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BSI Standards Publication

Food analysis — Determination of furan in coffee and coffee products by headspace gas chromatography and mass spectrometry (HS GC-MS)

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

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

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Food analysis - Determination of furan in coffee and coffee products by headspace gas chromatography and mass spectrometry (HS GC-MS)

Analyse des produits alimentaires - Dosage du furane dans le café et les produits à base de café par chromatographie en phase gazeuse avec espace de tête couplée à la spectrométrie de masse (ET-CPG-SM)

Lebensmittelanalytik - Bestimmung von Furan in Kaffee und Kaffee-Erzeugnissen mit Headspace-Gaschromatographie und Massenspektrometrie (HS GC-MS)

This European Standard was approved by CEN on 7 February 2015.

EUROPEAN COMMITTEE FOR STANDARDIZATION
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EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 16620:2015) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2015 and conflicting national standards shall be withdrawn at the latest by October 2015.

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1 Scope

This European Standard specifies a method for the determination of furan in coffee and coffee products with headspace-gas chromatography-mass spectrometry (HS-GC-MS), see [1] and [2]. Coffee products in the scope of this method are extracts which have been spray-dried, agglomerated or freeze-dried. The method has been validated in an interlaboratory study via the analysis of naturally contaminated samples of spray-dried coffee, freeze-dried coffee and ground roasted coffee ranging from 264 µg/kg to 2 840 µg/kg.

2 Normative references

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

EN ISO 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

Coffee and coffee products are weighed into headspace vials, diluted with water and incubated at 50 °C for 30 min. The furan content in the headspace is measured with HS-GC-MS. Quantification is done by using a standard addition curve, taking into account deuterated furan (*d4*-furan) as internal standard.

4 Reagents

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. Solvents shall be of quality for HPLC analysis.

4.1 Furan, (CAS 110-00-9), mass concentration ρ = 0,936 g/ml.

4.2 *d4*-Furan, (CAS 6142-90-1), ρ = 0,991 g/ml.

4.3 Methanol.

4.4 Potassium hydroxide, aqueous solution, ρ = 250 g/l.

4.5 Stock solutions

4.5.1 Furan stock solution, ρ approximately 2,50 mg/ml.

Place 20 ml of methanol in a headspace vial and seal the vial. Weigh the sealed vial. Using a chilled syringe, transfer 50 µl of furan through the septum of the vial into the methanol. Reweigh the sealed vial. Mix the contents well. The solution is stable for 2 weeks at 4 °C. Pierced headspace vials shall be re-capped.

4.5.2 *d4*-Furan stock solution, ρ approximately 2,50 mg/ml.

Place 20 ml of methanol in a headspace vial and seal the vial. Weigh the sealed vial. Using a chilled syringe, transfer 50 µl of *d4*-furan through the septum of the vial into the methanol. Reweigh the sealed vial. Mix the contents well. The solution is stable for 2 weeks at 4 °C. Pierced headspace vials shall be re-capped.

4.6 Standard solutions

4.6.1 General

The amount of added solutions is calculated by differential weighing considering the densities. The solutions and additional dilutions should be kept cooled when working. Adapt the concentration of standard and internal standard solutions to the expected values in the samples. If necessary, prepare additional dilutions according to the working range. All furan and *d4*-furan solutions should be prepared in different rooms. Use separate syringes without plastic material.

4.6.2 **Furan standard solution 1**, *ρ* approximately 25 µg/ml.

Place 10 ml of water in a headspace vial and seal the vial. Weigh the sealed vial. Using a chilled syringe, transfer 100 µl of furan stock solution (4.5.1) through the septum of the vial into the water. Reweigh the sealed vial. Mix the contents well. Prepare the solution daily.

4.6.3 ***d4*-Furan standard solution 1**, *ρ* approximately 25 µg/ml.

Place 10 ml water in a headspace vial and seal the vial. Weigh the sealed vial. Using a chilled syringe, transfer 100 µl of *d4*-furan stock solution (4.5.2) through the septum of the vial into the water. Reweigh the sealed vial. Mix the contents well. Prepare the solution daily.

4.6.4 **Furan standard solution 2**, *ρ* approximately 250 ng/ml.

Place 10 ml water in a headspace vial and seal the vial. Weigh the sealed vial. Using a chilled syringe, transfer 100 µl of furan standard solution 1 (4.6.2) through the septum of the vial into the water. Reweigh the sealed vial. Mix the contents well. Prepare the solution daily.

