INTRODUCTION

- Twelve major isoflavones were found in plants of soy (Glycine max), red clover (Trifolium pretense), and Kudzu (Pueraria lobata). These hormone-like compounds are often used in remedies to reduce pre- and postmenopausal symptoms.
- Standard methods for isoflavones in dietary supplements use reverse-phase LC with C18 columns and ultraviolet and visible light (UV-Vis) spectroscopy for analysis and quantitation. The chromatographic run time is over 75 minutes long.

This work demonstrates the transfer of the USP isoflavone method onto an ACQUITY Arc™ system and an ACQUITY UPLC-H Class system. The benefits of Mass Spectrometry in method transfer and sample analysis are highlighted.

METHODS

- Transfer the USP isoflavone HPLC method to faster UHPLC and UPLC methods to achieve higher sample analysis throughputs.

GOAL

- To develop a fast analysis method for isoflavones in dietary supplements.

RESULTS

1) USP method transfer and optimization

The USP method (isoﬂavone powder extract) was transferred to an ACQUITY Quanta system with ACQUITY UPLC and ACQUITY QDa Detector (Performance). The system was optimized for 10.5 minutes using mobile phase A and B (background subtracted) from the ACQUITY Arc system at atmospheric pressure. The mass spectrum at a front end from the USP isoflavone method is different from the method at a front end. This ensures that the peak area is caused by a compound other than the between.

2) Analysis of isoflavones in dietary supplements

- The isoflavone contents in three isoflavone dietary supplement samples were analyzed by the UPLC method. The accuracy for the daidzein, genistein, and glycitein was better than 0.998. The relative standard deviation (RSD) in area ratio and the standard concentrations (ppm) for all compounds were within 10% of the expected values.

3) Analysis of isoflavones in dietary supplements

- The isoflavone contents in three isoflavone dietary supplement samples were measured by the UPLC method. The accuracy for the daidzein in NIST 3238 SRM was 102%. The quantitation error that is caused by the USP method was less than 0.08.

4) Mass detection is extremely useful in the analysis of complex samples.

CONCLUSION

- The USP isoflavone method was successfully transferred to UPLC on ACQUITY Arc system and UPLC on ACQUITY H Class system.

- The total run time is shortened from 74 min (HPLC) to 18 min (UPLC) to 8 min (UHPLC).

- Analysis results for the NIST reference material showed good agreement between the reference values and the expected results.

- Mass detection is extremely useful in the analysis of complex samples, and the mass retention time is invaluable in troubleshooting.

- Daily supplementation samples have been analyzed using the UPLC and the UPLC methods. One sample that had its total isoflavone content much less than their label claim.

REFERENCES

- The United States Pharmacopeial Convention. USP 38 NF 33 (2015), chapter 242, "Preparation of Samples for Procedures Using Waters QDa Detector."