Primaide

HITACHI   Primaide
1310 Column Oven

■ Simultaneous Analysis of Water-Soluble Vitamins

Vitamins are essential nutrients, and are classified into water-soluble vitamins and fat soluble vitamins. We have simultaneously analyzed 9 water-soluble vitamin components by separation through a reverse phase column and diode array detector (DAD). By using DAD, detected peaks can be identified from absorption spectra. Thus, food and other samples containing many contaminants can be analyzed effectively. Please take care to note that since vitamin C and erythorbic acid are unstable, they are easily decomposed during sample preparation or with the passage of time, and that it is difficult to obtain linearity and reproducibility. Therefore, these analysis conditions are suitable for qualitative analysis. For quantitative analysis, the use of an individual test method for each vitamin is recommended.

■ Standard Samples and Structural Formulas

Component
1. Vitamin B_1 (thiamine) *
2. Vitamin B_6 (pyridoxine) *
3. Nicotinamide
4. Vitamin B_{12} (cyanocobalamin)
5. Ascorbic glucoside
6. Vitamin C (ascorbic acid)
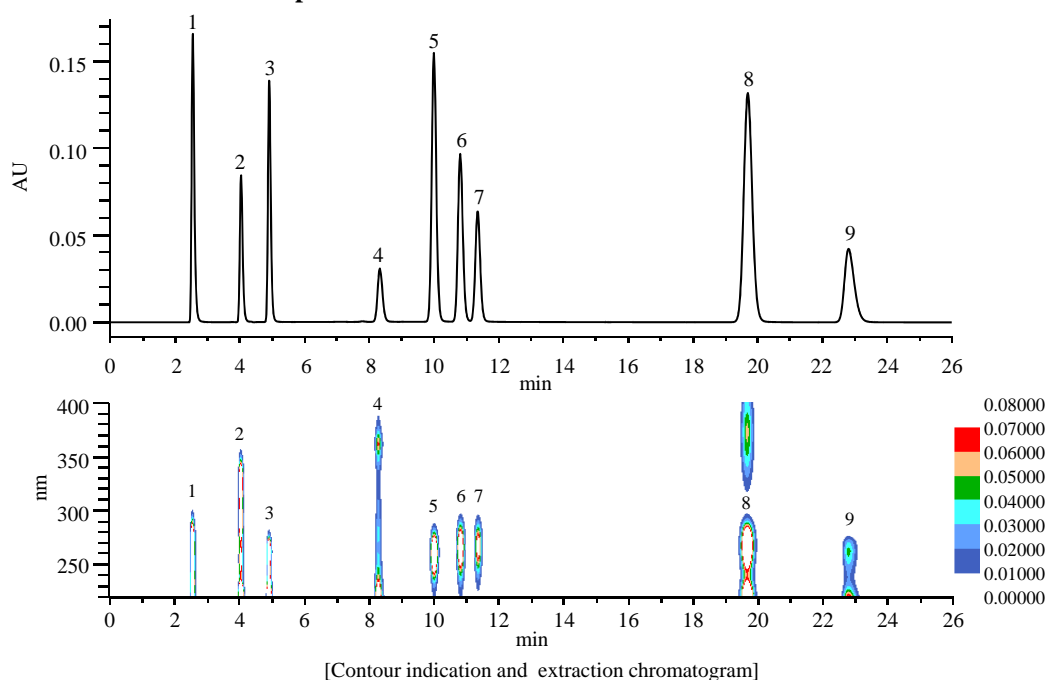7. Erythorbic acid
8. Vitamin B_2 (riboflavin)
9. Nicotine acid

(*) hydrochloride salt used.
Concentration of 50 mg/L for each.
Standard stock / standard solution diluted using eluent.

