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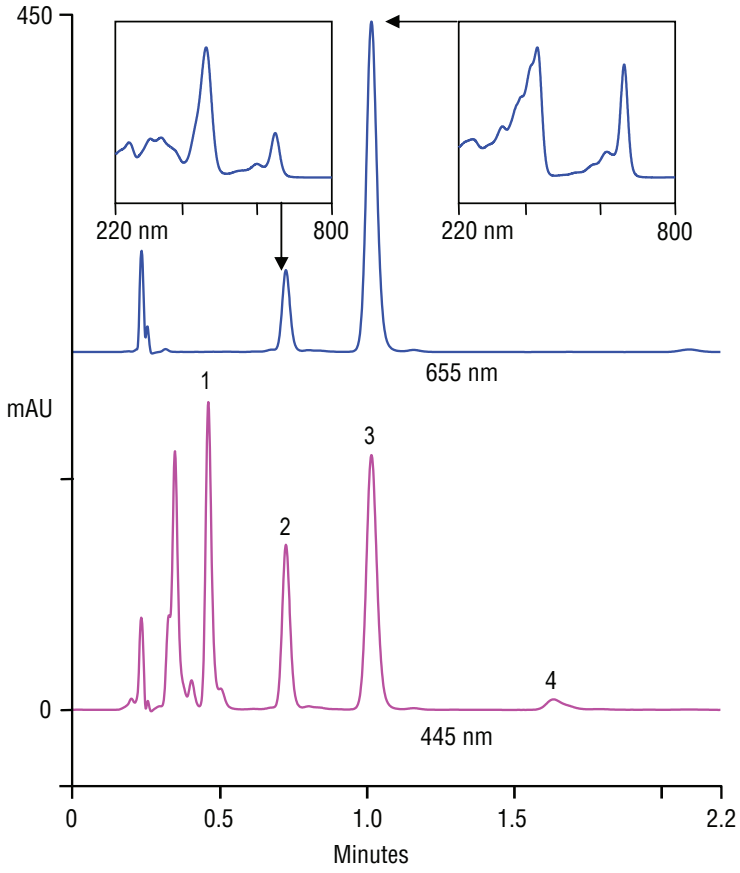
Chlorophyll on a Thermo Scientific™ Acclaim™ RSLC PolarAdvantage II (PA2) Column



Column:	Thermo Scientific™ Acclaim™ RSLC PolarAdvantage II (PA2), 2.2 µm
Dimension:	2.1 × 50 mm
HPLC System:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
Mobile Phase:	50/50/0.1 v/v/v acetonitrile/methanol/ 2 M ammonium acetate pH 5.4
Flow Rate:	0.60 mL/min
Pressure:	300 bar
Inj.Volume:	2 µL
Temperature:	30 °C
Detection:	Visible at 445 and 655 nm (shown); spectra 220–800 nm 10 Hz data rate, 0.5 sec time constant
Sample:	Acetone extract of spinach leaf
Peaks:	1. Lutein 2. Chlorophyll b 3. Chlorophyll a 4. beta-Carotene

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Chlorophyll is a green pigment found in most plants, algae, and cyanobacteria. Chlorophyll is vital for photosynthesis, which allows plants to obtain energy from light. Chlorophyll a and chlorophyll b are present in plants. Because of their high hydrophobicity, they can be analyzed by HPLC using a reversed-phase column under normal-phase condition (high organic solvent). The Acclaim RSLC PA2 column stationary phase is based on bonding silyl ligands containing a hydrophobic alkyl chain with a polar amide-embedded group, onto high-purity, spherical, porous, 2.2 micron silica particles. As the result, a 2.1 × 50 mm Acclaim RSLC PA2 column offers suitable selectivity for high-speed, baseline separation of chlorophylls in spinach leaf. The small amount of buffer ensures good reproducibility and symmetrical peaks. It was observed that in this application, acetonitrile is the weak solvent for the polar pigments, and methanol is the strong solvent.