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 B₁ (thiamine) *
  - Vitamin B₂ (pyridoxine) *
  - Nicotinamide
  - Vitamin B₁₂ (cyanocobalamin)
  - Ascorbic glucoside
  - Vitamin C (ascorbic acid)
  - Erythorbic acid
  - Vitamin B₆ (riboflavin)
  - Nicotinic acid

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

- Measurement examples for standard samples

[Analysis conditions]
- Elute:Phosphate buffer (pH 5.2) / CH₃CN = 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 mm I.D. x 250 mm)
- Column temperature: 40°C
- 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.
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**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 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.**

**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.

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