

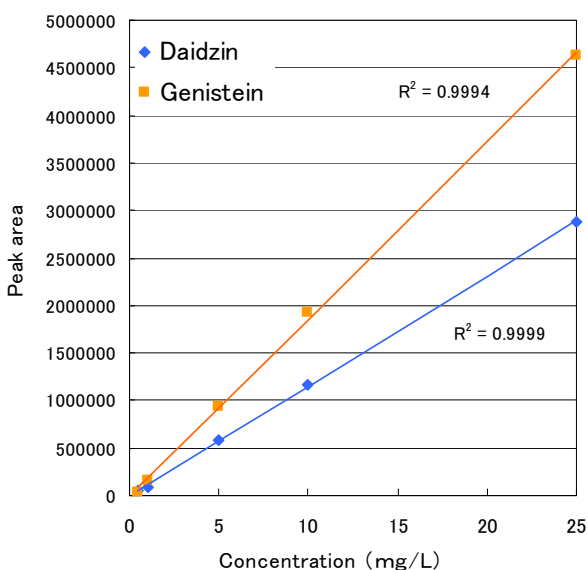
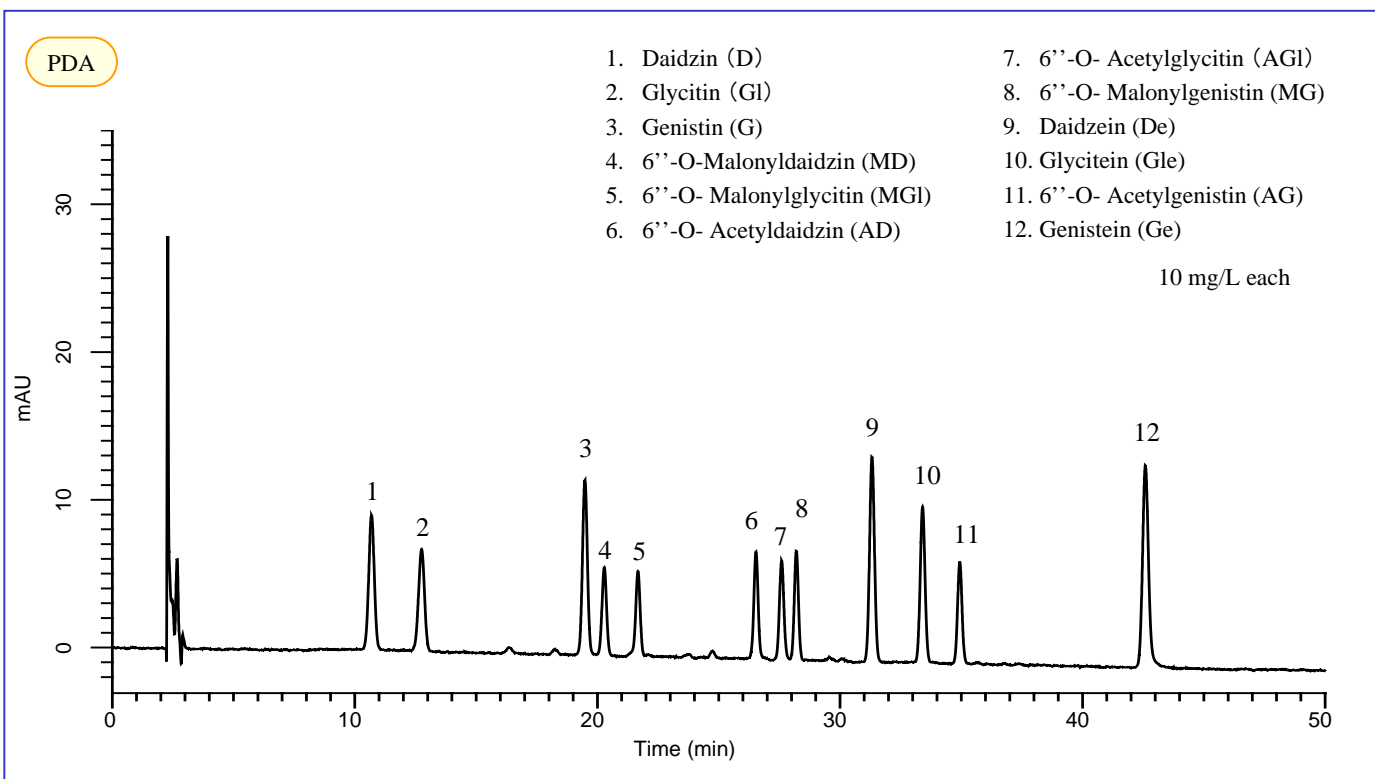
Isoflavones are a type of flavonoid and found in a number of legumes, grains, and, vegetables. Among them, soybean is by far the most concentrated source of isoflavones. As their chemical structures are very similar to those of endogenous estrogens, isoflavones are known to have estrogen-like effect and provide several health

