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 B₁ (thiamine) *
2. Vitamin B₂ (pyridoxine) *
3. Nicotinamide
4. Vitamin B₆ (cyanocobalamin)
5. Ascorbic glucoside
6. Vitamin C (ascorbic acid)
7. Erythorbic acid
8. Vitamin B₂ (riboflavin)
9. Nicotinic acid

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

Analysis result of standard samples

[Contour indication and extraction chromatogram]

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

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, Eruthorubic 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

By using DAD, detected peaks can be identified from comparing the absorption spectrum with the spectrum of the standard.

1. Vitamin B$_1$ (thiamine)
2. Vitamin B$_6$ (pyridoxine)
3. Nicotinamide

#### Nutritional Supplement Sample

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

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

[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.

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.