Vitamin B12 Analysis in Food by HPLC

Analytical methods for vitamins include microbiological assay and high-performance liquid chromatography (HPLC). Vitamin B12 analysis in food is usually performed by microbiological assay because its content correspond to just a few micrograms /100g, and a HPLC method won`t be 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.

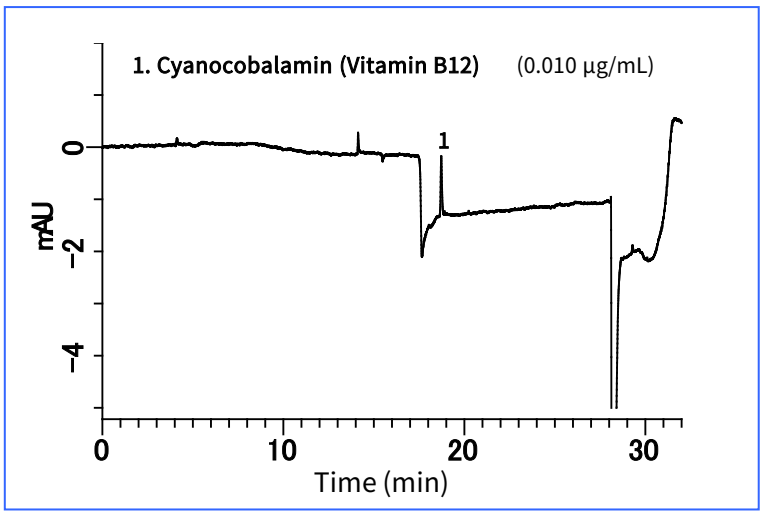
Here we discuss the analysis of vitamin B12 in powdered milk using the HPLC method with reference to the AOAC analysis method*. Large-volume injections using the HPLC method resolved the sensitivity-related problems, and the method was further refined by size-exclusion chromatography (SEC) and injection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

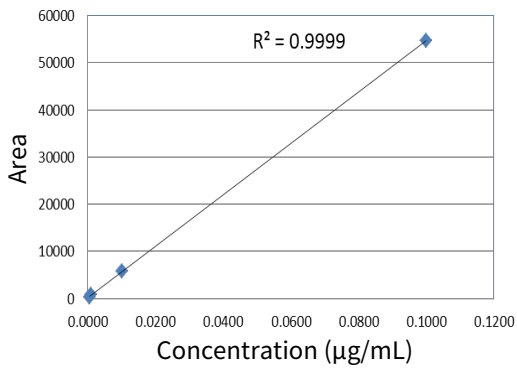
(K. Kanno)

*Reference
KAREN SCHIMPF, RENEE SPIEGEL, and LINDA THOMPSON
Determination of Vitamin B12 in Infant Formula and Adult Nutritionals by HPLC: First Action 2011.10
JOURNAL OF AOAC INTERNATIONAL VOL. 95, NO. 2, 2012

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 a SEC column maintains peak shape and provides increased sensitivity even for large 2 mL injection volumes.





Calibration curve
(0.001 µg/mL -0.100 µg/mL)

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

Time [min]	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

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.

GL Sciences

Example of vitamin B12 pretreatment in feeds

Sample
- Powdered milk 10 g

Enzyme treatment
- Water 25 mL
- 1 mL of 6 % takadiastase
- Static 30 min

Extraction
- 30 mL of Sodium acetate buffer solution, pH 4.5
- 1 mL of 1 % potassium cyanide
- 105 ˚C , 60 min - 120 min
- Ice cooling
- 100 mL volume is fixed with water.

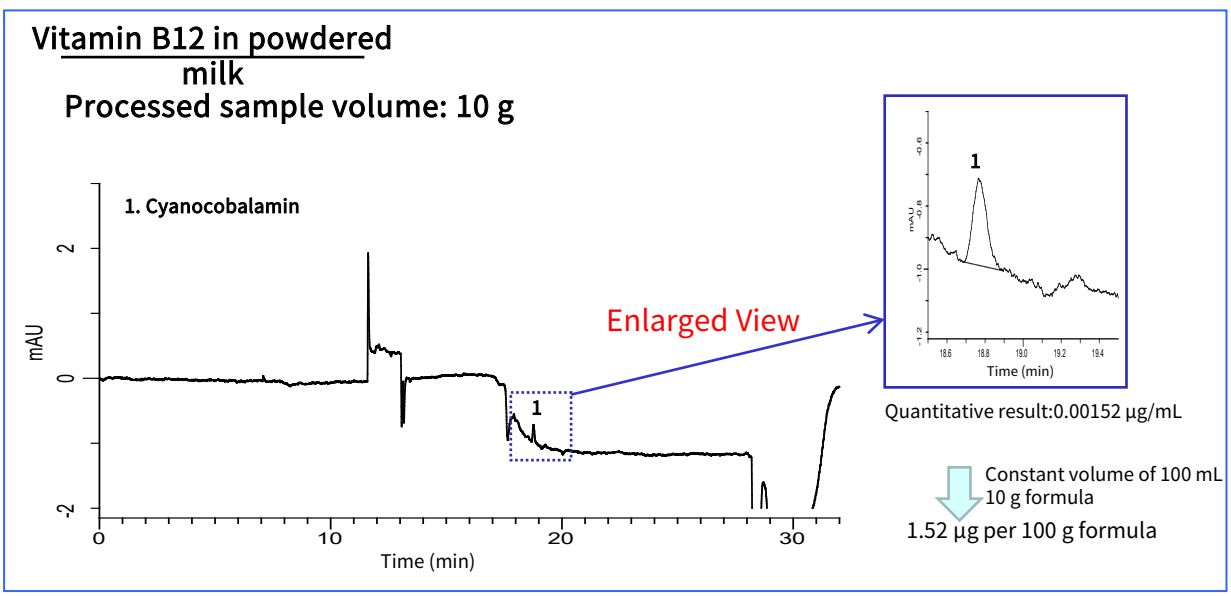
Filtration
- Filtration (0.45 µm)

