Nicotinic acid content Assay kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4844

Sizes： 50T/48S

Product Description：

Niacin is a water-soluble B vitamin, also known as vitamin B3, is one of the 13 vitamins necessary for the human body, its natural products mainly exist in animal viscera and muscle tissue, widely used in feed additives, pharmaceutical intermediates and other fields.

Niacin has ultraviolet absorption at 210 nm, and its content can be determined by ultraviolet detector.

Reagents and Equipment Required but Not Provided：

High-efficiency liquid chromatograph (C18 column (4.6×250 mm), ultraviolet detector (VWD)), desktop centrifuge, adjustable pipette, mortar/ homogenizer, EP tube (1.5 mL), syringe filters (water), syringe, suction filter, filter membrane (organic, water), brown injection bottle, carbinol (chromatographically pure), ultrapure water.

Product Composition:

Extract solution: Liquid 30 mL×1. Storage at 2-8℃.

Reagent I: Liquid 5 mL×1. Storage at 2-8℃.

Reagent II: Liquid 1.5 mL×1. Storage at 2-8℃.

Reagent III: Powder×2. Storage at 2-8℃.

Standard： Powder×1. Store at 2-8℃. Before use, 1 mL distilled water was added to prepare 5 mg/mL Nicotinic acid standard solution, which was sealed and stored at 2-8℃, away from direct sunlight.

Preparations before the experiment:

1. Dissolve 1 bottle of Reagent III into 1000 mL of ultra-pure water, then add 0.55 mL of Reagent II and mix well to obtain mobile phase A.
2. Filter 1000 mL of prepared mobile phase A and methanol (chromatographically pure) with filter membrane. (The prepared mobile phase A was filtered by 0.22μm aqueous filter membrane, and methanol was filtered by 0.45μm organic filter membrane).
3. Ultrasound the filtered mobile phase for 20 min to remove bubbles.
4. Preparation of standard products: 5 mg/mL Nicotinic acid standard solution is diluted with distilled water into500 μg/mL、100 μg/mL、20 μg/mL、4 μg/mL、0.8 μg/mL Nicotinic acid standard solution. (The standard concentration is for reference only and can be adjusted according to the actual sample concentration). Store (sealed) at 4°C away from light, filter into brown sample bottle with water needle filter before test, to be tested.

Procedure

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I. Pyridoxamine extraction:

1. Tissue: According to the mass (g): Extract solution volume (mL) 1:5~10 ratio, it is recommended to weigh 0.1g sample (Fresh sample: chopped; Drying sample: Grinding and sifting) and add 0.6 mL Extract solution to fully homogenize), seal, mix evenly, and soak in a water bath at 60℃ for 30 min. Cool to room temperature, add 0.1 mL of Reagent I, 0.3 mL distilled water, mix well, let stand for 2 min. Centrifuge at 10000 rpm for 10 min, take the supernatant (if there is still turbidity, it can be centrifuged again), filter it into the brown sample bottle using a water-based needle filter before the test, and then filter it again to be tested (if the color of the supernatant is too dark or the concentration is too high, it can be diluted and filtered again to be tested).

2. Cells: According to the number of cells (10^4): the Extract solution volume (mL) is 10-50 million :1 ratio, it is recommended to take 50 million cells, add 0.6 mL of the Extraction solution, ultrasonic crushing cells (power 200W, ultrasonic 3s, intermittent 9s, repeat 30 times, total time 6 min), seal and mix well. Soak in a water bath at 60℃ for 30 min. Cool to room temperature, add 0.1 mL of Reagent I, 0.3 mL of distilled water, mix well, leave for 2 min. Centrifuge at 10000 rpm for 10 min, take the supernatant (if there is still turbidity, it can be centrifuged again), and filter it into the brown sample bottle using a water-based needle filter before testing.

3. Serum: According to the serum volume (mL) : Extract solution volume (mL) 1~5:1 ratio, it is recommended to take 0.5 mL of serum, add 0.1 mL of Extract solution, seal and mix, and soak in a water bath at 60℃ for 30 min. Cool to room temperature, add 0.1 mL of Reagent I, 0.3 mL of distilled water, mix well, leave for 2 min. Centrifuge at 10000 rpm for 10 min, take the supernatant (if there is still turbidity, it can be centrifuged again), and filter it into the brown sample bottle using a water-based needle filter before testing.

II. Determination procedure：

1. Turn on the computer, turn on the switch buttons of each module of the HPLC, install the chromatographic column, open the software, and set the injection volume in the method group to 10 μL, column temperature: 30°C, flow rate 1 mL/min, and the ultraviolet detector wavelength to 210 nm. The sampling time of a single sample is 10 minutes, and the preservation method group is set.
2. Use the corresponding mobile phase to clean the column, balance the column with mobile phase A, and start adding samples after the baseline is stable.
3. Test the standard solution to be measured, the sample size is 10 μL, Nicotinic acid can be separated within 10 min, and the retention time of Nicotinic acid is about 5.5 min (the retention time is different with the system, column, mobile phase pH, temperature, etc., and is only for reference).
4. Test the sample solution to be measured, the injection volume is 10 μL, and test the peak area of Folic acid at the corresponding retention time.
5. Complete sequence sampling table: (including the cleaning and rebalancing process of the column after the determination of a single sample is completed)

Time (t)	Carbinol (%)	Mobile phase A (%)

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0 min	0	100
1 min	0	100
1.1 min	3	97
10 min	3	97
10.1 min	60	40
20 min	60	40
20.1 min	0	100
30 min	0	100

III. Calculations：

The standard curve y=kx+b was drawn with the standard concentration (μg /mL) as the horizontal coordinate x and the peak area as the vertical coordinate y. The peak area of the sample was substituted into the standard curve to calculate the concentration x (μg /mL) of Nicotinic acid in the Extraction solution.

1. Tissue sample：

Nicotinic acid content (μg/g)＝x ×V_E÷W×F=x÷W×F

V_E: Add the total volume of Extraction solution, 1 mL (0.6mL Extraction solution +0.1mL Reagent I+0.3mL distilled water); W: Sample quality, g; F: dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio.

2. Cell sample：

Nicotinic acid content (μg /10^4cell)＝x ×V_E÷N×F=x÷N×F

V_E: Add the total volume of Extraction solution, 1 mL (0.6mL Extraction solution +0.1mL Reagent I+0.3mL distilled water); N: Cell number, 10^4; F: dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio.

3. Serum samples：

Nicotinic acid content (μg /mL)＝x ×V_E÷V_S×F=2x×F

V_E: Add the total volume of Extraction solution, 1mL (0.5mL Serum+0.1mL Extraction solution +0.1mL Reagent I+0.3mL distilled water); V_S: Add sample volume, 0.5mL; F: dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio.

Note:

Precautions:

1. After the test is completed, it is necessary to flush the column with a high concentration of ultra-pure water phase (about 20-30 column volumes) to prevent blocking the column, and then flush the column with a high concentration of organic phase, and finally flush according to the type of column specification to prevent damage to the column.

2. The dilution of the standard product should be determined according to the concentration of niacin in the sample, and the peak area of niacin in the sample must be within the peak area of the standard solution of different concentrations, and the dilution of the standard product is only a reference. If the concentration of niacin in the sample is too high, it is recommended to dilute it with distilled water and then measure it.
3. If the sample size is too large, it is recommended to test the standard solution once a day (one

concentration of the standard solution can be) to determine the corresponding retention time, and the solution to be tested must be placed at room temperature before testing.
4. In order to exclude the influence of gradient elution baseline drift, a blank detection can be performed.

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