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CONFERENCE

57th ASMS Conference on Mass Spectrometry

TITLE

Quantitation of Free Quercetin in Human Whole Blood by LC-MS/MS

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ABSTRACT

Novel Aspect: Quantitative analysis of quercetin in human whole blood

Introduction

As one important member of flavonoid family, quercetin has shown indications of many biological activities, e.g, anti-inflammatory, and anti-oxidant activities. An effective quantitative analysis is crucial for studying the pharmacokinetics and toxicity of quercetin. Previous studies exclusively measured the plasma concentrations of quercetin. However, it was reported that quercetin can be quickly taken up by human red blood cells. Therefore, quercetin concentrations should preferably be determined in whole blood instead of plasma. In this study, we report an HPLC-MS/MS method to quantify quercetin in human whole blood and evaluate the stability of quercetin in human whole blood under various conditions. We also evaluate the possible interference on the assay performance from quercetin glucuronides (major metabolite) expected in study samples.

Methods

To stabilize quercetin, human whole blood samples were treated with ascorbic acid (0.6% in water, w/v). Quercetin and a structural analogue internal standard were extracted from human whole blood by liquid-liquid extraction using methyl t-butyl ether. The reconstituted extracts were then injected into an LC/MS/MS system for quantitative analysis. The liquid chromatography was performed through a Synergi Hydro-RP column (4µm, 50x2.0 mm, Phenomenex) on a Shimadzu LC-10AD LC system. A gradient elution programs was used to chromatographically separate quercetin and the internal standard from matrix components including conjugated quercetins. The MS/MS detection was performed on a Sciex API4000 with negative Turbo Ion Spray mode.

Preliminary results

The method for qualitative analysis of quercetin in human whole blood by HPLC-MS/MS was successfully validated for an assay range of 5 to 5,000 ng/mL. The assay performance was characterized by an intra-day precision of 1.0 to 14.9%, intra-day accuracy of -9.8 to 6.0%, inter-day precision of 3.0 to 10.9%, and inter-day accuracy of -0.4 to 2.6%. The average extraction recovery of quercetin and internal standard was 93.5 and 96.7%, respectively. The method was found selective and reliable, and not subject to interference from conjugated quercetins. The stability of quercetin in human whole blood was evaluated and, using ascorbic acid as the stabilizer, quercetin was found to be stable in human whole blood for up to 6 hours stored on wet ice, for 6 freeze/thaw cycles from -70°C to ~4°C on wet ice, and for 28 days of storage at -70°C.