DRAFT FOR DEVELOPMENT

DD CEN/TS
13130-10:2005

Materials and articles in contact with foodstuffs — Plastics substances subject to limitation —

Part 10: Determination of acrylamide in food simulants

ICS 67.250

BSI
British Standards

National foreword

This Draft for Development is the official English language version of CEN/TS 13130-10:2005.

This publication is not to be regarded as a British Standard.

It is being issued in the Draft for Development series of publications and is of a provisional nature because the method was not evaluated using recognized ring trial procedures. As a consequence there are no reproducibility data for the method. It should be applied on this provisional basis, so that information and experience of its practical application may be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the European organization responsible for its conversion to a European standard. A review of this publication will be initiated 2 years after its publication by the European organization so that a decision can be taken on its status at the end of its 3-year life. Notification of the start of the review period will be made in an announcement in the appropriate issue of *Update Standards*.

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into a European Standard, to extend the life of the Technical Specification or to withdraw it. Comments should be sent in writing to the Secretary of BSI Subcommittee CW/47/1 Migration from plastics, at British Standards House, 389 Chiswick High Road, London W4 4AL, giving the document reference and clause number and proposing, where possible, an appropriate revision of the text.

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

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the *BSI Catalogue* under the section entitled "International Standards Correspondence Index", or by using the "Search" facility of the *BSI Electronic Catalogue* or of British Standards Online.

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Summary of pages

This document comprises a front cover, an inside front cover, the CEN/TS title page, pages 2 to 15 and a back cover.

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TECHNICAL SPECIFICATION

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English version

Materials and articles in contact with foodstuffs - Plastics substances subject to limitation - Part 10: Determination of acrylamide in food simulants

Matériaux et objets en contact avec les denrées alimentaires - Substances dans les matières plastiques soumises à des limitations - Partie 10 : Détermination de l'acrylamide dans les simulants d'aliments

Werkstoffe und Gegenstände in Kontakt mit Lebensmitteln - Substanzen in Kunststoffen, die Beschränkungen unterliegen - Teil 10: Bestimmung von Acrylamid in Prüflebensmitteln

This Technical Specification (CEN/TS) was approved by CEN on 16 December 2004 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

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Contents

page

Foreword

This document (CEN/TS 13130-10:2005) has been prepared by Technical Committee CEN/TC 194 “Utensils in contact with food”, the secretariat of which is held by BSI.

This part of EN 13130 has been prepared within the Standards, Measurement and Testing project, MAT1-CT92-0006, “*Development of Methods of Analysis for Monomers*” and has been prepared by Subcommittee (SC 1) of TC 194 "Utensils in contact with food" as one of a series of test methods for plastics materials and articles in contact with foodstuffs.

This standard is intended to support Directives 2002/72/EC [1], 89/109/EEC [2], 82/711/EEC [3] and its amendments 93/8/EEC [4] and 97/48/EC [5], and 85/572/EEC [6].

At the time of preparation and publication of this part of EN 13130 the European Union legislation relating to plastics materials and articles intended to come into contact with foodstuffs is incomplete. Further Directives and amendments to existing Directives are expected which could change the legislative requirements which this standard supports. It is therefore strongly recommended that users of this standard refer to the latest relevant published Directive(s) before commencement of a test or tests described in this standard.

This part of EN 13130 should be read in conjunction with EN 13130-1.

Further parts of EN 13130, under the general title *Materials and articles in contact with foodstuffs - Plastics substances subject to limitation*, have been prepared, and others are in preparation, concerned with the determination of specific migration from plastics materials into foodstuffs and food simulants and the determination of specific monomers and additives in plastics. The parts of EN 13130 are as follows.

