

CALCIUM 5'-RIBONUCLEOTIDES

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 18th JECFA (1974).

SYNONYMS

Calcium ribonucleotides, INS No. 634

DEFINITION

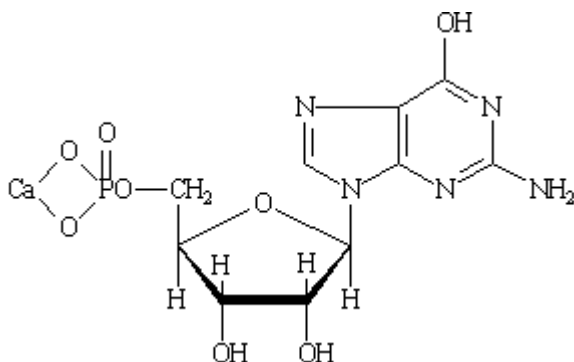
Chemical names

(Mixture of) calcium inosine-5'-monophosphate and calcium guanosine-5'-monophosphate

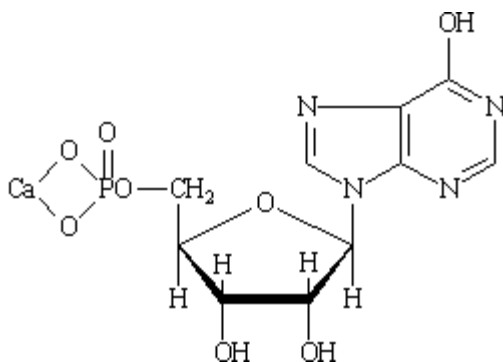
Chemical formula

$C_{10}H_{11}CaN_4O_8P \cdot x H_2O$ and
 $C_{10}H_{12}CaN_5O_8P \cdot x H_2O$

Structural formula



Calcium 5'-guanylate



Calcium 5'-inosinate

Assay

Not less than 97% and not more than the equivalent of 102% of $C_{10}H_{11}CaN_4O_8P$ and $C_{10}H_{12}CaN_5O_8P$, calculated on the anhydrous basis. The proportion of $C_{10}H_{11}CaN_4O_8P$ or $C_{10}H_{12}CaN_5O_8P$ to the sum of them is between 47% and 53%.

DESCRIPTION

Odourless, white or off-white crystals or powder

FUNCTIONAL USES

Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

<u>Solubility</u> (Vol. 4)	Sparingly soluble in water
<u>Test for ribose</u> (Vol. 4)	Passes test
<u>Test for organic phosphate</u> (Vol. 4)	Passes test Test 5 ml of a 1 in 2,000 solution
<u>Test for inosinic acid</u>	To 2 ml of a 1 in 2,000 solution add 2 ml of 10% hydrochloric acid and 0.1 g of zinc powder, heat in a water bath for 10 min, and filter. Cool the filtrate in ice water, add 1 ml of a 3 in 1,000 sodium nitrite solution, shake well, and allow to stand for 10 min. Add 1 ml of a 1 in 200 ammonium sulfamate solution, shake well, and allow to stand for 5 min. Add 1 ml of a 1 in 500 N-(1-naphthyl)-ethylenediamine dihydrochloride solution. A violet red colour is produced.
<u>Test for guanylic acid</u>	To 1 ml of a 1 in 5,000 solution add 1 ml of 10% hydrochloric acid, heat in a water bath for 10 min, cool, and add 0.5 ml of Folin-Ciocalteu TS and 2 ml of saturated sodium carbonate solution. A blue colour is produced.
<u>Test for calcium</u> (Vol. 4)	Passes test

PURITY

<u>Water</u> (Vol. 4)	Not more than 23% (Karl Fischer Method)
<u>pH</u> (Vol. 4)	7.0 - 8.0 (1 in 2,000 soln)
<u>Water soluble matter</u>	To 1 g of the sample, add 50 ml of water, allow to stand for 10 min with occasional shaking, filter through analytical grade filter paper (Whatman No. 42 or equivalent). Evaporate a 25 ml portion of the solution to dryness on a water bath and dry the residue at 105° for 1 h. Residue weighs less than 80 mg.
<u>Amino acids</u>	Not detectable by the following test: To 5 ml of a 1 in 2,000 solution add 1 ml of ninhydrin TS and heat for 3 min. No colour is produced.
<u>Related foreign substances</u>	Chromatographically not detectable Test 5 µl of a 1 in 1,000 soln. Only inosinic acid and 5'-guanylic acid are detected
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Calculate the contents of calcium inosine-5'-monophosphate (I) and calcium guanosine-5'-monophosphate (G) in the sample by the following equation, using values for I_{ca} and G_{ca} obtained as described below.

$$\text{Content (\%)} = \frac{I_{ca} + G_{ca}}{100 - \text{water (\%)}} \times 100$$

Weigh accurately about 650 mg of the sample, and dissolve in water to make 500 ml (Solution A).

