BS EN 14152:2014

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Foodstuffs — Determination of vitamin B2 by high performance liquid chromatography

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Foodstuffs - Determination of vitamin B2 by high performance liquid chromatography

Produits alimentaires - Détermination de la teneur en vitamine B2 par chromatographie liquide haute performance

Lebensmittel - Bestimmung von Vitamin B2 mit Hochleistungs-Flüssigchromatographie

This European Standard was approved by CEN on 17 April 2014.

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Foreword

This document (EN 14152:2014) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2014 and conflicting national standards shall be withdrawn at the latest by December 2014.

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This document supersedes EN 14152:2003.

Annexes A, B and C are informative.

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1 Scope

This European Standard specifies a method for the determination of vitamin B_2 in food by high performance liquid chromatography (HPLC) and fluorescence detection. This method has been validated in two interlaboratory studies. The first study was for the analysis of samples of milk powder and pig's liver ranging from 1,45 mg/100 g to 10,68 mg/100 g. The second study was for the analysis of samples of tube feeding solution, baby food, powdered milk, meal with fruits, yeast, cereal and chocolate powder ranging from 0,21 mg/100 g to 87,1 mg/100 g. Vitamin B_2 is the mass fraction of total riboflavin including its phosphorylated derivatives.

For further information on the validation, see Clause 8 and Annex B.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

3 Principle

Riboflavin is extracted from food after acid hydrolysis followed by dephosphorylation using an enzymatic treatment, and separated by HPLC, and detected by fluorometric detection. An external standard is used for quantification. For further information see [1] to [11].

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of at least grade 1 according to EN ISO 3696, or double distilled water.

4.1 Methanol, mass fraction $w(CH_3OH) \geq 99{,}8$ %, HPLC grade.

4.2 Sodium acetate trihydrate, $w(CH_3COONa \cdot 3H_2O) = 99$ %.

4.3 Sodium acetate solution, substance concentration $c(CH_3COONa \cdot 3H_2O) = 0{,}1$ mol/l.

4.4 Sodium acetate solution, $c(CH_3COONa \cdot 3H_2O) = 2{,}5$ mol/l.

4.5 Glacial acetic acid, $w(CH_3COOH) = 99{,}8$ %.

4.6 Acetic acid solution, $c(CH_3COOH) = 0{,}02$ mol/l.

4.7 Hydrochloric acid, $w(HCl) = 36$ %.

4.8 Hydrochloric acid, $c(HCl) = 0{,}1$ mol/l.

4.9 Hydrochloric acid, $c(HCl) = 0{,}01$ mol/l.

4.10 Sulfuric acid, $c(H_2SO_4) = 0{,}05$ mol/l.

4.11 Sodium hydroxide, $w(NaOH) \geq 99$ %.

4.12 Sodium hydroxide solution, $c(NaOH)$ = 0,5 mol/l.

4.13 Phosphorous pentoxide, $w(P_2O_5)$ = 98 %.

4.14 Enzyme or enzyme mixture, with the ability to liberate vitamin B_2 from foods as free riboflavin.

NOTE For the precision data in Table B.1, Taka-Diastase from Pfaltz and Bauer[1)] has been used. For the precision data in Table B.2 and Table B.3 an enzyme mixture of β-amylase from barley and Taka-Diastase from Serva[1)] have been used.

4.15 HPLC Mobile phase

Examples of appropriate mixtures of e.g. 10 % to 50 % methanol (4.1) in water or using phosphate or acetate buffer are given in Annex A and Annex C. The possibility of using ion-pairing agents is also given.

4.16 Phosphate buffer (pH = 3,5), $c(KH_2PO_4)$ = 9,0 mmol/l.

4.17 Tetraethylammoniumchloride, $w(C_8H_{20}ONCl) \geq 98$ %.

4.18 Sodium heptanesulfonate, $w(C_7H_{15}NaO_3S) \geq 98$ %.

4.19 Standard substances

4.19.1 Riboflavin, $w(C_{17}H_{20}N_4O_6)$ = 98 %.

Vitamin B_2 can be obtained as riboflavin from various suppliers. The purity of the riboflavin standard may vary. It is therefore necessary to determine the concentration of the calibration solution by UV-spectrometry (see concentration test in 4.20.3).

4.19.2 Riboflavin-5'-phosphate, $w(C_{17}H_{20}N_4NaO_9P)$ = 95 %.

Riboflavin-5'-phosphate sodium salt (for check of enzyme and retention time in chromatogram).

4.20 Stock solution

4.20.1 Precautions

Vitamin B_2 is very sensitive to light. Measures shall be taken to protect the vitamin B_2 and the corresponding solutions during the whole sample preparation procedure e.g. by using generally brown glassware.

4.20.2 Riboflavin stock solution, M = 376,36, $\rho(C_{17}H_{20}N_4O_6) \approx 100$ µg/ml.

Dissolve an amount of riboflavin standard substance (4.19.1) previously dried and stored in dark in a desiccator possibly under vacuum and/or over phosphorous pentoxide (4.13), weighed to the nearest milligram, e.g. approximately 50 mg in a defined volume, e.g. 500 ml in an appropriate solvent e.g. diluted acetic acid (4.6) using brown volumetric flasks. This solution can be stored at 4 °C in the dark for 2 months.

Riboflavin is sparingly soluble. To facilitate dissolution warm with approximately 300 ml diluted acetic acid (4.6), on a steam bath with constant stirring until dissolved, cool and add diluted acetic acid (4.6) to make 500 ml. Alternatively add 5 ml of sodium hydroxide solution (4.12) to the standard substance in a 500 ml volumetric flask. Due to the instability in alkaline solutions immediately after dissolution add 1,5 ml of glacial acetic acid (4.5) and dilute to volume with diluted acetic acid (4.6), or another appropriate acid. The concentration of the freshly prepared and if necessary also stored solution should be tested (4.20.3).

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1) The information of the suppliers of Taka-Diastase, Pfaltz & Bauer, Waterbury, CT 06708, USA (No T00040), and Serva is given for the convenience of users of this European standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

4.20.3 Concentration test

Mix 20 ml of the riboflavin stock solution, (4.20.2) in a 200 ml volumetric flask with 3,5 ml sodium acetate solution (4.3) and dilute with water to the mark. For the preparation of the blank solution, mix 20 ml of acetic acid solution (4.6) with 3,5 ml of sodium acetate solution (4.3) in a 200 ml volumetric flask and dilute to the mark with water. Take these solutions for the spectrometric measurement.

Measure the absorbance of the riboflavin solution at the maximum wavelength of about 444 nm (A_{444}) in a 1 cm cell with a spectrometer (5.1) against the blank solution as reference. Calculate the mass concentration, ρ, of riboflavin in micrograms per millilitre, of the stock solution (4.20.2) according to Formula (1):

$$\rho = \frac{A_{444} \cdot M \cdot 1\,000}{\varepsilon} \qquad (1)$$

where

ε is the molar absorption coefficient of riboflavin at the maximum wavelength of about 444 nm. The value is 12 340 $l \cdot mol^{-1} \cdot cm^{-1}$. This value is calculated from the extinction coefficient, $E_{1cm}^{1\%}$ = 328, in acetate buffer (pH = 3,8) at 444 nm [9] and the molar mass, M = 376,36. The value is given with four significant figures;

M is the molar mass, in grams per mol. The value is 376,36;

A_{444} is the absorption value of the riboflavin solution.

4.21 Standard solutions

4.21.1 Riboflavin standard solution, $\rho(C_{17}H_{20}N_4O_6) \approx 10$ µg/ml.

Prepare a 1:10 dilution of the riboflavin stock solution (4.20.2), e.g. pipette 10 ml of the riboflavin stock solution, into a 100 ml brown volumetric flask and add diluted acetic acid (4.6) or another appropriate solvent to make 100 ml. Prepare this solution fresh every day.

4.21.2 Riboflavin standard test solution, $\rho(C_{17}H_{20}N_4O_6) \approx$ 0,1 µg/ml to 1 µg/ml.

Pipette corresponding volumes e.g. 1,0 ml to 10,0 ml of the standard solution (4.21.1), into brown volumetric flasks e.g. 100 ml and dilute with the mobile phase (4.15) to the mark. Prepare this solution fresh every day.

