codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 18 A **CX/FAC 02/31 Add.1**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD ADDITIVES AND CONTAMINANTS
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METHODS OF ANALYSIS FOR THE DETERMINATION OF FOOD ADDITIVES AND CONTAMINANTS IN FOOD

The following comments have been received from Germany

GERMANY

Background
At its 32nd meeting, CCFAC agreed to invite comments for additional methods of analysis for the determination of food additives and contaminants in foods for discussion at its 33rd Session (ALINORM 01/12, para. 139). Proposals for additional methods of analysis for the determination of food additives and contaminants in foods were requested under CL 2001/13-FAC on the basis of criteria established at the 28 th Session of the CCFAC (ALINORM 01/12, para 137). Germany submitted relevant comments. However, this was done so late that they could not be dealt with at the 33rd Session of the CCFAC. Therefore Germany now wishes to come back to the issue with updated comments to be found below.
The attached methods of analysis for the determination of food additives and contaminants have been validated nationally or internationally according to ISO 5725, ISO/IUPAC/AOAC-protocol or equivalent requirements, and were subsequently standardized by the Technical Committee 275 "Food analysis - Horizontal methods" of the European Committee for Standardization (CEN). The list of methods has already been presented at the 23rd session of the CCMAS and, in agreement with the chairman of CCMAS and the CODEX procedures, it has been decided to address these methods at the responsible CODEX-Committee on food additives and contaminants.
It was noted that for some of the methods (e.g. fumonisins), no CODEX-standard exists for the time being. However, the methods are dispatched to CCFAC in order to inform CODEX if it is intended to fix levels for those substances in certain foodstuffs.
The attached extracts of the European standardized methods contain information on their scope, principle, validation data and some further aspects, if appropriate.

Proposal
Germany proposes to endorse the attached methods of analysis for the determination of food additives and contaminants in foods as CODEX-methods.

European Standards elaborated by CEN/TC 275 "Food analysis – Horizontal methods" which are proposed by the German delegation to be endorsed as CODEX-methods by the CCFAC

Additives

Determination of sulfites

EN 1988-1 : 1998-02	Foodstuffs - Determination of sulfite - Part 1: Optimized Monier-Williams method	Type III
EN 1988-2 : 1998-02	Foodstuffs - Determination of sulfite - Part 2: Enzymatic method	Type III

Determination of intense sweeteners

EN 1376 : 1996-09 (confirmed 2001)	Foodstuffs - Determination of saccharin in table top sweetener preparations – Spectrometric method	Type III
EN 1377 : 1996-09 (confirmed 2001)	Foodstuffs - Determination of acesulfame K in table top sweetener preparations – Spectrometric method	Type II
EN 1378 : 1996-09 (confirmed 2001)	Foodstuffs - Determination of aspartame in table top sweetener preparations - Method by high performance liquid chromatography	Type II
EN 1379 : 1996-09 (confirmed 2001)	Foodstuffs - Determination of cyclamate and saccharin in liquid table top sweetener preparations - Method by high performance liquid chromatography	Type II
EN 12856 : 1999-04	Foodstuffs - Determination of acesulfame-K, aspartame and saccharin - High performance liquid chromatographic method	Type II
EN 12857 : 1999-04	Foodstuffs - Determination of cyclamate - High performance liquid chromatographic method	Type II

Determination of vitamins

EN 12821 : 2000-02	Foodstuffs - Determination of vitamin D by high performance liquid chromatography - Measurement of cholecalciferol (D_3) and ergocalciferol (D_2)	Type III
EN 12822 : 2000-02	Foodstuffs – Determination of vitamin E by high performance liquid chromatography - Measurement of a-, ß-, ?-, and d-tocopherols	Type III
EN 12823-1 : 2000-02	Foodstuffs – Determination of vitamin A by high performance liquid chromatography - Part 1: Measurement of all-trans-retinol and 13-cis-retinol	Type III
EN 12823-2 : 2000-02	Foodstuffs – Determination of vitamin A by high performance liquid chromatography - Part 2: Measurement of - ß-carotene	Type III

Contaminants

Determination of PCBs

EN 1528-1: 1996-10 (confirmed 2001)	Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 1: General considerations	Type III
EN 1528-2: 1996-10 (confirmed 2001)	Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 2: Extraction of fat, pesticides and PCBs and determination of fat content	Type III
EN 1528-3: 1996-10 (confirmed 2001)	Fatty food – Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 3: Clean-up methods	Type III
EN 1528-4: 1996-10 (confirmed 2001)	Fatty food – Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 4: Determination, confirmatory	Type III

	tests, Miscellaneous	

Determination of nitrates/nitrites (partly also to be assessed as additives)

EN 12014-1:1997-04	Foodstuffs - Determination of nitrate and/or nitrite content - Part 1: General considerations	Type III
EN 12014-2:1997-04	Foodstuffs - Determination of nitrate and/or nitrite content - Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products	Type II
ENV 12014-3:1998-06	Foodstuffs - Determination of nitrate and/or nitrite content - Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite	Type III
ENV 12014-4:1998-06	Foodstuffs - Determination of nitrate and/or nitrite content - Part 4: Ion-exchange chromatographic (IC) method for the determination of nitrate and nitrite content of meat products	Type III
EN 12014-5:1997-04	Foodstuffs - Determination of nitrate and/or nitrite content - Part 5: Enzymatic determination of nitrate content of vegetable-containing food for babies and infants	Type II
EN 12014-7:1998-06	Foodstuffs - Determination of nitrate and/or nitrite content - Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after cadmium reduction	Type III

Determination of mycotoxins

EN 12955 : 1999-07	Foodstuffs - Determination of aflatoxin B_1, and the sum of aflatoxins B_1, B_2, G_1 and G_2 in cereals, shell-fruits and derived products - High performance liquid chromatographic method with post column derivatization and immunoaffinity column clean up	Type III
EN 13595 : 2001 - 11	Foodstuffs – Determination of fumonisins B_1 and B_2 in maize – HPLC method with solid phase extraction clean-up	Type I
EN ISO 15141-1:1998-10	Foodstuffs - Determination of ochratoxin A in cereals and cereal products - Part 1: High performance liquid chromatographic method with silica gel clean up (ISO 15141-1:1998)	Type II
EN ISO 15141-2:1998-10	Foodstuffs - Determination of ochratoxin A in cereals and cereal products - Part 2: High performance liquid chromatographic method with bicarbonate clean up (ISO 15141-2:1998)	Type III

Additives

Sulfites

EN 1988 Determination of Sulfites

Introduction

Sulfite can be used as a preservative of foodstuffs. In order to minimize possible negative health effects, many countries have regulated the use of sulfite in foods. This has resulted in the development of several methods of analysis to detect the presence of and quantify sulfite in a great variety of foods.

This standard is divided in the following parts:

Part 1: Optimized Monier-Williams Method

Part 2: Enzymatic method

Part 1: Optimized Monier-Williams Method

1 Scope

This European Standard specifies a distillation method for the determination of the sulfite content, expressed as sulfur dioxide, in foodstuffs, in which the content of sulfite is at least 10 mg/kg. The method is applicable in the presence of other volatile sulfur compounds. It is not applicable to cabbage, dried garlic, dried onions, ginger, leeks and soy proteins. It has been shown that the analysis of isolated soy protein leads to false positive results in the range of 20 mg/kg to 30 mg/kg expressed as sulfur dioxide. Therefore, when analysing foodstuffs containing isolated soy proteins a proportional enhancement of the result may be obtained and is taken into account.
Specific products, for which European Standards exist, are excluded from the scope of this horizontal European Standard.

2 Principle

Free sulfite plus reproducible portion of bound sulfites (such as carbonyl addition products) in foods are measured. The test portion is heated with refluxing solution of hydrochloric acid to convert sulfite to sulfur dioxide. A stream of nitrogen is introduced below the surface of refluxing solution to sweep sulfur dioxide through water-cooled condenser and, via bubbler attached to condenser, into hydrogen peroxide solution, where sulfur dioxide is oxidized to sulfuric acid. The generated sulfuric acid is titrated with standardized sodium hydroxide solution. The sulfite content is directly related to the generated sulfuric acid. See [1], [2].

3 Precision data

In accordance with ISO 5725 : 1986 (see [3]), the following parameters have been identified in an inter-laboratory test (see [2]). The test was conducted of the Food and Drug Administration (FDA).

Sample	Hominy	Fruit juice	Sea food
Year of inter-laboratory test	1986	1986	1986
Number of laboratories	21	21	21
Number of samples	9	9	9
Number of laboratories retained after eliminating outliers	18	21	20
Number of outliers	3	0	1
Number of accepted results	39	42	41
Mean value (x)	9,17 mg/kg	8,05 mg/l	10,41 mg/kg
Repeatability standard deviation (s_r)	1,33 mg/kg	1,36 mg/l	1,47 mg/kg
Repeatability relative standard deviation (RSD_r)	14,49 %	16,90 %	14,13 %
Repeatability limit (r)	3,72 mg/kg	3,81 mg/l	4,12 mg/kg
Reproducibility standard deviation (s_R)	1,42 mg/kg	1,62 mg/l	2,77 mg/kg
Reproducibility relative standard deviation (RSD_R)	15,50 %	20,14 %	26,62 %
Reproducibility limit (R)	3,98 mg/kg	4,54 mg/l	7,76 mg/kg

[1] Association of Official Analytical Chemists (AOAC International): Official Methods of Analysis (1995) 16th Edition, method 990.28, *47.3.43.*
[2] Hillary et al: J. Assoc. Off. Anal. Chem. (Vol. 72, NO. 3, 1989), p 470.
[3] ISO 5725:1986, Precision of test methods – Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

Part 2: Enzymatic method

1 Scope

This European Standard specifies an enzymatic method for the determination of the sulfite content, expressed as sulfur dioxide, in foodstuffs. Other sulfur-containing substances such as sulfate, sulfide or thiosulfate do not interfere with the determination. Carbonyl-sulfite complexes react as free sulfites. Isothiocyanates occurring in, e.g. mustard interfere with the determination. The method is not applicable to cabbages, dried garlic, dried onions, ginger, leeks and soy protein. It has been shown that the analysis of isolated soy protein leads to false positive results in the range of 20 mg/kg to 30 mg/kg expressed as sulfur dioxide. Therefore, when analysing foodstuffs containing isolated soy proteins a proportional enhancement of the result may be obtained which is taken into account
Specific products, for which European Standards exist, are excluded from the scope of this horizontal European Standard.

2 Principle

Oxidation of sulfite to sulfate in the presence of sulfite oxidase with the liberation of hydrogen peroxide at the same time.

$$SO_3^{2-} + O_2 + H_2O \xrightarrow{\text{sulfite oxidase}} SO_4^{2-} + H_2O_2$$

Reduction of hydrogen peroxide and conversion of NADH to NAD^+ in the presence of NADH peroxidase.

$$H_2O_2 + NADH + H^+ \xrightarrow{\text{NADH peroxidase}} 2\,H_2O + NAD^+$$

Conversion of NADH to NAD^+ is determined spectrometrically and is proportional to the concentration of sulfite see [1] to [6].

3 Precision data

The precision of the method has been established by inter-laboratory tests carried out in accordance with ISO 5725 : 1986 [7] especially on samples with a low sulfite concentration [1], [4] and [5].
The study samples consisted of potato flakes, wine, juice, dried apples, sultanas and beer having a sulfite content between 0 mg/kg and 960 mg/kg. Eleven laboratories participated in the full study analysing twelve samples. Six laboratories analysed eight samples in a complementary study.

The results of the collaborative studies show that the method is well suited for the quantitative analysis of sulfite at levels well below 100 mg SO_2/kg. For very low concentrations of sulfite the type of food is of great importance. In the analysis of solid samples or when the sulfite has adhered to particles (e.g. in juice), a high coefficient of variation may be expected, especially if the analyst has little experience with enzymatic methods. Concentrations in wine of between 1 mg/l and 10 mg/l can be determined with good reliability.
The limit of detection of the method, expressed as absorbance, is 0,04. For a sample of 1 ml the limit of detection, calculated as the mean value of a representative number of blanks (n > 20) plus three times the coefficient of variation of the mean value (according to recommendations of the European Community) is 1,2 mg SO_2/kg.
The parameters given in Table 1 (matched pairs/blind duplicates) and Table 2 have been identified in inter-laboratory tests in accordance with ISO 5725 : 1986. The tests were conducted by the Swedish National Food Administration on potato flakes, wine, dried apples and juice [8].

