NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 1903.33-2022

National food safety standard - Food nutritional fortification substance - 5'-CMP

Issued on: June 30, 2022 **Implemented on: December 30, 2022**

Issued by: NHC; SAMR

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1 Scope

This standard applies to the food nutrition fortifier - 5'-cytidine monophosphate, which is produced by using yeast ribonucleic acid (RNA) as raw material, through nuclease enzymatic hydrolysis.

2 Chemical name, molecular formula, structural formula, relative molecular mass

2.1 Chemical name

5'-cytidine monophosphate (5'-cytidylic acid, 5'-CMP)

2.2 Molecular formula

C9H14N3O8P

2.4 Relative molecular mass

323.20 (by 2018 international relative atomic mass)

3.1 Sensory requirements

Sensory requirements shall meet the requirements of Table 1.

Appendix A Inspection method

A.1 General provisions The reagents and water used in this standard, unless other requirements are specified, refer to the analytical reagents and the grade-3 water, which is specified in GB/T 6682.

The standard titration solution, standard solution for impurity determination, preparations and products, which are used in the test, shall be prepared in accordance with the provisions of GB/T 601, GB/T 602, GB/T 603, unless otherwise specified. The solution used in the test refers to the aqueous solution, when the solvent is not specified.

A.2 Identification experiment It is carried out, according to the method specified in GB/T 6040. The infrared spectrum of the product shall be consistent with the infrared spectrum of the standard product (see Appendix B, for the infrared spectrum of the standard product).

A.3 Determination of 5'-cytidine monophosphate (C9H14N3O8P) (on a dry basis)

A.3.1 Reagents and materials A.3.1.1 Water: Grade-1 water in accordance with GB/T 6682-2008.

A.3.1.2 2 mol/L sodium hydroxide solution: Weigh 40 g of solid sodium hydroxide in a 500 mL volumetric flask. Use water to dissolve it. Dilute it to the mark. Shake well.

A.3.1.3 0.05 mol/L potassium dihydrogen phosphate solution: Weigh 6.8045 g of potassium dihydrogen phosphate (KH2PO4) into a 1 L volumetric flask. Use water to dissolve it AND dilute it to the mark. Shake well. Use a 0.45 μm microporous membrane for filtration. Perform ultrasonic degassing for 30 min, before use.

A.3.1.4 5'-cytidine monophosphate standard substance (purity >= 98%).

A.3.1.5 Standard stock solution of 5'-cytidine monophosphate: Accurately weigh 100.0 mg of 5'-cytidine monophosphate standard substance, in a 50 mL volumetric flask. Use water to dissolve it. Add 1 ~ 2 drops of 2 mol/L sodium hydroxide solution, to help dissolve. Make the volume to the mark. Mix well. Seal and store it in a 4 °C refrigerator, to prepare for later use.

A.3.1.6 Standard use solution of 5'-cytidine monophosphate: Accurately pipette 1.0 mL of 5'-cytidine monophosphate standard stock solution, into a 50 mL volumetric flask.

Use water to dilute it to the mark. Shake well. Use a 0.45 μm microporous membrane for filtration, before injection.

A.3.2 Instruments and equipment A.3.2.1 High performance liquid chromatography (ultraviolet detector).

A.3.2.2 Analytical balance (sensitivity 0.0001 g).

A.3.3 Reference liquid chromatography conditions A.3.3.1 Chromatographic column: C18 (4.6 mm x 250 mm, 5 μm) or equivalent type column.

A.3.3.2 Mobile phase: 0.05 mol/L KH2PO4 solution + methanol = 95 + 5.

A.3.3.3 Injection volume: 20 μL.

A.3.3.4 Flow rate: 0.8 mL/min.

A.3.3.5 UV detection wavelength: 254 nm.

A.3.3.6 Column oven temperature: 25 °C.

A.3.4 Preparation of specimen Sample stock solution: Accurately weigh 100.0 mg of the sample into a 50 mL volumetric flask. Use water to dissolve it. Add 1 ~ 2 drops of 2 mol/L

sodium hydroxide solution, to help dissolve. Make the volume to the mark. Mix well.

Sample use solution: Accurately pipette 1.0 mL of the sample stock solution, into a 50 mL volumetric flask. Use water to make the volume reach to the mark. Shake well. Use a 0.45 um microporous membrane for filtration, before injection.

A.3.5 Determination According to the chromatographic conditions of A.3.3, inject the 5'-cytidine monophosphate standard solution and the sample use solution, respectively. Based on the retention time of the standard, determine the chromatographic peak of 5'-cytidine monophosphate in the sample. According to the peak area of the sample, use the external standard method, to calculate the content of 5'-cytidine monophosphate in the sample.

A.3.6 Result calculation The mass fraction w1 of 5'-cytidine monophosphate content (on a dry basis) is calculated, according to formula (A.1).

Where: