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(Acts whose publication is not obligatory)

COMMISSION

FIRST COMMISSION DIRECTIVE

of 28 July 1981

laying down Community methods of analysis for verifying that certain additives used in foodstuffs satisfy criteria of purity

(81/712/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to the Council Directive of 23 October 1962 on the approximation of the laws of the Member States concerning the colouring matters authorized for use in foodstuffs intended for human consumption ⁽¹⁾, as last amended by Directive 78/144/EEC ⁽²⁾, and in particular Article 11 (2) thereof,

Having regard to Council Directive 64/54/EEC of 5 November 1963 on the approximation of the laws of the Member States concerning the preservatives authorized for use in foodstuffs intended for human consumption ⁽³⁾, as last amended by Directive 79/40/EEC ⁽⁴⁾, and in particular Article 8 (2) thereof.

Having regard to Council Directive 70/357/EEC of 13 July 1970 on the approximation of the laws of the Member States concerning the antioxidants author-

ized for use in foodstuffs intended for human consumption ⁽⁵⁾, as last amended by Directive 78/143/EEC ⁽⁶⁾, and in particular Article 5 (2) thereof.

Whereas these provisions lay down that Community methods of analysis shall be established for verifying that these additives satisfy general and specific criteria of purity;

Whereas a first series of methods for which the studies have been completed should now be adopted;

Whereas the measures provided for in this Directive are in accordance with the opinion of the Standing Committee on Foodstuffs,

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Member States shall prescribe that the analyses necessary for verifying that certain additives used in foodstuffs satisfy the general and specific criteria of

⁽¹⁾ OJ No 115, 11. 11. 1962, p. 2645/62.

⁽²⁾ OJ No L 44, 15. 2. 1978, p. 20.

⁽³⁾ OJ No 12, 27. 1. 1964, p. 161/64.

⁽⁴⁾ OJ No L 13, 19. 1. 1979, p. 50.

⁽⁵⁾ OJ No L 157, 18. 7. 1970, p. 31.

⁽⁶⁾ OJ No L 44, 15. 2. 1978, p. 18.

purity shall be carried out according to the methods described in Annex II, the scope of which is laid down in Annex I.

Article 3

This Directive is addressed to the Member States.

Article 2

The Member States shall bring into force the laws, regulations or administrative provisions necessary to comply with this Directive not later than 20 February 1983. They shall forthwith inform the Commission thereof.

Done at Brussels, 28 July 1981.

For the Commission

Karl-Heinz NARJES

Member of the Commission

ANNEX I**SCOPE OF THE COMMUNITY METHODS OF ANALYSIS FOR VERIFYING THAT
CERTAIN ADDITIVES USED IN FOODSTUFFS MEET PURITY CRITERIA****I. INTRODUCTION**

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II. COLOURING MATTERS

- II.1. Determination of substances extractable with diethyl ether from water-soluble sulphonated organic colouring matters used in foodstuffs using Annex II, method 1.

III. PRESERVATIVES

- III.1. Determination of formic acid, formates and of other oxidizable impurities in acetic acid (E 260), potassium acetate (E 261), sodium diacetate (E 262) and calcium acetate (E 263) using Annex II, method 2.
- III.2. Determination of non-volatile substances in propionic acid (E 280) using Annex II, method 3.
- III.3. Determination of the loss of mass on drying of sodium nitrite (E 250) using Annex II, method 4.
- III.4. Limit test for salicylic acid in ethyl *p*-hydroxybenzoate (E 214), ethyl *p*-hydroxybenzoate, sodium salt (E 215), *n*-propyl *p*-hydroxybenzoate (E 216), *n*-propyl *p*-hydroxybenzoate, sodium salt (E 217), methyl *p*-hydroxybenzoate (E 218) and methyl *p*-hydroxybenzoate, sodium salt (E 219) using Annex II, method 5.
- III.5. Determination of free acetic acid in sodium diacetate (E 262) using Annex II, method 6.
- III.6. Determination of sodium acetate in sodium diacetate (E 262) using Annex II, method 7.
- III.7. Limit test for determination of aldehydes in sorbic acid (E 200) in sodium, potassium and calcium sorbates (E 201, E 202, E 203) and in propionic acid (E 280) using Annex II, method 8.

IV. ANTIOXIDANTS

- IV.1. Determination of the number of peroxide groups of lecithins (E 322) using Annex II, method 9.
- IV.2. Determination of toluene-insoluble substances in lecithins (E 322) using Annex II, method 10.
- IV.3. Limit test for reducing substances in sodium, potassium and calcium lactates (E 325, E 326 and E 327) using Annex II, method 11.
- IV.4. Determination of volatile acids in orthophosphoric acid (E 338) using Annex II, method 12.

- IV.5. Limit test for nitrates in orthophosphoric acid (E 338) using Annex II, method 13.
- IV.6. Determination of water-insoluble substances in mono-, di- and tri-sodium orthophosphate and mono-, di- and tri-potassium orthophosphates (E 339(i), E 339(ii), E 339(iii), E 340(i), E 340(ii), E 340(iii)) using Annex II, method 14.

V. GENERAL

- V.1. Determination of pH in food additives using Annex II, method 15.
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ANNEX II**METHODS OF ANALYSIS RELATING TO THE CRITERIA OF PURITY OF FOOD ADDITIVES****INTRODUCTION****1. Preparation of the analysis sample****1.1. General**

The mass of the laboratory sample intended for analysis must normally be 50 g unless a larger quantity is required for a specific determination.

1.2. Sample preparation

The sample shall be made homogeneous prior to analysis.

1.3. Preservation

The prepared sample shall always be kept in an air-tight and moisture-tight container and stored so that deterioration is prevented.

2. Reagents**2.1. Water**

2.1.1. Wherever mention is made of water for solution, dilution or washing purposes, distilled water, or demineralized water of at least equivalent purity, is intended.