Table 1 : Inter-laboratory data on wine, dried apples and lemon juice

Sample	Wine	Dried apples	Dried apples	Lemon juice
Year of inter-laboratory test	1991	1990	1990	1990
Number of laboratories	6	11	11	11
Number of laboratories retained after eliminating outliers	6	10	10	10
Number of outliers (laboratories)	0	1	1	1
Number of samples	1	1	1	2
Number of accepted results	6	7	7	10
Mean value (x) mg/kg	75	800	960	270
Repeatability standard deviation (s_r) mg/kg or mg/l	3	106	128	13
Repeatability relative standard deviation (RSD_r) %	4	13	13	5
Repeatability limit (r) mg/kg	8	298	358	37
Reproducibility standard deviation (s_R) mg/kg	6	111	133	28
Reproducibility relative standard deviation (RSD_R) %	8	14	14	10
Reproducibility limit (R) mg/kg	16	311	374	79
The unit for wine and lemon juice is mg/l, the unit for dried apples is mg/kg				

Table 2 : Inter-laboratory data on potato flakes

Sample	Potato flakes	Potato flakes
Year of inter-laboratory test	1990	1990
Number of laboratories	11	11
Number of laboratories retained after eliminating outliers	10	10
Number of outliers (laboratories)	1	1
Number of samples	1	1
Number of accepted results	6	7
Mean value (x) mg/kg	28,3	110
Reproducibility standard deviation (s_R) mg/kg	13	15
Reproducibility relative standard deviation (RSD_R) %	45	13
Reproducibility limit (R) mg/kg	36	42

In accordance with ISO 5725 : 1986 [7], the parameters in table 3 have been identified in inter-laboratory tests. The tests were conducted by the Max von Pettenkofer Institute of the Federal Health Office, Food Chemistry Department, Berlin, BRD, on sultanas and beer [4], [5].

Table 3: Inter-laboratory data on sultanas and beer

Sample	Sultanas	Beer
Year of inter-laboratory test	1986	1986
Number of laboratories	14	16
Number of laboratories retained after eliminating outliers	13	14
Number of outliers (laboratories)	1	2

Number of samples	1	1
Number of accepted results	74	70
Mean value (x)	260 mg/kg	4,9 mg/l
Repeatability standard deviation (s_r)	16 mg/kg	0,3 mg/l
Repeatability relative standard deviation (RSD_r)	6 %	5,8 %
Repeatability limit (r)	45 mg/kg	0,8 mg/l
Reproducibility standard deviation (s_R)	46 mg/kg	0,6 mg/l
Reproducibility relative standard deviation (RSD_R)	18 %	11,6 %
Reproducibility limit (R)	129 mg/kg	1,6 mg/l

[1] Nordic Committee on Food Analysis, No 135 (1990).
[2] Methods for the enzymatic food analysis, published by Boehringer, Mannheim.
[3] Beutler, H O: Food Chemistry 15, 157 - 164 (1984).
[4] Official collection of methods of food analysis according to § 35 LMBG (German food and commodities regulations), Beuth, Berlin, 1993, Method L 30.00-1.
[5] Official collection of methods of food analysis according to § 35 LMBG (German food and commodities regulations), Beuth, Berlin 1993, Method L 36.00-8.
[6] Journal of the Institute of Brewing, European Brewery Convention, Vol. 98, 1992, Method 9.12.3.
[7] ISO 5725:1986, Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.
[8] Edberg, U.: Journal of AOAC International, 76 (1993) 53-58.

Intense sweeteners

EN 1376 Foodstuffs - Determination of saccharin in table top sweetener preparations - Spectrometric method

1 Scope

This European Standard specifies a spectrometric method for the determination of sodium saccharin and saccharin content in solid table top sweetener preparations prepared from cyclamate/saccharin or saccharin. An inter-laboratory test has been carried out on sweetener tablets [1].

2 Principle

Preparation of the sample test solution by dissolving table top sweetener preparation in sodium hydroxide solution. Photometric determination of the sodium saccharin content at the absorption maximum of about 265 nm.

3 Precision data

The following parameters have been defined in an inter-laboratory test in accordance with ISO 5725 : 1986 [2]. The test was conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany [1].

Sample	**saccharin-cyclamate tablets**
Year of inter-laboratory test	1986
Number of laboratories	8
Number of samples	1
Number of laboratories retained after eliminating outliers	7
Number of outliers (laboratories)	1
Number of accepted results	41
Mean value $\bar{x}$	5,80 mg/100 mg
Repeatability standard deviation s_r	0,15 mg/100 mg
Repeatability relative standard deviation RSD_r	2,59 %
Repeatability limit *r*	0,42 mg/100 mg
Reproducibility standard deviation s_R	0,30 mg/100 mg
Reproducibility relative standard deviation RSD_R	5,23 %
Reproducibility limit *R*	0,85 mg/100 mg

[1] Food Analysis: Determination of sodium saccharin and saccharin content in sweetener tablets L 57.22.99-2, 1988-05. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office, Loose leaf edition, as of 1994-05 Vol. I., Berlin, Köln: Beuth Verlag GmbH.

[2] ISO 5725 : 1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

EN 1377 Foodstuffs – Determination of acesulfame K in table top sweetener preparations – Spectrometric method

1 Scope

This European Standard specifies a spectrometric method for the determination of acesulfame K in solid table top sweetener preparations containing it.
An inter-laboratory test has been carried out on sweetener tablets [1].

2 Principle

Preparation of the sample test solution by dissolving table top sweetener preparation in water. Photometric determination of the acesulfame K content at the absorption maximum of about 227 nm.

3 Precision data

The following parameters have been defined in an inter-laboratory test in accordance with ISO 5725 : 1986 [2]. The test was conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany [1].

Sample	commercially available acsulfame tablets
Year of inter-laboratory test	1986
Number of laboratories	8
Number of samples	1
Number of laboratories retained after eliminating outliers	7
Number of outliers (laboratories)	1
Number of accepted results	38
Mean value $\bar{x}$	98,0 mg/100 mg
Repeatability standard deviation s_r	1,1 mg/100 mg
Repeatability relative standard deviation RSD_r	1,2 %
Repeatability limit *r*	3,2 mg/100 mg
Reproducibility standard deviation s_R	1,3 mg/100 mg
Reproducibility relative standard deviation RSD_R	1,3 %
Reproducibility limit *R*	3,7 mg/100 mg

[1] Food Analysis: Determination of acesulfam-K content in sweetener tablets containing it, L 57.22.99-3, 1989-05. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office). 1 (Loose leaf edition, as of 1994-05 Vol. I.) Berlin, Köln: Beuth Verlag GmbH.

[2] ISO 5725 : 1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

EN 1378 Foodstuffs – Determination of aspartame in table top sweetener preparations - Method by high performance liquid chromatography

1 Scope

This European Standard specifies a high performance liquid chromatography (HPLC) method for the determination of aspartame in table top sweetener preparations.
An inter-laboratory test has been carried out on sweetener tablets [1].

2 Principle

Determination of aspartame in an appropriate solution of table top sweetener preparation in water by HPLC and subsequent photometric detection in the ultraviolet (UV) range. Identification of the aspartame on the basis of the retention time and determination by the external standard method using peak areas or peak heights.

3 Precision data

The following parameters have been defined in an inter-laboratory test in accordance with ISO 5725 : 1986 [2]. The test was conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany [1].

Sample	aspartam tablets
Year of inter-laboratory test	1988
Number of laboratories	8
Number of samples	1
Number of laboratories retained after eliminating outliers	7
Number of outliers (laboratories)	1
Number of accepted results	35
Mean value $\bar{x}$	19,04 mg/100 mg

Repeatability standard deviation s_r Repeatability relative standard deviation RSD_r	0,23 mg/100 mg 1,24 %
Repeatability limit *r*	0,66 mg/100 mg
Reproducibility standard deviation s_R Reproducibility relative standard deviation RSD_R	0,45 mg/100 mg 2,40 %
Reproducibility limit *R*	1,28 mg/100 mg

[1] Food Analysis: Determination of aspartame content of sweetener tablets L 57.22.99-4, 1989-12. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office) Loose leaf edition, as of 1994-05 Vol. I, Berlin, Köln: Beuth Verlag GmbH.

[2] ISO 5725 : 1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

EN 1379 Foodstuffs - Determination of cyclamate and saccharin in liquid table top sweetener preparations - Method by high performance liquid chromatography

1 Scope

This European Standard specifies a high performance liquid chromatography (HPLC) method for the determination of sodium cyclamate and saccharin in liquid table top sweetener preparations. It also allows the determination of sorbic acid in liquid table top sweetener preparations.
An inter-laboratory test has been carried out with liquid table top sweetener preparation [1].

2 Principle

Determination of sodium cyclamate, saccharin and sorbic acid in an appropriate dilution of a liquid table top sweetener preparation in water by HPLC and subsequent photometric detection in the ultraviolet (UV) range. Identification on the basis of the retention times, and quantitative determination by the external standard method using peak areas or peak heights.

3 Precision data

The following parameters have been defined in an inter-laboratory test in accordance with ISO 5725 : 1986 [2]. The test was conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany [1].

Sample	**liquid table top sweetener preparations**		
	sodium cyclamate	**saccharin**	**sorbic acid**
Year of inter-laboratory test	1989	1989	1989
Number of laboratories	8	8	8
Number of samples	1	1	1
Number of laboratories retained after eliminating outliers	6	7	7
Number of outliers (laboratories)	2	1	1
Number of accepted results	30	35	35
Mean value $\bar{x}$	12,02 g/100 ml	1,06 g/100 ml	30 mg/100 ml
Repeatability standard deviation s_r	0,24 g/100 ml	0,01 g/100 ml	0,6 mg/100 ml
Repeatability relative standard deviation RSD_r	2,05 %	1,01 %	2,38 %
Repeatability limit *r*	0,69 g/100 ml	0,33 g/100 ml	2 mg/100 ml
Reproducibility standard deviation s_R	0,35 g/100 ml	0,03 g/100 ml	3 mg/100 ml
Reproducibility relative standard deviation RSD_R	2,97 %	2,70 %	9,52 %
Reproducibility limit *R*	1,0 g/100 ml	0,08 g/100 ml	8 mg/100 ml

[1] Food Analysis: Determination of the contents of sodium cyclamate, saccharin and sorbic acid in liquid table top sweeteners L 57.22.99-5, 1990-12. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office, Loose leaf edition, as of 1994-05 Vol. I. Berlin, Köln: Beuth Verlag GmbH.

[2] ISO 5725 : 1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

EN 12856 Determination of acesulfame K, aspartame and saccharin - High performance liquid chromatographic method

1 Scope

This European Standard specifies an high performance liquid chromatographic (HPLC) method for the determination of acesulfame-K, aspartame and saccharin, see [1], [2] and [3]. It also allows the determination of caffeine, sorbic acid and benzoic acid in foodstuffs.

Inter-laboratory tests have been carried out with acesulfame-K in marzipan, yogurt, fruit yogurt, orange juice beverage, cola, cream and jam, with aspartame in marzipan, fruit yogurt, orange juice beverage, orange flavoured beverage, cola, jam, and preparation for flan, and with sodium saccharin in marzipan, yogurt, fruit yogurt, orange juice, orange juice beverage, cola, cream and jam.

2 Principle

The sample is extracted or diluted with water. If necessary, the sample solution with the intense sweeteners is purified on a solid phase extraction column or with Carrez reagents. The intense sweeteners in the sample test solution are separated on an HPLC-reversed phase chromatography column and determined spectrometrically at a wavelength of 220 nm.

3 Precision data

The following data were obtained in inter-laboratory tests according to ISO 5725:1986 [4] conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany* on marzipan, fruit yoghurt, cola and orange juice beverage, see [1]. Further inter-laboratory tests were conducted by the French Institute for Beverages, Brewing and Malting (IFBM)** on cola, orange flavoured beverage, jam, and preparation for flan, see [3].

Further inter-laboratory tests were conducted by the Ministry of Agriculture, Fisheries and Food, MAFF***, Norwich Research Park, UK on orange juice beverage, cola, cream, yoghurt and orange juice, see [2].

