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Determination of Vitamin B_{12} in Health Foods

保健食品中维生素 B_{12} 的测定

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Determination of Vitamin B_{12} in Health Foods

1 Scope

This Standard specifies the determination of vitamin B_{12} in heath foods.

This Standard is applicable to the determination of vitamin B_{12} in heath foods, such as tablets, capsules, powders and functional beverages.

When this method adopts solid-phase extraction to treat the sample, the sampling amount is 2.0g, and the detection limit of the method is 1.0μg/g. When taking the immunoaffinity method to treat the sample, the sampling amount for tablets, capsules and powders is 2.0g, the detection limit of the method is 0.05μg/g; while the sampling amount for functionable beverage is 20mL, and the detection limit of the method is 3.0μg/L.

2 Principle

Adopt the solid-phase extraction or immunoaffinity chromatography to enrich the vitamin B_{12} in the sample extracting solution and remove the impurities; then analyze by high performance liquid chromatography.

3 Reagents

Unless otherwise specified, all reagents used in this Standard are of analytical reagent. The experimental water is Class-1 laboratory water; the conductivity (25°C) is 0.01mS/m.

3.1 Acetonitrile (C_2H_3N): chromatographic pure.

3.2 Methanol (CH_4O): guaranteed reagent.

3.3 Ethanol (C_2H_6O).

3.4 Tetrabutylammonium chloride ($C_{16}H_{36}NCl$).

3.5 5% tetrabutylammonium chloride ($C_{16}H_{36}NCl$) solution: take 5.0g of tetrabutylammonium chloride; add water to dissolve and dilute to 100mL.

3.6 Chloroform ($CHCl_3$).

(EASI-EXTRACT® VITAMINE B_{12}) [1)]

5 Analytical Procedures

5.1 Solid-phase extraction method (pretreatment method I) operation procedures

5.1.1 Specimen treatment

Evenly mix 20 pieces of tablet and capsule samples; take 5~10 packets of powder samples to mix thoroughly.

5.1.2 Extraction

Take 4g~10g (equivalent to about 4μg of vitamin B_{12}, accurate to 0.001g) of specimens into 50mL centrifuge tube; add 10mL~15mL water; mix evenly; place it into the ultrasonic cleaner. Ultrasonic extraction is performed for about 10min and then centrifuge at 4000r/min for 5min. Pipette the supernatant into another 50mL centrifuge tube. Add about 10mL of water to the residue each time according to the above procedure; repeat the extraction twice; combine the extract solutions into a 50mL centrifuge tube.

5.1.3 Purification

Add 1mL of 5% tetrabutylammonium chloride and about 20mL of chloroform into the extracting solution; use vortex mixer to mix them evenly; centrifuge at 1000r/min for 3min in the centrifuge machine. Transfer the water layer into the evaporating dish; place it onto the water bath to heat and evaporate to dryness. Dissolve the residue with ethanol; transfer into the centrifuge tube; ultrasound-dissolve, centrifuge, and pipette the supernatant into the evaporating dish. Repeat the extraction for twice, combine the extracting solutions into the evaporating dish. Evaporate the ethanol; quantitatively transfer the specimen by 5mL of 5% acetonitrile solution to the test tube for later-use on the solid-phase extraction column.

5.1.4 Solid-phase extraction

Firstly, use 3mL of methanol to activate the solid-phase extraction column; then use 3mL of water to balance the solid-phase extraction column; the speed is 1 drop/s. Add the appropriate amount of the above-treated specimen onto the solid-phase extraction column. After loading the specimen, use 5ml of 5% acetonitrile solution as the elution solvent to rinse the interference materials off the solid-phase extraction column. finally, use 25% acetonitrile solution to elute off the vitamin B_{12}; collect 0.5mL of elate.

[1)] This information is given for the convenience of the users of this Standard and does not imply endorsement of the product. If other equivalent products have the same effect, then these equivalent products can be used.

5.2 Immunoaffinity method (pretreatment method II) operation procedures

5.2.1 Specimen treatment

Carbonated functional beverage: take 150mL of sample solution and degas for 10min in ultrasonic wave for later-use.

Fruit or juice-type functional beverage: adjust the pH to 7.0 with 1mol/L sodium hydroxide; centrifuge in a centrifuge machine at 4000r/min for 10min; filter; and take filtrate for later-use.

Tablets, capsules, powders: take 20 pieces of tablet and capsule specimens to crush or mix evenly. Mix evenly the 5 ~ 10 packets of powder specimens.

Take 10g~50g of specimen (equivalent to 25μg~2500μg of vitamin B_{12}; accurate to 0.001g) into 250mL volumetric flask; add 100mL of water; shake and mix evenly. Place it into the ultrasonic cleaner; after 15-minute ultrasonic extraction, make constant volume by water; dilute the concentration of the sample solution till the vitamin B_{12} content in the sample is 25μg/mL. If the pH of the sample solution is 7.0 above; use citric acid to adjust the pH to be 4.5~7.0; if the pH of the sample solution is 4.5 below; use phosphate buffer instead of water as the extracting solution to repeat the above procedures.

5.2.2 Enrichment and purification

Firstly, use 10mL of water to wash the unbound compound in the small column of vitamin B_{12} immunoaffinity purification column; pipette 20mL (5.2.1) of extracting solution to the purification column; then use 3mL of methanol to elute the vitamin B_{12} to the evaporating dish; the speed during the whole process is 1 drop/s. Evaporate the solvent in the 60°C~70°C water bath; use the 1mL of 0.025% trifluoroacetic acid solution to dissolve; pass the solution through 0.45μm aqueous filter membrane for the later-use on the high performance liquid chromatograph.

5.3 Reference and analysis conditions of the liquid chromatography

5.3.1 Chromatographic column: C_{18} (4.6mm×250mm, 5μm) reversed phase column.

5.3.2 Mobile phase: use the gradient elution method, see Table 1.

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