LT166

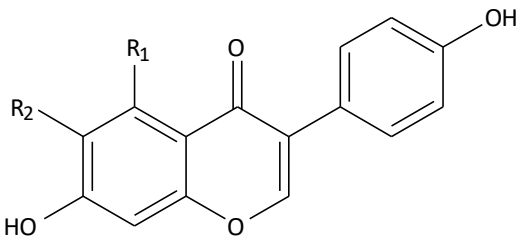
GL Sciences Inc.

Simultaneous Analysis of 12 Soybean Isoflavones

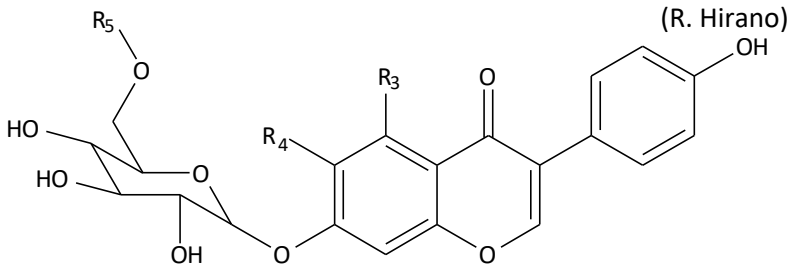
Isoflavones are contained in several products made of soybeans. They are known to be functional compounds with antioxidant properties and for their metabolites for having estrogenic properties. Soybean isoflavones contain 15 types of isoflavones: 3 types of isoflavones (daidzein, glycitein and genistein)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

(R. Hirano)

