ANALYSIS OF SOY ISOFLAVONES IN A DIETARY SUPPLEMENT USING UPLC-PDA-SQD

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INTRODUCTION

The consumption of soy products has been linked to many health benefits, as they contain isoflavones:

- **Reduction of breast & prostate cancers**
- **Cardiovascular disease**
- **Reduced symptoms of diabetes**
- **Postmenopausal bone loss**

Isoflavones are commonly known as phytoestrogens & 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 [1] (their structures are shown in Figure 1).

The approval by the US Food & Drug Administration (US-FDA) in 1999 allowing the food industry to promote soy protein for heart health [2] led to an escalation in sales of soyfoods as functional foods.

Journals have been published where the soy isoflavones are now being incorporated in pasta, cookies, fruit juices, chocolate and dietary supplements.

The poster describes a rapid method using reversed phase UPLC to detect and characterise the isoflavone glucoside conjugates present in a commercial soy nutritional supplement using PDA and MS detection.

METHOD

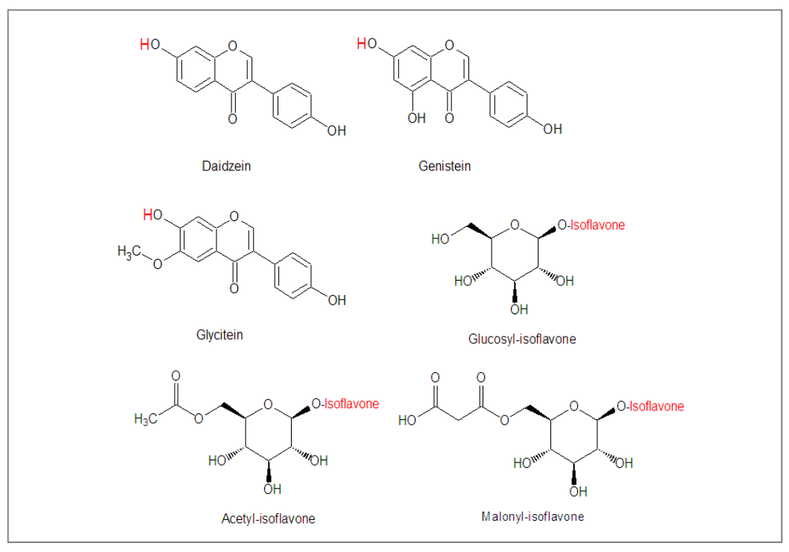

Figure 1. Structures of three soy isoflavones: daidzein, genistein and glycitein and their conjugates.

LC conditions

LC System:	Waters® ACQUITY UPLC® System
Column:	ACQUITY UPLC BEH C8 Column 2.1 x 100 mm, 1.7 µm
Flow Rate:	500 µL/min
Mobile Phase A:	0.2% Formic acid in Water
Mobile Phase B:	Methanol

PDA conditions

PDA System:	Acquity 2996 PDA

MS conditions

MS System:	Waters SQD™ Mass Spectrometer
Ionization Mode:	ESI Positive

Full scan settings:

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

SIR settings:

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

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

Table 1. SIR settings showing cone voltages used for each m/z value.

RESULTS

Figure 3 shows the rapid analysis time achieved by using Acquity UPLC.

The analysis was first performed looking at the full scan data. Then, using the full scan data it was 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 260nm from the PDA detector.

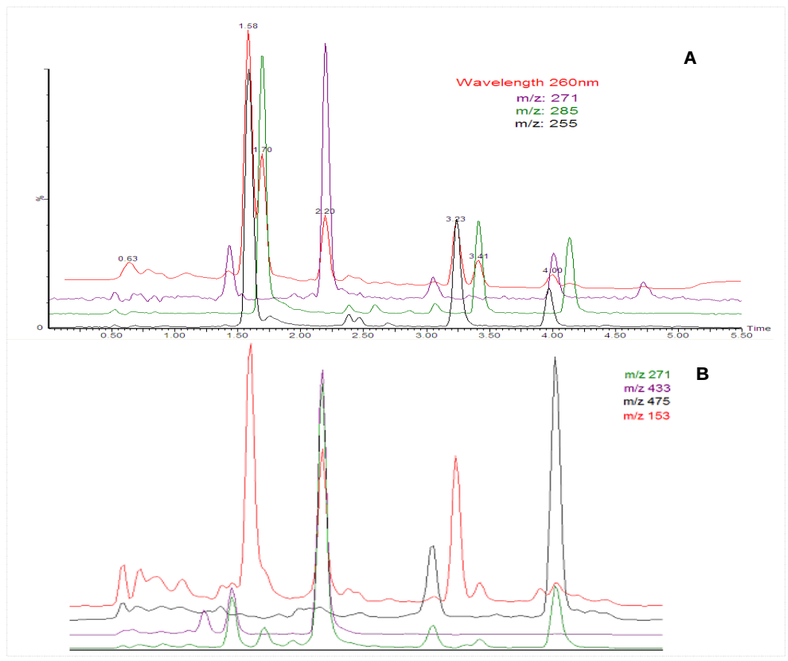

*Figure 3. **A**: Full scan MS, with m/z ions (extracted from the TIC) for daidzein, genistein & glycitein, & wavelength 260nm and **B**: SIR method, and the ions for genistein & it's conjugates in the soy supplement.*

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 the discussion section).