4.6.5 ***d4*-Furan standard solution 2**, *ρ* approximately 250 ng/ml.

Place 10 ml water in a headspace vial and seal the vial. Weigh the sealed vial. Using a chilled syringe, transfer 100 µl of *d4*-furan standard solution 1 (4.6.3) through the septum of the vial into the water. Reweigh the sealed vial. Mix the contents well. Prepare the solution daily.

5 Apparatus

Usual laboratory glassware and equipment and, in particular, the following:

5.1 Positive displacement pipette.

5.2 Microlitre syringes.

5.3 Hand crimper and de-crimper.

5.4 Homogenizer or mill.

5.5 Test tube shaker.

5.6 Gas chromatograph for capillary gas chromatography, with split/splitless injector and headspace autosampler.

5.6.1 Fused silica capillary column, suitable for analysing volatile organic compounds e.g. non-polar polysiloxane with 5 % phenyl. The following column has been shown to be suitable: J&W DB-5 from Agilent[1)] length of 60 m, internal diameter (i.d.) of 0,32 mm and film thickness (d_f) of 1 µm.

5.6.2 PLOT column for separation of very volatile compounds, e.g. HP® Plot Q from Agilent[1)], length of 15 m, i.d. of 0,32 mm and d_f of 20 µm coupled to a restriction column HP-5MS from Agilent[1)], length of 30 m, i.d. of 0,25 mm and d_f of 0,25 µm. This column is an alternative for the column given under 5.6.1.

5.6.3 Rt®Q-Bond.[2)]

5.6.4 Carrier gas, helium.

5.6.5 Mass spectrometer with capability of single ion monitoring, coupled to gas chromatograph.

5.7 Headspace vials, 20 ml, with aluminium crimp seals and PTFE-faced silicone septa.

6 Procedure

6.1 Sample preparation

The samples shall be stored refrigerated in unopened original packages. Immediately prior to analysis the original packages should be opened and weighed with minimal delay to avoid losses of furan. Powdered samples should be weighed without further treatment. Coffee beans should be milled under conditions that do not raise the temperature and weighed. Although not tested in the interlaboratory study, the use of cooled milling equipment is recommended for milling in order to avoid losses of furan. Milling tests minimizing losses of furan should be performed ahead of the sample preparation.

6.2 Estimation of furan content

The furan content in a sample is quantified by means of standard addition.

The amount of furan and/or *d4*-furan which is added to the test portions is based on a rough estimation (x0) in relation to the initial sample weight.

This rough estimation (x_0) is done as follows: to 0,5 g to 5 g of the sample (normally 1 g) water is added (normally 5 ml), an amount of *d4*-furan (internal standard) in ng is added (approximately corresponding to the expected amount of furan in the sample) and is analysed with HS-GC-MS as described in 6.4.

Using the peak area ratio furan/*d4*-furan (m/z = 68/72, see 7.2) and the amount of added internal standard in ng the furan content in relation to the initial weight of the sample solution can be estimated (equivalent to a simple isotope dilution analysis).

In order to avoid the forming of furan from precursors, the pH value is adjusted to ca. pH = 10. For this 5 drops to 6 drops of potassium hydroxide solution (4.4) are added into the headspace vial.

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1) These are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

2) Rt®-Q-Bond is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

6.3 Standard addition analysis

In 9 headspace vials each 0,5 g to 5 g of the sample (normally 1 g) are weighed in, water is added (normally 5 ml) and *d4*-furan standard solution in relation to the order of magnitude of the estimated amount of furan (see 6.2) into the solution. Three headspace vials are capped immediately. The others are fortified with 0,5, 1,0 and 2,0 times of estimated furan content. The fortifying volume should not exceed 10 % of the total volume. For each new sample the concentration of the furan- and *d4*-furan standard solution (see 4.6) shall be adapted.

For an estimated furan content in the sample of 2,5 mg/kg (2,5 µg/g) and an initial weight of 1 g, the furan standard solution 1 and *d4*-furan standard solution 1 (see 4.6) can be used. Table 1 gives an example of a standard addition for an estimated furan content of 2,5 µg/g.

