DRAFT FOR DEVELOPMENT

DD CEN/TS
13130-14:2005

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

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

ICS 67.250



BSI
British Standards

# National foreword

This Draft for Development is the official English language version of CEN/TS 13130-14:2005.

**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.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

**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.

The BSI copyright notice displayed in this document indicates when the document was last issued.

**Amendments issued since publication**

| Amd. No. | Date | Comments |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

This Draft for Development was published under the authority of the Standards Policy and Strategy Committee on 16 September 2005



**ISBN 0 580 46072 X**

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

# CEN/TS 13130-14

February 2005

ICS 67.250

English version

# Materials and articles in contact with foodstuffs - Plastics substances subject to limitation - Part 14: Determination of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indoline in food simulants

Matériaux et objets en contact avec les denrées alimentaires - Substances dans les matières plastiques soumises à des limitations - Partie 14 : Détermination de la 3,3-bis(3-méthyl-4-hydroxyphènyl)-2-indolinone dans les simulants d'aliments

Werkstoffe und Gegenstände in Kontakt mit Lebensmitteln - Substanzen in Kunststoffen, die Beschränkungen unterliegen - Teil 14: Bestimmung von 3,3-Bis(3-Methyl-4-Hydroxyphenyl)-2-Indolinon 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.

CEN members are the national standards bodies of 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.

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

**Management Centre: rue de Stassart, 36 B-1050 Brussels**



Ref. No. CEN/TS 13130-14:2005: E

# Contents

page

# Foreword

This document (CEN/TS 13130-14:2005) 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

3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone, PM/Ref. 13600, $C_{22}H_{19}NO_3$, is a monomer used in the manufacture of certain plastics materials and articles intended to come into contact with foodstuffs. After manufacture residual 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone can remain in the finished product and may migrate into foodstuffs coming into contact with that product.

The method has been pre-validated by collaborative trial with two laboratories.

# 1 Scope

This document, part of EN 13130, specifies an analytical procedure for the determination of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in the food simulants water, 3 % w/v aqueous acetic acid, 15 % v/v aqueous ethanol and olive oil or approved substitute. The level of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone determined, is expressed as milligrams of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone per kilogram of food or food simulant. The method is applicable to the quantitative determination of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in an approximate analyte concentration range of 0,18 mg/kg to 4/ kg of food simulants.

NOTE 1 The method should also be applicable to other aqueous food simulants as well as to the other fatty food simulants e.g. a mixture of synthetic triglycerides or sunflower oil.

NOTE 2 The suitability of the fat simulant should be assessed prior to setting up migration tests. Olive oil has been found to give unacceptable interference in some cases.

# 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 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in aqueous food simulants is determined by reverse phase high performance liquid chromatography (HPLC) with UV detection at 235 nm. Fat simulants are extracted with 80 % aqueous acetonitrile prior to HPLC analysis. Quantification is achieved by calibration against samples of relevant food simulants, fortified with known amounts of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone. The level of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone determined, is expressed as milligrams per kilogram of food simulant.

Confirmation of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone is carried out by reverse phase HPLC using an analytical column of different polarity with UV detection at 280 nm.

# 4 Reagents

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

## 4.1 Analyte

**3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone**, ($C_{22}H_{19}NO_3$) purity greater than 98 % w/w.

## 4.2 Chemicals

### 4.2.1 Ethanol, absolute

### 4.2.2 Acetonitrile, chromatography grade

### 4.2.3 Water, HPLC grade

### 4.2.4 Orthophosphoric acid, 80 %

## 4.3 Solutions

### 4.3.1 Stock solution of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in ethanol (approximately 2 500 mg/l)

Weigh to the nearest 0,1 mg approximately 0,06 g of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone (4.1) into a tared 25 ml volumetric flask. Half fill the flask with ethanol (4.2.1), shake thoroughly and make the volume up to the mark with ethanol (4.2.1).

Calculate the exact concentration of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in milligrams per litre.

Repeat the procedure to provide a second stock solution.

NOTE The stock solutions may be stored in the dark at room temperature for up to 3 months in stoppered glass volumetric flasks.

### 4.3.2 Intermediate standard solutions of 3,3-bis(3-methyl-4 -hydroxyphenyl)-2-indolinone in ethanol

Into six 25 ml volumetric flasks, add by pipette 0 ml, 0,1 ml, 0,5 ml, 1,0 ml, 2,0 ml and 4,0 ml of the stock solution (4.3.1). Dilute to the mark with ethanol (4.2.1) to give standard solutions containing nominal concentrations of approximately 0 mg/l, 10 mg/l, 50 mg/l, 100 mg/l, 200 mg/l and 400 mg per litre of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone. Calculate the exact concentrations of the standard solutions in milligrams per litre.

