Determination of Nicotinic Acid and Niacinamide by HPLC with Post-Column Photochemical Derivatization and Fluorescence Detection

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Niacin, or Vitamin B3, is the generic descriptor for the combined vitamers: nicotinic acid (pyridine 3-carboxylic acid) and nicotinamide (pyridine 3-carboxylic acid amide). Biologically, the amide form has the same activity as nicotinic acid. Niacin is an essential human nutrient.

The Lahély et al (1) method has been approved as a British and European Standard (2). The method evaluation presented here utilizes the Aura PHRED™, a versatile and economical in-line photochemical reactor unit (Figure 1). The method employs post-column derivatization of nicotinic acid and niacinamide in the presence of hydrogen peroxide and copper (II) ions to yield a fluorophore. The reaction resembles a hydroxyl radical reaction, however the fluorophore structure is unknown (3).

This method and apparatus provide excellent separation of the nicotinic acid and niacinamide peaks eluting at 4.07 and 7.20min. respectively (Figure 2). When the photolysis unit was off, no peaks were observed at the corresponding retention times. This demonstrates the high specificity of the present method. We achieved a linear correlation with an R^2 value of 0.999 and 0.9982 for Niacinamide and Nicotinic acid respectively (Figure 3). The lower limit of detection, calculated as 3 times background noise, was found to be 0.50ppm for niacin and 0.44ppm for niacinamide. New standards and mobile phase must be prepared daily.

HPLC Parameters:

Column: reversed phase Supelco C18 Column, 15 cm x 4.6 mm, 3 µm particles, with Aura CJB-10 column jacket.
Temperature: 40°C
Mobile Phase: 4.83 g NaH_2PO_4 dissolved in 400 mL H_2O, add 0.5 ml $CuSO_4$ stock solution (stock solution: 0.12g $CuSO_4$ dissolved in 100 mL H_2O), add 3.8 mL 30% H_2O_2, bring to 500 mL with H_2O.
Flow rate: 1.0 mL/min
Injection Volume: 50 µL
Flow restrictor on waste line to avoid possible in-line bubbles (optional): 40psi restrictor

Photochemical reactor:

PHRED™ with knitted reactor coil 10 m, ID 0.5mm (KRC 10-50)
366 nm black light bulb (BLB-366)

Detector:

Fluorescence detector. Excitation: 322nm, Emission: 370nm

Chemicals:

Nicotinic acid and niacinamide standards (Sigma-Aldrich) were diluted with distilled water to concentrations ranging from 0.4 – 250ppm. Poland Spring® Brand distilled water was used for reagent and sample preparations.