2.1.2. Wherever reference is made to 'solution' or 'dilution' without further indication of a reagent, an aqueous solution is intended.

2.2. Chemicals

All chemicals shall be of analytical reagent quality except where otherwise specified.

3. Equipment**3.1. List of equipment**

The list of equipment contains only those items with a specialized use and items with a particular specification.

3.2. Analytical balance

Analytical balance means a balance with a sensitivity of 0.1 mg or greater.

4. Expression of results**4.1. Results**

The result stated in the official analysis report shall be the mean value of at least two determinations the repeatability of which is satisfactory.

4.2. *Calculation of percentage*

Unless otherwise stated the results shall be expressed as a percentage by mass of the original sample as received at the laboratory.

4.3. *Number of significant figures*

The number of significant figures in the result so expressed shall be governed by the precision of the method.

METHOD 1

DETERMINATION OF SUBSTANCES EXTRACTABLE WITH DIETHYL ETHER FROM WATER-SOLUBLE SULPHONATED ORGANIC COLOURING MATTERS INTENDED FOR FOODSTUFFS

1. **Scope and field of application**

The method determines substances extractable with diethyl ether in water soluble sulphonated organic colouring matters which have not been mixed with any support.

2. **Definition**

Substances extractable with diethyl ether: the content of material as determined by the method specified.

3. **Principle**

Extract the colouring matter with diethyl ether and weigh the extracted residue after evaporation of the ether.

4. **Reagents**

- 4.1. Diethyl ether, dry, peroxide-free (dried with the aid of freshly calcined calcium chloride).

5. **Apparatus**

- 5.1. Soxhlet apparatus with flask.
- 5.2. Desiccator, containing freshly activated silica gel or equivalent desiccant with a water content indicator.
- 5.3. Analytical balance.
- 5.4. Oven, thermostatically controlled at $85 \pm 2^\circ\text{C}$.

6. **Procedure**

Accurately weigh, to the nearest 10 mg, about 10 g of the sample of the colouring matter on a piece of filter paper. Fold the paper, put it into a paper thimble and close the latter with some fat-free cotton wool. Extract for six hours with diethyl ether (4.1) in a Soxhlet extraction apparatus (5.1). Evaporate the ether at as low a temperature as possible. Place

the Soxhlet flask, which has been previously weighed, with the residue in the oven (5.4) at $85 \pm 2^\circ\text{C}$ for 20 minutes to dry. Transfer the flask to a desiccator (5.2), cover with a loose-fitting lid and allow to cool. Weigh the flask and residue.

Repeat the drying and weighing until two successive weighings differ by less than 0.5 mg. Should an increase in mass occur, the lowest recorded reading will be used in the calculation.

7. Expression of results

7.1. Formula and method of calculation

The content of substances extractable with ether, as a percentage of the sample, is given by:

$$\frac{m_1 \times 100}{m_0}$$

where:

m_1 = mass in grams of the residue after evaporation,

m_0 = initial mass in grams of the sample taken.

7.2. Repeatability

The difference between the results of two determinations when carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 20 mg per 100 g of sample.

METHOD 2

DETERMINATION OF FORMIC ACID, FORMATES AND OTHER OXIDIZABLE IMPURITIES IN ACETIC ACID (E 260), POTASSIUM ACETATE (E 261), SODIUM DIACETATE (E 262) AND CALCIUM ACETATE (E 263)

1. Scope and field of application

The method determines formic acid, formates and other oxidizable impurities, expressed as formic acid in:

- acetic acid (E 260),
- potassium acetate (E 261),
- sodium diacetate (E 262),
- calcium acetate (E 263).

2. Definition

Formic acid, formates and other oxidizable impurities content: the content of formic acid, formates and other oxidizable impurities as determined by the method specified.

3. Principle

The solution of the sample is treated with excess of standard potassium permanganate in alkaline conditions to form manganese dioxide. The manganese dioxide and excess potassium permanganate are determined iodometrically in acid conditions and the concentration of oxidizable impurities calculated and expressed as formic acid.

4. Reagents

- 4.1. Potassium iodide.
- 4.2. Potassium permanganate, 0.02 mol/l.
- 4.3. Sodium carbonate (anhydrous).
- 4.4. Sodium thiosulphate, 0.1 mol/l.
- 4.5. Starch solution (approximately 1 % m/v).
- 4.6. Dilute sulphuric acid: add 90 ml of sulphuric acid ($\rho_{20} = 1.84$ g/ml) to water and dilute to 1 l.

5. Apparatus

- 5.1. Water bath, boiling.
- 5.2. Analytical balance.

6. Procedure

If the test sample is the free acid, accurately weigh, to the nearest 10 mg, about 10 g of the sample and dilute with 70 ml of water and add a solution containing 10 g of anhydrous sodium carbonate (4.3) in 30 ml of water. If the sample is a salt, accurately weigh, to the nearest 10 mg, about 10 g of the sample and dissolve in 100 ml of water. Add 1 g anhydrous sodium carbonate (4.3) and shake to dissolve. Add 20 ml of 0.02 mol/l potassium permanganate (4.2) and heat on a boiling water bath for 15 minutes. Cool the mixture. Add 50 ml of dilute sulphuric acid (4.6) and 0.5 g of potassium iodide (4.1). Swirl the mixture until all precipitated manganese dioxide has redissolved. Titrate with 0.1 mol/l sodium thiosulphate (4.4) until the solution becomes pale yellow in colour. Add a few drops of starch solution (4.5) and continue the titration until the solution becomes colourless.

7. Expression of results**7.1. Formula and method of calculation**

The percentage of formic acid, formates and of other oxidizable impurities, expressed as formic acid, is given by:

$$\frac{2.3b}{m_0} \times \left(\frac{100a}{b} - V \right)$$

where:

- a = molarity of potassium permanganate,
- b = molarity of sodium thiosulphate,
- m_0 = initial mass in grams of the sample taken,
- V = volume in millilitres of 0.1 mol/l sodium thiosulphate used in the titration.

