BEYOND MEASURE

SCION INSTRUMENTS

Analysis of Catechins by HPLC-UV

Application Note

AN0063

INTRODUCTION

Hot beverages are one of the most widely consumed drinks worldwide, with tea becoming increasingly more popular. Camellia Sinensis, the leaves used during the production of tea. Studies have shown that tea provides several health benefits such as protecting against cardiovascular disease and the management of cholesterol and obesity [1]. The main antioxidants found in tea are catechins.

The composition of catechins in commercial teas vary due to the species of Camellia Sinensis used, horticultural conditions but most importantly, the degree of oxidation during the manufacturing process. Natural processes such as sun drying or steaming the leaves, therefore preventing oxidation, not only protects the tea flavour but also results in high catechin concentrations with lower caffeine amounts whereas harsh leaf processing results in lower catechins and higher caffeine concentrations. Due to the variability in the composition of catechins in tea, it is vital that catechins can be easily identified in a variety of tea products.

SCION Instruments developed a method for the identification of eight catechins commonly found in tea products as well as caffeine, by HPLC-UV Vis.

EXPERIMENTAL

A SCION 6000 HPLC with UV was used with a C18 reverse phase column for the simultaneous identification of nine target compounds. An analytical standard containing all target analytes was analysed to demonstrate identification and separation of all compounds. Samples included commercially available bottled green tea and a green tea catechin supplement. Table 1 details the analytical conditions of the HPLC-DAD.

Table 1. Analytical conditions of HPLC-UV

Conditions	
Column	C18 5µm x 4.6mm ID x 150mm
Column Temp	40°C
Mobile Phase	A 0.05% Phosphoric Acid pH 2.4 B Methanol: Acetonitrile (3:2)
Gradient	0 min B:10% A:90% 15min: B25% A75% 25min: B60% A 40%
Flow Rate	1mL/min
Injection Vol	10µL
UV	280nm

RESULTS

Table 2 identifies all target compounds and associated abbreviations used throughout this application note.

Table 2. Target analytes and associated abbreviations

Compound	Abbreviation
Gallocatechin	GC
Gallocatechin Gallate	GCG
Epigallocatechin	EGC
Epigallocatechin Gallate	EGCG
Epicatechin	EC
Epicatechin Gallate	ECG
Catechin	C
Catechin Gallate	CG
Caffeine	CA

All target compounds were analysed in a concentration range of 1-50mg/L with the exception of caffeine (1-200mg/L). All target compounds gave a correlation coefficient of >0.999. Figures 1 and 2 show the calibration curve of EGC and CA, the two compounds with the lowest and highest R^2 value.

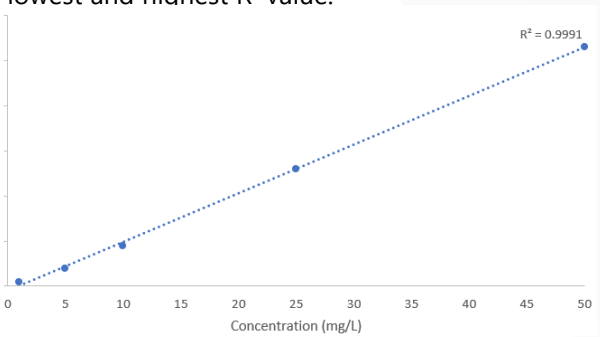

Figure 1. Calibration curve of epigallocatechin (EGC) 1-50mg/L

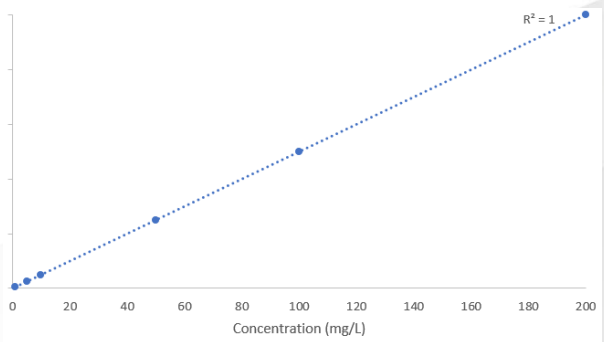

Figure 2. Calibration curve of caffeine (CA) 1-200mg/L

Following the excellent linearity observed of all target compounds, both the green tea and green tea supplement were analysed. Figures 3 and 4 show the obtained chromatograms including peak identification.

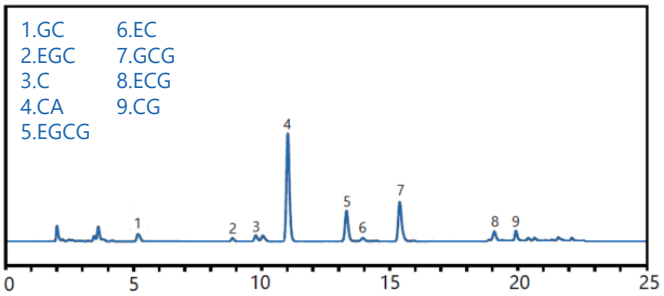

Figure 3. Chromatogram and compound identification of green tea sample

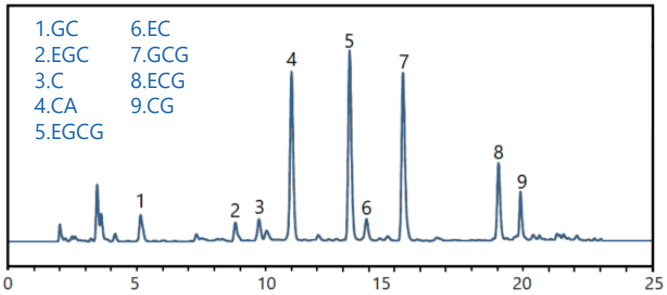

Figure 4. Chromatogram and compound identification of the supplement

As shown in the above figures, the green tea and the green tea supplement exhibit the same catechin profile but at varying concentrations. The naturally derived green tea drink gave considerably lower catechin and caffeine concentrations compared to the supplement sample, which exhibited high levels of CA, EGCG and GCG.

CONCLUSION

SCION Instruments offers an easy solution for the simultaneous identification of eight target catechins plus caffeine using the SCION 6000 HPLC-UV system. Excellent separation and linearity was observed for all target compounds using a C18 column at 280nm wavelength.

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