Table 1

acesulfame-K	marzipan * mg/kg	fruit yogurt* mg/kg	orange juice* beverage mg/l	jam** mg/kg
Year of inter-laboratory test	1992	1992	1992	1993
Number of laboratories	8	8	13	9
Number of samples	1	1	1	1
Number of laboratories retained after eliminating outliers	7	7	10	9
Number of outliers (laboratories)	1	1	3	0
Number of accepted results	38	38	53	9
Mean value	256,6	230,8	172,0	60
Repeatability standard deviation s_r	18,7	7,7	2,1	2,9
Repeatability relative standard deviation RSD_r %	7,3	3,4	1,2	4,8
Repeatability limit r	52,0	21,8	5,8	8
Reproducibility standard deviation s_R	28,1	22,9	5,0	10,7
Reproducibility relative standard deviation RSD_R %	11,1	10,0	3,0	17,8
Reproducibility limit R	79,6	64,7	14,3	30
Horrat value	1,6	1,5	0,5	2,2

Table 2

acesulfame-K	orange juice beverage *** mg/l	cola*** mg/l	cream *** mg/kg	yogurt*** mg/kg	orange juice*** mg/kg
Year of inter-laboratory test	1995	1995	1995	1995	1995
Number of laboratories	12	12	11	11	11
Number of samples	2	2	2	2	2
Number of laboratories retained after eliminating outliers	11	11	8	10	7
Number of outliers (laboratories)	1	1	3	1	4
Number of accepted results	22	22	16	20	14
Mean value	370	351	316	264	24,3
Repeatability standard deviation s_r	10,9	7,3	5,4	12,4	1,9
Repeatability relative standard deviation RSD_r %	3	2	2	5	8
Repeatability limit r	30	20	15	35	6
Reproducibility standard deviation s_R	23,5	19,7	49,3	47,6	12,2
Reproducibility relative standard deviation RSD_r %	6	6	16	18	50
Reproducibility limit R	66	55	138	133	34
Horrat value	1,0	0,8	2,3	2,6	5,1

Table 3

aspartame	marzipan* mg/kg	fruit yogurt* mg/kg	orange juice beverage* mg/l	cola* mg/l
Year of inter-laboratory test	1992	1992	1992	1991
Number of laboratories	8	8	13	8
Number of samples	1	1	1	1
Number of laboratories retained after eliminating outliers	7	8	13	8
Number of outliers (laboratories)	1	0	0	0
Number of accepted results	35	43	68	43
Mean value	845,2	468,0	308,0	270,7
Repeatability standard deviation s_r	14,6	10,6	5,0	3,8
Repeatability relative standard deviation RSD_r %	1,7	2,3	1,6	1,4
Repeatability limit r	41,2	29,9	14,2	10,7
Reproducibility standard deviation s_R	58,6	38,4	36,8	14,7
Reproducibility relative standard deviation RSD_R %	7,0	8,3	12,1	5,5
Reproducibility limit R	165,7	108,6	104,2	41,5
Horrat value	1,2	1,4	1,5	0,8

Table 4

aspartame	cola** mg/l	orange flavoured beverage** mg/l	jam ** mg/kg	preparation for flan** mg/kg
Year of inter-laboratory test	1993	1993	1993	1993
Number of laboratories	9	9	5	9
Number of samples	1	1	1	1
Number of laboratories retained after eliminating outliers	9	9	4	8
Number of outliers (laboratories)	0	0	1	1
Number of accepted results	9	9	4	8
Mean value	185	301	26	3100
Repeatability standard deviation s_r	3,9	8,9	4,6	214
Repeatability relative standard deviation RSD_r %	2,1	3,0	17,7	6,9
Repeatability limit r	11	25	13	600
Reproducibility standard deviation s_R	13,6	31,4	7,1	821
Reproducibility relative standard deviation RSD_R %	7,3	10,4	27,5	26,5
Reproducibility limit R	38	88	20	2300
Horrat value	1,0	1,4	2,8	5,5

Table 5

sodium saccharin	marzipan* mg/kg	fruit yogurt* mg/kg	orange juice beverage* mg/l	cola** mg/l	jam** mg/kg
Year of inter-laboratory test	1992	1992	1992	1993	1993
Number of laboratories	8	8	13	9	9
Number of samples	1	1	1	1	1
Number of laboratories retained after eliminating outliers	6	8	12	8	8
Number of outliers (laboratories)	2	0	1	1	1
Number of accepted results	30	46	63	8	8
Mean value	228,0	116,0	50,8	75	60
Repeatability standard deviation s_r	10,0	2,7	1,2	1,4	1,8
Repeatability relative standard deviation RSD_r %	4,4	2,4	2,4	1,9	3,0
Repeatability limit r	28,2	7,7	3,4	4	5
Reproducibility standard deviation s_R	13,5	16,1	8,1	12,1	16,8
Reproducibility relative standard deviation RSD_R %	5,9	14,0	16,2	16,2	28,0
Reproducibility limit R	37,9	45,5	23,0	34	47
Horrat value	4,1	1,8	2,0	1,7	2,8

Table 6

sodium saccharin	orange juice beverage*** mg/l	cola*** mg/l	cream*** mg/kg	yogurt*** mg/kg	orange juice*** mg/kg
Year of inter-laboratory test	1995	1995	1995	1995	1995
Number of laboratories	12	12	11	11	11
Number of samples	2	2	2	2	2
Number of laboratories retained after eliminating outliers	10	11	10	10	8
Number of outliers (laboratories)	2	1	1	1	3
Number of accepted results	20	22	20	20	16
Mean value	82	64,9	68,4	71,4	16,1
Repeatability standard deviation s_r	2,0	2,0	5,5	8,9	2,3
Repeatability relative standard deviation RSD_r %	2	3	8	12	14
Repeatability limit r	6	5	15	25	6
Reproducibility standard deviation s_R	6,7	10,6	11,3	15,8	6,9
Reproducibility relative standard deviation RSD_R %	8	16	17	22	43
Reproducibility limit R	19	30	32	44	19
Horrat value	1,0	1,9	1,9	2,6	4,1

[1] Food Analysis: Determination of acesulfame-K, aspartame and sodium saccharin content in foodstuffs L 00.00-28, 1994-05. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office. Loose leaf edition, as of 1994-05 Vol. I., Berlin, Köln: Beuth Verlag GmbH.

[2] Willetts, P., Hawkins, S., Brereton, P., and Wood, R.: Determination of intense sweeteners in foodstuffs. Collaborative trial, J Assoc Publ Analysts, 1996, 32,53 - 97.

[3] Determination of Aspartam, Acesulfam-K and Saccharin in Foodstuff, Collaborative study, May 1993, IFBM, France.

[4] ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

EN 12857 Foodstuffs - Determination of cyclamate - High performance liquid chromatographic method

1 Scope

This draft European Standard specifies a high performance liquid chromatographic (HPLC) method for the determination of sodium cyclamate in foodstuffs [1], [2], [3]. The method has been validated in an inter-laboratory test according to ISO 5725 : 1986 [4] which has been carried out with lemonade, orange juice beverage, fruit yoghurt and spray cream.

In addition to the matrices investigated in the inter-laboratory test, experiences have shown that the method is also applicable to various foodstuffs, such as gherkins, canned morello cherries, pineapple, orange nectar, apricot jam, blackberry jam, cherry nectar, hard candy, mixed fruit yoghurt, strawberry yoghurt, fruit quark, rice-pudding-apple-raisins, chocolate custard powder, cream dessert vanilla powder, vanilla ice, passion fruit-orange ice cream, [2], [3].

2 Principle

Sodium cyclamate is extracted from the sample with water, converted to N, N-dichlorocyclohexylamine and determined by HPLC on a reversed-phase column using UV detection at a wavelength of 314 nm.

3 Precision data

The following data were obtained in inter-laboratory tests according to ISO 5725:1986 [4] conducted by the Netherlands on lemonade and the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany [1] on orange juice beverage, spray cream, and fruit yogurt.

Table 1

cyclamate	lemonade	orange juice beverage	spray cream	fruit yogurt
Year of inter-laboratory test	1992	1994	1994	1994
Number of laboratories	4	10	10	10
Number of samples	1	1	1	1
Number of laboratories retained after eliminating outliers	4	9	9	9
Number of outliers	0	1	1	1
Number of accepted results	8	48	48	48
Mean value	435,9 mg/l	178,3 mg/l	280,9 mg/kg	647,6 mg/kg
Repeatability standard deviation s_r	6,0 mg/l	5,5 mg/l	9,2 mg/kg	15,2 mg/kg
Repeatability relative standard deviation RSD_r %	1,4	3,1	3,3	2,3
Repeatability limit *r*	16,7 mg/l	15,4 mg/l	26,0 mg/kg	42,4 mg/kg
Reproducibility standard deviation s_R	13,6 mg/l	8,6 mg/l	17,6 mg/kg	59,5 mg /kg
Reproducibility relative standard deviation RSD_R %	3,1	4,9	6,3	9,3
Reproducibility limit *R*	38,1 mg/l	24,4 mg/l	49,7 mg/kg	168,4 mg/kg
Horrat value	0,5	0,7	0,9	1,5

[1] Food Analysis: Determination of Cyclamate in foodstuffs L 00.00-29 1996-02. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office, Loose leaf edition, as of 1996 - 02 Vol. I. Berlin, Köln: Beuth Verlag GmbH.

[2] Lehr, M. and Schmid, W.: Simple and specific HPLC procedure for determination of cyclamate in fruit juice beverages after conversion to N,N-dichlorocyclohexylamine. (Einfaches und spezifisches HPLC-Verfahren zur Bestimmung von Cyclamat in fruchtsafthaltigen Getränken nach Derivatisierung zu N,N-Dichlorcyclohexylamin) In: Z Lebensm Unters Forsch (1991), 192, pp. 335 – 338.

[3] Lehr, M. and Schmid, W.: Application of solid phase extraction for the determination of intense sweeteners in foodstuffs by HPLC. (Anwendung der Festphasenextraktion bei der Bestimmung von Süßstoffen in Lebensmitteln mittels HPLC) In: Dtsch. Lebensm. Rdsch. (1993), 89, Heft 2; pp 43 - 45.

[4] ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

Vitamins

EN 12821 Foodstuffs - Determination of vitamin D by high performance liquid chromatography - Measurement of cholecalciferol (D_3) and ergocalciferol (D_2)

1 Scope

This European Standard specifies a method for the determination of vitamin D in foodstuffs by high performance liquid chromatography (HPLC).

In the majority of foodstuffs vitamin D is naturally present as cholecalciferol, vitamin D_3, and this is the form of the vitamin determined. Vitamin D_2, ergocalciferol, is sometimes present in fortified foodstuffs and can also be determined using this European Standard. Some foods will contain both vitamin D_3 and D_2. This method is not applicable to these samples.

2 Principle

Vitamin D_3 and D_2 are saponified in the foodstuffs using alcoholic potassium hydroxide solution and extracted by a solvent. The determination of vitamin D_3 or D_2 in an appropriate sample extract solution is carried out by semi-preparative normal phase HPLC followed by reversed-phase analytical HPLC. If vitamin D_3 is to be determined, then vitamin D_2 is used as an internal standard. If vitamin D_2 is to be determined, then vitamin D_3 is used as an internal standard.

Vitamin D is detected by ultraviolet (UV) spectrometry and peaks are identified on the basis of retention times and additionally by UV spectral profile if diode-array detection is used. The determination is carried out by the internal standard procedure using peak areas or peak heights, see [1] to [9].

3 Precision data

In accordance with the EU MAT Certification Study Guidelines, the following parameters have been defined in an inter-laboratory test [9]. The study was organized by the Laboratory of the Government Chemist, UK.

Sample	**Margarine (CRM 122)**	**Milk powder (CRM 421)**
Year of inter-laboratory test	1994	1994
Number of laboratories	11	11
Number of samples	5	5
Number of laboratories retained after eliminating outliers	11	11
Number of outliers (laboratories)	0	0
Number of accepted results	48	47
Mean value $\bar{x}$, µg/100 g	12,30	14,30
Repeatability standard deviation s_r, µg/100 g	0,82	0,74
Repeatability relative standard deviation RSD_r	6,7 %	5,2 %
Repeatability limit r (2,83 x s_r), µg/100 g	2,32	2,09
Reproducibility standard deviation s_R, µg/100g	0,94	0,78
Reproducibility relative standard deviation RSD_R	7,6 %	5,5 %
Reproducibility limit R (2,83 x s_R), µg/100 g	2,66	2,21

In accordance with ISO 5725 : 1986 [10], the following validation data have been defined in an inter-laboratory test on a method using a calculation based on external standard. The test was conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany [5].

Sample	Porridge	Milk powder
Year of the inter-laboratory test	1989	1989
Number of laboratories	13	13
Number of samples	5	5
Number of laboratories retained after elimination of outliers	12	10
Number of outliers (laboratories)	1	3
Number of accepted results	68	56
Mean value $\bar{x}$, µg/100 g	9,81	9,95
Repeatability standard deviation s_r, µg/100 g	0,876	0,82

Repeatability relative standard deviation RSD_r	8,9 %	8,2 %
Repeatability value r ($2{,}83 \times s_r$) µg/100 g	2,48	2,32
Reproducibility standard deviation s_R, µg/100 g	1,27	1,35
Reproducibility relative standard deviation RSD_R	13,0 %	13,6 %
Reproducibility value R ($2{,}83 \times s_R$), µg/100 g	3,61	3,83

[1] Johnsson, H., Hessel, H., and Thorzell, K. Internat. J. Vitamin & Nutr. Res., 1987, **57**, 357-365.

[2] Johnsson, H., Halen, B., Hessel, H., Nyman, A., and Thorzell, K., Internat. J. Vitamin & Nutr. Res.. 1989, **59**, 262-268.

[3] Reynolds, S.L., and Judd, H.J. The Analyst, 1984, **109**, 489-492.

[4] Bognar, A., Z. Lebensm. Unters. Forsch., 1992, **194**, 469-475.

[5] Food Analysis: Determination of vitamin D in dietetic foodstuffs L 49.00-1, June 1991. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office. Loose leaf edition, 1994-05 Vol. I. Berlin, Köln: Beuth Verlag GmbH.

[6] Van Den Berg, J. Agric. Fd. Chem., 1986, **34**, 264-268.

[7] Mattila,P., Piironen, V., Bäckman, C., Asunmaa, A., Uusi-Rauva, E. and Koivistoinen, P., J. Food Comp. Anal., 1992, **5**, 281-290.

