ISOFLAVONES—USE AS COUNTERPARTS TO ACQUITY ARC SYSTEM WITH MASS DETECTOR

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INTRODUCTION

Isoflavones are found primarily in plants of soy (Glycine max), red clover (Trifolium pretense), and Kudzu (Pueraria lobata). 12 major isoflavones found in these plants are daidzein, genistein, and their respective glucoside and malonyl-glucoside derivatives. The structures of 12 isoflavones and their respective glucosides are shown in Figure 1. These hormone-like compounds are often used in remedies to reduce menopausal and post-menopausal symptoms.

Standard methods for isoflavones in dietary supplements have been established by standard organizations such as USP28 and AOAC25. These methods use reversed-phase LC with C18 columns, ultraviolet and visible light (UV-Vis) spectroscopy for separation and quantitation. Because of the close structural similarity of this group of compounds, the chromatographic run times of these methods are over 70 minutes long. It is highly desired to develop a fast isoflavone analysis method.

This study demonstrates the method transfer of the USP method on an ACQUITY Arc system. The analysis time, including column wash and regeneration, is only 18 minutes. Waters QDa mass detector was used for peak identification and method optimization.

EXPERIMENTAL

Sample preparation:

Samples from commercial dietary supplements, soy isolate, glucoside, genistein, daidzin, glycitin, 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 2238 was purchased from NIST (Gaithersburg, MD). Isoflavone dietary supplement samples from major brands were purchased from online retail stores.

The standard and sample solutions were prepared the same way as in the USP isoflavone method 25, except that the sample solutions were further diluted with acetonitrile water mixture (2/3 by volume) to various levels to fit the calibration range. The retenion time of internal standard was always maintained at 4.0 min.

1) USP method transfer and optimization: The USP method (isoflavone powder extract 15) was transferred to an ACQUITY Arc system with a CORTECS C18 column (2.7 µm x 100 mm, 2.7 µm particle size). The CORTECS column 2.7 µm packing material is solid-core particle material, which provides higher separation efficiency and lower back pressure than full porous particle equivalent gene size. The USP method’s 74 minutes long mobile phase elution program was converted to a 18 minutes program using Waters UPLC Column Calculator. The mobile phase elution program was changed from phosphoric acid to formic acid, which is a mass spectrometry friendly additive. The factory default QDa instrument parameters were used without any modification. The mobile phase was optimized to 10% acetonitrile to meet the USP sensitivity criteria on peak resolution. Figure 2 shows the chromatograms of the USP defatted soy RS, unheated, and treated, and their Single Ion Recording (SIR) traces that were obtained from the QDa mass detector. The QDa detector was used to confirm peak identities.

2) QDa detector in method transfer and optimization: The use of QDa detector for compound identification. The acetyl and malonyl isoflavone standards were not available. The peak assignment of blank soy RS run was further aided with acetonitrile water mixture (2/3 by volume) to various levels to fit the calibration range. The retenion time of internal standard was always maintained at 4.0 min.

A total of 12 isoflavone peaks were monitored. The peak intensity was compared to the internal standard (Methyl-13C3-4-hydroxyestrone). The relative intensity was calculated and compared to the peak area ratio in Table 3. Excellent recovery of 98% to 101% were obtained. The results were within the ±11% for genistin, glycitin, and daidzin. The contents of daidzein, malonyl genistin, and apigenin in NIST sample were below the LOQ, their quantity was analyzed in the SRM system. The contents prior to and post to the spiking in an isoflavone sample were determined and shown in Table 4. Excellent recovery of 98% to 101% were obtained.

RESULTS AND DISCUSSION

3) Method performance characteristics:

Table 3 shows the UV/Vis calibration results of the standards. Apigenin was used as the internal standard for calibration and quantitation of all compounds. The square of the correlation coefficient 

Table 3. Comparison of determined isoflavone values to the certified and reference values of NIST 3238 SRM

Table 4. Recovery results from a spiking experiment

CONCLUSIONS

- The USP method for isoflavones was successfully transferred to an ACQUITY Arc system with a 2998 PDA detector.
- The total analysis time per injection on Arc system is 18 minutes.
- This method offers a three time increase in the analysis throughput, and a 75% solvent cost savings.
- Waters QDa detector offered high confidence in peak identification and shortened the method optimization time.
- Waters QDa offers high confidence in new and challenging samples analysis with a potential interference risk is unknown or high.

References
