Rapid Separation of Catechins in Tea Using Core-Shell Columns

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Key Words
Green Tea, Antioxidants, Polyphenols, Accucore HPLC Columns, Flavonoids

Goal
Evaluate use of the core-shell column to determine catechins in different varieties of tea.

Introduction
Catechins are flavonoid phytochemical compounds found primarily in green tea and—in smaller amounts—in grapes, black tea, chocolate, and wine. Catechins are considered potent antioxidants that provide protection against certain diseases, such as cardiovascular disease and cancer. In North America, the consumption of green tea products has increased due to the reported health benefits associated with it. Next to water, tea is the most widely consumed beverage in the world, and can be found in almost 80% of all U.S. households, according to the Tea Association of the USA.[1] However, commercially available teas show a high variability in catechin content; therefore, simple and rapid methods are needed to evaluate product quality.[2]

Dionex (now part of Thermo Scientific) Application Note (AN) 275 demonstrated a sensitive method to determine catechins in a variety of commercially available teas using a Thermo Scientific Acclaim Rapid Separation Liquid Chromatography (RSLC) 120 C18 column.[3] Catechins present in tea were separated in 15 min at a flow rate of 0.45 mL/min and a system backpressure of ~6200 psi during the gradient.

This study evaluates a Themo Scientific Accucore C18 High-Performance LC (HPLC) column to rapidly (<6 min) determine catechins in three different types of tea. Core-shell particles improve mass transfer kinetics, and therefore separation efficiency, by restricting intraparticle diffusion to the thin, porous shell while maintaining the hydraulic permeability associated with the total particle diameter.[4-7] This work demonstrates how core-shell columns can be used to increase separation efficiency with improved mass transfer kinetics without significantly increasing pressure. The Accucore™ C18 HPLC column enables faster separation of catechins than when using the Acclaim™ C18 column per the method described in AN 275.

Equipment
Thermo Scientific Dionex UltiMate 3000 RSLC System, including:

- SRD-3600 Integrated Solvent and Degasser Rack (P/N 5035.9230)
- EO Eluent Organizer including pressure regulator and 2 L glass bottles for each pump, eluents maintained under helium or nitrogen headspace (5–8 psi)
- HPG-3400RS Pump with Solvent Selector Valves (P/N 5040.0046)
- WPS-3000TRS Well Plate Sampler, Thermostatted (P/N 5840.0020)
- Sample Loop, 25 µL (P/N 6820.2415)
- TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
- DAD-3000RS Diode Array Detector (P/N 5082.9920)
- Semi-Micro Flow Cell for DAD-3000 and MWD-3000 Series, SST, 2.5 µL Volume, 7 mm Path Length (P/N 6082.0300)

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Sample Preparation

Prepare each of the tea samples by weighing 60 mg of solid and adding 7 mL of 0.05% formic acid in 70% methanol. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min. Collect the supernatant in a glass vial and add an additional 7 mL of solvent to the pellet. Vortex the mixture again to mix, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min. Add the supernatant to the first 7 mL to make a total volume of 14 mL. Filter the samples using 0.2 µm cellulose acetate sterile syringe filters and dilute 1:5 with 0.05% formic acid in 70% methanol prior to analysis.

Figure 1 shows the standard with the predominant catechins in tea. In addition, free gallic acid and caffeine are naturally present in tea, and therefore were included in the mixed standard. The retention times are 0.61 min for gallic acid, 1.2 min for gallocatechin, 2.6 min for epigallocatechin (EGC), 2.7 min for catechin, 2.9 min for caffeine, 3.2 min for epicatechin (EC), 3.3 min for epigallocatechin gallate (EGCG), 3.5 min for gallocatechin gallate, and 5.0 min for epicatechin gallate, with all analytes at a concentration of 100 µg/mL with the exception of gallic acid at 50 µg/mL and caffeine at 35 µg/mL.

All catechins with the exception of EC and EGCG were baseline resolved in <6 min. If a better separation of EC and EGC is desired, the 15 min separation described in AN 275 using an Acclaim RSLC C18 column can be used.

Column:	Accucore C18, 2.6 µm, Analytical (150 × 2.1 mm)
Eluent:	A: 2.5% Acetonitrile in water B: 0.1% TFA in acetonitrile
Gradient:	0.0–1.0 min, 0% B 1.0–5.0 min, 10% B 5.0–6.0 min, 0% B
Flow Rate:	0.8 mL/min
Inj. Volume:	2.0 µL
Temperature:	42 °C
Detection:	Absorbance, UV 280 nm

Peaks:
1. Gallic Acid
2. Gallocatechin
3. Epigallocatechin
4. Catechin
5. Caffeine
6. Epicatechin
7. Epigallocatechin Gallate
8. Gallocatechin Gallate
9. Epicatechin Gallate

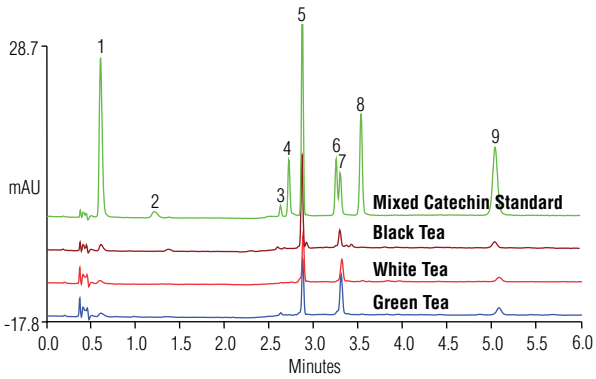

Figure 1. Separation of catechins in a mixed standard and three different commercially available teas using an Accucore C18 HPLC column.

The samples investigated in this study included green, white, and black teas. White tea is minimally processed and is expected to be very high in catechin content. However, green tea showed the highest catechin content, suggesting that the quality of the white tea was poor and the tea was possibly adulterated with more processed varieties of teas.

Conclusion

This work describes a simple and rapid method to determine catechins in different commercially available teas with a simple solvent extraction. The method uses an Accucore C18 HPLC column and absorbance detection at a wavelength of 280 nm to separate and detect catechins in <6 min. The method described in this study is ideal for routine and rapid screening of catechins in different tea products.

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