CAPSULES notes and applications from BASi
December 2001
364

Analysis of Isoflavones in Natural Sources and Nutritional Supplements by LC and Multi-Channel EC Detection

Purpose

Separation, identification and determination of four isoflavones, daidzin, genistin, daidzein and genistein, in various natural sources and dietary supplements by LC and multi-channel EC detection.

Isoflavones, a subclass of flavonoids, are a group of plant polyphenolic compounds. They have attracted a great deal of public attention because of their potential in the prevention and treatment of a number of chronic diseases such as cardiovascular disease, osteoporosis, and hormone-related cancers. Four isoflavones (**F1**) occur both in the free state and as glycosides.

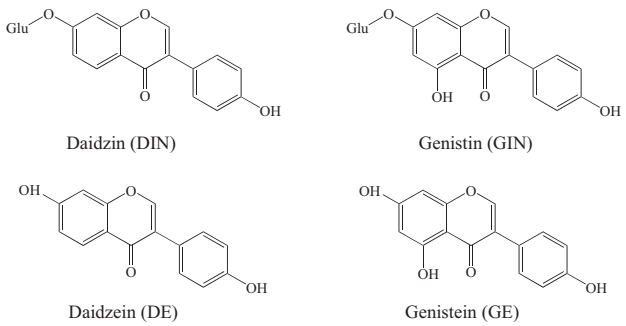

Figure 1. The structures of four isoflavones.

Conditions

LCEC System: BAS 480e chromatograph with a multi-channel amperometric detector (epsilon™, BAS, West Lafayette, IN) and ChromGraph version 2.00 software

Electrode: Four 2 mm glassy carbon working electrodes

Potential: +1100, 950, 850 and 750 mV vs. Ag/AgCl

Column: C_8 5 µm column (150 x 2.0 mm, BAS, West Lafayette, IN)

Mobile phase:

A: 9.3% acetonitrile, 5.9% methanol and 84.8% aqueous buffer (25 mM ammonium acetate, pH4.3, 0.25 mM EDTA)

B: 19.6 % acetonitrile, 12.0% methanol and 68.4% aqueous buffer (25 mM ammonium acetate, pH4.3, 0.25 mM EDTA)

Gradient cycle: 100% A for 1 min, 100% A to 80% A over 8 min, 80% A to 0% A over 1 min, 0% A for 10 min, 0% A to 100% A over 1 min, 100% A for 6 min.

Flow rate: 0.6 ml/min

Sample Preparation

A nutritional supplement sample consisted of 15 tablets or capsules randomly chosen and pooled. The various dry foods or supplements were ground into a fine powder using a mortar and pestle. A 0.25 g portion of each powdered sample was added to 10 mL of extraction solvent (methanol:water, 8:2, v/v) and immediately sonicated in ice water for 30 minutes. The sample was vortexed and a 200 µL aliquot of solution was transferred into a microcentrifuge filter (0.45 µm) and centrifuged at 6000 g for two minutes. Following centrifugation, a 50 µL aliquot of clear solution was diluted appropriately to make sure its concentration fell into the range of the standard curve. A 20 µL volume was then injected into the LC system.

Notes

The multi-channel electrochemical detector allows for the simultaneous application of different oxidation potentials on four separate channels *(1)*. Based on the HDV's of the standard isoflavones, the four channels were set at oxidation potentials of +1100, 950, 850, and 750 mV, respectively, for all subsequent determinations carried out in this study. The chromatograms of a soy product and standard isoflavones are shown in **F2**. Peak height ratios between different oxidation potentials of a standard can be used for peak identification of the retention-equivalent peak in an unknown sample *(2-4)*. Such peak height ratios of standard and sample from **F2** are listed in **T1**. The close correlation confirms peak assignment in the sample. Full scan MS/MS experiments confirm the identification of four isoflavones in soy products.

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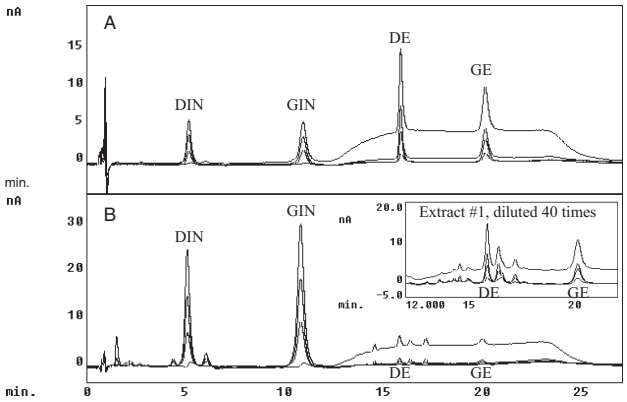

Figure 2. Chromatograms of standard mixture (A) and a diluted (200 times) extract (B) at +1100, 950 850 and 750 mV, respectively.