Part 1: *Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants*

Part 2: *Determination of terephthalic acid in food simulants*

Part 3: *Determination of acrylonitrile in food and food simulants*

Part 4: *Determination of 1,3-butadiene in plastics*

Part 5: *Determination of vinylidene chloride in food simulants*

Part 6: *Determination of vinylidene chloride in plastics*

Part 7: *Determination of monoethylene glycol and diethylene glycol in food simulants*

Part 8: *Determination of isocyanates in plastics*

Part 9: *Determination of acetic acid, vinyl ester in food simulants*

Part 10: *Determination of acrylamide in food simulants*

Part 11: *Determination of 11-aminoundecanoic acid in food simulants*

Part 12: *Determination of 1,3-benzenedimethanamine in food simulants*

Part 13: *Determination of 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A) in food simulants*

Part 14: *Determination of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indoline in food simulants*

Part 15: *Determination of 1,3-butadiene in food simulants*

Part 16: *Determination of caprolactam and caprolactam salt in food simulants*

Part 17: *Determination of carbonyl chloride in plastics*

Part 18: *Determination of 1,2-dihydroxybenzene, 1,3-dihydroxybenzene, 1,4-dihydroxybenzene, 4,4'-dihydroxybenzophenone and 4,4'dihydroxybiphenyl in food simulants*

Part 19: *Determination of dimethylaminoethanol in food simulants*

Part 20: *Determination of epichlorohydrin in plastics*

Part 21: *Determination of ethylenediamine and hexamethylenediamine in food simulants*

Part 22: *Determination of ethylene oxide and propylene oxide in plastics*

Part 23: *Determination of formaldehyde and hexamethylenetetramine in food simulants*

Part 24: *Determination of maleic acid and maleic anhydride in food simulants*

Part 25: *Determination of 4-methyl-pentene in food simulants*

Part 26: *Determination of 1-octene and tetrahydrofuran in food simulants*

Part 27: *Determination of 2,4,6-triamino-1,3,5-triazine in food simulants*

Part 28: *Determination of 1,1,1-trimethylolpropane in food simulants*

Parts 1 to 8 are European Standards. Parts 9 to 28 are Technical Specifications.

WARNING All chemicals are hazardous to health to a greater or lesser extent. It is beyond the scope of this Technical Specification to give instructions for the safe handling of all chemicals, that meet, in full, the legal obligations in all countries in which this Technical Specification may be followed. Therefore, specific warnings are not given and users of this Technical Specification should ensure that they meet all the necessary safety requirements in their own country.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this CEN Technical Specification: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Introduction

Acrylamide, PM/Ref. No 10630, is a monomer used in the manufacture of certain plastics materials and articles intended to come into contact with foodstuffs. After the manufacture, residual acrylamide can remain in the polymer and may migrate into foodstuffs coming into contact with that product.

The method has been pre-validated in a validation experiment only by one laboratory (developing laboratory).

NOTE 1 The analytical method described in this part of EN 13130 is the result of a study for the determination of the migration of acrylamide from plastic materials into food simulants. In the course of the study several problems were encountered and solutions for these problems were incorporated in the final method. The most suitable and straightforward method is described in this part of EN 13130. The method was successfully pre-validated by the developing laboratory, using the four official EU food simulants to establish the precision data at the restriction criterion. Also migration tests were performed with samples containing acrylamide as monomer in contact with the four simulants for 10 d at 40 °C. Recovery of acrylamide from fortified simulants at the restriction level was found satisfactory. On testing of the method by a second laboratory it appeared that the testing laboratory was not able to achieve the required detection limit of 0,01 mg/kg. Therefore determination of the reproducibility was not performed by the testing lab. In addition the testing laboratory experienced difficulties with the stability of the base line when operating the HPLC UV detector at 202 nm. Based on the problems encountered by the testing laboratory the method description was modified to make critical points more clear.

NOTE 2 The confirmation method as given in this method description could be followed by the testing laboratory for water and olive oil, although again the detection limit could not be achieved. The lowest possible level was approximately 0,02 mg/kg. In the determination of acrylamide in 15 % v/v aqueous ethanol and 3 % w/v aqueous acetic acid, separation of the simulant and acrylamide was problematic. The testing laboratory concluded that the method was not suitable for the intended purpose. When however, the migration experiment is carried out in a more favourable ratio of contact area to simulant then the level of determination can be increased by -at most- a factor of five. In that case problems with detection limit and base line stability will be much less, and the method may appear to be suitable for the intended purpose.

Within the scope of the Standards, Measurement and Testing project “Monomers” it was not possible to re-test the method and therefore the method described is considered to be a useful analytical method, with limited validation data. Further testing is required to demonstrate that the method can be applied with the required accuracy and limit of detection.

1 Scope

This document, part of EN 13130, specifies an analytical procedure for the determination of acrylamide in the food simulants water, 3 % w/v aqueous acetic acid, 15 % v/v aqueous ethanol and fat simulant. The level of acrylamide monomer determined is expressed as milligrams of acrylamide/kg of food simulant. The method is appropriate for the quantitative determination of acrylamide in approximate analyte concentration range of 0,01mg/kg to 0,1 mg/kg of food simulants.

The method should also be applicable to other fat simulants.

NOTE The suitability of the fat simulant should be assessed prior to setting up migration tests - it may be found necessary to use sunflower oil or a mixture of synthetic triglycerides if unacceptable interferences are found with olive oil.

2 Normative references

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

EN 13130-1:2004, *Materials and articles in contact with foodstuffs – Plastics substances subject to limitation – Part 1: Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants.*

3 Principle

The level of acrylamide in food simulants is determined by direct injection of aqueous food simulants for analysis by high performance liquid chromatography with an ion exclusion column and ultra violet, UV, detection. Fat simulants are extracted with water and the aqueous extracts than analyzed by high performance liquid chromatography (HPLC). Quantification is achieved using external standards.

Confirmation of the identity of acrylamide is established by means of reversed phase HPLC using a column of different polarity but the same detection as used in the quantitative determination.

4 Reagents

NOTE All reagents should be of recognized analytical quality unless otherwise stated.

4.1 Analyte

Acrylamide, CH_2:CH $CONH_2$, molecular weight 71,08, purity greater than 99 %.

4.2 Chemicals

4.2.1 Acetonitrile HPLC grade, suitable for low UV wavelength applications.

4.2.2 Methanol HPLC grade

4.2.3 Water HPLC grade

4.2.4 Sulfuric acid 0,05 mol/l in water

4.3 Solutions

4.3.1 Stock solution of acrylamide in methanol (500 μg/ml)

Weigh to the nearest 0,1 mg approximately 0,05 g of acrylamide into a 100 ml volumetric flask. Dissolve the acrylamide in methanol and fill up to the mark with methanol. Close and mix thoroughly.

Calculate the actual concentration in μg acrylamide/ml solution.

Repeat the procedure to provide a second stock solution.

Store the stock solutions at 5 °C for up to 3 months protected from light in septum capped glass vials with minimum headspace.

4.3.2 Diluted stock solution (10 μg/ml)

Using a graduated pipette, transfer 1,0 ml of the acrylamide stock solution (4.3.1) to a 50 ml volumetric flask and fill to the mark with methanol. This solution contains nominally 10 μg per millilitre of acrylamide.

Repeat the procedure using the second stock solution.

4.3.3 Intermediate standards

Using graduated pipettes, transfer 0 ml, 0,5 ml, 1,0 ml, 2,0 ml, 3,0 ml and 4,0 ml of the 10 μg/ml diluted stock solution (4.3.2) to a series of 10 ml volumetric flasks. Dilute to the mark with methanol and mix. These standards correspond nominally to 0 μg/ml, 0,5 μg/ml, 1,0 μg/ml, 2,0 μg/ml, 3,0 μg/ml and 4,0 μg/ml acrylamide.

4.3.4 HPLC mobile phase

Using a measuring cylinder, transfer 70 ml of 0,05 mol/l sulfuric acid (4.2.4) to a 1 litre volumetric flask and dilute to about 500 ml with water (4.2.3). Add, using a measuring cylinder, 70 ml of acetonitrile (4.2.1) and dilute to the mark with water (4.2.3).

NOTE This solution may require de-gassing prior to use.