benefits.

In this note, a simultaneous determination method for 12 major isoflavones (daidzein, genistein, glycitein, and their glycosides) in soy foods is described. The HPLC separation of isoflavones was achieved by using Inertsil ODS-SP as a separation column.

(K.Suzuki)

## A chromatogram obtained from standard solution



	R <sup>2</sup>
1. D	: 0.9994
2. GI	: 1.000
3. G	: 1.000
4. MD	: 1.000
5. MGI	: 1.000
6. AD	: 0.9999
7. AGI	: 0.9999
8. MG	: 1.000
9. De	: 0.9999
10. Gle	: 1.000
11. AG	: 1.000
12. Ge	: 0.9999

### Conditions

**Column** : Inertsil ODS-SP  
(5 μm, 250 x 4.6 mm I.D.)  
Cat.No. 5020-02746

**Eluent** : A) 0.1 % acetic acid in CH<sub>3</sub>CN  
B) 0.1 % acetic acid in H<sub>2</sub>O  
A/B = 15/85 – 8 min  
– 15/85 – 42 min – 35/65  
(equilibration 10 min), v/v  
(Mixed by a gradient mixer)

**Flow rate** : 1.5 mL/min

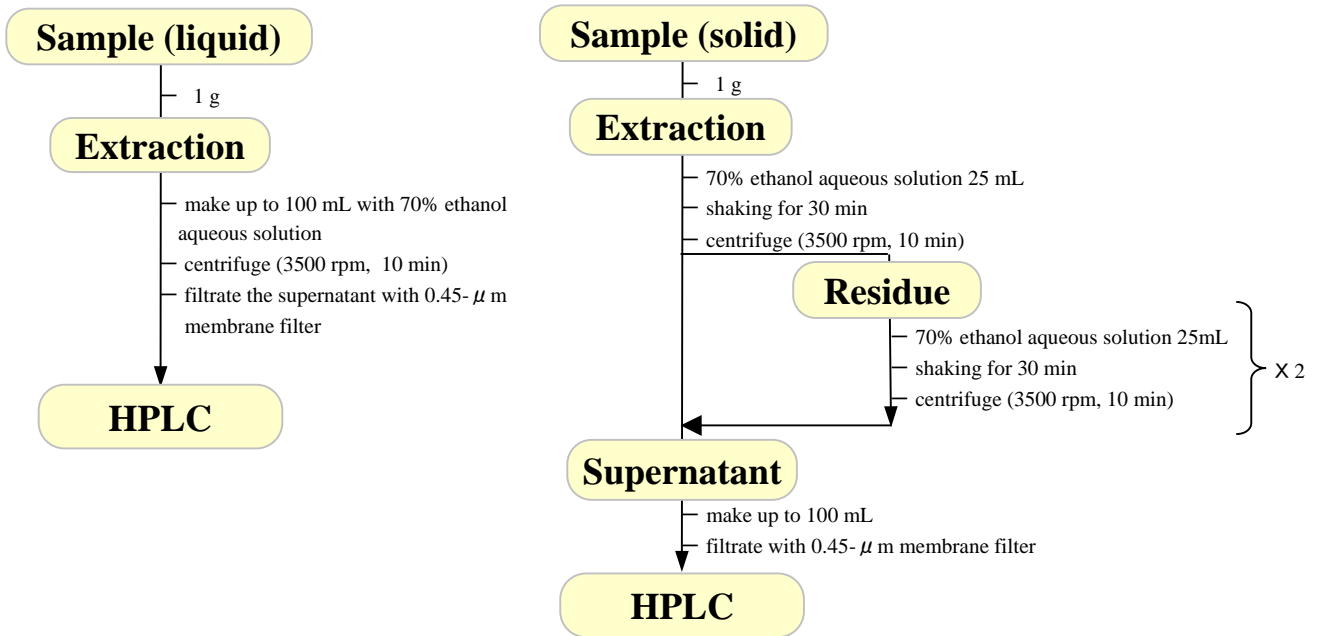
**Col. Temp.** : 35 °C

**Detection** : PDA 254 nm

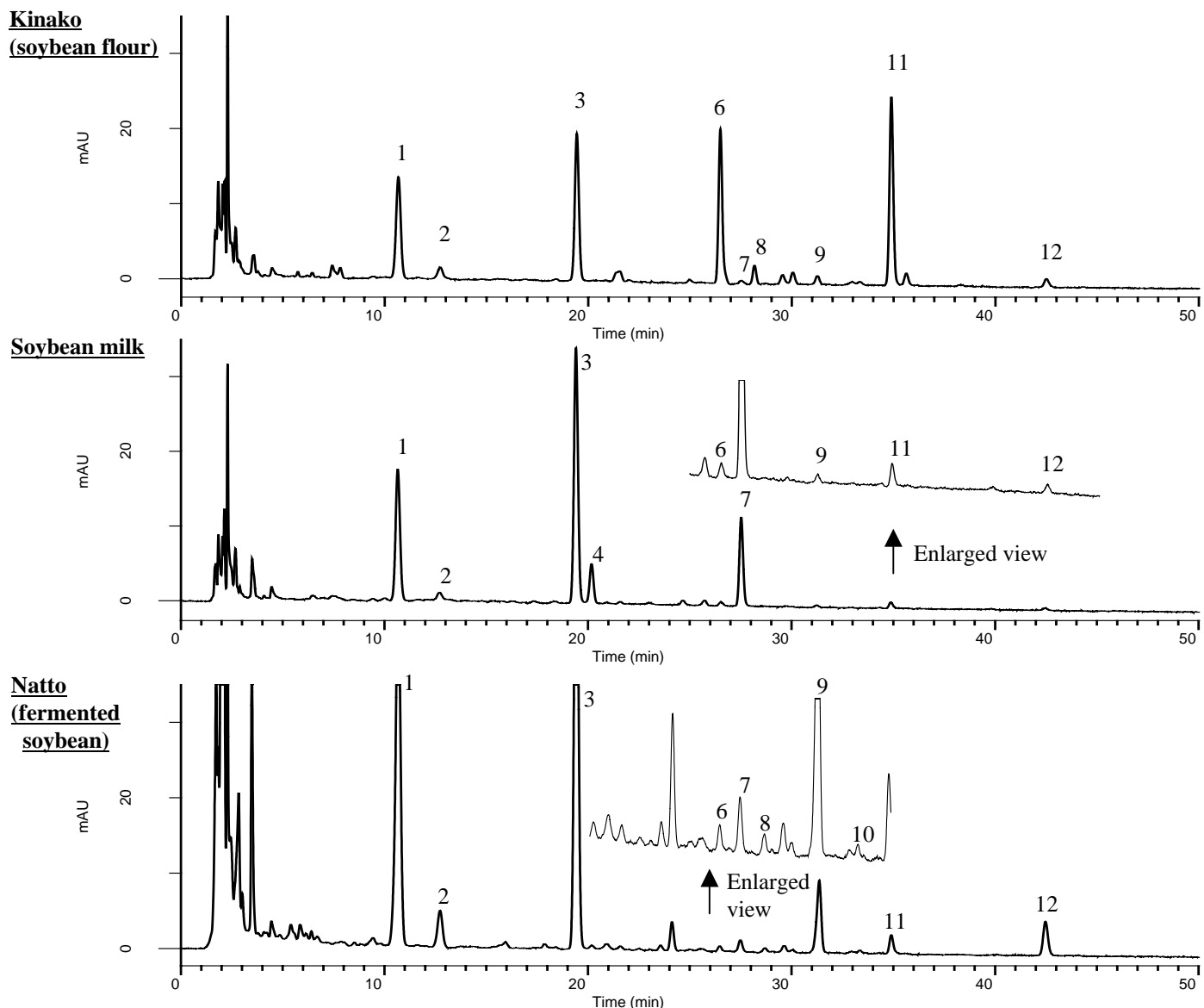
**Inj. Vol.** : 10 μL

Calibration curves and their correlation coefficients

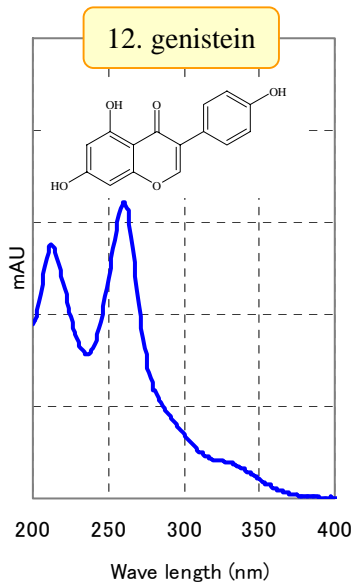
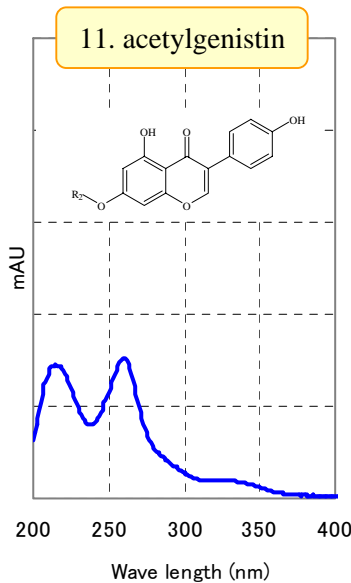
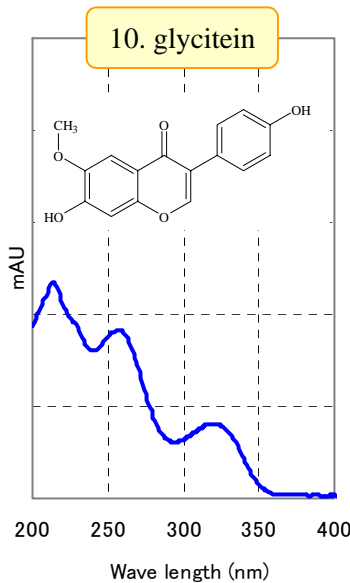
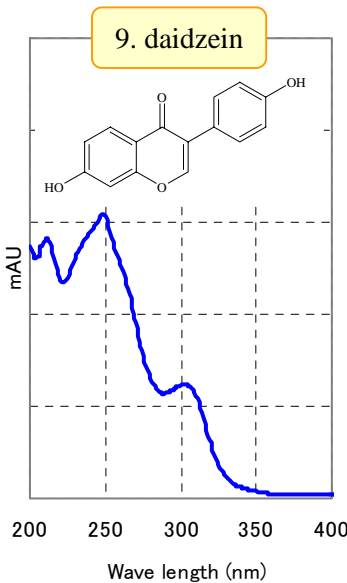
