■ 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 detection (DAD). By using DAD, detected peaks can be identified from absorption spectra. Thus, food and other samples containing many contaminants can be analyzed particularly 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.

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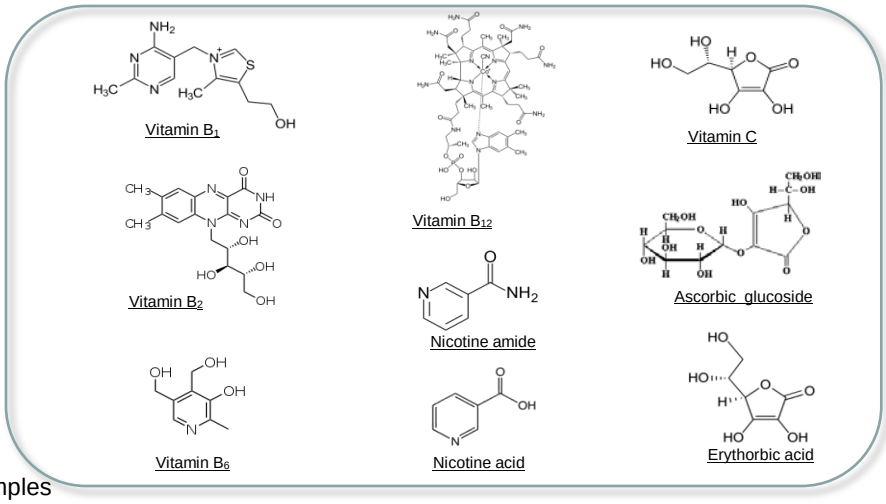
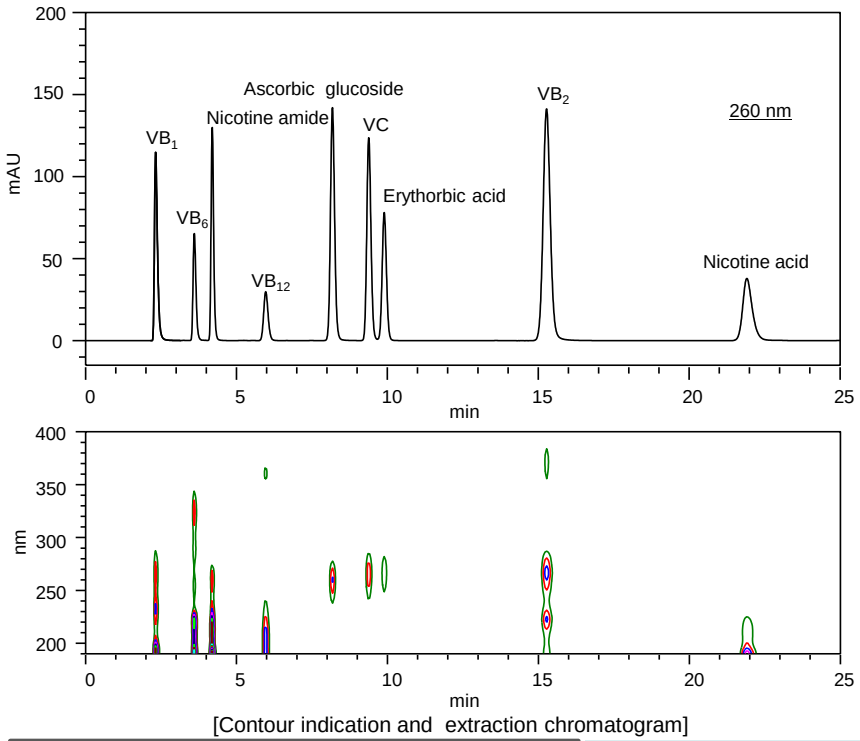
■ Standard Samples
Concentrations and Structural Formulas

Component
Vitamin B_1 (thiamine) *
Vitamin B_6 (pyridoxine) *
Nicotinamide
Vitamin B_{12} (cyanocobalamin)
Ascorbic glucoside
Vitamin C (ascorbic acid)
Erythorbic acid
Vitamin B_2 (riboflavin)
Nicotine acid

