Carotenoid Characterization by HPLC

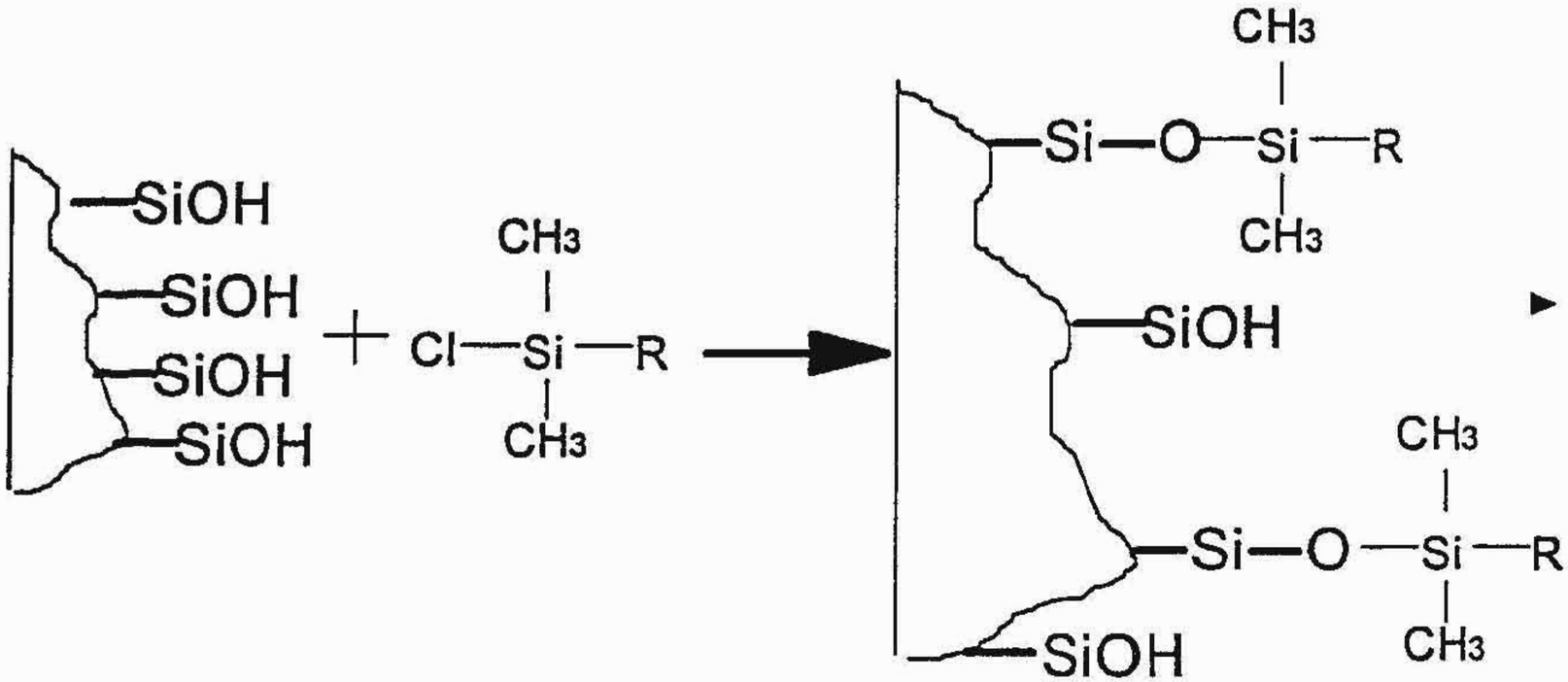
YMC AMERICA, INC.

Introduction

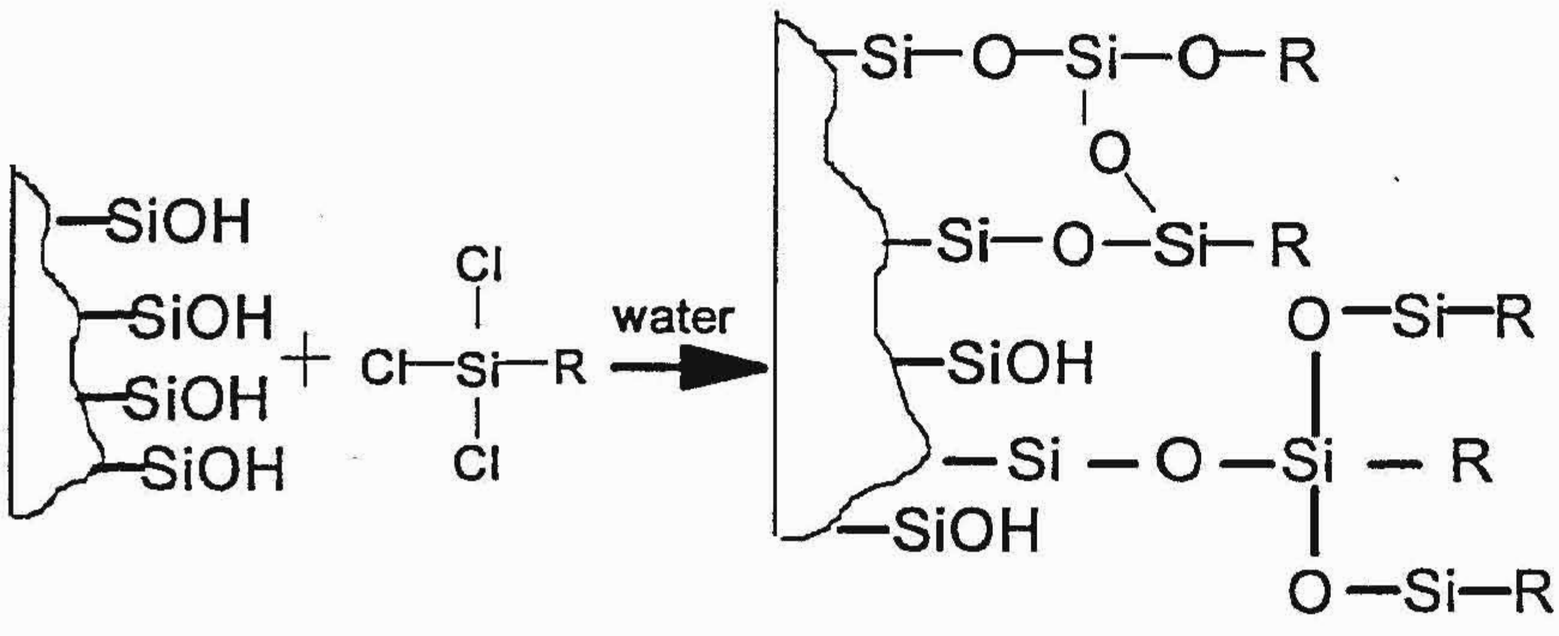
Interest in the perceived health benefits of carotenoids has led to the separation, purification and quantification of materials containing these compounds. Carotenoids constitute a diverse class of molecules with properties such as the non-polar carotenes and the polar xanthophylls. Very subtle molecular differences exist within these compound types to make up the many geometric and positional isomers of carotenoids. Because of the complexity of carotenoid extracts and the minor shape differences among carotenoid isomers, separation of individual species has been very challenging.

