Annex 2 – Formaldehyde

The 2 methods are analytical procedures for the determination of formaldehyde CH2O, PM/Ref. No 17260, and hexamethylenetetramine (HMTA) C6H12N4, PM/Ref. No 18670, in food simulants 3 % w/v aqueous acetic acid. The methods are appropriate for the quantitative determination of formaldehyde in approximate analyte concentration range of 3,0 mg to 30 mg formaldehyde per kilogram of food simulant, which corresponds to 2,3 mg to 23,3 mg hexamethylenetetramine per kilogram of food simulant. They are interchangeable for use as determination and confirmation. Both are spectrophotometric. One uses chromotropic acid in the presence of sulphuric acid, and the other pentane-2,4-dione in the presence of ammonium acetate.

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1 PROTOCOL A

1.1 Scope and principle

HMTA is treated with acid and heated to release formaldehyde.
Formaldehyde in food simulant is determined by spectrophotometry. Formaldehyde reacts with chromotropic acid in the presence of sulphuric acid. This solution is measured in a spectrophotometer at the wavelength of 574 nm, with cells of an optical path length of 10 mm. Quantification is achieved using an external standard. The method has been pre-validated by collaborative trial with two laboratories.

1.2 Materials and chemicals

Reagents and solvents shall be of analytical quality.

1.2.1 Analytes and chemicals

1.2.1.1 Formaldehyde solution, CH_2O, minimum 37 % (w/v), stabilised with about 10 % methanol

1.2.1.2 Water deionised

1.2.1.3 Acetic acid glacial 100%

1.2.1.4 Ethanol absolute

1.2.1.5 Chromotropic acid disodium salt (dihydrate), 4,5-dihydroxy-2,7-naphthalene-disulphonic acid, $C_{10}H_6Na_2O_8S_2.2H_2O$

1.2.1.6 Sulphuric acid, density 1.84 g/cm^3

1.2.1.7 Starch, soluble

1.2.2 Solutions

1.2.2.1 Stock solution of formaldehyde in water containing approximately 1,5 mg formaldehyde per millilitre

Weigh to the nearest 1 mg approximately 0,8 g formaldehyde into a 200 ml volumetric flask and make up to the mark with water.

Determine the strength of this solution as follows:

Pipette 10,0 ml formaldehyde stock solution into a beaker, add 25,0 ml of a 0,05 mol standard iodine solution and 10,0 ml of 1 mol sodium hydroxide solution to the beaker. Allow to stand for 5 min. Acidify with 11,0 ml of 1 mol hydrochloric acid and determine the excess iodine by titration with a 0,1 mol standard sodium thiosulphate solution using 0,1 ml of the starch solution as indicator.

Make a blank test on the corresponding amount of standard iodine solution.

1 ml of 0,1 N iodine consumed is equivalent to 1,5 mg formaldehyde.

Calculate the concentration in mg formaldehyde per millilitre solution.

Repeat the procedure to obtain a second stock solution.

Store the formaldehyde stock solution for up to 3 months in a refrigerator.

1.2.2.2 Diluted standard solutions of formaldehyde in aqueous food simulants

Pipette into a series of five 100 ml volumetric flasks, 100 μl, 400 μl, 700 μl, 1000 μl and 2000 μl of the stock solution. Make up to the mark with the appropriate food simulant. The standard solutions thus obtained shall contain approximately 1,5 mg/l, 6,0 mg/l, 10,5 mg/l, 15 mg/l and 30 mg formaldehyde per litre.

Calculate the exact concentration in milligrams of formaldehyde per litre of solution corresponding to mg/kg food simulant.

Repeat the procedure using the second standard stock solution to obtain a second set of diluted standard solutions.

Store the solutions in well closed container for a maximum period of 3 months in a refrigerator

1.2.2.3 Iodine in aqueous solution, 0,05 M

1.2.2.4 Sodium hydroxide in aqueous solution solution, NaOH, 1 M.

1.2.2.5 Hydrochloric acid in aqueous solution solution, HCl, 1 M.

1.2.2.6 Sodium thiosulphate in aqueous solution solution, $Na_2S_2O_3.5H_2O$. 0.1 M..

1.2.2.7 0,5 % chromotropic acid disodium salt (dihydrate)
Weigh 500 mg of chromotropic acid into a 100 ml volumetric flask and make to the mark up with water. This solution shall be prepared freshly on the day of use.

1.2.2.8 Sulphuric acid solution
Measure 100 ml water and make up with sulphuric acid to 400 ml.

1.2.2.9 Sulphuric solution (4M)
Add carefully 222 ml sulphuric acid (1.2.1.6) to a flask containing approximately 700 ml of water. After cooling to room temperature, make up to 1 000 ml with water.

1.2.2.10 Starch solution
Dissolve 1 g soluble starch in 50 ml boiling water.

1.2.3 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.

Spectrophotometer, preferably with a double beam.

