Determination of Nicotinamide and Nicotinic Acid by HPLC

Assay Parameter:	Niacinamide, Nicotinic Acid		
Principle:	HPLC		
Products to which applicable:	Multivitamin Preparations and Vitamin premixes		
Assay range:	a) For pure Substance	:	98.0 – 101.0%
	b) For premixes	:	95.0 – 105.0%
			of the declared content

Reagents (and sources where applicable)

1. Methanol (HPLC Grade)
2. Niacinamide for biochemical purposes
3. Nicotinic Acid for biochemical purposes
4. Potassium dihydrogen phosphate, AR

Apparatus (and sources where applicable)

1. HPLC with UV absorbance detector and integrator.
2. Sintered glass filtration apparatus for preparation of solvents (filter 0.45µm)
3. 10 ml syringe with microsyringe filter hold (filter 0.45µm)
4. Ashless filter paper. show filter rate (1500 s, filtration time)
5. Medium fast filtration rate (140 s filtration time)

Standard Solutions

1. For niacinamide: dissolve 1 g niacinamide and 30 mg nicotinic acid – accurately weighed - in 1 000 ml water. Transfer 10.0 ml of this solution to 100 ml volumetric flask and make up to the mark with water. 1 ml of this solution contains approximately 0.1 mg / niacinamide and 3.0 µg nicotinic acid.
2. For Nicotinic acid: dissolve 1 g nicotinic acid – accurately weighed – in 1 000 ml water. Transfer 10.0 ml of this solution to a 100 ml volumetric flask and make up to the mark with water. 1 ml of this solution contains approximately 0.1 mg nicotinic acid.

1. Semi - quantitatively approximate the composition of the sample using a qualitative method of analysis. Prepare a standard solution for calibration with a similar concentration of substance as determined by the quantitative analysis. Finally, carry out the quantitative determination.

   a) Qualitative Analysis

      Dissolve approximately 10 mg niacinamide / nicotinic acid sample in 100 ml mobile Phase (90 % 0.05 mole / l potassium dihydrogen phosphate solution, 9 % methanol, 1 % acetic acid.)

      The identity of the sample (niacinamide, nicotinic acid or mixtures) is determined by comparison of the retention times with standards.

   b) Quantitative Analysis (calibration)

      Accurately weight 1 g of substance in the proportions determined in the qualitative analysis (niacinamide, nicotinic acid) as a calibration standard into 200 ml volumetric flask 1. Dissolve the calibration standard in the mobile phase and make up to mark. Prepare 1:1100 dilution of this stock solution with the mobile phase. 10 µl of this solution are used for the HPLC analysis.

2. Quantitative Analysis (Measurement)

   Prepare 1 g sample (niacin amide, nicotinic acid or mixture of both) as in the calibration step. 10 µl of the diluted sample is injected into the HPLC system.

3. Sample

   Niacinamide and (or nicotinic acid in premixes: Accurately weight the finely ground sample (corresponding to a niacinamide or nicotinic acid content of approximately 10 mg) into a 100 ml volumetric flask and make up to the mark with water. Add a magnetic stirring bar and stir the mixture for 15 minutes. Let the solution settle and then filter the supernatant liquid through a 0.45 µm filter. 1 ml of this solution contains approximately 0.1 mg niacinamide / nicotinic acid.

4. HPLC Conditions

Column:	RP 18, 25 cm x 4.6 mm (particle size 7 µm)
Mobile phase :	90 % 0.05 mol / l KH_2PO_4 solution 9 % methanol 1 % acetic acid filtered through 0.45 µm solvent filter
Flow rate :	1.0 ml / min.
Temperature:	Room temperature
Volume of sample:	10 µl
Detection:	UV at 264 nm
Retention times :	nicotinic acid at about 5 minutes niacinamide at about 9 minutes

Calculations

1. Calibration

$$RFc = \frac{Ac \times DF}{Wc}$$

Where:

RFc	=	response factor for niacinamide or nicotinic acid
Ac	=	peak area of calibration peak
DF	=	dilution factor
Wc	=	weight of calibration substance in mg
C	=	calibration substance (niacinamide or nicotinic acid)

2. Measurement

$$\text{Content of pure Substance (\%)} = \frac{As \times DF \times 100}{RFc \times Ws}$$

Where

As	=	peak area of sample
DF	=	dilution factor
RFc	=	response factor from calibration
Ws	=	weight of sample in mg
S	=	sample (niacinamide, nicotinic acid or mixtures of both)

The active content of nicotinic acid and niacinamide mixture is calculated from the sum of the individual contents. The content of the pure substance may be determined in one measurement (see Fig. 1).

The difference between the molar masses (amide: 122.1 g/mole and acid: 123.1 g/mole) is disregarded.

Variations or exceptions for different product: None

Special advice or precautions: None