	750mV/850mV		850mV/950mV		950mV/1100mV	
	Standards	Extract #1	Standards	Extract #1	Standards	Extract #1
DIN	0.10	0.11	0.49	0.47	0.64	0.63
GIN	0.10	0.09	0.53	0.52	0.68	0.65
DE	0.32	0.32	0.59	0.61	0.62	0.63
GE	0.38	0.38	0.74	0.74	0.67	0.67

Table 1. Comparison of peak height ratios for standards and retention-equivalent peaks in Extract #1. *Data from F2.*

Under the described chromatography conditions, all analytes of interest are separated from other components in the extract (**F3**). Peak heights at +950 mV were plotted against varying concentrations of standards. The calibration curves were obtained by linear regression on the data sets. Linearity was found in the range of 125-4000 nM for daidzin and genistin, and 60-2000 nM for daidzein and genistein. Correlation coefficients (r^2) were greater than 0.9996 in all cases. The detection limit was determined to be 33 nM for daidzin and genistin, and 16 nM for daidzein and genistein, based on a signal-to-noise ratio of 3:1. The content of daidzein, genistein, daidzin and genistin in various dry food and nutritional supplements was measured (**T2**).

References

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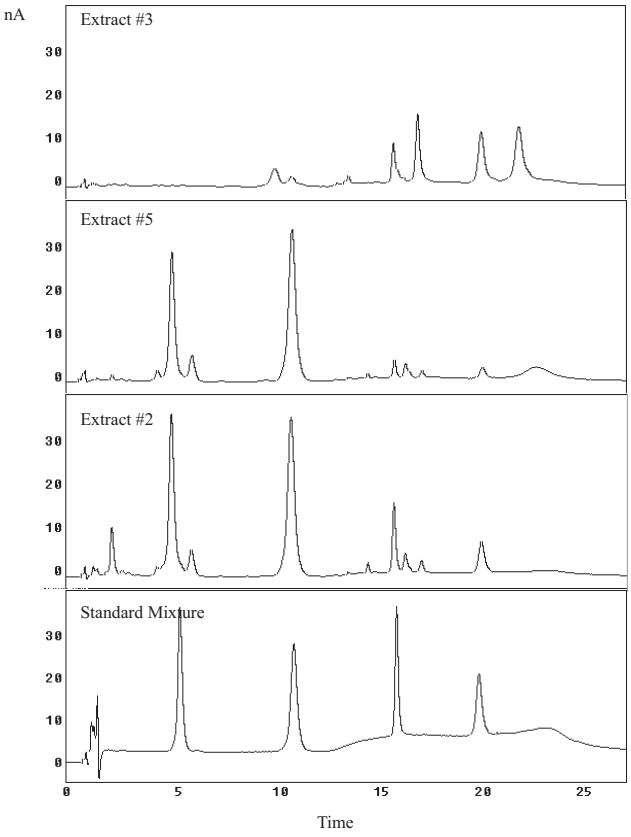

Figure 3. Chromatograms of standard mixture and representative extracts at an applied potential of +950 mV.

Sample	DIN	GIN	DE	GE
Puraria	212 (6.7)	14.0 (1.5)	182 (2.2)	33.2 (1.1)
Dried soybean	138 (13)	158 (9.7)	2.8 (0.5)	4.6 (0.3)
Soy flour	94.3 (2.5)	117 (14)	*	3.0 (0.9)
Regular rat food	34.3 (4.0)	56.2 (3.7)	*	4.9 (0.3)
NS #1	6340 (95)	10000 (104)	118 (3.6)	185 (17)
NS #2	9810 (309)	11500 (1160)	1210 (91)	1390 (78)
NS #3	67.1 (17)	199 (37)	663 (113)	1810 (189)
NS #4	991 (224)	125 (23)	453 (50)	54.0 (9.5)
NS #5	4810 (483)	7570 (1764)	287 (49)	429 (36)
NS #6	3020 (428)	5330 (223)	209 (28)	366 (23)

*Table 2. Content of isoflavones (µg/g) in natural sources and nutritional supplements. Standard deviations (n=3, independent sampling and sample preparation) are listed in parentheses. NS: nutritional supplement. *: Not detected.*