Repeat the procedure using the second stock solution prepared in 4.3.1 to give a second set of intermediate standard solutions.

Store the standard solutions of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone stored for up to 3 months in the stoppered glass flasks, protected from light, at room temperature.

### 4.3.3 Solvent A, HPLC mobile phase

Dissolve 0,7 ml of 80 % orthophosphoric acid (4.2.4) in 620 ml of water (4.2.3) and shake thoroughly to mix. Add 380 ml of acetonitrile (4.2.2) to the mixture.

### 4.3.4 Acetonitrile in water, 80 %

Transfer using a measuring cylinder 100 ml of water (4.2.3) and 400 ml of acetonitrile (4.2.2) to a 500 ml screw cap bottle, shake to mix thoroughly.

## 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**, capable of pumping a binary mobile phase gradient, equipped with an ultraviolet detector (UV) and fitted with an injection valve with a 10 µl injection loop.

**5.2 High performance liquid chromatography analytical column**, packed with a bonded, reverse phase, fully end capped C18, 25 cm x 4,6 mm I.D, 5 µm particle size silica based packing maintained at a constant temperature of 20 °C ± 1 °C. The analytical column should be allowed to equilibrate at the correct flow rate for an hour. The analytical column shall be capable of resolving 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone from any interferences in the food simulant.

NOTE The retention of the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone on the analytical column is sensitive to the proportion of acetonitrile in the mobile phase. The mobile phase should be prepared with care and an adjustment of ± 5 % v/v of acetonitrile is usually sufficient to obtain a suitable retention time.

For guidance, details of a column and chromatographic conditions, which have been found suitable, are given below:

| | | |
|---|---|---|
| Column | Spherisorb ODS 2, C18, 25 cm x 4,6 mm id, 5 µm | |
| Mobile phase | Reservoir A: | Solvent A (4.3.3). |
| | Reservoir B: | 100 % acetonitrile |
| Gradient | 100 % A held for 8 min; raised over 1 min to 100 % B, held for 5 min, returned over 1 min to 100 % A, held for 5 min. | |
| Flow rate | 1,5 ml/min | |
| Injector | 10 µl loop | |
| Detection | 235 nm | |
| Run time | 20 min | |

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

**5.3** **Vials**, glass, 20 ml capacity.

**5.4** **Caps**, screw on, polytetrafluoroethylene (PTFE) lined.

**5.5** **Cartridges**, $C_{18}$ reverse phase solid phase extraction, containing at least 850 mg of sorbent.

**5.6** **Syringe**, 100 µl fitted with a repeatable dispensing adapter.

**5.7** **Glass sample vials**, 1 ml capacity, with PTFE stoppers.

# 6 Samples

## 6.1 Preparation of test samples

### 6.1.1 General

Laboratory samples of the food simulant to be analyzed shall be obtained as described in EN 13130-1.

NOTE Samples should be analyzed as soon as possible, but may be stored for 10 d at room temperature in sealed glass containers, protected from light.

3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone-free simulants of the same type as those to be analyzed shall be prepared for calibration purposes.

### 6.1.2 Aqueous food simulants

Pipette 10,0 ml of the aqueous food simulant obtained from the migration experiment (see EN 13130-1) into the 20 ml glass vials (5.3). Add using the 100 µl syringe (5.6) 0,10 ml of the intermediate standard solution containing no 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone (4.3.2) into the glass vial. Seal the vial using a PTFE lined screw on cap (5.4), and shake thoroughly to mix.

### 6.1.3 Fat simulant

Transfer 10,0 g ± 0,1 g, accurately weighed, of fat simulant obtained from the migration experiment (see EN 13130-1) into a 20 ml glass vial (5.3). Add using the 100 µl syringe (5.6) 0,10 ml of the intermediate standard solution containing no 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone (4.3.2) into the vial. Swirl the vial to thoroughly mix the standard solution with the oil. Add by pipette 5,0 ml of the 80 % aqueous acetonitrile (4.3.4). Seal the vial using a PTFE lined screw on cap (5.4). Shake thoroughly for two min. Allow the phases to separate for a least 1 h. Transfer approximately 1 ml into a HPLC injection vial. Seal the sample vial. Inject the solution onto the HPLC analytical column as soon as possible.

If the extract with 80 % acetonitrile contains interfering oil constituents, remove them, for example, by passing the acetonitrile solution through a $C_{18}$ solid phase extraction cartridge as follows:

Withdraw approximately 2 ml of the upper acetonitrile phase using a 5 ml glass syringe, and pass the extract through a $C_{18}$ solid phase extraction cartridge (5.5) into a 1 ml HPLC glass sample vial (5.7).

