FIRST SUPPLEMENT
TO THE
FOURTH EDITION

FOOD CHEMICALS CODEX

INSTITUTE OF MEDICINE

FIRST SUPPLEMENT
TO THE
FOURTH EDITION

FOOD CHEMICALS CODEX

Effective December 1, 1997

COMMITTEE ON FOOD CHEMICALS CODEX
Food and Nutrition Board
Institute of Medicine
National Academy of Sciences

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This study is supported by U.S. Food and Drug Administration Contract No. 223-92-2250.

COMPLIANCE WITH FEDERAL STATUTES The fact that an article appears in the *Food Chemicals Codex* or its supplements does not exempt it from compliance with requirements of acts of Congress, with regulations and rulings issued by agencies of the United States Government under authority of these acts, or with requirements and regulations of governments in other countries that have adopted the *Food Chemicals Codex*. Revisions of the federal requirements that affect the *Codex* specifications will be included in *Codex* supplements as promptly as practicable.

EFFECTIVE DATE The specifications in this supplement become effective December 1, 1997.

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The image adopted as a logotype by the Institute of Medicine is based on a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

FIRST SUPPLEMENT TO THE FOURTH EDITION

FOOD CHEMICALS CODEX

INSTITUTE OF MEDICINE

* Member, Institute of Medicine

NEW MONOGRAPHS IN THIS FIRST SUPPLEMENT TO THE *FOOD CHEMICALS CODEX*, FOURTH EDITION

Calcium Lignosulfonate
beta Cyclodextrin
Dimethyl Dicarbonate
Glyceryl Palmitostearate
4-Hexylresorcinol
Magnesium Phosphate, Dibasic, Mixed Hydrates
Manganese Citrate
Olestra
Sodium Lignosulfonate
Sucrose Fatty Acid Esters
Sugar Beet Fiber
Vitamin K
Whey Protein Concentrate
Whey, Reduced Lactose
Whey, Reduced Minerals
Yeast, Autolyzed

Flavors

omega-Pentadecalactone
1,3,5-Undecatriene
Veratraldehyde

CHANGES IN TITLES OF *FOOD CHEMICALS CODEX* MONOGRAPHS IN THIS SUPPLEMENT

Fourth Edition Title	*First Supplement Title*
Sodium Carboxymethylcellulose	Cellulose Gum
Enzyme-Modified Milkfat	Enzyme-Modified Fats

[*] Member, Institute of Medicine
[†] Member, National Academy of Sciences

This First Supplement to the fourth edition of the *Food Chemicals Codex* contains additions and changes to certain sections of the original text. This supplement contains each new and revised monograph in its entirety, each preceded by a boxed explanatory section. The exception is *Section 3/ Flavor Chemicals*, in which the boxed sections follow each entry. The revised monographs supersede those in the fourth edition. However, changes in the section on analytical *General Tests and Assays* (Appendixes I through X in section 5 of the fourth edition) are specific to the relevant individual test or assay. The entire appendix in which the revised test or assay appears is not reprinted and it is necessary to still refer to the fourth edition for unchanged portions.

An errata section to the fourth edition is located just before the Index.

Users of this Supplement are reminded to consult the general policies and guidelines given in *Section 1/General Provisions and Requirements Applying to Specifications, Tests, and Assays of the Food Chemicals Codex*, fourth edition, pages 1 through 7. The policies and guidelines given therein remain in effect and are pertinent to this Supplement.

Contents

2 / *Monograph Specifications*

Revision: Add ***3-Chloropropane-1,2-diol*** and ***1,3-Dichloro-2-propanol*** specifications; correct ***Potassium*** and ***Sodium*** tests.

Acid Hydrolysates of Proteins

Acid-Hydrolyzed Proteins; Hydrolyzed Vegetable Protein (HVP); Hydrolyzed Plant Protein (HPP); Hydrolyzed (Source) Protein Extract; Acid-Hydrolyzed Milk Protein

DESCRIPTION

Acid Hydrolysates of Proteins are composed primarily of amino acids, small peptides (peptide chains of five or fewer amino acids), and salts resulting from the essentially complete hydrolysis of peptide bonds in edible proteinaceous materials catalyzed by heat and/or food-grade acids. Cleavage of peptide bonds typically ranges from a low of 85% to essentially 100%. In processing, the protein hydrolysates may be treated with safe and suitable alkaline materials. The edible proteinaceous materials used as raw materials are derived from corn, soy, wheat, yeast, peanuts, rice, or other safe and suitable vegetable or plant sources, or from milk. Individual products may be in liquid, paste, powder, or granular form.

Functional Use in Foods Flavoring agent; flavor enhancer; adjuvant.

REQUIREMENTS

Calculate all analyses on the dried basis. In a suitable tared container, evaporate liquid and paste samples to dryness on a steam bath, then, as for the powdered and granular forms, dry to constant weight at 105° (see the *General Provisions*).

Assay (Total Nitrogen; TN) Not less than 4.0% total nitrogen.
α-Amino Nitrogen (AN) Not less than 3.0%.
α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Not less than 62.0% and not more than 85.0%, when calculated on an ammonia nitrogen-free basis.
Ammonia Nitrogen (NH_3-N) Not more than 1.5%.
3-Chloropropane-1,2-diol (3-CPD) Not more than 1 mg/kg, calculated on the dried basis.
1,3-Dichloro-2-propanol (DCP) Not more than 0.05 mg/kg, calculated on the dried basis.
Glutamic Acid Not more than 20.0% as $C_5H_9NO_4$ and not more than 35.0% of the total amino acids.
Heavy Metals (as Pb) Not more than 10 mg/kg.
Insoluble Matter Not more than 0.5%.
Lead Not more than 5 mg/kg.
Potassium Not more than 30.0%.
Sodium Not more than 20.0%.

