Vitamin Analyzer

Vitamins are unstable compounds, which are easily oxidized and destroyed during sample preparation procedures. For that reason, a simple assay method that reduces degradation of vitamins needs to be established in order to produce accurate results.

Typically, vitamins have been analyzed by spectrophotometric and microbiological procedures, which involve lengthy processes due to complicated sample preparation procedures, and often result in poor reproducibility.

Recently, HPLC has been used for analyses of vitamins in order to overcome these problems, and produce accurate and reproducible results.

YL Vitamin Analyzer is dedicated to analyze water-soluble and fat-soluble vitamins.

• Useful Information

- **Water-soluble vitamin**
  Vitamin C (Ascorbic acid), B1 (Thiamine), B2 (Rivoflavin), B3 (Niacin), B6 (Pyridoxine), B12 (Cyanocobalamin), Folic acid, Pantothenic acid

- **Fat-soluble vitamin**
  Vitamin A (Retinol), D, E, K (K1, K2, K3)

Sample preparation procedures and the analysis of Vitamins

(1) Sample preparation procedure of Vitamins

- **Sample preparation procedure for water-soluble vitamins**

Sample 100g
↓
Add 100 ml of purified water and homogenize the sample for 10 min
↓
Adjust the pH to 4.5 with phosphoric acid
↓
Add 250 mg of bromelain or protease, and 2.0 g of α-amylase
↓
Incubate the sample at 45 °C for 3 hrs
↓
Drop the temperature to ambient room temperature
↓
Precipitate the protein by adding 10 ml of 20 % metaphosphoric acid
↓
Centrifuge at 10,000 rpm for 30 min at 5 °C
↓
Repeat the protein precipitation procedure three times, and bring the sample solution to 100 ml by adding purified water.
↓
Filter the sample through a C18 Sep-pak column which has already been cleansed with 5.0 ml of methanol
↓
Collect the solution passed through the column with 7.0 ml of 1 % phosphoric acid and 10.0 ml of 0.1 N NaOH, and 3.0 ml of a methanol:purified water (1:1) solution
↓
Collect all solutions and bring the sample solution to 100 ml
↓
Filter the sample with a 0.25 um membrane filter
↓
Inject 10 µl of sample to HPLC

- **Sample preparation procedure for fat-soluble vitamins**

Sample 10g
↓
To the sample, add 75 ml of 70 % Ethanol , 25 ml of 17 N NaOH and 0.5 g of ascorbic acid
↓
Incubate the sample overnight in a dark location (about 8~16 hrs) after fudging with nitrogen gas
↓
Extract the sample with n-Hexane
↓
Rinse the sample with water until it reaches pH 7.0 (check the solutions by adding pp)
↓
Extract the sample with acetonitrile after passing the sample through a Sep-pak (alumina, silica, C18) column
↓
Adjust the final volume of the sample to 10 ml
↓
Inject 10 µl of sample to HPLC

- **Recommended Columns**
  - Water-soluble vitamins : Aqueous C8 (4.6 mm, 150 mm)
  - Fat-soluble vitamins : C8 (4.6 mm, 150 mm)

• Application

- Vitamins in powdered milk
- Vitamins in beverage
- Vitamins in natural foods
- Vitamins in a medicine promoting nutrition

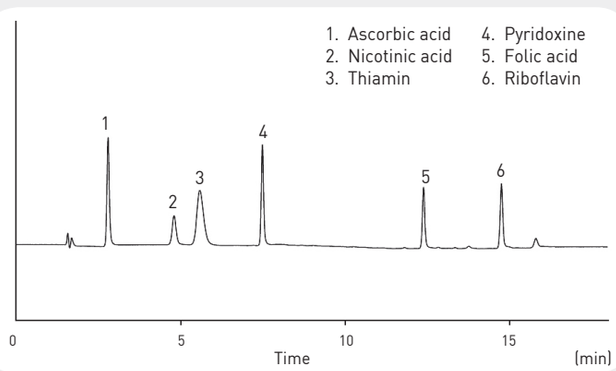
■ Water Soluble Vitamins

• Mobile phase : A- Ultrapure water (10 mM NaH2PO4 + 10 mM Na2HPO4 + H3PO4 0.1 %)
B- MeOH

• Gradient program

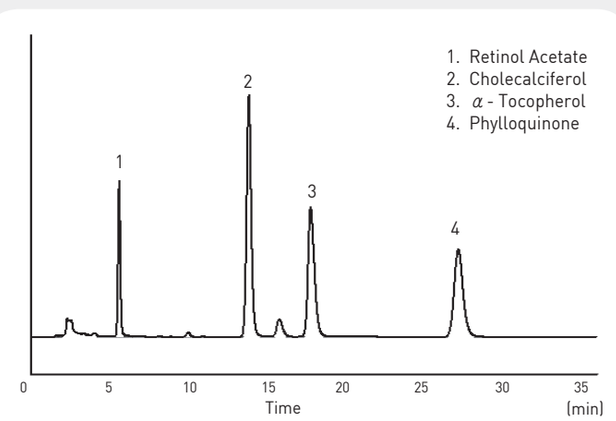
Time (min)	Flow Rate (ml/min)	Mobile Phase	
		A (%)	B (%)
0	1.0	100	0
2	1.0	100	0
15	1.0	40	60
16	1.0	40	60

• Column oven : 35 °C
• Detector : UV/Vis 280 nm
• Column : Atlantis dC18 (4.6 mm, 150 mm, 5 µm)
• Injection volume : 20 µl sample loop



■ Fat Soluble Vitamins

• Mobile phase : ACN:MeOH = 75:25
• Flow rate : 1.0 ml/min
• Column oven : 35 °C
• Detector : UV/Vis 280 nm
• Column : C18 (4.6 mm, 250 mm, 5 µm)
• Injection volume : 20 µl sample loop



■ Fat Soluble Vitamins E (α - Tocopherol, Tocopherol Acetate)

• Mobile phase : MeOH 49 % : Water 1 %
• Flow rate : 1 ml
• Column oven : 25 °C
• Detector : UV 284 nm
• Column : Xbridge C18 (4.6 mm, 150 mm, 5 µm)