[8] Lumley, I.D. and Lawrance, P.R. J. Micronutrient Anal., 1990, **7**, 301-313.

[9] Finglas, P.M., van den Berg, H. and de Froidmont-Görtz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg.

[10] ISO 5725 : 1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

EN 12822 Foodstuffs - Determination of vitamin E by high performance liquid chromatography - Measurement of *a* -, *b*-, *g*- and *d*-tocopherols

Introduction

As this European Standard method deals with the measurement of the mass fraction of α-, β-, γ- and δ-tocopherol in foodstuffs, reference is made to the literature for the calculation and expression of the vitamin E content in terms of biological activities [1], [2], [3].

1 Scope

This European Standard specifies a method for the determination of Vitamin E in foodstuffs by high performance liquid chromatography (HPLC). The determination of Vitamin E content is carried out by measurement of α-, β-, γ- and δ-tocopherol.

The vitamin E activity can be calculated from the tocopherol content assuming appropriate factors as given in the introduction.

2 Principle

Determination of α-, β-, γ- and δ-tocopherol in an appropriate sample solution by high performance liquid chromatographic (HPLC) separation and subsequent photometric (UV-range) or preferably fluorometric detection. In most cases a saponification of the test material followed by an appropriate extraction is necessary. Identification is carried out on the basis of the retention times, and quantitative determination by the external standard method using peak areas or peak heights. Internal standard methods can also be used if the corresponding recovery tests have proven the same behaviour of the internal standard during the analysis as the analyte itself [4] to [13].

3 Precision Data

The following parameters of different methods for the determination of Vitamin E (α-tocopherol) have been defined in an international comparison study organised by the European Commissions Standard, Measurement and Testing program [14].

	Margarine(CRM 122)	Milkpowder(CRM 421)
Analyte	*a*-tocopherol	*a*-tocopherol
Year of the inter-laboratory test	1994	1994
Number of laboratories	9	10
Number of samples	1	1
Number of laboratories retained after elimination of outliers	9	10
Number of outliers	0	0
Number of data sets	9	10
Number of replicate measurement	45	50
Mean value, x , mg/100 g	24,09	9,89
Repeatability standard deviation, s_r, mg/100 g	0,977	0,399
Repeatability relative standard deviation RSD_r	4,1 %	4,0 %
Repeatability value r ($2{,}83 \times s_r$) mg/100 g	2,765	1,130
Reproducibility standard deviation, s_R, mg/100 g	1,477	0,693
Reproducibility relative standard deviation RSD_R	6,1 %	7,0 %
Reproducibility value, R ($2{,}83 \times s_R$), mg/100 g	4,180	1,960

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.

In accordance with ISO 5725 : 1986 [15], the following validation data have been defined in an inter-laboratory test. The test was conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany [16].

	Milk powder

Analyte	*a*-tocopherol	*b*-tocopherol	*g*-tocopherol	*d*-tocopherol
Year of the inter-laboratory test	1993	1993	1993	1993
Number of laboratories	13	12	13	10
Number of samples	5	5	5	5
Number of laboratories retained after elimination of outliers	12	9	11	8
Number of outliers	1	3	2	2
Number of accepted results	66	51	65	40
Mean value, $\bar{x}$, mg/100 g	10,2	0,081	1,989	0,280
Repeatability standard deviation, s_r, mg/100 g	0,301	0,009	0,110	0,029
Repeatability relative standard deviation RSD_r	3,0 %	11,1 %	5,5 %	10,4 %
Repeatability value, r ($2{,}83 \times s_r$), mg/100 g	0,853	0,025	0,311	0,082
Reproducibility standard deviation s_R, mg/100 g	1,31	0,016	0,346	0,047
Reproducibility relative standard deviation RSD_R	12,8 %	19,8 %	17,4 %	16,8 %
Reproducibility value R ($2{,}83 \times s_R$), mg/100 g	3,705	0,046	0,978	0,134

	Oat powder	
Analyte	*a*-tocopherol	*b*-tocopherol
Year of the inter-laboratory test	1993	1993
Number of laboratories	13	13
Number of samples	5	5
Number of laboratories retained after elimination of outliers	12	11
Number of outliers	1	2
Number of accepted results	70	64
Mean value, x, mg/100 g	0,279	0,057
Repeatability standard deviation s_r, mg/100 g	0,025	0,006
Repeatability relative standard deviation RSD_r	9,0 %	10,5 %
Repeatability value r ($2{,}83 \times s_r$), mg/100 g	0,071	0,016
Reproducibility standard deviation s_R, mg/100 g	0,047	0,011
Reproducibility relative standard deviation RSD_R	16,8 %	19,3 %
Reproducibility value R ($2{,}83 \times s_R$), mg/100 g	0,133	0,030

[1] Subcommittee on the Tenth Edition of the RDA s, Food and Nutrition Board, Commission on Life Sciences, National Research Counsil, Recommended Dietary Allowances, 10th Edition, National Acadamy Press, Washington, D.C. 1989.

[2] Deutsche Gesellschaft für Ernährung (DGE): Empfehlungen für die Nährstoffzufuhr; 5. Überarbeitung 1991.

[3] Brubacher, G. and Wiss, O. (1972), The Vitamins, eds Sebrell & Harris, 5th Edition, Academic Press, New York, 255.

[4] Brubacher, G. et al., (1985). Methods for the Determination of Vitamins in Food, Elsevier App. Science Publishers, London, 97-106.

[5] Gertz, C. and Kerrman, K. (1982). Z. Lebensmittelunters. Forsch., **174,** 390-394.

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[7] Determination of Tocopherols in fats and oils. L-13.03/04. September 1987. Collection of official methods under article 35 of the German Federal Foods Act, Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office. Loose leaf edition as of 1998-09, Vol.1) Berlin, Köln: Beuth Verlag GmbH

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[9] Speek, A.J., Schrijver, J. and Schreurs, W.H.P. (1985), J. Food Sci. **50,** 121-124.
[10] Manz, U. and Philipp, K. (1981). Internat. J. Vit. Nutr. Res., **51,** 342-348.
[11] Pocklington, W.D. and Diefenbacher, A. (1988). Pure & Appl. Chem. **60,** 877-892.
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[14] Finglas, P.M., van den Berg, H. and de Froidmont-Gortz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg.
[15] ISO 5725 : 1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.
[16] Food analysis – Determination of tocopherols and Tocotrienols in dietetic foodstuffs L 49.00-5 September 1998 Collection of official methods under article 35 of the German Federal Foods Act, Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office. Loose leaf edition as of 1998-09, Vol.1) Berlin, Köln: Beuth Verlag GmbH

EN 12823 Foodstuffs - Determination of vitamin A by high performance liquid chromatography

Introduction

This European Standard provides the base for the analytical methods. It is intended to serve as a frame in which the analyst can define his own analytical work in accordance to the standard procedure.
As this draft European Standard deals with the measurement of total-*b*-carotene in foodstuffs, reference is made to the literature for the calculation and expression of *b*-carotene as vitamin A equivalents [1], [2].
Vitamin A activity can be calculated from the *b*-carotene data assuming appropriate factors.
This European Standard Foodstuffs - Determination of vitamin A by high performance liquid chromatography consists of two parts:
Part 1: Measurement of all-trans-retinol & 13-cis-retinol.
Part 2: Measurement of *b*-carotene.

[1] World Health Organisation, Expert Committee on Biological Standardisation, Techn. Report No. 147, 11, WHO, Geneva, 1958 and Techn. Report 222, 10, 51-52, WHO, Geneva, 1961.
[2] Int. Union of Pure and Applied Chemistry (IUPAC), The Vitamin A Potency of β-Carotene, Butterworths Scientific Publications, London 1959

EN 12823 Part 1: Measurement of all-trans-retinol and 13-cis-retinol

1 Scope

This European Standard specifies a method for the determination of vitamin A in foodstuffs by high performance liquid chromatography (HPLC). The determination of vitamin A content is carried out by the measurement of all-trans-retinol, 13-cis-retinol and *b*-carotene. This part covers the measurement of all-trans-retinol and 13-cis-retinol.
The extract obtained after saponification in this method can be used for the determination of *b*-carotene, as described in prEN 12823-2:1999 Measurement of *b*-carotene.

2 Principle

Retinol (i.e. the sum of all-trans-retinol and 13-cis retinol) is saponified by using methanolic or ethanolic potassium hydroxide solution and extracted by an appropriate solvent. The determination is carried out by high performance liquid chromatography (HPLC) with either fluorometric (F) or ultra-violet (UV) detection. The substances are identified on the basis of the retention times and determined by the external standard procedure using peak areas or heights, see [1] to [4].

3 Precision data

In accordance with the EU MAT Certification Study Guidelines, the parameters given in Table.1 have been defined in an inter-laboratory test [3]. The study was organised by the Institute of Food Research, Norwich, United Kingdom

Sample:	Margarine (CRM 122) *		Milk Powder (CRM 421) *	
	all-trans-retinol	13-cis-retinol	all-trans-retinol	13-cis-retinol
Year of inter-laboratory test	1994	1994	1994	1994
Number of laboratories	9	5	8	5
Number of samples	2	2	2	2
Number of laboratories retained after eliminating outliers	9	5	6	5
Number of outliers (laboratories)	0	0	2	0
Number of accepted results	45	25	40	21
Mean value $\bar{x}$ µg/100 g	729	39	653	30
Repeatability standard deviation s_r µg/100g	28	3	14	1,5
Repeatability relative standard deviation *RSD*r %	3,8	7,7	2,1	5,0
Repeatability limit *r* µg/100 g	78,4	8,4	39,2	4,2
Reproducibility standard deviation s_R µg/100 g	73	12	22	7,2
Reproducibility relative standard deviation RSD_R %	10,0	30,8	3,4	24,0
Reproducibility limit *R* µg/100 g	204,4	33,6	61,6	20,2
* CRM = Certified reference material				

[1] Bourgeois, C.F. and Ciba, N., 1988. Disposal cartridge extraction of retinol and alpha-tocopherol from fatty samples. J.A.O.A.C 71 (1), 12-15.

[2] Leth, T., 1993. Vitamin A in Danish pig, calf and ox liver. J. Food Composition & Analysis 6, 3-9.

[3] Finglas, P.M., van den Berg, H. & de Froidmont-Gortz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg.

[4] Food Analysis: Determination of vitamin A in dietetic foodstuffs L 49.00-3, 1985. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office. Loose leaf edition, as of 1996-02 Vol. I. Berlin, Köln: Beuth Verlag GmbH.

EN 12823 Part 2: Measurement of *b*-carotene

1 Scope

This European Standard specifies a method for the determination of total-*b*-carotene in foodstuffs by high performance liquid chromatography (HPLC).

2 Principle

Determination of the sum of *b*-carotene isomers in an appropriate sample solution by HPLC and spectrometric detection in the visible range. The extract obtained after saponification as described in prEN 12823 -1 may be used for quantification. Identification on the basis of the retention times, and determination by the external standard method using peak areas or peak heights, see [1] to [5].

Internal standard methods may also be used if the corresponding recovery tests have proven the same behaviour of the internal standard during the analysis as the analyte itself.

3 Precision Data

Statistical results and precision data in the following table for the determination of total-*b*-carotene were established by an inter-laboratory-test according to ISO 5725-2:1994 [6] organised by W. Schüep and J. Schierle [7].

Sample	Margarine	Vitamin Drink	Pudding Powder	Mixed Vegetables
Analyte	Total *b*-carotene	Total *b*-carotene	Total *b*-carotene	Total *b*-carotene
Year of the inter-laboratory test	1995	1995	1995	1995

Number of laboratories	13	13	13	13
Number of samples	1	1	1	1
Number of laboratories retained after elimination of outliers	12	12	11	12
Number of outliers	1	1	2	1
Number of accepted results	55	59	51	56
Mean value mg/100 g	0,253	2,248	1,531	18,05
Repeatability standard deviation s_r mg/100 g	0,011	0,065	0,085	0,71
Repeatability relative standard deviation RSD_r	4,5 %	2,9 %	5,6 %	3,9 %
Repeatability value r (2,83 x s_r) mg/100 g	0,032	0,19	0,24	2,0
Reproducibility standard deviation s_R mg/100 g	0,024	0,15	0,14	2,7
Reproducibility relative standard deviation RSD_R	9,7 %	6,5 %	9,3 %	15 %
Reproducibility value R (2,83 x s_R) mg/100 g	0,069	0,41	0,40	7,6

The validation data given in the following table have been defined in an inter-laboratory test which was conducted under the European Commissions Standard, Measurement and Testing program organised by Institute of Food Research in 1996 on the measurement of all-trans-*b*-carotene in a mixed vegetable sample [8].

