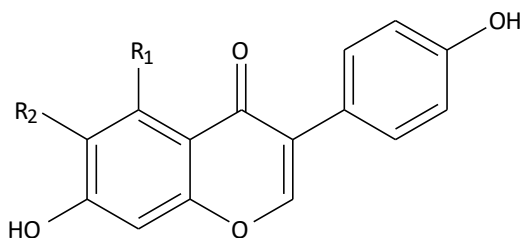


Simultaneous Analysis of 12 Soybean Isoflavones

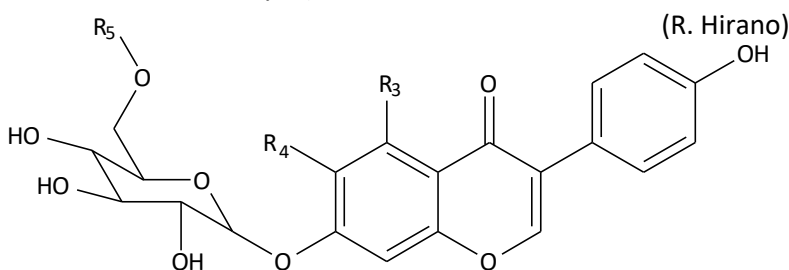
Isoflavones are contained in several products made of soybeans. They are known to be functional compounds with antioxidant properties and for their metabolites for having estrogenic properties. Soybean isoflavones contain 15 types of isoflavones: 3 types of isoflavones (daidzein, glycitein and genistein) called Aglycone (non-glycoside),

3 types of glycosides (daidzin, glycitin, genistin), 3 types of acetylated glycosides, 3 types of malonylated glycosides and 3 types of succinylated isoflavone glycosides.

In this note InertSustainSwift C18 column was used to analyze 12 types of soybean isoflavones (the 3 types of succinylated isoflavone glycosides are not been considered in this analysis).



Isoflavone



Soybean Isoflavone Glycosides

HPLC Conditions

Column	InertSustainSwift C18 (5 μ m, 250 x 4.6 mm I.D.)
Flow rate	1.0 mL/min
Column temp.	35 $^{\circ}$ C
Detection	PDA 254 nm (PD7752 PDA Detector)
Injection Vol.	10 μ L