TESTS

Assay (Total Nitrogen) Proceed as directed under *Nitrogen Determination*, Appendix IIIC.
α-Amino Nitrogen Transfer 7 to 25 g, accurately weighed, into a 500-mL volumetric flask with the aid of several 50-mL portions of warm ammonia-free water, dilute to volume with water, and mix. Neutralize 20.0 mL of the solution with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide, using phenolphthalein TS as indicator, and add 10 mL of freshly prepared phenolphthalein–formol solution (50 mL of 40% formaldehyde containing 1 mL of 0.05% phenolphthalein in 50% alcohol neutralized exactly to pH 7 with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide). Titrate with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide to a distinct red color, add a small, but accurately measured, volume of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide in excess, and back titrate to neutrality with 0.2 *N* hydrochloric acid. Conduct a blank titration using the

same reagents, with 20 mL of water in place of the test solution. Each mL of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide is equivalent to 2.8 mg of α-amino nitrogen.

α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Calculate by dividing the percent α-amino nitrogen (*AN*) by the percent total nitrogen (*TN*) as corrected for ammonia nitrogen (NH_3-*N*) according to the formula

$$100[(AN - NH_3\text{-}N)/(TN - NH_3\text{-}N)].$$

Ammonia Nitrogen (**Caution**: Provide adequate ventilation.) (**Note**: All reagents should be nitrogen free, where available, or otherwise very low in nitrogen content.) Transfer from 700 mg to 2.2 g of the sample to a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass, wrapping the sample, if solid or semi-solid, in nitrogen-free filter paper to facilitate the transfer if desired.

Add about 200 mL of water, and mix. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets, or a 2 in 5 sodium hydroxide solution, down the inside of the flask so that it forms a layer under the solution, using a sufficient amount (usually about 25 g of solid NaOH) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of a measured excess of 0.5 *N* hydrochloric or sulfuric acid contained in a 500-mL flask. Add from 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and then heat until all of the ammonia has distilled, collecting at least 150 mL of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 *N* sodium hydroxide. Perform a blank determination, substituting 2 g of sucrose for the sample, and make any necessary correction (see the *General Provisions*). Each mL of 0.5 *N* acid consumed is equivalent to 7.003 mg of ammonia nitrogen.

> **Note**: If it is known that the substance to be determined has a low nitrogen content, 0.1 *N* acid and alkali may be used, in which case each mL of 0.1 *N* acid consumed is equivalent to 1.401 mg of nitrogen.

Calculate the percent ammonia nitrogen by dividing the weight of ammonia nitrogen, in mg, by the weight of the sample, in mg, times 100.

3-Chloropropane-1,2-diol (3-CPD)

3-CPD Stock Solution Transfer 12.5 mg, accurately weighed, of reagent-grade 3-chloropropane-1,2-diol (3-CPD) into a 100-mL volumetric flask; dilute to volume with ethyl acetate, and mix.

Dilute 3-CPD Solution Dilute 5 mL of *3-CPD Stock Solution* to 100 mL with ethyl acetate to yield a solution containing 6.25 µg/mL.

Internal Standard Solution Transfer 50 mg of 1-chlorotetradecane into a 50-mL volumetric flask, and dilute to volume with ethyl acetate. Dilute 1 mL of this solution to 100 mL with ethyl acetate to yield a solution containing 10 µg/mL.

Standard Solutions

A. Pipet 2 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask, dilute to volume with ethyl acetate, and mix. The resulting *Standard Solution* contains 0.5 µg/mL of 3-CPD.

B. Pipet 8 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask, dilute to volume with ethyl acetate, and mix. The resulting *Standard Solution* contains 2.0 µg/mL of 3-CPD.

C. Pipet 16 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask, dilute this mixture to volume with ethyl acetate, and mix. The resulting *Standard Solution* contains 4.0 µg/mL of 3-CPD.

Chromatographic System Use a gas chromatograph equipped with an electrolytic conductivity detector operated in the halogen mode. The gas chromatograph is fitted with either a capillary injector operated in the splitless mode or a purged, packed injector with a glass insert. Use a 30-m × 0.53-mm id fused silica column coated with 1-µm Supelcowax 10 or an equivalent bonded carbowax column. The column is fitted with a 50-cm retention gap of 0.53 mm deactivated, fused silica. Use helium as the carrier gas at a flow-rate of 8 mL/min. Set the column temperature to 170° for 5 min, then raise the temperature at a rate of 5°/min to 250°, and hold it at that temperature for 10 min. The injector temperature is maintained at 225°.

Use hydrogen as the reactant gas at a flow-rate of 30 mL/min, and use 1-propanol as the solvent at a flow-rate through the cell of 0.5 mL/min or the manufacturer's specified flow-rate for the optimum operation of the electrolytic conductivity detector. The reactor temperature should be 900°, with a base temperature of 275°. Minimize contamination of the reaction tube by venting flow from the column at all times, except for the time during which compounds of interest elute.

Calibration Inject 1 µL each of standard solutions *A*, *B*, and *C* into the gas chromatograph. Calculate the response area ratios of 3-CPD to the *Internal Standard* for each standard solution. Plot the response area ratios versus the µg of 3-CPD in each standard solution to obtain the standard curve.

Procedure Adjust an accurately weighed sample of Acid Hydrolysates of Proteins, as needed, with 20% aqueous sodium chloride to obtain a solution with a solids content of 36%. Weigh a 20-g aliquot of the solution directly into a 20-mL Extrelut column (EM Science, Gibbstown, NJ, or equivalent), and allow it to equilibrate for 15 min. Elute the column with 150 mL of ethyl acetate, collecting the eluent in a 250-mL, short-neck, round-bottom flask with a 24/40 joint. Concentrate the eluent to a volume of approximately 3 mL using a rotary evaporator at 50°. Add 0.5 mL of *Internal Standard Solution* to the eluent, transfer this mixture to a 4-dram screw-cap vial, and dilute to a volume of 5.0 mL. Inject 1 µL into the gas chromatograph, measure its response area ratio of 3-CPD to the *Internal Standard*, and determine from the standard curve the µg of 3-CPD in the 20-g aliquot taken.

1,3-Dichloro-2-propanol (DCP)

Eluent Transfer 850 mL of chromatographic-grade pentane and 150 mL of chromatographic-grade diethyl ether into a suitable container, and mix well.

DCP Stock Solution Transfer 50 mg, accurately weighed, of reagent-grade 1,3-dichloro-2-propanol (DCP) into a 50-mL volumetric flask, dilute to volume with *Eluent*, and mix.

Dilute DCP Solution Stepwise and quantitatively dilute the *DCP Stock Solution* with *Eluent* to obtain a final solution containing 1 µg/mL of DCP.

