

ANALYSIS OF SOY ISOFLAVONES IN A DIETARY SUPPLEMENT USING UPLC-UV-MS

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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.

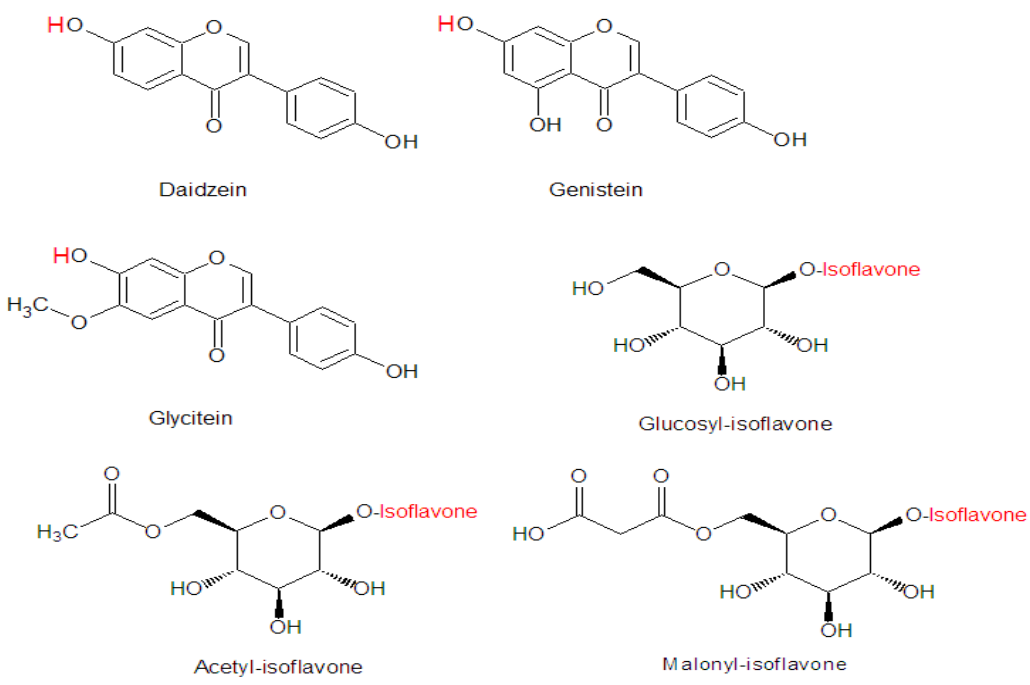


Figure 1 Structures of three soy isoflavones: daidzein, genistein and glycitein and their conjugates.

EXPERIMENTAL SET-UP

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: Waters® 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



