Title

Simultaneous quantitative analysis of six major grape polyphenols in mouse liver using LC-MS/MS

Introduction

There are many well know health benefits of grape consumption such as: antioxidant activity, anti-cancer activity, decrease of plasma protein oxidation, improvement of the endothelial function and inhibition of platelet aggregation. Two major sites of grape polyphenols metabolism are the liver and the colonic flora. A highly sensitive, efficacious and robust analytic methods are required to support the preclinical studies which was to check health benefits of grape after the conducted mice groups daily consumption. Here we explore the use of a high performance triple quadrupole mass spectrometer for the quantitation of of major grape polyphenols including catechin, epicatechin, trans-resveratrol, quercetin, isorhamnetin, and kaempferol in various mouse samples at low concentration levels with protein precipitation extraction method.

Methods

Triple quadrupole mass spectrometer (Sciex API 4000) was used with multiple reaction monitoring (MRM) in negative ionization mode for mass detection. Separation was achieved by Shimadzu LC-20AD XR UFLC system. Major grape polyphenols including catechin, epicatechin, *trans*-resveratrol, quercetin, isorhamnetin, and kaempferol and the internal standards were spiked in mouse liver samples and extractied with protein precipitation method. The liquid chromatography was optimized on Waters Xselect HSS Cyano column (2.5 µm,4.6 × 50 mm) using 0.1% formic acid containing MeOH and water in gradient mode at flow rate of 1 ml/min. Liver samples were homogenised using 0.1% w/v of ascorbic acid containing MeOH.

Preliminary data

The MS method deveopment fund each transitions for analyte to abundant product ions as follows: m/z 288.8 to m/z 244.8 for catechin and its isomer, epicatechin; m/z 227.0 to m/z 184.4 for resveratrol; m/z 315.0 to 299.8 for isorhamnetin; m/z 284.8 to 184.8 for kaempferol; m/z 300.8 to 150.4 for quercetin; and m/z 271 to 151 for the internal standard naringenin. Collision energies for each MRM were optimized at 21 V, 20 V, 25 V, 37V, 34V, 43V and 29 V, respectively.

For tissue sample analysis, various extraction procedure like protein precipitation extraction (PPE), liquid-liquid extraction (LLE) and solid phase extraction (SPE) were evaluated. Protein precipitation methods was selected. 20 µl of tissue homogenate was extracted with 380 µl of MeOH with 10 ng/ml of myricetin (Internal standard) and 0.1% formic acid. The supernatant was evaporated under slow nitrogen stream and reconstituted in 80 µl of 50:50 (MeOH:water) before analysis. This validated according to US Food and Drug Administration (USFDA) bioanalytical parameters like selectivity, linearity, precision, accuracy, limits of detection (LODs), limits of quantification (LOQs). This assay has a wide dynamic range ($R2 > 0.998$) for all the analytes (~ 3 orders of magnitude). Recovery of grape analytes was found in between 60-95%. Accuracy and precision were found within the limits (< 15% error). LOD and LOQ were lower than 0.25 ng/ml and 0.5 ng/ml for most of the analytes. This methodology was then applied for the mice liver samples obtained at fixed time point after feeding grape powder containing chow.

Novel aspect

A bioanalytical method was developed and validate to simultaneous quantify six major grape polyphenols in mouse tissue using LC-MS/MS.