Internal Standard Solution Transfer 50 mg of trichlorobenzene into a 50-mL volumetric flask, dilute to volume with *Eluent*, and mix. Use a 1 in 1000 dilution of this solution as the *Internal Standard Solution*.

Standard Solutions Into separate 50-mL volumetric flasks pipet 1-, 2-, 3-, and 4-mL portions of *Dilute DCP Solution*. To each add 1.0 mL of *Internal Standard Solution*, dilute to volume with *Eluent*, and mix.

Sample Preparation Dissolve 5.0 g, accurately weighed, of Acid Hydrolysates of Proteins in a minimum volume of 20% aqueous sodium chloride solution. Transfer this solution quantitatively to an Extrelut column (EM Science, Gibbstown, NJ, or equivalent). After 15 min, elute the column with three 20-mL portions of *Eluent*, collecting all the eluate. Carefully evaporate the eluate to less than 4 mL. Add 1.0 mL of *Internal Standard Solution*, and dilute with *Eluent*, as necessary, to bring the final volume to 5.0 mL.

Chromatographic System Use a gas chromatograph equipped with a split injector and a nickel electron-capture detector. The gas chromatograph is fitted with a 50-m × 0.2-mm id fused silica column coated with dimethylpolysiloxane (Carbowax 20M, or equivalent). Use nitrogen as the carrier gas at a flow-rate of 8 mL/min. Before use, precondition the column by heating it at 200° and the detector at 300° for 24 h. Set the injector temperature at 250° and the electron-capture detector at 300°, and program the column temperature as follows: Maintain for 10 min at 115°, raise rapidly at 30°/min to 200°, and maintain at 200° for 12 min.

Calibration Inject 1.0 µL of each of the four *Standard Solutions* into the gas chromatograph. Calculate the response area ratios of DCP to the *Internal Standard Solution* for each *Standard Solution*. Plot the response area ratios versus the µg of DCP in each *Standard Solution* to obtain the standard curve.

Procedure Similarly, inject 1.0 µL of *Sample Preparation*. Measure its response ratio, and determine from the standard curve the µg of DCP in the sample taken.

Glutamic Acid

Apparatus Use an ion-exchange amino acid analyzer, equipped with sulfonated polystyrene columns, in which the effluent from the sample is mixed with ninhydrin reagent and the absorbance of the resultant color is measured continuously and automatically at 570 and 440 nm by a recording photometer.

Standard Solution Weigh 1250 ± 2 mg of glutamic acid, reagent grade, and place in a 500-mL volumetric flask. Fill the flask half-full with water, and add 5 mL of hydrochloric acid to help dissolve the amino acid, dilute to volume with water, and mix. Prepare the standard for analysis by diluting 1 mL of this solution with 4 mL of 0.2 *N* sodium citrate, pH 2.2, buffer. This *Standard Solution* contains 0.5 mg of glutamic acid per mL (C_S).

Sample Preparation Accurately weigh 5 mg of the sample and dilute to exactly 5 mL with 0.2 *N* sodium citrate, pH 2.2, buffer. Remove any insoluble material by centrifugation or filtration.

Procedure Using 2-mL aliquots of the *Standard Solution* and *Sample Preparation*, proceed as directed according to the apparatus manufacturer's instructions. From the chromatograms thus obtained, match the retention times produced by the *Standard Solution* with those produced by the *Sample Solution*, and identify the peak produced by glutamic acid. Record the area of the glutamic acid peak from the sample as A_U, and that from the standards as A_S.

Calculations Calculate the concentration, C_A, in mg/mL, of glutamic acid in the *Sample Preparation* by the formula

$$A_U \times C_S/A_S,$$

in which C_S is the concentration, in mg/mL, of the glutamic acid in the *Standard Solution*.

Calculate the percentage of glutamic acid, on the basis of total amino acids, by the formula

$$100C_A/6.25N_T,$$

in which N_T is the percentage of total nitrogen determined in the *Assay*.

Calculate the percentage of glutamic acid in the sample by the formula

$$100 \times C_A/S_W,$$

in which S_W is the weight of the sample taken, in mg.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Matter Transfer about 5 g, accurately weighed, into a 250-mL Erlenmeyer flask, add 75 mL of water, cover the flask with a watch glass, and boil gently for 2 min. Filter the solution through a tared filtering crucible, dry at 105° for 1 h, cool, and weigh.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 µg of lead ion (Pb) in the control.

Potassium

Spectrophotometer Use any suitable atomic absorption spectrophotometer.

Standard Solution Transfer 38.20 mg of reagent-grade potassium chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with deionized water, and mix. Transfer 5.0 mL of this solution to a 1000-mL volumetric flask, dilute to volume with deionized water, and mix. Each mL contains 1.0 µg of K.

Sample Solution Transfer 1.00 ± 0.05 g of previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 1000-mL volumetric flask. Wash the filter paper with hot water, dilute to volume, and mix. Use a 1 to 300 dilution as the *Sample Solution*.

Procedure Determine the absorbance of each solution at 766.5 nm, following the manufacturer's instruction for optimum

operation of the spectrophotometer. The absorbance produced by the *Sample Solution* does not exceed that of the *Standard Solution.*

Sodium

Spectrophotometer Use any suitable atomic absorption spectrophotometer.

Standard Solution Transfer 25.42 mg of reagent-grade sodium chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with deionized water, and mix. Transfer 5.0 mL of this solution to a 1000-mL volumetric flask, dilute to volume with deionized water, and mix. Each mL of the final *Standard Solution* contains 0.5 µg of Na. Using water as the solvent, prepare a 1 in 100 dilution of this solution to obtain the final working *Standard Solution.*

Sample Solution Transfer 1.00 ± 0.05 g of previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 100-mL volumetric flask. Wash the filter paper with hot water, dilute to volume, and mix. Using water as the solvent, prepare a 1 in 4000 dilution of this solution to obtain the final *Sample Solution.*

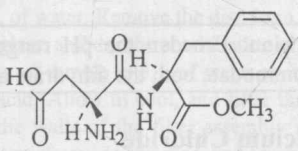
Procedure Determine the absorbance of each solution at 589.0 nm, following the manufacturer's instructions for optimum operation of the spectrophotometer. The absorbance produced by the *Sample Solution* does not exceed that of the *Standard Solution.*

Packaging and Storage Store in well-closed containers.