Table 1 — Example of a standard addition for an estimated furan content x_0 of 2,5 µg/g

Sample solution	*d4*-Furan standard solution µl	*d4*-Furan standard solution µg	Furan standard solution µl	Furan standard solution µg
P_{11} (0 x_0)	100	2,5	-	-
P_{12} (0 x_0)	100	2,5	-	-
P_{13} (0 x_0)	100	2,5	-	-
P_{21} (0,5 x_0)	100	2,5	50	1,25
P_{22} (0,5 x_0)	100	2,5	50	1,25
P_{31} (1,0 x_0)	100	2,5	100	2,5
P_{32} (1,0 x_0)	100	2,5	100	2,5
P_{41} (2,0 x_0)	100	2,5	200	5,0
P_{42} (2,0 x_0)	100	2,5	200	5,0

With the above fortifying procedure the *d4*-furan is added to the sample solutions according to the estimated furan content of the sample (here 2,5 µg/kg).

If necessary more than 2 or 3 headspace vials can be prepared for each fortifying level.

6.4 Determination with headspace-GC-MS

The following parameters should be kept:

— Incubation temperature: 50 °C

— Incubation time: 30 min

Headspace parameters:

The following headspace parameters are examples and shall be adopted to the used device:

— Needle temperature: 52 °C to 55 °C

— Shaker speed: 300 min^{-1}

— Filling speed: 200 µl/s

— Pull up delay: 500 ms

— Injection speed: 500 µl/s

— Preinject delay: 500 ms

— Postinject delay: 500 ms

— Scavenging time: 3 min

GC-MS parameters:

In combination with the recommended column (5.6.1) the following parameter settings have been proven useful:

— GC-column gas flow: 1,7 ml/min Helium (constant flow)

— Gas saver: off

— Injector: split 2:1

— Injection temperature: 200 °C

— Injection volume: 1 000 µl

— Temperature program: 50 °C, 10 °C/min to 225 °C, 225 °C for 12,5 min, run time 30 min

— Coupling to MS: direct

In combination with the recommended column (5.6.2) the following parameters have been proven themselves:

— GC-column gas flow: 1,0 ml/min Helium (constant flow)

— Gas saver: off

— Injector: split or splitless

— Injection temperature: 200 °C

— Injection volume: 1 000 µl

— Temperature program: 50 °C, 10 °C/min to 225 °C, 225 °C for 6,5 min, run time 24 min

— Coupling to MS: direct

MS parameters:

— Ionization energy: 70 eV

— SIM (single ion monitoring)

— Mode:

— Furan: m/z = 68 (100 ms) and m/z = 39 (20 ms)

— *d4*-Furan: m/z = 72 (100 ms) and m/z = 42 (20 ms)

— Alternatively: Scan m/z 35>75

— MS source temperature: 230 °C

— MS quad temperature: 150 °C

— MS transfer line: 225 °C

Table 2 gives information on ions for identification and quantification of furan for confirmation purposes.

Table 2 — Ions for identification and quantification of furan (for more information see [3])

Name	Qualifier Ion Q_1 m/z	Qualifier Ion Q_2 m/z	Peak area ratio Q_1/Q_2	Peak area ratio - lower limit	Peak area ratio - upper limit
Furan	68	39	1,56	1,40	1,71

7 Calculation

7.1 General

Quantify the furan content with standard addition and the method of internal standard via integrated peak areas of m/z = 68 for furan and m/z = 72 for *d4*-Furan. Use at least 7 points of the standard addition (see 6.3) for the calculation.

7.2 For a multipoint measurement of the sample with internal standard correction, determine the area ratio of m/z = 68 for furan and m/z = 72 for the internal standard *d4*-furan using Formula (1):

$$Q = \frac{A_A}{A_S} \tag{1}$$

where

A_A is the peak area m/z = 68 furan;

A_S is the peak area m/z = 72 *d4*-furan.

7.3 For the standard calibration graph, plot the resulting peak area ratios (Q, see 7.2) against the fortified furan amount in ng according to the standard addition series.

Calibration graph $Q = bx + a$ or rather

$$x = \frac{(Q - a)}{b} \tag{2}$$

where

x is the mass of furan in ng in the sample;

a is the intercept of the calibration graph;

b is the slope of the calibration graph.

NOTE Calculation is done automatically by any modern chromatography software (mode: calculation with internal standard).

7.4 Calculate the furan content of the sample, in µg/kg, by determination of the point of intersection with the prolonged calibration graph and the axis of abscissae.

