

Analytical methods for vitamins include microbiological assay and high-performance liquid chromatography (HPLC). Vitamin B12 analysis in foods is usually performed by microbiological assay because it is present at trace levels of a few micrograms /100g, and an HPLC method is not sufficiently sensitive and may also be affected by contaminants. However, microbiological assays require culture procedures and takes longer to obtain quantitative results than HPLC assays.

Here we discuss the analysis of vitamin B12 in powdered milk using the HPLC method with reference to the AOAC analysis method*. Large-volume injections using the HPLC method resolved the sensitivity-related problems, and the method was further refined by size-exclusion chromatography (SEC) and introduction onto the analysis column using a heart-cut method, thereby reducing the impact of contamination and large-volume injections on peak shape.

*Reference

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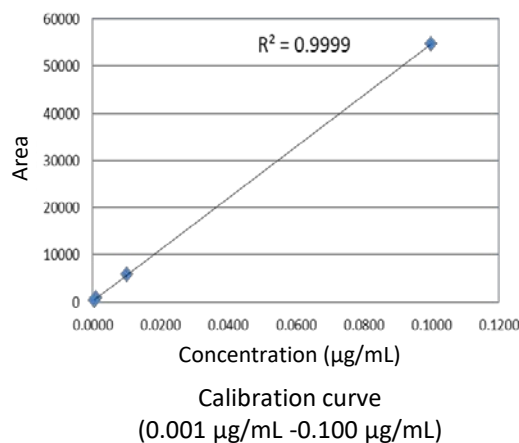
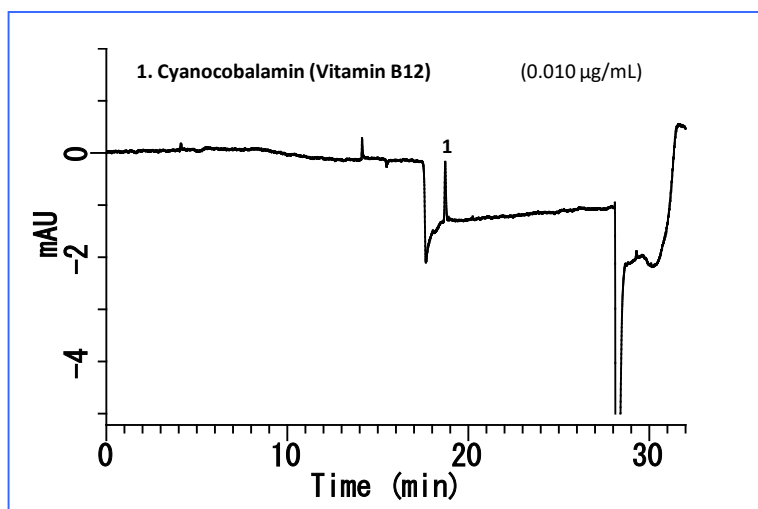
Determination of Vitamin B12 in Infant Formula and Adult Nutritionals by HPLC: First Action 2011.10

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(K. Kanno)

Example of Vitamin B12 Reference Standard Analysis

After a large volume of sample is injected onto an SEC column for purification, a selection valve is then switched using the timing of the elution of cyanocobalamin, and injected onto an ODS column using a heart-cut method. The use of an SEC column maintains peak shape and provides increased sensitivity even for large 2 mL injection volumes.



HPLC conditions

Columns

Analytical column :Inertsil ODS-4 (5 µm, 150 x 4.6 mm I.D.)

Pretreatment column :Inertsil Diol (5 µm, 250 x 7.6 mm I.D.)

Temperature :40 °C

Detector :VIS 550 nm

Injection volume :2.0 mL

Flow rate

Main column :1.0 mL/min

Pretreatment column :1.0 mL/min

• Example of valve switching timing

0-7 minutes position 0

7-11 minute position 1

Position 0 for 11-32 minutes

*The valve switching timing must be adjusted for each pretreatment column.

