

# Analysis of Soy Isoflavones from a Dietary Supplement Using UPLC® with PDA and SQ Detection

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The consumption of soy products has been linked to many health benefits, as they contain isoflavones. Isoflavones are commonly known as phytoestrogens and 12 isoflavones found in soybeans are daidzein (De), glycitein (Gle) and genistein (Ge) and their respective malonyl (6''-O-malonyl-β-glucoside-), acetyl (6''-O-acetyl-β-glucoside-) and glucosyl (β-glucoside-) forms<sup>1</sup>. Their structures are shown in Figure 1.

Many research studies have indicated that consumption of isoflavone-containing functional foods are associated with a wide variety of health benefits, including prevention of breast and prostate cancers, cardiovascular disease and reduced symptoms of diabetes and postmenopausal bone loss<sup>2-6</sup>. These functional foods include soy milk and soy flour.

The approval by the U.S. Food and Drug Administration in 1999, allowing the food industry to promote soy protein for heart health<sup>7</sup>, led to an escalation in sales of soy foods as functional foods. These foods are also being promoted for their isoflavones content.

This application note provides a rapid method for using reversed-phase UPLC® to detect and characterize the isoflavone glucoside conjugates present in a commercial soy nutritional supplement using PDA and MS detection.

## Experimental

The isoflavones were extracted from the soy capsules using 9:1 methanol/water liquid extraction and sonification for 10 minutes. The extract was filtered through a 0.45 μm filter.

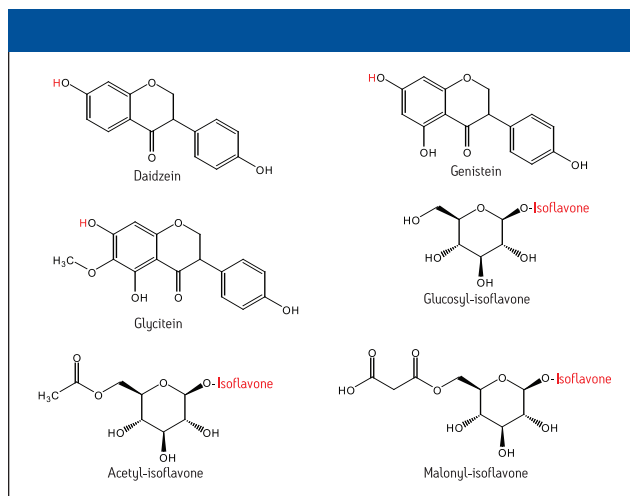
The mass spectrometer was used in two modes: Full scan (m/z: 50 to 550) and single ion recording (SIR) mode.

### UPLC conditions

LC System:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C <sub>8</sub> 2.1 x 100 mm, 1.7 μm
Temperature:	35 °C
Flow Rate:	500 μL/min
Mobile	
Phase A:	0.2% formic acid in water
Mobile	
Phase B:	Methanol
Gradient:	25% B for 0.4 min, 25-40%/1.1 min Hold for 0.8 min

### PDA conditions

PDA:	ACQUITY UPLC
Wavelength	
Range:	205 to 450 nm



**Figure 1:** Structures of three soy isoflavones: daidzein, genistein and glycitein and their conjugates.

Resolution: 1.2 nm  
Sampling Rate: 20 spectra/s

### MS conditions

MS: SQ Detector  
Ionization  
Mode: ESI Positive  
Capillary  
Voltage: 2000 V

Desolvation  
Temp: 400 °C  
Desolvation  
Gas: 1000 L/Hour  
Source Temp: 130 °C

