TDS

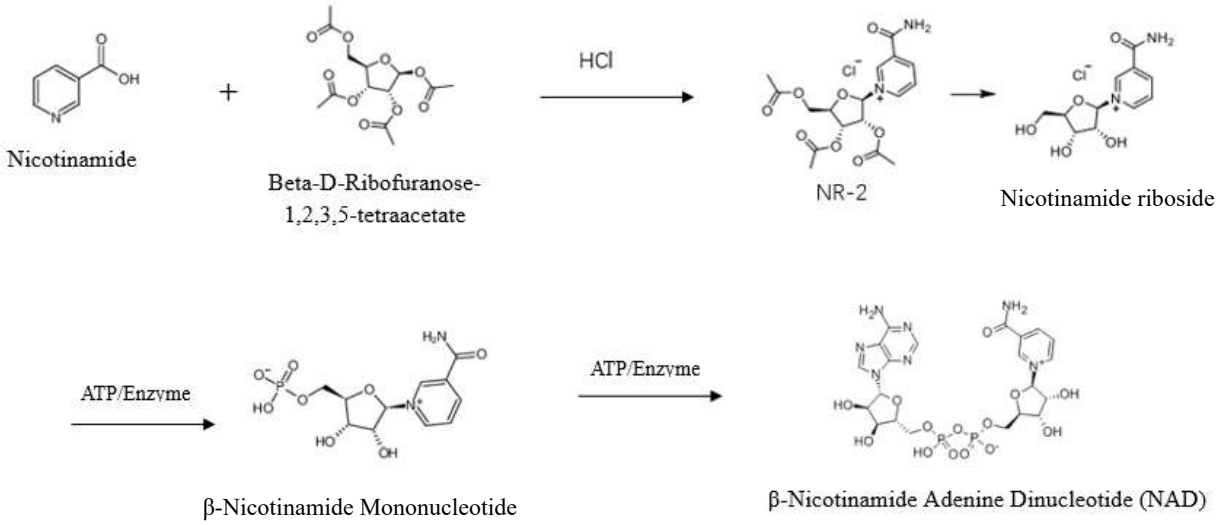
Product Name: β - Nicotinamide Adenine Dinucleotide