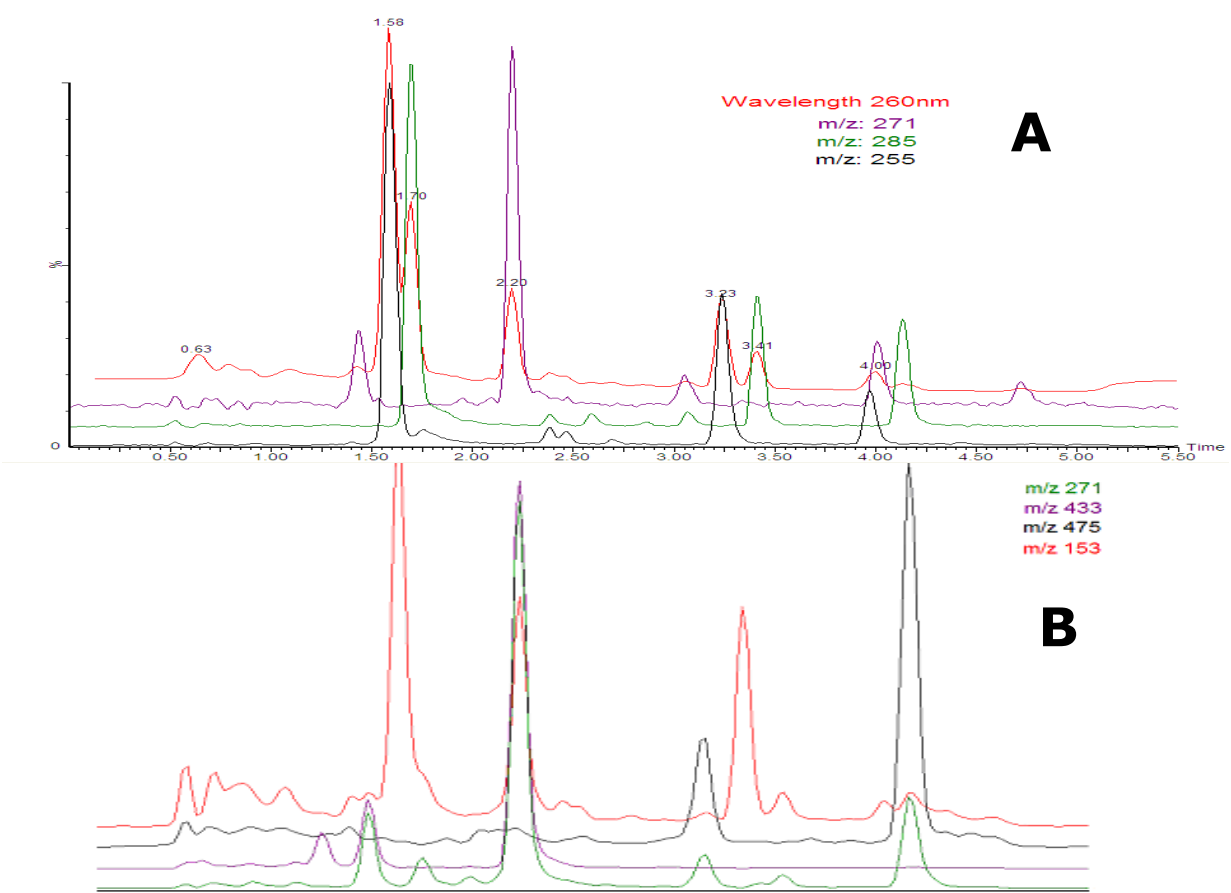


Figure 2 A: Full scan MS, with m/z ions (extracted from the TIC) for daidzein, genistein & glycitein, & wavelength 260nm (from PDA) and B: SIR method, & the m/z ions for genistein & it's conjugates in a soy supplement.

Figure 2B shows the selected ions for genistein and the genistein conjugates. The m/z 153 is a product ion from the isoflavone structure. Full scan provides spectral information from the fragmentation patterns (Figure 3) , which can help with structural determination and is useful when identifying unknown compounds and examples of daidzein MS spectra can be seen below.

For the conjugated isoflavone systems the ion of the conjugate ([M+H]⁺: m/z 417) was present. Also present in the MS of these compounds is the positively charged ion related to the loss of the glucose group (m/z 255).