Revision: Delete ***Transmittance*** specification.

Aspartame

N-L-α-Aspartyl-L-phenylalanine 1-Methyl Ester; APM



$C_{14}H_{18}N_2O_5$ Formula wt 294.31

INS: 951 CAS: [22839-47-0]

DESCRIPTION

A white, odorless, crystalline powder having a sweet taste. It is sparingly soluble in water and slightly soluble in alcohol. The pH of a 0.8% solution is between about 4.5 and 6.0.

Functional Use in Foods Sweetener; sugar substitute; flavor enhancer.

REQUIREMENTS

Identification The infrared absorption spectrum of a potassium bromide dispersion of Aspartame exhibits maxima only at the same wavelengths as that of a similar preparation of USP Aspartame Reference Standard.

Assay Not less than 98.0% and not more than 102.0% of $C_{14}H_{18}N_2O_5$, calculated on the dried basis.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid Not more than 1.5%.

Heavy Metals (as Pb) Not more than 10 mg/kg.

Loss on Drying Not more than 4.5%.

Other Related Substances Not more than 2.0%.

Residue on Ignition Not more than 0.2%.

Specific Rotation $[\alpha]_D^{20^\circ}$: Between +14.5° and +16.5°, calculated on the dried basis.

TESTS

Assay Transfer about 300 mg of the sample, accurately weighed, to a 150-mL beaker, dissolve in 1.5 mL of formic acid (96%), and add 60 mL of glacial acetic acid. Add crystal violet TS, and titrate immediately with 0.1 *N* perchloric acid to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 *N* perchloric acid is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

> **Note**: Use 0.1 *N* perchloric acid previously standardized to a green endpoint. A blank titration exceeding 0.1 mL may be due to excessive water content and may cause loss of visual endpoint sensitivity.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid

Mobile Phase Weigh 5.6 g of potassium phosphate monobasic into a 1-L flask, add 820 mL of water, and dissolve. Adjust the pH to 4.3 using phosphoric acid, add 180 mL of methanol, and mix. Filter through a 0.45-µm disk, and de-gas.

Diluting Solvent Add 200 mL of methanol to 1800 mL of water, and mix.

Impurity Standard Preparation Transfer about 25 mg of USP 5-Benzyl-3,6-dioxo-2-piperazineacetic Acid Reference Standard, accurately weighed, into a 100-mL volumetric flask. Add 10 mL of methanol, and dissolve. Dilute to volume with water, and mix. Pipet 15 mL of this solution into a 50-mL volumetric flask, dilute to volume with *Diluting Solvent*, and mix. Use a freshly prepared solution.

Sample Preparation Transfer about 50 mg of the Aspartame sample, accurately weighed, to a 10-mL volumetric flask. Dilute to volume with *Diluting Solvent*, and mix. Use a freshly prepared solution.

Chromatographic System Use a suitable high-pressure liquid chromatograph equipped with a detector measuring at 210 nm and a 250- × 4.6-mm column packed with octadecyl silanized silica (10-µm Partisil ODS-3, or equivalent) and operated under isocratic conditions at 40°. The flow rate of the *Mobile Phase* is about 2 mL/min.

System Suitability The area responses of three replicate injections of the *Impurity Standard Preparation* show a relative standard deviation of not more than 2.0%.

Procedure Separately inject equal 20-µL portions of the *Impurity Standard Preparation* and the *Sample Preparation* into the chromatograph, and record the chromatograms (the approximate retention time of 5-benzyl-3,6-dioxo-2-piperazineacetic acid is 4 min, and the approximate retention time of Aspartame is 11 min). Measure the peak area response of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in each chromatogram. Calculate the percentage of 5-benzyl-3,6-dioxo-2-piperazine-acetic acid in the sample by the formula

$$1000(A_U C_S)/(A_S W_U),$$

in which A_U and A_S are the peak area responses of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the *Sample Preparation* and in the *Impurity Standard Preparation*, respectively; C_S is the concentration, in mg/mL, of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the *Impurity Standard Preparation*; and W_U is the weight, in mg, of Aspartame taken for the *Sample Preparation*.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, Appendix IIC Dry at 105° for 4 h.

Other Related Impurities Proceed as directed in the test for *5-Benzyl-3,6-dioxo-2-piperazineacetic Acid*, except to use the following in place of the *Standard Preparation*:

Other Related Substances Standard Preparation Pipet 2 mL of the *Sample Preparation* from the test for *5-Benzyl-3,6-dioxo-2-piperazineacetic Acid* into a 100-mL volumetric flask, dilute to volume with the *Diluting Solvent*, and mix.

Procedure Inject about 20-µL portions of the *Other Related Substances Standard Preparation* and the *Sample Preparation* into the chromatograph, and record the chromatogram for a time equal to twice the retention time of Aspartame. In the chromatogram obtained from the *Sample Preparation*, the sum of the responses of all secondary peaks, other than that for 5-benzyl-3,6-dioxo-2-piperazineacetic acid, is not more than the response of the Aspartame peak obtained in the chromatogram from the *Other Related Substances Standard Preparation*.

Residue on Ignition Ignite a 1-g sample as directed in the general method, Appendix IIC.

Specific Rotation, Appendix IIB Determine in a solution containing 4 g of sample in sufficient 15 *N* formic acid to make 100 mL. Make the determination within 30 min of preparation of the sample solution.

Packaging and Storage Store in well-closed containers in a cool, dry place.

Revision: Change ***Specific Rotation*** to ***Angular Rotation***.

Black Pepper Oil

CAS: [8006-82-4]

DESCRIPTION

The volatile oil obtained by steam distillation from the dried, unripened fruit of the plant *Piper nigrum* L. (Fam. *Piperaceae*). It is an almost colorless to slightly greenish liquid having the characteristic odor of pepper and a relatively mild taste. It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is sparingly soluble in glycerin.

Functional Use in Foods Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum in the section on *Infrared Spectra* (*Series A: Essential Oils*), using the same test conditions as specified therein.

Angular Rotation Between –1° and –23°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.479 and 1.488 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.864 and 0.884.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical (Specific) Rotation*, Appendix IIB.

