「Application Note」

The Efficient Method for Determination of Biotin (Vitamin B_7) by ChroZen HPLC with Switching Valve

- HPLC Application

Abstract

As one of the water-soluble B complex vitamins, Biotin (vitamin B_7) is an essential nutrient that works as a coenzyme of carboxylase in important metabolic pathways such as gluconeogenesis, fatty acid synthesis and amino acid catabolism. Since biotin takes a vital role in DNA synthesis, a deficiency of biotin can cause to stunted growth for infants and children and hair loss, skin problems and depression for adults. But, It is rare to be deficient in biotin because intestinal bacteria produce biotin more than the daily requirements, and many types of food contain it as well.

Because vitamins are unstable compounds that can easily be degraded by exposure of oxygen or UV light. It is required to speed up the sample preparation and use an efficient system that can ensure both accuracy and sensitivity. Saponification and Liquid-Liquid Extraction (LLE) are generally used sample preparation methods for the determination of vitamins.

ChroZen HPLC with switching valve system employs a focusing column to concentrate the target compounds and 2-stage separation to enhance the resolution. In this study, the determination of vitamin B_7 (Biotin) at trace level was conducted by ChroZen HPLC with a switching valve referring to the standard methods for the analysis of food ingredients at trace level.

Instruments and Software

Item	Description	Part No.
Pump	ChroZen HPLC Quaternary Gradient Pump with Vacuum degasser 2 set	9421011020
Autosampler	ChroZen HPLC Autosampler	5421011020
Column Compartment	ChroZen HPLC Column Oven for Analytical scale	3421011020
Detector	ChroZen HPLC UV/Vis Detector with dual wavelength	7411011020
Install. Option	HPLC Performance Kit	1601011890
CDS	YL-Clarity software of Young In Chromass HPLC for single instrument control	5301011000
	Autosampler control of YL-Clarity	5301011040
Column	Agilent ZORBAX Eclipse XDB-C8 (4.6 mm x 150 mm, 5 μm) Agilent Eclipse Plus C18 (4.6 mm x 50 mm, 5 μm) Agilent InfinityLab Poroshell EC-C18 (2.1 mm x 250 mm, 4 μm)	-
Switching Valve	6-port 2-position valve, SS/PEEK, 40MPa , 0.4, 10-32 for ChroZen HPLC	3421012020

Reagents and Standards

- Acetonitrile, HPLC Grade
- Biotin, ≥ 99.0 %
- Methanol, HPLC grade
- Phosphoric acid, 85.0 %
- PBS(Phosphate buffered saline)
- Potassium phosphate dibasic, ≥ 98.0 %
- Potassium phosphate monobasic, ≥ 99.0 %
- Ultrapure water, 18.2 MΩ-cm resistivity

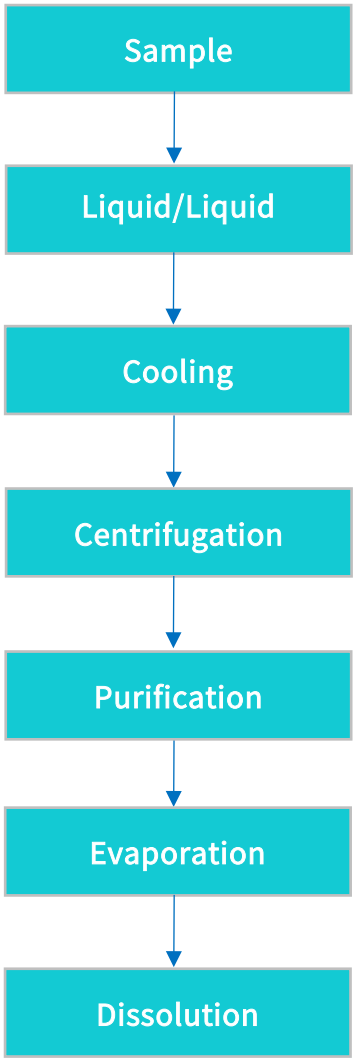
Figure 1. ChroZen HPLC with 6-port valve

Preparation of Standard Solution

① Add 100 mg of biotin to 1000 mL volumetric flask, then dilute to the 1000 mL mark with 0.01M of KH_2PO_4 (pH 4.8).