Vitamin B_1
Vitamin C
Vitamin B_2
Vitamin B_{12}
Ascorbic glucoside
Vitamin B_6
Nicotine amide
Nicotine acid
Erythorbic acid
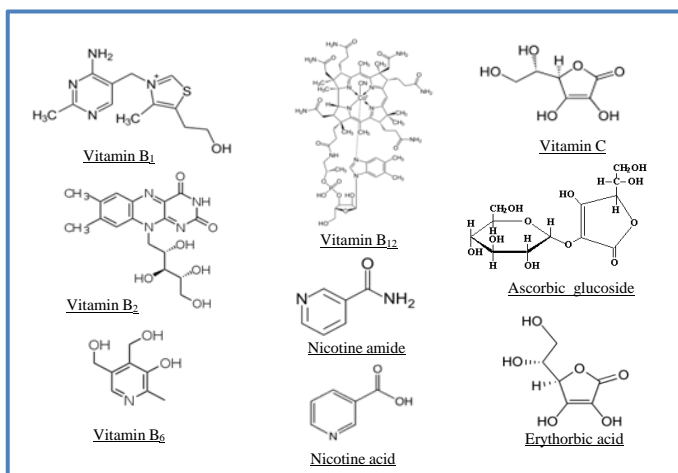
■ Analysis result of standard samples

[Contour indication and extraction chromatogram]

[Analytical conditions]
Column   : HITACHI LaChrom C18 (5 μm)
                 4.6 mm I.D. × 250 mm
Eluent   : Phosphate buffer, pH 5.2*/ CH_3CN = 90 / 10 (v/v)
                 *consists of 10 mM tetrabutylammonium hydroxide
                  and 10 mM KH_2PO_4, adjusted the pH using H_2PO_4
Flow rate   : 0.8 mL/min
Column temp. : 40 ℃
Detection   : DAD 260 nm
Injection vol. : 10 μL

This analysis uses an ion-pair reagent (tetrabutylammonium) as eluent.
As it is difficult to remove the ion-pair reagent completely from the column, use of a column that is exclusive for water-soluble vitamins is recommended.

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■ Linearity

2. VB$_6$

$r^2 = 1.0000$
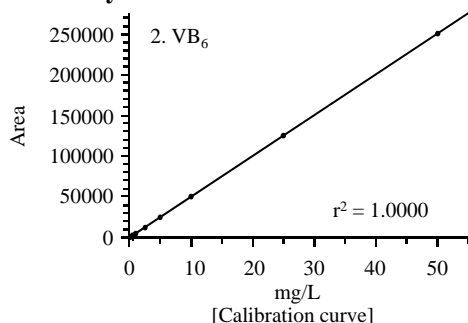
[Calibration curve]

All of the calibration curves (all components in the range from 0.1 to 50 mg/L) exhibited high linearity, with $r^2 = 0.996$ or more.
However, Vitamin C, Eruthoribic acid, and Vitamin B$_{12}$ are unstable when diluted by this eluent.
It is important to use fresh sample for obtaining good linearity.
Please note this procedure should be used mainly for qualitative purposes.

■ Analysis example of samples
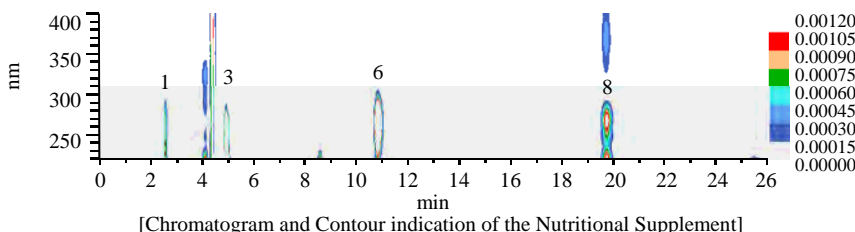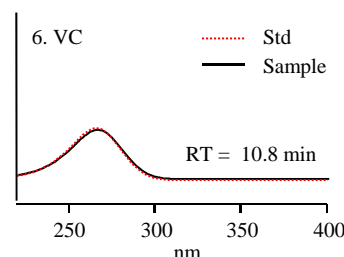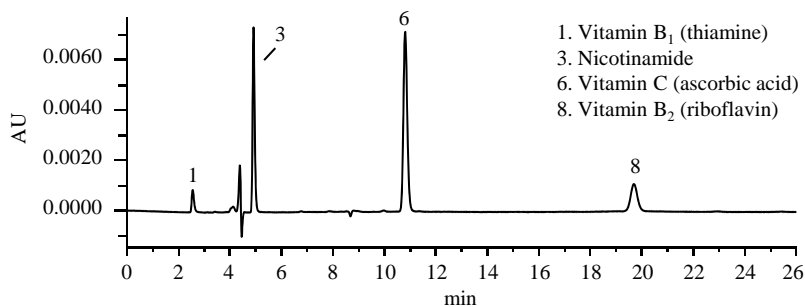
Health Drink Sample