7.2. Repeatability

The difference between the results of two determinations when carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 5 mg per 100 g of sample.

8. Notes

- 8.1. A volume of 11.3 ml of 0.1 mol/l sodium thiosulphate is equivalent to 0.2 % formic acid in a 10 g sample.
- 8.2. If there is no formate present, the volume required will be 20 ml, but if there is more than 0.27 % (m/m) of formic acid present, there will be insufficient excess of potassium permanganate and a fixed minimum volume of 8 ml will be obtained. In this case repeat the determination using a smaller sample weight.

METHOD 3**DETERMINATION OF NON-VOLATILE SUBSTANCES IN PROPIONIC ACID (E 280)****1. Scope and field of application**

The method determines non-volatile substances in propionic acid (E 280).

2. Definition

The content of non-volatile material in propionic acid: the content of non-volatile material as determined by the method specified.

3. Principle

The sample is evaporated and then dried at $103 \pm 2^\circ\text{C}$ and the residue determined gravimetrically.

4. Apparatus

- 4.1. Evaporation vessel, silica or platinum and of sufficient size to contain 100 g of sample.
- 4.2. Oven, electrically heated, thermostatically controlled at $103 \pm 2^\circ\text{C}$.
- 4.3. Analytical balance.
- 4.4. Water bath, boiling.
- 4.5. Desiccator, containing freshly activated silica gel or equivalent desiccant with water content indicator.

5. Procedure

Weigh, to the nearest 0.1 g, 100 g of the sample of propionic acid into a previously dried and weighed vessel (4.1). Evaporate over a boiling water bath in a fume cupboard (4.4). When all the propionic acid has evaporated, place in an oven (4.2) at $103 \pm 2^\circ\text{C}$ for one hour. Place in a desiccator and allow to cool and then weigh. Repeat the heating, cooling and weighing operations until the difference between two successive weighings is less than 0.5 mg. Should an increase in mass occur the lowest recorded reading will be used in the calculation.

6. Expression of results**6.1. Formula and method of calculation**

The non-volatile matter content, calculated as a percentage of the sample, is given by:

$$\frac{100 \times m_1}{m_0}$$

where:

m_1 = mass in grams of the residue after evaporation,

m_0 = mass in grams of the sample taken.

6.2. Repeatability

The difference between the results of two determinations, carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 5 mg per 100 g of sample.

METHOD 4**DETERMINATION OF THE LOSS OF MASS ON DRYING OF SODIUM NITRITE
(E 250)****1. Scope and field of application**

The method determines the loss of mass on drying of sodium nitrite (E 250).

2. Definition

The moisture content of sodium nitrite; the loss of mass on drying as determined by the method specified.

3. Principle

The loss of mass on drying is obtained by heating in an oven at $103 \pm 2^\circ\text{C}$, weighing and calculation of the loss in mass.

4. Apparatus

- 4.1. Oven, electrically heated, thermostatically controlled at $103 \pm 2^\circ\text{C}$.
- 4.2. Weighing dish, flat-bottomed, glass, of diameter 60 to 80 mm and depth at least 25 mm, with loose-fitting lid.
- 4.3. Desiccator, containing freshly activated silica gel or equivalent desiccant with water content indicator.
- 4.4. Analytical balance.

5. Procedure

Remove the lid from the weighing dish (4.2) and heat dish and lid in the oven (4.1) at $103 \pm 2^\circ\text{C}$ for one hour. Replace the lid and place the dish (4.2) with its lid in the desiccator (4.3) and allow to cool to room temperature. Weigh the covered dish (4.2) to the nearest

10 mg. Accurately weigh, to the nearest 10 mg, approximately 10 g of sample into the covered dish. Remove the lid and place both dish and lid in the oven (4.1) for one hour at $103 \pm 2^\circ\text{C}$. Replace the lid and allow the covered dish to cool to room temperature in the desiccator (4.3). Weigh it to the nearest 10 mg. Repeat the heating, cooling and weighing until the difference between two successive weights is less than 10 mg. Should an increase in mass occur, the lowest recorded reading will be used in the calculation.

6. **Expression of results**

6.1. *Formula and method of calculation*

The loss of mass on drying, calculated as a percentage by mass of the sample, is given by:

$$\frac{100 \times (m_2 - m_3)}{(m_2 - m_1)}$$

where:

m_1 = mass in grams of the dish,

m_2 = mass in grams of the dish and sample before drying,

m_3 = mass in grams of the dish and sample after drying.

6.2. *Repeatability*

The difference between the results of two determinations, carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 100 mg per 100 g of sample.

METHOD 5

LIMIT TEST FOR SALICYLIC ACID IN ETHYL *p*-HYDROXYBENZOATE (E 214), ETHYL-*p*-HYDROXYBENZOATE, SODIUM SALT (E 215), *n*-PROPYL *p*-HYDROXYBENZOATE (E 216), *n*-PROPYL *p*-HYDROXYBENZOATE, SODIUM SALT (E 217), METHYL *p*-HYDROXYBENZOATE (E 218), METHYL *p*-HYDROXYBENZOATE, SODIUM SALT (E 219)

1. **Scope and field of application**

The method detects salicylic acid in ethyl *p*-hydroxybenzoate (E 214), *n*-propyl *p*-hydroxybenzoate (E 216), and methyl *p*-hydroxybenzoate (E 218) and in their sodium salts (E 215, E 217 and E 219).

2. **Definition**

The detection of the limit test concentration of salicylic acid: the limit test result as determined by the method specified.

3. **Principle**

A violet colouration is produced from the reaction of ammonium iron (III) sulphate with a solution of the sample. Its intensity is compared with that produced by a reference solution.