Ensure that all of the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone is eluted from the cartridge. If loss of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone is observed, flush the cartridge with 100 % acetonitrile. To assure quantitative results, bring exactly 1,0 ml of the oil extract into the cartridge, and then flush it with pure acetonitrile until a final volume of 2,0 ml is collected.

## 6.2 Blank sample preparation

Follow the procedure described in 6.1.2 and 6.1.3 employing 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone-free food simulant.

## 6.3 Calibration sample preparation

### 6.3.1 Aqueous simulants

Pipette 10,0 ml of the blank 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone-free aqueous food simulant into a series of 20 ml glass vials or flasks. Add using the 100 µl syringe (5.6) 0,10 ml of each of the intermediate standard solutions prepared in (4.3.2). Seal the vials using a PTFE lined screw on cap (5.4). Shake thoroughly to mix. In this way nominal concentrations of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in the calibration solutions of approximately 0 mg/l, 0,1 mg/l, 0,5 mg/l, 1,0 mg/l, 2,0 mg/l and 4,0 mg per litre are obtained.

Calculate the exact concentrations of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in the calibration samples in milligrams per litre. Assuming a density of 1,0 kg/l gives a calibration graph in milligrams per kilogram.

NOTE Commission Directive 2002/72/EC [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.

Repeat the procedure using the second set of intermediate standard solutions prepared in 4.3.2 to give a second set of calibration samples.

### 6.3.2 Fat simulant

Weigh accurately approximately 10,0 g ± 0,05 g of blank fatty food simulant into a series of 20 ml glass vial. Add using the 100 µl syringe (5.6), 0,10 ml of each of the standard solutions (4.3.2). Swirl the vial to thoroughly mix the standard solutions with the fat food simulant. The nominal concentrations of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in the calibration solutions correspond to approximately 0 mg/kg, 0,1 mg/kg, 0,5 mg/kg, 1,0 mg/kg, 2,0 mg/kg and 4,0 mg of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone per kilogram of fat simulant.

Calculate the exact concentrations of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in the calibration samples in milligrams per kilogram. Treat the calibration samples thus obtained as described in 6.1.2.

Repeat the procedure using the second set of intermediate standard solutions prepared in 4.3.2 to give a second set of calibration samples.

# 7 Procedure

## 7.1 HPLC analysis

When starting measurements, examine the baseline stability and response linearity of the detector.

The same operating conditions of the HPLC system shall be maintained throughout the measurements of all samples prepared in 6.1 to 6.3. Each sample shall be determined at least in duplicate, i.e. as a pair of measurements or injections.

NOTE Under the conditions given in 5.2 the retention time obtained for 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone was approximately 7 min.

### 7.2 Sample treatment and execution of the determination

Inject the samples as prepared in 6.1 and 6.2 in duplicate onto the HPLC column using the conditions given in 5.2. Identify the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone peak on the basis of the retention time and measure the peak area.

### 7.3 Calibration

Inject the calibration samples prepared in 6.3.1 and 6.3.2 in duplicate on to the HPLC column under conditions used in 5.2. Measure the peak areas of the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone. Construct the calibration line by plotting the mean of duplicate peak areas (PA) against the concentration, in milligrams per kilogram, of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in the calibration samples.

Repeat for the second set of calibration samples (6.3) to obtain a second calibration line.

The calibration lines shall be rectilinear and the correlation coefficients shall be 0,996 or better.

If the correlation coefficients are less than 0,996, fresh intermediate standard solutions and calibration solutions shall be prepared from the original stock solutions.

If the levels of the two independently prepared stock solutions do not correspond to within ± 5 %, both stock solutions, intermediate and working standards shall be discarded. Fresh stock solutions of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone shall be prepared and the entire procedure repeated from the beginning.

### 7.4 HPLC interferences

If the HPLC chromatogram of the blank sample (5.2) shows an interfering peak in the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone region, different chromatographic conditions and/or alternative food simulants (e.g. a mixture of synthetic triglycerides instead of sunflower oil) shall be used.

NOTE No interferences with the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone peak arising from water, 3% w/v aqueous acetic acid, 15% v/v aqueous ethanol and sunflower oil food simulants, have so far been observed using the method and the HPLC conditions specified in (4.2). Some batches of olive oil have shown unacceptable interferences which could not be resolved from the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone. However, sunflower oil has been used to overcome this problem.

## 8 Expression of results

### 8.1 Determination of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone concentration in the test samples

NOTE The following calculations assume that for all measurements exactly the same weight or volume of food simulant has been used.

