

## Simultaneous Analysis of Vitamins B1, B2, B3 and B6 in Protein Powders and Supplements

B vitamins are a group of water soluble vitamins that play an important role in cell metabolism. This group consists of a number of compounds including Thiamine (Vitamin B1), Riboflavin (Vitamin B2), Niacin and Nicotinamide (Vitamin B3) and Pyridoxine and Pyridoxal (Vitamin B6). B vitamins are found in plant and animal food sources, such as legumes, nuts, green leafy vegetables, red meat and poultry. Many commercial food products are fortified with vitamin B complex and supplementation with multi-vitamins is common to fight deficiencies.

Pickering Laboratories offers a method for simultaneous determination of Vitamins B1, B2, B3 and B6 in supplements and protein powders. The method uses chemical and photochemical post-column derivatization with Fluorescence detection that increases sensitivity and selectivity of analysis. Photochemical derivatization is required for Niacin and Nicotinamide and chemical derivatization is needed for Thiamine. Vitamins B2 and B6 have natural fluorescence.

### Method

#### Calibration

##### Calibration Ranges:

Thiamine: 0.03 – 10 ug/mL; Niacin: 0.125-10 ug/mL;  
Nicotinamide: 0.3 – 100 ug/mL; Riboflavin: 0.03-10 ug/mL;  
Pyridoxal: 0.125-10 ug/mL, Pyridoxine: 0.125-10 ug/mL.

##### To Make Riboflavin Stock Solution:

Dissolve 20 mg of Riboflavin in 5 mL of 0.1 M NaOH and immediately add 50 mL of 0.1 M HCl and make up the solution with DI water to 500 mL. Make working standards by diluting the stock solution with 0.01 M HCl. Store protected from light.

Stock and working standards for Thiamine, Niacin, Nicotinamide, Pyridoxal and Pyridoxine are made in 0.01 M HCl and stored protected from light.

#### Instrument set up

##### Connect the Instruments in the Following Order:

Onyx PCX post-column derivatization instrument  
– UVE™ photochemical reactor – Fluorescence detector.

#### Sample Preparation

##### For Protein Powders:

To 0.5 g of samples add 50 mL of extraction buffer (0.1 N NaOH adjusted to pH 2 with Phosphoric acid). Homogenize using hand held homogenizer for 30 sec and heat on a water bath at 100 °C for 30 min. Cool the solution down, filter through 0.45 um nylon filter and inject. Protect from light.

##### For Multi-Vitamins Supplements Tablets:

Blend at least 10 tablets to a fine powder and mix the entire sample thoroughly. Weigh 250 mg of sample and add 90 mL of DI water acidified to pH 2.6 with 0.1 N HCl. Stir using magnetic stirring plate for 2 hours, protecting from light. Make the volume up to 100 mL with acidified water. Filter the sample through 0.45 um nylon filter and inject.

#### Analytical Conditions

**Analytical Column:** Thermo Hypersil, Aquasil C<sub>18</sub> (4.6x150 mm)

**Column Temperature:** 40 °C

**Flow Rate:** 1 mL/min

##### Mobile Phase:

**Solvent A:** Dissolve 4.77 g of Potassium Phosphate Monobasic in 900 mL of DI water, adjust pH to 5.9 with KOH. Bring volume to 1 L with DI water.

**Solvent B:** Acetonitrile. See Table 1 for gradient conditions.

**Injection Volume:** 20 uL

#### Post-Column Conditions

**Post-Column Derivatization System:** Onyx PCX, Pinnacle PCX

**Reactor Volume:** 0.5 mL

**Reactor Temperature:** 30 °C

**Reagent:** Dissolve 10 g of Sodium Hydroxide in 500 mL of DI water, add 2 g of Sodium Sulfite, mix till fully dissolved

**Reagent Flow Rate:** Initial flow rate 0 mL/min. See Table 2 for pump program

**Detection:** FLD, see Table 3 for detector program  
UVE™ Photochemical Reactor

Table 1. HPLC Gradient		
Time	Solvent A %	Solvent B %
0	100	0
8	100	0
15	90	10
22	90	10
30	40	60
35	40	60
35.1	100	0
45	100	0

