PICKERING LABORATORIES

Analysis of Bioavailable Niacin (Vitamin B3) by HPLC with Post-column Photochemical Derivatization in Foods and Supplements

Niacin (Nicotinic acid) is an essential nutrient important to human health. Free Nicotinic Acid and Nicotinamide have similar biological activity and both are used as dietary supplements to prevent Vitamin B3 deficiency. In supplements, the use of Nicotinamide is often preferred due to absence of common side effects of Nicotinic Acid, such as skin flushing.

The European Committee for Standardization has approved the HPLC method with post-column photochemical derivatization to measure Vitamin B3 in foodstuff. UV irradiation converts Nicotinic Acid and Nicotinamide into highly fluorescent derivatives. The addition of Pickering Laboratories UVE™ Photochemical reactor to any HPLC system allows for highly sensitive and selective analysis of both Nicotinic Acid and Nicotinamide in a variety of different matrices.

Method

Calibration

- *Calibration Range:* Nicotinic Acid: 0.1 – 50 ug/mL, R2=0.999; Nicotinamide: 0.1 – 50 ug/mL, R2=0.998

Sample Preparation

The sample preparation described below is designed to measure bioavailable (or free) Nicotinic Acid and Nicotinamide in foods. Different procedures can be employed to prepare samples for the analysis of total Niacin.

- *Food Samples:* To 5 g of sample, add 30 mL of 0.1 N HCl. Blend at high speed for 2-3 min and heat the mixture at 100 °C for 1 h. Cool the mixture to room temperature, transfer into a graduated cylinder and adjust the volume to 50 mL with DI water. Dilute further with water to fit the calibration curve as needed. Filter the solution through a 0.45 um filter.
- *For High Protein / High Fat Matrices:* Proceed as directed above. After filtering, pipette 4 mL of the solution into a centrifuge tube. Add 1 mL of 50% (w/v) solution of Trichloroacetic Acid in water to precipitate proteins. Cool the mixture in an ice water bath for 5 min. Centrifuge and filter through a 0.45 um syringe filter.
- *Dietary Supplement:* Thoroughly mix the content of at least 10 finely ground capsules / tablets. Dissolve 100 mg portion in 100 mL of DI water. Dilute further with water to fit the calibration curve as needed. Filter through a 0.45 um filter.

Analytical Conditions

Analytical Column: ThermoHypersil, Aquasil C_{18} (150 x 4.6 mm)

Temperature: 40 °C

Flow Rate: 1 mL/min

Mobile Phase: Methanol / Phosphate buffer (0.035 mol/L of Potassium Phosphate Monobasic adjusted to pH 4.45). See Table 1 for gradient conditions.

Injection Volume: 20 uL

Post-column Conditions

UVE™ Photochemical Reactor

Detection: FLD, Ex 322 nm, Em 370 nm

Table 1. HPLC Gradient

Time, Min	Phosphate Buffer	Methanol
0	100	0
25	100	0
25.1	0	100
29	0	100
29.1	100	0
35	100	0

Table 1. Niacin Analysis in Foods

Sample	Fresh Peas		Fresh Tomatoes		Raw Ground Pork		Oat Cereal	
	Nicotinic Acid	Nicotinamide	Nicotinic Acid	Nicotinamide	Nicotinic Acid	Nicotinamide	Nicotinic Acid	Nicotinamide
Found in the sample	2.77 ug/g	5.35 ug/g	1.30 ug/g	3.33 ug/g	2.48 ug/g	60.95 ug/g	7.91 ug/g	243.30 ug/g
RSD, N=4	1.8%	1.5%	1.6%	2.0%	2.3%	0.7%	3.8%	0.6%
Spike	10 ug/g	10 ug/g	10 ug/g	10 ug/g	10 ug/g	50 ug/g	20 ug/g	300 ug/g
Recoveries	93%	94%	98%	92%	70%	102%	109%	96%
RSD, N=3	3.8%	2.0%	1.1%	1.4%	0.8%	1.5%	3.2%	2.1%

