

## Application Note

# Fast Analysis of Isoflavones in Dietary Supplements – Benefits of Mass Detection in Method Transfer and Sample Analysis

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## Abstract

This application note demonstrates the benefits of the ACQUITY QDa Detector for method transfer and routine analysis of isoflavones in dietary supplements. The focus of this application note is on the qualitative data analysis.

### Benefits

- Reduced method development and transfer times
- Improved data quality and simplified peak detection with the ACQUITY QDa Mass Detector
- Minimize false peak assignments and co-elution errors in routine analyses.

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## Introduction

LC method development is always a challenging task, especially for dietary supplements from plant sources, as the target compounds are often structurally similar to each other, and the standards are not always commercially available. The UV/Vis detection method may not provide adequate detection selectivity when needed, which makes the method development and method transfer process time consuming.

Recently, an industry standard isoflavone method<sup>1</sup> was successfully transferred onto a Waters ACQUITY Arc UHPLC System equipped with a 2998 PDA Detector.<sup>2</sup> The analysis time per injection was shortened from 74 minutes to 18 minutes. During the method transfer, an ACQUITY QDa Mass Detector was simultaneously used, which helped to shorten the method development and transfer time.

The ACQUITY QDa Mass Detector is a single quadrupole mass spectrometer (MS) detector designed for routine analysis. It comes factory pre-optimized, so little MS expertise is required to operate it. The QDa is ready to operate minutes after pressing the power button. It fits neatly into Waters Alliance HPLC, ACQUITY Arc UHPLC, and ACQUITY UPLC stacks, and it can be controlled by either Empower CDS or MassLynx MS software.

This application note demonstrates the benefits of the ACQUITY QDa Detector for method transfer and routine analysis of isoflavones in dietary supplements. The focus of this application note is on the qualitative data analysis. The benefits for the quantitative data analysis will be evaluated in a future application note.<sup>3</sup>



The structures of isoflavones analyzed in this study are in Figure 1.