5 Apparatus

Use laboratory apparatus, glassware, and, in particular, the following:

5.1 UV spectrometer, UV spectrometer capable of measuring absorption at defined wavelengths (444 nm), with appropriate cells, e.g. of 1 cm length.

5.2 Autoclave or heating device, autoclave for extraction purpose, e.g. pressure cooker type, with pressure or temperature reading device, electrical heating device or water bath.

5.3 HPLC system, consisting of a pump, a sample injecting device, a fluorescence detector with an excitation and emission wavelength set at e.g. 468 nm and 520 nm, respectively (see Annex C), and an evaluation system such as an integrator.

5.4 HPLC column

Analytical reversed phase column, e.g. of diameter 4,0 mm to 4,6 mm, length 100 mm to 250 mm, filled with particle size 3 µm to 10 µm. Other systems (see Annex A) can be used providing that a satisfactory separation of riboflavin from other co-extractives is achieved.

Other particle sizes or column dimensions than those specified in this European Standard may be used. Separation parameters shall be adapted to such materials to guarantee equivalent results.

5.5 Filter device

Filtering of the mobile phase as well as of the sample solution through a membrane filter, e.g. a pore size of 0,45 µm, prior to use or injection will increase longevity of the columns.

6 Procedure

6.1 Precautions

Vitamin B_2 is very sensitive to light. Measures shall be taken to protect the sample and the corresponding solutions during the whole procedure e.g. by using generally brown glassware.

6.2 Preparation of the test sample

Homogenize the test sample. Grind coarse material with an appropriate mill and mix again. Measures such as pre-cooling shall be taken to avoid exposing to high temperature for long periods of time.

6.3 Preparation of the sample test solution

6.3.1 Extraction

Weigh an appropriate amount of the sample to the nearest mg, e.g. 2 g to 10 g in a beaker or a conical flask. Add a defined volume ranging from 50 ml to 200 ml of hydrochloric acid (4.8), or sulfuric acid (4.10). The pH of the solution should not be more than 2,0. Cover the container with a watch glass and either autoclave the test portion at 121 °C for 30 min, or heat it at 100 °C for 60 min.

The data from the BCR study have shown that a wide range of conditions for the acid hydrolysis can be applied (temperature 95 °C to 130 °C, time 15 min to 60 min). The higher the temperature is, the shorter the time should be. However, prolonged heating of riboflavin and riboflavin-5'-phosphate can cause losses. It has been shown that, notably for chocolate foods, the extraction efficiency could drop when pH was above 2.

6.3.2 Enzyme treatment

After cooling to room temperature adjust the extract to the optimal pH for the enzyme used with sodium acetate solution (4.4) and add a suitable amount of dephosphorylating enzyme (4.14) to the sample. Incubate the mixture at the optimal time and temperature for the enzyme(s) used. After cooling to room temperature transfer to a light protected volumetric flask using diluted acetic acid (4.6) or another appropriate solvent and dilute to a defined volume (V_E).

For each enzyme used, optimal pH, incubation time and incubation temperature shall be checked.

To ensure an optimal dephosphorylation, the enzymatic step shall be checked, for example by analysis of samples spiked with riboflavin-5'-phosphate sodium salt (4.19.2), or a material similar in sample type as the test sample. This material should be a reference material.

The amount of riboflavin possibly brought in with the enzyme shall be considered in the calculation of the result.

NOTE For determination of the precision data given in Table B.1, Table B.2 and Table B.3, Taka-Diastase (Table B.1) or Taka-Diastase combined with β-amylase from barley (Table B.2 and Table B.3) were used for dephosphorylation under the following conditions. The extract was adjusted to pH = 4,0 and pH = 4,5, respectively, with sodium acetate solution (4.4) and 100 mg of Taka-Diastase and 10 mg β-amylase per gram of sample was added. The mixture was incubated at 37 °C to 45 °C for 4 h to 24 h, see [9], [10] and [13].

6.3.3 Sample test solution

If necessary, filter the sample solution (6.3.2) through a filter paper or a 0,45 µm membrane filter. Centrifugation at appropriate *g* level may also be used. If appropriate, dilute an aliquot (V_A) to a defined volume (V) with a solvent mixture, which is compatible with the HPLC system used. As an example, dilute 1,0 ml of the extract (6.3.2) with 1,0 ml of methanol (4.1). This is the sample test solution for the HPLC analysis.

