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First Commission Directive 81/712/EEC of 28 July 1981 laying down Community methods of analysis for verifying that certain additives used in foodstuffs satisfy criteria of purity

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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 authorized 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 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.

Article 3

This Directive is addressed to the Member States.

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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III. PRESERVATIVES

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V. GENERAL

V.1. Determination of pH in food additives using Annex II, method 15.

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 71 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 ± 2 ºC.

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 ± 2 ºC 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 75 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: >PIC FILE= "T0020608">

where:

m1 = mass in grams of the residue after evaporation,

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 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 >PIC FILE= "T0020908">

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 0 702 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 75 g of potassium iodide (4.1). Swirl the mixture until all precipitated manganese dioxide has redissolved. Titrate with 0 71 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: >PIC FILE= "T0020609">

where:

a = molarity of potassium permanganate,

b = molarity of sodium thiosulphate,

m0 = initial mass in grams of the sample taken,

V = volume in millilitres of 0 71 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 73 ml of 0 71 mol/l sodium thiosulphate is equivalent to 0 72 % 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 0 727 % (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 ± 2 ºC 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 ± 2 ºC.

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 71 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 ± 2 ºC 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 75 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: >PIC FILE= "T0020610">

where:

m1 = mass in grams of the residue after evaporation,

m0 = 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 ± 2 ºC, weighing and calculation of the loss in mass.

4. Apparatus 4.1. Oven, electrically heated, thermostatically controlled at 103 ± 2 ºC.

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 ± 2 ºC 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 ± 2 ºC. 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: >PIC FILE= "T0020611">

where:

m1 = mass in grams of the dish,

m2 = mass in grams of the dish and sample before drying,

m3 = 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 72 % m/v. Prepare by dissolving 0 72 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 71 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 71 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 71 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 71 % 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: >PIC FILE= "T0020612">

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 acetate acid, before titration, with standard perchloric acid, using crystal violet as indicator.

4. Reagents >PIC FILE= "T0020613">

>PIC FILE= "T0020614">

5. Apparatus 5.1. Analytical balance.

6. Procedure

Weigh, to the nearest 0 75 mg, about 0 72 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 71 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: >PIC FILE= "T0020615">

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 75 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 aid (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 (0 701 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 71 % 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 71 mol/l or 0 701 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 73 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 71 mol/l or 0 701 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: >PIC FILE= "T0020616">

where:

V1 = volume in millilitres of thiosulphate solution required for the titration of the sample (6.2),

V2 = volume in millilitres of thiosulphate solution required for the titration of the blank (6.3),

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

m0 = 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 75 (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 75 ml of 0 71 mol/l sodium thiosulphate is required, repeat the determination using 0 701 mol/l sodium thiosulphate.

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

>PIC FILE= "T0020617">

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 ± 2 ºC.

5.3. Water bath, operating at a temperature not exceeding 60 ºC.

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 ± 2 ºC (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 ± 2 ºC 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 75 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: >PIC FILE= "T0020618">

where:

m1 = mass in grams of the empty crucible (6.1),

m2 = mass in grams of the crucible and residues (6.4),

m0 = 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 : 6 793 g of copper sulphate pentahydrate is dissolved in water and made up 100 ml with water.

4.2. Fehling's solution B : 34 76 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, 0 701 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 0 701 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: >PIC FILE= "T0020619">

where:

V = volume in millilitres of 0 701 mol/l sodium hydroxide solution used for neutralization,

m0 = 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 >PIC FILE= "T0020911">

5. Procedure

Measure 2 ml of the sample and dilute to 10 ml with the sodium chloride solution (4.2). Add 0 71 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-xylenol spectrophometric 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 ± 2 ºC.

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 ± 2 ºC 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 75 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: >PIC FILE= "T0020620">

where:

m1 = mass in grams of the residue after drying,

m0 = 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 6 788 at 20 ºC, consisting of equal volume 0 705 mol/l potassium dihydrogen phosphate (KH2PO4) and 0 705 mol/l disodium hydrogen ortho phosphate dyhidrate (Na2HPO4 7 2H2O).

4.1.2. Buffer solution pH 4 at 20 ºC, consisting of 0 705 mol/l potassium hydrogen phthalate (C8H5KO4).

4.1.3. Buffer solution pH 9 722 at 20 ºC, consisting of 0 705 mol/l sodium borate (Na2B4O7 7 10H2O).

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 0 701 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 ºC.

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 6 788 (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 9 722 (4.1.3) and pH 6 788 (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 ºC 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 0 705 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.