Fast analysis of isoflavonoids in food

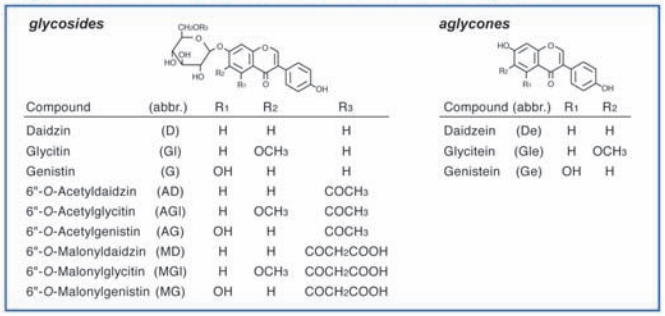
by ***N. Shoji, M. Moriyama, Dr. N. Kuriyama*** *- YMC Co. Ltd. YMC Karasuma-Gojo Bld. 284 Daigo-cho, Karasuma Nishiiru Gojo-dori Shimogyo-ku, Kyoto 600-8106, Japan*

C. Stammel *- YMC Europe GmbH, Schöttmannshof 19, D-46539 Dinslaken, Germany*

As soy is the most important source of vegetable oil worldwide, it contributes essentially to a balanced diet. Secondary components such as isoflavonoids have a significant positive effect on the hormonal balance. However, adverse effects can occur. The following method for a fast and robust separation of isoflavonoids will facilitate the analysis of these food ingredients.

Post-menopausal disorders due to hormonal imbalance are often reduced by phytoestrogenes. Theses hormones are highly effective in the prophylaxis of hot flushes, osteoporosis and atherosclerosis and especially beneficial on decreasing the risk of cancer. One natural source of these natural products is soybeans. Epidemiological studies indicate that Japanese and Chinese women suffer less from the effects of the post-menopausal disorders due to their high consumption of soy products. It is likely that isoflavonoids from soybeans reduce the side effects of the menopause. Apart of the positive effects, recent studies have revealed that adverse side effects can occur. Women affected by breast cancer or even with just a higher risk of breast cancer should avoid phytoestrogenes. As a result, a healthy recommended daily allowance should be carefully calculated, especially for children. For these reasons, it is necessary to develop an easy, reliable and fast method for the determination of isoflavonoids on a routine base.

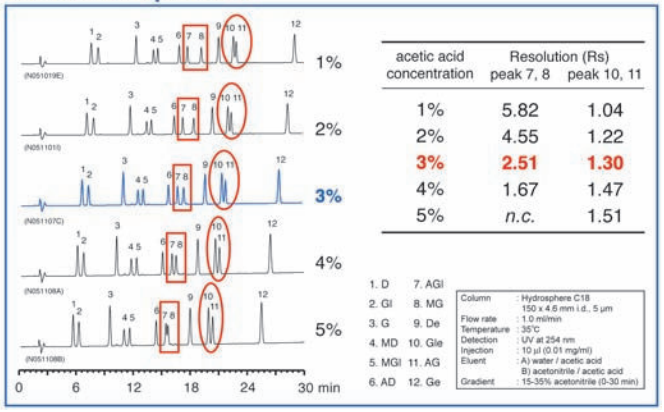
Figure 1: Structures of 12 isoflavones in soybeans



Compound	(abbr.)	R_1	R_2	R_3
Daidzin	(D)	H	H	H
Glycitin	(GI)	H	OCH_3	H
Genistin	(G)	OH	H	H
6"-*O*-Acetyldaidzin	(AD)	H	H	$COCH_3$
6"-*O*-Acetylglycitin	(AGI)	H	OCH_3	$COCH_3$
6"-*O*-Acetylgenistin	(AG)	OH	H	$COCH_3$
6"-*O*-Malonyldaidzin	(MD)	H	H	$COCH_2COOH$
6"-*O*-Malonylglycitin	(MGI)	H	OCH_3	$COCH_2COOH$
6"-*O*-Malonylgenistin	(MG)	OH	H	$COCH_2COOH$

Compound	(abbr.)	R_1	R_2
Daidzein	(De)	H	H
Glycitein	(Gle)	H	OCH_3
Genistein	(Ge)	OH	H