Sample	Mixed vegetables
Year of the inter-laboratory test	1996
Number of laboratories	14
Number of samples	1
Number of laboratories retained after elimination of outliers	14
Number of outliers	0
Number of data sets	14
Number of accepted results	60
Mean value mg/100 g	2,37
Repeatability standard deviation s_r mg/100 g	0,159
Repeatability relative standard deviation RSD_r	6,7%
Repeatability value *r* (2,83 x s_r) mg/100 g	0,450
Reproducibility standard deviation s_R mg/100 g	0,241
Reproducibility relative standard deviation RSD_R	10,2 %
Reproducibility value *R* (2,83 x s_R) mg/100 g	0,682

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

[1] Bushway, R.J.: Separation of Carotenoids in Fruits and Vegetables by High-Performance Liquid Chromatography, J. Liq. Chromatogr. **8**, 1985, 1527-47.

[2] Quackenbush, F.W.: Reversed Phase HPLC Separation of Cis- and Trans-Carotenoids and its Application in Food Materials, J. Liq. Chromatogr. **10**, 1987, 643-653.

[3] O Neil, C.A., Schwartz, S.J. , Catignani, G.L.: Comparison of Liquid Chromatographic Methods for Determination of Cis-Trans Isomers of β-Carotene, J. Assoc. Off. Anal. Chem. **74**, 1991, 36-42.

[4] Saleh, H.M., Tan, B.: Separation and Identification of Cis/Trans Carotenoid Isomers, J. Agric. Food Chem., **39**, 1991, 1438-1443.

[5] Lumley, I. D.: in The Technology of Vitamins in Food, ed. by P. B. Ottaway, Blacie Academic & Professional, Glasgow, 1993, 183-186.

[6] ISO 5725-2:1994 Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility for a standard measurement method.

[7] Schüep, W. and Schierle, J., 1997. Determination of β-Carotene in Commercial Foods: Inter-laboratory Study. Journal of AOAC International Vol. 80 No. 5, 1057-1064.

[8] Finglas, P.M., Scott, K.J., Wilthöft, C. M., van der Berg, H. & de Froidmont – Görtz, I., 1999. The certification of the mass fractions of vitamins in four reference materials: wholemeal flour (CRM 121),

milk powder (CRM 421), lyophilized mixed vegetables (CRM 485) and lyophilized pig's liver (CRM 487). EUR-Report DOC/BCR/01/98. Commission of the European Union, Luxembourg.

Contaminants
PCBs

EN 1528 Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) –

General Introduction
EN 1528 consists of the following Parts:
Part 1 "General" presents the scope of the standard and describes general considerations with regard to reagents, apparatus, gas chromatography etc., applying to each of the analytical methods selected.
Part 2 "Extraction of fat, pesticides and PCBs, and determination of fat content" presents a range of analytical procedures for extracting the fat portion containing the pesticide and PCB residues from different groups of fat-containing foodstuffs.
Part 3 "Clean-up methods" presents the details of methods A to H for the clean-up of fats and oils or the isolated fat portion, respectively, using techniques such as liquid/liquid partition, adsorption or gel permeation column chromatography.
Part 4 "Determination, confirmatory tests, miscellaneous" gives guidance on some recommended techniques for the determination of pesticides and PCBs in fatty foodstuffs and on confirmatory tests and lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.
This European Standard comprises a range of multi-residue methods of equal status: no single method can be identified as the prime method because, in this field, methods are continuously developing. The methods selected for inclusion in this standard have been validated and are widely used throughout Europe. Any variation in the methods used should be shown to give comparable results.

EN 1528 Part 1: General considerations
1 Scope
This European Standard specifies methods for the determination of residues of pesticides and polychlorinated biphenyls (PCBs) in fatty food.
Each method described in this European Standard is suitable for identifying and quantifying a definite range of those non-polar organochlorine and/or organophosphorus pesticides which occur as residues in fats and oils as well as in the fat portion of fat-containing foodstuffs, both of either animal or vegetable origin. The PCB indicator congeners usually selected for the enforcement of maximum residue limits (MRLs) are determined along with the organochlorine pesticides.
This European Standard contains the following clean-up methods that have been subjected to interlaboratory studies and are adopted throughout Europe:
- Method A: Liquid-liquid partitioning with acetonitrile and clean-up on a Florisil® column (AOAC) [1]
- Method B: Liquid-liquid partitioning with dimethylformamide and clean-up on a Florisil® column (Specht) [2]
- Method C: Column chromatography on activated Florisil® (AOAC) [3]
- Method D: Column chromatography on partially deactivated Florisil® (Stijve) [4]
- Method E: Column chromatography on partially deactivated aluminium oxide (Greve & Grevenstuk) [5]
- Method F: Gel permeation chromatography (GPC) (AOAC) [6]
- Method G: Gel permeation chromatography (GPC) and column chromatography on partially deactivated silica gel (Specht) [7]
- Method H: High performance gel permeation chromatography (HPGPC) (MAFF) [8]

The applicability of the eight methods A to H for residue analysis of organochlorine pesticides, PCB indicator congeners, and organophosphorus pesticides, respectively, is given in table 1. Where no + sign is shown, there are no data available in literature, but this does not necessarily exclude the applicability.

Table 1: Applicability of methods A to H according to reference given in literature

Compound[2])	Method							
	A [1]	B [2]	C [3]	D [4]	E [5]	F [6]	G [7]	H [8]
Organochlorine pesticides								
aldrin (HHDN)	+	+		+	+	+	+	+
cis-chlordane		+		+	+	+	+	+
trans-chlordane		+		+	+	+	+	+
o, p'-TDE (DDD)	+			+		+	+	+

p, p'-TDE (DDD)	+	+	+	+	+	+	+	+
o, p'-DDE	+				+		+	+
p, p'-DDE	+	+	+	+	+	+	+	+
o, p'-DDT	+	+		+	+	+	+	+
p, p'-DDT	+	+	+	+	+	+	+	+
dieldrin (HEOD)	+	+	+	+	+	+	+	+
a-endosulfan					+		+	
ß-endosulfan							+	
endrin	+	+		+	+	+	+	+
hexachlorobenzene (HCB)		+		+	+	+	+	+
a-HCH	+	+		+	+	+	+	+
ß-HCH	+	+		+	+	+	+	+
?-HCH (lindane)	+	+		+	+	+	+	+
d-HCH	+	+		+			+	+
heptachlor	+	+		+	+	+	+	+
heptachlor epoxide	+	+		+	+		+	+
methoxychlor	+	+		+	+	+	+	
mirex	+					+	+	+
oxychlordane		+		+	+		+	+
camphechlor (toxaphene)				+	+	+	+	
PCB indicator congeners	+	+	+	+	+		+	+
Organophosphorus pesticides								
bromophos		+		+			+	+
bromophos-ethyl							+	+
carbophenothion		+					+	+
chlorfenvinphos		+					+	+
chlorpyrifos				+			+	+
chlorpyrifos-methyl								+
crotoxyphos								+
diazinon	(+)	+					(+)	+
dichlorvos								+
ethion	(+)			+			+	+
famphur								+
fenitrothion							+	+
fenchlorphos (ronnel)	(+)	+		+			+	
fenthion								+
iodofenphos				+			+	+
malathion	(+)	+					+	+
phosmet								+
pirimiphos-methyl							+	+
parathion	(+)	+					+	
parathion-methyl	(+)						+	
phenkapton				+				
tetrachlorvinphos								+

Key: + applicable, (+) validated for special cases, see [1]

[2]) For the full chemical names and structures, see ISO 1750 Pesticides and other agrochemicals – Common names.

Table 2: PCB indicator congeners

Chemical name	Number
1) 2, 4, 4'-trichlorobiphenyl	28
2) 2, 2', 5, 5'-tetrachlorobiphenyl	52
3) 2, 2', 4, 5, 5'-pentachlorobiphenyl	101
4) 2, 2', 3, 4, 4', 5'-hexachlorobiphenyl	138
5) 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl	153
6) 2, 2', 3, 4, 4', 5, 5'-heptachlorobiphenyl	180

2 Principle

The methods described in this European Standard are based on a four-stage process (in some cases two stages may be combined, in whole or in part), as described in 3.2 to 3.5.

- Extraction

Extraction of the residues from the sample matrix by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the residue and minimum co-extraction of any substances which can give rise to interferences in the determination.

NOTE: Methods for extraction of fat are recommended which are simultaneously applicable for the extraction and determination of fat and the residue analysis in the fat portion.

- Clean-up

Maximum removal of interfering substances with minimal loss of analyte from the sample extract, so as to obtain a solution of the extracted residue in a solvent which is suitable for quantitative examination by the selected method of determination.

- Determination

Gas chromatography (GC) with various detectors, e.g. electron-capture detector (ECD), the thermionic detector (P- or N/P- mode), the flamephotometric detector (FPD), the Hall detector or mass spectrometry (MS) as appropriate.

- Confirmation

Procedures to confirm the identity and quantity of observed residues, particularly in those cases where it would appear that the maximum residue limit has been exceeded.

[1] Cunniff, P. (Ed.): Official Methods of Analysis of AOAC INTERNATIONAL, 16th edition, Arlington VA USA 1995, Vol. 1, Chapter 10, pp. 1-10, Method No. 970.52.

[2] Specht, W.: Organochlorine and organophosphorus pesticides. In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim 1987, Vol. 1, pp. 309-319, Method S 10.

[3] Cunniff, P. (Ed.): Official Methods of Analysis of AOAC INTERNATIONAL, 16th edition, Arlington VA USA 1995, Vol. 1, Chapter 10, pp. 11-12, Method No. 983.21.

[4] Stijve, T.: Organochlorine and organophosphorus pesticides. In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim 1987, Vol. 1, 5, pp. 297-308, Method S 9.

[5] Greve, P.A., and Grevenstuk, W.B.F.: Meded. Fac. Landbouwwet. (Gent) 40, pp. 1115-1124 (1975), cited in: Analytical Methods for Residues in Foodstuffs, 5th edition, The Hague 1988, Vol.1, pp. 12-15, Multi-Residue Method 1, submethod 5.

[6] Cunniff, P. (Ed.): Official Methods of Analysis of AOAC INTERNATIONAL, 16th edition, Arlington VA USA l995, Vol. 1, Chapter 10, pp. 12-13, Method No. 984.21.

[7] Specht, W.: Organochlorine, organophosphorus, nitrogen-containing and other pesticides. In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim 1987, Vol. 1, pp. 75-78 and pp. 383-400, Cleanup Method 6 and Method S 19.

[8] UK Ministry of Agriculture, Fisheries and Food: Analysis of pesticide residues in products of animal origin, Method FScLPest-1, (23.4.91).

EN 1528 Part 2: Extraction of fat, pesticides and PCBs and determination of fat content General considerations

1 Scope

This Part of EN 1528 specifies a range of analytical procedures for extracting the fat portion containing the pesticide and polychlorinated biphenyl (PCB) residues from different groups of fat-containing foodstuffs.

2 Principle

Extraction of the residues from the sample matrix by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the residue and minimum co-extraction of any substances which can give rise to interferences in the determination. Removal of the solvents by evaporation and, optionally, determination of the fat content by weighing out the mass of the remainder.

EN 1528 Part 3: Clean-up methods

1 Scope

This Part of EN 1528 specifies the details of methods A to H for the clean-up of fats and oils or the isolated fat portion, respectively, using techniques such as liquid/liquid partition, adsorption or gel permeation column chromatography. The applicable usage of the methods A to H is given in detail in each method described.

NOTE: See also EN 1528-4 which lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

2 Principle

Removal of interfering materials from the sample extract to obtain a solution of the extracted residue in a solvent which is suitable for quantitative examination by the selected method of determination.

EN 1528 Part 4: Determination, confirmatory tests, Miscellaneous

1 Scope

This Part of EN 1528 gives guidance on some recommended techniques for the determination of pesticides and polychlorinated biphenyls (PCBs) in fatty foodstuffs and on confirmatory tests and lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

2 Principle

The methods described in this Part of EN 1528 permit the residues present to be provisionally identified and quantified, by gas chromatographic methods using selective detectors.

All positive results require confirmation of identity and quantity.

The procedures listed for confirmation such as alternative GC columns, alternative GC detectors, thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), column fractionation, derivatization, spectral measurements, etc., are all of value. Results obtained using mass spectrometry (MS) present definitive evidence for confirmation/identification purposes.

Nitrate/Nitrite

EN 12014 Foodstuffs – Determination of nitrate and/or nitrite content

Introduction

Due to existing and possible future legislation for nitrate and/or nitrite content of foodstuffs, and especially to avoid trade barriers, there is a need for European Standards for the determination of nitrate and/or nitrite in the following foodstuffs:

- vegetables and vegetable products,
- meat products,
- food for babies and infants,
- milk and milk products.

It was decided to not consider any method involving the use of open sources of spongy cadmium on the grounds of its potential threat to the environment. As a result, the only methods available for inclusion in this standard were vertical methods for the particular substrates of interest.