### Full scan settings

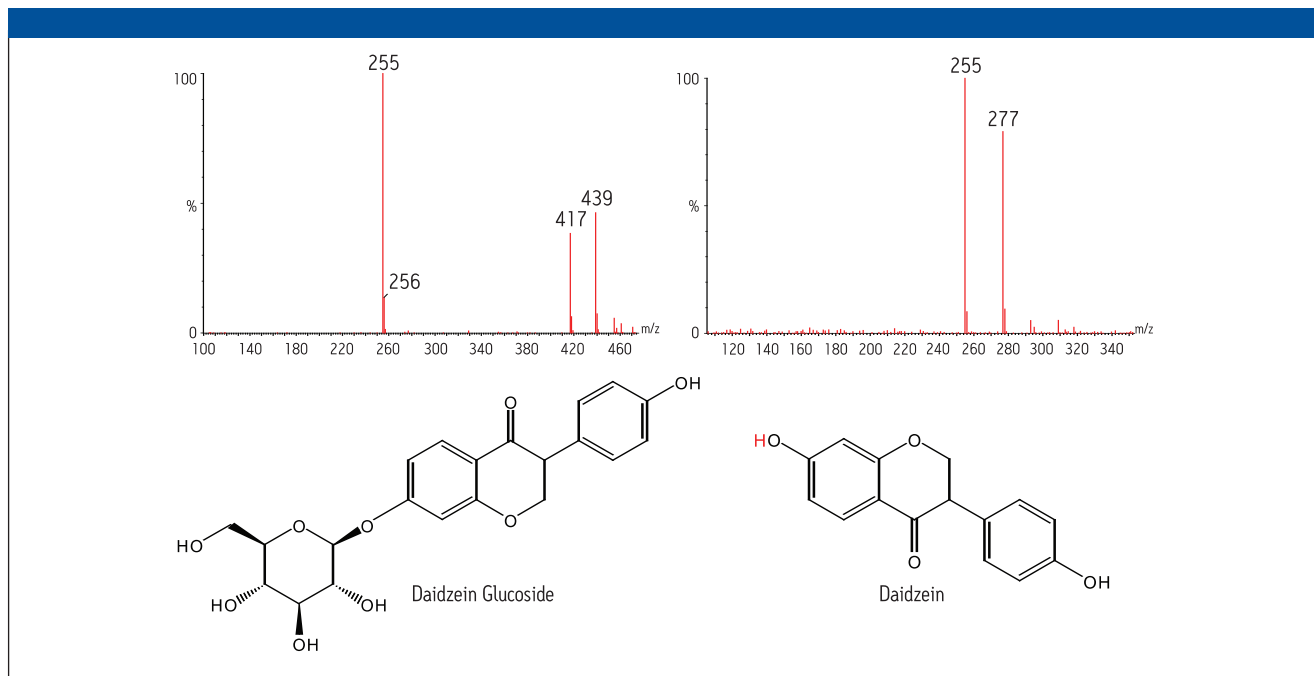
Cone Voltage: 37 V  
Acquisition  
Range: 50 to 550 m/z

### SIR settings

A dwell time of 10ms was used for each SIR and a delay of 5ms.

## Results

The relative elution order is: glucosyl, malonyl, acetyl and isoflavone and the retention times are noted in Table II. The ACQUITY UPLC C<sub>8</sub> phase was compared to the equivalent C<sub>18</sub> column: C<sub>8</sub> was found to give better separation using this solvent method.



**Figure 2:** Spectra for daidzein glucoside (daidzin) and daidzein where the parent ions in positive ESI are 417 and 255, respectively.

In this application note there are two methods described: one using full scan and the other an SIR experiment, where ions indicative of the target compounds of interest have been selected.

Full scan provides spectral information (Figure 2) from the fragmentation patterns, which can help with structural determination and is useful when identifying unknown compounds.

Examples of daidzein MS spectra using the full scan method can be seen in Figure 2. For the conjugated isoflavone systems the parent ion of the conjugate ( $[M+H]^+$ ;  $m/z$  417) was present along with the parent ion of the isoflavone ( $[M+H]^+$ ;  $m/z$  255). The  $m/z$  439 and 277 may be attributed to  $[M+Na]^+$ .

Using the full scan data it is possible to extract the ions of interest and this procedure has been performed in Figure 3A for  $m/z$  255, 271 and 285. The same procedure was performed for 260 nm from the PDA detector.