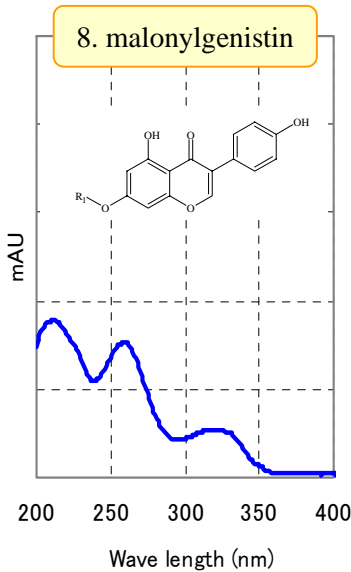
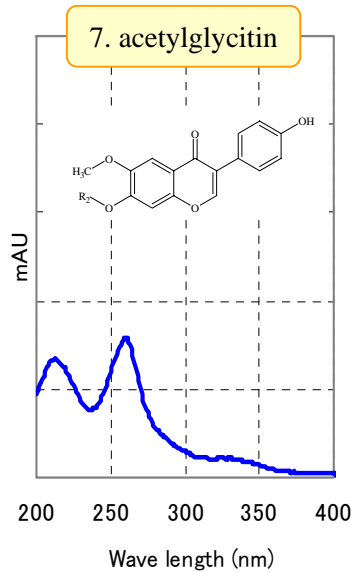
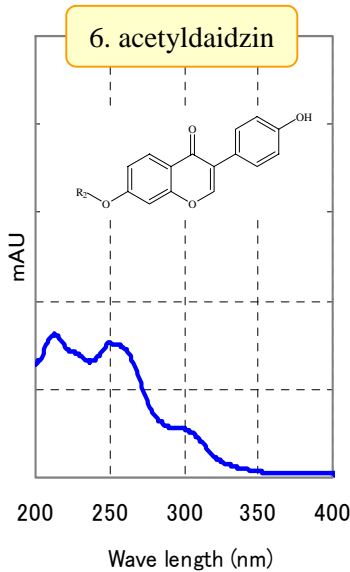
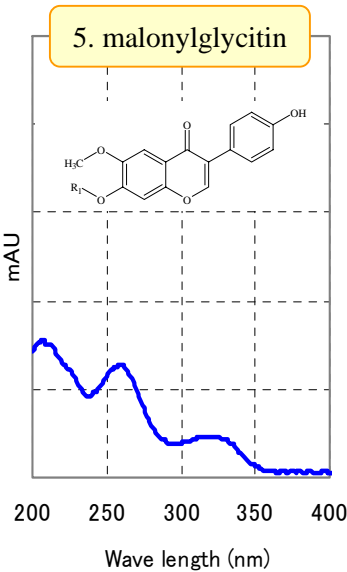
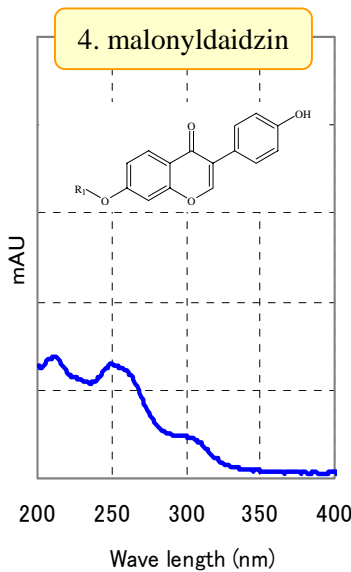
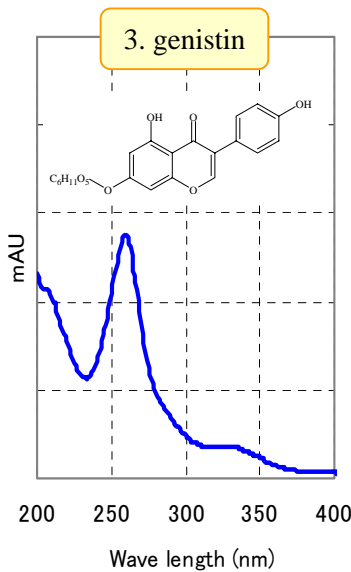
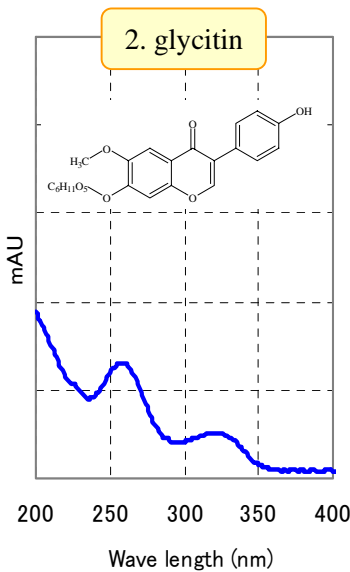
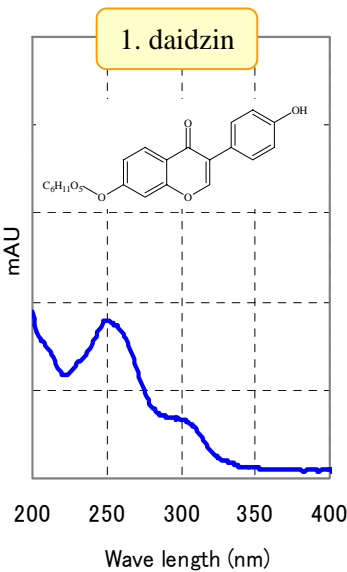
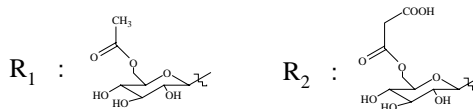
## Examples of sample pretreatment