This EN is divided in the following parts:

Part 1: General considerations

Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products

Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite

Part 4: IC method for the determination of nitrate and nitrite content of meat products

Part 5: Enzymatic determination of nitrate content of vegetable-containing food for babies and infants

Part 6: (Method for milk and milk products – to be elaborated by CEN/TC 302)

Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after cadmium reduction

EN 12014 Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products

1 Scope

This European Standard specifies a high performance liquid chromatography (HPLC)/ion-exchange high performance liquid chromatography (IC) method for the determination of nitrate contents of vegetables and vegetable products. This method is applicable to nitrate contents in the range of 50 mg/kg to 3000 mg/kg.

2 Principle

Extraction of nitrate from the food with hot water and removal of interfering substances by clarification with Carrez reagents or by purification with solid phase extraction columns. Determination by reversed phase HPLC with ultraviolet (UV) detection or IC with conductivity detection [1].

3 Precision data

In accordance with ISO 5725 : 1986 [2], the following parameters have been defined in two inter-laboratory tests [1]. The tests were conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany.

	Spinach	**Beetroot juice**	**Spinach product**		**Carrot product**	
Year of test	1989	1989	1988	1988	1988	1988
Number of samples	1	1	1	1	1	1
Number of laboratories	9	9	6	8	8	6
Number of laboratories retained after eliminating outliers	8	7	6	6	6	6
Number of outliers	1	2	0	2	2	0
Number of accepted results	65	50	56	54	55	56

Mean value ($\bar{x}$)	2 899 mg/kg	1163 mg/l	197 mg/kg	98 mg/kg	76 mg/kg	62 mg/kg
Repeatability standard deviation (s_r)	32 mg/kg	17 mg/l	5 mg/kg	1,8 mg/kg	1,8 mg/kg	2,1 mg/kg
Repeatability relative standard deviation (RSD_r) [%]	1,1 %	1,4 %	2,5 %	1,8 %	2,4 %	3,5 %
Repeatability limit (*r*)	90 mg/kg	47 mg/l	14 mg/kg	5 mg/kg	5 mg/kg	6 mg/kg
Reproducibility standard deviation (s_R)	88 mg/kg	34 mg/l	7,9 mg/kg	9,3 mg/kg	3,9 mg/kg	3,6 mg/kg
Reproducibility relative standard deviation (RSD_R)[%]	3,1 %	3,0 %	4,0 %	9,5 %	5,2 %	5,8 %
Reproducibility limit (*R*)	248 mg/kg	97 mg/l	22 mg/kg	26 mg/kg	11 mg/kg	10 mg/kg

[1] Food Analysis: Determination of nitrate content of vegetable products: L 26.00-1 1989-12 and Determination of nitrate in mashed vegetables for babies and infants L 48.03.05-2. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office. Loose leaf edition, as of 1993 - 08 Vol. I. Berlin, Köln: Beuth Verlag GmbH.

[2] ISO 5725 : 1986 "Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests".

ENV 12014 Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite

1 Scope

This European Standard specifies a spectrometric method for the determination of nitrate and nitrite content of meat products and has been validated for a total nitrite and nitrate content of 25 mg/kg as nitrite ion. Experiences have shown that the method is also applicable for total nitrite and nitrate content from 10 mg/kg up to 50 mg/kg as nitrite ion. For further information on applicability, see [1].

2 Principle

Nitrite in an aqueous extract of the analytical sample is treated with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride. A red compound is produced which is measured spectrometrically at a wavelength of 540 nm [2].

Nitrate in an aqueous extract of the analytical sample is converted into nitrite by nitrate reductase. Treatment of this nitrite together with the nitrite which is already in the analytical sample with sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride. Photometric measurement of the colour intensity of this red compound at a wavelength of 540 nm. The nitrate content is calculated from the difference between the spectrometric measurements.

3 Precision data

In accordance with ISO 5725 : 1986 [3], the following parameters have been identified in inter-laboratory tests. The test was conducted by the Federal Health Office, (BGA), Germany on sausages [2] and the values are expressed as sodium nitrite.

Sample	**Sausage**
Year of inter-laboratory test	1988
Number of samples	1
Number of laboratories	19
Number of laboratories retained after eliminating outliers	19
Number of outliers	0
Number of accepted results	95
Mean value $\bar{x}$	37 mg/kg

Repeatability standard deviation s_r	2 mg/kg
Repeatability relative standard deviation RSD_r	5,8 %
Repeatability limit r	6 mg/kg
Reproducibility standard deviation s_R	3 mg/kg
Reproducibility relative standard deviation RSD_R	7,7 %
Reproducibility limit R	8 mg/kg

[1] Arneth, W.; Herold, B. Nitrat/Nitrit-Bestimmung in Wurstwaren nach enzymatischer Reduktion (nitrate/nitrite determination in sausages after enzymatic reduction). Fleischwirtschaft, 1988:68, No. 6, page 761.

[2] Food Analysis: Determination of nitrite and nitrate content of sausage products after enzymatic reduction: L 08.00-14 1990-12. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods (...). Loose leaf edition, as of 1993 - 08 Vol. I. Berlin, Köln: Beuth Verlag GmbH.

[3] ISO 5725 : 1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

ENV 12014 Part 4: IC method for the determination of nitrate and nitrite content of meat products

1 Scope

This European Prestandard specifies an ion-exchange chromatographic method for the determination of the nitrate and nitrite contents of meat products having a nitrate content of 50 mg/kg to 300 mg/kg as nitrate ions and a nitrite content of approximately 40 mg/kg as nitrite ion.

NOTE: Validation data obtained from inter-laboratory studies show that this method may also be applied to the determination of nitrate in vegetables and baby food, see [1], [2]. Furthermore, the method may be applied for the determination of nitrite in meat products having a nitrite content of greater than 40 mg/kg.

2 Principle

Nitrate and nitrite are extracted from the test sample with hot water. The aqueous solution is treated with acetonitrile to remove any interfering substance. The nitrate and nitrite contents of the solution are then determined by ion-exchange chromatography (IC) and ultraviolet (UV) detection at a wavelength of 205 nm.

3 Precision data

In accordance with ISO 5725:1986 [3], the following parameters have been defined in an inter-laboratory test. The test was conducted by the Centre Technique de la Salaison, de la Charcuterie et des Conserves de Viandes (CTSCCV), France [2].

	Corned beef NO *)	Corned beef NO	Corned beef NO	Corned beef NO
Year of inter-laboratory test	1994	1994	1994	1994
Number of laboratories	16	16	16	16
Number of samples	1	1	1	1
Number of laboratories retained after eliminating outliers	14	13	14	13
Number of outliers	2	3	2	3
Number of accepted results	42	39	42	39
Mean value $\bar{x}$ mg/kg	7	60,8	38,9	289,6
Repeatability standard deviation s_r	1,2	2,1	1,5	8,6
Repeatability relative standard deviation RSD_r %	17,0	3,5	4,0	3,1
Repeatability limit *r* mg/kg	3,3	6,0	4,4	25,0
Reproducibility relative standard deviation s_R	2,3	9,8	3,7	9,4
Reproducibility relative standard deviation RSD_R %	2,9	16,1	9,4	3,3
Reproducibility limit *R* mg/kg	6,5	27,7	10,3	26,6
*) According to Horwitz [4], the precision data for low nitrite concentrations are assessed as unacceptable and are only given for information purposes.				

In accordance with ISO 5725:1986 [3], the following parameters have been defined in an inter-laboratory test. The test was conducted by the Centre Technique de la Salaison, de la Charcuterie et des Conserves de Viandes (CTSCCV), France.

	Spinach (NO)	Carrots (NO)	Baby food (NO)
Year of inter-laboratory test	1994	1994	1994
Number of laboratories	17	17	17
Number of samples	1	1	1
Number of laboratories retained after eliminating outliers	15	15	14
Number of outliers	2	2	3
Number of accepted results	45	44	70
Mean value $\bar{x}$ mg/kg	1347,1	63,3	83,2
Repeatability standard deviation s_r mg/kg	19,6	4,3	2,5
Repeatability relative standard deviation RSD_r %	1,5	6,7	3,0
Repeatability limit *r* mg/kg	55,6	12,1	7,0
Reproducibility relative standard deviation s_R mg/kg	69,6	9,6	5,9
Reproducibility relative standard deviation RSD_R %	5,2	15,2	7,1
Reproducibility limit *R* mg/kg	196,9	27,3	16,7

[1] Bulletin de liaison du Centre Technique de la Salaison, de la Charcuterie et des Conserves de Viandes, CTSCCV 7, Avenue du General de Gaulle, F-94700 Maisons Alfort, Vol. 5, No 1, 1995.

[2] Congrès Sep 95 chromatographies et techniques apparentées, Poster A 90, available from Euradif-B.P.11-38243 Meylan Cedex, France.

[3] ISO 5725 : 1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

[4] Horwitz, W.: International coordination and validation of analytical methods. In: Food Additives Contaminants, 1993, Vol 10, No 1, 61 – 69.

EN 12014 Part 5: Enzymatic determination of nitrate content of vegetable-containing food for babies and infants

1 Scope

This European Standard specifies an enzymatic method for the determination of vegetable-containing food for babies and infants [1], [2]. This method is applicable to nitrate contents in the range of 50 mg/kg to 200 mg/kg

2 Principle

Enzymatic determination in an aqueous sample extract by measuring the amount of NADPH used up in the following reaction:

$$\text{Nitrate} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{nitrate reductase}} \text{Nitrite} + \text{NADP}^+ + \text{H}_2\text{O}$$

where the amount of NADPH used up is equivalent to the quantity of nitrate [3].

3 Precision data

In accordance with ISO 5725 : 1986 [4], the following parameters have been defined in two inter-laboratory tests [1], [2]. The tests were conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany.

Sample	Spinach	Carrot juice
Year of inter-laboratory test	1988	1992
Number of samples	1	1
Number of laboratories	19	9
Number of laboratories retained after eliminating outliers	16	7
Number of outliers	3	2
Number of accepted results	80	35
Mean value $\bar{x}$	64 mg/kg	200 mg/l
Repeatability standard deviation s_r	5,5 mg/kg	4 mg/l
Repeatability relative standard deviation RSD_r	8,7 %	2,1 %
Repeatability limit *r*	15,5 mg/kg	12,2 mg/l
Reproducibility standard deviation s_R	8,7 mg/kg	6 mg/l
Reproducibility relative standard deviation RSD_R	13,6 %	3,3 %
Reproducibility limit *R*	24,5 mg/kg	17,7 mg/l

[1] Food Analysis: Determination of nitrate in vegetable juices: L 26.26-2 1988-05. Collection of official methods under article 35 of the German Federal Foods Act; Methods of Sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office. Loose leaf edition, as of 1993 - 08 Vol. I. Berlin, Köln: Beuth Verlag GmbH.

[2] Food Analysis: Determination of nitrate in mashed vegetables for babies and infants: L 48.03.05-1 1988-05. Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office. Loose leaf edition, as of 1993 - 08 Vol. I. Berlin, Köln: Beuth Ver lag GmbH.

[3] Beutler, H.-O., Wurst, B., and Fischer, S.,: "Eine neue Methode zur enzymatischen Bestimmung von Nitrat in Lebensmitteln" (A new method for the enzymatic determination of nitrate in foodstuffs), In: Dt. Lebensm. Rundsch. 82, 1986, 283 – 289.

[4] ISO 5725 : 1986 "Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests".

EN 12014 Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after cadmium reduction

1 Scope

This European Standard specifies a continuous flow method (CF-method) for the determination of nitrate content of vegetables and vegetable products having a nitrate content of 900 mg/kg to 5200 mg/kg (calculated as nitrate ion).

NOTE: Experiences have shown that the method may also be applied for vegetables and vegetable products having a nitrate content of greater than 50 mg/kg (calculated as nitrate ion).

2 Principle

Test portions are extracted with water and filtered. The filtrate is transferred to the dializer of the continuous flow (CF) system [1]. An aliquot portion of the nitrate ions diffuses in the dializing unit with a hydrophilic membrane into a slightly alkaline buffer solution in which the nitrates are reduced to nitrite by metallic cadmium. The nitrite ions react with sulfanilamide and N-1-naphthylethylenediamine to a give reddish-purple azo dye.

The absorbance of this dye is determined spectrometrically at a wavelength between 520 nm and 540 nm, preferably at its maximum.

NOTE: The CF method is an automated version of the manual procedure for nitrate determinations in leafy vegetables as prescribed by the Official Dutch Food Act [2]. With the automated method the cadmium reductor may be used for a longer period of time without any appreciable loss of its reducing capacity. Also ready-made cadmium columns from commercial suppliers are available, minimizing the main objection of working with this toxic element.

3 Precision data

In accordance with with ISO 5725 : 1986 [3], the following parameters have been defined in an inter-laboratory test conducted by the laboratories of the Netherland's Food Inspection Service with levels of nitrate ions between 900 mg and 5200 mg/kg, in samples of frozen vegetables by the laboratories of the Food Inspection Services, Netherlands. Statistical evaluation of the collaborative study data was performed according to the International Union of Pure and Applied Chemistry (IUPAC)/International Organization for Standardization (ISO 5725 : 1986)/AOAC protocol for a uniform-level study as described by Pocklington [4].

Two different sets of equipment have been used for the inter-laboratory trial.