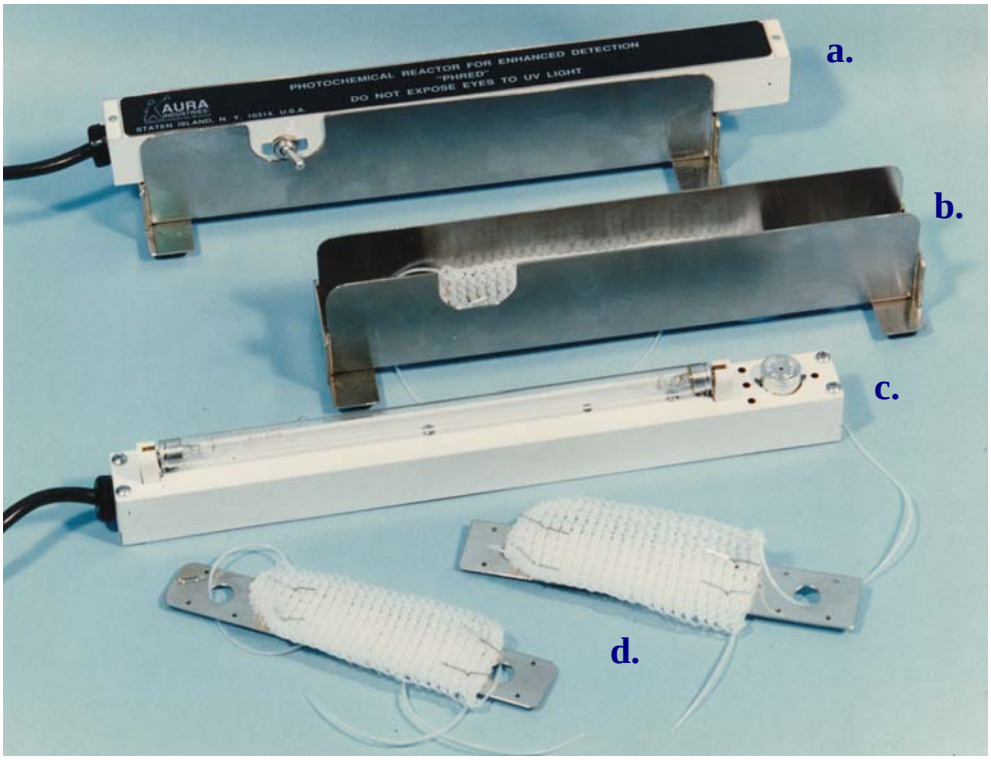


Figure 1. a. Aura Industry PHRED™ Photochemical Reactor for Enhanced Detection unit. b. Stainless steel bottom casing with knitted reactor coil inside. c. Lamp holder outfitted with a 254 nm low-pressure mercury lamp (for this analysis however we used a 366 nm black light bulb). d. 2 polished support plates displayed with attached knitted reactor coils which when assembled fit inside the stainless steel bottom casing (b) and the lamp holder (c) fits snuggly on top to reduce excess radiation to form a completed unit (a).

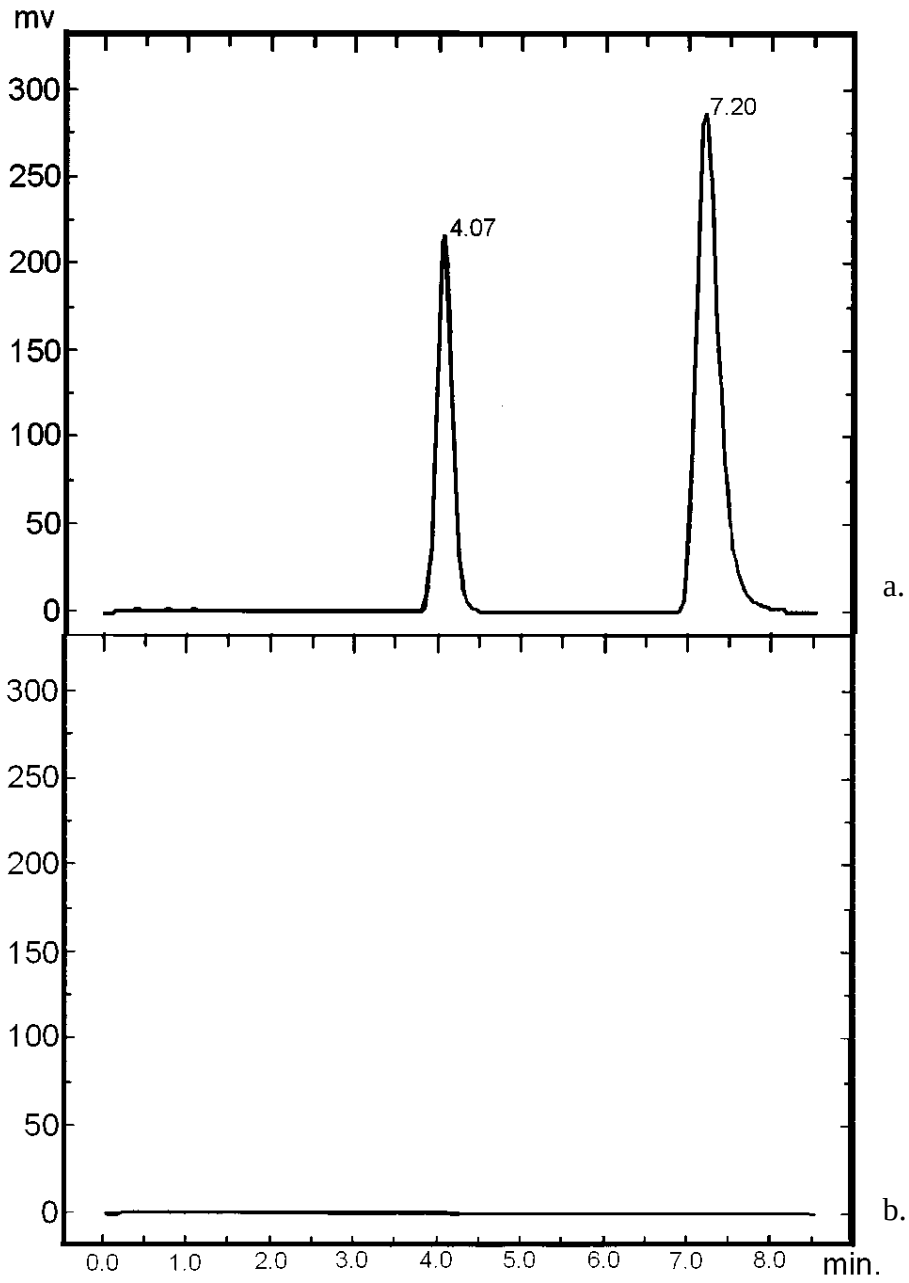


Figure 2. a. Chromatogram of nicotinic acid, eluting at 4.07 min and niacinamide, eluting at 7.20min. with photolysis unit on. b. chromatogram of nicotinic acid and niacinamide with photolysis unit off.