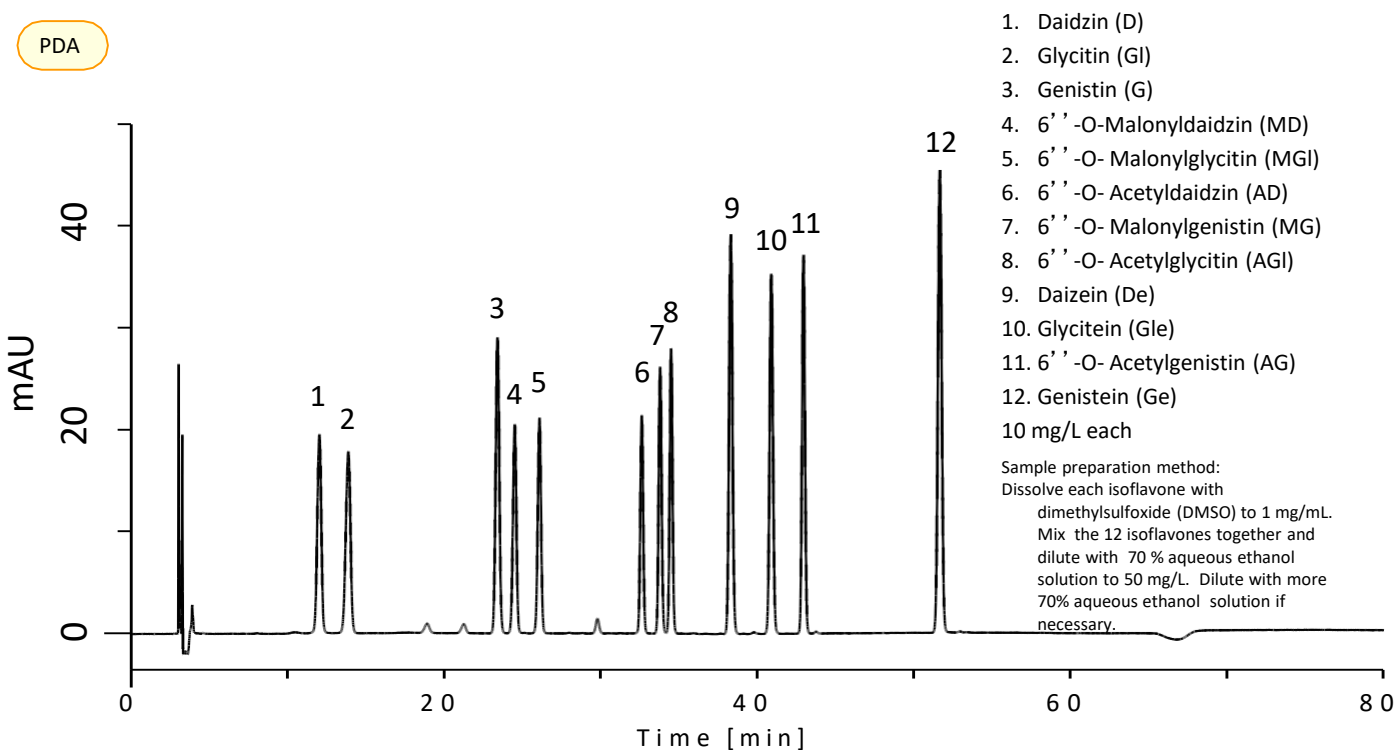
Eluent

- : A) CH₃CN/H₂O/CH₃COOH = 150/850/1, v/v/v
 B) CH₃CN/H₂O/CH₃COOH = 350/650/1, v/v/v