② Dilute ① solution with 0.01M of KH_2PO_4 to make each concentration for calibration curve.

Preparation of Sample Solution



Prepare 1~5 g of homogenized sample

+ 0.15M KH_2PO_4 (pH 7.0) 25 mL
→ Sonication extraction at room temperature for 5 min →
Sonication extraction in 100 °C of water bath for 30 min

Cool down the solution to room temperature
Add 0.15 M KH_2PO_4 (pH 7.0) and make the solution to 50 mL

Centrifuge at 22,000 xg for 20 min → Filtration

Remove the buffers and load 10 mL of extraction solution in immunoaffinity column → Remove impurities (PBS solution 10 mL → D.W. 10 mL)

Elute biotin combined antibody in the column using 4 mL of methanol and evaporate at 70°C with nitrogen

Add 1mL of 0.01M KH_2PO_4 (pH 4.8) in the solution and dissolve

Instrument conditions

ChroZen HPLC system		
Mobile phase / Flow rate	Pump 1 – ACN : 10mM KH_2PO_4 (0.1 % H_3PO_4) = 5 : 95, 0.3 mL/min Pump 2 – ACN : 10mM KH_2PO_4 (0.1 % H_3PO_4) = 15 : 85, 0.2 mL/min	
Column	Pre-separation : Agilent ZORBAX Eclipse XDB-C8 (4.6 mm x 150 mm, 5 µm) Focusing : Agilent ZORBAX Eclipse Plus C18 (4.6 mm x 50 mm, 5 µm) Analytical : Agilent InfinityLab Poroshell EC-C18 (2.1 mm x 250 mm, 4 µm)	
Temperature	40 °C	
Injection volume	200 µL	
Detection	UV/Vis detector 200 nm	
Valve program	Time (min)	Valve
	Initial	Position 2
	3.1	Position 1
	3.7	Position 2

Summary of Test Method

This method requires the configuration of HPLC, 6-port switching valve, an additional pump and 3 types of columns to determine trace level of biotin (Vit B_7). There are 3 steps in valve position and [Fig.2] shows how to determine the right time to switch the valve for the separation of biotin.

< Determination for valve switching time>

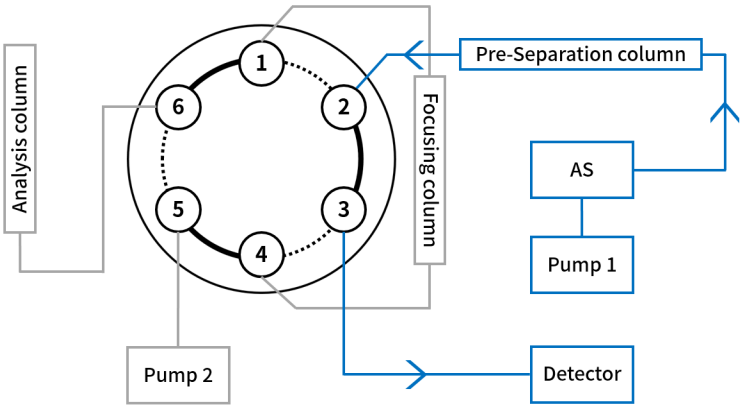


Figure 2. Valve diagram – Determination for valve switching time

The sample flows from the pre-separation column (C8 column) to the detector and the retention time of biotin will be determined to estimate the right time of valve switching.