Determine the furan content in the sample in µg/kg mathematically using Formula (3):

$$x = \frac{(Q - a)}{b} \quad (3)$$

with

Q = 0 becomes x = |-a/b |

$\rho = x/W$

where

ρ is the amount of furan in the sample (ng/g = µg/kg);

x is the mass of furan in ng in the sample (see 7.3);

W is the initial sample weight in g (see 6.1).

Result is given with 3 significant digits.

8 Precision

8.1 General

Details of the interlaboratory test of the precision of the method are summarized in Annex B. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and/or matrices other than those given in Annex B.

8.2 Repeatability

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

The values for spray-dried coffee are:

$\bar{x}$ = 264 µg/kg r = 53,5 µg/kg (naturally contaminated)

The values for freeze-dried coffee are:

$\bar{x}$ = 1 140 µg/kg r = 238 µg/kg (naturally contaminated)

The values for roasted coffee are:

$\bar{x}$ = 2 840 µg/kg r = 678 µg/kg (naturally contaminated)

8.3 Reproducibility

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

The values for spray-dried coffee are:

$\bar{x}$ = 264 µg/kg *R* = 142 µg/kg (naturally contaminated)

The values for freeze-dried coffee are:

$\bar{x}$ = 1 140 µg/kg *R* = 517 µg/kg (naturally contaminated)

The values for roasted coffee are:

$\bar{x}$ = 2 840 µg/kg *R* = 1 530 µg/kg (naturally contaminated)

9 Test report

The test report shall contain the following data:

a) all information necessary for the identification of the sample (type of sample, origin and designation of the sample);

b) a reference to this European Standard;

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

d) the date of receipt;

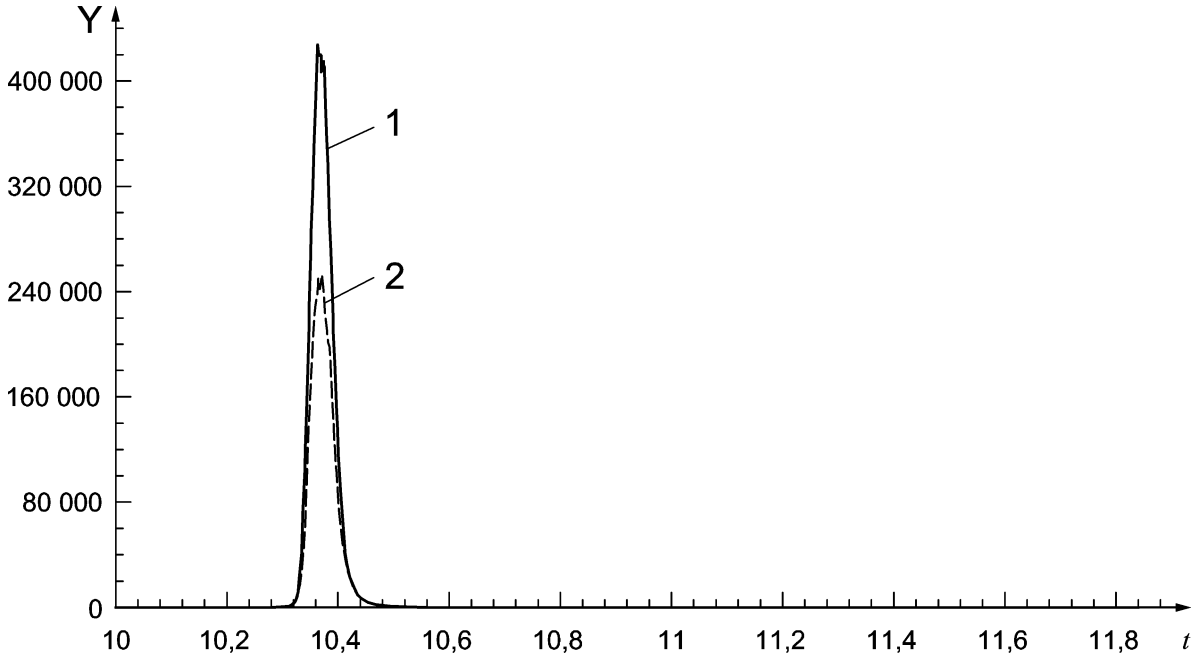
e) the date of test;