To determine I_{ca} (calcium inosine-5'-monophosphate), take a 1-ml portion of Solution A, add 4 ml of 6 N hydrochloric acid and water to make to 10 ml. Heat in a water bath for 40 min, cool, add 0.4 g of zinc powder, allow to stand for 50 min, shaking occasionally and vigorously, and add water to make to 20 ml. Filter through filter paper. To a 10-ml portion of the filtrate add 1 ml of 6 N hydrochloric acid, and add 1 ml of a 1 in 1,000 sodium nitrite solution, cooling in an ice-water bath. Shake well, allow to stand for 10 min, add 1 ml of a 1 in 200 ammonium sulfamate solution, shake well, and allow to stand for 5 min. Add 1 ml of a 1 in 500 N-(1-naphthyl)-ethylenediamine dihydrochloride solution, shake well, allow to stand for 15 min at room temperature, and add water to make to 20 ml (Sample solution). For the control, prepare in the same manner as the sample, using 1 ml of water instead of Solution A. Determine the absorbance of the sample solution at 515 nm against the control solution.

To prepare calibration curves, weigh accurately about 3 mg each of disodium 5'-inosinate and disodium 5'-guanylate, and dissolve respectively in 100 ml of 0.01 N hydrochloric acid. Determine the absorbance at 250 nm on the solution of disodium 5'-inosinate and at 260 nm on the solution of disodium 5'-guanylate, using 0.01 N hydrochloric acid as the control. Determine the molecular extinction coefficients E_I and E_G , and calculate the contents of (I) disodium inosine-5'-monophosphate and (G) disodium guanosine-5'-monophosphate by the equations:

$$\text{Content (\%)} \text{ of } I = \frac{E_I}{12,160} \times 100$$

$$\text{Content (\%)} \text{ of } G = \frac{E_G}{11,800} \times 100$$

Weigh accurately a quantity of each which is equivalent to about 50 mg, combine and dissolve in water to make 200 ml (Solution B). To 1-ml, 2-ml and 3-ml portions of Solution B add 4 ml of 6 N hydrochloric acid and make each to 10 ml with water. Prepare Standard Solutions in the same manner as directed for preparing Sample Solution from Solution A. Determine the absorbance of each Standard Solution at 515 nm and prepare the calibration curve. For the control, use the control solution used for Sample Solution. Calculate the content of I (disodium inosine-5'-monophosphate) from the calibration curve and the absorbance of Sample Solution.

From the content of I, calculate the content of I_{ca} (calcium inosine 5'-monophosphate) as $0.985 \times I$.

To determine G_{ca} (calcium guanosine-5'-monophosphate), take 1 ml of Solution A, add 4 ml of 2 N hydrochloric acid and water to make to 10 ml.

Heat in a water bath for 30 min, cool, add 2 ml of Folin-Ciocalteu TS and 5 ml of a 4 in 5 sodium carbonate solution. Allow to stand for 15 min, and add water to make to 50 ml. Centrifuge if necessary, and use the supernatant for the test (Sample Solution).

Prepare the control in the same manner as the Sample Solution, using 1 ml of water instead of Solution A. Determine the absorbance of the Sample Solution at 750 nm.

To 1-ml, 2-ml and 3-ml portions of Solution B, add 4 ml of 2 N hydrochloric acid and make each to 10 ml with water. Prepare Standard Solutions in the same manner as directed in preparing Sample Solution. Determine the absorbance of each Standard Solution at 750 nm, and prepare the calibration curve. For the control, use the control solution used for Sample Solution. Calculate the content of G (disodium guanosine-5'-monophosphate) from the calibration curve and the absorbance of Sample Solution.

From the content of G calculate the content of G_{ca} (calcium guanosine-5'-monophosphate) as $0.986 \times G$.