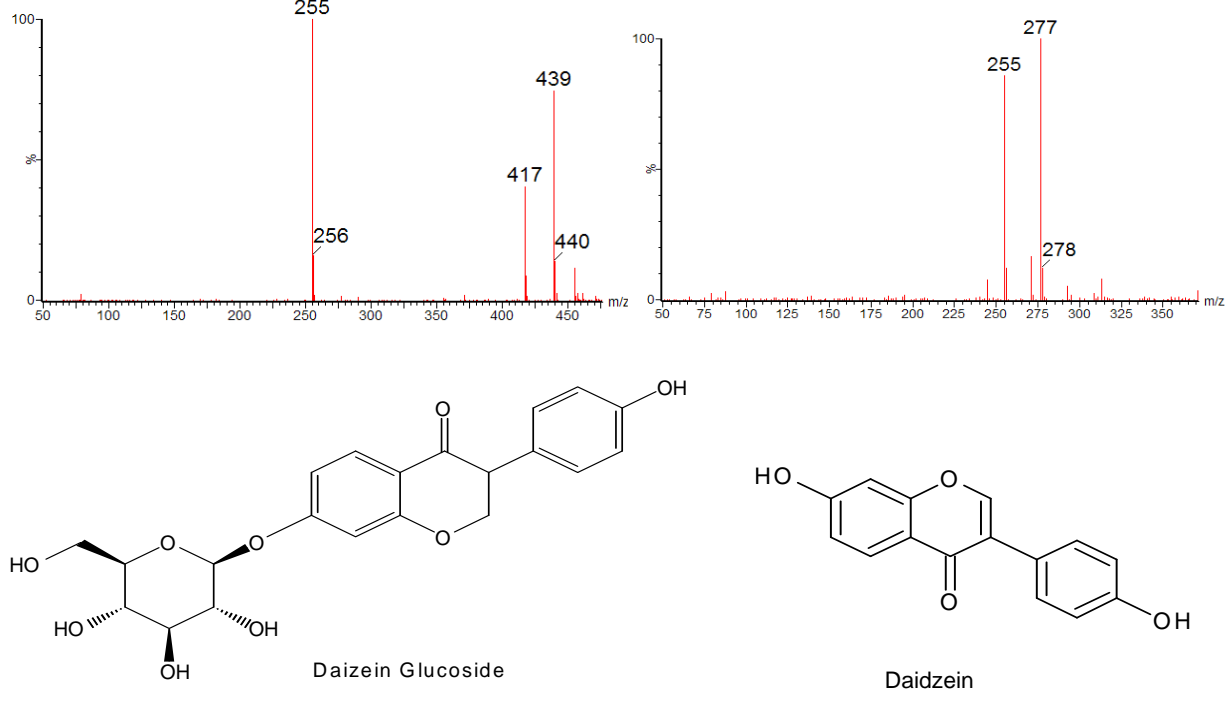


Figure 3 Mass spectra for daidzein glucoside (daidzin) and daidzein where the parent ions in positive ESI are 417 and 255 respectively

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	475
4.13	Glycitein	285
4.72	Genistein	271

Table 2 Retention times for the soy isoflavones and their conjugates.

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 4 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