## Chromatograms obtained from soy food samples



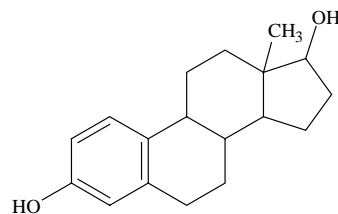
**The chemical structures and the absorption spectra**



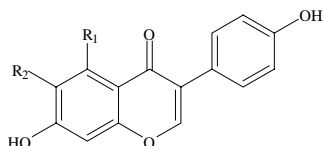
※ The absorbance scale of each spectrum was all the same.

Coffee Break

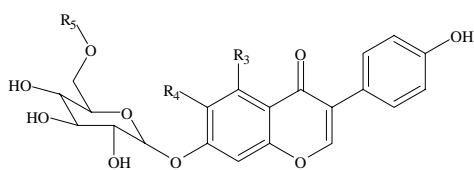
Since isoflavones are similar to estrogens (e.g. estradiol shown on the right) in chemical structure, isoflavones are also classified as phytoestrogen. It is suggested that isoflavones attenuate some estrogen-deficiency diseases such as osteoporosis due to their estrogen-like effect.



Estradiol



Isoflavones



Isoflavone glycosides

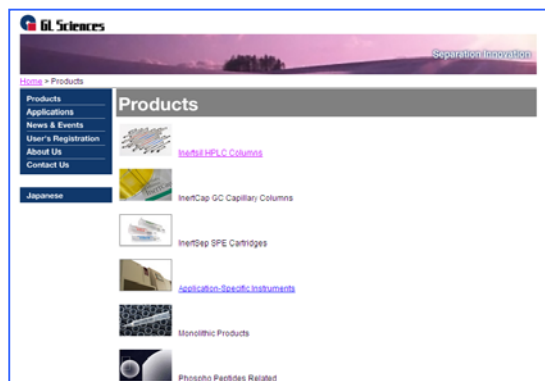
	R1	R2	R3	R4	R5
Daidzein	: H	: H	: H	: H	: H
Genistein	: OH	: H	: OH	: H	: H
Glycitein	: H	: OCH <sub>3</sub>	: H	: OCH <sub>3</sub>	: H
Daidzin	: H	: H	: H	: H	: H
Genistin	: OH	: H	: OH	: H	: H
Glycitin	: H	: OCH <sub>3</sub>	: H	: OCH <sub>3</sub>	: H
6''-O-Acetyldaidzin	: H	: H	: H	: H	: COCH <sub>3</sub>
6''-O-Acetylgenistin	: OH	: H	: OH	: H	: COCH <sub>3</sub>
6''-O-Acetylglycitin	: H	: OCH <sub>3</sub>	: H	: OCH <sub>3</sub>	: COCH <sub>3</sub>
6''-O-Malonyldaidzin	: H	: H	: H	: H	: COCH <sub>2</sub> COOH
6''-O-Malonylgenistin	: OH	: H	: OH	: H	: COCH <sub>2</sub> COOH
6''-O-Malonylglycitin	: H	: OCH <sub>3</sub>	: H	: OCH <sub>3</sub>	: COCH <sub>2</sub> COOH
6''-O-Succinyldaidzin	: H	: H	: H	: H	: COC <sub>2</sub> H <sub>4</sub> COOH
6''-O-Succinylgenistin	: OH	: H	: OH	: H	: COC <sub>2</sub> H <sub>4</sub> COOH

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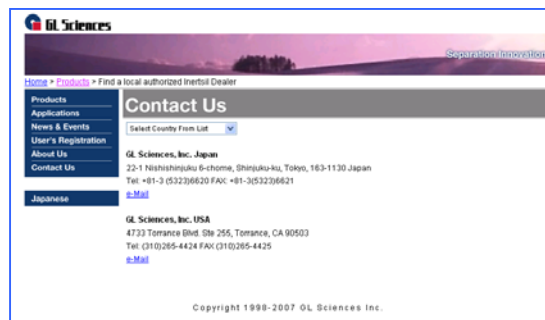
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