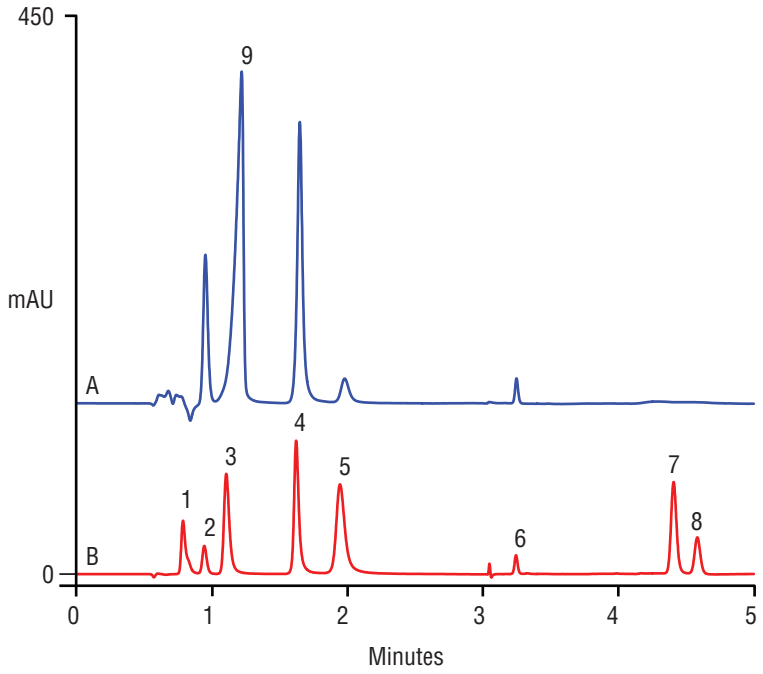
Thermo SCIENTIFIC

A Thermo Fisher Scientific Brand

Water-Soluble Vitamins on a Thermo Scientific™ Acclaim™ RSLC PolarAdvantage II (PA2) Column with TFA Mobile Phase Additive



Column:	Thermo Scientific™ Acclaim™ RSLC PA2, 2.2 µm
Dimensions:	2.1 × 100 mm
System:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC
Mobile Phases:	A: 30 mM H_3PO_4 + 4 mM TFA adjusted to pH 2.95 with NH_4OH B: Isopropanol

Gradient Times:

min	-4.0	0.0	0.8	3.0	5.0
%A	100	100	100	88	88
%B	0	0	0	12	12

Flow Rate:	0.41 mL/min
Pressure:	280–430 bar
Injection:	2 µL; bypass mode at 0.15 min
Temperature:	25 °C
Detection:	Diode array, UV 210 (shown), 246, 260, 375 nm; spectra 200-450 nm Baseline subtraction with water blank

Peaks:

1. Thiamine	25	µg/mL
2. Ascorbic acid	25	
3. Niacin	25	
4. Niacinamide	25	
5. Pyridoxine	25	
6. Pantothenic acid	25	
7. Folic acid	25	
8. Riboflavin	10	
9. Citrate	–	

A. Propel® (PepsiCo) lemon flavor drink mix, 0.40 g in 20 mL water, filtered
B. Standards in phosphate buffer

Propel is a registered trademark of Stokely-Van Camp, Inc.

26201

Water-soluble vitamins are a chemically heterogeneous group including acids, bases, zwitterions, and neutrals with different spectroscopic properties. The amounts in samples can vary from a few micrograms to hundreds of milligrams. Each matrix presents a unique set of interferences and sample preparation problems. The Acclaim PolarAdvantage II (PA2) column features an amide embedded functionality in the stationary phase, and provides unique selectivity and aqueous compatibility, making it suitable to separating water-soluble vitamins. The use of the 2.2 µm Acclaim RSLC column in 2.1 mm i.d. format allows fast analysis time with reduced solvent consumption. The diode-array detector confirms the identity and purity of each peak. In this example, trifluoroacetic acid (TFA) is used to improve the retention time and peak shapes of thiamine and pyridoxine. As the result, the Acclaim RSLC PA2 column baseline separates eight common water-soluble vitamins using a “green” method (iso-propanol as the organic modifier) in 5 min. Note that citric acid and other minor components can interfere with ascorbic acid or pyridoxine, which requires that the pH of the mobile phase be carefully adjusted.