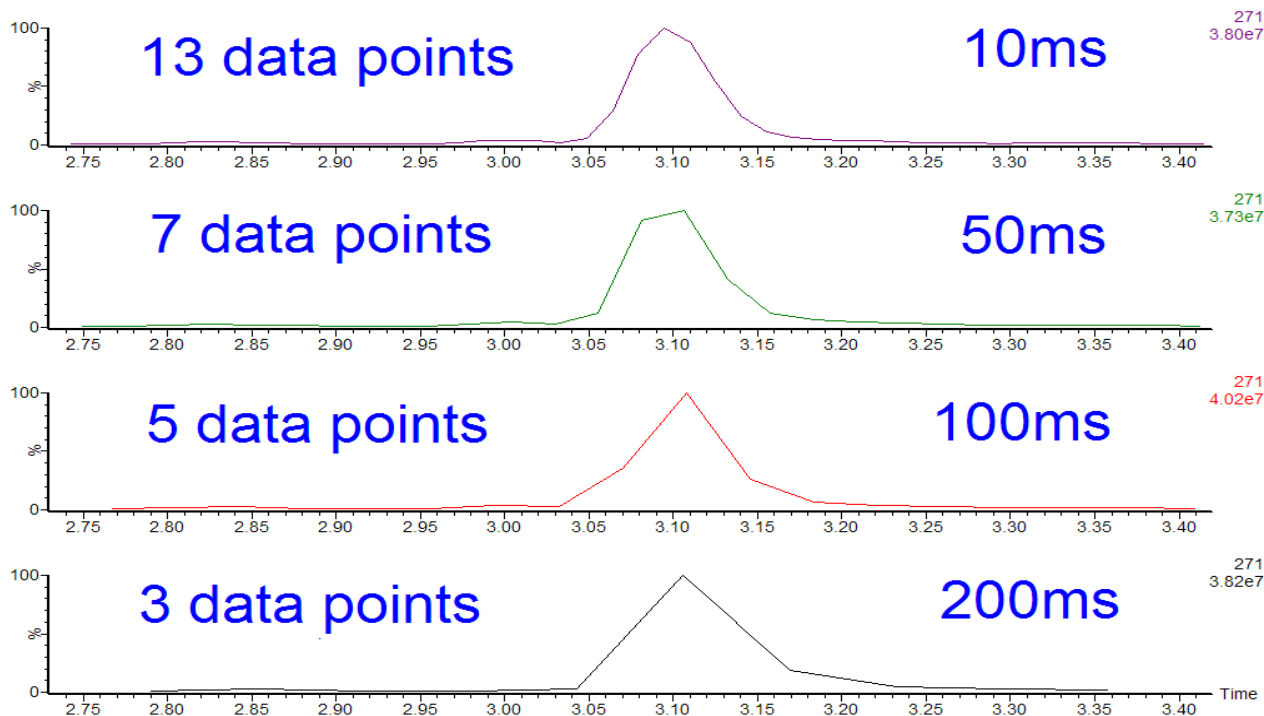


Figure 4 Comparison of dwell times and data points

Quantification

For quantification experiments, SIR is preferred as it provides more sensitivity (Figure 5) than the compared extracted ion full scan data.

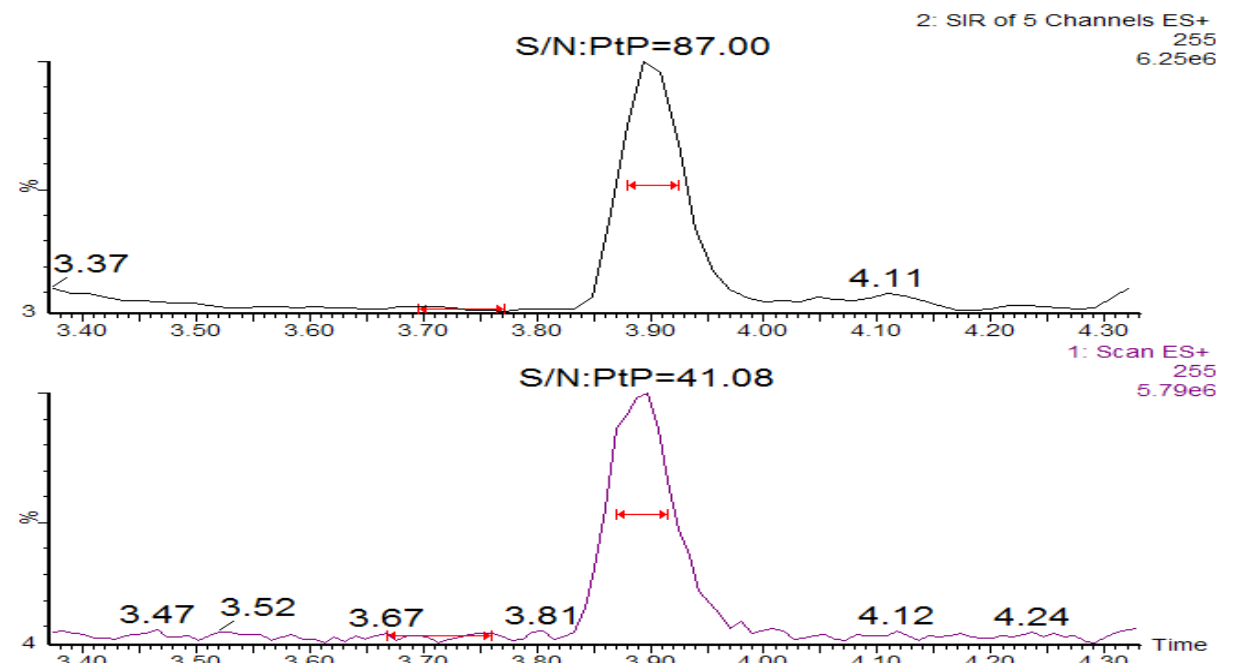


Figure 5 Comparison of S/N using SIR data (top) and extracted ion from full scan data (bottom)

CONCLUSION

A soy supplement has been used to look at the isoflavone content of the major compounds related to daidzein, genistein and glycitein.

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:

- A full scan method was used,

However, if quantification is required:

- 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.