Heavy Metals Shake 10 mL of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, Appendix IIB Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, Appendix VI. One mL dissolves in 3 mL of 95% alcohol.

Specific Gravity Determine by any reliable method (see Appendix VII).

Packaging and Storage Store in full, tight, glass containers in a cool place protected from light.

Revision: Broaden the pH range in the ***Description*** to accommodate both the dihydrate and anhydrous forms.

Calcium Chloride

$CaCl_2.2H_2O$ Formula wt, dihydrate 147.01
$CaCl_2$ Formula wt, anhydrous 110.98

INS: 509 CAS: dihydrate [10035-04-8]
CAS: anhydrous [10043-52-4]

DESCRIPTION

White, hard, odorless fragments, granules, or powder. It is deliquescent. It is anhydrous or the dihydrate. It is soluble in water and slightly soluble in alcohol. The pH of a 1 in 20 solution is between 4.5 and 10.5.

Functional Use in Foods Sequestrant; firming agent.

REQUIREMENTS

Labeling Indicate whether it is the dihydrate or anhydrous.

Identification A 1 in 10 solution gives positive tests for *Calcium* and for *Chloride*, Appendix IIIA.

Assay For the dihydrate, not less than 99.0% and not more than 107.0% of $CaCl_2.2H_2O$; for the anhydrous, not less than 93.0% and not more than 100.5% of $CaCl_2$.

Acid-Insoluble Matter (for the anhydrous) Not more than 0.02%; no particles per kg of sample greater than 2 mm in any dimension.

Arsenic (as As) Not more than 3 mg/kg.

Fluoride Not more than 0.004%.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 5 mg/kg.

Magnesium and Alkali Salts Not more than 4.0% for the dihydrate, and not more than 5.0% for the anhydrous.

TESTS

Assay Transfer about 1.5 g, accurately weighed, into a 250-mL volumetric flask, dissolve it in a mixture of 100 mL of water and 5 mL of 2.7 *N* hydrochloric acid, dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a suitable container, and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each mL of 0.05 *M* disodium EDTA is equivalent to 5.55 mg of $CaCl_2$ or 7.35 mg of $CaCl_2.2H_2O$.

Acid-Insoluble Matter (**Note**: The following test is intended for Anhydrous Calcium Chloride.) Place a 32-mm od lintine disc filter[1] in a suitable filter assembly comprising a 2.5-L screw-cap bottle cut in half horizontally and fitted with a rubber washer that is 35 mm od and 25 mm id, followed by the lintine disc, a 20-mesh stainless steel screen 35 mm od, and a bottle cap with a 25-mm hole in the top. With the filter at the bottom, wash the assembly with 100 mL of diluted acetic acid (1 in 300), followed by 100 mL of water. Remove the disc from the assembly, place on a watch glass, and dry the combination at 105° for 2 h.

Dissolve 1 kg of sample in 3 L of water containing 10 mL of glacial acetic acid. Allow to cool, and filter through the lintine disc. Rinse the walls of the filter assembly so that all insoluble matter is transferred to the disc, and wash with 100 mL of water. Place the disc on the same watch glass mentioned above, and dry at 105° for 2 h, being careful at all times not to lose any particles that may be on the disc. The difference in the two weights is the weight of the acid-insoluble matter.

Place the disc under a low-power magnifier (4× to 10× magnification). Using a millimeter rule, measure the largest dimension of each particle (or as many as may be necessary) on the disc. No particles greater than 2 mm in any dimension are present.

[1] Available from Filter Fabrics, Inc., 814 E. Jefferson, Goshen, IN 46526; 219/533-3114.

Arsenic A solution of 1 g in 10 mL of water meets the requirements of the *Arsenic Test*, Appendix IIIB.

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, Appendix IIIB.

Heavy Metals Dissolve 1 g in 2 mL of 1 *N* acetic acid, and add water to make 25 mL. This solution meets the requirements of the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 20 mL of water meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 µg of lead ion (Pb) in the control.

Magnesium and Alkali Salts Dissolve 1.0 g in about 50 mL of water, add 500 mg of ammonium chloride, mix, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add 6 *N* ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-mL cylinder, dilute with water to 100 mL, let it stand for 4 h or overnight, then decant the clear, supernatant liquid through a dry filter paper. To 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 20 mg for the dihydrate or 25 mg for the anhydrous.

Packaging and Storage Store in tight containers.

Revision: Decrease ***Lead*** limit.

Calcium Chloride Solution

DESCRIPTION

Calcium Chloride Solution occurs as a clear to slightly turbid, colorless or slightly colored liquid at room temperature. It is nominally available in a concentration range of about 35% to 45% of $CaCl_2$.

Functional Use in Foods Sequestrant; firming agent.

REQUIREMENTS

Identification When diluted to a concentration of about 1 to 10 ($CaCl_2$ basis), it gives positive tests for *Calcium* and for *Chloride*, Appendix IIIA.

Assay Not less than 90.0% and not more than 110.0%, by weight, of the labeled amount of calcium chloride, expressed as $CaCl_2$.

Alkalinity [as $Ca(OH)_2$] Not more than 0.3%.

Fluoride Not more than 0.004%, calculated on the $CaCl_2$ determined in the *Assay*.

Heavy Metals (as Pb) Not more than 0.002%, calculated on the $CaCl_2$ determined in the *Assay*.

Lead Not more than 5 mg/kg, calculated on the $CaCl_2$ determined in the *Assay*.

Magnesium and Alkali Salts Not more than 5.0%, calculated on the $CaCl_2$ determined in the *Assay*.

TESTS

Assay Transfer an accurately weighed amount of the solution, equivalent to about 1 g of $CaCl_2$, into a 250-mL volumetric flask, dissolve it in a mixture of 100 mL of water and 5 mL of 2.7 *N* hydrochloric acid, dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a suitable container, and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each mL of 0.05 *M* disodium EDTA is equivalent to 5.550 mg of $CaCl_2$.

Alkalinity Dilute an accurately weighed amount of the solution, equivalent to about 5 g of $CaCl_2$, to 50 mL with water, add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid. Each mL of 0.1 *N* hydrochloric acid is equivalent to 3.71 mg of $Ca(OH)_2$.