<Step 1. First separation>

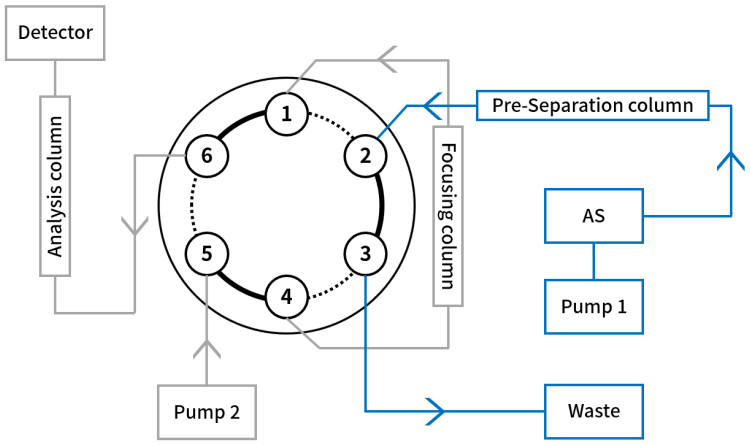


Figure 3. Valve diagram – Step 1

The sample is carried by a mobile phase from Pump 1 to the pre-separation column and will be drained to the waste before biotin is eluted while the mobile phase is flowing at 0.2 mL/min from Pump 2. In this procedure, the interference by the sample matrix can be reduced.

<Step 2. Concentration>

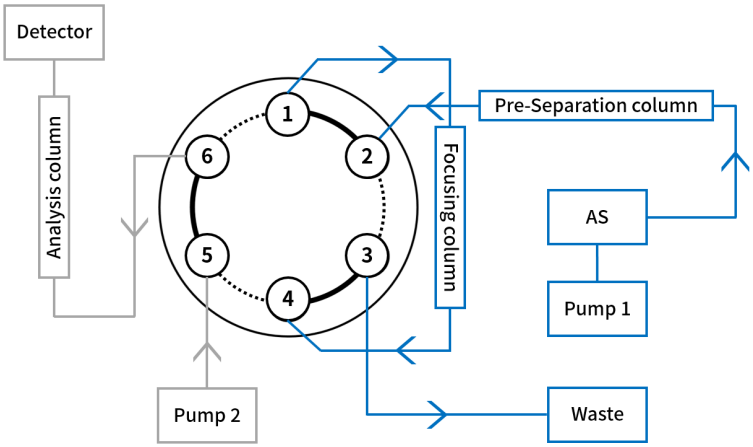


Figure 4. Valve diagram – Step 2

The valve switches the flow when biotin is eluted so the target compounds can remain in the focusing column for concentration. (Valve – Position 1)

<Step 3. Second separation>

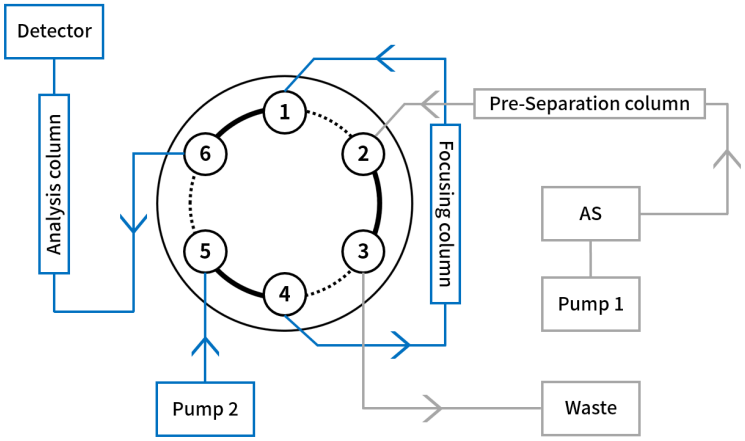


Figure 5. Valve diagram – Step 3

The valves switch the position back right before the complete elution of biotin so the target compounds remained in the focusing column flow to an analytical column to be detected by the detector. The smaller ID (Inner Diameter) of the analytical column is used for better sensitivity and purification. (Valve – Position 2)

