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SWEDISH STANDARDS INSTITUTE

Livsmedelsanalyser – Bestämning av bens[a]pyrene, bens[a] anthracene, chrysene and benso[b]fluoranthene i livsmedel med gaskromatografi/ mass spektrometri (GC-MS)

Food analysis – Determination of benzo[a]pyrene, benz[a] anthracene, chrysene and benzo[b]fluoranthene in foodstuffs by gas chromatography mass spectrometry (GC-MS)

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The European Standard EN 16619:2015 has the status of a Swedish Standard. This document contains the official English version of EN 16619:2015.

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

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16619

April 2015

ICS 67.050

English Version

Food analysis - Determination of benzo[a]pyrene, benz[a]anthracene, chrysene and benzo[b]fluoranthene in foodstuffs by gas chromatography mass spectrometry (GC-MS)

Analyse des produits alimentaires - Dosage du benzo(a)pyrène, benzo(a)anthracène, chrysène et benzo(b)fluoranthène dans les denrées alimentaires par chromatographie en phase gazeuse couplée à la spectrométrie de masse (CG-SM)

Lebensmittelanalytik - Bestimmung von Benzo[a]pyren, Benz[a]anthracen, Chrysen und Benzo[b]fluoranthen in Lebensmitteln mit Gaschromatographie und Massenspektrometrie (GC-MS)

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

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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 16619:2015) 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of an identical text or by endorsement, at the latest by October 2015 and conflicting national standards shall be withdrawn at the latest by October 2015.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 4 of the 16 EU priority polycyclic aromatic hydrocarbons (PAHs), identified as target PAHs. They are benz[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbF) and chrysene (CHR). The method allows their quantification in the presence of the other 12 EU priority PAHs (benzo[*j*]fluoranthene (BjF), cyclopenta[*cd*]pyrene (CPP), benzo[*k*]fluoranthene (BkF), dibenz[*a,h*]anthracene (DhA), benzo[*c*]fluorene (BcL), dibenzo[*a,e*]pyrene (DeP), benzo[*ghi*]perylene (BgP), dibenzo[*a,h*]pyrene (DhP), dibenzo[*a,i*]pyrene (DiP), dibenzo[*a,l*]pyrene (DlP), indeno[1,2,3-*cd*]pyrene (IcP), 5-methylchrysene (5MC)) in extruded wheat flour, smoked fish, dry infant formula, sausage meat, freeze-dried mussels, edible oil and wheat flour, by gas-chromatography mass-spectrometry (GC-MS). The extraction of PAHs from solid samples is performed by pressurized liquid extraction (PLE). Soxhlet extraction was applied by some participants in the method validation study by collaborative trial as alternative to PLE. The sample cleanup is performed by applying the following techniques in the reported sequence: size exclusion chromatography (SEC), and solid phase extraction (SPE).

This method complies with the performance characteristics specified in Commission Regulation (EU) No 836/2011 (see [1]). In particular the specifications for the limit of detection (LOD) and of the limit of quantification (LOQ) (0,30 µg/kg and 0,90 µg/kg respectively) were met.

The method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples, ranging from 0,5 µg/kg to 11,9 µg/kg. However, linearity of the instrument response was proven for the concentration range 0,5 µg/kg to 20 µg/kg.

For the determination of PAHs in edible fats and oils, two other standards are also available, EN ISO 22959 and EN ISO 15753, for more information see [2] and [3].

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

The sample is homogenized. A test portion is mixed with desiccant, sand and the stable isotope labelled internal standard solution. It is then extracted with *n*-hexane or cyclohexane by pressurized liquid extraction, or alternatively by Soxhlet extraction. If applicable, co-extracted water is separated from the organic phase of the extract. The organic extract is evaporated to a small volume, filtered and purified by SEC, using a mixture of ethyl acetate and cyclohexane as eluent.

After SEC, 200 µl of toluene are added as a keeper to the collected SEC fraction. The SEC fraction is evaporated to about 200 µl, and cleaned up by SPE on silica, using cyclohexane as eluent. The cleaned up sample extract is evaporated again to about 200 µl. Finally, an injection standard solution is added to the sample prior to measurement by GC-MS.

The injection is performed with a PTV, or split/splitless injection port. The chromatographic separation is obtained on a mid-polar capillary column with high selectivity for PAHs. The analytes are ionised by electron ionization (EI) at 70 eV. The target PAHs are recorded in Single Ion Monitoring (SIM) mode, and quantified by comparison with the stable isotope labelled analogues.

4 Reagents

4.1 General

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. All reagents and standard solutions shall be stored according to the specifications given by the supplier. The specifications given in this procedure for opened commercial solutions or for in-house prepared solutions aim to minimize solvent evaporation and to protect the analytes (PAHs) from degradation.