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, Appendix IIIB, using as the sample an accurately weighed amount of the solution equivalent to 1 g of $CaCl_2$.

Heavy Metals Dilute an accurately weighed amount of the solution, equivalent to 1 g of $CaCl_2$, to 25 mL with water. This solution meets the requirements of the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Dilute an accurately weighed amount of the solution, equivalent to 1 g of $CaCl_2$, to 10 mL with water. The resulting solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 µg of lead ion (Pb) in the control.

Magnesium and Alkali Salts Dilute an accurately weighed amount of the solution, equivalent to 1.0 g of $CaCl_2$, to 50 mL with water, add 500 mg of ammonium chloride, mix, and boil for about 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add 6 *N* ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture into a 100-mL cylinder, dilute with water to 100 mL, let it stand for 4 h or overnight, and then decant the clear, supernatant liquid through a dry filter paper. To 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 25 mg.

Packaging and Storage Store in tight containers.

Insert the following new monograph:

Calcium Lignosulfonate

CAS: [8061-52-7]

DESCRIPTION

Calcium Lignosulfonate is a brown, amorphous polymer obtained from the spent sulfite and sulfate pulping liquor of wood or from the sulfate (kraft) pulping process. It may contain up to 30% reducing sugars. It is soluble in water, but not in any of the common organic solvents. The pH of a 1 in 100 solution is between approximately 3 and 11.

Functional Use in Foods Binder; dispersant.

REQUIREMENTS

Identification

A. A 0.15-g/L solution of the sample gives positive tests for *Calcium*; Appendix IIIA.

B. Dissolve 100 mg of the sample in 50 mL of water. Add 1 mL each of 10% acetic acid and 10% sodium nitrite solutions to this solution. Mix the solution by swirling, and allow it to stand for 15 min at room temperature. A brown color appears.

C. The ultraviolet absorption spectrum of a 0.1 g/L solution of the sample at pH 5 exhibits a peak between 275 and 280 nm.

Assay Not less than 5.0% sulfonate sulfur.

Calcium Not more than 7.0%.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 10.0%.

Reducing Sugars Not more than 30.0%.

Residue on Ignition Not more than 20.0%.

Viscosity of a 50% Solution Not more than 3000 centipoises.

TESTS

Assay for Sulfonate Sulfur Dissolve 1.0 g, accurately weighed, of the sample in 400 mL of water in a beaker. Direct a gentle stream of nitrogen gas over the liquid's surface. Add 10 mL of nitric acid, and swirl the solution thoroughly until the reaction subsides. Add 10 mL of 70% perchloric acid; again swirl thoroughly. Place the uncovered beaker on a hot plate, and heat the contents vigorously until the center of the bottom of the beaker becomes clear. Remove the beaker, and cool to room temperature. Add 5 mL of hydrochloric acid, and heat again until white fumes appear. After cooling and diluting to approximately 100 mL with water, adjust to pH 6 ± 0.2 with 10% sodium hydroxide. Then heat the solution to boiling. Add 15 mL of 10% barium chloride solution, and place the solution in a beaker in a steam bath at 90° to 95° overnight. Filter through ashless filter paper (Whatman No. 42, or equivalent), and wash with 200 mL of warm water. Transfer the paper and precipitate to a tared crucible. Heat the crucible slowly on a Bunsen burner to expel moisture. Place the crucible and contents in a muffle furnace at 850° for 1 h. Let the crucible cool in a desiccator, and then

weigh the residue to the nearest 0.0001 g. Calculate the percent of sulfonate sulfur by the formula

$$(R/S) \times 13.7$$

in which R is the weight, in g, of the residue and S is the weight, in g, of the sample taken.

Calcium

Strontium Chloride Solution Add 164.7 g of 60% perchloric acid, with stirring, to 500 mL of distilled water in a 1-L beaker. Then add 15.2 g of strontium chloride hexahydrate, with stirring, until solution is complete. Transfer the solution to a 1-L volumetric flask, and dilute to volume at room temperature with distilled water. Mix the contents by inverting the stoppered flask several times.

Standard Solution Using a certified 1000 ppm Calcium Standard Solution (Mallinckrodt or equivalent), dilute quantitatively and stepwise to obtain a *Standard Solution* containing 0.7 mg of calcium per mL. The *Standard Solution* should be stored in polyethylene bottles due to its instability in glass.

Sample Solution Accurately weigh a previously dried 1-g sample, and dilute to 10.0 mL. If the initial *Sample Solution* is not particle free, filter through a 0.45-µm disposable Millipore filter, discarding the first few mL of filtrate. Pipet 5 mL of *Strontium Chloride Solution* into a 50-mL volumetric flask, and add 5.0 mL of the filtrate. Dilute to volume, and mix well.

Procedure Using a suitably calibrated atomic absorption spectrophotometer, determine the absorbance of the *Standard Solution* and the *Sample Solution* at 422.7 nm, following the manufacturer's instructions for optimum operation of the spectrophotometer. The absorbance produced by the *Sample Solution* is not greater than that produced by the *Standard Solution*.

Lead A sample solution from a 3-g sample prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 3 µg of lead ion (Pb) in the control.

Loss on Drying, Appendix IIC Dry at 105° for 24 h.

Reducing Sugars

Lead Subacetate Solution Dissolve 80 g in 220 mL of water. Stir overnight, and filter through Whatman No. 42 filter paper, or the equivalent. Dilute the supernatant solution to a specific gravity of 1.254 with freshly boiled water.

Copper Reagent Solution Dissolve 28 g of anhydrous sodium phosphate, dibasic, and 40 g of potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$) in 700 mL of water. Add 100 mL of 1 *N* sodium hydroxide and 8 g of copper sulfate pentahydrate, followed by 180 g of anhydrous sodium sulfate. Add 0.7134 g of potassium iodate, and dilute to 1 L. Allow to stand for several days, then filter the clear top part of the solution through a medium-porosity, sintered-glass funnel.

Dextrose Standard Solution Dissolve 140 mg of dried dextrose, accurately weighed, in 500 mL of water.

Dibasic Sodium Phosphate Solution Dissolve 19 g of sodium phosphate, dibasic, heptahydrate in 100 mL of water.