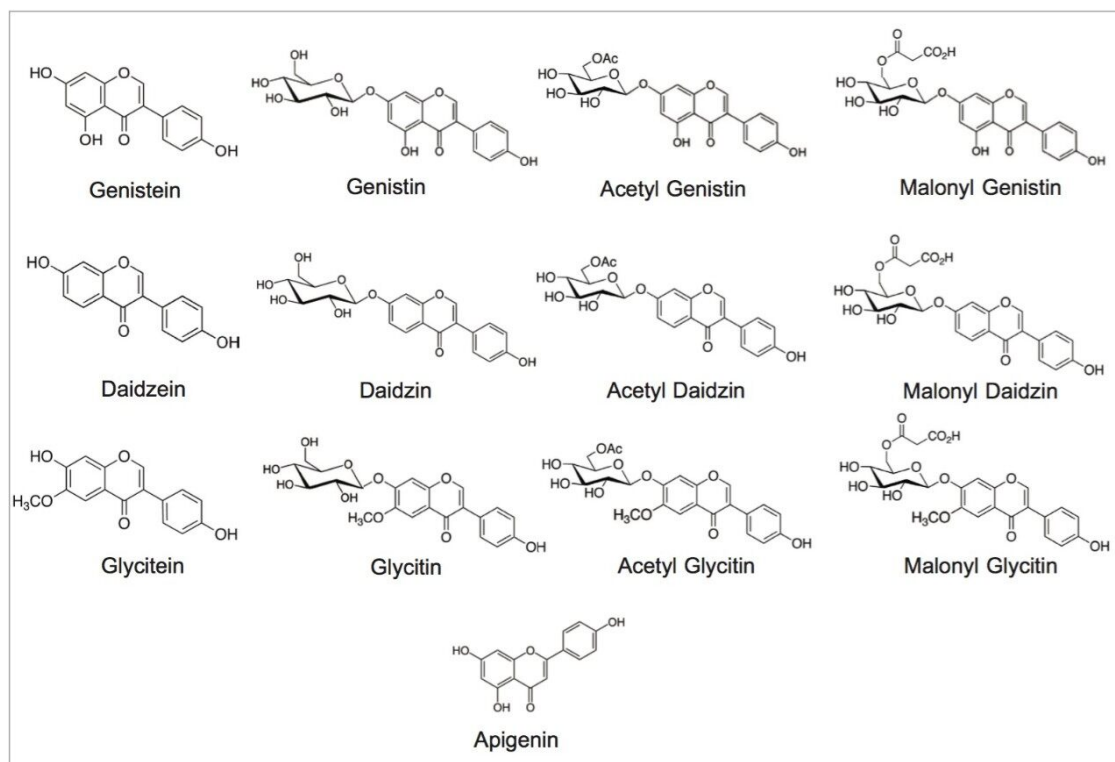


Figure 1. Structures of isoflavones in this study.

## Experimental

### Sample preparation

The standards: daidzin, glycitin, genistin, daidzein, glycitein, genistein, and apigenin, were purchased from ChromaDex (Irvine, CA) and INDOFINE Chemical (Hillsborough Township, NJ). Defatted powdered Soy RS was purchased from US Pharmacopeia (Rockville, MD). NIST SRM 3238 was purchased from NIST (Gaithersburg, MD). Isoflavone dietary supplement samples from major brands were purchased online.

The standard and sample solutions were prepared the same way as in the USP isoflavone method.<sup>1</sup> Sample solutions were further diluted with acetonitrile/water mixture (2/3 by volume) to various levels to fit into the calibration range (Table 2). The concentration of the internal standard was maintained at 4 ppm.



## UHPLC conditions

UHPLC system:	ACQUITY Arc
Detector:	2998 PDA
Software:	Empower 3
Column:	CORTECS C <sub>18</sub> 2.7 µm, 3.0 x 100 mm
Column temp.:	30 °C
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid
Injection volume:	2.0 µL
Flow rate:	1.08 mL/min
Run time:	18.0 min
UV detection:	260 nm
UV resolution:	1.2 nm

## Elution Gradient:

Time(min)	Flow rate(ml/min)	%A	Curve
Initial	1.08	90	6
14.40	1.08	70	6
14.50	1.08	10	6



Time(min)	Flow rate(ml/min)	%A	Curve
15.20	1.08	10	6
15.40	1.08	90	6
18.00	1.08	90	6

## MS conditions

MS system:	ACQUITY QDa (Performance)
Ionization mode:	ESI+
Capillary voltage:	0.8 kV
Cone voltage:	15 V
Probe temp.:	600 °C
SIR masses:	(Table 1)



Analyte	SIR mass (Daltons)
Daidzin	417.1
Glycitin	447.0
Genistin	433.1
Daidzein	254.9
Glycitein	285.0
Genistein	270.9
Apigenin (IS)	270.9
Malonyl Daidzin	503.4
Malonyl Glycitin	533.1
Acetyl Daidzin	459.1
Acetyl Glycitin	489.0
Malonyl Genistin	519.0
Acetyl Genistin	475.1

Table 1. Masses of isoflavone molecular ions.

