Separation of 10 Green Tea Components on an Agilent InfinityLab Poroshell 120 EC-C18, 1.9 µm Column

Application Note

Agriculture, Food Testing

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Abstract

Ten compounds commonly found in green tea were analyzed with an Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm column using a formic acid and acetonitrile gradient. Eight catechins, including four isomer pairs, plus gallic acid and caffeine were baseline resolved in 7 minutes, with a minimum resolution of 2.2.

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Introduction

Catechins are known to affect the color and flavor of tea, contributing to its characteristic bitterness. Particular attention focuses on epigallocatechin gallate (EGCG), as the most abundant polyphenol in tea extracts. While all teas originate from the same plant, *Camellia sinensis*, the amount of oxidation that a tea leaf undergoes following harvesting dictates what type of tea is produced. Oxidation breaks down chlorophyll and releases tannins so the leaves turn darker. The oxidation process is then stopped at different times by heating the leaves and deactivating the enzymes responsible for breaking down chlorophyll. Black tea is fully oxidized, oolong tea is semi-oxidized, and green tea is unoxidized [1,2].

This application note demonstrates the UHPLC performance of an Agilent InfinityLab Poroshell 120 EC-C18 1.9 µm column, and its ability to baseline-resolve 10 closely related compounds.

Experimental

An Agilent 1290 Infinity LC system was used in this experiment. The system was modified from its standard configuration to have low system volume and dispersion. Table 1 shows the configuration details, and the Agilent LC column used in this experiment. Table 2 shows the LC method parameters.

The 10 green tea components analyzed in this work were purchased from Sigma-Aldrich; their structures are shown in Figure 1. Formic acid was also purchased from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore).

Table 1. UHPLC System Configuration

Agilent 1290 Infinity LC System Configuration	
Agilent 1290 Infinity Binary Pump (G4220A)	35 µL solvent mixer: Jet Weaver, 35 µL/100 µL (G4220-60006)
Agilent 1290 Infinity High Performance Autosampler (G4226A)	Seat assembly, ultralow dispersion, for Agilent 1290 Infinity Autosampler (G4226-87030) Autosampler to heater: capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784) Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (5182-0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (5182-0717) Vial insert, 250 µL, glass with polymer feet, 100/pk (5181-1270)
Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)	Heat exchanger, low dispersion, 1.6 µL, double (G1316-60005) Heater to column: Agilent InfinityLab Quick Connect assembly, 105 mm, 0.075 mm (5067-5961) Column to flow cell: capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784)
Agilent 1290 Infinity Diode Array Detector (G4212A)	Agilent Ultralow dispersion Max-Light cartridge flow cell, 10 mm (G4212-60038)
Agilent OpenLAB CDS ChemStation Edition, revision C.01.05 [35]	G4220A: B.06.53 [0013] G4226A: A.06.50 [003] G1316C: A.06.53 [002] G4212A: B.06.53 [0013]
Agilent LC Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (699675-902)

Table 2. UHPLC Method Parameters

Column	Mobile phase	Flow rate (mL/min)	Gradient	Injection volume (µL)	Sample	Thermostated column compartment (°C)	Diode array detector
Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm	A) 0.2 % Formic acid in water B) Acetonitrile	0.5	5–16 %B in 7 minutes	1	0.06 mg/mL each of gallic acid, gallocatechin, epigallocatechin, catechin, caffeine, epicatechin, epigallocatechin gallate, gallocatechin gallate, epicatechin gallate, catechin gallate in water. Compounds are listed in elution order; see Figure 1 for structures.	30	240 nm, 80 Hz