Water-bath

Micro syringe 0 -1.0 ml

Tubes, glass, stoppered

1.3 Procedure

1.3.1 Preparation of samples

1.3.1.1 test samples

1.3.1.1.1 For the determination of HMTA,

Take 25 ml of the aqueous food simulant into a 50 ml volumetric flask and make up to the mark with the diluted sulfuric acid (4.3.11). Immerse the flask in a water bath at 90 °C for 30 min. Shake while cooling. Transfer 1,0 ml of this solution into a 12 ml glass stoppered tube. Treat the sample as described in the section sample treatment.

1.3.1.1.2 For the determination of formaldehyde

Transfer 1,0 ml of food simulant obtained from the migration experiment into a 12 ml glass stoppered tube. Treat the sample as described in the section sample treatment.

1.3.1.2 Preparation of blank samples
Treat simulants which have not been in contact with packaging material in the same way.

1.3.2 Preparation of calibration samples

Transfer 1,0 ml of each of the diluted standard solutions into a 12 ml glass stoppered tube and treat the calibration samples as described in the section sample treatment.

1.3.3 Spectrophotometric analysis

The test samples, blanks and calibration samples are submitted to a reaction procedure with chromotropic acid. The absorption of reaction product is measured over the range of 650 nm to 450 nm. The absorption at 574 nm is used for quantitative calculations. Each solution must be analysed at least in duplicate

1.3.3.1 Sample treatment
Add to each of the vials 1 ml chromotropic acid solution and 8 ml sulphuric acid 75 %. Place the vials in a water-bath at 60°C for 20 min. Remove the vials from the water-bath and allow the vials to cool for one hour at room temperature.

Record the absorption curve of the solution obtained from 650 nm to 450 nm using cells with an optical path length of 10 mm and a reagent blank solution or zero level of the calibration curve as a reference. The absorption at 574 nm is used for quantitative calculations.

1.3.3.2 Calibration
Measure the absorption of the formaldehyde/chromotropic acid complex at 574 nm of the standard solutions. Plot the absorption against the concentration of formaldehyde in the calibration samples 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.

The calibration curves shall be rectilinear and the correlation coefficient should 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 absorption measurement should agree to ± 5 % of one another.

1.3.4 Evaluation of data

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

1.3.4.1 Spectroscopic interferences
Possible interferences due to background colour in the sample are eliminated by the use of a reference solution.

1. Calculation of analyte level

1.3.4.2 Graphical determination:
Measure the absorbance at 574 nm and calculate the average of absorption values obtained from the test samples according to the section sample treatment. Read the formaldehyde concentration of the test samples from the calibration graph.

1.3.4.3 Calculation from the regression parameters:
If the regression line equation is

$$y = a * x + b \quad (1)$$

where

y is the absorbance of formaldehyde/chromotropic acid complex

a is the slope of the regression line;

x is the concentration of formaldehyde in the food simulant (mg/kg)

b is the intercept of the regression line.

then

$$C_{For,fs} = (y-b)/a \quad (2)$$

where

$C_{For,fs}$ is concentration of formaldehyde in milligrams per kilogram of food simulant

Both procedures yield directly the formaldehyde concentration in the food simulant in milligrams of formaldehyde per kilogram of food simulant

The method applying calculation from the regression parameters shall be the preferred one.

1.3.4.4 Calculation of the specific formaldehyde migration
Depending on the fill volume of the test material and on the surface area/food simulant, the formaldehyde concentration in the test sample may need mathematical transformation to calculate the specific migration value to be compared to the restriction.

1.4 Precision

1.4.1 Validation

1.4.1.1 Determination of HMTA

This method was pre-evaluated by a collaborative trial with three laboratories. In each laboratory awithin-laboratory precision experiment using the four official EU food simulants for establishment of precisiondata at the restriction criterion was carried out. Also within-laboratory migration tests were carried out with apolymer sample, manufactured using hexamethylenetetramine as a monomer, being in contact for 10 d at 40 °C with 15 % v/v aqueous ethanol and olive oil, respectively.

Evaluation of the within-laboratory precision experiment results according to ISO 5725, at a concentration of 11,7 mg HMTA per kilogram of food simulant, (corresponding to 15 milligrams of formaldehyde per kilogram for the 95 % probability level yielded the following performance characteristics (lab 1/lab 2/lab 3):

Repeatability:

r = 0,2/0,8/1,0 mg formaldehyde per kilogram in water;
r = 0,8/0,7/1,9 mg formaldehyde per kilogram in 3% w/v aqueous acetic acid;
r = 0,6/0,6/2,6 mg formaldehyde per kilogram in 15% v/v aqueous ethanol;
r = 0,4/1,1/4,2 mg formaldehyde per kilogram in olive oil.