HPLC

*The AOAC method uses HPLC samples after solid-phase treatment, but this Technical Note uses filtered analytical samples without solid-phase treatment. Contact GL Sciences for solid-phase assays that conform to the AOAC method.

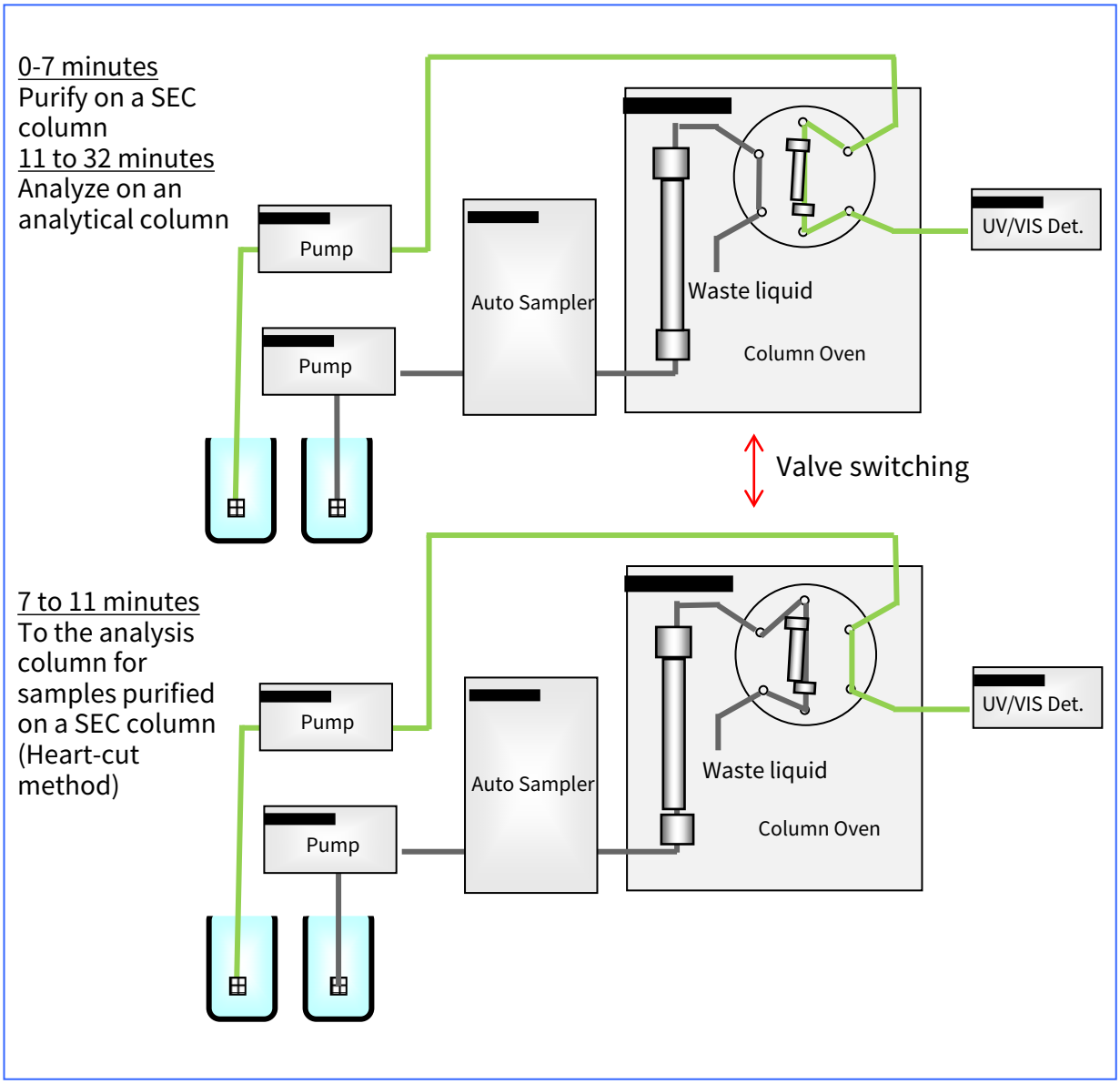
Example of real sample analysis

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



Flow diagram

When the peak of vitamin B12 elutes from the SEC column between 7 to 11 minutes, the valve is switched 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 Cat.No. 5040-28512

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