Simultaneous Analysis of Ten Water-Soluble Vitamins Using a Polymer-Based Reversed Phase Column - Shodex™ RSpak DE-413L

Introduction

Vitamins are micronutrients essential for the metabolism of living organisms. Since humans cannot produce vitamins, the intake of vitamins must come from the outside, food, etc. There are many commercial foods and drinks supplemented with vitamins for the nutrient enhancement purposes. Most processed foods contain daily recommended values of multiple vitamins.

Methods using microbiological assays, absorption spectrophotometry, and HPLC have been used to analyze vitamins, creating a time-consuming process. A typical HPLC method to separate and quantify vitamins can use an ODS column with an addition of ion-pair reagent. However, the ion-pair reagent tends to remain on the column and the flow-lines, resulting in increased background level and lowers the sensitivity.

Therefore, in this application, a simple method to simultaneously analyze various water-soluble vitamins was developed. Shodex™ RSpak DE-413L, a polymer-based reversed phase column, was used without a use of ion-pair reagent. Fig.1 shows the structures of ten water-soluble vitamins analyzed in the study. We further applied the developed method to quantify vitamins in a commercial multi-vitamin supplement.

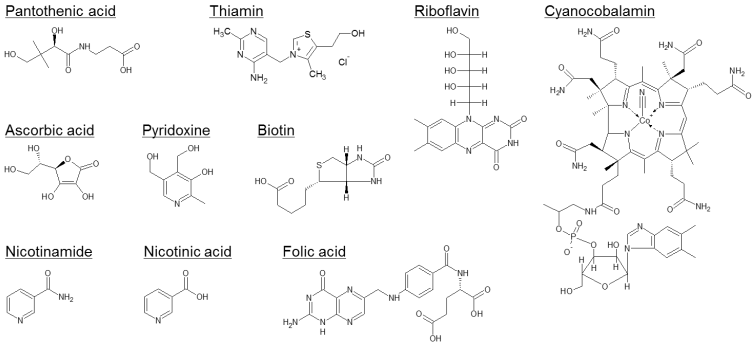

Fig.1 Ten water-soluble vitamins analyzed in the study

Experimental

1. Preparation of Standard Solutions

Ten vitamins (thiamin hydrochloride, pyridoxine hydrochloride, nicotinamide, ascorbic acid, nicotinic acid, calcium pantothenate, cyanocobalamin, folic acid, riboflavin, and biotin) were used as standards. 4-mM standard solution for biotin and 2-mM standard solutions for other vitamins. 250-mM phosphoric acid was used to dissolve most standards. Since folic acid, riboflavin, and biotin do not dissolve well in acidic solvents, they were first dissolved in a small amount of 10% ammonium water, and then diluted with 10-mM phosphoric acid to prepare the standard solution.

Five levels of multi-vitamin calibration standards were prepared using appropriate amounts of the original standard solutions and 250-mM phosphoric acid. We used 250-mM phosphoric acid to prevent the oxidation of ascorbic acid. However, with 250-mM phosphoric acid, precipitation of folic acid was observed after a few hours of preparation. 20-uM was set as the maximum concentration as it does not cause precipitation during folic acid calibrator.

2. Sample Preparation

One tablet of a commercial multi-vitamin supplement (210 mg) was dissolved in 5 mL of 250-mM phosphoric acid by ultra-sonication. This solution was used as the original sample solution.

Since concentrations of each vitamin in the supplement are different, x 200 diluted sample solution was prepared in addition to the original sample solution. A portion of the original sample solution was taken

immediately after mixing (while the solution is cloudy), diluted x 200 times with 250-mM phosphoric acid, and sonicated. The original samples and the x 200 diluted samples were centrifuged at 10,000 rpm for 5 minutes to let the insoluble substances settle. The supernatants were filtered with 0.5 um filter membranes and kept for measurement.

Folic acid required different sample preparation method. One supplement tablet was ground with a mortar and pestle and dissolved 10 mg of the powder in 50 µL of 10% ammonium water by ultra-sonication. Next, the sample was diluted with 250-mM phosphoric acid with 10-mg/mL ascorbic acid to make the total volume 5 mL. The ascorbic acid was added to prevent the oxidation of folic acid.[1] The dissolved solution was then sonicated, centrifuged, and filtered with 0.5 um filter membrane as for other test samples and kept for the measurement.