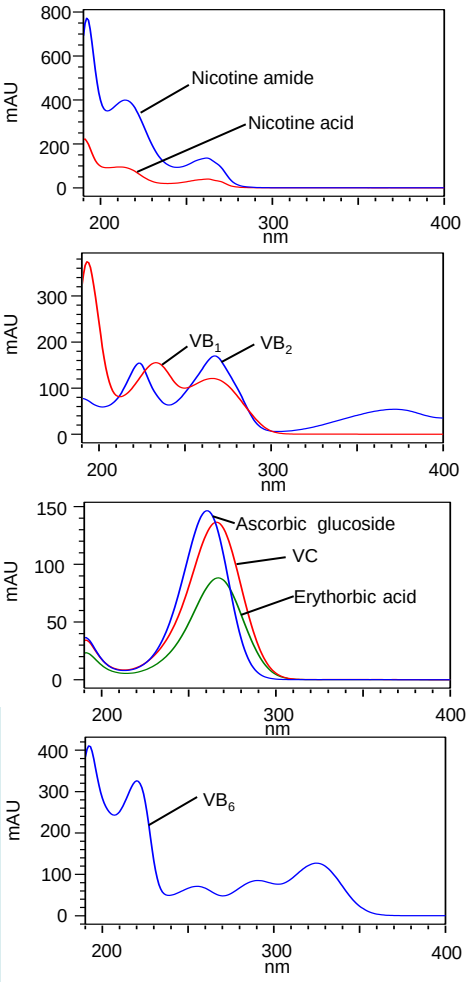
(*) hydrochloride salt used.
Concentration of 50 mg/L for each.
Standard stock/standard solution diluted using elute.



■ Measurement examples for standard samples



[Contour indication and extraction chromatogram]



[Water-Soluble Vitamin Spectra]

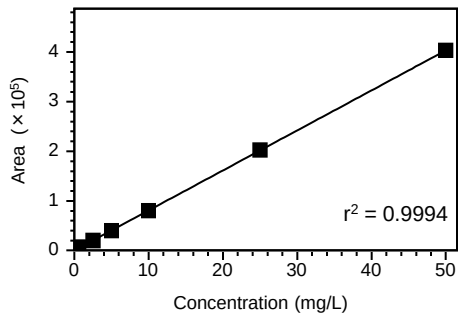
[Analysis conditions]
Elute:Phosphate buffer（pH 5.2）/ CH_3CN = 90 / 10 (v/v)
(containing tetrabutylammonium)
Flow rate: 0.8 mL/min
Injection vol.: 10 µL
Column: HITACHI LaChrom C18-PM (5 µm)
(4.6 mmI.D.× 250 mm)
Column temperature: 40ºC
Detection: DAD 260 nm

This analysis uses an ion-pair reagent (tetrabutylammonium) as elute.
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.

[Devices] Chromaster
5110 pump, 5210 automatic Sampler, 5310 Column Oven, 5430 Diode Array Detector, Data Processing System

■ Simultaneous Analysis of Water-Soluble Vitamins

■ Linearity

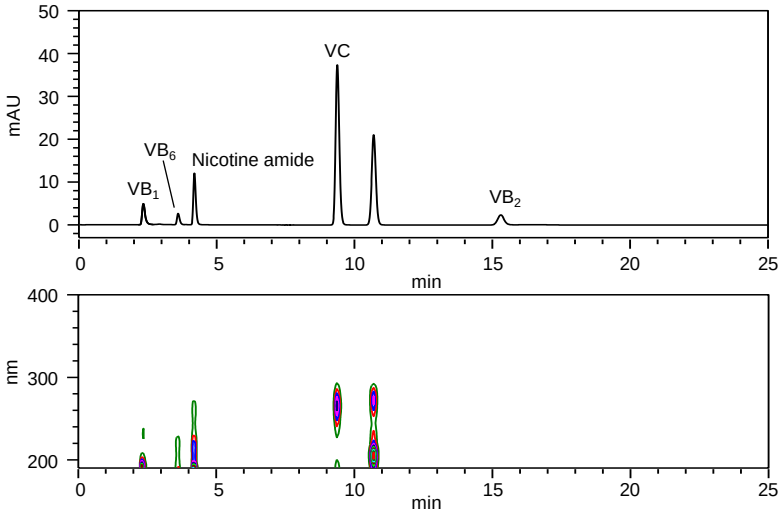


[VB_6 Calibration curve]