1.4.1.2 Determination of formaldehyde

This method was pre-evaluated in 1993 by collaborative trial with two laboratories. In each lab a within-laboratory precision experiment using the four official EU food simulants for establishment of precision data at the restriction criterion was carried out as well as migration testing with formaldehyde containing samples in contact for 10 d at 40 °C, with 15 % v/v aqueous ethanol and olive oil, respectively.

Evaluation (ISO 5725) of the results of the two within-laboratory precision experiments at a concentration of 15 mg formaldehyde per kilogram of food yielded the following performance characteristics at the 9 5% probability levels (lab1/lab2):

Repeatability:

r = 0,5/0,2 mg formaldehyde per kilogram in water;
r = 0,3/0,2 mg formaldehyde per kilogram 3 % w/v aqueous acetic acid;
r = 0,4/0,1 mg formaldehyde per kilogram in 15 % v/v aqueous ethanol;
r = 0,5/0,5 mg formaldehyde per kilogram in olive oil.

1.4.2 Detection limit

1.4.2.1 Determination of HTMA

The within-laboratory detection limits (WDL), based on the calibration curve method according to DIN 32645, were found to be in the range of 0,8 mg/kg to 3,0 mg/kg food simulant, depending on the type of food simulant. Thus the method is capable of quantitative detection of HMTA, as formaldehyde at a minimum level of 3,0 mg per kilogram food simulant.

1.4.2.2 Determination of Formaldehyde

The within-laboratory detection limits (WDL), based on the calibration curve method according to DIN 32645, were found to be in the range of 0,5 mg/kg to 3,0 mg/kg

food simulant, depending on the type of food simulant. Thus the method is capable of quantitative detection of formaldehyde at a minimum level of 3,0 mg/kg food simulant.

2 PROTOCOL B

In cases where the specific migration of formaldehyde exceeds the restriction, e.g. a specific migration of 15 mg/kg, the result of the determination shall be confirmed. The confirmation procedure is quantitative. In collaborative trials with three laboratories comparable results using the chromotropic acid method and the acetyl acetone method were found for the detection limits, repeatability, recovery and migration of formaldehyde. Therefore the method of determination and the confirmation procedure are interchangeable.

2.1 Principle

Formaldehyde is reacted with pentane-2,4-dione in the presence of ammonium acetate to form 3,5-diacetyl-1,4-dihydrolutidine. The absorbance of this complex is measured at 410 nm with a spectrophotometer.

2.2 Materials and chemicals

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

2.2.1 Analytes and Chemicals

2.2.1.1 Ammonium acetate, anhydrous

2.2.1.2 Pentane-2,4-dione (acetyl acetone), $C_5H_8O_2$, distilled

2.2.2 Solutions

2.2.2.1 Pentane-2,4-dione reagent
This reagent shall be freshly prepared on the day of use.

Dissolve 15 g ammonium acetate in a 100 ml volumetric flask containing approximately 75 ml of water. Add 0,2 ml pentane-2,4-dione and 0,3 ml acetic acid. Make up to 100 ml with water (pH of solution about 6,4)

2.2.2.2 Reagent without pentane-2,4-dione
Prepare the reagent solution while omitting the addition of pentane-2,4-dione.

2.3 Procedure

2.3.1 Preparation of test samples

2.3.1.1 For the determination of HMTA
Transfer into a 50 ml flask 10,0 ml of aqueous food simulant , add 5 ml of sodium hydroxide solution (1.2.2.4) and 15 ml of water. Check the pH of the solution, and if necessary adjust to pH 4 to pH 6 using a few drops of sodium hydroxide solution

(1.2.2.4) or sulfuric acid solution (1.2.2.9), as appropriate. Add 5 ml of pentane-2,4-dione reagent (2.2.1.2) and continue as described in sample treatment.

2.3.1.2 For the determination of formaldehyde
Transfer into a 50 ml flask 5,0 ml of aqueous food simulant, add 20,0 ml water and 5,0 ml of pentane-2,4-dione reagent and continue as described in sample treatment.

2.3.2 Preparation of blank samples

Treat simulants that have not been in contact with packaging material, as described in sample preparation.

2.3.3 Preparation of calibration samples

Transfer into a 50 ml flask 5 ml of diluted standard solutions, 20 ml water and add 5.0 ml of pentane-2,4-dione reagent.

2.3.4 Reference solution

Transfer into a 50 ml flask 5,0 ml aqueous food simulant, 20 ml water and 5,0 ml of the reagent without pentane-2,4-dione.

NOTE: Possible interferences due to background colour in the test sample are eliminated by the use of this reference solution.

2.3.5 Sample treatment

Shake the mixtures prepared. Immerse the flasks in a waterbath at 60 °C for exactly 10 min. Allow to cool for 2 min in a bath of iced water. Within 25 min from the moment when the flasks are placed in the water-bath measure the absorbance at 410 nm of the sample solution with the reference solution in the reference cell.

2.4 Evaluation of data

Evaluate the data following the procedure given for protocol A.