3. HPLC Settings

Column: Shodex™ RSpak DE-413L (4.6 mm I.D. x 250 mm, 4 µm)
Eluent: (A) 10 mM H_3PO_4 aq. / (B) CH_3CN
Linear gradient (high pressure); (B%) 0% (0 min) → 30% (5-10 min) → 0% (10.1-20 min)
Flow rate: 1.0 mL/min
Detector: PDA (190-400 nm)
Column temp.: 50 ºC
Injection vol.: 5 µL

Results and Discussion

1. Chromatograms and the Calibration Graphs of the Standards

Fig. 2 shows the UV chromatograms of the standards. Peaks of the ten vitamins were fully resolved using the developed method. Fig. 3 shows the calibration graphs of each vitamin. The UV absorbance was measured at 254 nm. However, since the absorbance of pantothenic acid and biotin at 254 nm were low, 210 nm was used for the measurement. The concentration range of each vitamin in the calibrators are indicated below their names in Fig. 3. The correlation coefficient of all ten vitamins were R^2>0.999, provided good linearity.

This simple method using phosphoric acid and acetonitrile as the eluents demonstrated a successful simulates analysis of ten water-soluble vitamins in 20 minutes, including the column equilibration time.

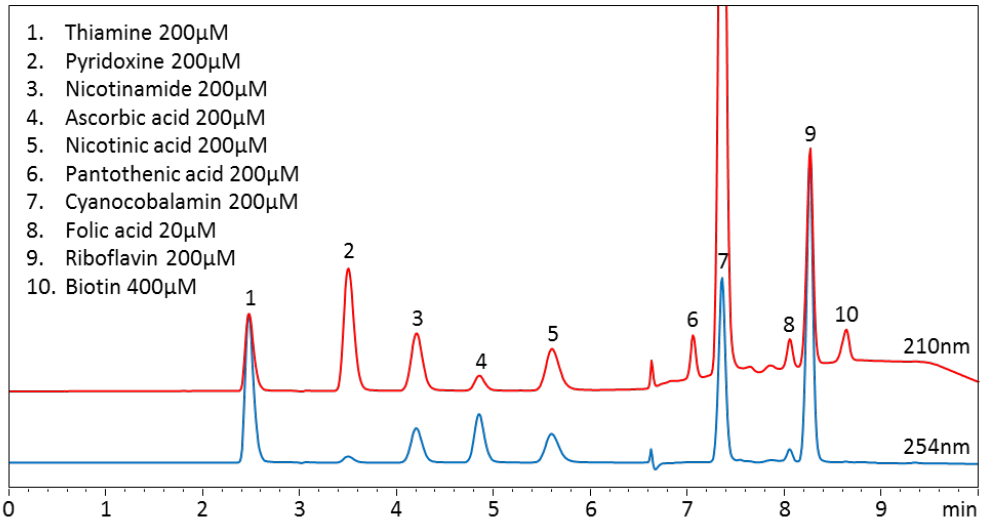

Fig 2. Sample UV chromatograms showing a simultaneous analysis of ten water-soluble vitamins

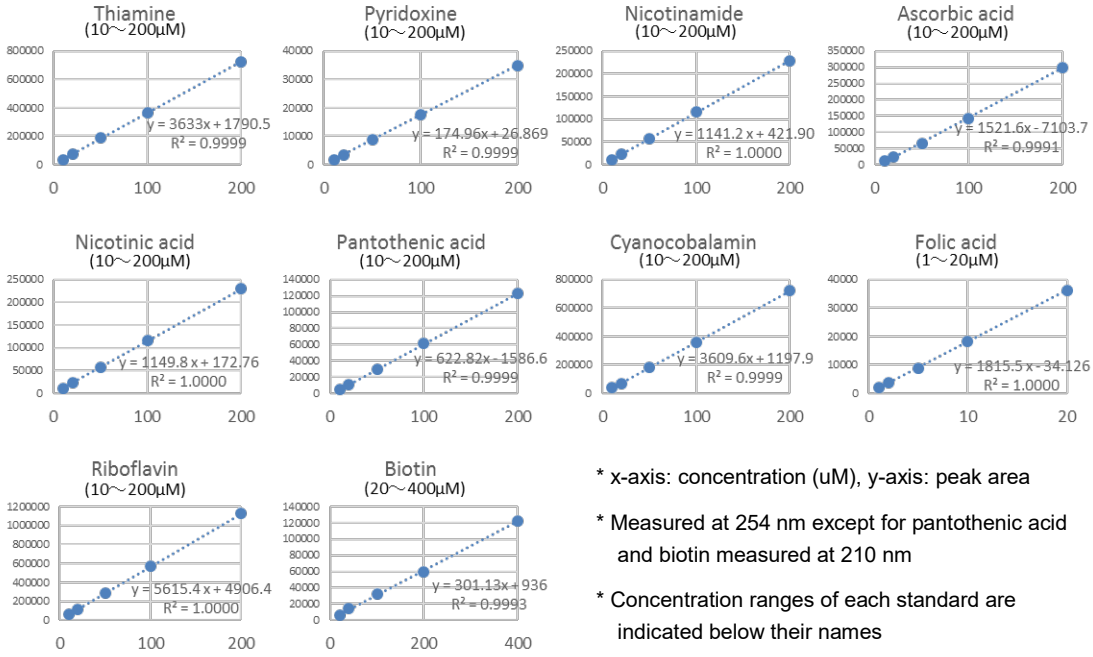

Fig 3. Calibration graphs of ten water-soluble vitamins

2. Quantification of water-soluble vitamins in a commercial multi-vitamin supplement

As an application, we analyzed the extract of a commercial multi-vitamin supplement. We used a guard column (Shodex™ RSpak DE-G 4A (4.6 mm I.D. x 10 mm, 10 μm)) during the sample analysis. Fig.4 shows the UV chromatograms from the analysis.