Figure 2: Influence of acetic acid concentration on soy isoflavone separation



acetic acid concentration	Resolution (Rs) peak 7, 8	peak 10, 11
1%	5.82	1.04
2%	4.55	1.22
3%	**2.51**	**1.30**
4%	1.67	1.47
5%	*n.c.*	1.51

1. D 7. AGI
2. GI 8. MG
3. G 9. De
4. MD 10. Gle
5. MGI 11. AG
6. AD 12. Ge

Column	: Hydrosphere C18 150 x 4.6 mm i.d., 5 µm
Flow rate	: 1.0 ml/min
Temperature	: 35°C
Detection	: UV at 254 nm
Injection	: 10 µl (0.01 mg/ml)
Eluent	: A) water / acetic acid B) acetonitrile / acetic acid
Gradient	: 15-35% acetonitrile (0-30 min)

Soybeans contain nine glycosidic and three aglycosidic isoflavonoids (see). The analysis of these compounds is difficult since they are structurally very similar. Common reversed phase media do not have the ability to separate these substances due to a poor selectivity towards polar analytes.

The method described here uses the well established YMC Hydrosphere C18. Due to its hydrophilic endcapping, this material is especially suited for the separation of polar compounds. The conventional HPLC was performed on a Shimadzu LC-VP-system with 3µm or 5 µm particle size column (4.6 mm id). The ultra-fast LC was performed with 2 µm YMC UltraHT Hydrosphere C18 on a JASCO X-LC-system. The isoflavonoids were extracted from the crude matrix by stirring with a 50:50 water/ethanol mixture at room temperature for one hour. After filtration (filter paper No. 5A) the samples were prepared for HPLC analysis by use of a syringe filter (0.2 µm). Initial experiments showed very quickly that the method would be successful using gradient elution with water/acetonitrile and acetic acid (see figure 2, chromatogram a). Further optimisation was achieved by varying the acetic acid content. Peaks 10 and 11 (Glyciteine and 6´´-O-acetylgenistine) were baseline separated with a high percentage of acetic acid. However, under these conditions the resolution of peaks 7 and 8 (6´´-O-Acetylglycitine and 6´´-O-Malonylgenistine) was poor. The concentration was then reduced to 3 %. The result was nearly baseline separation of all compounds as shown in figure 2, chromatogram c) (Column: YMC Hydrosphere C18, 5 µm, 150 x 4.6 mm id). The analysis time of 30 min could be reduced substantially by conventional means of reducing particle size and column dimension (3 µm, 50 x 4.6 mm id). To get the same results in terms of the chromatographic behaviour it is of importance to keep a constant gradient

Figure 3: Optimization of conventional LC conditions with 3 µm particles

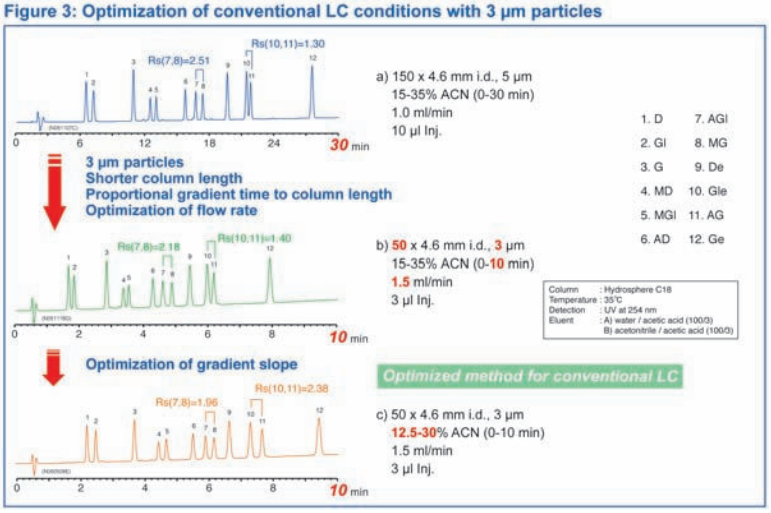


Figure 4: Method transfer from conventional LC with 3 µm to ultra-fast LC with 2 µm