4. Reagents

- 4.1. Ammonium iron (III) sulphate solution, 0.2 % m/v. Prepare by dissolving 0.2 g of ammonium iron (III) sulphate dodecahydrate in 50 ml of water, add 10 ml of nitric acid, 10 % v/v, and dilute to 100 ml with water.
- 4.2. Ethanol, 95 % v/v.
- 4.3. Salicylic acid solution, 0.1 g/l.
- 4.4. Sulphuric acid, 1 mol/l.

5. Apparatus

- 5.1. Nessler cylinders, graduated at 50 ml. Total volume approximately 60 ml.

6. Procedure**6.1. Ethyl, n-propyl and methyl p-hydroxybenzoate samples**

- 6.1.1. Weigh, to the nearest 1 mg, 0.1 g of the sample and dissolve in 10 ml of 95 % v/v ethanol (4.2). Transfer the solution to a graduated Nessler cylinder (5.1) and dilute to 50 ml with water. Stir and add 1 ml of ammonium iron (III) sulphate solution (4.1) while stirring. Allow to stand for one minute.
- 6.1.2. Prepare a comparison solution at the same time by repeating 6.1.1, but replacing the 0.1 g of sample by 1 ml of salicylic acid solution (4.3).
- 6.1.3. Compare the colouring in the sample solution with that appearing in the comparison solution.
- 6.2. *Sodium salts of ethyl, n-propyl and methyl p-hydroxybenzoate samples*
 - 6.2.1. Repeat 6.1.1 acidifying to pH 5 using 1 mol/l sulphuric acid (4.4) before dilution to 50 ml.
 - 6.2.2. Repeat 6.1.2.
 - 6.2.3. Repeat 6.1.3.

7. Expression of results**7.1. Limit test interpretation**

If the reddish-violet colour appearing in the sample solution tube is more intense than that appearing in the comparison solution tube, the test is positive and the sample contains more than 0.1 % salicylic acid.

7.2. Sensitivity

The limit of detection of the test is 30 mg of salicylic acid per 100 g of sample.

7.3. Observations

The results of two limit tests carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall be identical.

METHOD 6**DETERMINATION OF FREE ACETIC ACID IN SODIUM DIACETATE (E 262)****1. Scope and field of application**

The method determines acetic acid in sodium diacetate (E 262).

2. Definition

The acetic acid content: the content of acetic acid as determined by the method specified.

3. Principle

Direct titration of the acetic acid in the sample using standard sodium hydroxide solution and phenolphthalein indicator.

4. Reagents

4.1. Phenolphthalein solution 1% (m/v) in ethanol.

4.2. Sodium hydroxide, 1 mol/l.

5. Apparatus

5.1. Analytical balance.

6. Procedure

Weigh, to the nearest 1 mg, about 3 g of the test sample and dissolve in about 50 ml of water. Add two or three drops of phenolphthalein indicator solution (4.1) and titrate with 1 mol/l sodium hydroxide (4.2) until a red tint persists for five seconds.

7. Expression of results**7.1. Formula and method of calculation**

The acetic acid content, as a percentage of the sample mass, is given by:

$$\frac{6.005 \times V \times c}{m_0}$$

where:

V = volume in millilitres of sodium hydroxide (4.2) required,

c = concentration of the sodium hydroxide solution in mol/l,

m₀ = initial mass in grams of the sample taken.

7.2. Repeatability

The difference between the results of two determinations when carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 500 mg per 100 g of sample.

8. **Comment**

A volume of 20 ml is obtained when 3 g of a sample containing 40 % acetic acid is titrated with 1 mol/l sodium hydroxide.

METHOD 7**DETERMINATION OF SODIUM ACETATE IN SODIUM DIACETATE (E 262)**1. **Scope and field of application**

The method determines sodium acetate and water, expressed as sodium acetate, in sodium diacetate (E 262).

2. **Definition**

Sodium acetate content: the content of sodium acetate and water expressed as sodium acetate as determined by the method specified.

3. **Principle**

The sample is dissolved in glacial acetic acid, before titration, with standard perchloric acid, using crystal violet as indicator.

4. **Reagents**

- 4.1. Glacial acetic acid $\rho_{20^\circ\text{C}} = 1.049 \text{ g/ml}$ (for non-aqueous titrations).
- 4.2. Crystal violet, CI No 42555 indicator solution, 0.2 % (m/v) in glacial acetic acid.
- 4.3. Potassium hydrogen phthalate, $\text{C}_8\text{H}_5\text{KO}_4$.
- 4.4. Acetic anhydride $(\text{CH}_3\text{CO})_2\text{O}$.
- 4.5. Perchloric acid, 0.1 mol/l in glacial acetic acid. This must be prepared and standardized as follows:
Weigh P g of perchloric acid solution into a 1 000 ml volumetric flask fitted with a ground-glass stopper. The quantity P is calculated from the formula:

$$P = \frac{1\,004.6}{m}$$

where m is the concentration (per cent m/m) of perchloric acid determined by alkali-metric titration (70 to 72 % m/m acid is the most suitable). Add about 100 ml of glacial acetic acid and then a quantity, Q g, of acetic anhydride in successive small portion, stirring and cooling the mixture continuously during the additions. The quantity Q may be calculated from the formula:

$$Q = \frac{(567 \times P) - 5\,695}{a}$$

where P is the weighed amount of perchloric acid and a is the concentration (per cent m/m) of the acetic anhydride. Stopper the flask and allow to stand for 24 hours in a dark place, then add sufficient glacial acetic acid to produce 1 000 ml of solution. The solution prepared in this way is practically anhydrous. Standardize the solution against potassium hydrogen phthalate as follows:

Weigh accurately, to the nearest 0.1 mg, about 0.2 g of potassium hydrogen phthalate, previously dried at 110 °C for two hours, and dissolve in 25 ml of glacial acetic acid in a titration flask, warming gently. Cool, add two drops of a 0.2 % (m/m) crystal violet solution (4.2) in glacial acetic acid and titrate with the perchloric acid solution until the colour of the indicator changes to pale green. Carry out a blank titration using the same volume of solvent and deduct the value of the blank from the value found in the actual determination. Each 20.42 mg of potassium hydrogen phthalate is equivalent to 1 ml of 0.1 mol/l perchloric acid.