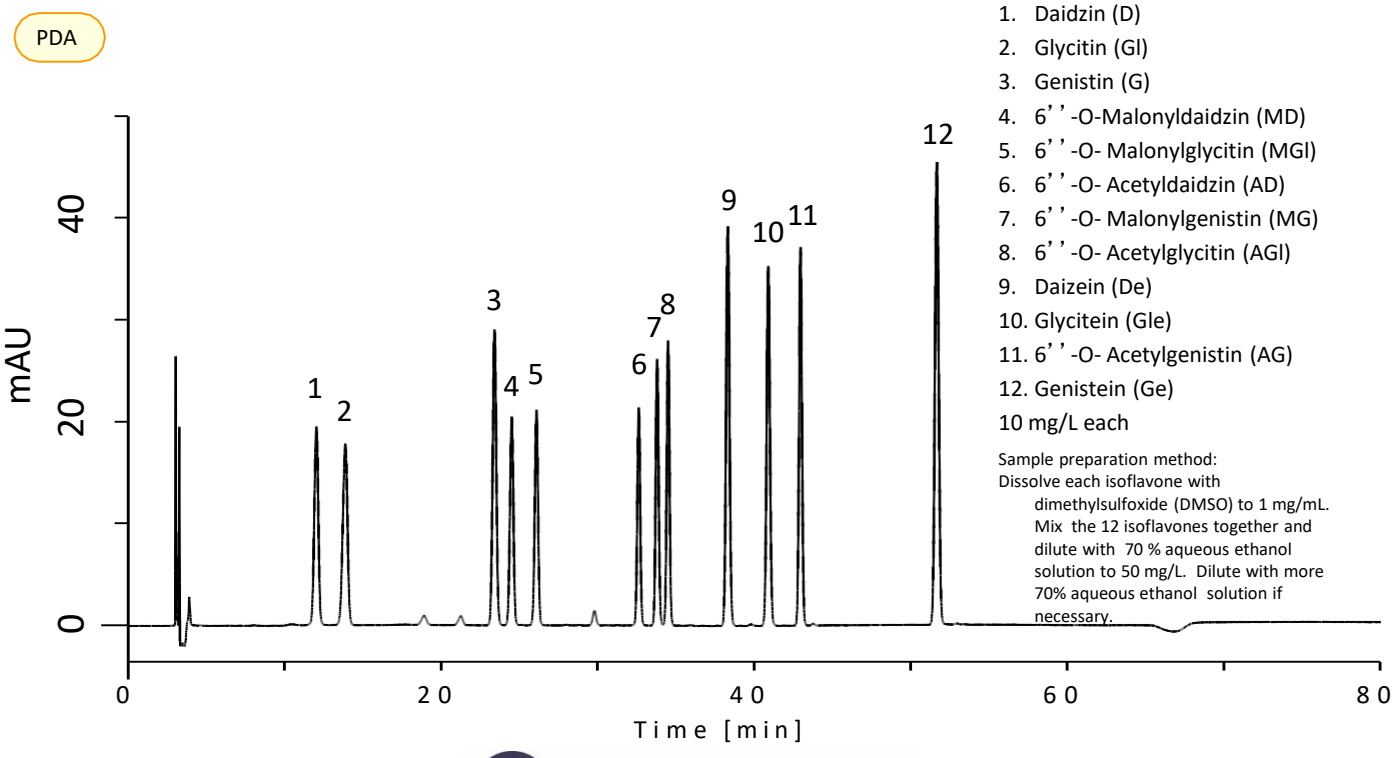
HPLC Conditions

Column : InertSustainSwift C18 (5 µm, 250 x 4.6 mm I.D).
Flow rate : 1.0 mL/min
Column temp. : 35 ℃
Detection : PDA 254 nm (PD7752 PDA Detector)
Injection Vol. : 10 µL

Eluent : A) $CH_3CN/H_2O/CH_3COOH$ = 150/850/1, v/v/v
B) $CH_3CN/H_2O/CH_3COOH$ = 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



1. Daidzin (D)
2. Glycitin (Gl)
3. Genistin (G)
4. 6'' -O-Malonyldaidzin (MD)
5. 6'' -O- Malonylglycitin (MGl)
6. 6'' -O- Acetyldaidzin (AD)
7. 6'' -O- Malonylgenistin (MG)
8. 6'' -O- Acetylglycitin (AGl)
9. Daizein (De)
10. Glycitein (Gle)
11. 6'' -O- Acetylgenistin (AG)
12. Genistein (Ge)

10 mg/L each

Sample preparation method:
Dissolve each isoflavone with dimethylsulfoxide (DMSO) to 1 mg/mL. Mix the 12 isoflavones together and dilute with 70 % aqueous ethanol solution to 50 mg/L. Dilute with more 70% aqueous ethanol solution if necessary.