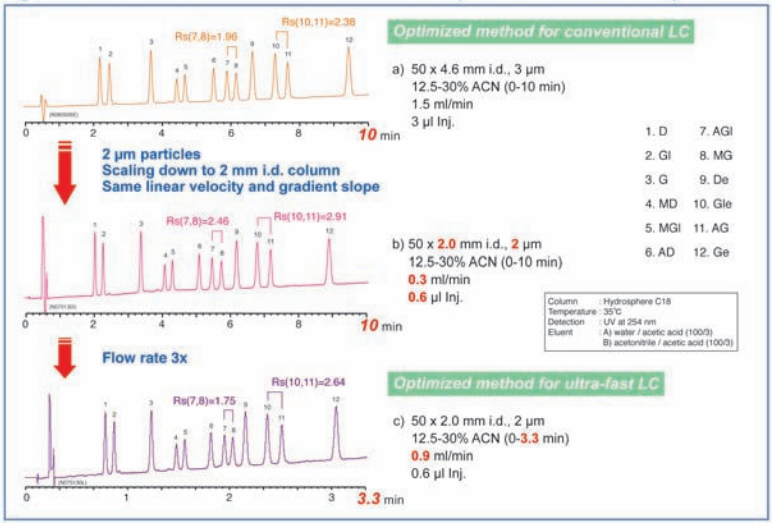
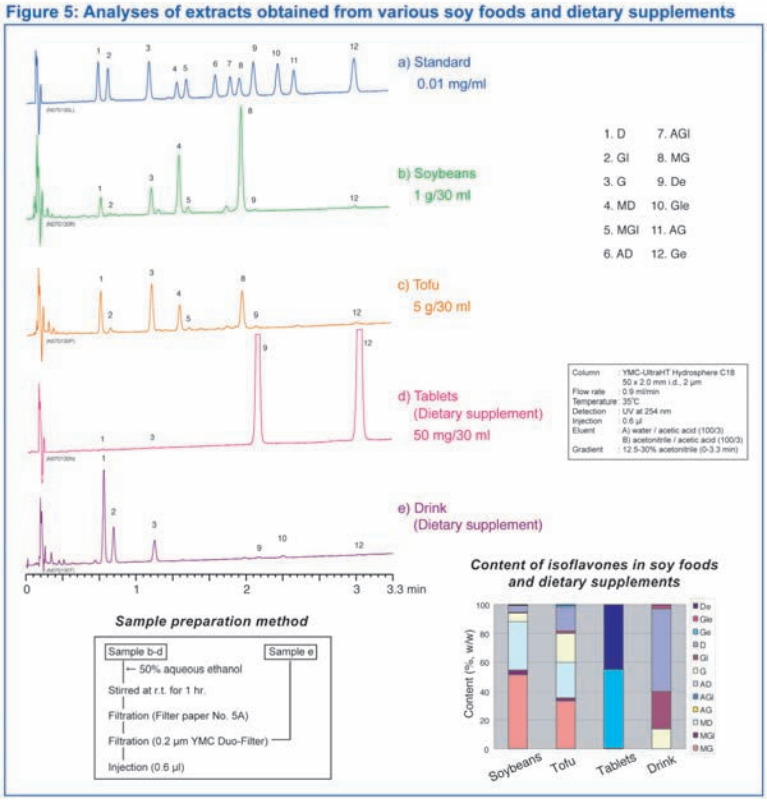


Figure 5: Analyses of extracts obtained from various soy foods and dietary supplements



volume. Figures 3a and 3b show the method transfer to a 50 x 4.6 mm id column. Increasing the flow rate to 1.5 mL/min was necessary to maintain the resolution and elution profile. Adjusting the gradient profile (figures 3b and 3c) led to a baseline resolution of the critical peak pair 10 and 11.

This conventional method was then transferred to ultra-fast analysis on a JASCO high pressure system using 2 µm particles. After modifying the chromatographic parameters the flow rate was again increased which reduced the analysis time in total by a factor of 10 (see figure 4).

Conclusion

The objective of this study was the development of a method for the determination of isoflavonoids in soy-containing foods. The method transfer from conventional to ultra-fast HPLC systems was successful when using YMC UltraHT Hydrosphere C18 with 2 µm particle size. The analysis of isoflavonoids was demonstrated by the determination of the isoflavonoid content of soybeans and Tofu. Figure 5 demonstrates that this method is suitable for the quantitative analysis of real samples.