Chromatogram

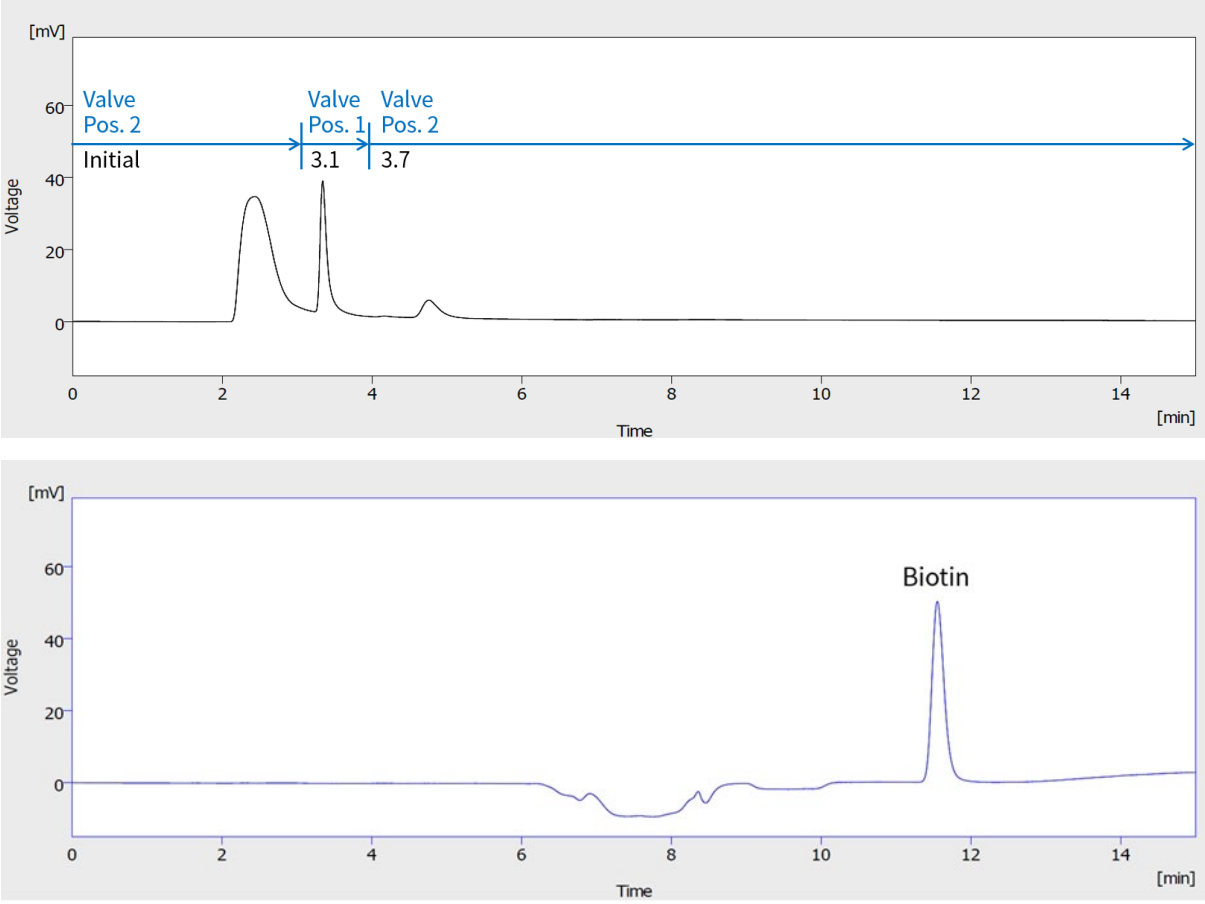


Figure 6. Chromatogram of biotin by ChroZen HPLC equipped with multiple column and switching valve system

