Analysis of Functional Ingredients in Dietary Supplements

Introduction

In Japan, previously, foods that were allowed to claim their functionalities were limited to the “Food for Specified Health Uses (FOSHU)”, individual foods that are officially approved by the Japanese Ministry of Health, and the “Functional Nutritional Food”, foods that met the national standard. However, in April 2015, the nation started the “Foods with Functional Claims (FFC)” system to help general consumers obtain correct information and wisely select foods. A FFC product package clearly shows that it is a FFC product and describes its claimed functionalities. The product producers obtain the FFC by submitting the necessary documents to the Consumer Affairs Agency. The submission does not require any national examination to prove ingredient’s safety nor functionality. FFC is approved, as long as the producer states the functionality of the food based on scientific evidences and be responsible for the statement. There are more than 2,000 submitted FFC, this number is now more than the approved FOSHU. It can be said that the FFC market is keep expanding.

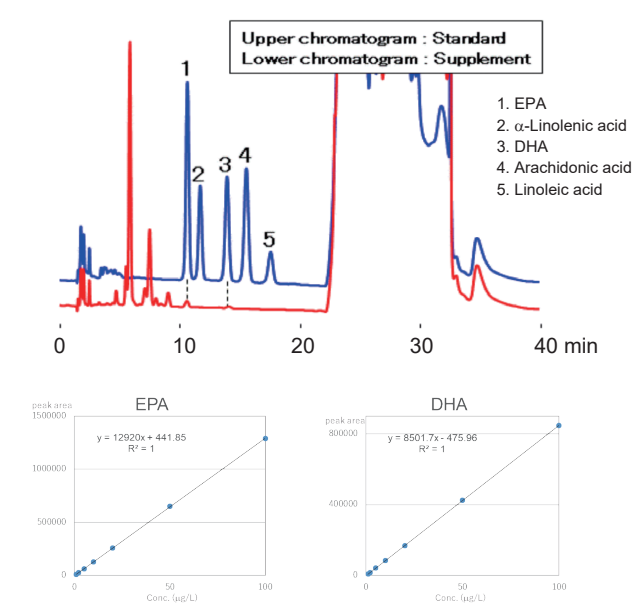
In this application, we optimized analytical conditions for the analyses of functional food ingredients that are registered as functional food ingredients in the database and which are used in commercial dietary supplement.

Experimental and Results

1. Analysis of DHA and EPA

Unsaturated fatty acids are expected to provide various positive health effects such as prevention of arteriosclerosis and thrombus or improve visual functions and allergic symptoms. Here, we analyzed unsaturated fatty acids including ω-3 fatty acids, such as docosahexanenoic acid (DHA) and eicosapentaenoic acid (EPA).

A polymer-based reversed phase column, Shodex Asahipak ODP-50 4D can separate five different unsaturated fatty acids by isocratic method. However, in addition to the unsaturated fatty acids, commercial dietary supplements contain other components. Therefore, we used step gradient method, increasing the acetonitrile concentration to 100 %, to let those components elute and separate from unsaturated fatty acids. The chromatograms obtained after 20 minutes show the baseline fluctuation caused by the step gradient (Fig. 1). Good linearlities were obtained for the calibration curves in the concentration range 1 to 100 µg/mL.



Sample : 20 µg/mL each (in ethanol), 5 µL
Column : Shodex ODP-50 4D (4.6 mm x 150 mm, 5 µm)
Eluent : (A) 0.1 % H_3PO_4 in (H_2O/CH_3CN = 35/65) (B) CH_3CN
Step gradient
0 B % (0 - 20 min)
→ 0 - 100 B % (20 - 20.01 min)
→ 100 B % (20.01 - 40 min)
→ 100 - 0 B % (30 - 30.01 min)
→ 0 B % (30.01 - 40 min)
Flow rate : 1.0 mL/min
Detector : UV (215 nm)
Column temp. : 40 °C

Fig. 1. Chromatograms of (blue) unsaturated fatty acid standards and (red) a dietary supplement

Below sample preparation method was used for the analysis of a commercial dietary supplement containing EPA and DHA.

Make a cut on a soft capsule of the dietary supplement and remove its content. The content of the supplement was viscous liquid. Measure 10 mg of the content and add 2-mL ethanol to dissolve the content. Filter the mixture using a membrane filter (0.45 µm) and use the filtrate for analysis.

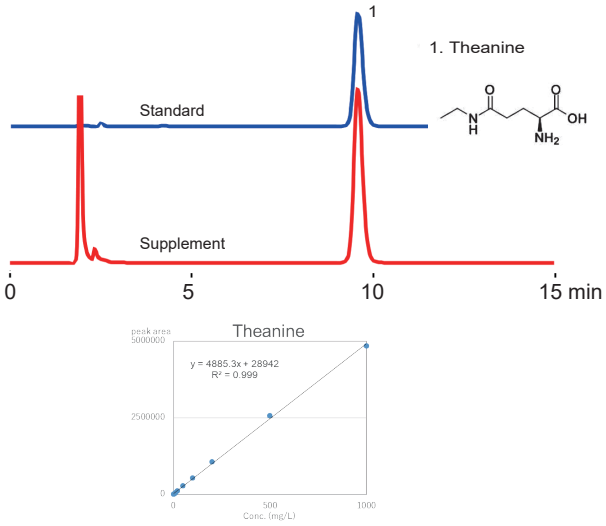
The optimized analytical condition was effective analyzing EPA and DHA with no influence from other components in the supplement. EPA and DHA quantified were 324 mg and 75 mg, respectively. The product claims to contain 350 mg and 80 mg of EPA and DHA, respectively. Based on this claim, the recovery rate of both compounds were more than 90 %, which demonstrated the method’s feasibility for quantifying those compounds.

