

Quantitative Analysis of Soy Isoflavones

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User benefit

- ◆ Eight soy isoflavones can be analyzed in 30 min.
- ◆ Conventional HPLC systems can be used with the developed method.

Introduction

In collaboration with the National Agriculture and Food Research Organization,



Shimadzu Corporation has been developing a simple, quick and accurate method of analyzing functional components in agricultural and food products. In this report, we introduce the method for analyzing isoflavones in soybeans and report the results of confirming the isoflavone content in one soybean variety.

Soy isoflavones are components that are contained in soybeans, especially soybean germ. Soy isoflavones usually have a sugar-bound structure. Aglycone is known to have an estrogen-like effect because its molecular structure resembles that of human estrogen (female hormone). Therefore, the maximum daily intake of soy isoflavone recommended is 75 mg/day in Japan. In this report, an analysis method of soy isoflavones shown in Table 1 was developed.

Table 1 Target Compounds

Compound
Genistein
Daidzein
Glycitein
Genistin
Daidzin
6''-O-malonyl genistin
6''-O-malonyl daidzine
6''-O-malonyl glycitin

Analytical Conditions

The analysis conditions were determined with reference to the existing analysis method.¹⁾ In the existing method, a mixture of water and acetonitrile, containing acetic acid, is used for mobile phases A and B.

In this method, ultra-pure water containing acetic acid for mobile phase A and acetonitrile containing acetic acid for mobile phase B were used to simplify mobile phase preparation.

A Shim-pack™ GIST HP (150 mm × 4.6 mm I.D., 3 μm) column was used for the analysis and the target soy isoflavones were separated in 30 min. The analytical conditions are shown in Table 2.

Table 2 Analytical Conditions

System	: Nexera X3
Column	: Shim-pack GIST-HP C18* ¹ (150 mm × 4.6 mm I.D., 3 μm)
Mobile Phases	: A) 0.1 % acetic acid in H ₂ O B) 0.1 % acetic acid in Acetonitrile
Gradient Program	: B conc. 15 % (0.00 min) - 25 % (5.00 min) - 35 % (20.00 min) - 100 % (20.01-25.00 min) - 15 % (25.01-30.00 min)
Flow rate	: 1.0 mL/min (Maximum pressure: 16.5 MPa)
Column Temp.	: 40 ° C
Injection volume	: 5 μL
Detection	: 254 nm

*1 P/N : 227-30041-05

Sample Pretreatment

The extraction was performed following the conditions determined with reference to the existing method¹⁾.

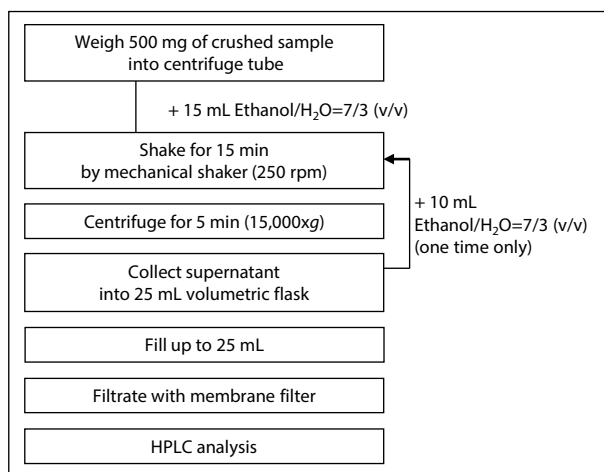


Fig. 1 Pretreatment Workflow

Analysis Results of Standard Samples

The linearities were determined by standards analysis. Fig. 2 shows a representative chromatogram, and Table 3 shows the dynamic range and the coefficients of determination. Good linearities were obtained with a coefficient of determination (R²) ≥ 0.997 for all compounds.

