



## High Speed Separation of Isoflavones in Soybean Milk

### Introduction

Isoflavones are polyphenolic compounds produced in Fabaceae such as soybeans. They act as phytoestrogens in mammals. They are also strong antioxidants and considered useful in treating cancer. Common isoflavones include daizin, genistin, glycitin, daidzein, genistein, and glycitein.

We have demonstrated, using extreme high-pressure liquid chromatography ( X-LC ) the analysis of isoflavones. The results were obtained using an X-PressPak C18S (2.1 mm I.D. x 50 mmL., 2  $\mu$ m) column combined with a high-pressure gradient method described below. Total analysis time was approximately 4 minutes.



## Experimental

The system used for the measurement consisted of two 3185PU pumps, 3080DG degasser, 3180MX mixing unit, 3067CO column oven, 3070UV UV/Vis detector 3059AS autosampler and a chromatography data system.

Soy milk (2 mL) is mixed with methanol (2 mL), incubated for 90 minutes at 60°C, mixed with hexane (1 mL), and centrifuged. The supernatant is filtered with 0.45 µm membrane filter. A portion of 1 µL is injected.

## Results and Discussion

Figure 1 shows the chromatogram of a standard mixture of isoflavones. Each component is sufficiently separated; the analysis time is 4 minutes and the time from injection to injection is 7.5 minutes.

Figure 2 shows the chromatogram of soy milk. Each component is well separated from unknown peaks.

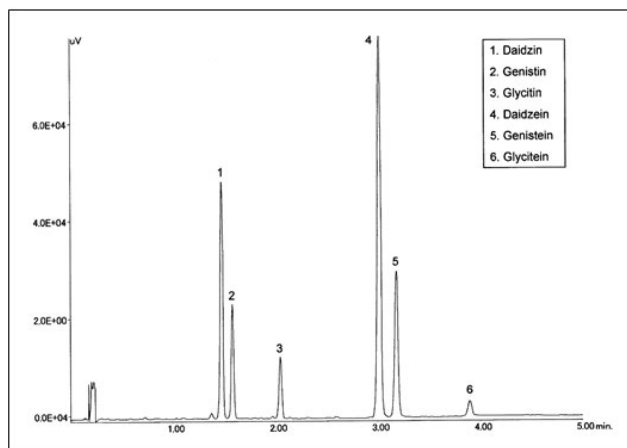


Figure 1. X-LC chromatogram of a standard mixture of isoflavone.

Conditions: column = X-PressPak C18S (2.1 mm I.D. x 50 mmL.), column temperature = 40°C, Mobile phase: A = acetonitrile/water/acetic acid (10/90/0.1), B = acetonitrile/water/acetic acid (40/60/0.1), 0 min = A/B(100/0) — 5 min = A/B(0/100) — 5.5 min = A/B(0/100) — 5.55 min = A/B(100/0), flow rate = 0.6 mL/min, detection wavelength = 254 nm, injection volume = 1 µL

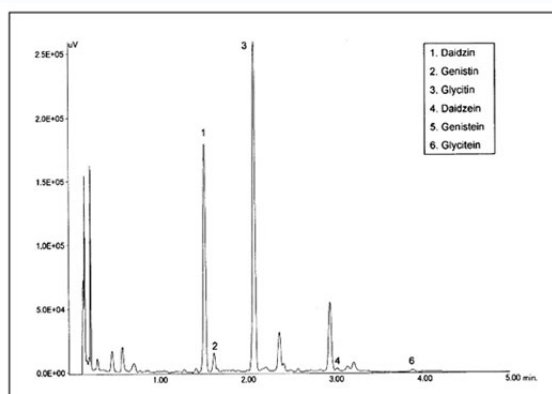


Figure 2 Chromatogram of soybean milk. The conditions are the same as in figure 1. Pretreatment: 1. Add methanol (2 mL) to soybean milk (2 mL), 2. Stand at 60°C for 1.5 hours, 3. Add Hexane (1 mL) and mix, 4. The hexane layer is filtered.