5. Apparatus

5.1. Analytical balance.

6. Procedure

Weigh, to the nearest 0.5 mg, about 0.2 g of the sample and dissolve in 50 ml of glacial acetic acid (4.1). Add a few drops of crystal violet indicator solution (4.2) and titrate to a pale green end-point, using standard 0.1 mol/l perchloric acid (4.5).

7. Expression of results

7.1. Formula and method of calculation

The sodium acetate content, as defined in section 2 (definition), expressed as a percentage by weight of the sample, is given by the following formula:

$$\frac{8.023 \times V \times c}{m_0}$$

where:

V = volume in millilitres of the standard perchloric acid (4.5) used,

c = molarity of the perchloric acid solution (4.5),

m₀ = initial mass in grams of the sample taken.

7.2. Repeatability

The difference between the results of two determinations when carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 1.5 g per 100 g of sample.

8. Observations

The reagents used in this method are toxic and explosive and need careful handling.

METHOD 8

LIMIT TEST FOR ALDEHYDES IN SORBIC ACID (E 200), SODIUM, POTASSIUM AND CALCIUM SORBATES (E 201, E 202, E 203) AND PROPIONIC ACID (E 280)

1. Scope and field of application

The method detects aldehydes, expressed as formaldehyde, in:

- sorbic acid (E 200),
- sodium, potassium and calcium sorbates (E 201, E 202, E 203),
- propionic acid (E 280).

2. Definition

The detection of the limit test concentration of aldehydes: the limit test result as determined by the method specified.

3. Principle

The aldehydes in the test solution, and the formaldehyde in a comparison solution, react with Schiff's reagent to produce red coloured complexes, the intensities of which are compared.

4. Reagents

4.1. Formaldehyde standard solution (0.01 mg/ml): prepared by dilution of concentrated formaldehyde solution (400 mg/ml).

4.2. Schiff's reagent.

5. Procedure

5.1. Weigh, to the nearest 1 mg, about 1 g of the sample, add to 100 ml of water and shake. Filter the solution if necessary and treat 1 ml of filtrate or sample solution with 1 ml of Schiff's reagent (4.2). At the same time, treat 1 ml of formaldehyde comparison solution (4.1) with 1 ml of Schiff's reagent (4.2).

5.2. Compare the colour in the sample solution with that appearing in the comparison solution.

6. Expression of results**6.1. Limit test interpretation**

If the red colour appearing in the sample solution tube is more intense than that appearing in the comparison solution tube, the test is positive and the sample contains more than 0.1 % aldehydes, expressed as formaldehyde.

6.2. Sensitivity

The limit of detection of this test is 30 mg of formaldehyde per 100 g of sample.

6.3. Observations

The result of two limit tests when carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall be identical.

METHOD 9**DETERMINATION OF THE PEROXIDE NUMBER IN LECITHINS (E 322)****1. Scope and field of application**

The method determines the peroxide number in lecithins (E 322).

2. Definition

Peroxide number of lecithins: the result obtained as determined by the method specified.

3. Principle

Oxidation of potassium iodide by the peroxides of lecithin and titration of the iodine liberated using standard sodium thiosulphate solution.

4. Reagents

- 4.1. Acetic acid glacial.
- 4.2. Chloroform.
- 4.3. Potassium iodide.
- 4.4. Sodium thiosulphate, 0.1 mol/l or 0.01 mol/l.
- 4.5. Starch solution (approximately 1 % m/v).

5. Apparatus

- 5.1. Analytical balance.
- 5.2. Apparatus, as shown in the figure, consisting of:
 - 5.2.1. round-bottomed flask, 100 ml;
 - 5.2.2. reflux condenser;
 - 5.2.3. glass tube, 250 mm long and 22 mm internal diameter, fitted with ground glass joints;
 - 5.2.4. micro beaker (external dimension of 20 mm diameter and 35 to 50 mm height).

6. Procedures

- 6.1. Place 10 ml of glacial acetic acid (4.1) and 10 ml of chloroform (4.2) in the 100 ml flask (5.2.1). Fit the glass tube (5.2.3) and reflux condenser (5.2.2) and gently boil the mixture for two minutes to expel all dissolved air. Dissolve 1 g of potassium iodide (4.3) in 1.3 ml of water and add this solution to the mixture in the flask (5.2.1) taking care that the boiling is not interrupted.

If a yellow colour appears at this stage the determination must be rejected and repeated using fresh reagents.

- 6.2. Accurately weigh, to the nearest 1 mg, about 1 g of the sample and, after a further two minutes of boiling, add the weighed sample to the contents of the flask (5.2.1) again taking care that the boiling remains continuous. For this purpose the sample should be contained in a micro-beaker (5.2.4) which may be lowered through the glass tube (5.2.3) with a glass rod, the bottom of which has been suitably shaped as shown in the diagram. The condenser (5.2.2) may be removed for a short time. Continue boiling for three to four minutes. Stop heating and immediately disconnect the condenser (5.2.2). Quickly add 50 ml of water through the glass tube (5.2.3). Remove the glass tube (5.2.3) and cool the flask (5.2.1) to room temperature under the water tap. Titrate with sodium thiosulphate (0.1 mol/l or 0.01 mol/l) (4.4) until the aqueous layer becomes pale yellow. Add 1 ml of starch solution (4.5) and continue the titration until the blue colour is discharged. Shake the flask (5.2.1) well during the titration to ensure the complete extraction of iodine from the non-aqueous layer.

- 6.3. Obtain a blank titration value by repeating the complete procedure 6.1 and 6.2, but without adding the sample.