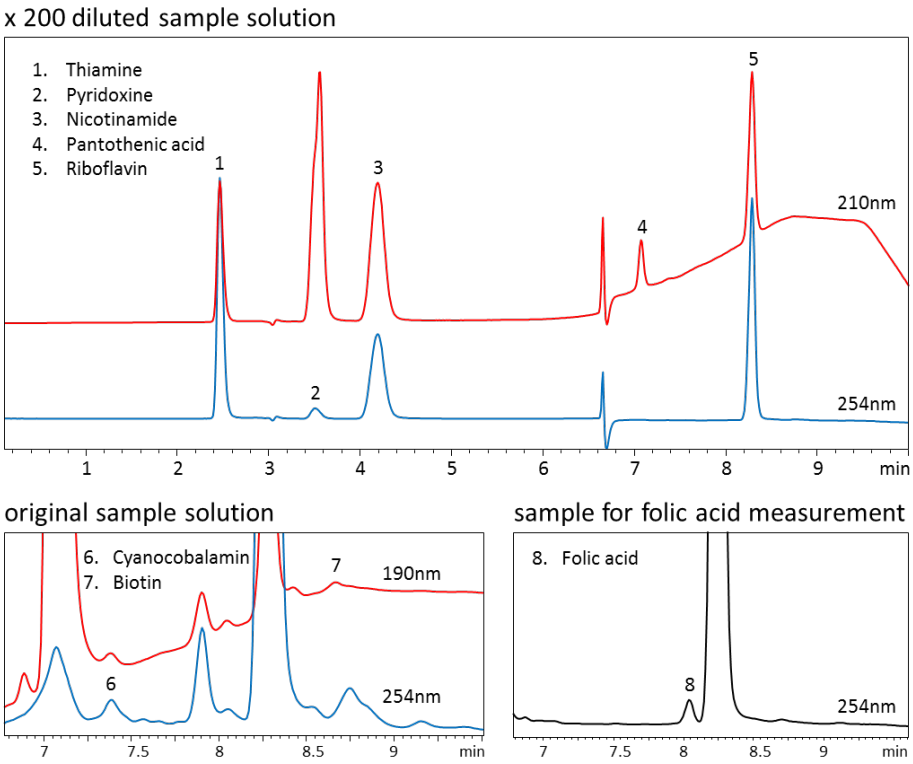

Fig. 4 UV chromatograms of the extract from a commercial multi-vitamin supplement

Table 1 summarizes the amount of water-soluble vitamins measured and the amount reported by the manufacturer. An overlap from another component was observed on the biotin peak monitored at 210 nm. The biotin peak measured at 190 nm had less influence from the other component – shown as a single component peak, but there was still a small portion of overlapping observed at the back part of the biotin peak. Thus, peak height was used instead of peak area for its quantification. The linearity of the calibration graph using the peak height was acceptable (correlation coefficient R^2= 0.9972).

Table 1. Summary of water-soluble vitamins in the supplement measured and from the manufacturer's report

	Thiamine	Pyridoxine	Nicotinamide	Pantothenic acid
Measured (mg/100 mg)	11	7.2	9.2	13
Manufacturer's Report (mg/100 mg)	10	7.5	10	10
	Cyanocobalamin	Folic acid	Riboflavin	Biotin
Measured (mg/100 mg)	0.0064	0.073	6.9	0.011
Manufacturer's Report (mg/100 mg)	0.005	0.05	7.5	0.0125

*Calculated based on the peak area observed at 254 nm, except for pantothenic acid observed at 210 nm, and for biotin, calculated using the peak height observed at 190 nm.

Conclusions

A method for a simultaneous analysis of ten water-soluble vitamins was developed using the Shodex™ RSpak DE-413L column. The eluents used was a simple mixture of an acid and acetonitrile. One sample measurement completes in 20 minutes. Good linearity was obtained for all ten vitamins calibration curves and the method was applicable for the quantification of water-soluble vitamins in a commercial multi-vitamin supplement.

The Shodex™ RSpak DE series column provides a stable analysis even under a highly aqueous eluent condition, without having a concern about the column deterioration. This is owing to the use of polymer-based materials compared to using silica-based material.

Reference

1) Xue Song Lianget al., Research on stability of synthetic folic acid, Advanced Materials Research Vol. 781-784 (2013) 1215-1218

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