6.4 Identification

Inject the same appropriate volumes of the solutions of standards, samples and blank into the HPLC system. Identify the riboflavin by comparison of the retention time of the peak in the chromatograms obtained with the sample test solution and with the standard solution. Adding small amounts of the appropriate standard solutions to the sample test solution can also perform peak identification.

NOTE The separation and the quantification have proven to be satisfactory if following experimental conditions are followed (see also Figure A.1). For alternative HPLC-systems see Table C.1.

Stationary phase:	Supelco® LC-18-DB[2)] 5 µm, 250 mm x 4,6 mm
Mobile phase:	Methanol (4.1): phosphate buffer, pH 3,5, (4.16) containing 1 g/l tetraethylammoniumchloride (4.17), and 5 mmol/l sodium heptanesulfonate (4.18) (35:65)
Flow rate:	1,0 ml/min
Injection volume:	20 µl
Detection:	Fluorometric: Excitation: 468 nm; Emission: 520 nm

6.5 Determination

Inject equal appropriate volumes (up to 100 µl) of the riboflavin solution (4.20.2) as well as of the sample test solution (6.3.3) into the HPLC-system. To carry out a quantitative determination by the external standard method, integrate the peak areas or determine the peak heights and compare the results with the corresponding values for the standard substance. Check the linearity of the calibration.

7 Calculation

Base the calculation on a calibration graph or use the corresponding programmes of the integrator or use the following simplified procedure. Calculate the mass fraction, w, of vitamin B_2 in mg/100 g of the sample using Formula (2):

$$w = \frac{A_S \cdot \rho \cdot V \cdot V_E}{A_{ST} \cdot m_S \cdot V_A \cdot 1\,000} \cdot 100 - \frac{m_E \cdot E}{m_S} \qquad (2)$$

where

A_S is the peak area or peak height for riboflavin obtained with the sample test solution (6.3.3), in units of area or height;

A_{ST} is the peak area or peak height for riboflavin obtained with the riboflavin solution (4.20.2), in units of area or height;

2) Supelco ® LC-18-DB is an example of a suitable product available commercially. This information is given for the convenience of the users of this standard and does not constitute an endorsement by CEN.

V is the total volume of the final sample test solution (6.3.3), in millilitre;

V_E is the volume of sample extract (6.3.2), in millilitre;

V_A is the volume of the aliquot used for the final dilution (6.3.3), in millilitre;

ρ is the mass concentration of riboflavin in the riboflavin solution (4.20.2), in microgram per millilitre;

m_S is the sample mass, in gram;

1 000 is the conversion factor for microgram to milligram;

100 is the conversion factor for the mass fraction per 100 g;

E is the mass fraction of riboflavin present in the enzyme, in mg/100 g;

m_E is the enzyme mass used in the analysis, in gram.

Report the result for vitamin B_2 in mg/100 g as riboflavin.

8 Precision

8.1 General

The precision data for the method is partly based on different HPLC methods applied for the determination of riboflavin in an international comparison study organized on behalf of the European Commission's Standards Measurement and Testing Programme on a sample of whole meal flour (CRM 121), milk powder/spray dried milk (CRM 421), freeze-dried mixed vegetables (CRM 485) and freeze-dried lyophilised pig liver (CRM 487). The study provided the statistical information shown in Annex B, Table B.1. Furthermore, the precision data include the results of a French collaborative study in tube feeding solution, baby food with vegetables, powdered milk, meal with fruits, yeast, cereal, chocolate powder and food supplement. The results from this study are shown in Annex B, Table B.2 and Table B.3.

8.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit r in not more than 5 % of the cases.