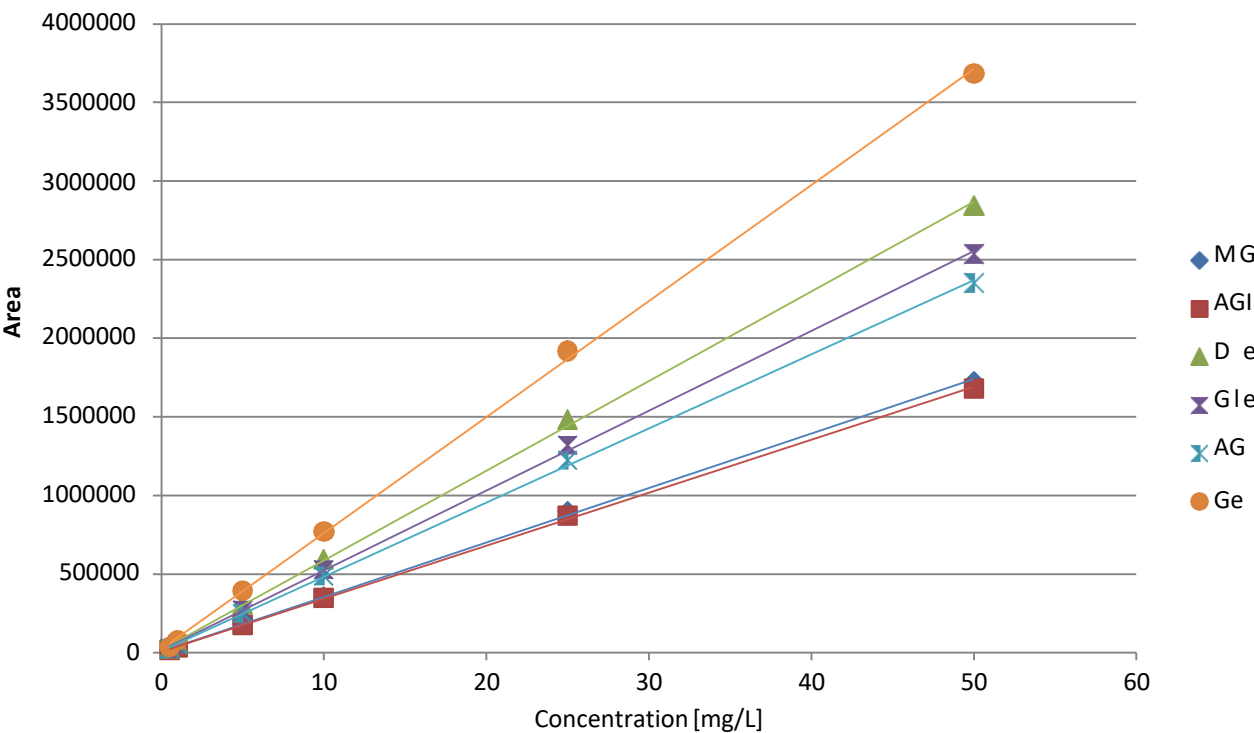
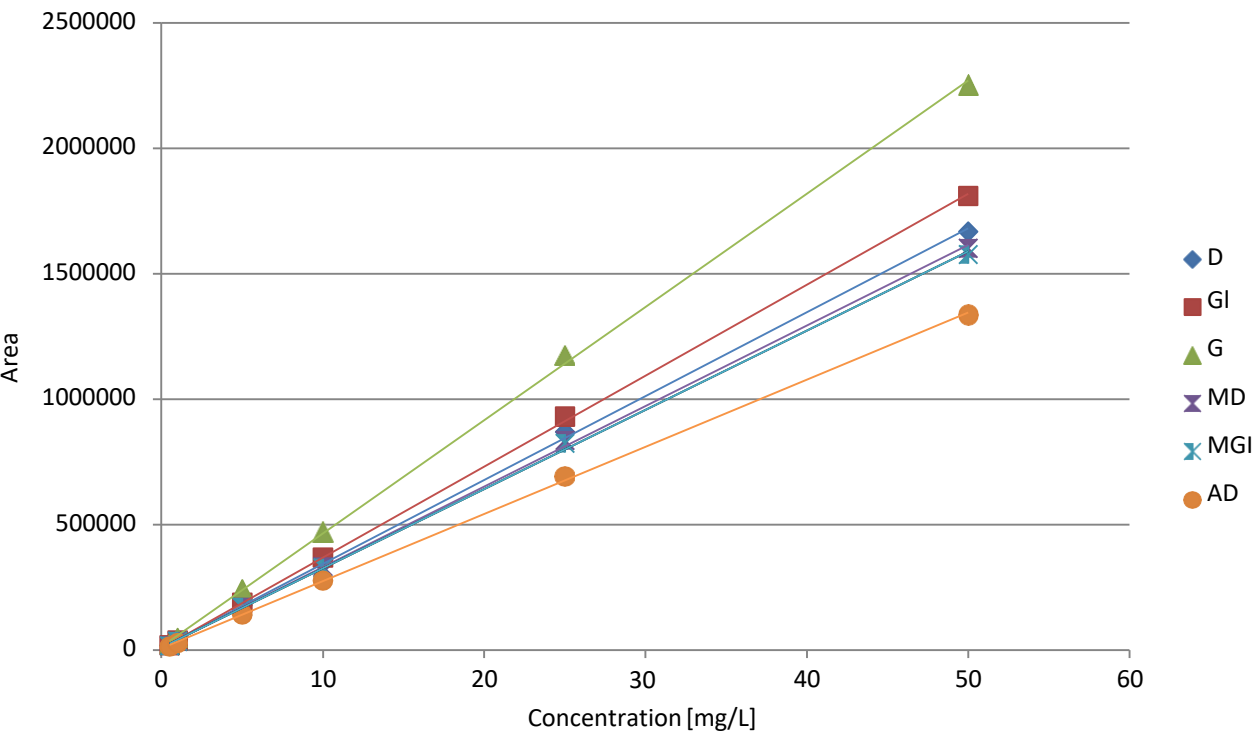
Time (min.)	Eluent A (%)	Eluent B (%)
0	100	0
10	100	0
60	0	100
61	100	0
80	100	0

