

The Separation of Carotenoids Using Segmented Column Technology (SCT)

Chemists: K. O'Connor and
G. Vella
037

Conditions:

Column: Nova-Pak™ C₁₈, 6 μm,
25 mm ID PrepPak® Cartridges

Eluent: Acetonitrile/Methanol/
Tetrahydrofuran (58:35:7),
isocratic

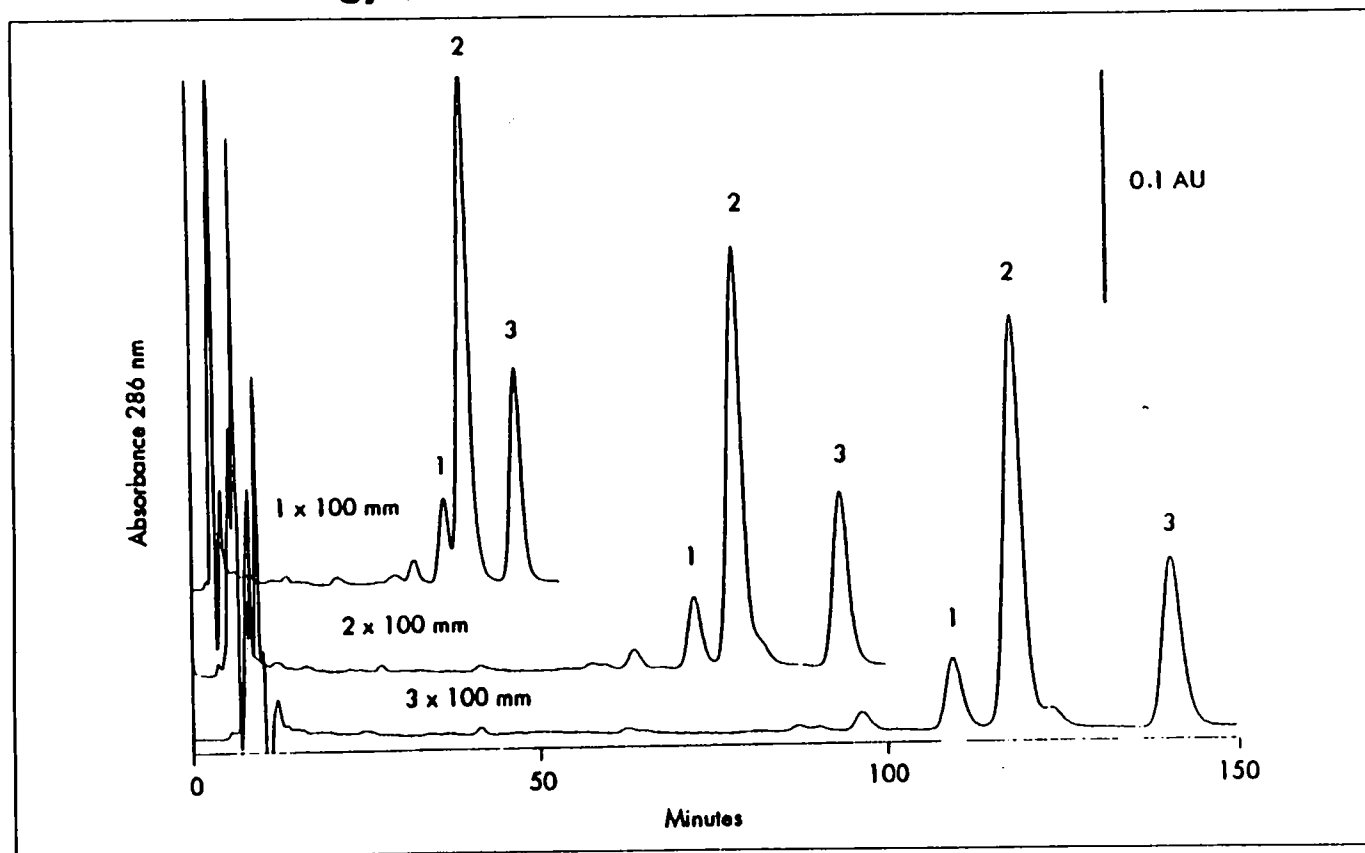
Flow Rate: 10 ml/min.

Injection Volume: 1.5 ml

Sample Concentration: 8 mg/ml
dissolved in Toluene/Eluent
(25:75)

Peak ID's:

1. Alpha-carotene
2. Trans-Beta-carotene
3. Phytoene



Segmented Column Technology employs cartridge columns which provide new high resolution options which are cost effective and flexible.

Objective:

The separation of carotenoids from a crude carrot extract is used to demonstrate that efficiencies are additive as column length is extended from 100 mm to 300 mm lengths. Column configuration (length) can be tailored to the separation need to provide sufficient resolution yet conserve time and solvent.

Details:

The table below summarizes efficiency (5-sigma using phytoene), selectivity (alpha- and trans-beta-carotene) and resolution (alpha- and trans-beta-carotene) as column length is increased from 100 to 300 mm using Segmented Column Technology.

The sample preparation involved blending raw carrots with methanol to remove the water, then filtering and discarding the filtrate. The carrot pulp was extracted with tetrahydrofuran repeatedly until the pulp was colorless. This mixture was evaporated to dryness and redissolved in a minimal amount of toluene. The sample was prepared for chromatographic analysis by mixing the concentrated toluene mixture with the mobile phase, acetonitrile/methanol/tetrahydrofuran (58:35:7), to arrive at a final solution that was 25% toluene in mobile phase prior to injection. This amount of toluene was necessary to maintain sample solubility.

Column Length (mm)	Plates (5 sigma)	Selectivity	Resolution
100	3708	1.08	0.89
200	7784	1.08	1.38
300	11962	1.08	1.67

System:

The system used consisted of a 600E Multisolvent Delivery System, 490 Programmable Multi-Wavelength UV/Visible Detector, U6K Injector with a 10 ml loop and 860 Expert Ease Data System.

References:

1. Kevin O'Connor and George Vella, "Non-Aqueous Reverse Phase Purification of Carotenes on a Small Particle Preparative Packing.", Presented as a poster at FASEB/ASBMB Meeting, April 22-25, 1991, Atlanta, GA, USA
2. Kevin O'Connor, "Non-Aqueous Reverse Phase Purification of Carotenes Using a Small Particle Preparative Packing", Waters Column, Summer 1990, pages 1,2, and 11-13.