## Results and Discussion

### Use of Mass Detection

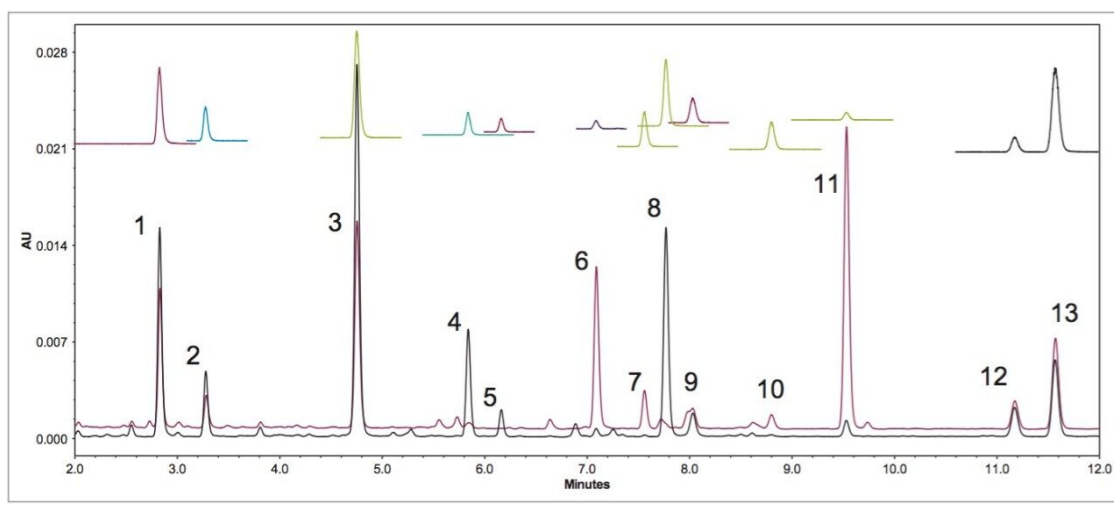
Little MS expertise is required to operate the ACQUITY QDa Detector. In this study, the factory default instrument parameters were used without any modification. The only change that was made to facilitate the MS detection was that the mobile phase additive was changed from phosphoric acid to formic acid. The mass spectra of the seven isoflavone standards (daidzin, glycitin, genistin, daidzein, glycitein, genistein, and apigenin) were obtained under the UHPLC conditions (see Experimental section), and it was confirmed that



their molecular ions were the most intense ions. Individual standards of the acetyl and the malonyl isoflavones, acetyl daidzin, acetyl glycitin, acetyl genistin, malonyl daidzin, malonyl glycitin, and malonyl genistin, were not commercially available. Their mass spectra were obtained from the USP reference materials (USP defatted powdered soy RS), which contained all the acetyl and the malonyl isoflavones. The molecular ions of these compounds are listed in Table 1 and were used in the MS SIR setup.

## Benefits of MS - Confirmation of Peak Assignments

MS greatly simplifies the peak identification. The original USP method uses a pattern matching method and a reference material (USP defatted powdered soy RS) for the acetyl and the malonyl isoflavones peak identification in UV chromatograms, since these standards are not commercially available. Figure 2 shows the UV and the SIR chromatograms of defatted soy under the final optimized LC conditions. The SIR chromatograms of each compound are free of interference, which makes peak identification easier.



*Figure 2. UV chromatograms of unheated defatted soy (black, the bottom trace) and heated defatted soy (red trace next to the bottom one), and the corresponding mass detector SIR traces for every compound. Peak ID: 1. Daidzin; 2. Glycitin; 3. Genistin; 4. Malonyl Daidzin; 5. Malonyl Glycitin; 6. Acetyl Daidzin; 7. Acetyl Glycitin; 8. Malonyl Genistin; 9. Daidzein; 10. Glycitein; 11. Acetyl genistin; 12. Genistein; and 13. Apigenin.*

Figure 3 demonstrates the major advantage of MS data over UV/Vis spectroscopy data in confirming peak identifications. Figure 3 was obtained on a CORTECS C<sub>18</sub> Column (2.7  $\mu$ m, 3 x 100 mm), using an initial 18 minutes gradient elution program, which was converted from the USP method directly prior to further optimization.<sup>2</sup> The SIR chromatograms of the acetyl glycitin and the malonyl genistin clearly show a co-



elution. It was difficult to discern this co-elution issue from the UV (260 nm) chromatogram. Even with the PDA peak purity test (Figure 4), it was difficult since the UV spectra across the peak were identical to the peak apex UV spectrum. The ACQUITY QDa data made the co-elution issue apparent during the early stages of the method transfer, which prevented future issues or an inappropriately transferred method.

