SINGLE LABORATORY VALIDATION OF UHPLC-MS/MS ASSAY OF RED CLOVER

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OBJECTIVES

• Develop and validate UHPLC-MS/MS assay for red clover isoflavones in dietary supplements and human serum
• Apply the validated method to standardize red clover extract and dietary supplement products, and analyze serum samples from clinical trials

BACKGROUND

With their origins in traditional medicine, botanical dietary supplements remain in widespread use. For example, preparations of red clover (Trifolium pratense L.) are commonly used to treat respiratory problems (asthma, whooping cough and bronchitis), skin disorders (eczema and psoriasis), and menopausal issues in women, especially vasomotor symptoms [1]. The active isoflavones are concentrated in the aerial parts of red clover. Daidzein and genistein are estrogenic, whereas biochanin A and formononetin can be metabolized to genistein and daidzein, respectively, and are considered proestrogens. In contrast, the isoflavones irinone and prunetin in red clover have progestosterone activity [3]. See chemical structures in Figure 1.

RESULTS

For safe use and to ensure reproducible effects, red clover dietary supplements should be chemically standardized to the active isoflavones. In this study, a fast UHPLC-MS/MS based method was developed and validated for the quantification of all 6 isoflavones in red clover dietary supplements using deuterium-labeled internal standards (Figure 1) to correct for variations during sample preparation and analysis. Additionally, the UHPLC-MS/MS assay was validated using human serum with the goal of supporting clinical research where serum samples will be measured for the various red clover isoflavones. References


METHODS

• Red clover isoflavones were purchased at >98% purity (Figure 1)
• Recovery, quality control and stability assays were carried out through protein precipitation as outlined in Figure 2

METHODS AND INSTRUMENTATION

• A UHPLC-MS/MS assay for the analysis of red clover isoflavones was developed and validated
• The standard curves were linear for both low and high isoflavone concentrations, recovery was excellent, and the isoflavones were stable for the duration of the assay and as well as storage
• The UHPLC-MS/MS assay is applicable to the standardization of red clover supplements and analysis of serum samples from clinical trials

CONCLUSIONS

• Apply the UHPLC-MS/MS assay for the analysis of commercial red clover supplements.

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