β-Nicotinamide Adenine Dinucleotide（NAD+）

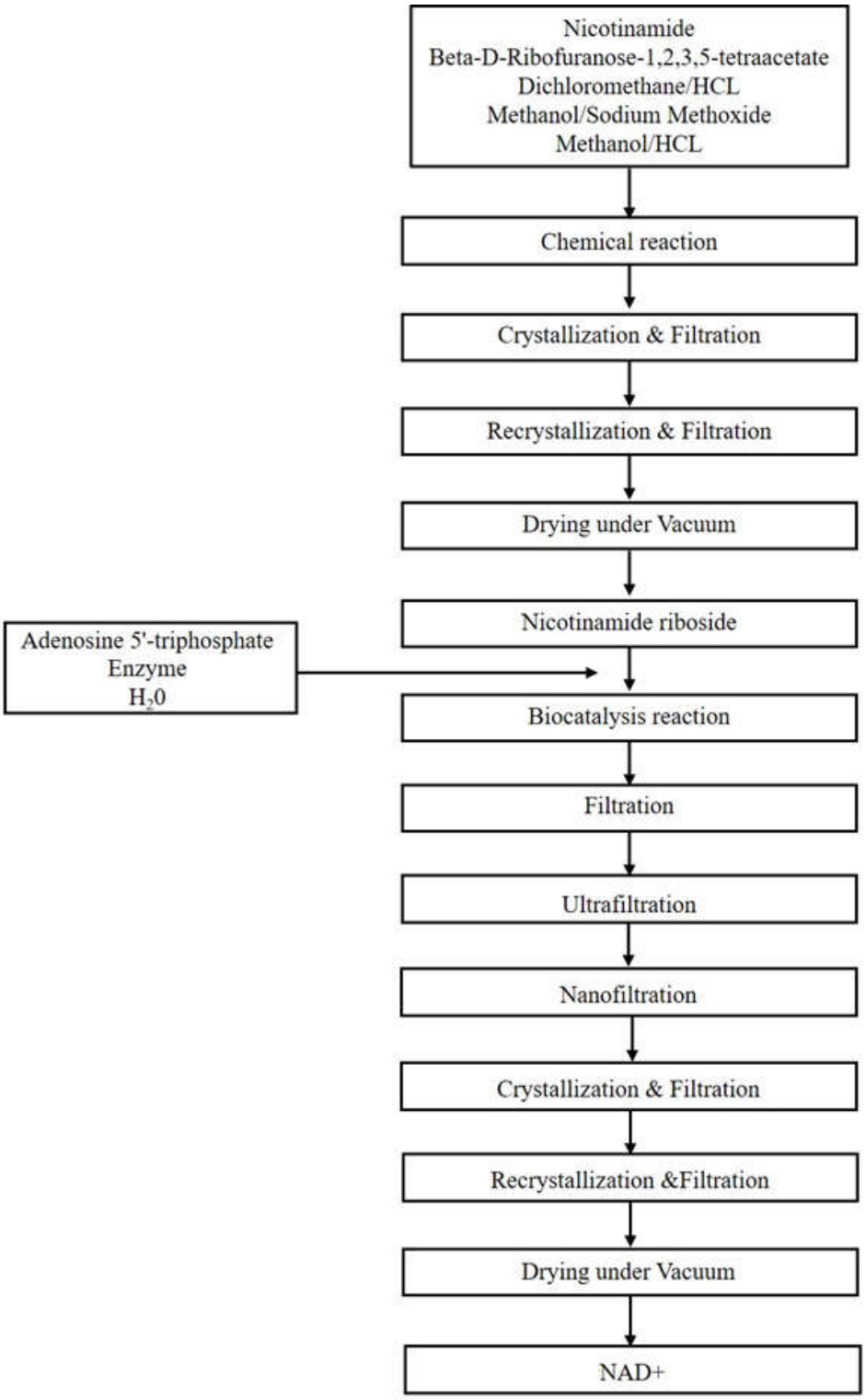
CAS No.：53-84-9

1、 Technical parameters

1. ROS of NAD+



2. NAD+process flowchart



3. Quality standards

Inspection items	Acceptance Criteria
Appearance	White almost white powder
Identification	HPLC retention time correspond to WRS
Loss on drying	≤8.0%
pH(1g/L)	2.0~4.0
lead Pb	≤0.5ppm
arsenic As	≤0.5ppm
mercury Hg	≤0.5ppm
cadmium Cd	≤0.5ppm
Total bacteria	≤750cfu/g
Yeast＆Molds	≤100cfu/g
Endotoxin	＜0.5EU/mg
Assay（Dry content）	≥95.0%
Purity (HPLC)	≥99.0%

4. Inspection method

4.1 Appearance

Take an appropriate amount of the test sample and visually inspect it. The result should be white or off white crystals or crystalline powder.

4.2 Related substances

4.2.1 Chromatographic conditions

instrument	hpLC
chromatographic column	Welch Ultimate AQ-C18 (250 * 4.6 mm, 5 μ m) or equivalent column
Mobile phase A	10 mMol PBS potassium salt buffer (pH=6.0) (1.36g potassium dihydrogen phosphate dissolved in water, adjusted to pH 6.0 with potassium hydroxide, diluted to 1L)
Mobile phase B	methanol
detection wavelength	254nm/uv

velocity of flow	1.0 mL/min		
Injection volume	20μL		
Column temperature	30℃		
thinner	water		
Gradient elution:	Time (minutes)	Mobile phase A (%)	Mobile phase B (%)
	0	95	5
	6	95	5
	13	90	10
	20	90	10
	20.1	95	5
	30	95	5

4.2.2 Solution Preparation

1) Blank solution: purified water.

2) Phosphate buffer solution: Dissolve 1.36g of potassium dihydrogen phosphate in water, adjust the pH to 6.0 with potassium hydroxide, dilute to 1L, and pass through the membrane.

3) β - nicotinamide adenine dinucleotide reference solution: Weigh 20mg of β - nicotinamide adenine dinucleotide reference solution and place it in a 100ml volumetric flask. Add diluent to dissolve and dilute to the mark. Ultrasonic dissolution is required to obtain the solution.

4) Test sample solution: Take 20mg of the test sample, place it in a 100ml volumetric flask, add diluent to dissolve and dilute to the mark, dissolve by ultrasound, and obtain.