Figure 3B shows the selected ions for genistein and the genistein conjugates. The  $m/z$  153 is a product ion from the isoflavone structure (see Discussion).

**Table I. SIR settings showing cone voltages used for each  $m/z$  value.**

SIR 1 (Daidzein)		SIR 2 (Genistein)		SIR 3 (Glycitein)	
$m/z$	Cone voltage	$m/z$	Cone voltage	$m/z$	Cone voltage
137	90	153	90	167	90
255	60	271	70	285	70
417	30	433	25	447	25
459	30	475	35	489	25
503	30	519	35	533	45

For quantification experiments, SIR is preferred as it provides more sensitivity (Figure 4) than the compared extracted ion full scan data.

## Discussion

Current interest in soy isoflavones is based on a vast literature reporting a wide range of biological properties for genistein and daidzein<sup>8-10</sup> and on clinical studies supporting their potential health benefits<sup>11, 12</sup>.

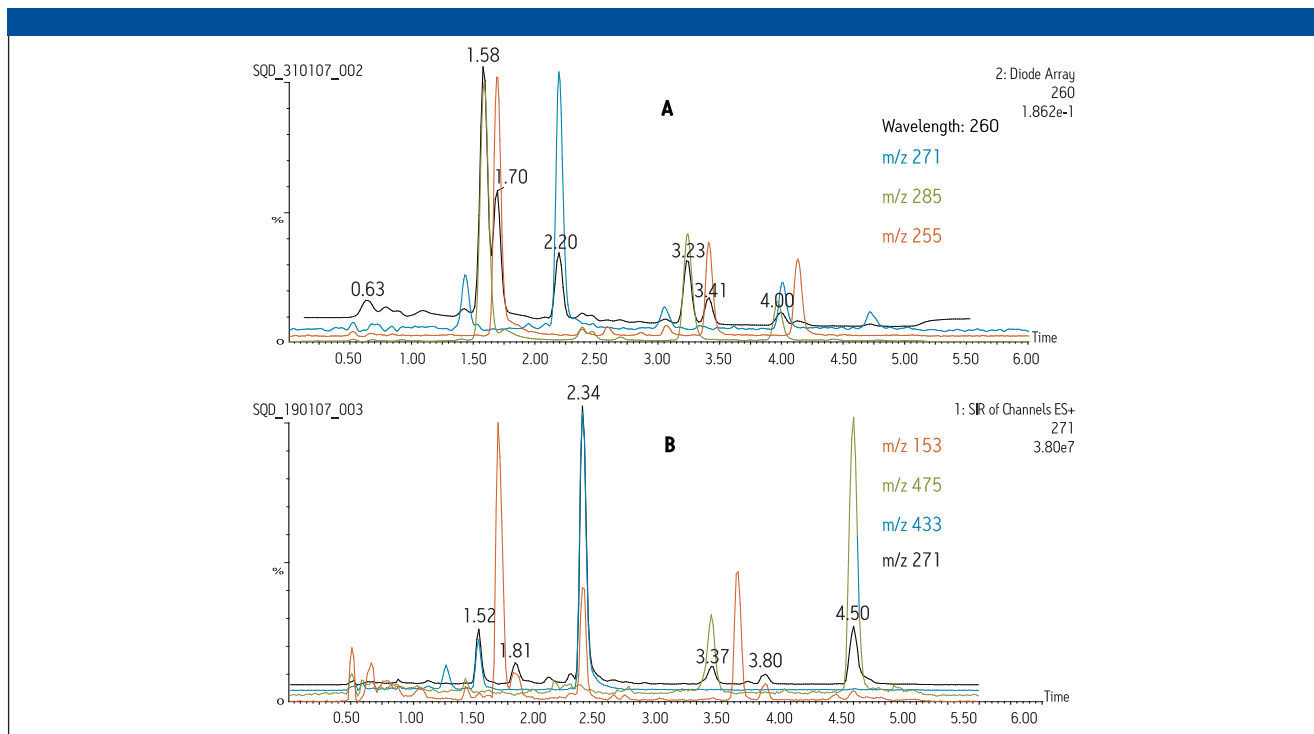
Studies using a tandem quadrupole MS have described that the isoflavones and their glucoside conjugates have a common product ion other than the  $[M+H]^+$  from the isoflavone. In the reaction pathway (Figure 5) this product ion may be assigned to  $[a+1]$ : for daidzein, genistein and glycitein the  $m/z$  values are 137, 153, and 167 respectively.

