

## Food/Nutraceutical

# Qualitative Analysis of Soy Isoflavone Compounds Using UHPLC/SQ MS



Isoflavones are non-steroidal compounds with estrogenic effects and can be found in many foods; the best known source is the soy bean (*Glycine max*). Recent studies have demonstrated that isoflavones have potent antioxidant properties which can reduce the long-term risk of cancer by preventing free radical damage to DNA. They can also be used for treating menopause and osteoporosis.

A quantitative UHPLC/SQ MS method is presented to detect isoflavone aglycones (genistein, daidzein and glycitein) and isoflavone glycosides (genistin, daidzitin and glycitin) using a single quadrupole MS detector and its front-end Collision Induced Dissociation (CID) capability.

## Experimental Conditions

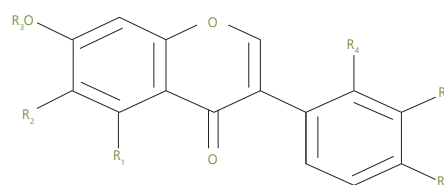
Target Analytes: Daidzitin, glycitin, genistin, daidzein, glycitein, genistein

### Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar™ FX-15		
Column:	PerkinElmer Brownlee™ Analytical C18 column (2.1 mm x 100 mm, 3 μm)		
Mobile Phase:	A: acetonitrile with 5 mM acetic acid B: water with 5 mM acetic acid		
Flow Rate:	0.5 mL/min		
Injection Volume:	2.5 μL		
Gradient:	Time (min)	%A	%B
	0	15	85
	6	45	55

### Mass Spectrometer Conditions

Ionization:	Ultraspray™ ESI – Positive and Negative modes
Selected Ion Monitoring (SIM) Mode:	Reported in Figure 1; dwell time of 100 ms each
Capillary Exit Voltage:	100 V



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	[M-H] <sup>-</sup>	[M+H] <sup>+</sup>
Daidzitin	H	H	Glucose	H	H	OH	415	417
Glycitin	H	OCH <sub>3</sub>	Glucose	H	H	OH	445	447
Genistin	OH	H	Glucose	H	H	OH	431	433
Daidzein	H	H	H	H	H	OH	253	255
Glycitein	H	OCH <sub>3</sub>	H	H	H	OH	283	285
Genistein	OH	H	H	H	H	OH	269	271

Figure 1. Structures of isoflavones.

## Results

A 6 minute UHPLC/SQ MS method was developed for measurement of 6 isoflavone compounds (Figure 2). This study determined that negative ion mode is more suitable than positive ion mode for 4 out of 6 isoflavone compounds (Figure 3) analyzed (genistein, genistin, diadzein and daidzin). The other two compounds (glycitein and glycitin), which contain a methoxy group, have comparable sensitivity in both ionization modes. Using this optimized method, the detection limit of 6 isoflavone compounds ranged from 0.56 to 2.29 pg.

## Reference

ASMS 2010 Poster: Effect of Ion Polarity on the Determination of Specific Isoflavone Compounds Using LC-MS

*Avinash Dalmia; et al.*

PerkinElmer Inc., Shelton, CT 06484

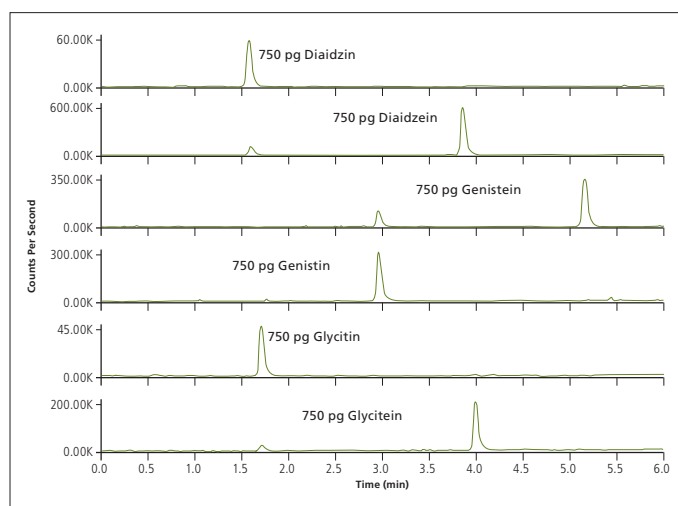


Figure 2. SIM chromatograms of isoflavones in negative ion mode.

Negative vs. Positive Ion Sensitivity				
Compound	S/N Position Ion Mode	S/N Negative Ion Mode	LOD (pg) Positive Ion Mode	LOD (pg) Negative Ion Mode
Genistein	1282	2318	1.75	1.03
Diadzein	1329	3023	1.69	0.74
Glycitein	1054	1008	2.13	2.23
Genistin	1016	3978	2.21	0.56
Daidzin	668	1084	3.38	2.07
Glycitin	861	982	2.61	2.29

Figure 3. Negative ion mode is more sensitive than positive ion mode for 4 out of 6 isoflavone compounds. The other two compounds (glycitein and glycitin) have comparable sensitivity in both ionization modes. S/N has been calculated for a concentration of 750 pg each.