Analytical Method for NMN

1. Purity And Assay:

Purity: HPLC:

T=25℃;

λ=254nm;

Flow rate: 0.8mL/min;

Column: Waters symmetryShield™ RP8 4.6*250mm 5μm;

Phase A: Weigh 11 mM KH_2PO_4 + 2.7 mM Tetrabutylammonium hydrogen sulfate dissolved in 500 mL of water;

Phase B: Methanol;

Sample: 10 mg dissolved in 10 mL H_2O and Injection volume: 10μL.

Gradient elution table:

Time(min)	Flow rate(mL/min)	A%	B%
0	0.8	100	0
5	0.8	95	5
10	0.8	90	10
15	0.8	80	20
20	0.8	70	30
25	0.8	60	40
30	0.8	50	50
35	0.8	80	20
40	0.8	100	0

Assay: Test condition is same with purity testing;

Calculation formula:

$$W = \frac{m_1 \times w_1 \times S_2}{m_2 \times S_1} \times 100\%$$

Among:m_1 is the quality of the standard,g;

w_1 is the assay of the standard,%;

S_1 is the area of the standard;

m_2 is the quality of the sample,g;

S_2 is the area of the sample.

2. Identification：

^{1}H-NMR &^{13}C-NMR: Weigh 15mg of NMN and dissolved in 3mL D_2O to run the NMR test.

LCMS: 1mg of NMN dissolved in 2 mL of water.

LC Analytical Method: Agilent Zorbax 300 SB C8 column (150*4.6mm, 5μm). Mobile phase A (acetonitrile), Mobile phase B (0.1 %CF3COOH in water). Gradient elution: 0-5min, A: 0-5%; 5- 10min, A: 5-15%; 10-15min: A 15-20%. Detection wavelength λ=254nm. Injection volume: 10μL. Column temperature: 35 ^{o}C. Flow rate: 1mL/min.

MS Analytical Method: ESI ion source, positive ion detection mode. Spray Voltage: 3.8KV, Scan Range 100-2000 m/z. Resolution: 200000.

3. Appearance: Visual. Odor & taste: Organoleptic.

Appearance of solution: 5% w/v of NMN in water. (0.5g NMN in 10mL H2O) by visual.

4. Water Content: Karl Fischer.

5. PH (10mg/mL, 25℃): 100mg NMN dissolved in 10mL H2O and analyzed by PH meter. CP<0631>.

6. Bulk Density: USP<616>.

7. Chemical Control: Heavy Metals: ICP-MS. USP<231>.

8. Microbiological Control: Follow the procedure listed in USP40.

Yours sincerely

Authorized signatory

Name: Jianjun Yu

Signature:

Designation: QA