HPLC-MS/MS Analysis of Anthocyanins in Human Urine and Plasma with Rapid Sample Preparation Methods
J. Liu, J. Huang, J. Song, J. Fang
University of Saskatchewan

Purpose
Anthocyanins constitute the largest group of water-soluble pigments in plants and are responsible for the blue, purple, and red color of many fruits, flowers, and leaves. Many investigations have associated the intake of anthocyanins with a reduced incidence of diseases such as cardiovascular disease, diabetes mellitus, and cancer.

This report describes an HPLC-MS/MS method for the analysis of anthocyanins in human urine and plasma using dilute-and-shoot and protein precipitation sample preparation methods, respectively.

Methods
Sample preparations: Plasma or urine samples (100 μl) were mixed with 50 μl trifluoroacetic acid water solution (50 μl, 20% v/v) and then centrifuged at 12,000 g for 15 minutes to remove protein and solid particles. The supernatants were injected (30 μl) directly for HPLC-MS/MS analysis.

HPLC-MS/MS conditions: The high performance liquid chromatography (HPLC) method employed a Synergi RP-Max column (250 x 4.6 mm, 4 μm, Phenomenex, Torrance, CA) and an API 4000 mass spectrometer (Applied Biosystem Sciex). Gradient system: phase A consisted of water/1% formic acid and mobile phase B was acetonitrile. The gradient was initiated at 5% mobile phase B, increased to 21% B at 20 min, and then increased to 40% B at 24 min.

Human study: Minced saskatoon berries were administered to healthy volunteers after overnight fasting. Blood samples were taken before and at 0.5, 1.5 and 3.5 hours after the supplements.

Results
The blank urine and plasma samples confirmed the absence of interference from endogenous compounds. Our API 4000 mass spectrometer demonstrated excellent selectivity, sensitivity and reproducibility for the analysis of these compounds in urine and plasma samples.

The method has been used to analyze anthocyanin concentrations in urine and plasma samples from volunteers administered saskatoon berries. Cyanidin-3-galactoside (Cy-3-gal), cyanidin-3-glucoside (Cy-3-glc), cyanidin-3-arabinoside (Cy-3-ara), cyanidin-3-xyloside (Cy-3-xyl), and quercetin-3-galactoside (Qu-3-gal) were identified and quantified in plasma and urine samples (Figure 1).

Conclusion
The analysis of anthocyanins presents a challenge because of their poor stability during sample preparation. Most existing HPLC-MS/MS methods involve time-consuming sample preparation procedures. Most liquid-liquid and solid phase extraction procedures require an evaporation step, which has been shown to decompose anthocyanins. In this method, the degradation of anthocyanins was minimized using dilute-and-shoot and protein precipitation sample preparation methods for urine and plasma, respectively.

Our sample preparation approaches are particularly helpful for identifying and measuring new metabolites whose extraction efficiencies are unknown with conventional extraction methods.