7. **Expression of results**

7.1. *Formula and method of calculation*

The peroxide number in the sample, in milliequivalents per kilogram, is given by:

$$\frac{1\,000 \times a \times (V_1 - V_2)}{m_0}$$

where:

V_1 = volume in millilitres of thiosulphate solution required for the titration of the sample (6.2),

V_2 = volume in millilitres of thiosulphate solution required for the titration of the blank (6.3),

a = concentration of sodium thiosulphate solution in mol/l,

m_0 = initial mass in grams of the sample taken.

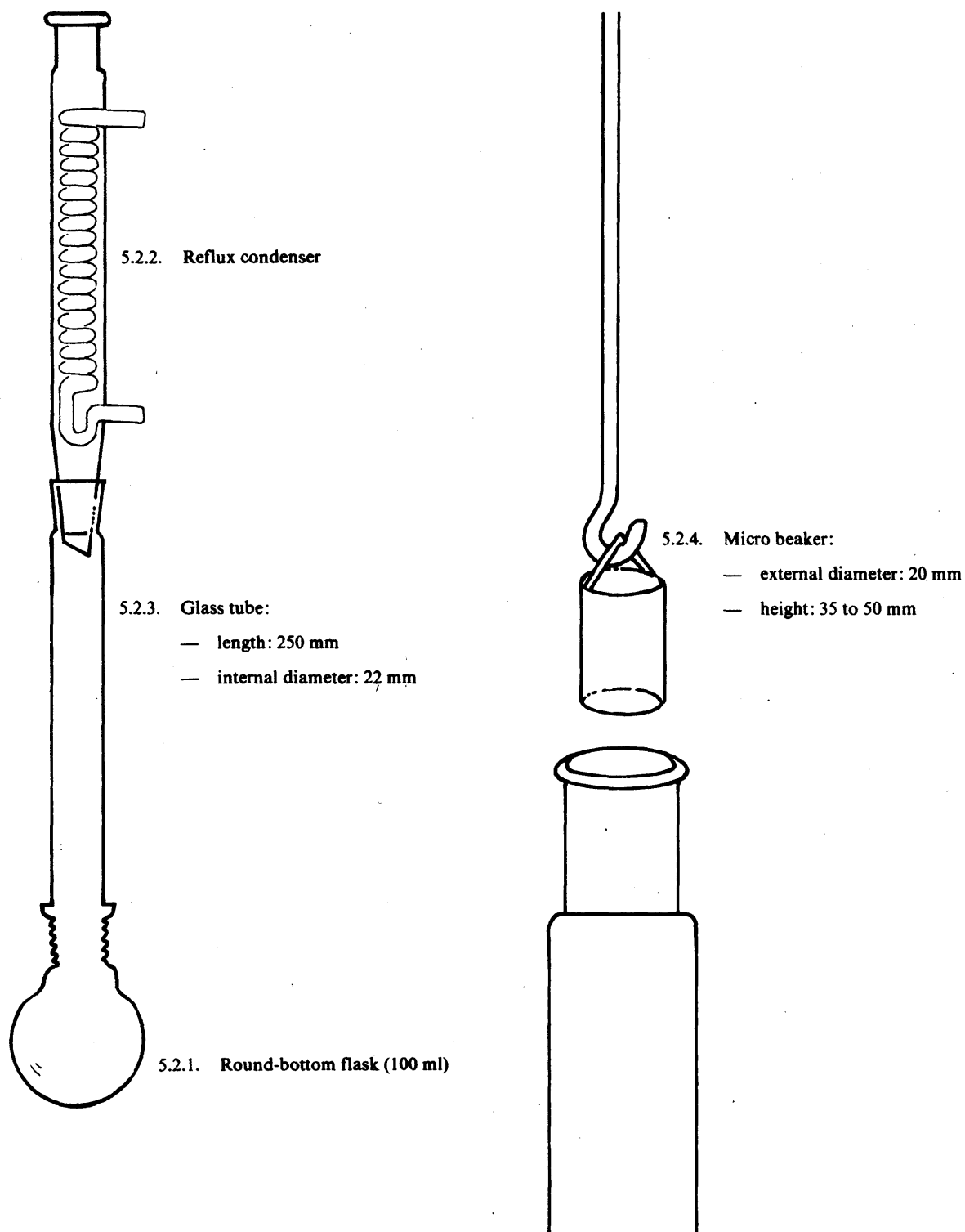
7.2. *Repeatability*

The difference between the results of two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 0.5 (expressed as a peroxide number in milliequivalents per kilogram of sample).

8. **Notes**

- 8.1. The choice of the concentration of the sodium thiosulphate used depends on the anticipated titration value. If less than 0.5 ml of 0.1 mol/l sodium thiosulphate is required, repeat the determination using 0.01 mol/l sodium thiosulphate.

- 8.2. The determination should not be carried out in strong light.



Apparatus for the determination of peroxide number in lecithins

METHOD 10**DETERMINATION OF TOLUENE-INSOLUBLE MATTER IN LECITHINS (E 322)****1. Scope and field of application**

The method determines the toluene-insoluble matter in lecithins (E 322).

2. Definition

The toluene-insoluble matter content: the content of toluene-insoluble matter as determined by the method specified.

3. Principle

The sample is dissolved in toluene, filtered, and the residue dried and weighed.