Standard Solution

PDA



Calibration Curve (0.5 - 50 mg/L)



Correlation Coefficient (R²)

	D	GI	G	MD	MGI	AD	MG	AGI	De	Gle	AG	Ge
R²	0.9996	0.9998	0.9996	0.9995	0.9995	0.9997	0.9996	0.9996	0.9996	0.9996	0.9996	0.9996

Example of Pretreatment Method**Liquid Sample**

— 1 g

Extraction

- dilute with 70% of aqueous ethanol solution to 100 mL
- centrifuge at 10,000 rpm for 10 min and collect the supernatant
- filtrate the supernatant by GL Chromato Disk 25P 0.45 μ m

HPLC**Solid Sample**

— 1 g

Extraction

- dilute with 25mL of 70 % aqueous ethanol solution
- shake for 30 min
- centrifuge at 10,000 rpm for 10min
- collect the supernatant

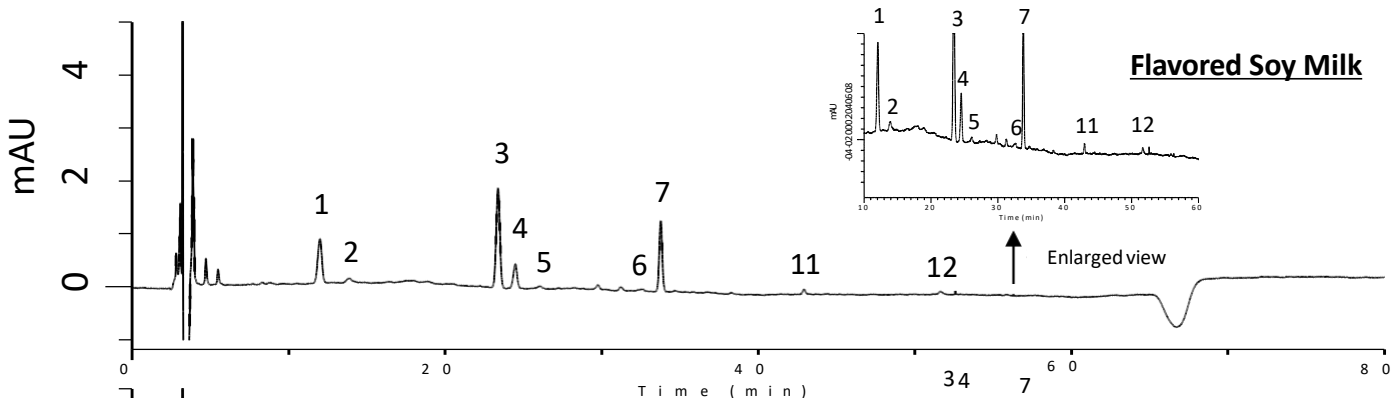
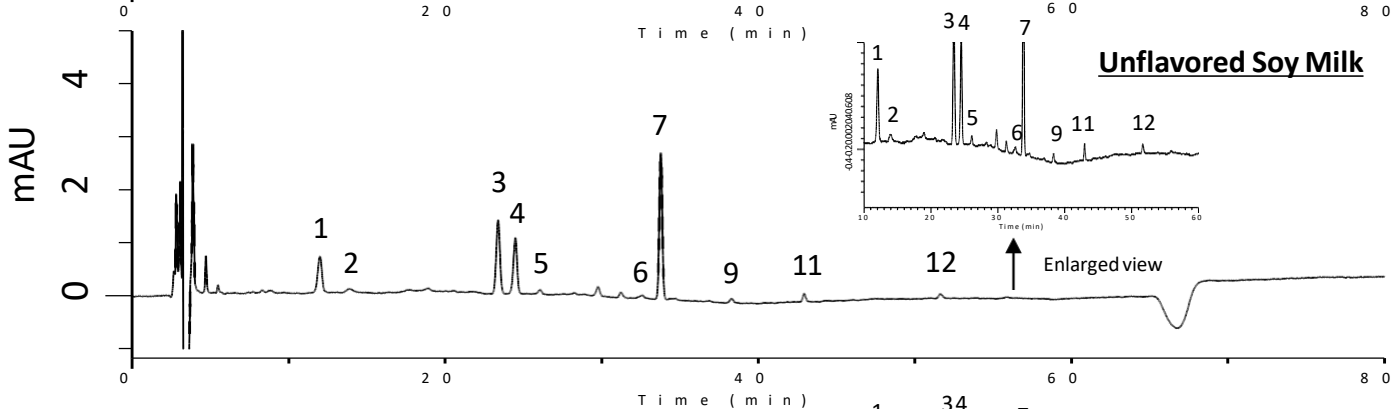
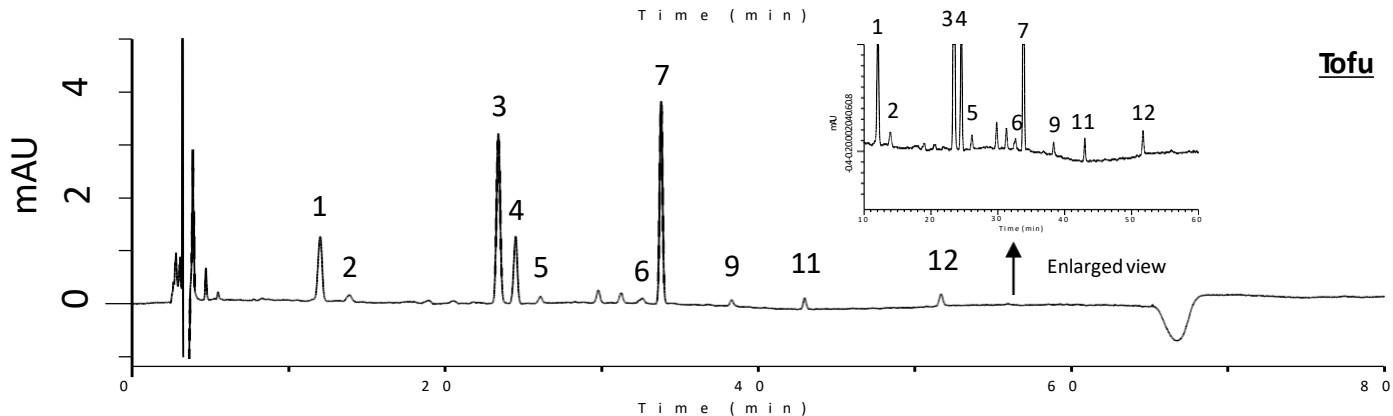
Residue

