.4.3 Grubbs' test

The application of the Grubbs' test to the cell means gives the values of the test statistic G shown in Table E.59.

Table E.59 — Erythrosine content — Application of Grubbs' test to cell means

Level *j*	Number of laboratories, *p*	Single low	Single high	Double low	Double high
A	8	1,397	1,276	0,530 2	0,480 6
B	11	1,874	1,446	0,446 1	0,514 7
C	10	2,413	1,210	0,143 7	0,749 2
D	10	1,918	1,181	0,397 3	0,652 0
E	8	1,599	1,255	0,355 6	0,503 9
Stragglers (5 %)					
p = 8		2,126	2,126	0,110 1	0,110 1
p = 10		2,290	2,290	0,186 4	0,186 4
p = 11		2,355	2,355	0,221 3	0,221 3
Outliers (1 %)					
p = 8		2,274	2,274	0,056 3	0,056 3
p = 10		2,482	2,482	0,115 0	0,115 0
p = 11		2,564	2,564	0,144 8	0,144 8

The double Grubbs' test indicates a straggler for level C. On further enquiry, the cell mean for laboratory 11 was much lower than other laboratories. Considering the instability of Erythrosine at low level, it was decided that the pairs of test results had to be rejected.

Without these test results, the double low of Grubbs' test statistic was 0,336 4 at level C, which was then compared with the critical value for 9 laboratories (0,149 2 at 5 %). This no longer appeared as a straggler and was retained.

E.2.10.5 Calculation of the general mean and standard deviation

Calculation of the general mean (m) and standard deviation (s_r and s_R) of Erythrosine content in each sample gives the values shown in Table E.60.

Table E.60 — Erythrosine content — Computed values of m, s_r, s_R

Parameter	Level j mg/kg				
	A	B	C	D	E
Number of laboratories, p	8	11	9	10	8
General mean, m	0,372	7,37	1,89	3,68	0,761
Repeatability standard deviation, s_r	0,013 1	0,157 2	0,088 7	0,148 9	0,015 6
Reproducibility standard deviation, s_R	0,018 1	0,546 1	0,094 5	0,223 2	0,021 9

E.2.10.6 Dependence of precision on general mean, m

Table E.60 shows that s_r and s_R have a strong positive correlation with m. The actual calculation demonstrates a linear correlation between $\log s_r$ (or $\log s_R$) with $\log m$, as follows:

$\log s_r = 0{,}973\ 4\ \ \log m - 1{,}501\ 4$, $R^2 = 0{,}905\ 3$

$\log s_R = 1{,}212\ 7\ \ \log m - 1{,}350\ 4$, $R^2 = 0{,}972\ 3$

E.2.10.7 Final values of precision

The precision of the Erythrosine content measurement method should be given as follows:

— $s_r = 0{,}032 + m^{0{,}97}$

— $s_R = 0{,}045 + m^{1{,}21}$

E.3 Conclusion

The conclusion was drawn from a uniform level experiment involving 11 laboratories. No straggler data were reported.

The final precision value given by the statistical work can be used to determine the repeatability and reproducibility standard deviation of the test method.

The final precision value confirms that the test method described in this document is reliable, since a good consistency was shown between the reported test values from all the participating laboratories.

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