1. Vitamin B$_1$ (thiamine)
2. Vitamin B$_6$ (pyridoxine)
3. Nicotinamide
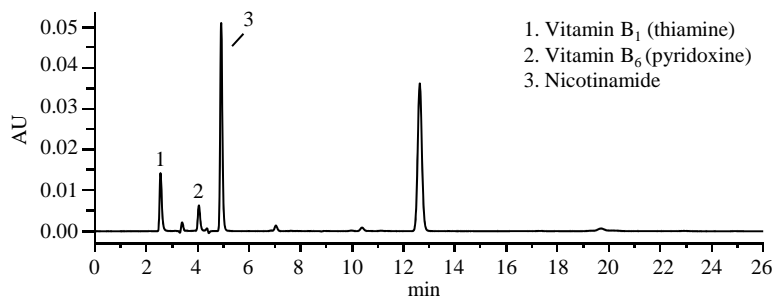
By using DAD, detected peaks can be identified from comparing the absorption spectrum with the spectrum of the standard.

3. Nicotinamide   ┈┈┈┈ Std   ──── Sample

RT = 4.9 min
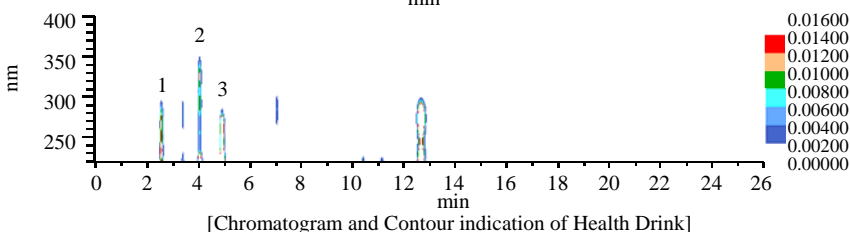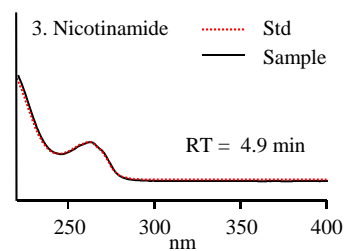
[Preparation of the Health Drink]
The sample was diluted 10-fold with eluent and filtered through a 0.45 µm filter.

[Chromatogram and Contour indication of Health Drink]

Nutritional Supplement Sample

1. Vitamin B$_1$ (thiamine)
3. Nicotinamide
6. Vitamin C (ascorbic acid)
8. Vitamin B$_2$ (riboflavin)

6. VC   ┈┈┈┈ Std   ──── Sample

RT = 10.8 min

[Preparation of the Nutritional Supplement]
A sample of 2 mg was weighed, dissolved in eluent to a volume of 10 mL, and filtered through a 0.45 µm filter.

[Chromatogram and Contour indication of the Nutritional Supplement]

System configuration : Primaide 1110 Pump, 1210 Auto Sampler, 1310 Column Oven, 1430 DAD

NOTE : These data are an example of measurement; the individual values cannot be guaranteed.
The system is for research use only, and is not intended for any animal or human therapeutic or diagnostic use.