2. Analysis of theanine

Theanine is a type of amino acid contained in tea leaves. It has anti-stress and relaxing effects.

We used a polymer-based HILIC column, Shodex Asahipak NH2P-50 4E, to analyze theanine in a commercial dietary supplement. HILIC mode is one type of LC separation mode which is created by using a column packed with high polar gels and high polar eluent to analyze hydrophilic compounds.

Theanine was analyzed under isocratic method (Fig. 2). Good linearity was obtained for the calibration curve of theanine in the concentration range 0.001 to 1 mg/mL. The recovery rate obtained against the value claimed by the supplement manufacturer was about 90 %.



Sample : 0.1 mg/mL theanine (in eluent), 20 µL
Column : Shodex Ashahipak NH2P-50 4E (4.6 mm x 250 mm, 5 µm)
Eluent : 20 mM NaH_2PO_4 + 30 mM H_3PO_4 aq. /CH_3CN = 25/75
Flow rate : 1.0 mL/min
Detector : UV (210 nm)
Column temp.: 40 ºC

Fig. 2. Chromatograms of (blue) theanine standard and (red) a dietary supplement

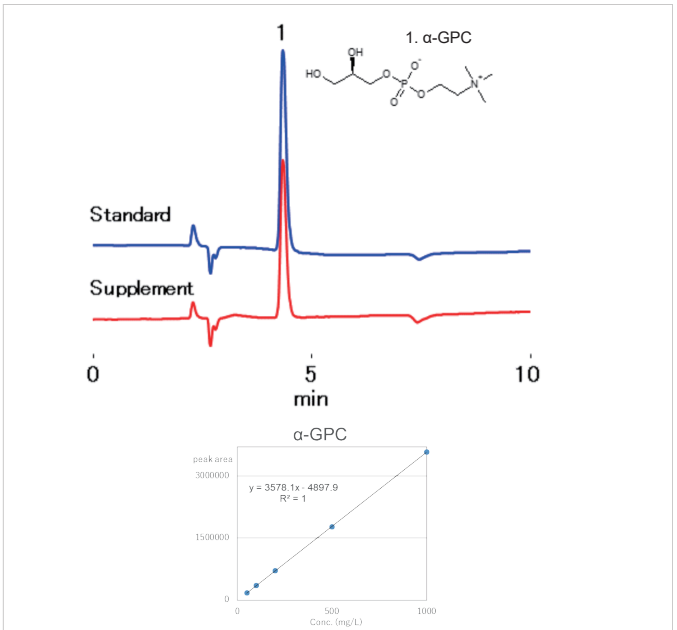
Below sample preparation method was used for the analysis of commercial dietary supplement containing theanine.

Make a cut on a soft capsule of the dietary supplement and remove its content. The content of the supplement was viscous liquid. Measure 5 mg of the content and add 5-mL eluent, ultrasonicate for 10 minutes to dissolve the content. Filter the mixture using a membrane filter (0.45 µm) and use the filtrate for analysis.

3. Analysis of α-GPC

L-α-Glycerylphosphorylcholine (α-GPC) is a naturally existing choline derivative. It is an acetylcholine precursor that stimulates the parasympathetic nerves, and has a potential of improving brain function in the treatment of Alzheimer's disease.

Similar to the analysis of theanine, we used a polymer-based HILIC column, Shodex Asahipak NH2P-50 4E, for the analysis of α-GPC. Figure 3 shows the chromatograms of α-GPC standard and a commercial supplement containing α-GPC. An isocratic method was feasible analyzing the α-GPC. Good linearity was obtained for the calibration curve of α-GPC in the concentration range 0.05 to 1 mg/mL. The recovery rate against the value claimed by the supplement manufacturer was almost 100%. Most content was found to be insoluble in the eluent.



Sample : 1 mg/mL α-GPC (in eluent), 20 µL
Column : Shodex Ashahipak NH2P-50 4E (4.6 mm x 250 mm, 5 µm)
Eluent : 50 mM NaH_2PO_4 aq./CH_3CN = 40/60
Flow rate : 1.0 mL/min
Detector : RI
Column temp.: 40 ºC

Fig. 3. Chromatograms of (blue) α-GPC standard and (red) a dietary supplement

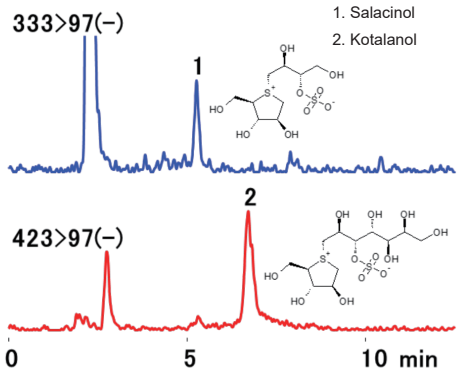
Below sample preparation method was used for the analysis of commercial dietary supplement containing α-GPC.