Prior to the collaborative trial a robustness test was performed. Also recovery experiments were conducted, see [5] and [6].

	Beetroot		**Lettuce**			**Endive**	**Spinach**
Year of test	1994	1994	1994	1994	1994	1994	1994
Number of samples	1	1	1	1	1	1	1
Number of laboratories	13	13	13	13	13	13	13
Number of labs retained after eliminating outliers	12	12	12	12	12	12	12
Number of outliers [1])	1	1	1	1	1	1	1
Number of accepted results	24	24	24	22 [2])	24	24	22 [2])
Mean value $\bar{x}$ (mg/kg)	901	2655	1319	2738	4021	1981	5197
Repeatability standard deviation s_r (mg/kg)	50	88	65	90	97	34	122
Repeatability relative standard deviation RSD_r (%)	5,5	3,3	5,0	3,3	2,4	1,7	2,4
Repeatability limit r (mg/kg)	139	246	183	252	271	94	342
Reproducibility standard deviation s_R (mg/kg)	53	121	72	90	150	74	205
Reproducibility relative standard deviation RSD_R (%)	5,9	4,6	5,4	3,3	3,7	3,8	3,9
Reproducibility limit R (mg/kg)	149	338	201	252	420	209	573

[1]) One laboratory showed outliers in 2 samples and standard solutions and was eliminated from all data.

[2]) Because of damage of sample in transport.

[1] Snyder, L., Levin, J., Stoy, R., and Conetta, A.: Automated Chemical Analysis: Update on Continuous-Flow Approach. In: Anal. Chem 38 (6), (1976), 942a - 956.

[2] Dutch Food Act (1984). Vol.2. Offical methods of analysis. Determination of Nitrate Content in Vegetables. General decree CII.1, Lelystad, The Netherlands.

[3] ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

[4] Pocklington, W.D.: Harmonized Protocols for the Adoption of Standardized Analytical Methods and for the Presentation of their Performance Characteristics. Pure & Applied Chemistry, 1990, **62**, 149.

[5] Beljaars, P.R., van Dijk, R., and van der Horst, G.M.: Determination of Nitrate in Vegetables by Con[5]tinuous Flow. Collaborative Study. In: J.A.O.A.C., 1994, **77**, 1522.

[6] Beljaars, P.R., van Dijk, R., and van der Horst, G.M.: Determination of Nitrate in Vegetables by Continuous Flow: Collaborative Study. In: De Ware(n)-Chemicus, 1993, **23**, 206-222.

Mycotoxins

EN 12955 Foodstuffs - Determination of aflatoxin B_1, and the sum of aflatoxins B_1, B_2, G_1 and G_2 in cereals, shell-fruits and derived products - High performance liquid chromatographic method with post column derivatization and immunoaffinity column clean up

1 Scope

This European Standard specifies a method for the determination of aflatoxin contents of greater than 8 µg/kg.

The method has been successfully validated in an inter-laboratory study according to ISO 5725:1986 [1] on maize containing 24,5 µg/kg, peanut butter containing 8,4 µg/kg and raw peanuts containing 16 µg/kg of total aflatoxins.

Some laboratory experiences have shown that this method can be used to several types of cereals, oilseed products, shell-fruits, dried fruits and derived products, after in-house validation.

2 Principle

The test sample is extracted with a mixture of methanol and water. The sample extract is filtered, diluted with water, and applied to an affinity column containing antibodies specific for aflatoxins B_1, B_2, G_1 and G_2. The aflatoxins are isolated, purified and concentrated on the column then removed from the antibodies with methanol. The aflatoxins are quantified by reverse-phase high performance liquid chromatography (HPLC) with fluorescence detection and postcolumn iodine derivatization.

WARNING: The method described requires the use of solutions of aflatoxins. Aflatoxins are carcinogenic to humans. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO) [2] [3].

3 Precision data

The following data were obtained in an inter-laboratory study organised by the AOAC and IUPAC in accordance with ISO 5725:1986. Samples of maize, peanuts and peanut butter, naturally contaminated and spiked at 10 µg/kg, 20 µg/kg and 30 µg/kg total aflatoxins in the ratio 7:1:3:1 of B_1, B_2, G_1 and G_2, respectively, have been investigated.

Table 1 — Precision data for maize

Aflatoxin	B_1	B_2	G_1	G_2	Total
Year of inter-laboratory test	1989	1989	1989	1989	1989
Number of laboratories	10	10	10	10	10
Number of samples	1	1	1	1	1
Number of laboratories retained after eliminating outliers	9	9	9	10	9
Number of outliers	1	1	1	0	1
Number of accepted results	18	18	18	20	18
Mean value $\bar{x}$ µg/kg	14,88	1,38	7,18	1,05	24,49
Repeatability standard deviation s_r, µg/kg	0,68	0,35	0,68	0,20	1,79
Repeatability relative standard deviation RSD_r, %	5,8	25	9,5	19	7,3
Repeatability limit r (r = 2,8 x s_r), µg/kg	2,4	0,98	1,90	0,56	5,0
Reproducibility standard deviation s_R, µg/kg	1,50	0,41	0,68	0,53	2,86
Reproducibility relative standard deviation RSD_R, %	10	30	9,5	51	11,7
Reproducibility limit R [R = 2,8 x s_R], µg/kg	4,20	1,15	1,90	1,48	8,01
Recovery, %	85	55	96	42	81

Table 2 — Precision data for peanut butter

Aflatoxin	B_1	B_2	G_1	G_2	Total
Year of inter-laboratory test	1989	1989	1989	1989	1989

Number of laboratories	10	10	10	10	10
Number of samples	1	1	1	1	1
Number of laboratories retained after eliminating outliers	10	9	10	10	10
Number of outliers	0	1	0	0	0
Number of accepted results	20	18	20	20	20
Mean value $\bar{x}$ µg/kg	5,26	0,58	2,34	0,24	8,42
Repeatability standard deviation s_r, µg/kg	0,78	0,12	0,55	0,19	1,45
Repeatability relative standard deviation RSD_r, %	14,9	21	24	79	17
Repeatability limit r (r = 2,8 x s_r), µg/kg	2,2	0,34	1,54	0,53	4,06
Reproducibility standard deviation s_R, µg/kg	1,56	0,22	0,71	0,24	2,54
Reproducibility relative standard deviation RSD_R, %	30	38	31	101	30
Reproducibility limit R (R = 2,8 x s_R), µg/kg	4,37	0,62	1,99	0,67	7,11
Recovery, %	90	70	93	29	84

Table 3 — Precision data for peanuts

Aflatoxin	B_1	B_2	G_1	G_2	Total
Year of inter-laboratory test	1989	1989	1989	1989	1989
Number of laboratories	10	10	10	10	10
Number of samples	1	1	1	1	1
Number of laboratories retainedafter eliminating outliers	9	10	10	10	9
Number of outliers	1	0	0	0	1
Number of accepted results	18	20	20	20	18
Mean value $\bar{x}$ µg/kg	9,71	1,07	4,54	0,65	16
Repeatability standard deviation s_r, µg/kg	0,53	0,25	0,28	0,27	0,83
Repeatability relative standard deviation RSD_r, %	5,5	23	6,2	42	5,2
Repeatability limit r (r = 2,8 x s_r), µg/kg	1,48	0,70	0,78	0,76	2,3
Reproducibility standard deviation s_R, µg/kg	1,62	0,41	0,66	0,50	2,58
Reproducibility relative standard deviation RSD_R, %	17	38	15	77	16
Reproducibility limit R (R = 2,8 x s_R), µg/kg	4,54	1,15	1,85	1,4	7,22
Recovery, %	83	64	91	39	80

[1] ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory test.

[2] Laboratory decontamination and destruction of aflatoxins B_1, B_2, G_1 and G_2 in laboratory wastes. Castegnaro, M., Hunt D.C., Sansone E.B., Schuller P.L., Siriwardana M.G., Telling G.M., van Egmond H.P. and Walker E.A. IARC Scientific publication no. 37, International Agency for Research on Cancer (WHO), Lyon (France), 1980, 59 p.

[3] Laboratory decontamination and destruction of carcinogens in laboratory wastes: some mycotoxins. Castegnaro M., Barek J., Fremy J.M., Lafontaine M., Miraglia M., Sansone E.B. and Telling G.M. IARC publication no. 113, International Agency for Research on Cancer (WHO), Lyon (France), 1991, 63 p.

EN 13585 Foodstuffs - Determination of fumonisins B_1 and B_2 in maize – HPLC method with solid phase extraction clean-up

1 Scope

This European Standard specifies a method for the determination of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) in maize using high performance liquid chromatography (HPLC).

The method has been successfully validated in an interlaboratory study according to AOAC Guidelines for Collaborative Studies [1] on maize containing 405 µg/kg to 6732 µg/kg fumonisin B_1 and 152 µg/kg to 2619 µg/kg fumonisin B_2. The method works well with maize or minimally processed maize (e.g. fresh, dried and milled maize), but does not provide reliable results with most maize-based processed products.

2 Principle

Fumonisins are extracted from the sample of maize with a mixture of methanol and water. The filtered extract is purified on a strong-anion-exchange (SAX) solid-phase extraction (SPE) cartridge, and the fumonisins are eluted with a mixture of acetic acid and methanol. The extract is evaporated and the residue is redissolved in methanol and o-phthaldialdehyde/2-mercaptoethanol (OPA/MCE) is added to form fluorescent fumonisin derivatives. The derivatives are analysed by reverse-phase high performance liquid chromatography (HPLC) with fluorescence detection.

3 Recovery and relative standard deviation

Table 1 shows recovery and relative standard deviation of HPLC determination of fumonisins B_1 and B_2 at different spiked levels in maize. In IUPAC/AOAC Interlaboratory Collaborative Study with 12 participating laboratories, nine laboratory data were elaborated. This method was used in the IUPAC/AOAC Interlaboratory Study for the HPLC determination of fumonisins B_1, B_2 and B_3 in maize [1]. It has been adopted by AOAC International as official first action method [2].

Table 1 - Recovery and relative standard deviation data

Spiking level (µg/kg)	Mean recovery $\bar{x}$ (%)	RSD_r (%) [a)]	RSD_R (%) [b)]
Fumonisin B_1			
500	81,1	7,1	13,9
1000	81,3	5,8	15,7
2000	81,1	7,7	16,1
4000	81,1	6,2	15,2
8000	84,2	10,9	16,3
4246 [c)]	-	13,2	22,2
Fumonisin B_2			
200	75,9	8,4	16,3
400	78,3	8,5	15,8
800	77,3	11,9	19,3
1600	80,9	7,2	17,2
3200	81,9	12,2	17,9
1234 [d)]	-	17,5	26,7

a) RSD_r is the repeatability relative standard deviation.
b) RSD_R is the reproducibility relative standard deviation.
c) Maize naturally contaminated with fumonisin B_1 at a mean concentration of 4246 µg/kg.
d) Maize naturally contaminated with fumonisin B_2 at a mean concentration of 1234 µg/kg.

4 Precision data

The following data were obtained in interlaboratory tests according to AOAC Guidelines for Collaborative Studies [3] conducted by IUPAC/AOAC [1].

Table 2 - Precision data

	Fumonisin B_1	Fumonisin B_2	Fumonisin B_1	Fumonisin B_2
Year of inter-laboratory test	1994	1994	1994	1994
Number of laboratories	9 [a)]	9 [a)]	9 [a)]	9 [a)]
Number of laboratories retained after eliminating outliers	8 1	8 1	9 -	9

Number of outliers (laboratories) Number of accepted results	16	16	18	18
Mean value $\bar{x}$, µg/kg	405	152	813	313
Repeatability standard deviation s_r, µg/kg	28,8	12,7	47,4	26,6
Repeatability relative standard deviation RSD_r, %	7,1	8,4	5,8	8,5
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	80,6	35,6	132,7	74,5
Reproducibility standard deviation s_R, µg/kg	56,2	24,7	127,4	49,4
Reproducibility relative standard deviation RSD_R, %	13,9	16,3	15,7	15,8
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	157,4	69,2	356,7	138,3
Recovery, %	81,1	75,9	81,3	78,3
a) Three laboratories out of the original 12 participants were removed because judged invalid.				