4. Reagents**4.1. Toluene.****5. Apparatus****5.1. Sintered glass crucible, 30 ml capacity, G 3 or equivalent porosity.****5.2. Drying oven, electrically heated and thermostatically controlled at $103 \pm 2^\circ\text{C}$.****5.3. Water bath, operating at a temperature not exceeding 60°C .****5.4. Desiccator, containing freshly activated silica gel or equivalent desiccant with a water content indicator.****5.5. Conical flask of 500 ml.****5.6. Vacuum pump.****5.7. Analytical balance.****6. Procedure****6.1. Dry a 30 ml sintered glass crucible (5.1) in an oven at $103 \pm 2^\circ\text{C}$ (5.2). Transfer the crucible to desiccator (5.4), allow to cool and then weigh.****6.2. Thoroughly mix the sample of lecithins, if necessary after warming in a water bath (5.3). Weigh, to the nearest 1 mg, about 10 g of the sample into a conical flask (5.5). Add 100 ml of toluene (4.1) and swirl the mixture until all the lecithin has apparently dissolved. Filter the solution through the sintered glass crucible (5.1). Wash the conical flask (5.5) with 25 ml of toluene (4.1) and pass the washings through the crucible (5.1). Repeat this process with another 25 ml of toluene (4.1). Remove excess toluene from the crucible (5.1) by suction.**

- 6.3. Dry the crucible (5.1) in the drying oven (5.2) at $103 \pm 2^\circ\text{C}$ for two hours. Place in desiccator (5.4) and allow to cool. Weigh the crucible and residue when cool.
- 6.4. Repeat 6.3 until the difference in weight between two successive weighings is less than 0.5 mg.

Should an increase in mass occur, the lowest recorded reading will be used in the calculation.

7. **Expression of results**

7.1. *Formula and method of calculation*

The content of toluene insoluble substances is given by:

$$\frac{100 (m_2 - m_1)}{m_0}$$

where:

- m_1 = mass in grams of the empty crucible (6.1),
 m_2 = mass in grams of the crucible and residues (6.4),
 m_0 = initial mass in grams of the sample taken.

7.2. *Repeatability*

The difference between the results of two determinations carried out in simultaneous or rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 30 mg per 100 g of sample.

METHOD 11

LIMIT TEST FOR REDUCING SUBSTANCES IN SODIUM, POTASSIUM AND CALCIUM LACTATES (E 325, E 326, E 327)

1. **Scope and field of application**

The test detects qualitatively reducing substances in:

- sodium lactate (E 325),
- potassium lactate (E 326),
- calcium lactate (E 327).

2. **Definition**

The detection of the limit test concentration of reducing substances: the limit test result as determined by the method specified.

3. **Principle**

Fehling's solution is reduced by substances capable of exhibiting reducing action. Such substances will normally be reducing sugars.

4. **Reagents**

- 4.1. Fehling's solution A: 6.93 g of copper sulphate pentahydrate is dissolved in water and made up 100 ml with water.
- 4.2. Fehling's solution B: 34.6 g of potassium sodium tartrate and 10 g of sodium hydroxide are dissolved in water and made up to 100 ml with water.

5. Procedures

Weigh, to the nearest 1 mg, about 1 g of the sample and dissolve in 10 ml of warm water. Add 2 ml of Fehling's solution A (4.1) and 2 ml of Fehling's solution B (4.2) and then boil the mixture for one minute and observe whether a colour change occurs. The precipitation of calcium sulphate, which sometimes occurs, does not interfere.

6. Expression of results**6.1. Limit test interpretation**

If there is a colour change after boiling (5), the test is positive and the presence of reducing substances is indicated.

6.2. Sensitivity

The limit of detection for reducing substances reacting is 100 mg glucose per 100 g of sample.

6.3. Observations

6.3.1. The results of two limit tests carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall be identical.

6.3.2. All of the Fehling solutions react if 2 % glucose is present in the sample.

METHOD 12**DETERMINATION OF VOLATILE ACIDS IN ORTHOPHOSPHORIC ACIDS (E 338)****1. Scope and field of application**

The method determines volatile acids, expressed as acetic acid, in orthophosphoric acid (E 338).

2. Definition

Volatile acid content: the content of volatile acids, expressed as acetic acid, as determined by the method specified.

3. Principle

Water is added to the sample and the solution is distilled. The distillate is titrated against standard sodium hydroxide solution and the acidity calculated and expressed as acetic acid.

4. Reagents

4.1. Pheolphthalein solution, 1 % (m/v) in ethanol.

4.2. Sodium hydroxide, 0.01 mol/l.

5. Apparatus

5.1. Distillation apparatus including a spray trap.

6. Procedure

Weigh, to the nearest 50 mg, about 60 g of the sample and place the weighed sample and 75 ml of freshly boiled cooled water in the distillation flask fitted with the spray trap (5.1). Mix and then distil about 50 ml.

Titrate the distillate with standard 0.01 mol/l sodium hydroxide (4.2) using phenolphthalein (4.1) as indicator. Continue the titration until the first red tint in the solution persists for 10 seconds.

7. Expression of results**7.1. Formula and method of calculation**

The content of volatile acids, expressed as milligrams per kilogram of acetic acid, is given by:

$$\frac{600 \times V}{m_0}$$

where:

V = volume in millilitres of 0.01 mol/l sodium hydroxide solution used for neutralization,

m_0 = mass in grams of the orthophosphoric acid sample.

7.2. Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 1 mg per 100 g of sample.

METHOD 13**LIMIT TEST FOR NITRATE IN ORTHOPHOSPHORIC ACID (E 338)****1. Scope and field of application**

This method detects nitrates in orthophosphoric acid (E 338).

2. Definition

The detection of the limit test concentration of nitrate, expressed as sodium nitrate; the limit test result as determined by the method specified.

3. Principle

The sample is added to indigo carmine solution in a concentrated sulphuric acid medium. The blue colouration present is discharged by oxidizing agents including nitrates.

4. Reagents

4.1. Indigo carmine solution, 0.18 % (m/v): dissolve 0.18 g of sodium indigotin disulphonate in water and make up to 100 ml with water.

4.2. Sodium chloride solution, 0.05 % (m/v).

4.3. Sulphuric acid concentrated ($\rho_{20} = 1.84$ g/ml).

5. Procedure

Measure 2 ml of the sample and dilute to 10 ml with the sodium chloride solution (4.2). Add 0.1 ml of carmine indigo solution (4.1) and then slowly add 10 ml of concentrated sulphuric acid (4.3), cooling during the addition. Note whether the blue colouration of the solution persists for five minutes.