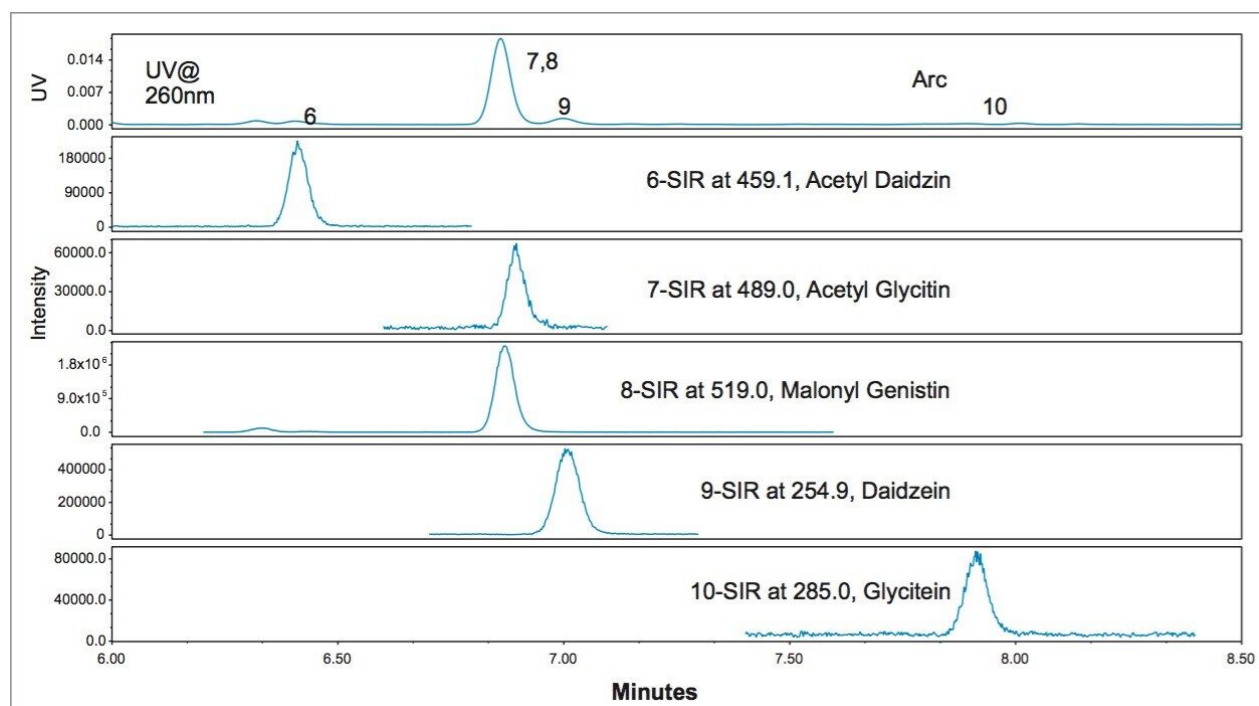


Figure 3. Sections of the UV and the SIR chromatograms of USP defatted powdered soy RS under the initial LC conditions without optimization. The LC conditions were converted from the USP method using the ACQUITY Column Calculator. Co-elution between Acetyl Glycitin and Malonyl Genistin was obvious in the SIR chromatograms.