4.2.3 Sample determination

Serial Number	Sample name	Number of injection needles
1	Blank solution (diluent)	1 or more
2	reference solution	1
3	test solution	1

4.3 Content

Result calculation: Calculate the content of nicotinamide adenine dinucleotide in the sample based on peak area using the reference standard external standard method.

$$X = \frac{A_x \times c_i \times P \times V}{A_i \times m} \times 100\%$$

In the formula:

X - Content of nicotinamide adenine dinucleotide in the sample,%;

Ax - peak area of nicotinamide adenine dinucleotide in the chromatogram of the test solution;

Ai - peak area of nicotinamide adenine dinucleotide in the chromatogram of the reference solution;

C_i- concentration of nicotinamide adenine dinucleotide in the reference solution, mg/mL；

P - content of reference substance,%;

V - dilution volume of the test solution, mL；

M - Weigh the test sample, mg。

4.4 Purity

Subtract the blank and calculate the purity using the area normalization method.

4.5 Related substances

Same purity determination method. Calculate relevant impurities using the area normalization method.

4.6 pH

Accurately weigh 1g of β - nicotinamide adenine dinucleotide sample into a 1000 mL volumetric flask, dissolve it in deionized water and dilute to the mark. Measure the pH value using a pHS-3C pH meter.

4.7 Determination of drying loss

Refer to the Chinese Pharmacopoeia 0831 Dry Loss Determination Method.

Take the test sample, mix it evenly (if it is a large crystal, it should be quickly crushed into small particles of less than 2 mm), take about 1 gram or the weight specified under each variety item, place it in a flat weighing bottle dried to constant weight under the same conditions as the test sample, weigh it accurately, and unless otherwise specified, dry it to constant weight at 105 ° C. Calculate the drying loss of the test sample based on the weight loss and sampling amount. When drying the test sample, it should be spread flat in a flat weighing bottle with a thickness not exceeding 5 mm. If it is a loose substance, the thickness should not exceed 10 mm. When placing it in an oven or dryer for drying, the bottle cap should be removed and placed next to the weighing bottle, or the bottle cap should be half opened for drying; When taking it out, the M bottle cap must be securely closed. The test sample dried in the oven should be taken out and placed in a dryer for cooling after drying, and then weighed.

4.8 Heavy metal detection (Hg, Pb, As, Cd)

Mercury is detected according to the national standard GB5009.17

Cadmium is tested according to the national standard GB5009.15

Lead is tested according to the national standard GB5009.12

Arsenic is detected according to the national standard GB5009.11

4.9 Microorganisms

Determine according to the method specified in Chapter 5 of the "Technical Specification for Safety of Cosmetics" (2015 edition).

4.10 endotoxin testing

General Rule 1143 of the Chinese Pharmacopoeia 2020 Edition, Part Four.

Test reagents:

Select a horseshoe crab reagent with a sensitivity of 0.125 EU/mL, and the endotoxin content in the water used for detection should be less than 0.015 EU/mL.

MVC (Minimum Effective Dilution Concentration) calculation MVC=λ/L=0.125/0.2=0.625mg/mL.

Conduct the experiment according to the standard operating procedure for bacterial endotoxin testing

The preparation of test solutions for each group is shown in the following table:

Number	Concentration of endotoxins/solution with added endotoxins	Number of parallel tubes
A	No/test solution available	2
B	2 λ/test solution	2
C	2 λ/inspection water	2
D	No/Check water usage	2

Bacterial endotoxin solution: Take 1 vial of bacterial endotoxin working standard at a rate of 10 EU/vial, dissolve it in 1 mL of test water, and mix well.

Solution A (test sample group): Weigh 63mg of the test sample and dissolve it in 10mL of bacterial endotoxin test water. Mix 1mL of the above solution with 9mL of bacterial endotoxin test water to obtain the solution.

B solution (positive test group): Transfer 0.1mL of bacterial endotoxin solution and mix it with 0.9mL of A solution; Mix 1mL of the above solution with 1mLA solution; Mix 1mL of the above solution with 1mLA solution.

C solution (positive control group): Transfer 0.1mL of bacterial endotoxin solution and mix it with 0.9mL of test water; Mix 1mL of the above solution with 1mL of inspection water; Mix 1mL of the above solution with 1mL of inspection water.

Note: Each dilution step should be mixed evenly on a vortex mixer for 30 seconds.

D solution (negative control group): water for bacterial endotoxin detection.

Take 8 tubes of dissolved horseshoe crab reagent,

Two of them were added with 0.1mL of the test solution as test tubes;

Add 0.1mL of positive control solution to 2 tubes as positive control tubes;

Add 0.1mL of test water to 2 tubes as a negative control tube;

Add 0.1mL of positive control solution to 2 tubes as positive control tubes for the test sample. After adding the sample, gently shake and mix the solution in the test tube, seal the tube mouth to avoid bubbles, and place it vertically in a 37 ℃± 1 ℃ thermostat for 60 ± 2 minutes.