- dilute with 25mL of 70% aqueous ethanol solution
- shake for 30 min
- centrifuge at 10000 rpm for 10 min
- collect the supernatant

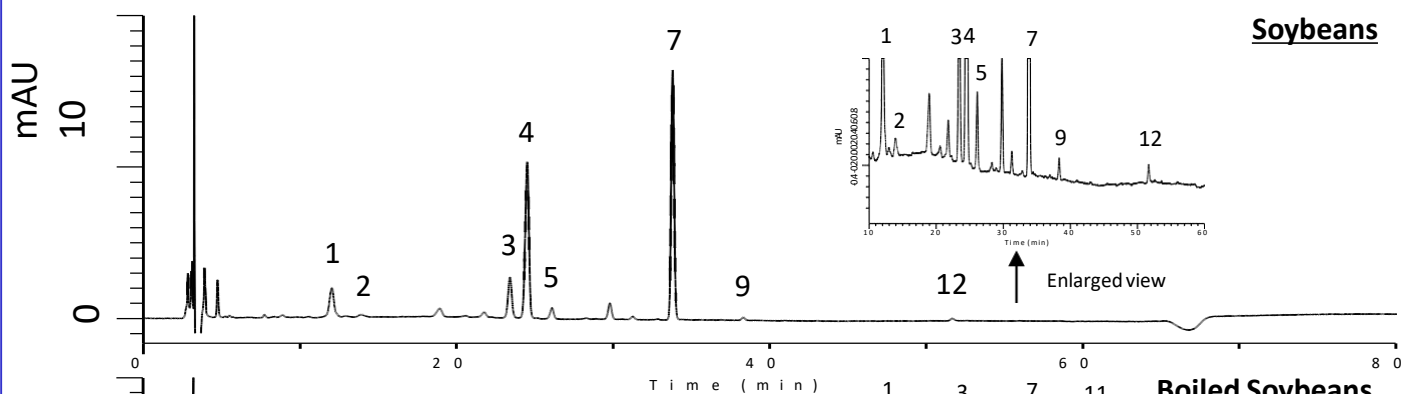
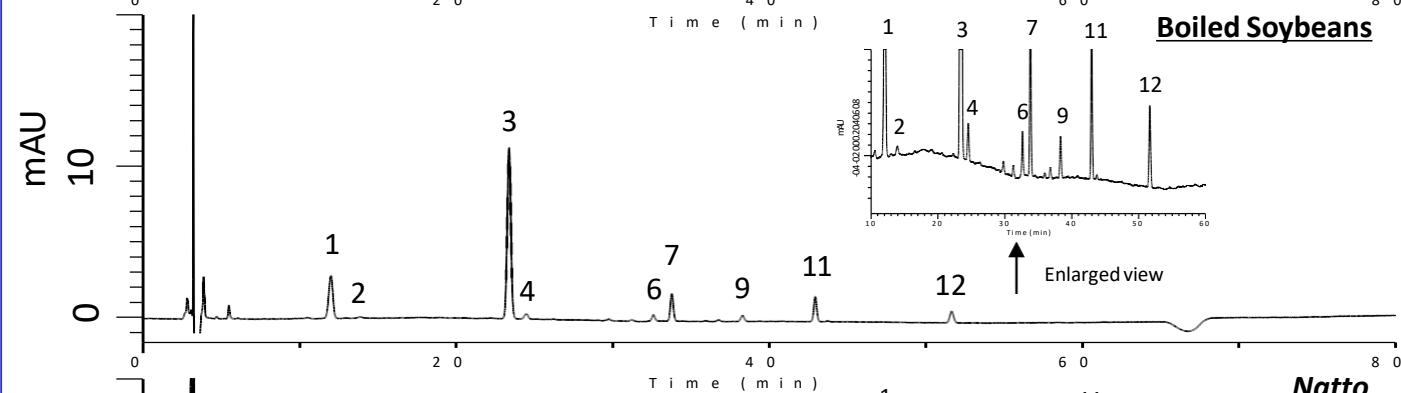
Repeat this procedure twice