Table 3 - Precision data

	Fumonisin B_1	Fumonisin B_2	Fumonisin B_1	Fumonisin B_2
Year of inter-laboratory test	1994	1994	1994	1994
Number of laboratories	9 [a)]	9 [a)]	9 [a)]	9 [a)]
Number of laboratories retained after eliminating outliers	9	9	9	9
	-	-	-	-
Number of outliers (laboratories) Number of accepted results	18	18	18	18
Mean value $\bar{x}$, µg/kg	1621	618	3245	1294
Repeatability standard deviation s_r, µg/kg	124,7	73,4	199,7	93,1
Repeatability relative standard deviation RSD_r, %	7,7	11,9	6,2	7,2
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	349,2	205,5	559,2	260,7
Reproducibility standard deviation s_R, µg/kg	261,1	119,7	494,1	222,3
Reproducibility relative standard deviation RSD_R, %	16,1	19,3	15,2	17,2
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	731,1	335,2	1383,5	622,4
Recovery, %	81,1	77,3	81,1	80,9
a) Three laboratories out of the original 12 participants were removed because judged invalid				

Table 4 - Precision data

Sample	Fumonisin B_1	Fumonisin B_2	Fumonisin B_1 [b)]	Fumonisin B_2 [b)]
Year of inter-laboratory test	1994	1994	1994	1994
Number of laboratories	9 [a)]	9 [a)]	9 [c)]	9 [c)]
Number of laboratories retained after eliminating outliers	9	9	9	9
Number of outliers (laboratories)	-	-	-	-
Number of accepted results	18	18	18	18
Mean value $\bar{x}$, µg/kg	6732	2619	4296	1234
Repeatability standard deviation s_r, µg/kg	736,6	318,4	561,7	215,4
Repeatability relative standard deviation RSD_r, %	10,9	12,2	13,2	17,5
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	2062,5	891,5	1572,8	603,1
Reproducibility standard deviation s_R, µg/kg	1099,7	467,7	944,1	329,4
Reproducibility relative standard deviation RSD_R, %	16,3	17,9	22,2	26,7
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	3079,2	1309,6	2643,5	922,3
Recovery, %	84,2	81,9	- [b)]	- [b)]

a) Three laboratories out of the original 12 participants were removed because judged invalid.

b) naturally contaminated maize

c) Three laboratories out of the original 12 participants were removed because judged invalid.

[1] E.W. Sydenham, G.S. Shepard, P.G. Thiel, S. Stockenstrom, P.W. Snijman, D.J. Van Schalkwyk. Liquid Chromatographic Determination of Fumonisins B_1, B_2 and B_3 in corn: AOAC-IUPAC Collaborative Study. Journal of AOAC International 1996, Vol. 79, No. 3, pp. 688-696.

[2] AOAC INTERNATIONAL Official Methods of Analysis, 17th Ed, 2000, Volume II, method 49.5.01 (995.15)

[3] AOAC Official Methods Program 1995, Associate Referee's Manual on development, Study, Review, and Approval Process, Part IV AOAC Guidelines for Collaborative Studies pp.23-51.

EN ISO 15141 Foodstuffs - Determination of ochratoxin A in cereals and cereal products

Introduction

This European Standard Foodstuffs - Determination of ochratoxin A in cereal and cereal products consists of two parts:

Part 1: High performance liquid chromatographic method with silica gel clean up

Part 2: High performance liquid chromatographic method with bicarbonate clean up

EN ISO 15141 Part 1: High performance liquid chromatographic method with silica gel clean up

1 Scope

This European Standard specifies a method for the determination of ochratoxin A at levels greater than 0,4 µg/kg.

The method has been successfully validated in 2 inter-laboratory studies according to ISO 5725:1996 [1] on wheat whole meal containing 0,4 µg/kg and 1,2 µg/kg of ochratoxin A.

NOTE: Numerous laboratory experiences have shown that this method is also applicable to cereals, dried fruits, oilseeds, pulses, wine, beer, fruit juices and raw coffee, see [2], [3], [4].

2 Principle

Ochratoxin A (OTA) is extracted with toluene after acidification with hydrochloric acid and after the ionic strength has been increased by adding magnesium chloride. The extract is purified using a mini silica gel column and ochratoxin A is determined by high performance liquid chromatography (HPLC) on a reversed phase column and detected by fluorescence. The result is verified, if required, by derivatization with boron trifluoride in methanolic solution [5], [6].

WARNING: Ochratoxin A causes kidney and liver damage and is a probable carcinogen. Observe appropriate safety precautions [7] for handling such compounds and in particular avoid handling in dry form as the electrostatic nature can result in dispersion and inhalation. Glassware can be decontaminated with 4 % sodium hypochlorite solution. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO) [8], [9].

3 Precision data

The following data were obtained in inter-laboratory tests according to ISO 5725 : 1986 [1] conducted by the Max-von-Pettenkofer-Institute of the Federal Health Office, Foodchemistry Department, Berlin, Germany on wheat whole flour [5], [6].

Sample	wheat whole flour	wheat whole flour
Year of inter-laboratory test	1993	1991
Number of laboratories	13	13
Number of samples	1	1
Number of laboratories retained after eliminating outliers	13	13
Number of outliers	0	0
Number of accepted results	65	65
Mean value $\bar{x}$ (µg/kg)	0,407	1,227
Repeatability standard deviation s_r (µg/kg)	0,062	0,248
Repeatability relative standard deviation RSD_r	15,32 %	20,21%
Repeatability limit r (µg/kg)	0,176	0,702
Reproducibility standard deviation s_R (µg/kg)	0,105	0,388
Reproducibility relative standard deviation RSD_R	25,80 %	31,62 %
Reproducibility limit R (µg/kg)	0,298	1,097
Recovery	90 % ± 15 %	80 % ± 15 %

[1] ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

[2] Majerus, P., Cutka, I., Dreyer, A., El-Dessouki, S., Eyrich, W., Reusch, H., Schurer, B., and Waiblinger, H.U.: Zur Belastungssituation von Ochratoxin A in Lebensmitteln pflanzlichen Ursprungs. In: Dt. Lebensm. Rundsch., 89, Vol 4 (1993) pp 112 ff.

[3] Jiao, Y., Blaas, W., Rühl, Ch., and Weber, R.: Ochratoxin A in Lebensmitteln pflanzlicher Herkunft. In: Dt. Lebensm. Rundsch., 90, Vol 10 (1994) pp 318 ff.

[4] Jiao, Y., Blaas, W., Rühl, Ch., and Weber, R.: Identification of ochratoxin A in food samples by chemical derivatization and gas chromatography - mass spectrometry. In: J. Chromat. 595 (1992) pp. 364 - 367.

[5] Untersuchung von Lebensmitteln: Bestimmung von Ochratoxin A: L 15.00-1 1992-12 (Food Analysis: Determination of Ochratoxin A in cereals and cereal products L 15.00-1 1992-12) in: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG: Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedarfsgegenständen/Bundesgesundheitsamt (In: Collection of official methods under article 35 of the German Federal Foods Act; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office) Loseblattausgabe, Stand Aug. 1993 Bd. 1(Loose leaf edition, as of 1993 - 08 Vol. I.) Berlin, Köln, Beuth Verlag GmbH.

[6] Majerus, P., Weber, R., and Wolff, J.,: Nachweis und Bestimmung von Ochratoxin A in Getreide und Getreideprodukten (Detection and determination of Ochratoxin A in cereals and cereal products) In: Bundesgesundheitsblatt (Journal of the Federal Health Office) 37, Nov. 1994, no 11, pp.454 - 458.

[7] Tauchmann, F.; Mintzlaff, H.-J.; Leistner, L.: Schutzmaßnahmen beim Arbeiten mit Mykotoxinen (Protective measures for working with mycotoxins) Alimenta 1972, 11, 85.

[8] Castegnaro, M., Hunt, D.C., Sansone, E.B., Schuller, P.L., Siriwardana, M.G., Telling, G.M., van Egmond, H.P., and Walker, E.A.: Laboratory decontamination and destruction of aflatoxins B_1 , B_2 , G_1 and G_2 in laboratory wastes. In: IARC Scientific publication no 37, International Agency for Research on Cancer (WHO), Lyon, France; 1980, 59p.

[9] Castegnaro, M., Barek, J., Fremy, J.M., Lafontaine, M., Miraglia, M., Sansone, E.B., and Telling, G.M.: Laboratory decontamination and destruction of carcinogens in laboratory wastes. In: IARC Scientific publication no 113, International Agency for Research on Cancer (WHO), Lyon, France; 1991, 63p.

EN ISO 15141 Part 2: High performance liquid chromatographic with bicarbonate clean up

1 Scope

This European Standard specifies a method for the determination of ochratoxin A (OTA) at levels greater than 3 µg/kg.

The method has been successfully validated in inter-laboratory studies according to ISO 5725:1986 [1] on whole barley containing 2,9 µg/kg, 3,0 µg/kg, 7,4 µg/kg and 14,4 µg/kg of ochratoxin A, on whole maize containing 8,2 µg/kg and 16,3 µg/kg of ochratoxin A as well as on wheat bran containing 3,8 µg/kg and 4,5 µg/kg of ochratoxin A.

NOTE: Numerous laboratory experiences have shown that this method is also applicable to wheat flour.

2 Principle

Ochratoxin A is extracted from grains with chloroform-aqueous phosphoric acid and isolated by liquid-liquid partitioning into aqueous bicarbonate solution. The solution is applied to a C_{18} cartridge, and ochratoxin A is eluted with ethyl acetate-methanol-acetic acid. Ochratoxin A is separated by reversed phase HPLC and identified and quantified by fluorescence. Chromatography of ochratoxin A methyl ester derivative confirms the identification [2] to [5].

WARNING: Ochratoxin A causes kidney and liver damage and is a probable carcinogen. Ob-serve appropriate safety precautions [6] for handling such compounds and in particular avoid handling in dry form as the electrostatic nature can result in dispersion and inhalation. Glassware can be decontaminated with 4 % sodium hypochlorite solution. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO) [7], [8].

3 Precision data

In accordance with ISO 5725 : 1986 [1], the following parameters have been defined in an inter-laboratory test. The test was conducted by AOAC International in Cupertino with the International Union of Pure and Applied Chemistry (IUPAC) and the Nordic Committee on Food Analysis (NMKL). A total of 16 laboratories in Europe, Canada and the United States participated in the study where 10 µg/kg and 20 µg/kg of ochratoxin A was added to barley and maize [4], [5].

Sample	barley	barley	maize	maize
Year of inter-laboratory test	1990	1990	1990	1990
Number of laboratories	16	16	16	16
Number of samples	1	2	1	2
Number of laboratories retained after eliminating outliers	15	14	15	15
Number of outliers (laboratories)	1	2	1	1
Number of accepted results	15	28	15	30
Mean value $\bar{x}$ (µg/kg)	7,4	14,4	8,2	16,3
Repeatability standard deviation s_r (µg/kg)	-	1,1	-	3,3
Repeatability relative standard deviation RSD_r	-	7,9 %	-	20,1 %
Repeatability limit r (µg/kg)	-	3,1	-	9,2
Reproducibility standard deviation s_R (µg/kg)	2,0	3,8	1,7	4,6
Reproducibility relative standard deviation RSD_R	27,2 %	26,5 %	20,7 %	28,4 %
Reproducibility limit R (µg/kg)	5,6	10,6	4,8	12,9
Recovery	74 %	72 %	82 %	82 %

In a second inter-laboratory study, 12 laboratories in Europe analysed samples of wheat-bran and rye to which ochratoxin A had been added and naturally contaminated barley samples. The concentrations of ochratoxin A ranged from 3 µg/kg to 9 µg/kg [4], [5]. Results from this study are listed hereunder

Sample	barley	barley	rye	rye	wheat bran	wheat bran
Year of inter-laboratory test	1993	1993	1993	1993	1993	1993
Number of laboratories	16	16	16	16	16	16
Number of samples	2	2	2	2	2	2
Number of laboratories retained after eliminating outliers	12	12	12	12	12	12
Number of outliers (laboratories)	4	4	4	4	4	4
Number of accepted results	24	24	24	24	24	24
Mean value $\bar{x}$ (µg/kg)	3,0	2,9	4,8	2,9	4,5	3,8
Repeatability standard deviation s_r (µg/kg)	0,46	0,49	0,78	0,64	0,77	0,80
Repeatability relative standard deviation RSD_r	15,2 %	17,1 %	16,2 %	22,5 %	17,1 %	20,8 %
Repeatability limit r (µg/kg)	1,28	1,37	2,18	1,80	2,16	2,23
Reproducibility standard deviation s_R (µg/kg)	0,68	0,62	1,11	0,84	1,20	0,92
Reproducibility relative standard deviation RSD_R	22,6 %	21,7 %	23,0 %	29,2 %	26,5 %	24,2 %
Reproducibility limit R (µg/kg)	1,90	1,74	3,10	2,34	3,35	2,58
Recovery	-	-	65 %	64 %	68 %	70 %

NOTE: The recovery of ochratoxin A added to rye samples does not meet the criteria adopted by CEN/TC 275/WG 5. Hence, this European Standard is not applicable to ochratoxin A in rye.

[1] ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.

[2] AOAC INTERNATIONAL Official Methods of Analysis 16th Ed 1995, AOAC INTERNATIONAL, Gaithersburg, MD, USA, Method 991.44.

[3] Nordic Committee on Food Analysis (1992) No 143. Ochratoxin A. Liquid chromatographic determination in barley and maize.

[4] Nesheim, S., Stack, M.E., Trucksess, M.W., Eppley, R.M. and Krogh, P.: Rapid solvent-efficient method for liquid chromatographic determination of ochratoxin A in corn, barley and kidney: Collaborative study. J. Assoc. Off. Anal. Chem. 1992, 75, 481-487.

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