



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 1903.43-2020

**National food safety standard - Food Nutritional Fortification
Substance - Cyanocobalamin cobione**

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Market Regulation**

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1 Scope

This Standard is applicable to cyanocobalamin, a food nutritional fortification substance obtained through transformation and crystallization of fermentation broth containing cobalamin produced by microbial fermentation.

2 Molecular Formula, Structural Formula and Relative

Molecular Mass

2.1 Molecular formula

C₆₃H₈₈CoN₁₄O₁₄P

2.3 Relative molecular mass

Cyanocobalamin: 1355.38 (according to 2018 international relative atomic mass)

Appendix A Test Methods

A.1 Warning Some reagents used in the test methods of this Standard are toxic or corrosive, and appropriate safety and protective measures shall be taken during operation.

A.2 General rules Unless otherwise specified in this Standard, the purity of the used reagents shall be analytically pure. The standard titration solution, standard solution for impurity determination, preparations and products shall be prepared in accordance with the provisions of GB/T 601, GB/T 602, and GB/T 603. The water used in the laboratory shall comply with the provisions of Class-3 water specified in GB/T 6682. The solution used in the test refers to an aqueous solution when it is not specified which solvent is used to prepare it.

A.3 Identification test A.3.1 Reagents and materials A.3.1.1 Acetone.

A.3.1.2 Chloroform.

A.3.1.3 Ether.

A.3.1.4 Hydrochloric acid.

A.3.1.5 Sodium acetate.

A.3.1.6 Potassium bromide.

A.3.1.7 Sodium fluoride.

A.3.1.8 Potassium hydrogen sulfate.

A.3.1.9 Hypophosphorous acid.

A.3.1.10 Phenolphthalein indicator.

A.3.1.11 Acetic acid (1mol/L).

A.3.1.12 Sodium hydroxide solution (100g/L): Take 10g of sodium hydroxide; add water to dissolve and make constant volume to 100mL.

m₀ - the mass of the weighing bottle after drying, in g.

The calculation result retains 2 significant digits.

Take the arithmetic mean of the parallel determination results as the determination result; and the absolute difference between the two parallel determination results shall be no more than 1.0%.

A.6 Related substances A.6.1 Principle of the method After the specimen is dissolved in the mobile phase; it is detected by a liquid chromatograph and quantified by the peak area comparison method.

A.6.2 Reagents and materials A.6.2.1 Chloramine T.

A.6.2.2 Methanol.

A.6.2.3 Disodium hydrogen phosphate.

A.6.2.4 Phosphoric acid.

A.6.2.5 Hydrochloric acid.

A.6.2.6 Disodium hydrogen phosphate solution: 0.028mol/L. Take 10.03g of disodium hydrogen phosphate dodecahydrate; add water to dissolve and make constant volume to 1000mL.

A.6.2.7 Hydrochloric acid solution: 0.05mol/L. Take 4.5mL of hydrochloric acid; add water to 1000mL; and mix well.

A.6.2.8 Chloramine T solution: 0.1%. Take 0.1g of chloramine T; add water to dissolve and

make constant volume to 100mL.

A.6.3 Apparatus A.6.3.1 Balance: Sensitivity 0.0001g.

A.6.3.2 High performance liquid chromatograph: equipped with ultraviolet detector.

A.6.4 Analytical procedures A.6.4.1 Specimen solution: Take 10mg of specimen into a 10mL volumetric flask;

dissolve it with mobile phase and make constant volume to the mark; and mix well.

A.6.4.2 Control solution: Accurately pipette 1mL of specimen solution in a 100mL