5 Apparatus

NOTE An instrument or item of apparatus is listed only where it is special, or made to a particular specification, usual laboratory glassware and equipment being assumed to be available.

5.1 High performance liquid chromatograph, equipped with a ultraviolet detector (UV).

Appropriate operating conditions shall be established for the specific equipment used for the determination.

NOTE The HPLC pump should be able to deliver an almost pulse free flow. The detector should be capable of giving a stable baseline response after the column has been stabilized for a few hours with the mobile phase. The detector should preferably be capable of achieving a detection limit of 0,01 mg/kg acrylamide in food simulants, see 7.1.

5.2 Ion exclusion column, capable of the separation of acrylamide from substances present in the food simulants. The column packing is based upon a styrene divinylbenzene polymer with sulfonated (cationic ion-exchange) groupings.

NOTE The following column and chromatographic conditions have been found to be suitable:

Column	Dionex Ion Pac ICE-ASI 250 x 7,6 mm
Eluent	mobile phase as prepared in 4.3.4
Flow rate	1,5 ml/min
Detector	UV 202 nm
Injection	loop 25 µl
Temperature	stabilized at room temperature

5.3 Sample vials, glass 120 ml capacity with polytetrafluoroethylene (PTFE) coated crimp top closures.

5.4 Graduated pipettes, 1 ml, 2 ml, 5 ml.

5.5 HPLC disposable membrane filters, 0,2 µm.

6 Samples

6.1 General

The laboratory samples of food simulants to be analyzed shall be obtained as described in EN 13130-1. Samples shall be kept refrigerated at 4 °C in closed containers with the exclusion of light.

NOTE 1 Acrylamide-free simulants of the same type as those to be analyzed are also required for calibration purposes.

NOTE 2 Depending on the available equipment it may not be possible to achieve the required detection limit of 0,01 mg/kg food simulant. In that case migration experiments (see EN 13130-1) should be performed using a different ratio of contact area/food simulant than 6 dm^2/ kg. EN 13130-1 allows for a concentration factor up to 5 provided the saturation of the food simulant is not approached. The high solubility of acrylamide in aqueous simulants justifies migration experiment to be carried out at ratios of 6 dm^2/200 ml food simulant.

6.2 Test sample preparation

6.2.1 Aqueous food simulants

The aqueous food simulants require no pre-treatment except filtration, using an HPLC membrane filter, if cloudy.

6.2.2 Fat simulant

Weigh 50 g ± 0,5 g of fat simulant obtained from the migration test (see EN 13130-1) into a 120 ml vial. Add 1,0 ml of the 0 µg/ml intermediate standard using a graduated pipette and mix well. Add 25 ml ± 0,5 ml of

water and shake vigorously for 1 min. Upturn the vial and allow the phases to separate for about 20 min. Withdraw about 4 ml of the lower aqueous phase using a syringe and filter using an HPLC membrane filter.

6.3 Blank sample preparation

Treat food simulants, free of acrylamide and which have not been in contact with packaging material, in the same way as described in 6.2.1 and 6.2.2.

6.4 Calibration sample preparation

NOTE Calibration solutions should be adapted to the ratio of contact area/food simulant in case a deviating ratio was used in the migration experiments.

6.4.1 Aqueous simulants

Into a series of 50 ml volumetric flasks add, by pipette, 1 ml of each of the intermediate standards (4.3.3) and dilute to the mark with the appropriate acrylamide-free aqueous food simulant. Mix thoroughly. These standards correspond nominally to approximately 0,0 μg/ml, 0,01 μg/ml, 0,02 μg/ml, 0,04 μg/ml, 0,06 μg/ml and 0,08 μg acrylamide per millilitre of food simulant.

Calculate the exact concentration in micrograms acrylamide per millilitre food simulant.

Repeat the procedure for the second set of standard solutions.