Standard solutions are preferably prepared gravimetrically. Depending on the handled amount of substance a micro-balance (6.4) and/or an analytical balance (6.5) are used for the preparation of solutions of both native and stable isotope labelled PAHs. All concentrations are expressed as mass per mass. If necessary, the concentrations expressed as mass per volume could be obtained applying the density equation (Formula (1)).

$$\rho = \frac{m}{v} \tag{1}$$

where

ρ density (in g/ml);

m measured mass of the substance (in g);

v volume of the solution (in ml).

The density of toluene at 20 °C is 0,8669 g/ml. Comprehensive information on the density of solvents at various temperatures is given in [4].

All solutions and substances are used at room temperature.

WARNING 1 — Some PAHs are considered carcinogenic. Persons using this document should be familiar with normal laboratory practices. It is the responsibility of the user of this document to apply practices which are in agreement with applicable occupational safety and health practices.

WARNING 2 — Dispose chemical waste according to applicable environmental rules and regulations.

WARNING 3 — PAHs are degraded by UV light. Protect PAHs solutions from light (keep in the dark, use aluminium foil or amber glassware).

WARNING 4 — Some precaution is needed when using plastics as polypropylene or PTFE because the analytes may be absorbed onto these materials.

4.2 Helium purified compressed gas (purity equivalent to 99,995 % or better).

4.3 Nitrogen purified compressed gas (purity equivalent to 99,995 % or better).

4.4 Disodium sulfate, (Na_2SO_4), anhydrous, granular.

4.5 Poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide) granular, 90 µm to 850 µm particle size.

4.6 Sand, 50 mesh to 70 mesh particle size.

4.7 *n*-Hexane.

4.8 Acetone.

4.9 Cyclohexane.

4.10 Toluene.

4.11 Ethyl acetate.

4.12 SEC eluent

Mix 1 part per volume of cyclohexane (4.9) with 1 part per volume of ethyl acetate (4.11).

4.13 SPE column

For the solid phase extraction cleanup, a silica SPE column is used. Commercial cartridges of 500 mg – 4 ml or self-filled cartridges of the same size containing 500 mg activated silica are used. The surface area of the silica should be around 500 m^2/g.

NOTE Commercial SPE columns made of polypropylene were used in the method validation study by collaborative trial.

4.14 Reference material for quality control

A certified reference material, or any other suitable quality control material (e.g left over proficiency test material) may be applied for this purpose. The CITAC/Eurachem Guide to Quality in Analytical Chemistry may be consulted for guidance, see [5].

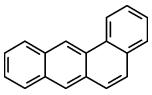
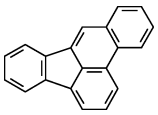
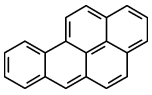
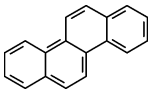
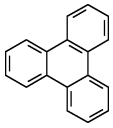
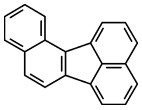
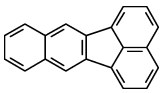
Analyse this material with every sample batch and use it to control the method performances along time (see 10.4).

4.15 Native reference substances - commercially available neat material or solutions of PAHs

The list of native substances analysed with this method is provided in Table 1. The target analytes are given in bold font. Commercially available, preferably certified, standard solutions are preferred due to the higher level of safety in handling.

Triphenylene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene are potentially interfering with the target analytes and are therefore used for evaluation of selectivity.

Table 1 — Names and structures of the native PAHs

Name[a]	CAS number	Structure	Name [a]	CAS number	Structure
Benz[*a*]anthracene (BaA)	56–55–3	 **Figure 1**	**Benzo[*b*]fluoranthene (BbF)**	205–99–2	 **Figure 2**
Benzo[*a*]pyrene (BaP)	50–32–8	 **Figure 3**	**Chrysene (CHR)**	218–01–9	 **Figure 4**
Triphenylene (TRP)	217–59–4	 **Figure 5**	Benzo[*j*]fluoranthene (BjF)	205–82–3	 **Figure 6**
Benzo[*k*]fluoranthene (BkF)	207–08–9	 **Figure 7**			

[a] The acronym is given in parenthesis, the target analytes are given in bold.

4.16 Stable isotope labelled reference standards (in the form of commercially available stable isotope labelled PAH solutions)

The stable isotope labelled analogues, applied for the quantification of the target PAHs are listed in Table 2. The commercial solutions used in the method validation study by collaborative trial contained the stable isotope labelled PAHs at a level of about 100 µg/kg in nonane.

Preference is given to ^{13}C labelled analogues as their chemical properties best match those of the native analytes.

However, alternatively to ^{13}C labelled substances, deuterated analogues of the target analytes may be applied. The concentration levels of these solutions should be similar to the levels specified for the ^{13}C labelled PAH solutions.

NOTE 1 Highly deuterated PAHs are separated on the specified GC-column at least partially from their native analogues.

NOTE 2 Both forms of benz[*a*]anthracene-$^{13}C_6$, which are displayed in Table 2, are equally suitable for the purpose of this standard. The ^{13}C labelled reference material might be even supplied as a mixture, which was the case in the method validation study by collaborative trial.