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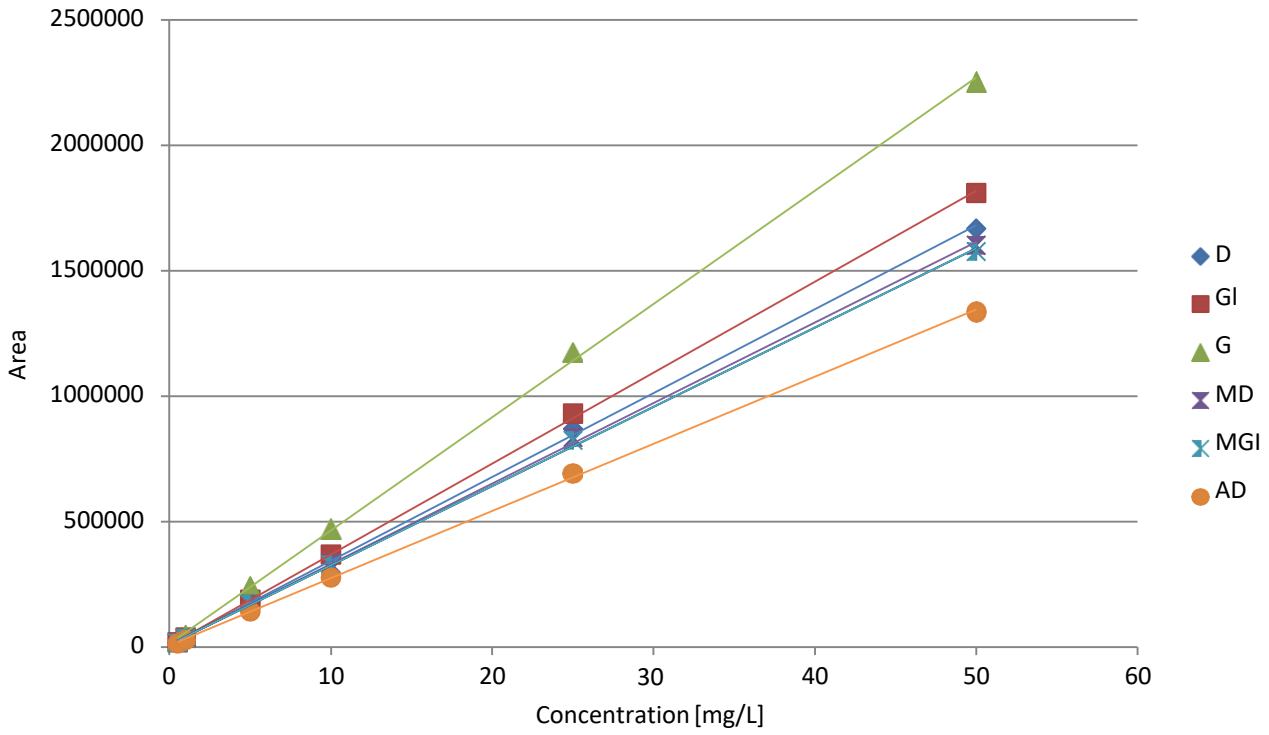
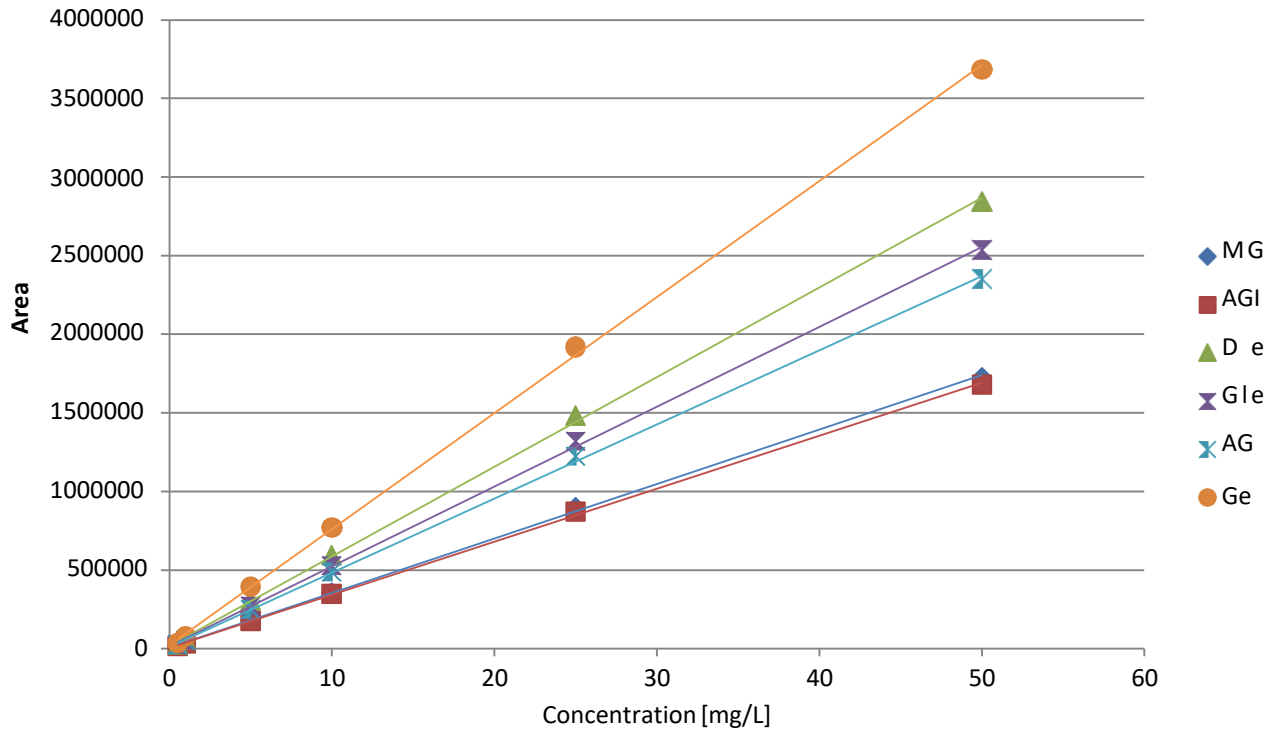
Calibration Curve (0.5 - 50 mg/L)





Correlation Coefficient (R^2)

	D	Gl	G	MD	MGI	AD	MG	AGI	De	Gle	AG	Ge
R^2	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 µm