LC separations of carotenes and xanthophylls have been developed using both monomeric and polymeric surface modification. This paper discusses the results from controlling the appropriate bonded phase variables and describes the development of a stationary phase tailored for the separation of carotenoids. The utility of this tailored phase is demonstrated for the separation of carotenoids in dietary supplements and vitamins with enhanced selectivity towards isomers and other groups of compounds with similar molecular structure.

Monomeric versus Polymeric Synthesis of the Bonded Phase



- Monomeric Synthesis (Monofunctional Silane)
  - Single point attachment.
  - High efficiency for small molecules.

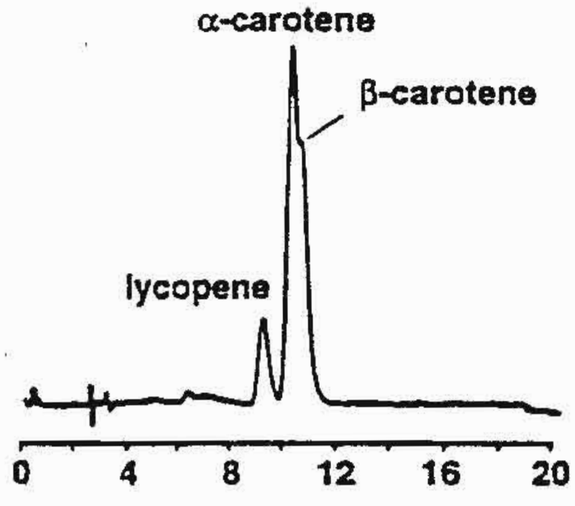


- Polymeric Synthesis (Trifunctional Silane)
  - Multipoint attachment.
  - Bridged ether linkage.
  - Highly selective for large long chained molecules.

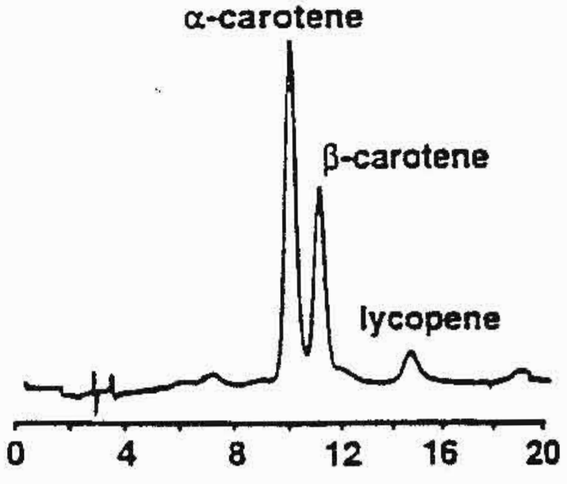
Chromatographic Comparison of Monomeric vs. Polymeric Synthesis of the Stationary Phase

- Selectivity is enhanced for the carotenoids with polymeric synthesis:
  - Lycopene is retained longer on the polymeric phase.
  - Alpha-carotene is retained less on the polymeric phase.

Monomeric

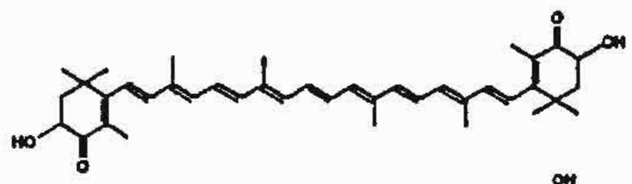


Polymeric

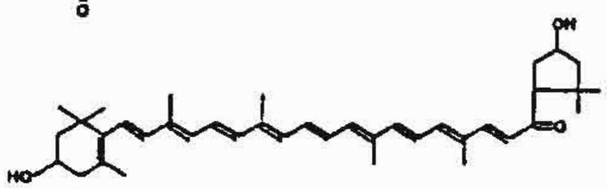


Carotenoid Structures

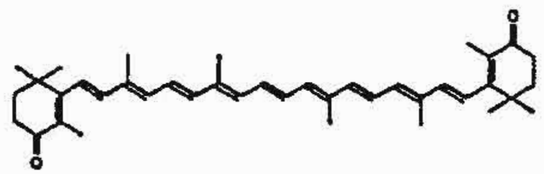
Xanthophylls



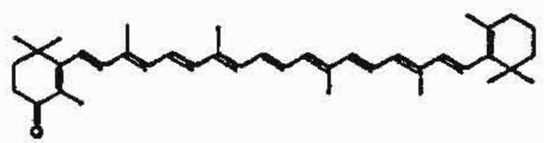
Astaxanthin



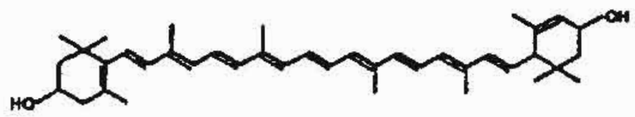
Capsanthin



Canthaxanthin

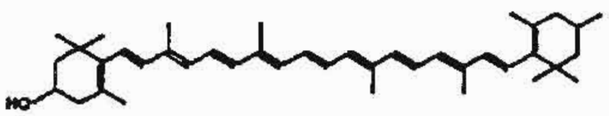


Echinenone



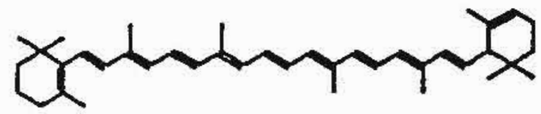
Lutein

Zeaxanthin

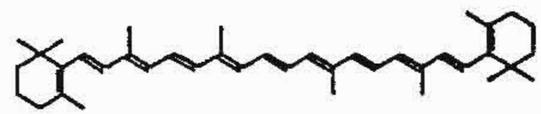


β-Cryptoxanthin

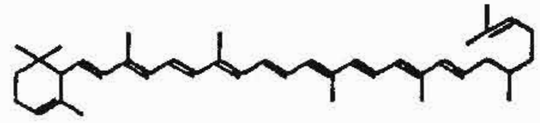
Hydrocarbons



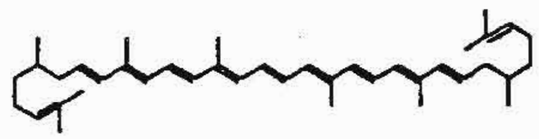
α-Carotene



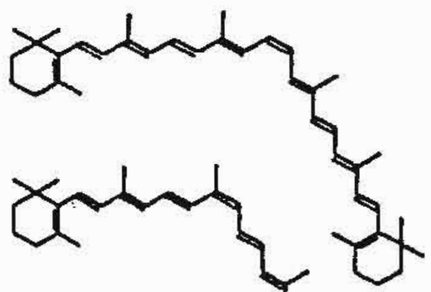
β-Carotene



δ-Carotene



Lycopene



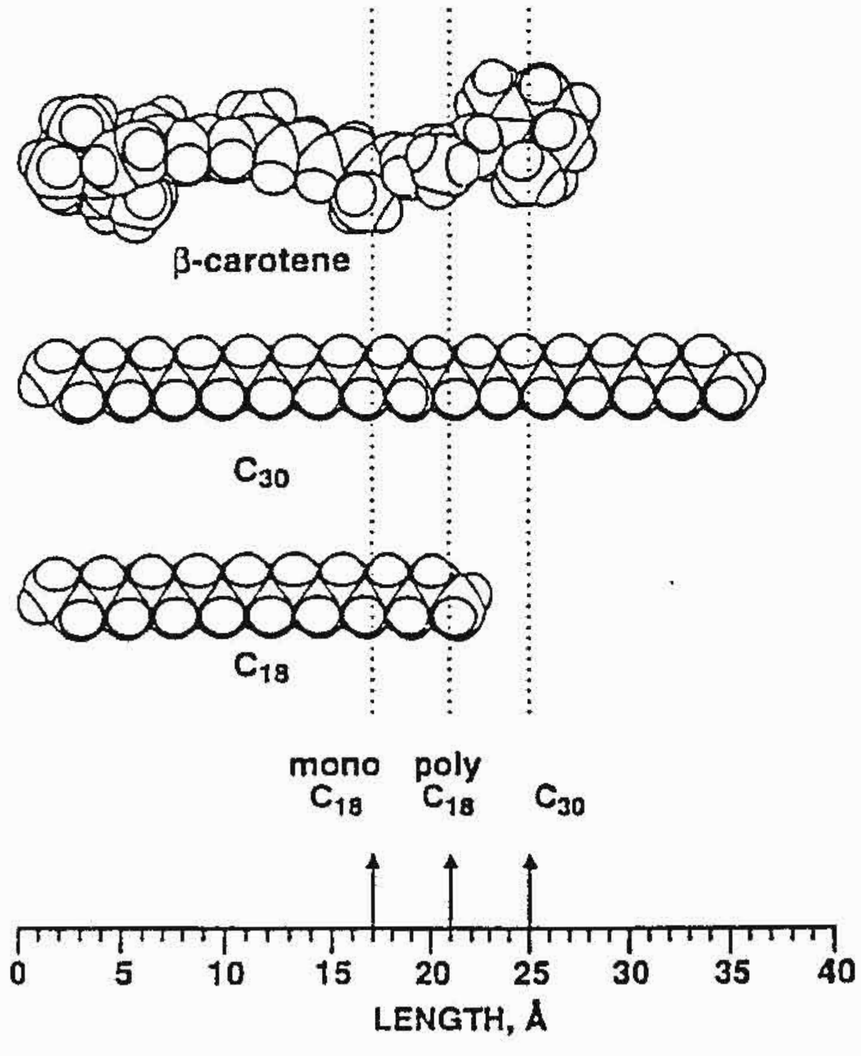
15-cis β-Carotene

13-cis β-Carotene

9-cis β-Carotene

Spacial Representation of Beta-carotene

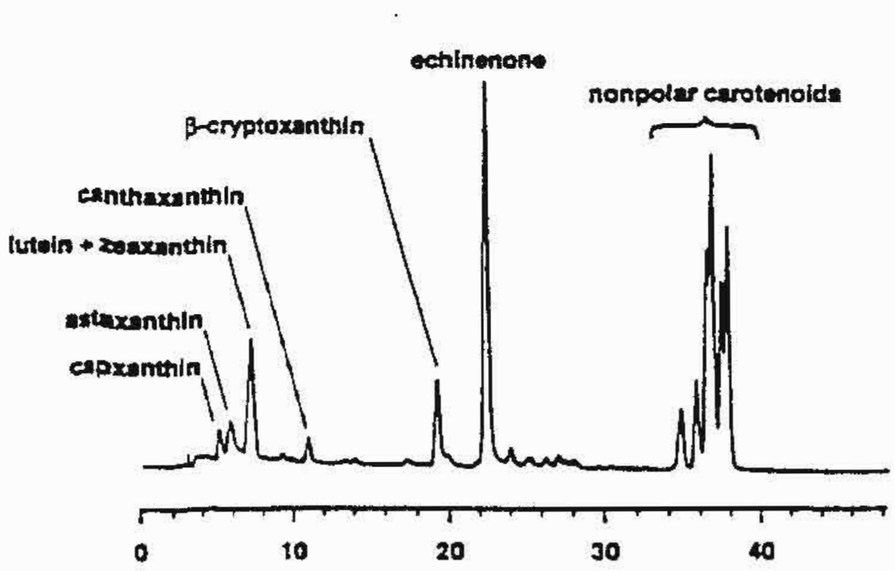
- C30 provides the greatest number of interaction sites for complete partitioning of the positional isomers.
- C30 enhances selectivity of these positional isomers.
- The shorter chain length of the C18 does not provide sufficient partitioning sites to resolve the structurally similar isomers.



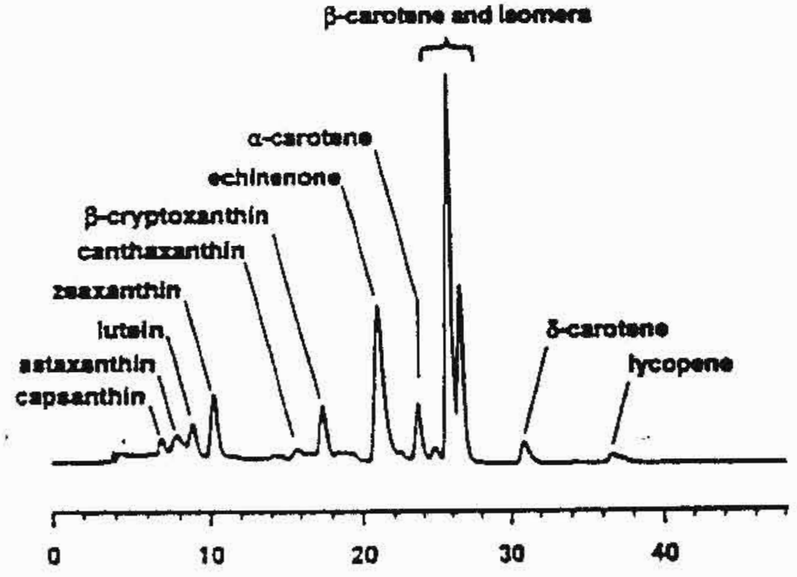
Monomeric C18 vs. Polymeric C18

- ► Monomeric Bonding
  - Incomplete resolution of the non-polar carotenoids.
  - Co-elution of lutein and zeaxanthin.
- ► Polymeric Bonding
  - Incomplete resolution of Beta-carotene and isomers.
  - Improved resolution of lutein and zeaxanthin.

Monomeric



Polymeric



Conditions:

Flow: 1 mL/min

Temp: 20 degrees C

Solvent A 81:15:4 MeOH:MTBE:H_2O

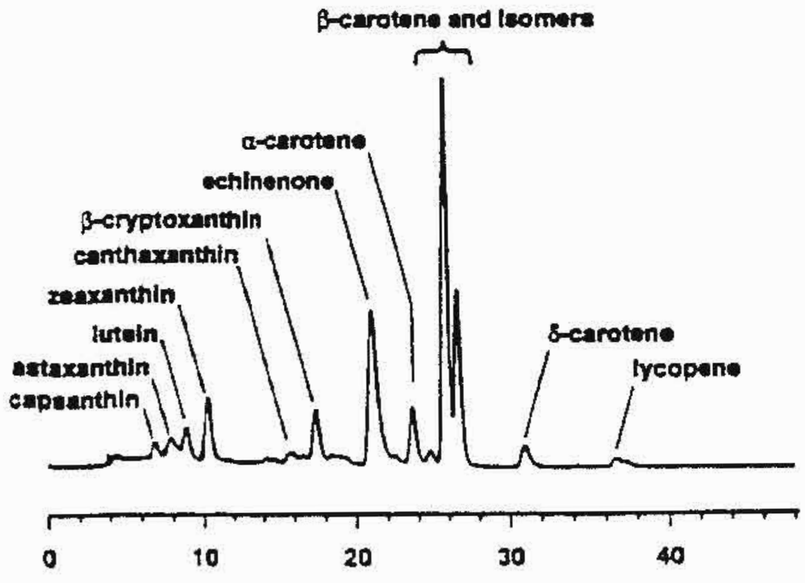
Solvent B 6:90:4 MeOH:MTBE:H_2O

100% A to 100% B in 90 minutes

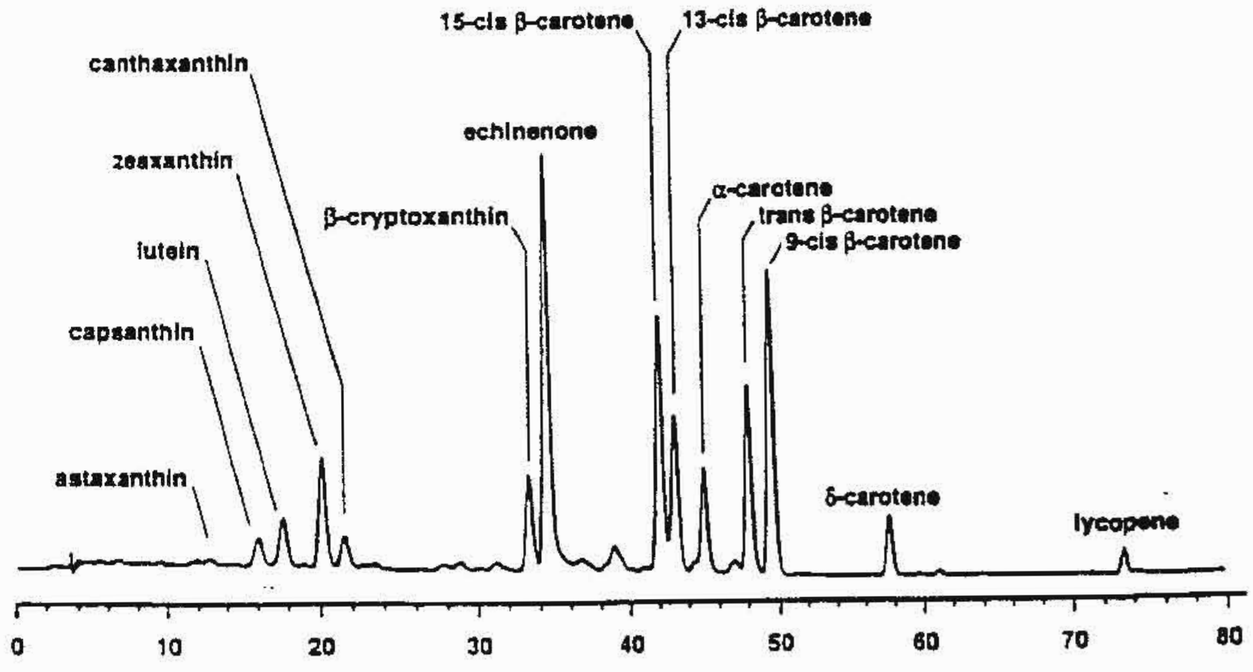
Polymeric C18 vs. Polymeric C30

- C30 gives longer retention and greater selectivity for the carotenoid isomers.
  - Lycopene elutes at 37 minutes on C18 versus 74 minutes on C30.
  - Beta-carotene isomers elute from 26-28 minutes on C18 versus 42-50 minutes on C30.
  - Lutein and zeaxanthin more fully resolved on C30 versus C18.

Polymeric C18



Polymeric C30



- Conditions:

  Flow: 1 mL/min

  Temp: 20 degrees C

  Solvent A 81:15:4 MeOH:MTBE:H_2O

  Solvent B 6:90:4 MeOH:MTBE:H_2O

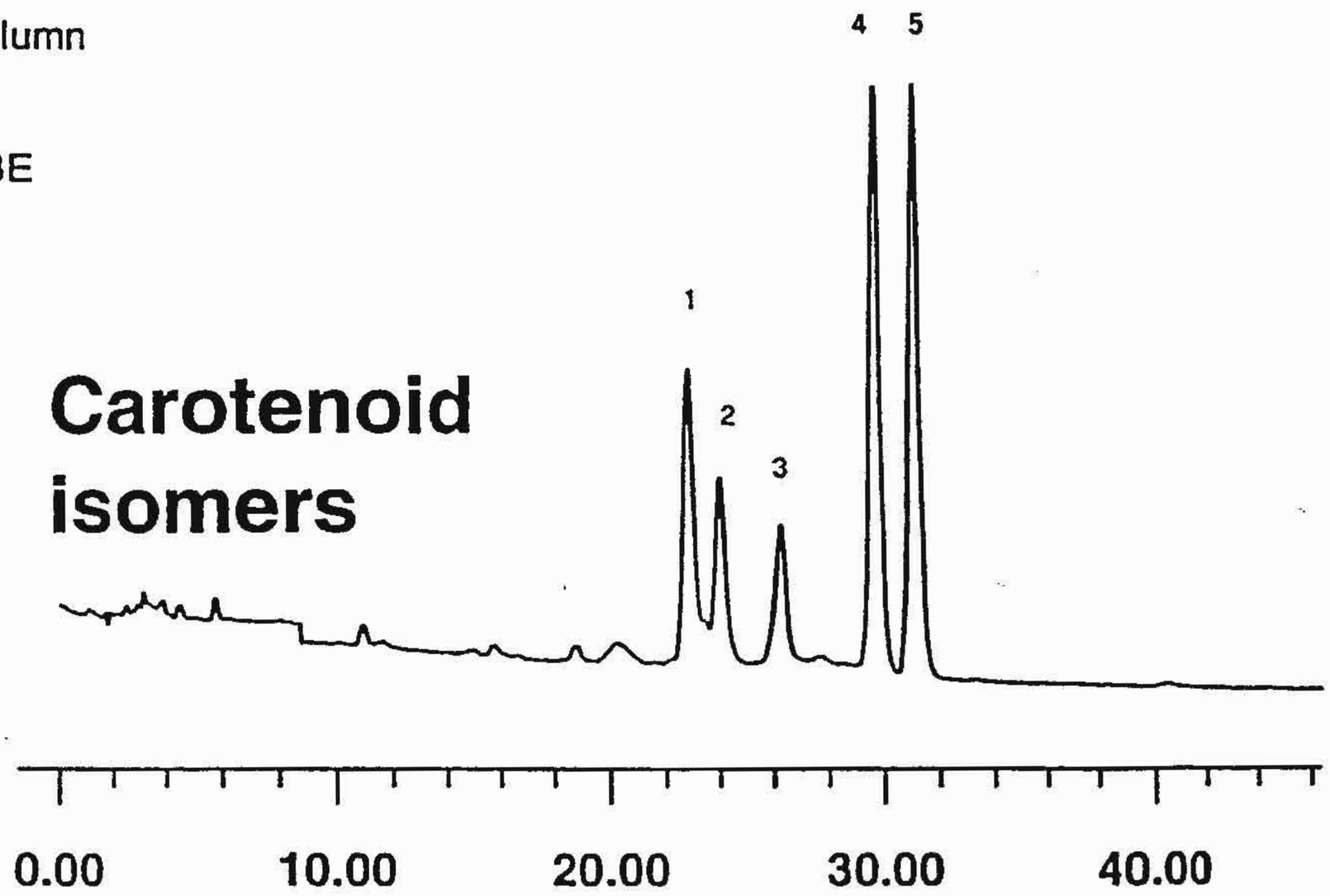
  100% A to 100% B in 90 minutes

Cis- and Trans-isomers of Beta-Carotene

► Conditions:
Column: YMC Carotenoid Column
Flow: 2 mL/min
Temp: ambient
Eluent: 80:20 MeOH:MTBE

► Peaks:

- 15-cis-β-carotene
- 13 cis-β-carotene
- all trans-α-carotene
- all trans-β-carotene
- 9-cis-β-carotene