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

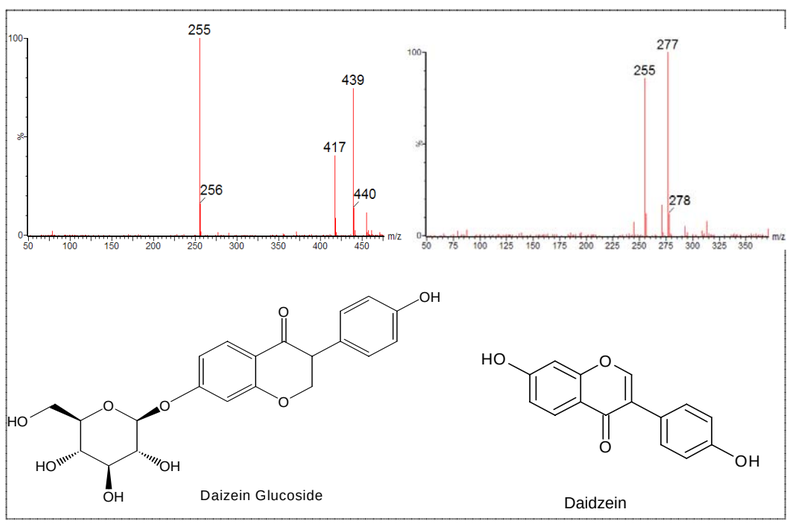

Figure 2. Spectra for daidzein glucoside (daidzin) and daidzein where the parent ions in positive ESI are 417 and 255 respectively

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

Table 2. 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
3.21	Genistein Malonyl Glucoside	519
3.23	Daidzein Acetyl Glucoside	459
3.41	Glycitein Acetyl Glucoside	489
3.98	Daidzein	255
4.00	Genistein Acetyl Glucoside	489
4.13	Glycitein	285
4.72	Genistein	271

DISCUSSION

Chromatographic Data Points

When using mass spectrometry, in particular for quantification it is important to have at least ten data points across a peak for repeatable peak integration. For UPLC-type 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 , a dwell time of 10ms was used to achieve the recommended data points for the compounds analysed

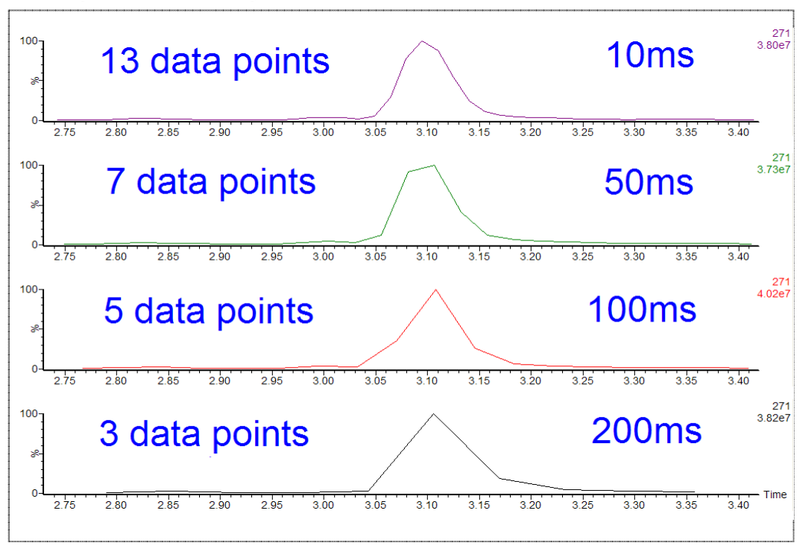

Figure 6. Comparison of dwell times and data points

Quantification

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

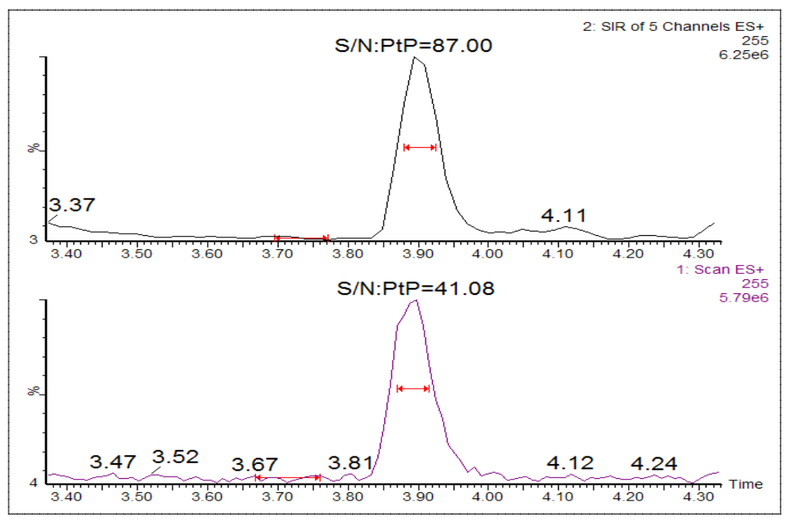

Figure 4. Comparison of S/N using SIR data (top) and extracted ion from full scan data (bottom)

CONCLUSION

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 method 5.5 minute method has been described using UV and MS data.

For structural information for the compounds a full scan method was used, however, if quantification is required, the SIR method is recommended as it provides better sensitivity.

References

[1]P.A. Murphy, T. Song, G. Buseman, K. Barua, G.R. Beecher, D. Trainer, J. Holden, J. Agric. Food Chem. 47 (1999) 2697

[2]Kim H, Peterson TG, Barnes S. Mechanisms of action of the soy isoflavone genistein: emerging role for its effects via transforming growth factor signaling pathways. Am J Clin Nutr 1998;68(suppl): 1418S–25S.

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