The values for riboflavin are:

Milk powder	$\bar{x}$ = 1,45 mg/100 g	r = 0,13 mg/100 g
Pig liver	$\bar{x}$ = 10,5 mg/100 g	r = 0,51 mg/100 g
Tube feeding solution	$\bar{x}$ = 0,21 mg/100 g	
Baby food with vegetables	$\bar{x}$ = 0,30 mg/100 g	r = 0,02 mg/100 g
Powdered milk	$\bar{x}$ = 1,13 mg/100 g	r = 0,08 mg/100 g
Meal with fruits	$\bar{x}$ = 0,60 mg/100 g	r = 0,06 mg/100 g
Yeast	$\bar{x}$ = 4,34 mg/100 g	r = 0,37 mg/100 g
Cereal	$\bar{x}$ = 0,43 mg/100 g	r = 0,06 mg/100 g
Cereal	$\bar{x}$ = 2,48 mg/100 g	r = 0,18 mg/100 g
Chocolate powder	$\bar{x}$ = 1,26 mg/100 g	r = 0,14 mg/100 g
Food supplement	$\bar{x}$ = 87,1 mg/100 g	r = 9,5 mg/100 g

8.3 Reproducibility

The absolute difference between two single test results obtained on identical material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5 % of the cases.

The values for riboflavin are:

Milk powder	$\bar{x}$ = 1,45 mg/100 g	*R* = 0,30 mg/100 g
Pig liver	$\bar{x}$ = 10,5 mg/100 g	*R* = 2,35 mg/100 g
Tube feeding solution	$\bar{x}$ = 0,21 mg/100 g	*R* = 0,02mg/100 g
Baby food with vegetables	$\bar{x}$ = 0,30 mg/100 g	*R* = 0,09 mg/100 g
Powdered milk	$\bar{x}$ = 1,13 mg/100 g	*R* = 0,27 mg/100 g
Meal with fruits	$\bar{x}$ = 0,60 mg/100 g	*R* = 0,09 mg/100 g
Yeast	$\bar{x}$ = 4,34 mg/100 g	*R* = 1,2 mg/100 g
Cereal	$\bar{x}$ = 0,43 mg/100 g	*R* = 0,19 mg/100 g
Cereal	$\bar{x}$ = 2,48 mg/100 g	*R* = 0,53 mg/100 g
Chocolate powder	$\bar{x}$ = 1,26 mg/100 g	*R* = 0,37 mg/100 g
Food supplement	$\bar{x}$ = 87,1 mg/100 g	*R* = 16,6 mg/100 g

9 Test report

The test report should comply with EN ISO/IEC 17025 [17] and shall contain at least the following data:

a) all information necessary for the identification of the sample;

b) a reference to this European Standard or to the method used;

c) the date and time of sampling procedure (if known);

d) the date of receipt;

e) the date of test;

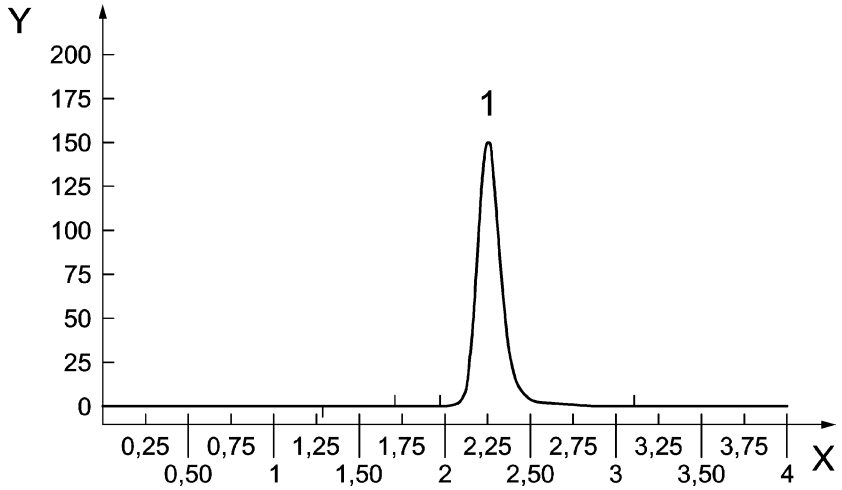
f) the results and the units in which the results have been expressed;

g) any particular points observed in the course of the test;