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

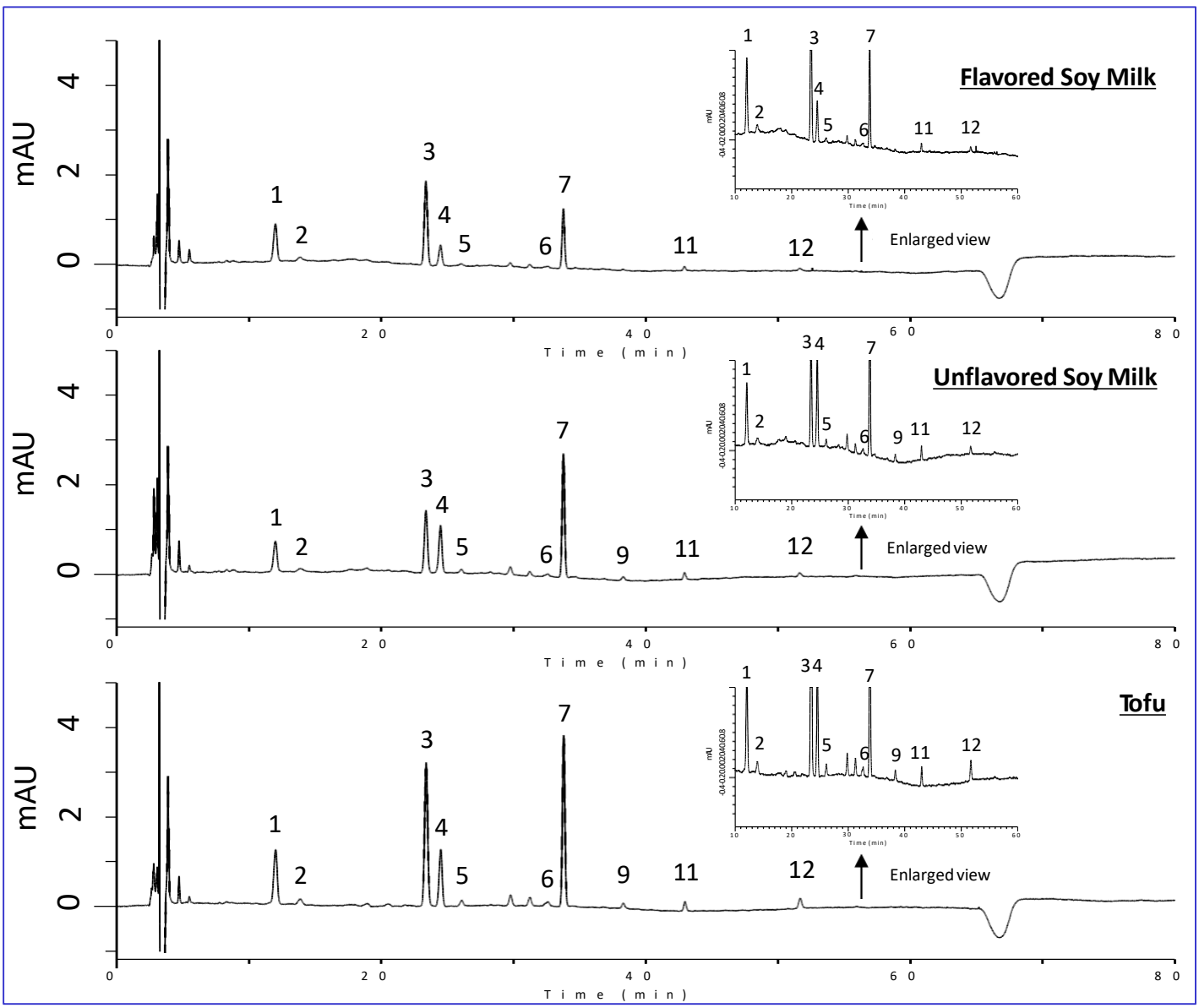
Repeat this procedure twice

Dilute to Volume

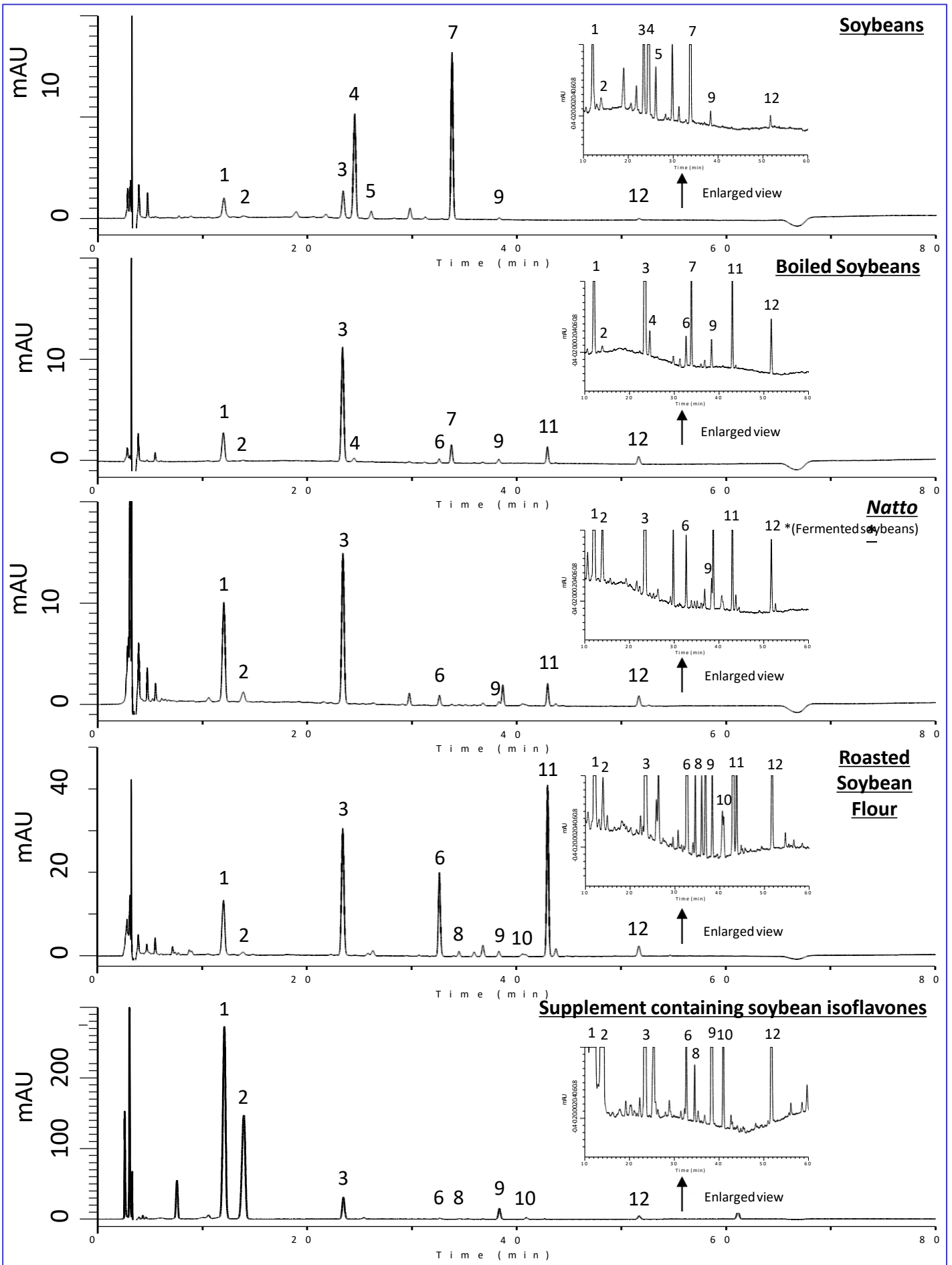
— dilute with aqueous ethanol solution to 100 mL
— filtrate by GL Chromato Disk 25P 0.45 µm

HPLC

Examples of Sample Analysis

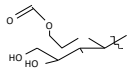


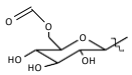
Examples of Sample Analysis



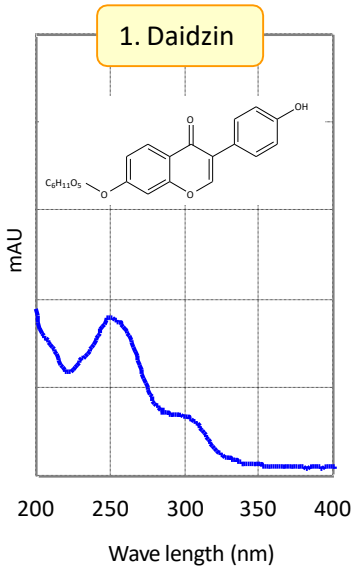
Chemical Structure and UV Spectrum

R_2 :