Open a dietary supplement capsule and remove its content (powder). Measure 10 mg of the content and add 5-mL eluent, ultrasonicate for 10 minutes to dissolve the content. Filter the supernatant using a membrane filter (0.45 µm) and use the filtrate for analysis.

4 Analysis of saracinol and kotalanol

Salacia extract is known to suppress blood glucose level elevation. It is also expected to prevent body fat deposition and to improve intestinal environment by preventing the absorption of sugar.

A water extract of a commercial dietary supplement containing Salacia essence was analyzed by an LC/MS/MS method with a polymer-based HILIC column, Shodex HILICpak VT-50 2D. An isocratic method using the VT-50 2D demonstrated its feasibility for analyzing salacinol and kotalanol (Fig. 4). Salacinol and kotalanol peaks detected were identified based on their molecular weights.



Sample : Salacia extract, 2 µL
Column : Shodex HILICpak VT-50 2D (2.0 mm x 150 mm, 5 µm)
Eluent : 100 mM $HCOONH_4$ aq./CH_3CN = 25/75
Flow rate : 0.3 mL/min
Detector : ESI-MS/MS MRM(-)
Column temp.: 40 ºC

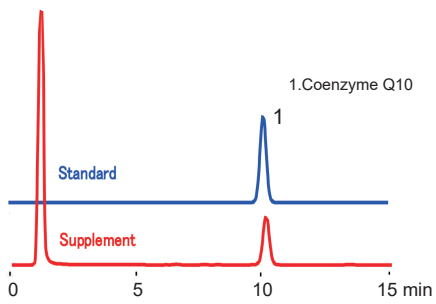
Fig. 4. Chromatograms of a Salacia extract containing dietary supplement

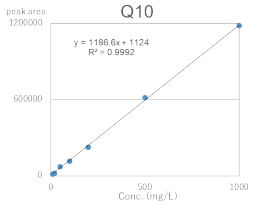
Below sample preparation method was used for the analysis of a commercial dietary supplement containing Salacia essence.

Grind a dietary supplement tablet in a mortar to make it into powders. Measure 0.1 g of the powder and add 1-mL ultrapure water, ultrasonicate for 10 minutes. Take the supernatant and add an equal volume of 100 % acetonitrile. Then, further dilute the mixture 10,000 times with 50 % acetonitrile and use the mixture for analysis.

5 Analysis of coenzyme Q10

Coenzyme Q10 is a fat soluble compound found in meat and seafoods. It is also called ubiquinone and known as a vitamin-like substance, expected to have antioxidant and anti-aging effects. We analyzed coenzyme Q10 using an ODS column, Shodex C18P 4D, under reversed phase mode. An isocratic method using the C18P 4D demonstrated its feasibility for the analysis of coenzyme Q10 (Fig. 5). A good linearity was obtained for the calibration curve of coenzyme Q10 in the concentration range 0.001 to 1 mg/mL. The recovery rate obtained against the value claimed by the supplement manufacturer was about 95 %. The chromatogram demonstrated a good separation of coenzyme Q10 and other components, proving the method's capability for the analysis of coenzyme Q10. The manufacturer of the supplement indicates that the supplement also contains vitamin C. A peak considere bserved at 1 minu





Sample : 1 mg/mL coenzyme Q10 (in eluent), 5 µL
Column : Shodex Silica C18P 4D (4.6 mm I.D. x 150 mm, 5 µm)
Eluent : CH_3OH/C_2H_5OH = 13/7
Flow rate : 1.2 mL/min
Detector : UV (275 nm)
Column temp.: 35 ºC

Fig. 5. Chromatograms of (blue) coenzyme Q10 standard and (red) a dietary supplement

Below sample preparation method was used for the analysis of a commercial dietary supplement containing coenzyme Q10.

Make a cut on a soft capsule of the dietary supplement and removed its content. The content of the supplement was viscous liquid. Measure 5 mg of the content and add 5-mL eluent, ultrasonicate for 10 minutes to dissolve the content. Filter the mixture using a membrane filter (0.45 µm) and use the filtrate for analysis.

Conclusions

This technical article demonstrated the Shodex columns' feasibility for the analysis of various functional ingredients in some dietary supplements. They were detected without the influence of other components present in the supplements.

In Japan, when applying to resister a food/food product as a FFC, which analytical method to analyse the target functional ingredients is up to the producer/claimer, as long as the method provides scientifically reliable result. Therefore, the methods introduced in this technical note using the Shodex columns may also be applicable for the analysis of those functional ingredients.

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