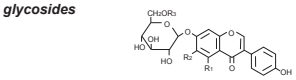
APPLICATION NOTE

YMC EUROPE GMBH

Fast analysis of isoflavonoids in food

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.

Structures of 12 isoflavones in soybeans

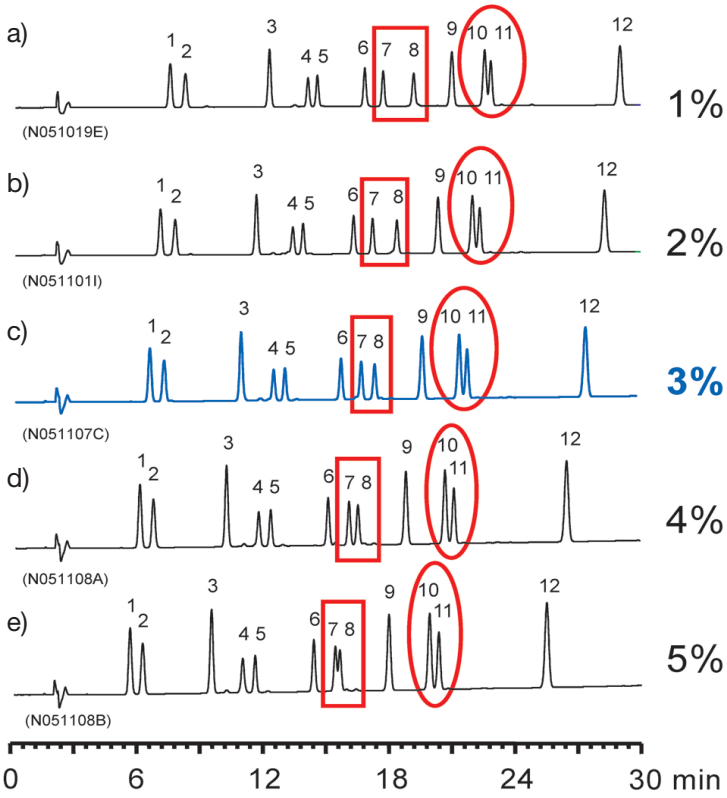


Compound	(abbr .)	R_1	R_2	R_3
Daidzin	(D)	H	H	H
Glycitin	(Gl)	H	OCH_3	H
Genistin	(G)	OH	H	H
6"-O-Acetyldaidzin	(AD)	H	H	$COCH_3$
6"-O-Acetylglycitin	(AGl)	H	OCH_3	$COCH_3$
6"-O-Acetylgenistin	(AG)	OH	H	$COCH_3$
6"-O-Malonyldaidzin	(MD)	H	H	$COCH_2COOH$
6"-O-Malonylglycitin	(MGl)	H	OCH_3	$COCH_2COOH$
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 1

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
2. Gl
3. G
4. MD
5. MGl
6. AD
7. AGl
8. MG
9. De
10. Gle
11. AG
12. Ge

Column:	YMC Hydrosphere C18 (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	HS12S051546WT
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% B (0-30 min)