All of the calibration curves (all components in the range from 0.1 to 50 mg/L) exhibited high linearity, with r^2= 0.995 or more. However, regarding Vitamin C, Erythorbic acid, and Vitamin B_{12}, take care to note that it is rather difficult to obtain good linearity.

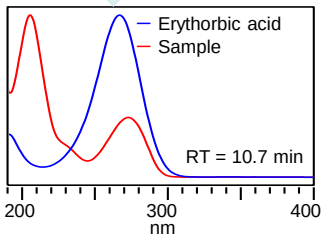
The peaks of both the Nutritional Supplement and Health Drink obtained at 10.7 min tended to be identified as Erythorbic acid, which has a similar retention time. However, it was found the they were completely different substances via spectral confirmation.
Thus, DAD, by which the spectral information was obtained, is effective for food and other samples containing many contaminants.

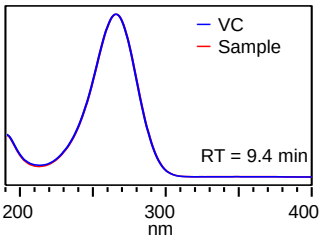
■ Analysis Example (1) : Nutritional Supplement Sample



[Chromatogram of the Nutritional Supplement]

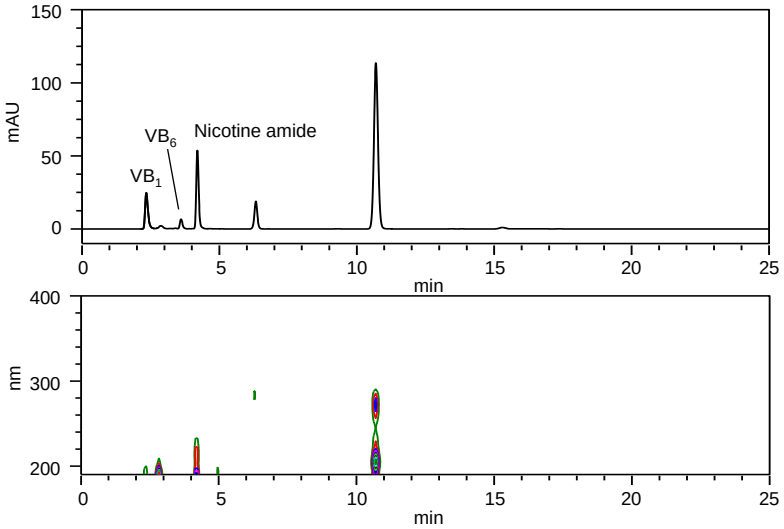
[Pretreatment of the Nutritional Supplement]
A sample of weight 2 mg was weighed out, dissolved in elute to a volume of 10 mL, and filtered through a 0.45 µm filter.





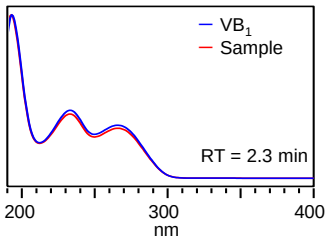
[Peaks and Spectra obtained for the standard sample]

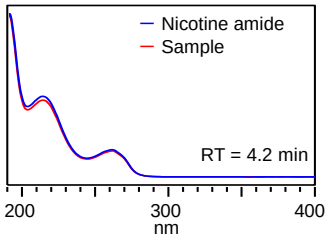
■ Analysis Example (2): Health Drink Sample



[Chromatogram and Contour indication of Health Drink]

[Pretreatment of the Health Drink]
The sample was diluted 10-fold with elute and filtered through a 0.45 µm filter.





[Peaks and Spectra obtained for the standard sample]

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.