Mobile phase

Pretreatment column :2.5 v% acetonitrile in water

Analytical column :A) Triethylamine-formic acid buffer/acetonitrile = 100/0, v/v

B) Triethylamine-formic acid buffer/acetonitrile = 75/25, v/v

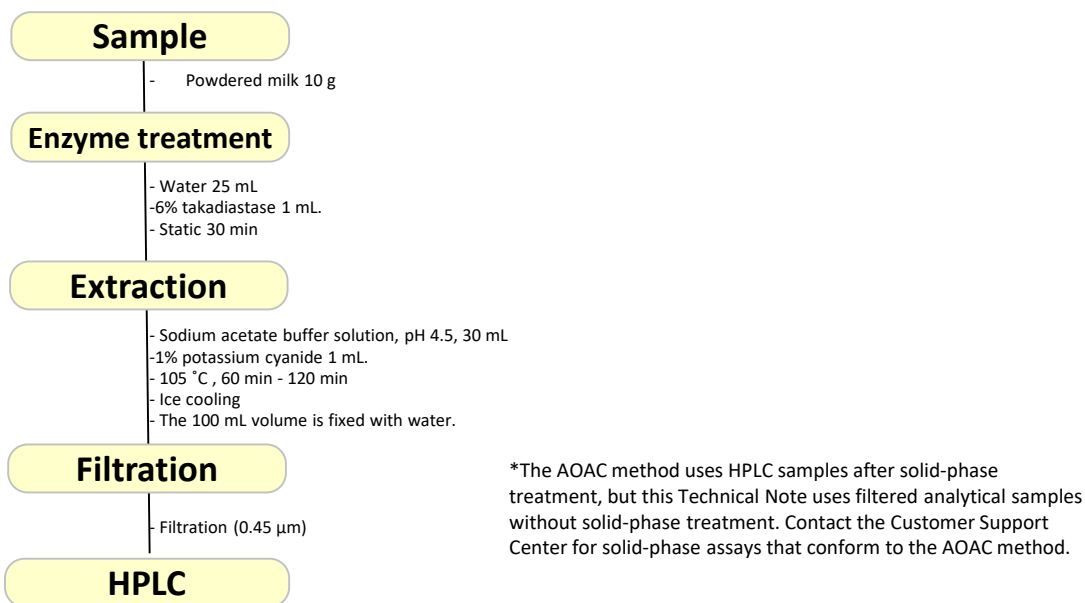
C) Triethylamine-formic acid buffer/acetonitrile = 25/75, v/v

Triethylamine-formic acid buffer:

4.0 mL of triethylamine was added to 1.0 L of water and adjusted to pH 5.0 with formic acid.

Time	A%	B%	C%
0.0	90	10	0
14.5	90	10	0
14.6	41	59	0
26.0	41	59	0
26.1	0	10	90
28.0	0	10	90
28.1	90	10	0
32.0	90	10	0

Example of vitamin B12 pretreatment in feeds

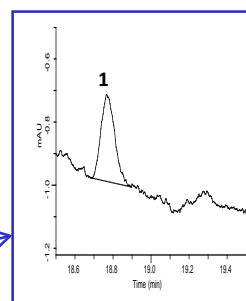
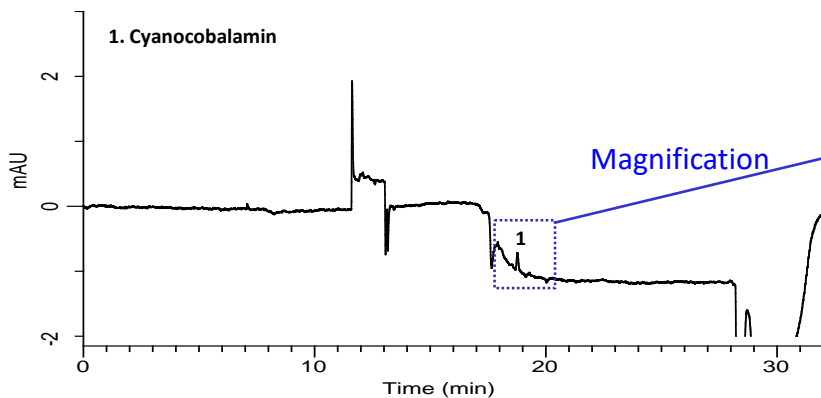


Example of real sample analysis

Commercial powdered milk containing 1.5 µg of vitamin B12 per 100 g were processed and analyzed in the aforementioned pretreatment flow.