6.4.2 Fat simulant

Weigh 50 g ± 0,5 g of acrylamide-free fat simulant into a series of 120 ml vials. Add 1,0 ml of each intermediate standard using a graduated pipette and mix well. Add 25 ml ± 0,5 ml of water and shake vigorously for 1 min. Upturn the vial and allow the phases to separate for about 20 min. Withdraw about 4 ml of the lower aqueous phase using a syringe and filter using an HPLC membrane filter. The standards correspond nominally to approximately 0,0 μg/g, 0,01 μg/g, 0,02 μg/g, 0,04 μg/g, 0,06 μg/g and 0,08 μg acrylamide per gram of fat simulant.

Calculate the exact concentration, in micrograms, of acrylamide per gram of fat simulant.

Repeat the procedure for the second set of standard solutions.

7 Procedure

7.1 HPLC analysis

When starting measurements, examine the baseline stability and response linearity of the detector, together with verification of the detection limit.

The same operating conditions of the HPLC system shall be maintained throughout the measurements of all samples prepared in 6.2 to 6.4.

Each sample shall be determined at least in duplicate, i.e. as a pair of measurements.

NOTE 1 Under the conditions given in 5.2 the retention time of acrylamide was found to be approximately 14 min.

Verify the detection limit for the method as being 0,01 mg/kg, or better, by injecting the 0,01 μg/ml and 0,01 μg/g standards prepared in 6.4 for analysis six times. Calculate the standard deviation, *Sd*, of the peak areas, and the concentration equivalent to 3 x *Sd* = detection limit. If this value is greater than 0,01 mg/kg, change the chromatographic parameters to improve the peak sharpness and repeat the procedure.

NOTE 2 If the migration experiments are carried out at a more favourable ratio than 6 dm²/kg simulant, the detection limit may be increased proportionately. This means that if the migration experiment was carried out by bringing into contact 6 dm^2 of plastic with 200 g of food simulant, the detection limit should be less than 0,05 mg/kg.

7.2 Sample treatment

The test samples, blanks, as well as calibration samples prepared in 6.2 and 6.3, shall be analyzed as they are without further treatment using conditions described in 5.2.

Inject the aqueous food simulant samples, the aqueous extracts of the fat simulant as well as the simulant blanks onto the HPLC column.

Identify the acrylamide peak on the basis of the retention time and measure the respective peak area.

7.3 Calibration

Calibration samples (6.4) shall be measured according to 7.2. Construct or calculate the calibration curve, plotting peak area values against the concentration of acrylamide in milligrams per kilogram of the food simulant.

NOTE Commission Directive 90/128/EEC [1] states that the specific gravity of all simulants should conventionally be assumed to be '1'. Milligrams of substance released per litre of simulant will thus correspond numerically to milligrams of substance released per kilogram of simulant and, taking into account of the provisions laid down in Directive 82/711//EEC [3], to milligrams of substance released per kilogram of foodstuff.

The calibration curves shall be rectilinear and the correlation coefficient shall be 0,996 or better.

The two sets of calibrant solutions made from independently prepared stock solutions shall be cross-checked by generating two calibration curves which on the basis of peak area measurement shall agree to within ± 5 % of one another.

7.4 Evaluation of data

NOTE The following calculation assumes that, for all measurements, exactly the same mass or volume of food simulant has been used.

Following the method described, for some batches of olive oil interferences have been observed in analysis of the olive oil extract. Suitable simulant should be sought which is found to be free from peaks interfering with the acrylamide peak or gives rise to insignificant interference equivalent to < 0,005 mg acrylamide/kg of food simulant.

8 Expression of results

8.1 Calculation of acrylamide concentration in the test samples

8.1.1 Graphical determination

Calculate the average of peak area values obtained from the test samples according to 7.2 and read the acrylamide concentration of the test sample from the calibration graph (7.3).

8.1.2 Calculation from the regression parameters

If the regression line equation is:

$$\mathrm{y[peak\ area]} = (a \times x) + b$$

where

y[peak area] is the peak area of acrylamide;

a is the slope of the regression line in milligram per kilogram;

x is the concentration of acrylamide in the food simulant in milligrams per kilogram;

b is the intercept of the regression line.

then the acrylamide concentration in the food simulant, $C_{\text{acrylamide, fs,}}$ is given by:

$$C_{\text{Tacrylamide, fs}} = \frac{y - b}{a}$$

where

$C_{\text{acrylamide, fs}}$ is the concentration of acrylamide expressed in milligrams per kilogram.