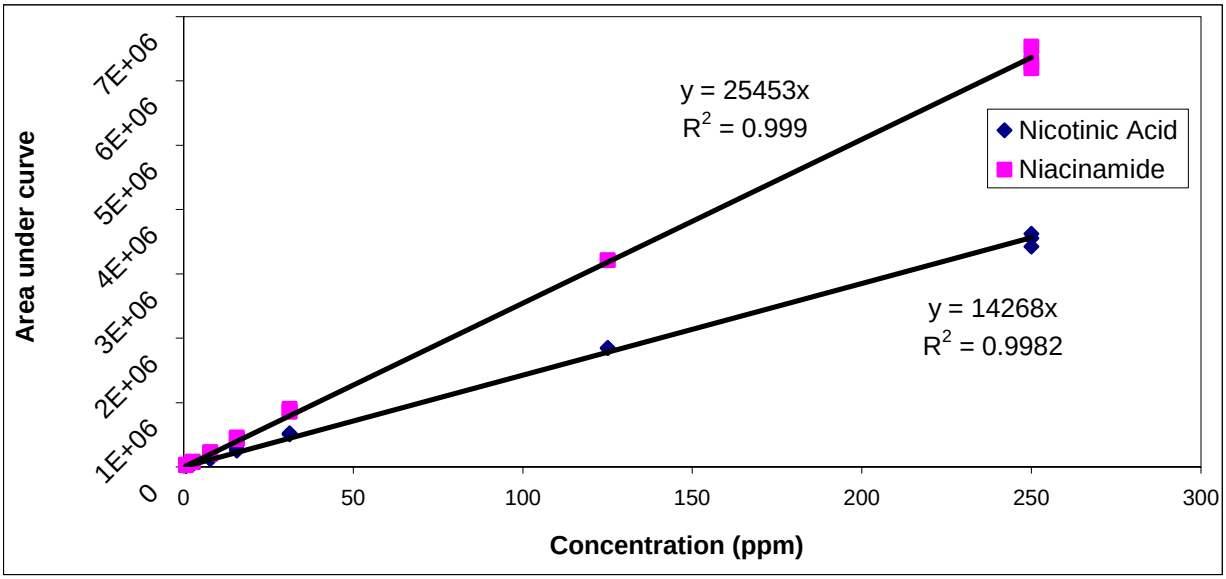
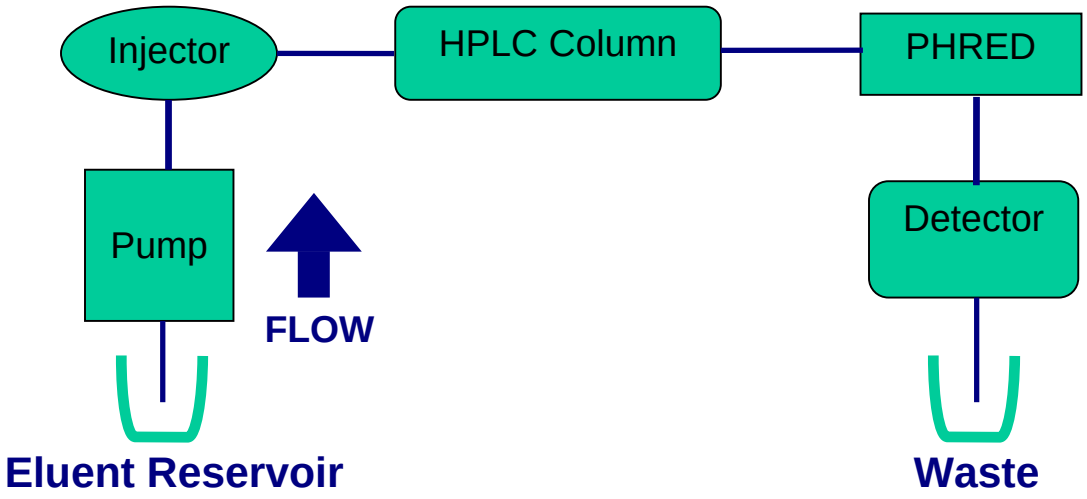


Figure 3. Calibration curve of nicotinic acid and niacinamide with a concentration range from 3ppm to 250 ppm. Linear regression lines and R2 values are displayed on the graph.

Schematic of Post-Column Photochemical Derivatization Set-Up



References:

(1) Lahély, S., Bergaentzlé, M., Hasselmann, C., 1999. Fluorimetric determination of niacin in foods by high-performance liquid chromatography with post-column derivatization. FOOD CHEMISTRY, 65,129-133.

(2) European Committee for Standardization. Foodstuffs – determination of niacin by HPLC. EN 15652:2009, 2009.

(3) Mawatari, K., Iinuma, F., Watanabe, M., 1991. Determination of Nicotinic Acid and Nicotinamide in Human Serum by High-Performance Liquid Chromatography with Postcolumn Ultraviolet=Irradiation and Fluorescence Detection, Analytical Science, 7,733-736.