Table 3. FLD Program		
Time	Excitation (nm)	Emission (nm)
0	322	400
17.9	322	400
18	370	440
25	370	440
25.1	350	470
35	350	470

Table 2. Post-Column Pump Program	
Time	Flow Rate (mL/min)
0	0
16.9	0
17	0.5
23	0.5
23.1	0
35	0

Table 4. Analysis of NIST Samples						
Compounds	Found in Protein Powder	RSD n=3	NIST Value for Protein Powder	Found in Vitamins Tablets	RSD n=3	NIST Value for Vitamins Tablets
<b>Nicotinamide</b>	282 ug/g	4.5%	258 ug/g	15288 ug/g	0.8%	13907 ug/g
<b>Thiamine</b>	12.6 ug/g	9.0%	15.8 ug/g	1145 ug/g	0.6%	1045 ug/g
<b>Riboflavin</b>	25.9 ug/g	11.2%	26.9 ug/g	1204 ug/g	1.8%	1302 ug/g

Run time: 35 min  
Equilibration time: 10 min

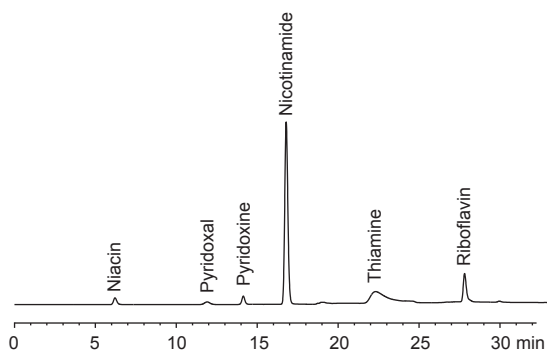


Fig 1. Chromatogram of mixed B Vitamins standard solution. Niacin, Riboflavin, Pyridoxine, Pyridoxal, Thiamine – 1 ug/mL; Nicotinamide – 10 ug/mL

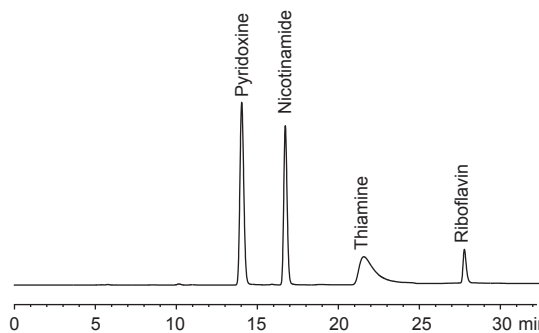


Fig 3. Chromatogram of soy protein shake powder sample

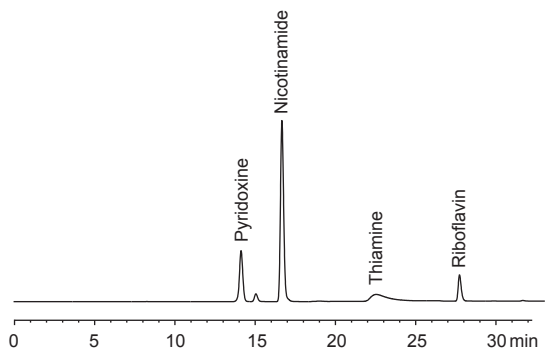


Fig 2. Chromatogram of NIST Multivitamin tablets sample

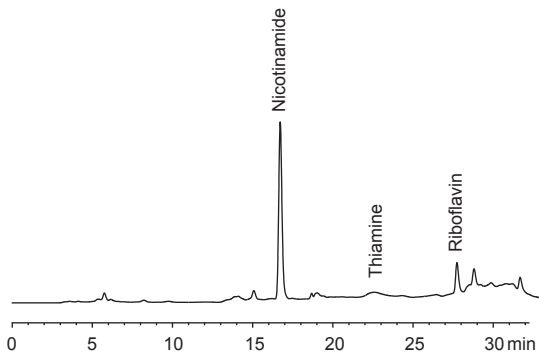


Fig 4. Chromatogram of NIST chocolate protein drink mix sample