It is possible to see the  $[a+1]$  ion using the SIR method, which allows a separate higher cone voltage energy to be selected for the three ions from Table I.

## Chromatographic data points

When using mass spectrometry, in particular for quantification, it is important to have at least 10 data points across a peak for repeatable peak integration. For UPLC-based experiments where the peak widths are much smaller than comparable HPLC peaks, MS acquisition rates have to be faster to achieve this. Figure 6 shows the comparison of the data points when the dwell time is changed in SIR mode.

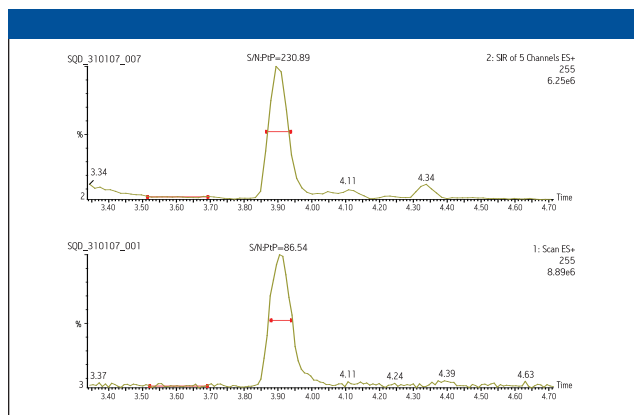
For the SIR experiment in this application note, a dwell time of 10 ms was used to achieve the recommended data points for the compounds analysed.



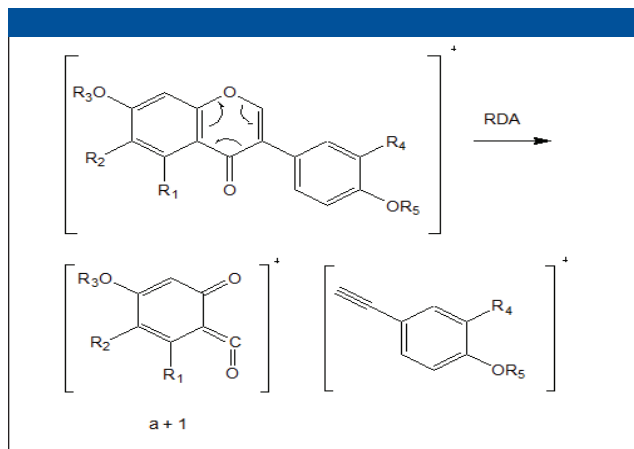
**Figure 3:** A: Selected wavelength of 260 nm and full scan MS data with  $m/z$  ions for daidzein, genistein and glycitein, extracted from the TIC and B: SIR method and the respective ions for genistein in the soy supplement.