Dilute to Volume

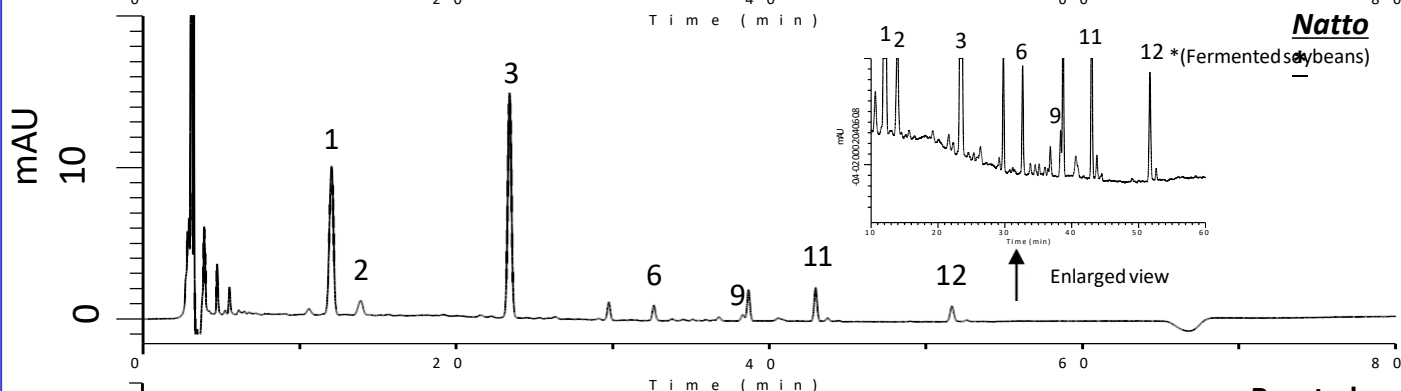
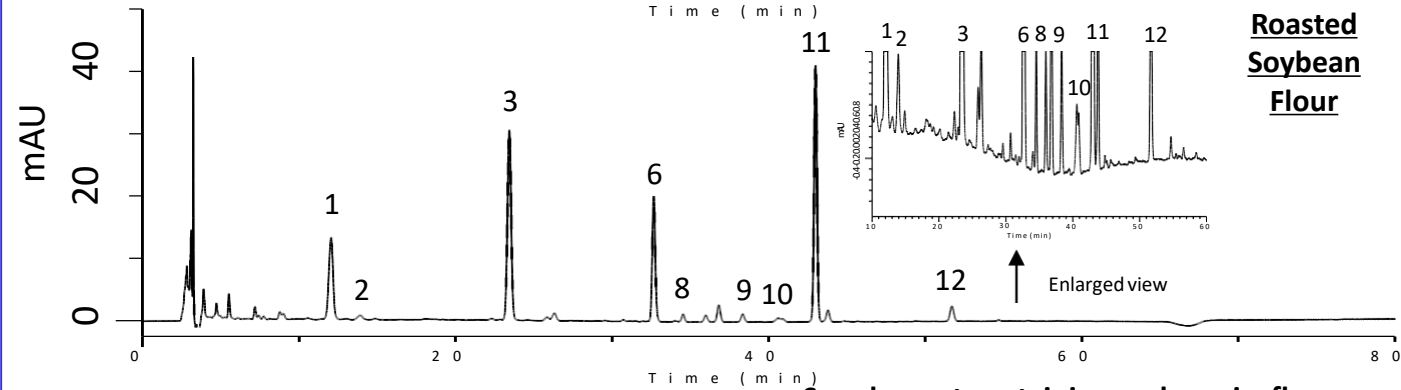
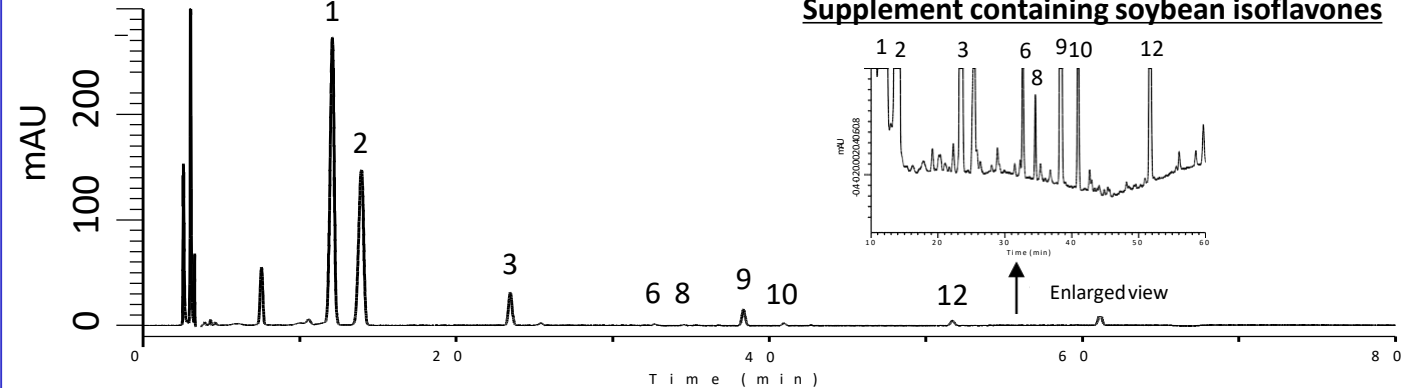
- dilute with aqueous ethanol solution to 100mL
- filtrate by GL Chromato Disk 25P 0.45 μ m