Result judgment:

When all tubes of solutions B and C are positive and all tubes of solution D are negative, the test is considered valid. At this point, if all tubes of tube A are negative, then the endotoxin of the test sample meets the requirements.

2、 Product performance characteristics

1. Characteristics

White powder, odorless.

2. Physical parameters

Melting point/freezing point: 140~142 º C, flash point: 90 ℃ (lit.),

Boiling point, initial boiling point, and boiling range: 151 ℃/21mmHg (lit.).

3. Main purpose

Used for making health food.

4. Characteristics

This product is non flammable, explosive, corrosive, non-toxic and harmless. It is not an oxidizing agent, narcotic, psychotropic drug, or raw material for the production of chemical weapons. It is non radioactive.

5. Ingredient Content

β - nicotinamide adenine dinucleotide ≥ 95.0%, water content ≤ 8.0%.

6. HS number

29349990.

7. Storage conditions

The retest period is 1 year at 2-8 ℃, and the storage period is 5 years at minus 20 ℃.

3、 Product Applicability

1. Overview of NAD

NAD or NAD+(Nicotinamide Adenine Dinucleotide) is a biological substance that transfers electrons. In Chinese, it is nicotinamide adenine dinucleotide and is an oxidized coenzyme I. This is a coenzyme among many dehydrogenases in the body, which functions to connect the tricarboxylic acid cycle and respiratory chain, transferring the hydrogen removed during metabolism to flavoprotein. Its reduced form is NADH, more precisely NADH and H+.

NAD+is an essential coenzyme required for life and cellular functions. Enzymes are catalysts that make biochemical reactions possible. Coenzymes are "helper" molecules required for enzymes to function. NAD+has always existed until recent years when it was utilized in conjunction with antioxidants to combat aging. NAD+is an essential energy producing factor in the human body. As the level of NAD+in the human body gradually decreases with age, it can lead to a decrease in brain cell vitality, affecting the energy required by cells to maintain health, and causing various physiological functions to deteriorate and aging symptoms.

NAD+is the most abundant molecule in the human body after water, without which organisms would die. NAD+is used by many proteins in the body, such as silencing regulatory proteins, which can repair damaged DNA. For mitochondria, they are also important as they serve as the power source for cells and generate the chemical energy used by the human body.

2. Potential efficacy of NAD

2.1 Anti aging

NAD+is the fuel that helps Sirtuins maintain genome integrity and promote DNA repair. Just like a car cannot drive without fuel, activation of Sirtuins requires NAD+. The results of animal studies have shown that increasing NAD+levels in the body can activate silencing regulatory proteins and prolong the lifespan of yeast, worms, and mice. Although animal studies have shown encouraging results in anti-aging properties, scientists are still studying how these results can be translated into humans.

2.2 Regulating metabolic disorders

NAD+is one of the key factors in maintaining healthy mitochondrial function and stable energy output. Aging and a high-fat diet can lower the levels of NAD+in the body. Taking NAD+supplements can also reduce weight gain related to diet and age, and improve physical activity. Other studies have even reversed the effects of diabetes, showing new strategies for combating metabolic diseases such as obesity.

2.3 Protecting Heart Function

Raising NAD+levels can protect the heart and improve heart function. Hypertension can cause heart enlargement and arterial blockage, leading to stroke. NAD+supplements supplement the NAD+levels in the heart and prevent damage to the heart caused by insufficient blood. Other studies have shown that NAD+supplements can protect against abnormal cardiac enlargement

2.4 Prevention of neurodegenerative diseases

In patients with Alzheimer's disease, increasing NAD+levels can reduce protein accumulation, thereby disrupting cellular communication in the brain and enhancing cognitive abilities. When there is not enough blood flow to the brain, increasing NAD+levels can also protect brain cells from death. Many studies have proposed new prospects for promoting brain health aging, preventing neurodegenerative diseases, and improving memory.

2.5 Regulating the immune system

As adults age, the weakened immune system makes them more susceptible to illness and more difficult to recover from seasonal flu or even COVID-19. Recent studies have shown that the level of NAD+plays an important role in regulating inflammation and cell survival during immune response and aging processes. This study emphasizes the therapeutic potential of NAD+for immune dysfunction.