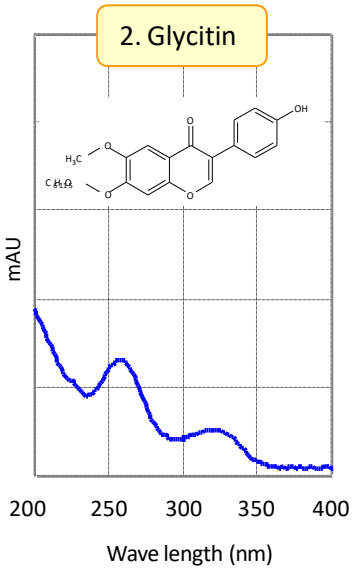




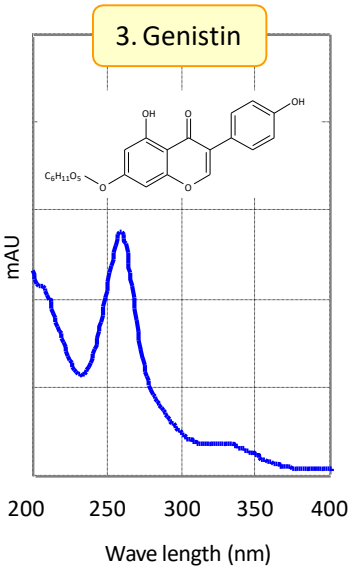
1. Daidzin



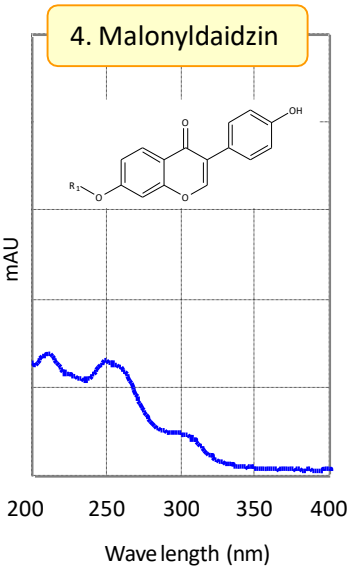
2. Glycitin



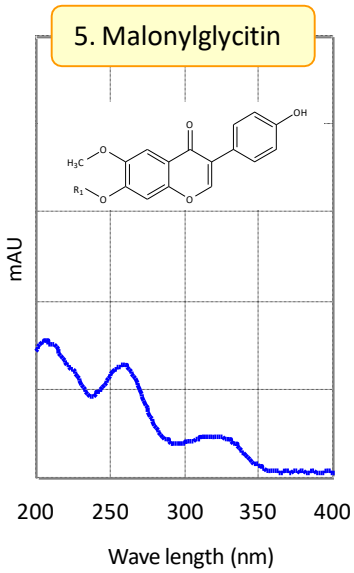
3. Genistin



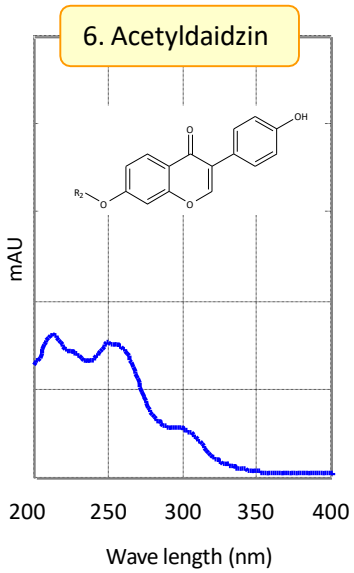
4. Malonyldaidzin



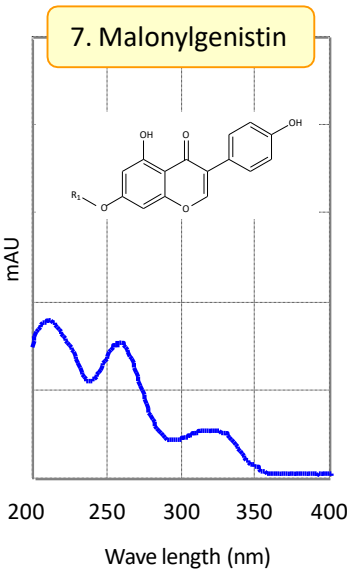
5. Malonylglycitin



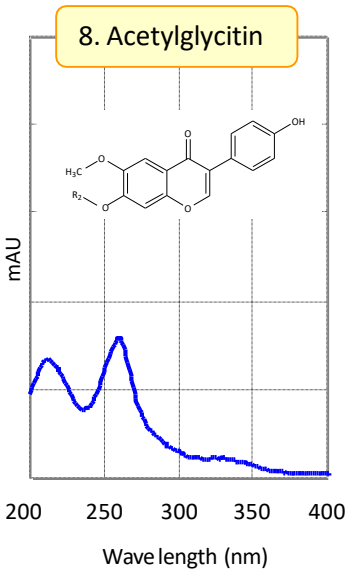
6. Acetyldaidzin



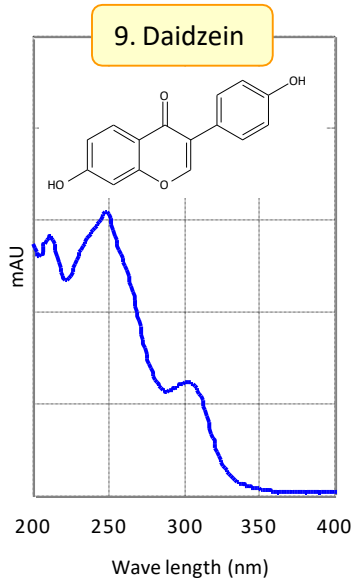
7. Malonylgenistin



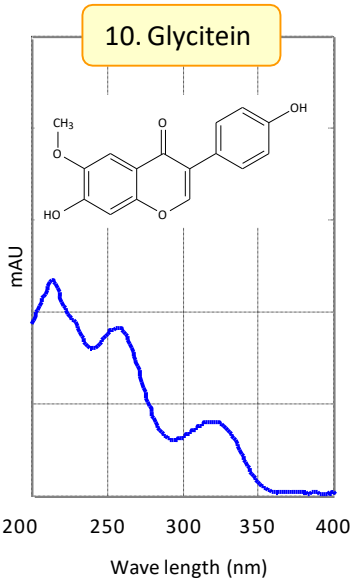
8. Acetylglycitin



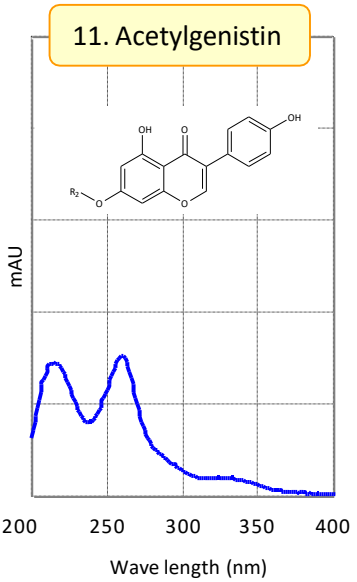