Figure 2

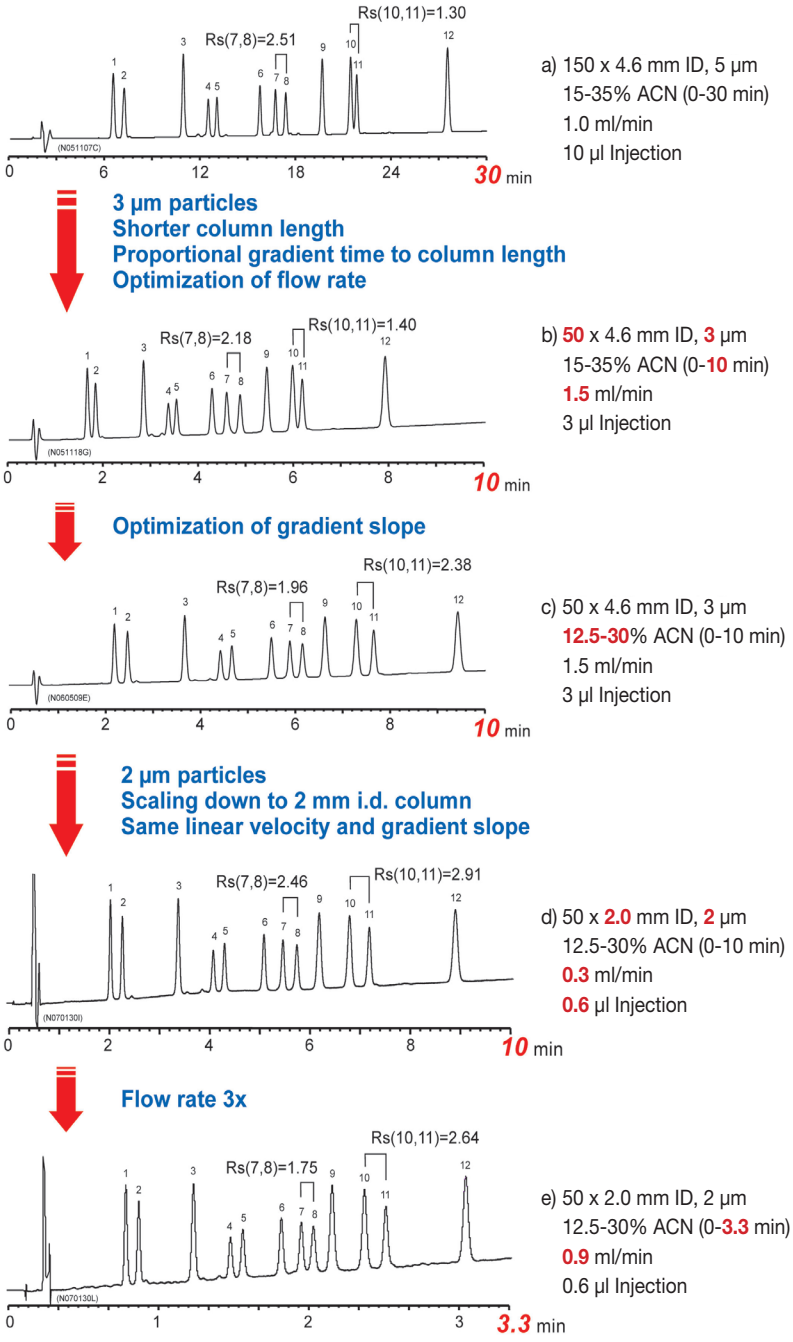
The isoflavonoids were extracted from the crude matrix by stirring with a 50:50 water/ethanol mixture at room temperature for one hour. After filtration (filter paper No. 5A) the samples were prepared for HPLC analysis by use of a syringe filter (0.2 µm). Initial experiments showed very quickly that the method would be successful using gradient elution with water/acetonitrile with acetic acid to control pH (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´´-OMalonylgenistine) was poor. Reduction of the acetic acid to 3% resulted in near baseline separation of all 7 compounds (see figure 2, chromatogram c).

APPLICATION NOTE

YMC EUROPE GMBH

Fast analysis of isoflavonoids in food

Method transfer from conventional LC to ultra-fast LC



a) 150 x 4.6 mm ID, 5 µm
15-35% ACN (0-30 min)
1.0 ml/min
10 µl Injection

b) 50 x 4.6 mm ID, 3 µm
15-35% ACN (0-10 min)
1.5 ml/min
3 µl Injection

c) 50 x 4.6 mm ID, 3 µm
12.5-30% ACN (0-10 min)
1.5 ml/min
3 µl Injection

d) 50 x 2.0 mm ID, 2 µm
12.5-30% ACN (0-10 min)
0.3 ml/min
0.6 µl Injection

e) 50 x 2.0 mm ID, 2 µm
12.5-30% ACN (0-3.3 min)
0.9 ml/min
0.6 µl Injection

Figure 3

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 volume. Figures 3a and b 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 c) 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 figures 3d and e).

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 (12 nm)
Temp.:	35 °C
Detection:	UV at 254 nm
Eluent:	A: water / acetic acid (100/3)
	B: acetonitrile / acetic acid (100/3)

Conclusion

The aim of this study was the development of an ultra-fast 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.

* Application data by courtesy YMC Co., Ltd.