6. Expression of results**6.1. Limit test interpretation**

If the blue colouration is discharged within five minutes the test is positive and the content of oxidizing agents, expressed as sodium nitrate, is greater than 5 mg/kg.

6.2. Observations**6.2.1. Carry out a blank test.**

6.2.2. The results of two limit tests when carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall be identical.

6.2.3. Indigo carmine solution should not be used if it has been prepared for more than 60 days.

6.2.4. If a positive result is obtained the sample may contain nitrates and other oxidizing agents and the test must be repeated using ISO Method 3709 (1976) 'Phosphoric acid for industrial use (including foodstuffs) — determination of oxides of nitrogen content — 3,4-xyleneol spectrophotometric method'.

METHOD 14

DETERMINATION OF WATER-INSOLUBLE SUBSTANCES PRESENT IN MONO-, DI- AND TRI-SODIUM ORTHOPHOSPHATES AND MONO-, DI- AND TRI-POTASSIUM ORTHOPHOSPHATES (E 339(i), E 339(ii), E 339(iii), E 340(i), E 340(ii) AND E 340(iii))

1. Scope and field of application

The method determines water-insoluble matter in:

- mono-sodium orthophosphate (E 339(i)),
- di-sodium orthophosphate (E 339(ii)),
- tri-sodium orthophosphate (E 339(iii)),
- mono-potassium orthophosphate (E 340(i)),
- di-potassium orthophosphate (E 340(ii)),
- tri-potassium orthophosphate (E 340(iii)).

2. Definition

Water insoluble matter: the content of water-insoluble matter as determined by the method specified.

3. Principle

The sample is dissolved in water and filtered through a suitable porcelain crucible. After washing and drying the residue is weighed and calculated as water-insoluble matter.

4. Apparatus

- 4.1. Sintered porcelain crucible, porosity G 3 or equivalent.
- 4.2. Desiccator, containing freshly activated silica gel with a water content indicator, or equivalent desiccant.
- 4.3. Oven, thermostatically controlled at $103 \pm 2^\circ\text{C}$.
- 4.4. Polypropylene beaker, 400 ml.
- 4.5. Water bath, boiling.

5. Procedure

Weigh, to the nearest 10 mg, about 10 g of the sample of phosphate and dissolve in 100 ml of hot water by bringing to the boil in a polypropylene beaker (4.4) and maintaining on a hot water bath (4.5) for 15 minutes. Filter the solution through a previously cleaned, dried and weighed crucible (4.1). Wash the insoluble residue with hot water. Place the crucible with residue in the oven (4.3) and dry at $103 \pm 2^\circ\text{C}$ for two hours.

Place the crucible in the desiccator and allow to cool and weigh the crucible.

Repeat the drying, cooling and weighing until the difference in weight of two successive weighings is less than 0.5 mg. Should an increase in mass occur the lowest recorded reading will be used in the calculation.

6. Expression of results**6.1. Formula and method of calculation**

The content of water-insoluble matter in the sample is given by:

$$\frac{m_1}{m_0} \times 100$$

where:

m_1 = mass in grams of the residue after drying,

m_0 = mass in grams of the sample taken.

6.2. Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 10 mg per 100 g of sample.

METHOD 15**DETERMINATION OF THE pH OF FOOD ADDITIVES****1. Scope and field of application**

The method outlines general instructions on how to determine the pH of food additives.

2. Definition

The pH of a food additive: the pH value as determined by the method specified.

3. Principle

The pH value of an aqueous solution of the dissolved or slurried sample is conventionally determined using a glass electrode, reference electrode and pH meter.

4. Reagents

4.1. Calibrate the instrument using the following buffer solutions:

4.1.1. Buffer solution pH 6.88 at 20 °C, consisting of equal volume 0.05 mol/l potassium dihydrogen phosphate (KH_2PO_4) and 0.05 mol/l disodium hydrogen ortho phosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$).

4.1.2. Buffer solution pH 4 at 20 °C, consisting of 0.05 mol/l potassium hydrogen phthalate ($\text{C}_8\text{H}_5\text{KO}_4$).

4.1.3. Buffer solution pH 9.22 at 20 °C, consisting of 0.05 mol/l sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$).

4.2. Saturated or 3 mol/l potassium chloride solution, or other suitable solution prescribed by the electrode manufacturer, to fill the reference electrode.

4.3. Distilled water, carbon dioxide-free, having a pH between 5 and 6.

5. Apparatus

5.1. pH meter, with an accuracy within 0.01 pH units.

5.2. Electrodes, either a combined glass electrode or single glass and reference electrodes together with suitable clamps to hold the electrodes.

5.3. Magnetic stirrer, with heater element.

5.4. Thermometer, calibrated over the range 0 to 100 °C.

6. Procedure**6.1. Standardization of the pH meter**

The glass electrodes must be set using the instructions given by the manufacturer. The pH reading from the electrodes must be regularly checked by comparison with buffer solutions of known pH.

Electrodes should be washed with water and then gently wiped with a soft tissue or should be rinsed with water and then twice with the next sample/standard solution before being placed in the next sample/standard solution to be used.

If the sample to be considered has an acid pH, the buffer solutions used to check the pH reading should be those of pH 4 (4.1.2) and pH 6.88 (4.1.1). If the sample to be analyzed has an alkaline pH, the buffer solutions to be used to check the pH reading should be those of pH 9.22 (4.1.3) and pH 6.88 (4.1.1).

6.2. Measurement of the sample solution

The concentration of sample to be used or the sample preparation procedure to be adopted is as prescribed in the appropriate Community food additive Directive.

Prepare the sample solution as directed using distilled water (4.3) and then adjust to 20 °C while stirring. Stop the stirring, place the glass electrodes in the solution and after two minutes note the pH on the pH meter (5.1).

7. Expression of results**7.1. Repeatability**

The difference between the results of two determinations when carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 0.05 pH unit.

8. Note

This method is only applicable to those pH requirements in Community food additives Directives where the food additive is dissolved or slurried in water.