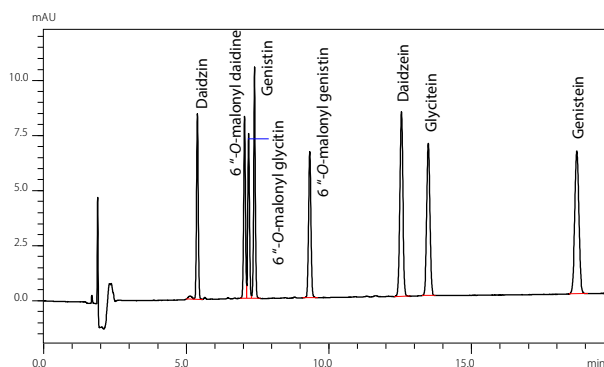


Fig. 2 Chromatogram of 2 μg/mL Standard Sample

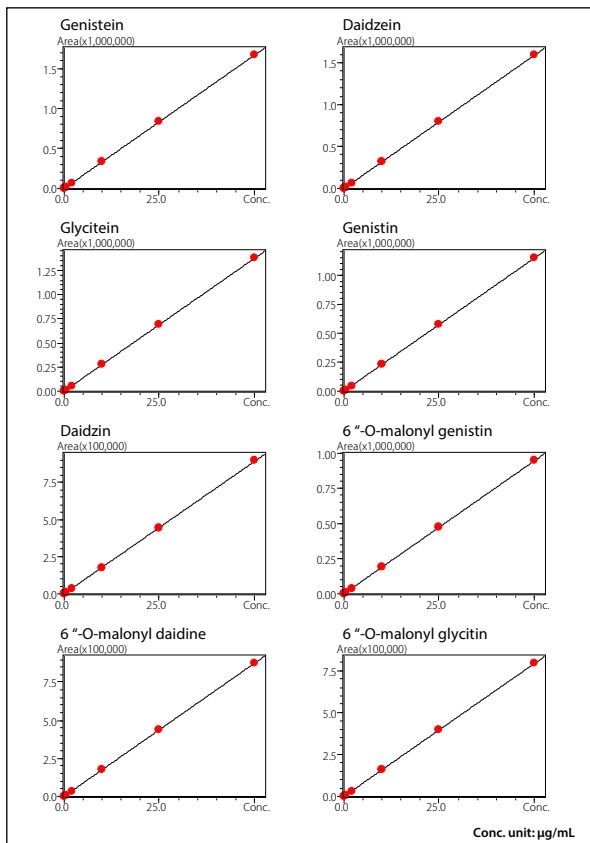


Fig. 2 Calibration Curves

Table 3 Linear Range and Coefficient of Determination (R²)

Compound	Linear range (µg/mL)	Coefficient of determination (R ²)
Genistein	0.10 - 50	0.9999
Daidzein	0.10 - 50	0.9999
Glycitein	0.10 - 50	0.9999
Genistin	0.10 - 50	0.9999
Daidzin	0.10 - 50	0.9998
6''-O-malonyl genistin	0.10 - 50	0.9999
6''-O-malonyl daidaine	0.10 - 50	0.9999
6''-O-malonyl glycitin	0.10 - 50	0.9999

Quantitative Results for Soybeans

The extract of soybean (Fukuyutaka) was analyzed to determine the content of isoflavones. Fig. 4 shows the chromatograms, and Table 5 shows the calculated content of each isoflavone in the soybean.

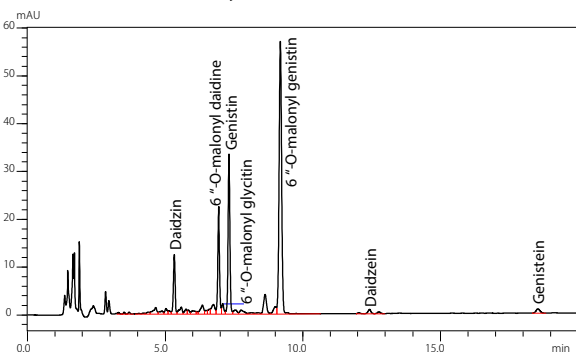


Fig. 4 Chromatograms of Soybean Extract

Table 4 Isoflavone Content in Soybean

Compound	Content (mg/100g)
Genistein	1.30
Daidzein	1.00
Glycitein	< LLOQ
Genistin	32.05
Daidzin	13.99
6''-O-malonyl genistin	85.35
6''-O-malonyl daidaine	28.31
6''-O-malonyl glycitin	3.35

Repeatability Test Results of Soybean Extract

Seven extracts were prepared from soybean (Fukuyutaka) and a repeatability test was performed to confirm the validity of the method. Table 5 shows the results. Good repeatability, %RSD ≤ 3%, was obtained for all detected compounds.

Table 5 Repeatability Test Results (n=7)

Compound	Repeatability (%RSD)
Genistein	1.19 %
Daidzein	1.32 %
Glycitein	- (< LLOQ)
Genistin	1.41 %
Daidzin	1.13 %
6''-O-malonyl genistin	1.21 %
6''-O-malonyl daidaine	1.49 %
6''-O-malonyl glycitin	2.56 %

Conclusion

- Simultaneous analysis of eight isoflavones was performed using the Nexera series.
- The content of isoflavones in soybean (Fukuyutaka) was determined using the developed method.

<References>

- 1) Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour, and Welfare, Government of Japan (PFSB), Guidelines for handling Foods for Specified Health Uses containing soy isoflavones, Food safety issue (Notice No. 0823001 of the Department of Food Safety)

This analytical method was developed in collaboration with the National Agriculture and Food Research Organization (scheduled from April, 2019 to March, 2022) at the Collaborative Research Laboratory for Analysis of Food Functionality in Shimadzu's Healthcare R&D Center. The analytical method and analysis data presented in this report were provided by Mr. Hironori Juichi and Ms. Yayoi Ichiki, researchers at the National Agriculture and Food Research Organization.

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