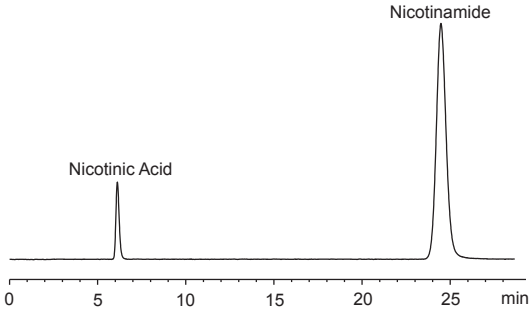

Fig 1. *Chromatogram of 1 ug/mL calibration standard of Nicotinic Acid and Nicotinamide*

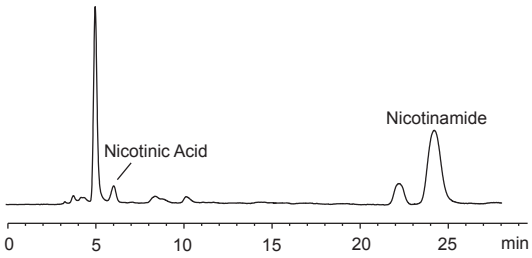

Fig 2. *Chromatogram of fresh peas sample*

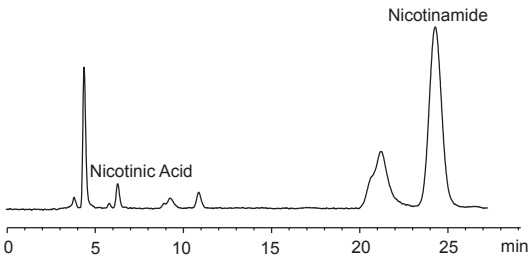

Fig 3. *Chromatogram of fresh tomatoes sample*

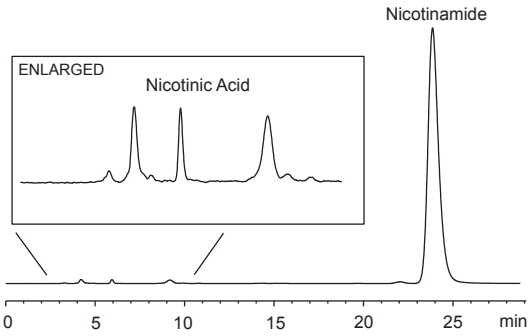

Fig 4. *Chromatogram of raw pork sample*

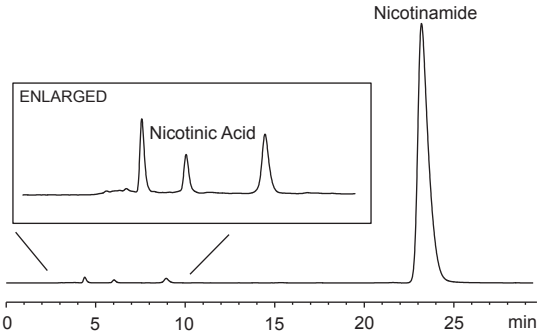

Fig 5. *Chromatogram of oat cereal sample*

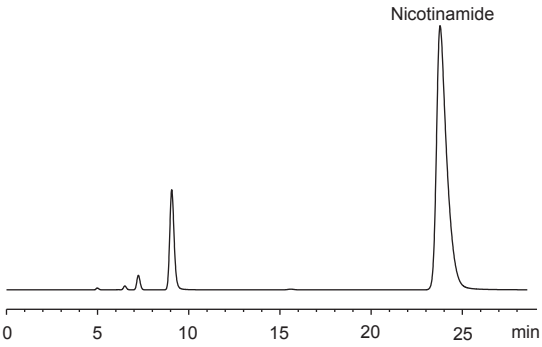

Fig 6. *Chromatogram of Vitamin B Complex dietary supplement*