Procedure Dissolve 1 g of sample, accurately weighed, in 150 mL of water, and adjust the pH to between 6.9 and 7.2 with sodium hydroxide solution or acetic acid. Add *Lead Subacetate Solution* in increments until no further precipitation is observed. Add water to 250.0 mL, and mix well. Centrifuge the mixture, pipet 10 mL of the supernatant to a 50-mL volumetric flask, and dilute to about 35 mL. Add 2 mL or more of *Dibasic Sodium Phosphate Solution* until no precipitation occurs. Dilute to 50 mL, and mix. Centrifuge at 2100 × gravity for 10 min. Pipet 5 mL of the supernatant solution, and mix with exactly 5 mL of *Copper Reagent Solution* in a test tube. Loosely plug the tube, and place it in a boiling water bath for 40 min ± 10 s. At the end of the heating period, cool the tube immediately in cold water. Add 2 mL of 2.5% potassium iodide solution and 1.5 mL of 2 *N* sulfuric acid. Mix well, and titrate with 0.005 *N* sodium thiosulfate, using starch indicator, noting the volume consumed as V_S. Repeat the above procedure with the dextrose standard (5 mL of *Dextrose Standard Solution* and 5 mL of *Copper Reagent Solution*), noting the volume consumed as V_D. For each of the above titrations, run a corresponding blank titration, V_B, using 5 mL of water and 5 mL of *Copper Reagent Solution*.

Calculate the percent of reducing sugars by the formula

$$(35V_B - V_S)/(V_B - V_D),$$

in which $V_B - V_S$ is the number of mL of 0.005 *N* sodium thiosulfate consumed by the 5-mL aliquot of the sample, and $V_B - V_D$ is the number of mL of 0.005 *N* sodium thiosulfate consumed by 5 mL of *Dextrose Standard Solution*.

Residue on Ignition Ignite 1 g as directed in the general method, Appendix IIC.

Viscosity of a 50% Solution Dissolve 200 g of sample, calculated on the dried basis and accurately weighed, in 200 mL of water contained in a 500-mL beaker. Equilibrate the solution at 25°, and measure its viscosity with a Brookfield viscometer A (model LVG, or equivalent), using a number 2 spindle at 20 rpm.

Packaging and Storage Store in well-closed containers.

Revision: Decrease ***Lead*** limit.

Calcium Phosphate, Dibasic

Dicalcium Phosphate

$CaHPO_4.2H_2O$ Formula wt, anhydrous 136.06
Formula wt, dihydrate 172.09

INS: 341(ii) CAS: anhydrous [7757-93-9]
CAS: dihydrate [7789-77-7]

DESCRIPTION

Dibasic Calcium Phosphate is anhydrous or contains two molecules of water of hydration. It occurs as a white, odorless, tasteless powder that is stable in air. It is practically insoluble in water, but is readily soluble in dilute hydrochloric and nitric acids. It is insoluble in alcohol.

Functional Use in Foods Leavening agent; dough conditioner; nutrient; dietary supplement; yeast food.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or the dihydrate.

Identification

A. Dissolve about 100 mg by warming with a mixture of 5 mL of 2.7 *N* hydrochloric acid and 5 mL of water, add 2.5 mL of 6 *N* ammonium hydroxide, dropwise, with shaking, and then add 5 mL of ammonium oxalate TS. A white precipitate is formed.

B. To 10 mL of a warm solution (1 in 100) in a slight excess of nitric acid add 10 mL of ammonium molybdate TS. A yellow precipitate of ammonium phosphomolybdate is formed.

Assay Not less than 97.0% and not more than 105.0% of Dibasic Calcium Phosphate ($CaHPO_4$) or of Dibasic Calcium Phosphate, Dihydrate ($CaHPO_4.2H_2O$).

Arsenic (as As) Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 0.0015%.

Lead Not more than 2 mg/kg.

Loss on Ignition $CaHPO_4$ (anhydrous): between 7.0% and 8.5%; $CaHPO_4.2H_2O$ (dihydrate): between 24.5% and 26.5%.

TESTS

Assay Dissolve about 250 mg of Dibasic Calcium Phosphate, accurately weighed, with the aid of gentle heat if necessary, in a mixture of 5 mL of hydrochloric acid and 3 mL of water contained in a 250-mL beaker equipped with a magnetic stirrer, and cautiously add 125 mL of water. With constant stirring, add, in the order named, 0.5 mL of triethanolamine, 300 mg of hydroxy naphthol blue indicator, and from a 50-mL buret, about 23 mL of 0.05 *M* disodium ethylenediaminetetraacetate. Add sodium hydroxide solution (45 in 100) until the initial red color changes to clear blue, then continue to add it dropwise until the color changes to violet, then add an additional 0.5 mL. The pH is between 12.3 and 12.5. Continue the titration dropwise with the 0.05 *M* disodium ethylenediaminetetraacetate to the appearance of a clear blue endpoint that persists for not less than 60 s. Each mL of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 6.803 mg of $CaHPO_4$ or to 8.604 mg of $CaHPO_4.2H_2O$.

Arsenic A solution of 1 g in 5 mL of 2.7 *N* hydrochloric acid meets the requirements of the *Arsenic Test*, Appendix IIIB.

Fluoride (**Note**: Prepare and store all solutions in plastic containers.)

Buffer Solution Dissolve 73.5 g of sodium citrate in water to make 250 mL of solution.

Standard Solution Dissolve an accurately weighed quantity of USP Sodium Fluoride RS quantitatively in water to obtain a solution containing 1.1052 mg/mL. Transfer 20.0 mL of the resulting solution to a 100-mL volumetric flask containing 50 mL of *Buffer Solution*, dilute with water to volume, and mix. Each mL of this solution contains 100 µg of fluoride ion.

Electrode System Use a fluoride-specific, ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ± 0.2 mV.

Standard Response Line Transfer 50.0 mL of *Buffer Solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals, add 100 µL, 100 µL, 300 µL, and 500 µL of *Standard Solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

Procedure Transfer 2.0 g of the specimen under test to a beaker containing a plastic-coated stirring bar, add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer Solution* and sufficient water to make 100 mL of test solution. Rinse and dry the electrodes, insert them into the test solution, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard Response Line* determine the concentration, *C*, in µg/mL, of fluoride ion in the test solution. Calculate the percentage of fluoride in the specimen taken by the formula

$$C \times 0.005.$$

Heavy Metals Warm 2.66 g with 5 mL of 2.7 *N* hydrochloric acid until no more dissolves, dilute to 50 mL with water, and filter. A 25-mL portion of the filtrate meets the requirements of the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A 10-g sample meets the requirements of the *APDC Extraction Method for Lead*, Appendix IIIB.