HPLC**Examples of Sample Analysis****Flavored Soy Milk****Unflavored Soy Milk****Tofu**

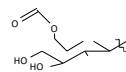
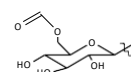
Examples of Sample Analysis

Soybeans**Boiled Soybeans****Natto**

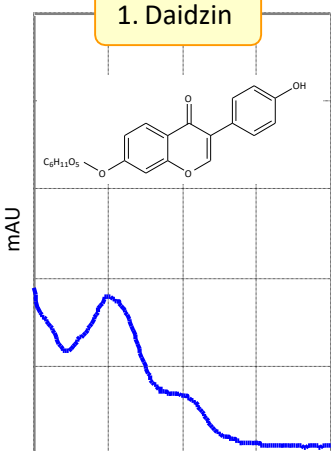
(Fermented soybeans)

**Roasted Soybean Flour****Supplement containing soybean isoflavones**

Chemical Structure and UV Spectrum

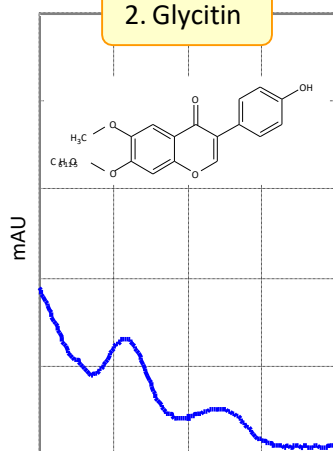
R₂ :

1. Daidzin



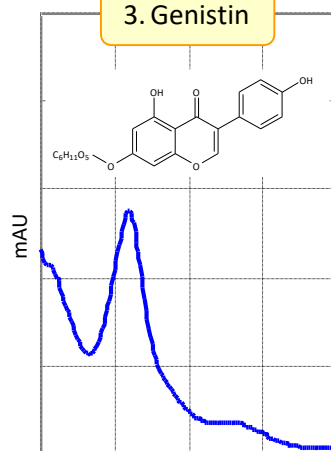
Wave length (nm)

2. Glycitin



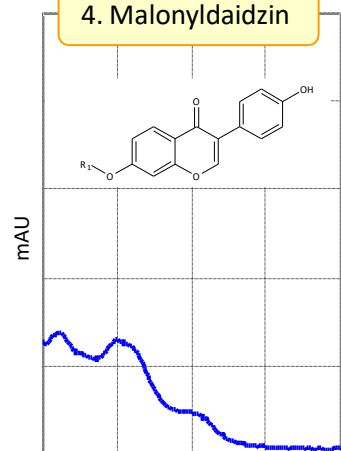
Wave length (nm)

3. Genistin



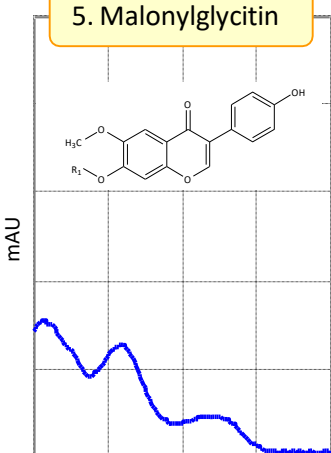
Wave length (nm)

4. Malonyldaidzin



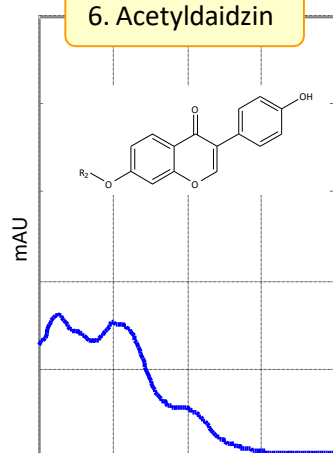
Wave length (nm)

5. Malonylglycitin



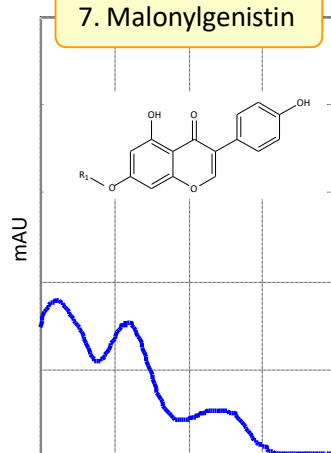
Wave length (nm)