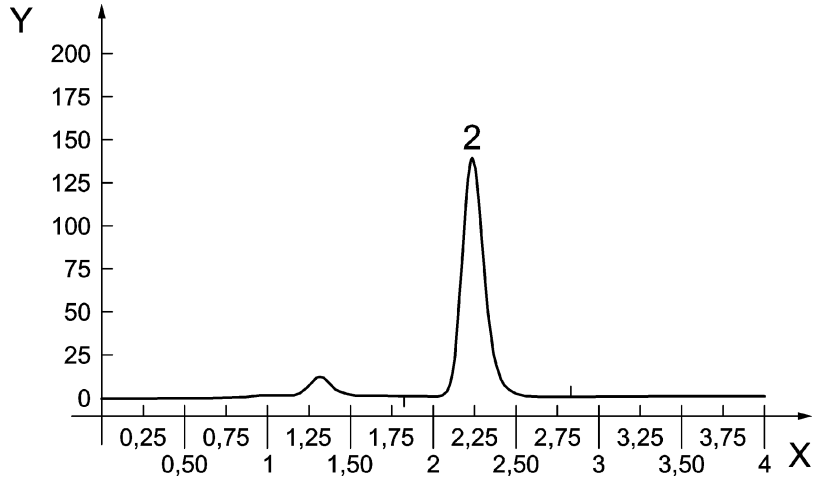
h) any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(informative)

Examples of HPLC chromatograms



a)



b)

Key

Y	fluorescence
X	time (min)
1	vitamin B_2 in riboflavin standard solution (c = 0,5 µg/ml) at 2,3 min
2	vitamin B_2 in infant formula at 2,3 min
Stationary phase:	SymmetryShield RP18, 5 µm, 150 mm x 3,0 mm
Mobile phase:	Methanol (4.1): sodium acetate (0,05 mol/l) (30:70)
Flow rate:	1,0 ml/min
Injection volume:	10 µl
Detection:	fluorometric: Excitation: 468 nm; Emission: 520 nm

Figure A.1 — Example for a HPLC separation of riboflavin standard solution a) and infant formula b)

Annex B
(informative)

Precision data

In accordance with the EU SMT Certification Study Guidelines, the data given in Table B.1 have been defined in an interlaboratory test [9]. The Institute of Food Research, Norwich, UK on behalf of the EU Community Bureau of Reference, conducted the study. The data given in Table B.2 and Table B.3 have been defined in a French interlaboratory test [10].

Table B.1 — Precision data for milk powder and pig liver

Sample	CRM 421 Milk Powder	CRM 487 Pig liver
Analyte	Riboflavin	Riboflavin
Year of interlaboratory study	1996	1996
Number of laboratories	13	11
Number of samples	1	1
Number of laboratories retained after elimination of outliers	12	11
Number of outliers	1	0
Number of accepted results	60	55
Mean value, $\bar{x}$, mg/100 g	1,45	10,5
Repeatability standard deviation s_r, mg/100 g	0,046	0,181
Repeatability coefficient of variation, %	3,2	1,7
Repeatability value, r [r = 2,83 x s_r], mg/100 g	0,130	0,511
Reproducibility standard deviation, $s_{R,}$ mg/100 g	0,106	0,832
Reproducibility coefficient of variation, %	7,3	7,9
Reproducibility value R, [R = 2,83 x s_R], mg/100 g	0,30	2,35
HorRat value, according to [14]	0,6	1,0

NOTE The data obtained in this international comparison study have been produced using established methods being identical with in-house routine assay procedures of the participating laboratories with the HPLC systems described in Annex C.

Table B.2 — Precision data for tube feeding solution, baby food with vegetables, powdered milk, meal with fruits and yeast

Sample	Tube feeding solution	Baby food with vegetables	Powdered Milk	Meal with fruits	Yeast
Year of interlaboratory study	1995	1995	1995	1995	1995
Number of laboratories	8	11	11	11	11
Number of samples	1	1	1	1	1
Number of laboratories retained after eliminating outliers	7	10	10	10	11
Number of outliers	1	1	1	1	0
Number of accepted results	14	20	20	20	22
Mean value, $\bar{x}$, mg/100 g	0,21	0,30	1,13	0,60	4,34
Repeatability standard deviation s_r, mg/100 g	< 0,01	0,01	0,03	0,02	0,13
Repeatability coefficient of variation, %	< 1	3	3	4	3
Repeatability value, *r,* [$r = 2{,}83 \times s_r$], mg/100 g	-	0,02	0,08	0,06	0,37
Reproducibility standard deviation s_R, mg/100 g	0,01	0,03	0,1	0,03	0,43
Reproducibility coefficient of variation, %	4	10	9	6	10
Reproducibility value *R* [$R = 2{,}83 \times s_R$], mg/100 g	0,02	0,09	0,27	0,09	1,2
HorRat value, according to [14]	0,3	0,7	0,8	0,5	1,1

