

Nederlandse norm

NEN-EN 16618

(en)

Voedingsmiddelenanalyse - Bepaling van
acrylamide in voedsel met
vloeistofchromatografie tandem
massaspectrometrie (LC-ESI-MS-MS)

Food analysis - Determination of acrylamide in
food by liquid chromatography tandem mass
spectrometry (LC-ESI-MS/MS)

Vervangt NEN-EN 16618:2013 Ontw.

ICS 67.050; 67.060; 67.080.20; 67.140.20

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 - EN 16618:2015,IDT

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EUROPEAN STANDARD

EN 16618

NORME EUROPÉENNE

EUROPÄISCHE NORM

April 2015

ICS 67.050; 67.060; 67.080.20; 67.140.20

English Version

Food analysis - Determination of acrylamide in food by liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS)

Analyse des produits alimentaires - Dosage de l'acrylamide dans les produits alimentaires par chromatographie en phase liquide couplée à la spectrométrie de masse en tandem (LC-ESI-MS/MS)

Lebensmittelanalytik - Bestimmung von Acrylamid in Lebensmitteln mit Flüssigchromatographie und Tandem-Massenspektrometrie (LC-ESI-MS/MS)

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Preview
 NEN-EN 16618:2015

Foreword

This document (EN 16618:2015) 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of an identical text or by endorsement, at the latest by October 2015 and conflicting national standards shall be withdrawn at the latest by October 2015.

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EN 16618:2015 (E)**1 Scope**

This European Standard specifies a method for the determination of acrylamide in bakery ware such as bread, toasted bread, crisp bread, butter cookies, and biscuits, as well as potato products such as potato chips, potato crisps, and potato pan cake and roasted coffee, by liquid chromatography in combination with electrospray ionization and tandem mass spectrometry (LC-ESI-MS/MS). This method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples, ranging from 14,3 µg/kg to 9 053 µg/kg. It was developed at the Swedish National Food Administration and validated in a study organized by the Directorate General Joint Research Centre (DG JRC), Swedish National Food Administration and the Nordic Committee on Food Analysis (NMKL), see [1] and [2].

The limit of quantification (LOQ) depends on the type of instrument used and on the actual performance of the instrument. The majority of the laboratories participating in the validation study were able to determine acrylamide in a butter cookie sample at a level of 14,3 µg/kg. Thus, the validation by interlaboratory study showed that LOQ can be expected to be in the range between below 15 µg/kg and 30 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 1042:1999, *Laboratory glassware - One mark volumetric flasks (ISO 1042:1998)*

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

3 Principle

Acrylamide is extracted with water and isotopic labelled acrylamide is added. The extract is centrifuged and the supernatant is cleaned up with two solid phase extraction (SPE) columns. The first SPE column contains silica based C18 groups as well as anion and cation exchangers, and since acrylamide is not retained by the column, the extract is just passed and collected. The reason for using this column is to retain as many matrix components as possible (non-polar compounds as well as anions and cations) without retaining acrylamide, i.e. this first SPE column is used as a chemical filter.

The second SPE column contains a polymer based phase with a relatively high capacity to bind acrylamide. The extract is loaded onto the column, the column is washed with water and finally eluted with a mixture of 60 parts per volume of methanol and 40 parts per volume of water. The purpose of this step, apart from further cleaning of the extract, is to concentrate the extract and to obtain low limits of quantification.

After evaporation of the methanol, the extract is analysed by LC-MS/MS. For this purpose an HPLC column with graphitized carbon as stationary phase is used, since the retention factor (k) is relatively high ($k = 4$ when no organic solvent is added in the mobile phase) compared to other commercially available columns.

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 Acrylamide (CAS 79-06-1), purity not less than 99,9 % mass fraction.

The chemical structure is:

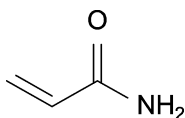


Figure 1 — Acrylamide

WARNING — Acrylamide has been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans. Protective equipment as laboratory coat, disposable gloves and safety glasses shall be used. All handlings of acrylamide and organic solvents shall be performed in a fume cupboard with adequate air flow.

4.2 Deuterium-labelled acrylamide – acrylamide-2,3,3-D₃ (CAS 122775-19-3).

The chemical structure is:

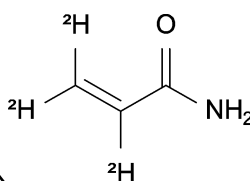


Figure 2 — Deuterium-labelled acrylamide

Alternatively, ¹³C-labelled acrylamide (acrylamide-¹³C₃, CAS 287399-26-2) may be used.

4.3 Methanol (CAS 67-58-2)

4.4 Glacial acetic acid (CAS 64-19-7).

4.5 *n*-Hexane (CAS 110-54-3).

Alternatively, cyclohexane (CAS 110-82-7) may be used.

4.6 Eluent for SPE column 2 (5.2.3)

Mix 60 parts per volume of methanol (4.3) with 40 parts per volume of water.