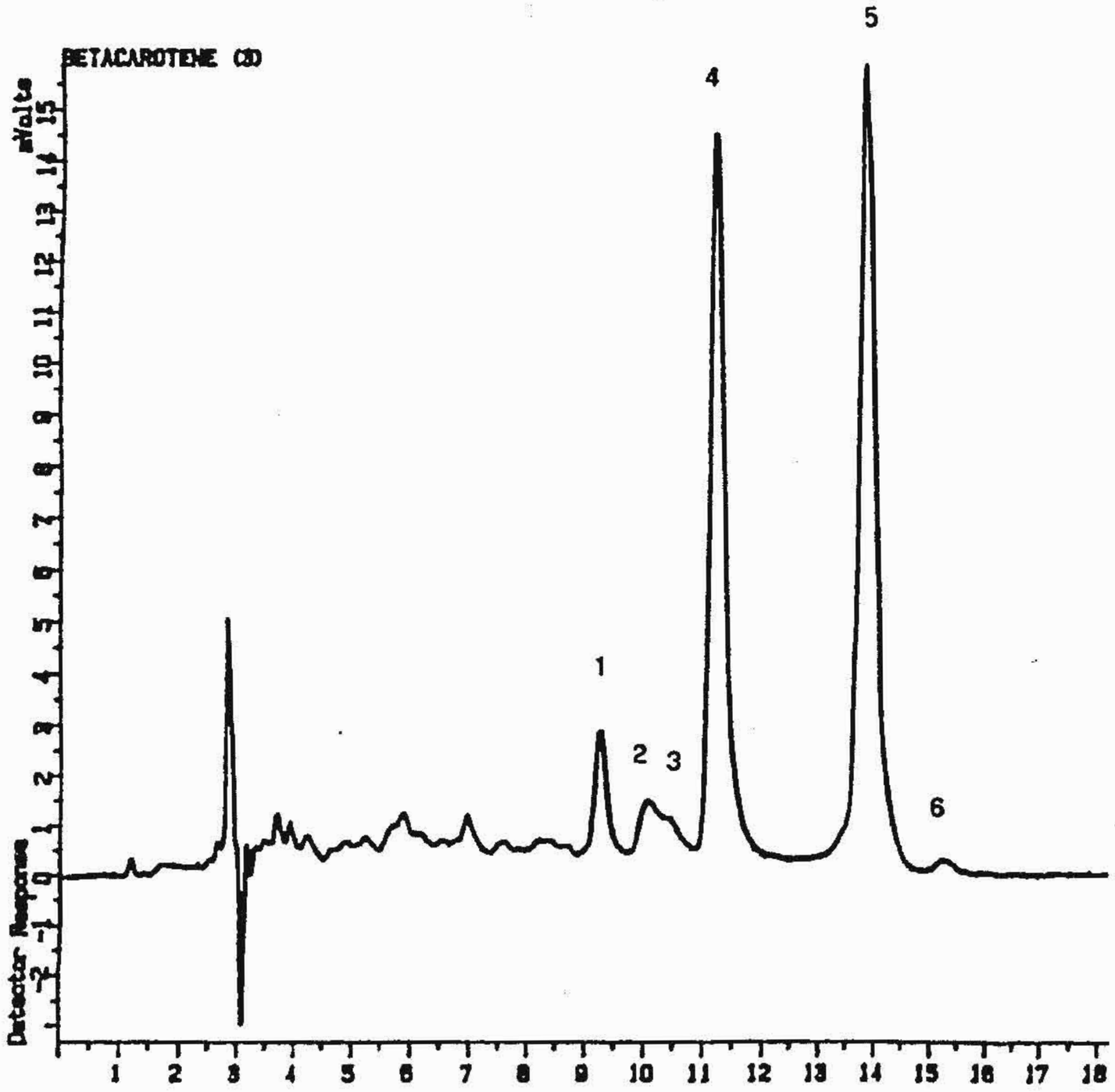


Carotenoid Isomers From Commercially Available Capsules

- Conditions:
  - Column: YMC Carotenoid Column
  - Flow: 1 mL/min
  - Temp: ambient
  - Eluent: 75:20:5 EtOH:MeOH:THF
  - Detection: UV @ 450nm

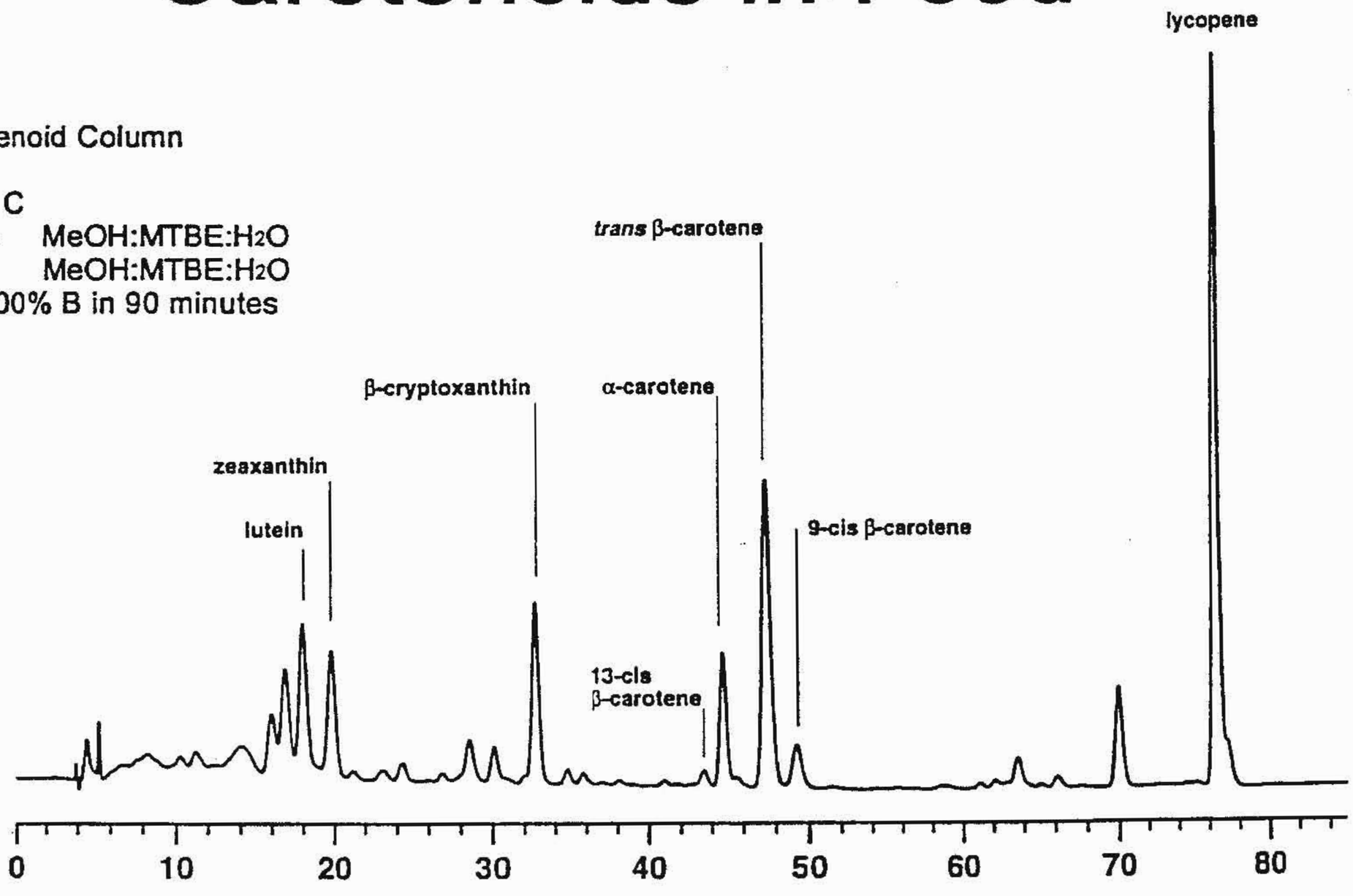
Peaks:

1. 15-cis-β-carotene
2. 13-cis-β-carotene
3. 13'-cis-β-carotene
4. α-carotene
5. β-carotene
6. 9-cis-β-carotene

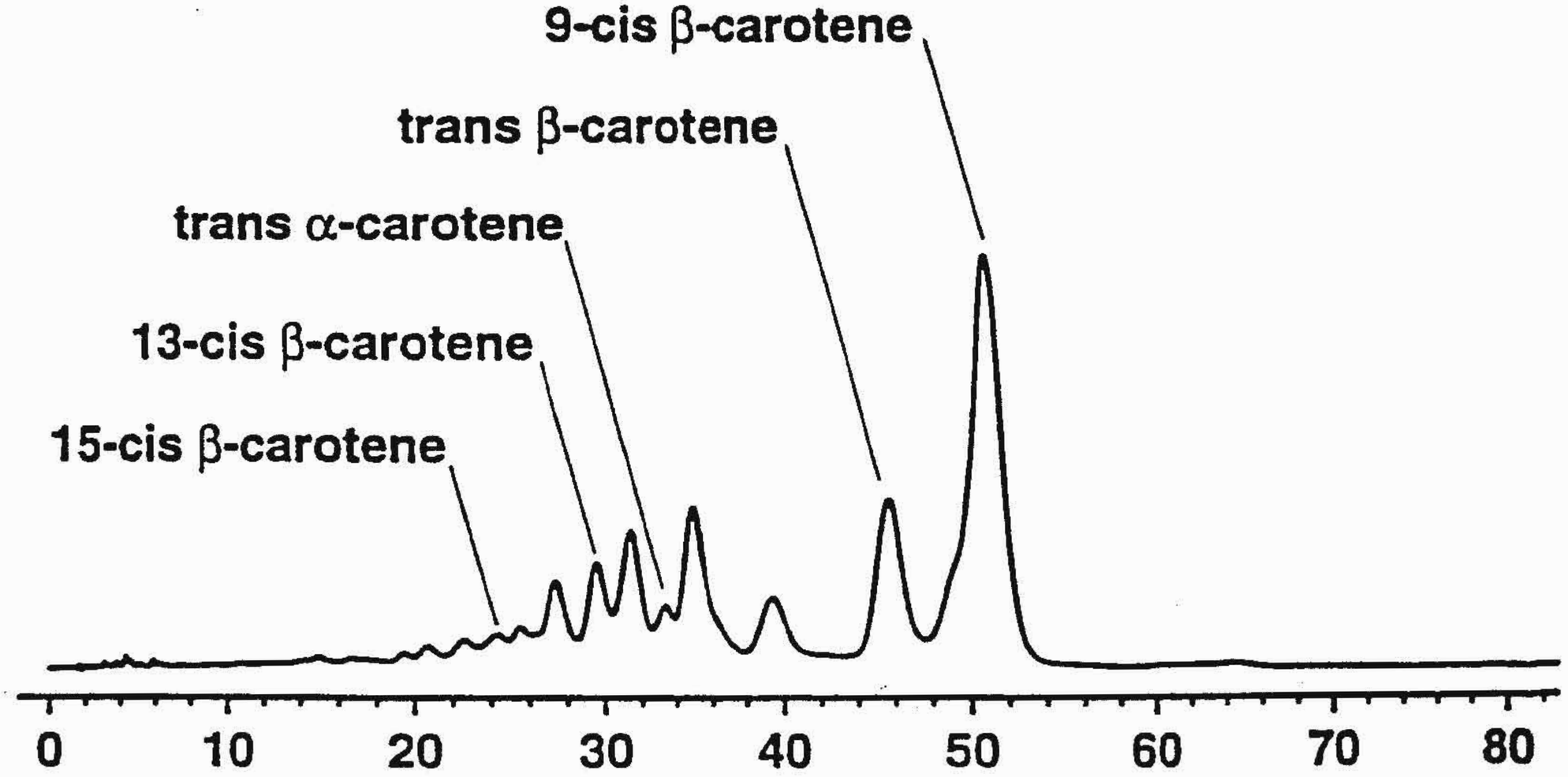


Separation of Carotenoids From An Extract of SRM 2383, Vitamins and Carotenoids in Food

Conditions:
Column: YMC Carotenoid Column
Flow: 1 mL/min
Temp: 20 degrees C
Solvent A 81:15:4 MeOH:MTBE:H_2O
Solvent B 6:90:4 MeOH:MTBE:H_2O
100% A to 100% B in 90 minutes



Separation of Carotenoids Found In Algae



► Conditions:

Column: YMC Carotenoid Column
Flow: 2 mL/min
Temp: 3 degrees C
Eluent: 80:20 MeOH:MTBE

Conclusion

- The YMC Carotenoid Column separates the many carotenoid isomers previously not resolved on shorter length media.

- Many separations are performed under isocratic conditions. The best results were typically obtained with non-aqueous conditions at room temperature.

- Natural product isomers of carotenoids are readily isolated with C30.

- Other applications for C30 may include polyaromatic hydrocarbons (PAH), prostaglandins, retinoids, leukotrienes and other long chained molecules.