Table B.3 — Precision data for cereal, chocolate powder and food supplement

Samples	Cereal	Cereal	Chocolate powder	Food supplement
Year of interlaboratory study	1995	1995	1995	1995
Number of laboratories	11	11	11	9
Number of samples	1	1	1	1
Number of laboratories retained after eliminating outliers	10	10	11	9
Number of outliers	1	1	0	0
Number of accepted results	20	20	22	18
Mean value, $\bar{x}$, mg/100 g	0,43	2,48	1,26	87,1
Repeatability standard deviation, s_r,, mg/100 g	0,02	0,07	0,05	3,4
Repeatability coefficient of variation, %	5	3	4	4
Repeatability value, *r,* [$r = 2{,}83 \times s_r$], mg/100 g	0,06	0,18	0,14	9,5
Reproducibility standard deviation, s_R, mg/100 g	0,07	0,19	0,13	5,9
Reproducibility coefficient of variation, %	16	8	11	7
Reproducibility value *R,* [$R = 2{,}83 \times s_R$], mg/100 g	0,19	0,53	0,37	16,6
HorRat value, according to [14]	1,2	0,8	1,0	1,2

Annex C
(informative)

Alternatives HPLC systems

The separation and quantification has been proven to be satisfactory if the following chromatographic conditions are being applied [2].

Table C.1 – Alternative HPLC conditions

Stationary Phase[a]	**Column Dimension** mm x mm	**Mobile Phase**	**Flow** ml/min	**Fluorometric Detection** (nm)
Hypersil® ODS, 5 µm	125 × 4,6	Methanol: water (50:50)	1,0	Ex: 462 Em: 520
Supelco® LC-18- DB, 5 µm	250 × 4,6	Methanol: phosphate buffer; (4.16) containing tetraethylammoniumchloride, $\rho(C_8H_{20}NCl)$ = 1 g/l and sodium heptanesulfonate, $c(C_7H_{15}NaO_3S)$ = 5 mmol/l (35:65)	1,0	Ex: 468 Em: 520
Lichrospher® RP 18, 5 µm	25 × 4 + 125 × 4	Methanol: 0,025 % ammonia (+ 1 g hexanesulfonic acid); (250: 500); pH 3,6	1,5l	Ex: 467 Em: 525
Apex® C18, 3 µm	250 × 4	Methanol: water (1: 1)	1,0l	Ex: 450 Em: 510
Bondapak® C18 radial-pak cartridges	100 × 8	Methanol: 5 mmol phosphate buffer pH 7 (35:65)	1,0	Ex: 440 Em: 520
Spherisorb® ODS2, 5 µm	250 × 4,6	Methanol: water (50: 50)	1,0	Ex: 450 Em: 510
µBondapak® C18, 10 µm	100 × 8	Methanol: 0,05 mol/l sodium acetate buffer pH 4,5 (40: 60)	1,0	Ex: 422 Em: 522
Kromasil® C-18, 5 µm	250 × 4,6	Methanol: water (40: 60)	1,0	Ex: 440 Em: 520
Eurospher® C18, 5 µm	250 × 4,6	Methanol: water (50: 50)	1,0	Ex: 445 Em: 530
Spherisorb® ODS, 5 µm	250 × 4,6	Methanol: water (50: 50)	1,0	Ex: 410 Em: 510
Spherisorb® ODS, 5 µm	250 × 4,6	KH_2PO_4: acetonitrile: methanol (60: 10: 30)	0,8	Ex: 450 Em: 520

[a] Trademarks of the listed products are examples of suitable products available commercially. This information is given for the convenience of the users of this European Standard and does not constitute and endorsement by CEN.

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