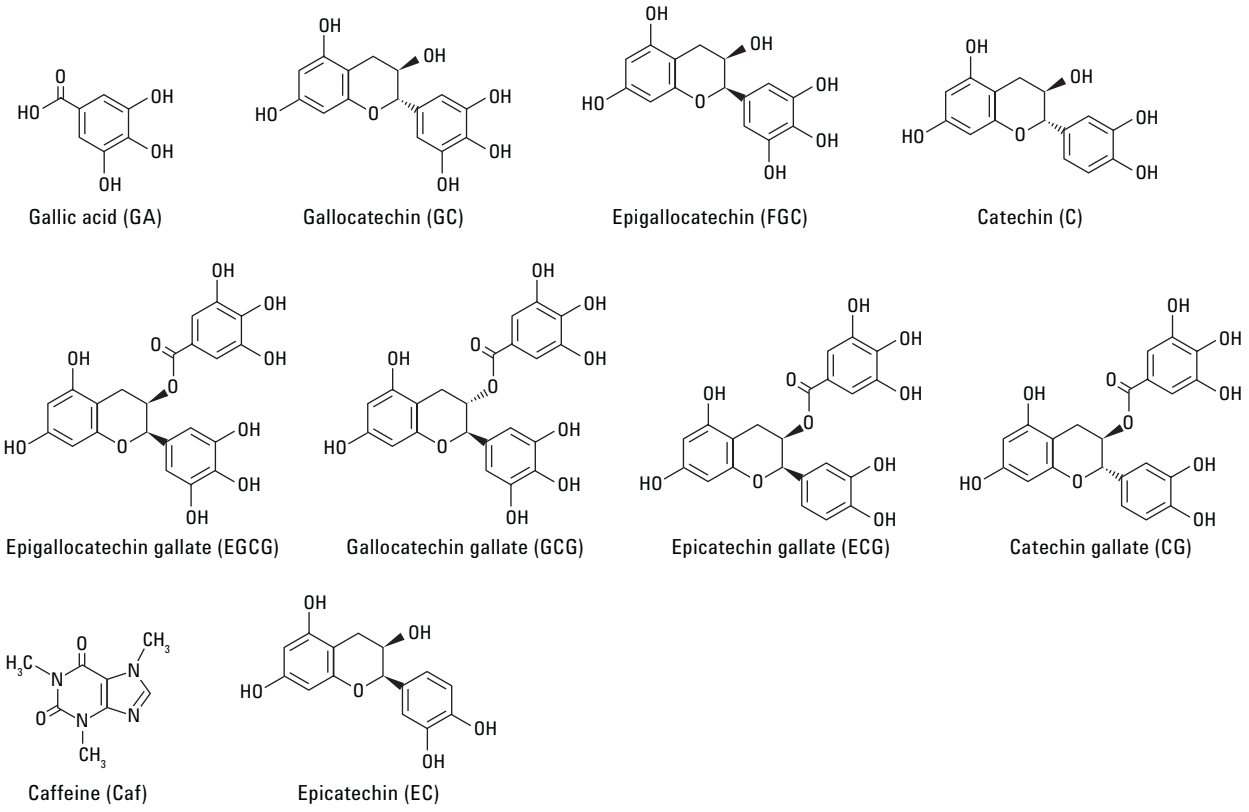

Figure 1. Compounds of interest.

Results and Discussion

Figure 2 shows the separation of 10 green tea components on an InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm column. All compounds were baseline-resolved, with a minimum resolution of 2.2, in seven minutes. The catechins were difficult to separate because they are structurally similar. The sample included four isomer pairs that can be challenging to resolve. However, the InfinityLab Poroshell 1.9 µm column had enough efficiency and resolving power, even in a short 50 mm format, to successfully separate this sample.

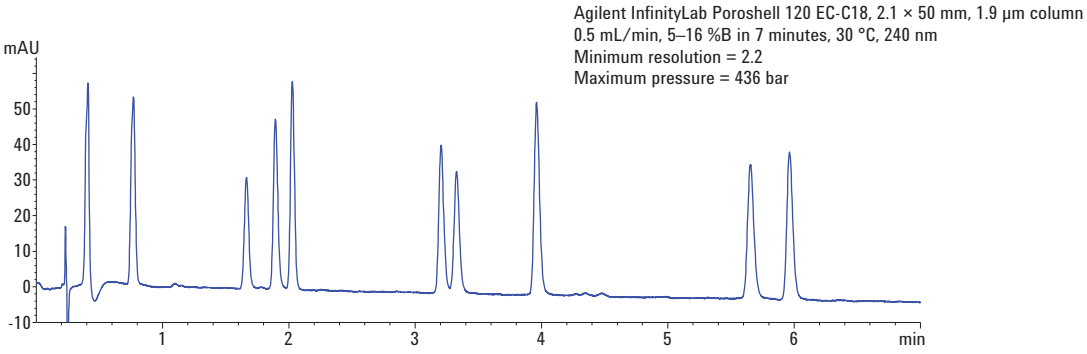

Figure 2. Separation of green tea catechins on an Agilent InfinityLab Poroshell 120 EC-C18, 1.9 µm column.

Conclusions

The Agilent InfinityLab Poroshell 120, 1.9 µm column was used to accomplish a challenging separation including eight isomer pairs, plus two additional compounds. The high efficiency of this small superficially porous particle column provided sufficient resolution to baseline-resolve the closely related catechin compounds on a short 2.1 × 50 mm column.

References

1. A. Mack, W. Long. Rapid Tea Analysis on Poroshell 120 SB-C18 with LC/MS. *Agilent Technologies Application Note*, publication number 5990-7824EN, **2011**.
2. T. Yoshida, R. Majors, H. Kumagai. "High-Speed Analysis using Rapid Resolution Liquid Chromatography on ZORBAX column packed 1.8 µm Particles" *J. Sep. Sci.* **2006**, 29(16), 2421-2432.

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