Vitamin B12 in powdered milk

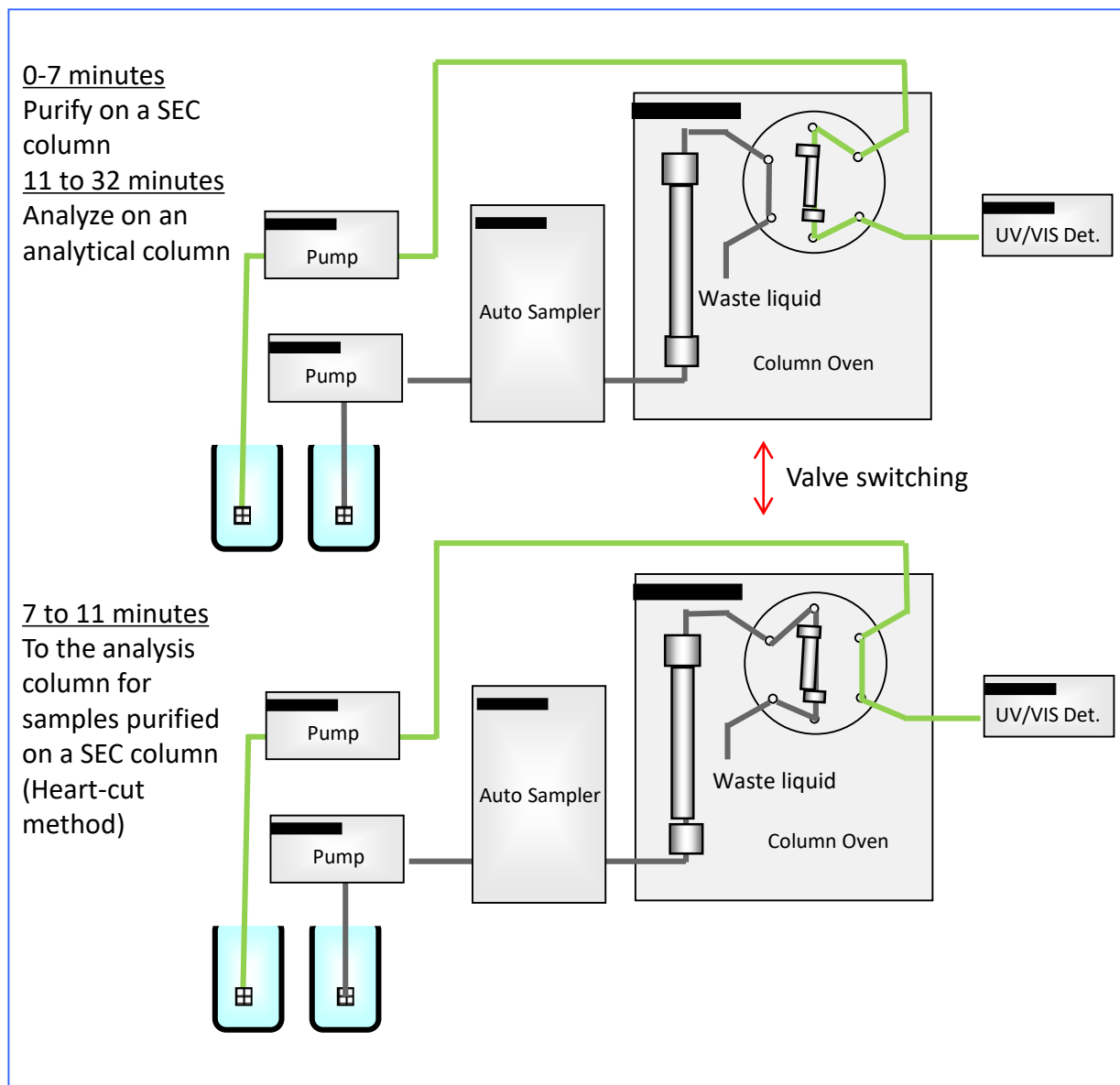
Processed sample volume: 10 g



Quantitative result:
 0.00152 µg/mL
 Constant volume of 100 mL
 10 g formula
 1.52 µg per 100 g
 formula

Flow diagram

The valve is switched from 7 to 11 minutes, when the peak of vitamin B12 elutes from the SEC column, and only the interval between the elution of the target compounds are injected onto the analysis column by a heart-cut method.



SEC column

Inertsil Diol 5 μm , 250 x 7.6 mm I.D. Cat.No. 5020-05666
 Inertsil WP300 Diol 5 μm , 250 x 7.6 mm I.D. Cat.No. 5020-05988

Inertsil® Diol

Columns for water-based and organic solvent-based SEC. The exclusion limit molecular weight is about 10,000, which is suitable for the separation of compounds with molecular weights of several hundred to thousands.

Inertsil® WP300 Diol

Columns for water-based and organic solvent-based SEC. The exclusion limit is 100,000 and is suitable for the separation of compounds with molecular weights of thousands to tens of thousands.

Analytical column

Inertsil ODS-4 5 μm , 150 x 4.6 mm I.D. Cat.No. 5020-03945

Cap with vial/septum

4 mL screw vial (brown) set 13-425100 sets Cat.No. 1030-54061

Filter

GL Chromatodisk Water 25A 0.45 μm , 100 pieces in Cat.No. 5040-28512

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