SVENSK STANDARD
SS-EN 16618:2015

Fastställd/Approved: 2015-05-06
Publicerad/Published: 2015-05-08
Utgåva/Edition: 1
Språk/Language: engelska/English
ICS: 67.050; 67.060; 67.080.20; 67.140.20

SWEDISH STANDARDS INSTITUTE

Livsmedelsanalyser – Bestämning av akrylamid i livsmedel med vätskekromatografi/tandem mass spektrometri (LC-ESI-MS/MS)

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

Standarder får världen att fungera

SIS (Swedish Standards Institute) är en fristående ideell förening med medlemmar från både privat och offentlig sektor. Vi är en del av det europeiska och globala nätverk som utarbetar internationella standarder. Standarder är dokumenterad kunskap utvecklad av framstående aktörer inom industri, näringsliv och samhälle och befrämjar handel över gränser, bidrar till att processer och produkter blir säkrare samt effektiviserar din verksamhet.

Delta och påverka
Som medlem i SIS har du möjlighet att påverka framtida standarder inom ditt område på nationell, europeisk och global nivå. Du får samtidigt tillgång till tidig information om utvecklingen inom din bransch.

Ta del av det färdiga arbetet
Vi erbjuder våra kunder allt som rör standarder och deras tillämpning. Hos oss kan du köpa alla publikationer du behöver – allt från enskilda standarder, tekniska rapporter och standard-paket till handböcker och onlinetjänster. Genom vår webbtjänst e-nav får du tillgång till ett lättnavigerat bibliotek där alla standarder som är aktuella för ditt företag finns tillgängliga. Standarder och handböcker är källor till kunskap. Vi säljer dem.

Utveckla din kompetens och lyckas bättre i ditt arbete
Hos SIS kan du gå öppna eller företagsinterna utbildningar kring innehåll och tillämpning av standarder. Genom vår närhet till den internationella utvecklingen och ISO får du rätt kunskap i rätt tid, direkt från källan. Med vår kunskap om standarders möjligheter hjälper vi våra kunder att skapa verklig nytta och lönsamhet i sina verksamheter.

Vill du veta mer om SIS eller hur standarder kan effektivisera din verksamhet är du välkommen in på www.sis.se eller ta kontakt med oss på tel 08-555 523 00.

Standards make the world go round

SIS (Swedish Standards Institute) is an independent non-profit organisation with members from both the private and public sectors. We are part of the European and global network that draws up international standards. Standards consist of documented knowledge developed by prominent actors within the industry, business world and society. They promote cross-border trade, they help to make processes and products safer and they streamline your organisation.

Take part and have influence
As a member of SIS you will have the possibility to participate in standardization activities on national, European and global level. The membership in SIS will give you the opportunity to influence future standards and gain access to early stage information about developments within your field.

Get to know the finished work
We offer our customers everything in connection with standards and their application. You can purchase all the publications you need from us - everything from individual standards, technical reports and standard packages through to manuals and online services. Our web service e-nav gives you access to an easy-to-navigate library where all standards that are relevant to your company are available. Standards and manuals are sources of knowledge. We sell them.

Increase understanding and improve perception
With SIS you can undergo either shared or in-house training in the content and application of standards. Thanks to our proximity to international development and ISO you receive the right knowledge at the right time, direct from the source. With our knowledge about the potential of standards, we assist our customers in creating tangible benefit and profitability in their organisations.

If you want to know more about SIS, or how standards can streamline your organisation, please visit www.sis.se or contact us on phone +46 (0)8-555 523 00

Europastandarden EN 16618:2015 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN 16618:2015.

The European Standard EN 16618:2015 has the status of a Swedish Standard. This document contains the official English version of EN 16618:2015.

Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS Förlag AB som även lämnar allmänna upplysningar om svensk och utländsk standard.

Information about the content of the standard is available from the Swedish Standards Institute (SIS), telephone +46 8 555 520 00. Standards may be ordered from SIS Förlag AB, who can also provide general information about Swedish and foreign standards.

Denna standard är framtagen av kommittén för Livsmedel och foder, SIS/TK 435.

Har du synpunkter på innehållet i den här standarden, vill du delta i ett kommande revideringsarbete eller vara med och ta fram andra standarder inom området? Gå in på www.sis.se - där hittar du mer information.

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16618

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 (CL-ESI-SM-SM)

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

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

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

WARNING — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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 9 083 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:

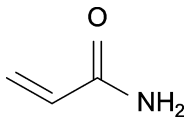

Figure 1 — Acrylamide

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-D_3 (CAS 122775-19-3).

The chemical structure is:

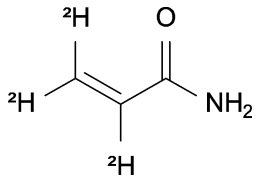

Figure 2 — Deuterium-labelled acrylamide

Alternatively, ^{13}C-labelled acrylamide (acrylamide-$^{13}C_3$, CAS 287399-26-2) may be used.

4.3 Methanol (CAS 67-56-1).

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 ρ = 1 000 µg/ml.

Weigh, to the nearest 0,05 mg, approximately 100 mg of acrylamide and acrylamide-2,3,3-D_3 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, ρ = 10 µg/ml.

Transfer 1 000 µl of the stock solution of acrylamide-2,3,3-D_3 (4.8) to a 100 ml volumetric flask and dilute to the mark with water.

4.10 Internal standard solution 2, ρ = 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, ρ = 100 µg/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, ρ = 10 µg/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, ρ = 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 µg/kg to 10 000 µg/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.