Overview

- An analytical method has been developed for the simultaneous quantification of eight green tea-derived catechins from human plasma.
- This uses a protein precipitation-based extraction method, incorporating an in-house synthesised internal standard with LC-MS/MS analysis using a sub 2 µm column.
- Challenges such as analyte stability have been addressed and the method has been validated.

Introduction

Catechins are polyphenols that are abundant in tea derived from Camellia sinensis. Green tea extract contains four major (Figure 1) and four minor catechins (Figure 2).

Catechin consumption (particularly of EGCg) has been suggested to have health benefits such as lower incidences of atherosclerosis, cancer, diabetes and neurodegenerative disorders. A low intake of tea is associated with a reduction in the incidence of diabetes and cancer.

Catechins are present in all parts of the plant, but the highest concentrations are in the young leaves. The catechin contents in tea leaves are highly dependent on the stage and condition of the plants. Catechins in tea are low in black tea and high in green tea, and highest in tea that is picked within a few days of芽め（bud）、avae (leaf). Extraction of the catechins from green tea is a challenge due to the presence of a large number of other compounds, which can interfere with the analysis.

Methods

Extraction

Liquid-liquid (L-L), solid phase extraction (SPE) and protein precipitation (PP) methods were evaluated. L-L proved non-specific with variable analyte recovery, SPE (using a variety of sorbents) showed marked differences in recovery of different catechin isomers (e.g. C and CG). A universal SPE method could not be established or recreated from literature methods. A PP extraction method was developed and is summarised below.

Reconstitution and analysis

Evaporation of supernatant

Protein precipitation

Figure 2: Catechin extraction procedure

Results

No cross-talk from metabolites was observed. Samples exceeding 70 samples (including calibration lines and quality controls) were analysed without encountering deterioration issues.

There is significant difference in the extent of matrix suppression of C, EC, GC and EGC relative to the standards.

Conclusions

- An analytical method has been developed for green tea catechins.
- Analyte stability has been improved using antioxidants.
- An LC-MS/MS method has been developed where catechins (including pairs of epimers) are separated in a relatively short runtime.
- Assay performance has been significantly improved by the use of a deactivated internal standard prepared in-house.
- The method has been validated.

References