Both procedures yield directly the acrylamide concentration in the food simulant in milligrams per kilogram.

The method applying calculation from the regression parameters is the preferred one. If relevant, correct values calculated for test samples with values calculated for blank simulants.

8.1.3 Calculation of the specific acrylamide migration

Depending on the fill volume of the test material and on the surface area/food simulant ratio, the acrylamide concentration in the test sample, as determined according to Clause 7, may need mathematical transformation to calculate the specific migration value to be compared with the specific migration limit (SML). For guidance see EN 13130-1:2004, Clause 13.

8.2 Precision

8.2.1 Validation

This method was pre-validated by a within-laboratory precision experiment using the four official EU food simulants for establishment of precision data at the restriction criterion as well as by carrying out within-laboratory migration tests with an acrylamide containing coated polymer film sample being in contact with water, 3 % w/v aqueous acetic acid, 15 % v/v aqueous ethanol and sunflower oil, respectively.

8.2.2 Repeatability

Evaluation of the within-laboratory precision experiment results, in accordance with ISO 5725, at a concentration of 0,01 mg/kg at the 95 % probability level yielded the following performance characteristics:

Repeatability r = 0,002 3 mg per kilogram of water;

r = 0,002 2 mg per kilogram of 3 % w/v aqueous acetic acid;

r = 0,001 9 mg per kilogram of 15 % v/v aqueous ethanol;

r = 0,001 5 mg per kilogram in sunflower oil.

8.2.3 Detection limit

The within-laboratory detection limits (WDL), based on the calibration curve method in accordance with DIN 32645, were found to be in the range 0,001 4 mg to 0,004 2 mg acrylamide per kilogram of food simulant (depending on the type of the food simulant). Thus the method is capable of quantitative detection at a minimum value of 0,01 mg acrylamide/kg.

The detection limits found in the confirmation procedure were in the range of 0,002 mg to 0,010 mg acrylamide per kilogram of food simulant.

9 Confirmation

9.1 Requirement for confirmation

If the specific migration of acrylamide into the food simulant, as determined in accordance with 8.1.3, exceeds the restriction criterion, e.g. SML = ND, DL = 0,01 mg/kg, the determination shall be confirmed by the method described in 9.2.

The confirmation is qualitative in the sense that it demonstrates the correct identity of the measured analyte and the absence of interferences. For the purposes of quantification the result as calculated according to 8.1.3 shall be taken as the true value.

The calibration procedure was found to be rectilinear while the detection limits calculated were only about two times higher than in the analytical method. Therefore the confirmation procedure is also suitable for the quantitative determination of acrylamide in the food simulants.

9.2 Confirmation by analysis using a column of different selectivity.

9.2.1 Principle

Food simulants, blanks and calibration samples are analyzed by HPLC using a hexylsiloxane column and UV detection at 202 nm.

NOTE The quantitative result for the analytical method and the confirmation method should agree to within ± 0,005 mg/l.

9.2.2 Reagents (Analytical grade)

NOTE Only additional chemicals required for the confirmation procedure are mentioned.

9.2.2.1 Sodium dihydrogen orthophosphate

9.2.2.2 Disodium hydrogen orthophosphate

9.2.3 Preparation of mobile phase

Dissolve 8,7g of disodium hydrogen orthophosphate and 6,1g of sodium dihydrogen orthophosphate in water and dilute with water to 1 l. Take 10 ml of this solution and dilute to 500 ml with water.

9.2.4 HPLC conditions

column	250 x 4,6 mm, packed with hexyl bonded spherical silica, 5 μm particles;
mobile phase	aqueous phosphate buffer see 9.2.2;
flow rate	1,1 ml/min;
detector	UV 202 nm;
injection	loop 10 μl.