6. Acetyldaidzin



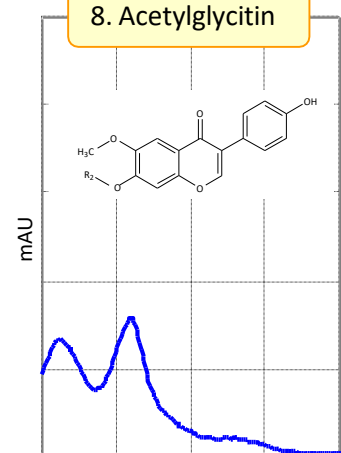
Wave length (nm)

7. Malonylgenistin



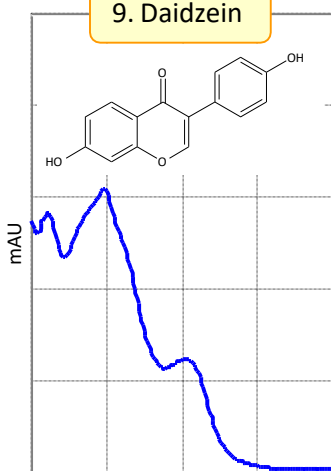
Wave length (nm)

8. Acetylglycitin



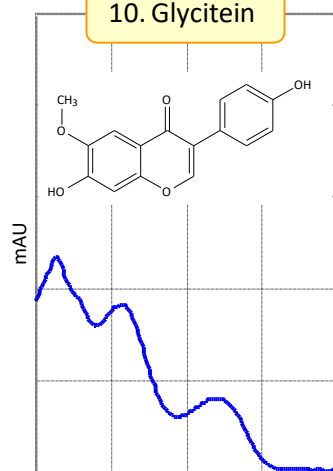
Wave length (nm)

9. Daidzein



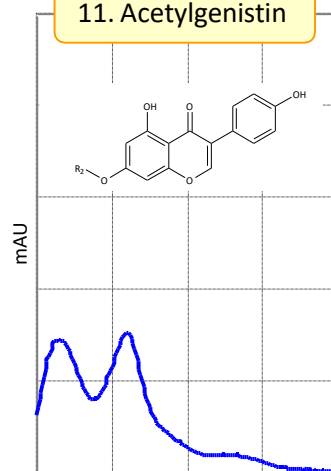
Wave length (nm)

10. Glycitein



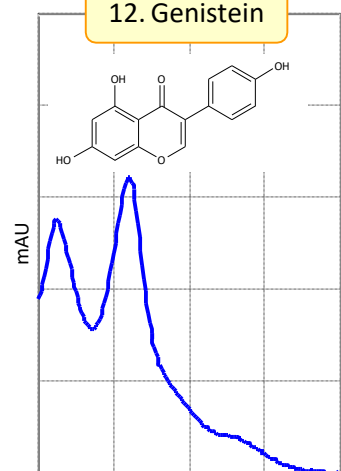
Wave length (nm)

11. Acetylgenistin



Wave length (nm)

12. Genistein



Wave length (nm)

✂ The absorbance scale (Y axis) is the same in every UV spectrum.

HPLC Column

InertSustainSwift C18 5 µm, 250 x 4.6 mm I.D.
Cat.No. 5020-88027

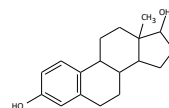
**GL Chromato Disk**

For both hydrophilic and
hydrophobic samples 25P
0.45 µm
Cat.No. 5040-28542

**Coffee Break**

The soybean isoflavones are one type of Polyphenols. They are also known as estrogen-like since the chemical structure of the isoflavones metabolites is similar to female hormones one (estrogens: e.g. estradiol) and some researches has also reported that their behavior is similar to that of female hormones. For this reason, among their applications they can also be used in the human body as substitutes of female hormones in case of deficiency.

	R1	R2
Daidzein	: H	: H
Genistein	: OH	: H
Glycitein	: H	: OCH ₃

**Estradiol**

	R3	R4	R5
Daidzin	: H	: H	: H
Genistin	: OH	: H	: H
Glycitin	: H	: OCH ₃	: H
6''-O-Acetyldaidzin	: H	: H	: COCH ₃
6''-O-Acetylgenistin	: OH	: H	: COCH ₃
6''-O-Acetylglycitin	: H	: OCH ₃	: COCH ₃
6''-O-Malonyldaidzin	: H	: H	: COCH ₂ COOH
6''-O-Malonylgenistin	: OH	: H	: COCH ₂ COOH
6''-O-Malonylglycitin	: H	: OCH ₃	: COCH ₂ COOH
6''-O-Succinyldaidzin	: H	: H	: COC ₂ H ₄ COOH
6''-O-Succinylgenistin	: OH	: H	: COC ₂ H ₄ COOH
6''-O-Succinylglycitin	: H	: OCH ₃	: COC ₂ H ₄ COOH

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