9. Daidzein



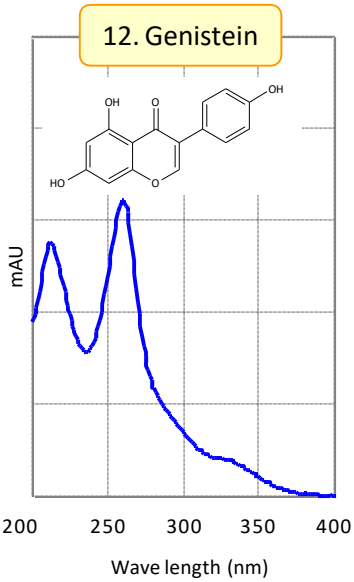
10. Glycitein



11. Acetylgenistin



12. Genistein



※ 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

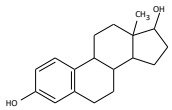
GL Chromato Disk

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	$COCH_2COOH$
6″-O-Malonylgenistin	OH	H	$COCH_2COOH$
6″-O-Malonylglycitin	H	OCH_3	$COCH_2COOH$
6″-O-Succinyldaidzin	H	H	COC_2H_4COOH
6″-O-Succinylgenistin	OH	H	COC_2H_4COOH
6″-O-Succinylglycitin	H	OCH_3	COC_2H_4COOH

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