Loss on Ignition Weigh accurately about 3 g, and ignite, preferably in a muffle furnace, at 800° to 825° to constant weight.

Packaging and Storage Store in well-closed containers.

Revision: Decrease ***Lead*** limit.

Calcium Phosphate, Monobasic

Monocalcium Phosphate; Calcium Biphosphate; Acid Calcium Phosphate

$Ca(H_2PO_4)_2.H_2O$

Formula wt, anhydrous 234.05
Formula wt, monohydrate 252.07

CAS: anhydrous [7758-23-8]
CAS: monohydrate [10031-30-8]

INS: 341(i)

DESCRIPTION

Monobasic Calcium Phosphate is anhydrous or contains one molecule of water of hydration, but, due to its deliquescent nature, more than the calculated amount of water may be present. It occurs as white crystals or granules or as a granular powder. It is sparingly soluble in water and is insoluble in alcohol.

Functional Use in Foods Buffer; dough conditioner; firming agent; leavening agent; nutrient; dietary supplement; yeast food; sequestrant.

REQUIREMENTS

Labeling Indicate the state of hydration.

Identification

A. Dissolve 100 mg by warming in a mixture of 2 mL of 2.7 *N* hydrochloric acid and 8 mL of water, and add 5 mL of ammonium oxalate TS. A white precipitate forms.

B. To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate TS. A yellow precipitate forms.

Assay $Ca(H_2PO_4)_2$ (anhydrous): not less than 16.8% and not more than 18.3% of Ca; $Ca(H_2PO_4)_2.H_2O$ (monohydrate): not less than 15.9% and not more than 17.7% of Ca.

Arsenic (as As) Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 0.0015%.

Lead Not more than 2 mg/kg.

Loss on Drying $Ca(H_2PO_4)_2.H_2O$ (monohydrate): between 14.0% and 15.5%.

Loss on Ignition $Ca(H_2PO_4)_2$ (anhydrous): not more than 1%.

TESTS

Assay Weigh accurately a portion of the sample equivalent to about 475 mg of $Ca(H_2PO_4)_2$, dissolve it in 10 mL of 2.7 *N* hydrochloric acid, add a few drops of methyl orange TS, and boil for 5 min, keeping the volume and pH of the solution constant during the boiling period by adding hydrochloric acid or water, if necessary. Add 2 drops of methyl red TS and 30 mL of ammonium oxalate TS, then add dropwise, with constant stirring, a mixture of equal volumes of 6 *N* ammonium hydroxide and water until the pink color of the indicator just disappears. Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through a sintered-glass crucible, using gentle suction. Wash the precipitate in the beaker with about 30 mL of cold (below 20°) wash solution, prepared by diluting 10 mL of ammonium oxalate TS to 1000 mL. Allow the precipitate to settle, and pour the supernatant liquid through the filter. Repeat this washing by decantation three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter with two 10-mL portions of cold (below 20°) water. Place the sintered-glass crucible in the beaker, and add 100 mL of water and 50 mL of cold dilute sulfuric acid (1 in 6). Add from a buret 35 mL of 0.1 *N* potassium permanganate, and stir until the color disappears. Heat to about 70°, and complete the titration with 0.1 *N* potassium permanganate. Each mL of 0.1 *N* potassium permanganate is equivalent to 2.004 mg of Ca.

Arsenic A solution of 1 g in 5 mL of 2.7 *N* hydrochloric acid meets the requirements of the *Arsenic Test*, Appendix IIIB.

Fluoride (anhydrous): determine as directed in *Method II* under the *Fluoride Limit Test*, Appendix IIIB; (monohydrate): proceed as directed under *Fluoride* in the monograph for *Calcium Phosphate, Dibasic*.

Heavy Metals Warm 2.66 g with 5 mL of 2.7 *N* hydrochloric acid until no more dissolves, dilute to 50 mL with water, and filter. A 25-mL portion of the filtrate meets the requirements of the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A 10-g sample meets the requirements of the *APDC Extraction Method for Lead*, Appendix IIIB.

Loss on Drying, Appendix IIC Dry $Ca(H_2PO_4)_2.H_2O$ (monohydrate) at 60° for 3 h.

Loss on Ignition Weigh accurately about 3 g of $Ca(H_2PO_4)_2$ (anhydrous), and ignite, preferably in a muffle furnace, at 800° for 30 min.

Packaging and Storage Store in well-closed containers.

Revision: Decrease ***Lead*** limit.

Calcium Phosphate, Tribasic

Tricalcium Phosphate; Precipitated Calcium Phosphate; Calcium Hydroxyapatite

INS: 341(iii) CAS: [7758-87-4]

DESCRIPTION

Tribasic Calcium Phosphate consists of a variable mixture of calcium phosphates having the approximate composition of $10CaO.3P_2O_5.H_2O$. It occurs as a white, odorless, tasteless powder that is stable in air. It is insoluble in alcohol and almost insoluble in water, but it dissolves readily in dilute hydrochloric and nitric acids.

Functional Use in Foods Anticaking agent; buffer; nutrient; dietary supplement; clouding agent.

REQUIREMENTS

Identification

A. To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate TS. A yellow precipitate forms.

B. Dissolve about 100 mg by warming with 5 mL of 2.7 *N* hydrochloric acid and 5 mL of water, add 1 mL of 6 *N* ammonium hydroxide, dropwise, with shaking, and then add 5 mL of ammonium oxalate TS. A white precipitate forms.

Assay Not less than 34.0% and not more than 40.0% of calcium (Ca).

Arsenic (as As) Not more than 3 mg/kg.

Fluoride Not more than 0.0075%.

Heavy Metals (as Pb) Not more than 0.0015%.

Lead Not more than 2 mg/kg.

Loss on Ignition Not more than 10.0%.

TESTS

Assay Proceed as directed in the *Assay* in the monograph for *Dibasic Calcium Phosphate*, using a 150-mg sample, accurately