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Title: Soy Isoflavones: An Automated and Validated UHPLC–MS/MS Method for the Determination of the Phase II Metabolites in Blood Plasma and Urine

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Abstract: The metabolic transformation of isoflavones (IF) by the gut microbiota as well as by endogenous drug metabolizing enzymes plays a crucial role in the understanding of their potential health promoting effects. However, analytical methods published so far do not include the direct determination of the phase II metabolites leading to the loss of important information. Our aim was to develop an automated method to simultaneously quantify the soy IF daidzein and genistein, their conjugative metabolites including glucuronides, sulfates, and sulfoglucuronides as well as their major microbial degradation products for the application in studies with larger sample sizes. An automated solid-phase extraction using 96well plates was established to extract the analytes from plasma or urine and was combined with a fast and selective UHPLC-MS/MS analysis. The method was validated based on the ICH analytical method validation guidelines and on the FDA recommendations to meet the requirements of international standards. All validation parameters including selectivity, accuracy, precision, recovery, limit of detection, linearity and robustness matched essentially the specifications given by the ICH and FDA. The applicability and efficiency of the new method was further demonstrated by the measurement of plasma and urine samples from animal and human intervention studies. In conclusion, a robust analytical approach was developed which allow the handling of large sample sizes but nevertheless provide detailed information on the IF metabolite profile. We assume that the application of our method may contribute to a better understanding and interpretation of experimental studies and in particular of clinical trials evaluating the biological effects of soy IF.

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