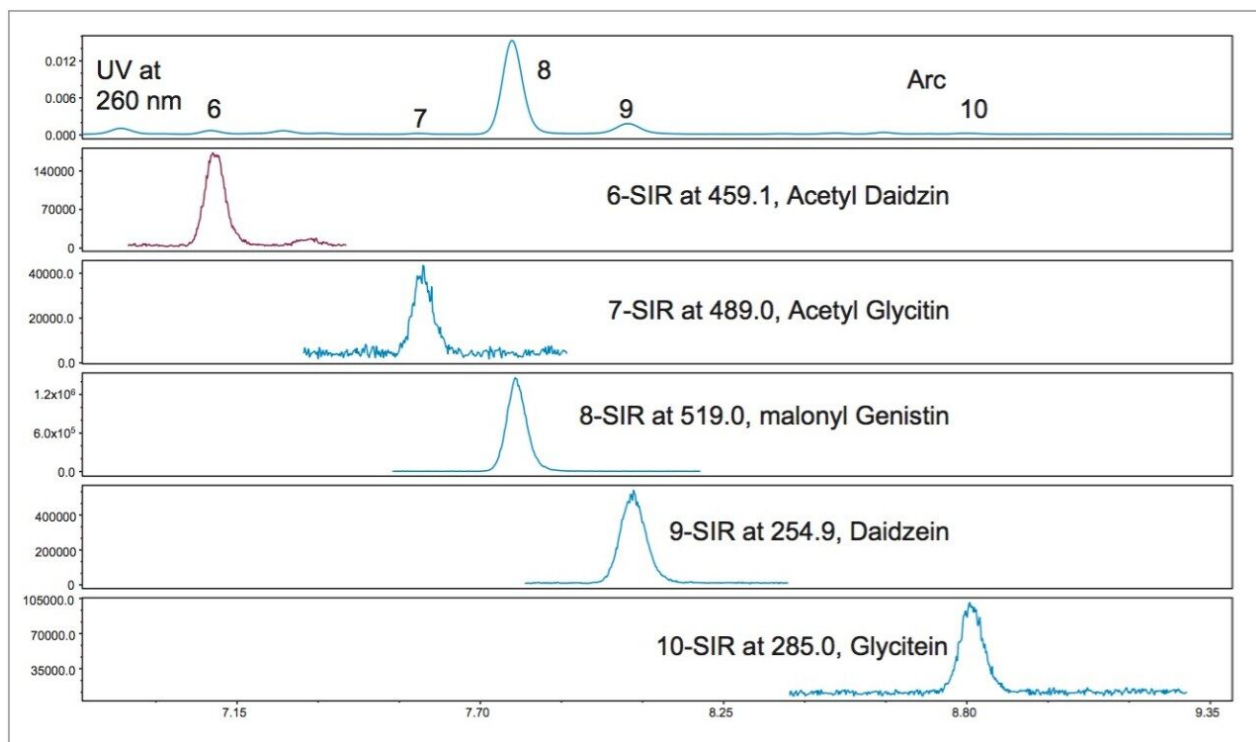


Figure 5. Sections of the UV and the SIR chromatograms of USP defatted powdered soy RS under the final optimized LC conditions. Co-elution between Acetyl Glycitin and Malonyl Genistin was solved (See UHPLC on page 3.)

## Benefits of MS - Errors in Routine Analysis

The ACQUITY QDa Mass Detector can also play an important role in routine sample analysis. Dietary supplements from plant sources often contain complex chemical matrices. The unknown matrix could interfere with the targeted compounds, and could cause error in quantitation, or even false peak assignment. Figure 6 shows the UV chromatogram of two partially co-eluting peaks for a dietary supplement sample. One of these peaks should be genistein, but it was hard to assign a peak ID because their RTs were both very close to the reference RT of genistein. The PDA data was not helpful in determining which one was genistein peak as their UV/Vis spectra were the same. Peak assignment was facile using data from the ACQUITY QDa Detector. The SIR channel of genistein (270.9 Dalton) clearly showed that the later eluting peak was genistein. This peak assignment and its purity were further confirmed and checked by inspecting the mass spectra extracted from the MS scan data at the front, the apex, and the back of the peak. Thus, the chance of false peak assignment or possible co-elution was greatly reduced.