#### 8.1.1 Graphical determination

Calculate the mean of the peak areas, PA, values (7.3) obtained from the two duplicate injections of each test samples in accordance with 7.2 and read the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone 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:

$$\mathrm{y\,[PA]} = (\mathrm{a} \times \mathrm{X}) + b$$

where

*y[PA]* is the peak area of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone;

*a* is the slope of the regression line in milligrams per kilogram;

*X* is the concentration of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in the food simulant in milligrams per kilogram;

*b* is the intercept of the regression line,

the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone concentration in the food simulant is given by:

$$\mathrm{C}_{\mathrm{indolinone}} = \frac{y - b}{a}$$

where

$C_{\mathrm{indolinone}}$ is the concentration of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone in the food simulant, in milligrams per kilogram.

Both procedures yield directly the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone concentration in the food simulant in milligrams per kilogram.

The method applying calculation from the regression parameters is the preferred method.

#### 8.1.3 Calculation of the specific 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone migration

Depending on the fill volume of the test material and on the surface area/food simulant ratio, the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone concentration in the test sample as determined according to 8.1.1 or 8.1.2 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 collaborative trial with two laboratories. In each laboratory a within-laboratory precision experiment using the four official EC food simulants for establishment of precision data at the restriction criterion was carried out as well as migration testing using a polymer film sample, manufactured using 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone as a monomer, being in contact with 15 % v/v aqueous ethanol and sunflower oil, respectively. In addition fortified food simulants were carried through the whole procedure of migration conditions and final determinations.

#### 8.2.2 Repeatability

Evaluation of the within-laboratory precision experiment according to ISO 5725, at a concentration of 1,8 mg/kg at the 95% probability level, yielded the following performance characteristics (lab 1/lab 2):

Repeatability r = 0,05/0,05 mg per kilogram in water;

r = 0,08/0,08 mg per kilogram in 3 % w/v aqueous acetic acid;

r = 0,05/0,05 mg per kilogram in 15 % v/v aqueous ethanol;

r = 0,18/0,08 mg per kilogram in sunflower oil.

#### 8.2.3 Detection limit

The within-laboratory detection limits (WDL), based on the calibration curve method according to DIN 32645, were found to be in the range 0,05 mg to 0,35 mg 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone 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 0,4 mg of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone per kilogram of food simulant.

## 9 Confirmation

### 9.1 Requirement for confirmation

If the specific migration of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone into the food simulant, determined as specified in 8.1.3, exceeds the restriction criterion, e.g. the specific migration limit (SML-value) is 1,8 mg/kg, the result of the determination shall be confirmed using 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.

### 9.2 Confirmation by analysis using reverse phase chromatography

#### 9.2.1 HPLC conditions

Remove the analytical column described in 5.2 from the HPLC. Install a Phenyl (silica bonded with Phenyl groupings) 25 cm x 4,6 mm id 5µm analytical column. Operate the chromatograph using the same mobile phase as used in the quantification procedure, and as described in 5.2. Operate the UV detector at 280 nm

NOTE The retention time of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone under these conditions is 4,5 min.

#### 9.2.2 Procedure for aqueous and fat food simulants

Re-inject the test samples, blanks and calibration samples, prepared in 6.1, 6.2 and 6.3 for HPLC analysis. Identify the 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone on the basis of retention time. Measure the peak area for obtaining quantitative results.

NOTE The peaks attributable to 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone should maximize within one half peak width (measured at half the peak height), or within 2 % of the absolute retention time of the standards, whichever is the smaller.

## 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 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone per kilogram of food simulant.

# Bibliography

[1] Commission of the European Communities, Commission Directive 2002/72/EC of 6 August 2002 relating to plastics materials and articles intended to come into contact with foodstuffs, Official Journal of the European Communities, 15 August 2002, no. L220, p18.

[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. L 40, 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.*

# BSI — British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

**Revisions**

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover. Tel: +44 (0)20 8996 9000. Fax: +44 (0)20 8996 7400.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

**Buying standards**

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services. Tel: +44 (0)20 8996 9001. Fax: +44 (0)20 8996 7001. Email: orders@bsi-global.com. Standards are also available from the BSI website at http://www.bsi-global.com.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

**Information on standards**

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact the Information Centre. Tel: +44 (0)20 8996 7111. Fax: +44 (0)20 8996 7048. Email: info@bsi-global.com.

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration. Tel: +44 (0)20 8996 7002. Fax: +44 (0)20 8996 7001. Email: membership@bsi-global.com.

Information regarding online access to British Standards via British Standards Online can be found at http://www.bsi-global.com/bsonline.

Further information about BSI is available on the BSI website at http://www.bsi-global.com.

**Copyright**



BSI
389 Chiswick High Road
London
W4 4AL