NOTE The retention time observed for acrylamide was 4,2 min.

9.2.5 Procedure for 15 % ethanol simulant

Dilute 10 ml of each calibration standard and the samples in 15 % v/v aqueous ethanol simulant, prepared in 6.2.1, 6.3 and 6.4.1, to 50 ml with water. Inject 10 µl for HPLC analysis. Follow the instructions given in 7.2 to 7.4 to determine the specific acrylamide migration.

9.2.6 Procedure for water, 3 % w/v aqueous acetic acid and fat simulant

Re-inject the calibration standards and samples, prepared in 6.2.1, 6.2.2, 6.3, 6.4.1 and 6.4.2, for HPLC analysis. Follow the instructions given in 7.2 to 7.4 to determine the specific acrylamide migration.

NOTE In some cases problems may be encountered with the 3 % w/v aqueous acetic acid simulant. The injection of acetic acid may influence the pH of the mobile phase. Addition of a few drops of sodium hydroxide solution to neutralize the major part of the acetic acid may be useful to obtain a better separation between acrylamide and acetic acid.

10 Test report

The test report shall include the following, where applicable:

a) all information necessary for complete identification of the sample, e.g. chemical type, trade mark, grade, batch number, thickness, etc.;

b) form of the plastics, e.g. film, bottle, pot, etc.;

c) use/class of food for which the sample is intended to contact, where known, and where possible food classification reference number; see Table 2 of EN 13130-1:2004;

d) intended conditions of use, where known e.g. time/temperature;

e) conditions of the test;

   1) part(s) of EN 13130 used;

   2) foodstuffs or food simulants used;

   3) duration and temperature, and relation with "Conditions of contact in worst foreseeable use", as given in Table 3 of EN 13130-1:2004;

   4) area and geometry of the test specimen;

   5) volume of foodstuff or food simulant used where appropriate;

f) any departures from the standard method, reasons for the departures;

g) any particular requirements of the parts of this document;

h) any relevant comments on the test results;

i) details of any confirmation procedure(s);

j) individual triplicate or quadruplicate test results, and the mean of these results expressed in milligrams of acrylamide per kilogram of food simulant.

Bibliography

[1] Commission of the European Communities, Commission Directive of 23 February 1990 relating to plastics materials and articles intended to come into contact with foodstuffs (90/128/EEC), Official Journal of the European Communities, 13 December 1990, no. L349, p26. Corrigendum of the previous publication, Official Journal of the European Communities, 21 March 1990, no. 75. p19.

[2] Commission of the European Communities, Council Directive of 21 December 1988 on the approximation of the laws of the Member States relating to materials and articles intended to come into contact with foodstuff (89/109/EEC), Official Journal of the European Communities, 11 February 1989, no. L40, p 38.

[3] Commission of the European Communities, Council Directive of 18 October 1982 laying down the basic rules necessary for testing migration of the constituents of plastics materials and articles intended to come into contact with foodstuffs (82/711/EEC), Official Journal of the European Communities, 23 October 1982, no. L 297, p 26.

[4] Commission of the European Communities, Commission Directive of 15 March 1993 amending Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of the constituents of plastics materials and articles intended to come into contact with foodstuffs (93/8/EEC), Official Journal of the European Communities, 14 April 1993, no. L 90, p 22.

[5] Commission of the European Communities, Commission Directive of 97/48/EC of 29 July 1997 amending Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of the constituents of plastics materials and articles intended to come into contact with foodstuffs, Official Journal of the European Communities, 12 August 1997, no. L 222, p 10

[6] Commission of the European Communities, Council Directive of 19 December 1985 laying down the list of simulants to be used for testing migration of constituents of plastics materials and articles intended to come into contact with foodstuffs (85/572/EEC), Official Journal of the European Communities, 31 December 1985, no. L372, p14.

[7] ISO 5725, *Accuracy (trueness and precision) of measurement methods and result.*

[8] DIN 32645, *Chemical analysis; decision limit; detection limit and determination limit; estimation in case of repeatability; terms, methods, evaluation.*

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