Furthermore, Figure 6 shows how easy and simple it is to use the SIR chromatograms for the quantitation of isoflavone contents. In the UV chromatogram, one has to use a deconvolution technique to minimize the



error from the interfering compound. By contrast, when using SIR chromatograms of isoflavones, there was much less chance of interference. The benefits of using MS in quantitation for isoflavone analysis will be further discussed in a separate application note.<sup>3</sup>

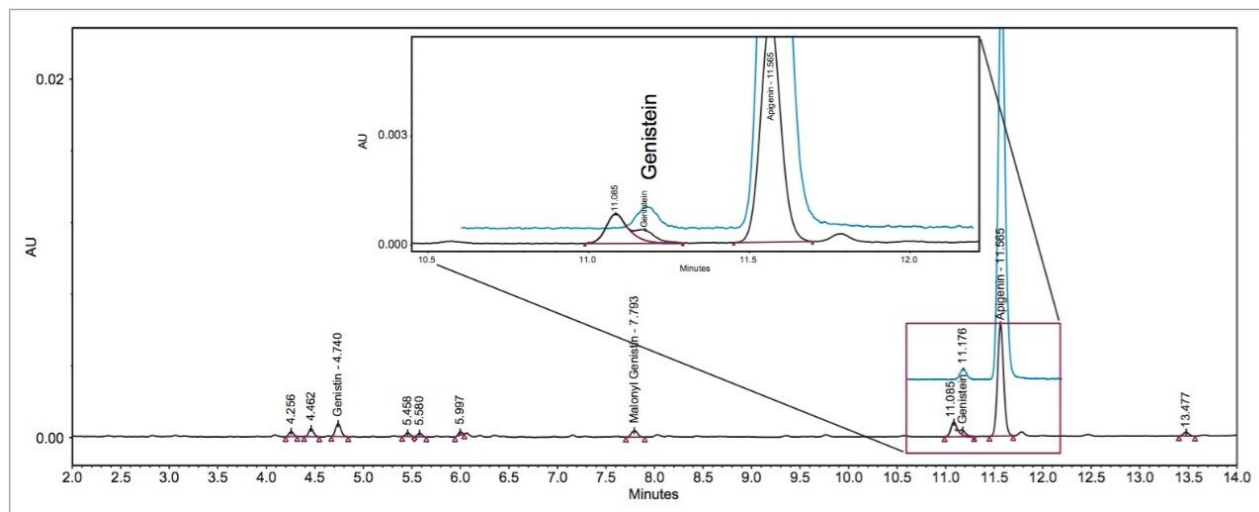


Figure 6. UV and SIR chromatograms of an unknown dietary supplement sample. The Genistein peak was identified with the help of its SIR (270.9 Dalton) chromatogram. Apigenin molecular ion has the same mass of 270.9 Daltons. The interfering peak (11.085 min) has the same UV/Vis spectrum as the genistein (not shown).

## Conclusion

The MS data, simultaneously generated during the isoflavone analysis brought ease and high confidence in peak identification, and facilitated fast method transfer of the USP isoflavones method onto a Waters ACQUITY Arc UHPLC System with a 2998 PDA Detector and ACQUITY QDa Mass Detector. In comparison to PDA detection, the addition of mass detection provided an increased capability for discerning co elution issues and confirming peak identifications.

## References



1. USP Monograph. Powdered Soy Isoflavones Extract, USP39–NF34 S1 [6841], The United States Pharmacopeial Convention.
2. J Yang, M Benvenuti, and G Cleland. Fast Analysis of Isoflavones in Dietary Supplements – USP Method Transfer on a UHPLC System with Mass Detection, Waters Application Note, no. 720005858en; 2016.
3. J Yang, M Benvenuti, and G Cleland. Fast Analysis of Isoflavones in Dietary Supplements: A Comparison of Mass Detection with UV Detection, Waters Application Note.

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## Featured Products

ACQUITY Arc System <<https://www.waters.com/134844390>>

ACQUITY QDa Mass Detector <<https://www.waters.com/134761404>>

2998 Photodiode Array (PDA) Detector <<https://www.waters.com/1001362>>

Empower 3 Chromatography Data Software <<https://www.waters.com/10190669>>

Available for purchase online:

CORTECS C18 Column, 90Å, 2.7 µm, 3 mm X 100 mm, 1/pkg <  
<https://www.waters.com/waters/partDetail.htm?partNumber=186007372>>

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