4.7 HPLC mobile phase

Mix 1 part per volume of glacial acetic acid (4.4) with 1 000 parts per volume of water.

4.8 Stock solutions of acrylamide and acrylamide-2,3,3-D₃, mass concentration $\rho = 1\ 000\ \mu\text{g/ml}$.

Weigh, to the nearest 0,05 mg, approximately 100 mg of acrylamide and acrylamide-2,3,3-D₃ respectively into separate 100 ml volumetric flasks, dissolve in water and dilute to 100 ml. Solutions can be stored at 4 °C for at least 3 months.

4.9 Internal standard solution 1, $\rho = 10\ \mu\text{g/ml}$.

Transfer 1 000 μl of the stock solution of acrylamide-2,3,3-D₃ (4.8) to a 100 ml volumetric flask and dilute to the mark with water.

EN 16618:2015 (E)**4.10 Internal standard solution 2, $\rho = 1\ 000$ ng/ml.**

Transfer 5 000 μl of the internal standard solution 1 (4.9) to a 50 ml volumetric flask and dilute to the mark with water.

4.11 Acrylamide standard solution 1, $\rho = 100$ $\mu\text{g/ml}$.

Transfer 5 000 μl of the stock solution of acrylamide (4.8) to a 50 ml volumetric flask and dilute to the mark with water.

4.12 Acrylamide standard solution 2, $\rho = 10$ $\mu\text{g/ml}$.

Transfer 5 000 μl of the acrylamide standard solution 1 (4.11) to a 50 ml volumetric flask and dilute to the mark with water.

4.13 Acrylamide standard solution 3, $\rho = 100$ ng/ml.

Transfer 1 000 μl of the acrylamide standard solution 2 (4.12) to a 100 ml volumetric flask and dilute to the mark with water.

4.14 LC-MS calibration solutions

Dilute aliquots from standard solutions (4.9), (4.11), (4.12) and (4.13) with water to give calibration solutions of e.g. 0 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 50 ng/ml, 100 ng/ml, 250 ng/ml, 500 ng/ml, 1 000 ng/ml, 2 000 ng/ml, 5 000 ng/ml and 10 000 ng/ml respectively of acrylamide, all containing 400 ng/ml of acrylamide-2,3,3- D_3 . Examples for the preparation of calibration solutions are given in Table 1. Table 2 indicates the relation between calibration solution concentrations and acrylamide contents of food samples. Calibration shall be performed on at least six concentration levels distributed properly over the working range. The analysis of an even higher number of calibration solutions should be analysed if such a broad range of concentrations (0 $\mu\text{g/kg}$ to 10 000 $\mu\text{g/kg}$) shall be covered.

Table 1 — Preparation of LC-MS calibration solutions

Calibration solution ng/ml	Volumetric flask ml	Internal standard solution (4.9) μl	Acrylamide standard solution μl
0	100	4 000	0
5	100	4 000	5 000 of (4.13)
10	100	4 000	10 000 of (4.13)
20	100	4 000	200 of (4.12)
50	100	4 000	500 of (4.12)
100	100	4 000	1 000 of (4.12)
250	100	4 000	2 500 of (4.12)
500	100	4 000	5 000 of (4.12)
1 000	100	4 000	1 000 of (4.11)
2 000	100	4 000	2 000 of (4.11)
5 000	100	4 000	5 000 of (4.11)
10 000	50	4 000	5 000 of (4.11)

Table 2 — Relation between acrylamide contents of calibration solutions and contents in food

Calibration solution ng/ml	Bakery and potato products µg/kg	Roasted coffee µg/kg
10	10	50

5 Apparatus

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

5.1 LC-MS/MS system

5.1.1 HPLC apparatus, comprising the following:

5.1.1.1 Thermostated column compartment.

5.1.1.2 Injection system, capable of injecting 10 µl of sample.

5.1.1.3 Mobile phase pump, capable of maintaining a mobile phase flow of 0,4 ml/min.

5.1.2 HPLC column

The stationary phase of the column is graphitized carbon^{1a)}, particle size 5 µm, 50 mm x 2,1 mm with a guard column^{1a)}, particle size 5 µm, 10 mm x 2 mm.

Alternative columns/stationary phases may be applied provided that similar performance to the graphitized carbon column can be demonstrated.

5.1.3 Mass spectrometer

Triple quadrupole mass spectrometer operating in positive electrospray and, selected reaction monitoring mode (SRM), set to obtain unit resolution.

5.1.4 Data acquisition and analysis system

Suitable data collection and evaluation software.

5.1.5 Divert valve (optional)

HPLC valve installed between HPLC column and mass spectrometer in order to direct the HPLC effluent either to waste or to the mass spectrometer, see 7.1.1.

5.2 Solid phase extraction system

5.2.1 Vacuum manifold for solid phase extraction

1) a) Hypercarb™ column, Thermo Hypersil-Keystone® column, b) ISOLUTE® Multimode SPE column and c) ISOLUTE® ENV+ SPE column from Biotage® 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. Equivalent products may be used if they can be shown to lead to the same results.

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