**Table II. Retention times for the soy isoflavones and their conjugates.**

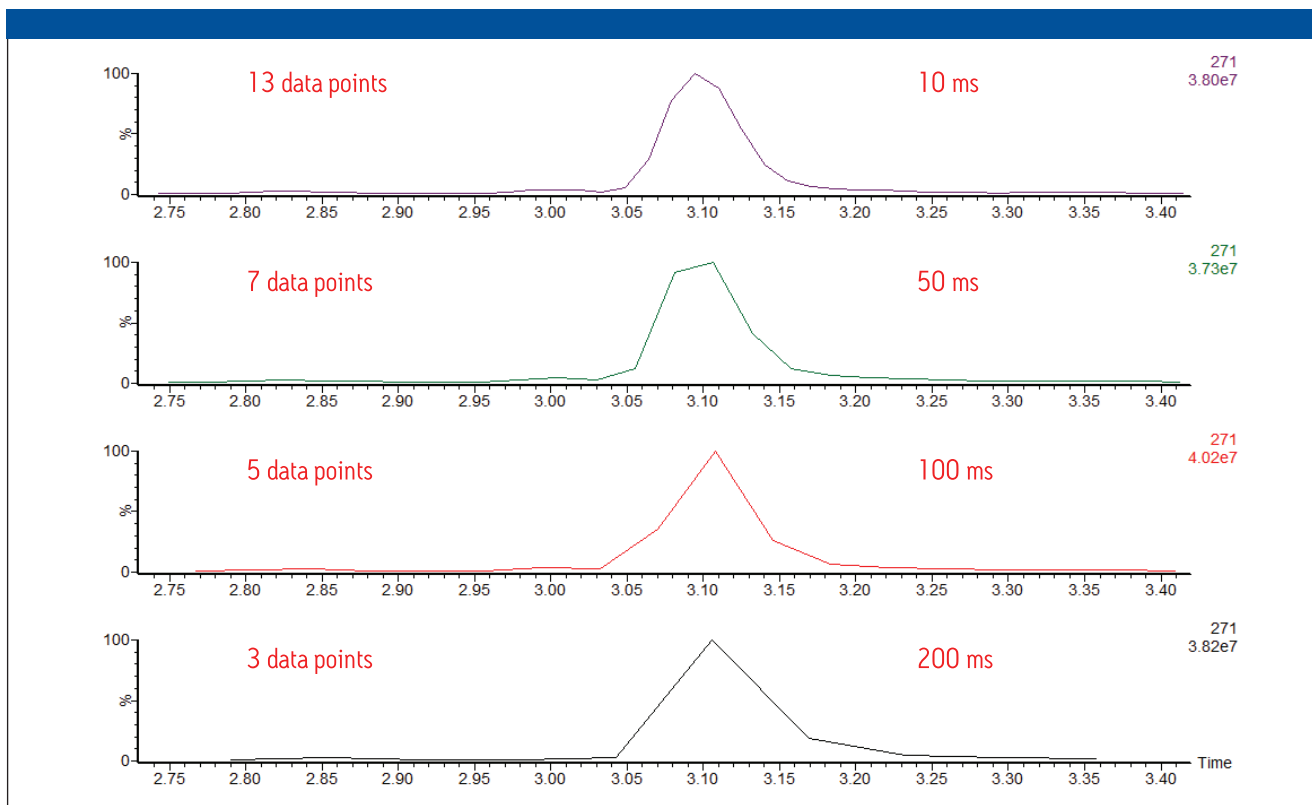
Ret. Time	Compound	[M+H]
1.59	Daidzein Glucoside	417
1.70	Glycitein Glucoside	447
2.20	Genistein Glucoside	433
2.69	Daidzein Malonyl Glucoside	503
2.86	Glycitein Malonyl Glucoside	533
3.23	Daidzein Acetyl Glucoside	459
3.41	Glycitein Acetyl Glucoside	489
3.98	Daidzein	255
4.00	Genistein Acetyl Glucoside	475
4.13	Glycitein	285
3.21	Genistein Malonyl Glucoside	519
4.72	Genistein	271



**Figure 4:** Comparison of S/N using SIR data (top) and extracted ion from full scan data (bottom).



**Figure 5:** Fragmentation pathway of daidzein.



**Figure 6:** Comparison of dwell times and data points.

## Conclusion

In this application note, a soy supplement has been used to look at the soy isoflavone content. With the increasing interest in functional foods and functional ingredients, it is also important to analyse for these compounds in the functional food and also their bio-availability in the body.

Here, a 5.5 minute method has been described using UV and MS data. For compound structural information a full scan method was used, however, if quantification is required, the SIR method is recommended as it provides better sensitivity.

## References

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