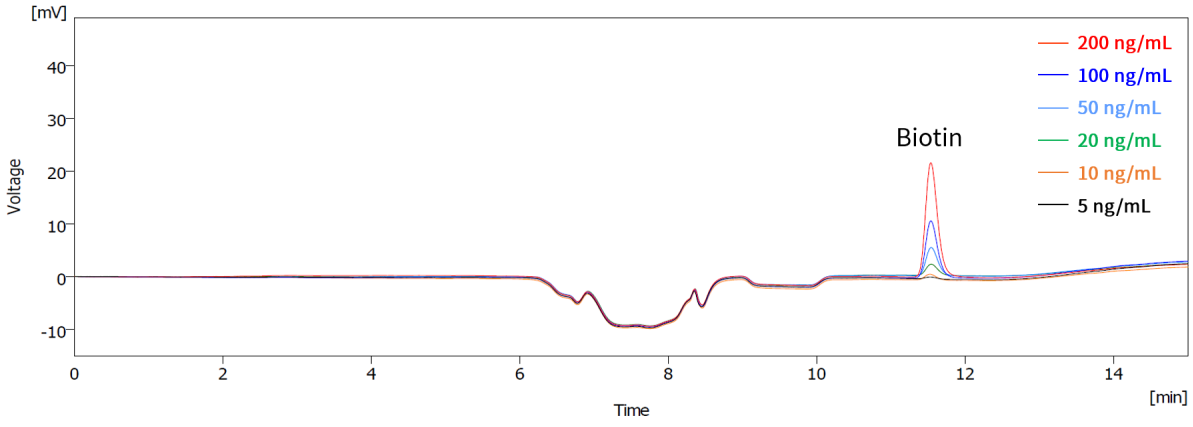
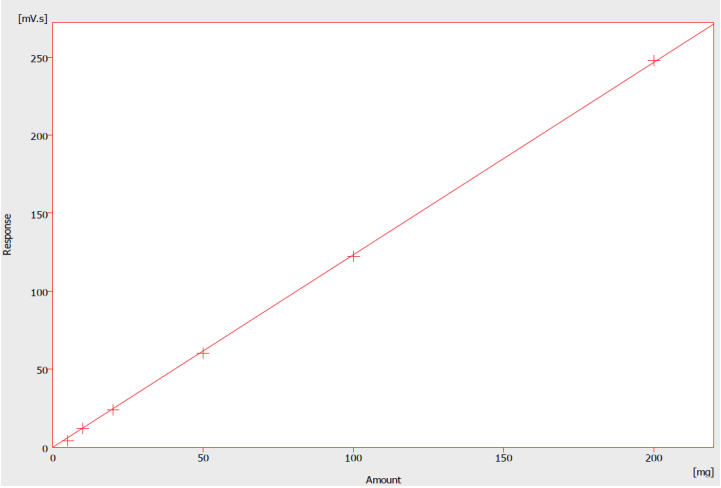


Figure 7. Overlay of biotin standards chromatogram



STD	Standard Concentration (ng/mL)	Area (mV.s)
1	5	3.9857
2	10	12.0833
3	20	23.9381
4	50	60.1531
5	100	122.4039
6	200	247.6947
Linearity		0.999954

Figure 8. Verification of calibration curve

Calculation

$$\text{Vitamin } B_7(Biotin) \text{ Content } (\mu g/100\ g) = C \times \frac{(a \times V_2 \times b)}{S \times V_1} \times 100$$

C = Concentration of Vitamin B_7 in Sample (μg/mL)

a = Total Solution Volume (mL)

V_1 = Volume of Loaded Solution in Immunoaffinity Column (mL)

V_2 = Volume of Eluted Solution from Immunoaffinity Column (mL)

b = Dilution Ratio

S = Sample Amount (g)

Conclusion

In this study, the determination of Biotin (Vit B7) was conducted by ChroZen HPLC referring to Korean standard methods for the analysis of food ingredients at trace level.

The R^2 value of greater than 0.999 was obtained in 5~200 ppb which verifies the validity of data. [Fig.8] This ensures ChroZen HPLC with switching valve is an efficient system for the analysis of Biotin at trace level.

Reference

- Korean standard methods for the analysis of food ingredients at trace level (Biotin)

- Simultaneous Determination of Vitamin D3 and K1 in Infant Formula by HPLC with column-switching
(Korean journal of food science and technology, Vol. 37, No; 6, 1024-1027)

- New nutritional concepts of some vitamins and minerals
(Korean Journal of Pediatrics, Vol. 48, No. 12)

- Adverse Effects on the skin by the deficiency of vitamins and minerals

(Korean Journal of Aesthetics and cosmetology, Vol. 10, No. 4)

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