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

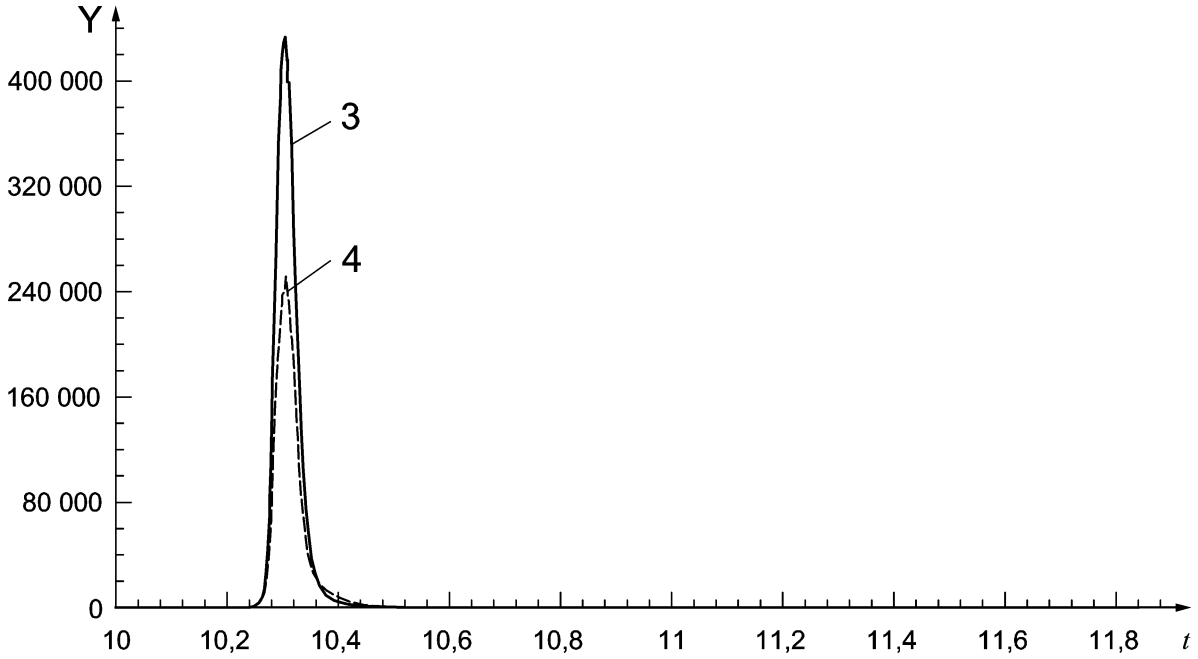
g) any operations not specified in the method or regarded as optional, which might have affected the results.

Annex A
(informative)

Typical chromatograms



a) Furan



b) *d4*-Furan

Key

t	time
Y	Abundance
1	m/z = 68
2	m/z = 39
3	m/z = 72
4	m/z = 42
Operating conditions	capillary column Rt®Q-Bond[3)] (30 m, 0,32 mm, 10 µm film thickness)
Temperature programme	50 °C (0,1 min), 10 °C/min to 190 °C (0 min), 40 °C/min to 225 °C (20 min).
Flow	2,0 ml/min

Figure A.1 — Typical chromatogram of furan and *d4*-furan

3) Rt®-Q-Bond is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

Annex B
(informative)

Precision data

The following data were obtained in an interlaboratory test according to ISO 5725-2 [4] for collaborative study procedures to validate characteristics of a method of analysis.

This method was worked out by the § 64 working group 'Furan-Analytik' of the 'Bundesamt für Verbraucherschutz und Lebensmittelsicherheit' and tested in an interlaboratory study with 15 participants.

Table B.1 — Precision data for furan in coffee with headspace GC-MS

Sample	Spray dried coffee	Freeze-dried coffee	Roasted coffee
Preparation of test material	naturally contaminated	naturally contaminated	naturally contaminated
Year of interlaboratory test	2008	2008	2008
Number of laboratories	15	15	15
Number of laboratories retained after eliminating outliers	13	14	15
Number of outliers (laboratories)	2	1	0
Number of accepted results	13	14	15
Mean value, $\bar{x}$, µg/kg	264	1 140	2 840
Repeatability standard deviation s_r, µg/kg	19,1	85,1	242,1
Repeatability relative standard deviation, RSD_r, %	7,2	7,5	8,5
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	53,5	238	678
Reproducibility standard deviation s_R, µg/kg	50,8	185	545
Reproducibility relative standard deviation, RSD_R, %	19,3	16,2	19,2
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	142	517	1 530
HorRat value, according to [5] and [6]	0,99	1,04	1,4

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