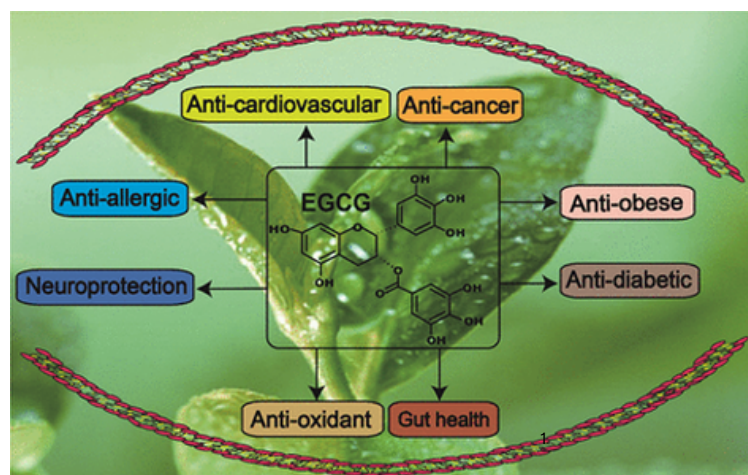


Natural Product Extraction with Stacking Reversed Phase SepaFlash® C18 Column, How to Get Four Active Ingredients in Green Tea in One Run

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Chromatography Application Note ANSS-014



Green tea is widely known for its superb health benefits such as anti-cancer, anti-inflammatory, antioxidant, anti-allergic, etc. Among the biologically active compounds, the main active/antioxidant agents are catechins.¹ Those natural polyphenolic phytochemicals exhibit the strong property of neutralizing reactive oxygen and nitrogen species.² The group of catechins derivatives in green tea includes: EGCG (Epigallocatechin gallate), ECG (Epicatechin gallate), EGC (Epigallocatechin), and EC (Epicatechin).

However, the effectiveness and the amount of those catechins dramatically depends on types of the green tea. Dietary supplementation with these active catechins have been widely used toward promoting human health. Therefore, a fast and convenient method to extract and separate individual catechins is in demand.

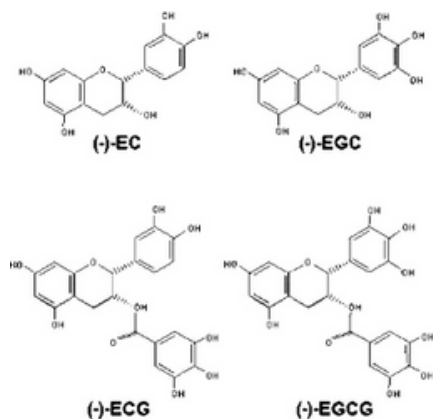


Figure 1 Structure of epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) used in this investigation.

In this study, researchers from Santai used the SepaBean™ chromatography system in conjunction with the SepaFlash® C18 high-performance spherical column to extract multiple components from tea polyphenols, obtaining four monomers of catechins, namely EGCG, ECG, EGC, and EC separately. This provides an economically efficient and rapid solution for the effective preparation and purification of this category of natural products.

The extraction of tea polyphenols was carried out using commercially available regular tea polyphenols capsule. After dissolving 5 g of tea polyphenols in 50 ml of 5% ethanol-water solution. The solution was treated with 5 g of active charcoal to absorb the impurities, which was allowed to sit still for 30 mins. The supernatant was separated from the sediments and treated with DCM; thus a solution of the active ingredients was acquired. An aliquot of 1 ml of the prepared DCM sample solution was filtered through a 0.45 μm microporous membrane. The clear liquid was loaded onto the SepaBean machine T with two stacked 40 g C18 reversed phase (SW-8222-SP) for the automatic separation process as shown in Figure 2.

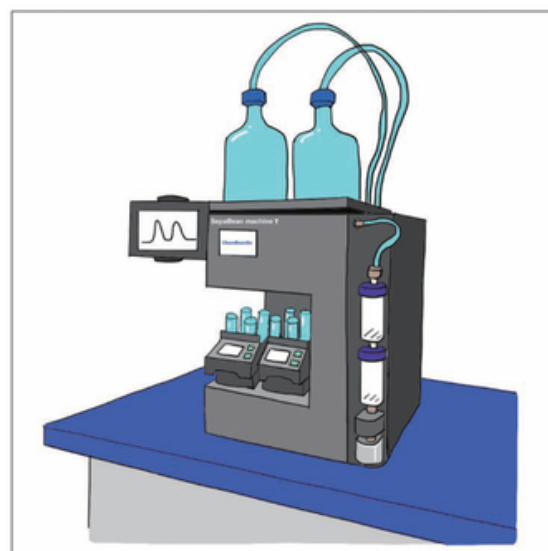


Figure 2 The schematic diagram of two stacking Flash column installed on a SepaBean machine T

¹ Musial C, Kuban-Jankowska A, Gorska-Ponikowska M. Beneficial Properties of Green Tea Catechins. *Int J Mol Sci.* 2020 Mar 4;21(5): 1744.

² Fan FY, Sang LX, Jiang M. Catechins and Their Therapeutic Benefits to Inflammatory Bowel Disease. *Molecules.* 2017 Mar 19;22(3):484

Instrument	SepaBean™ machine T
Columns	2pc, 40 g SepaFlash® Bonded C18 column (Spherical, 20-45 µm, 100Å, SW-8222-040)
Wavelength	275 nm 280 nm
Mobile phase	Solvent A: Water Solvent B: Methanol
Flow rate	30 mL/min
Sample loading	100 mg

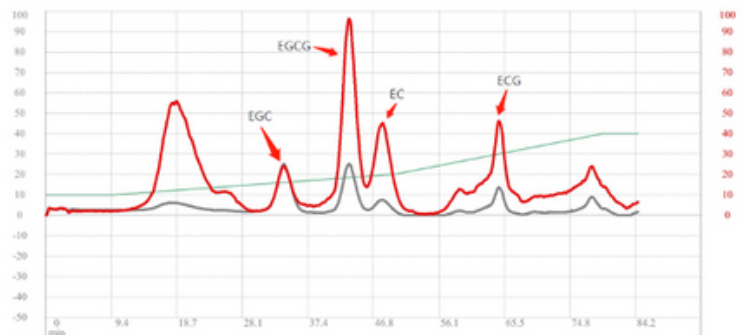


Figure 3. The flash chromatogram of the extraction of EC, ECG, EGC and EGCG from green tea by Santai reversed phase C18 column (SW-8222, 20-45 µm, 100 Å)

Table 1. The experimental setup for flash purification of catechins with two stacking columns.

As shown in Figure 3, the green tea extract mixture purification on two stacked 40g SepaFlash® C18 reversed phase column last 84 minutes, with all four target active ingredients nicely separated. It has shown that a blend of a certain percentage of the various green tea catechins could achieve the optimal health effect. With our SepaBean machine T, and our SepaFlash column, one simple run can yield four monomers of catechins from the crude extract of tea polyphenols¹, thus allow the researchers further their study of the synergy effect of various combination of catechins monomers and enable the formulation companies to provide the customers with optimized products.

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