Purification of Carotenoids via NP

Carotenoids are oil-soluble pigments that have a yellowish to reddish hue depending on their chemical structure. They provide antioxidant activity and serve as a precursor to vitamin A in humans. In addition, they are used as colourants in many foods and animal feeds. Therefore, they have to be produced on a large scale.

For some carotenoids, an efficient chemical synthesis route has been established. However, their chemical complexity and the formation of different isomers often make industrial synthesis uneconomical. Another method for the carotenoid production is via microbial cultivation, but due to their complex matrix the purification process is challenging.

Carotenoids are highly hydrophobic, so the method of choice for their purification is reversed phase chromatography.

To resolve geometrical or positional isomers C18 or C30 modifications are often used. However, the following method optimisation and linear scale-up process show that the use of unmodified silica provides an alternative approach. The aim of this study was to design a purification process for 5 carotenoids with a target purity of at least 95 %.

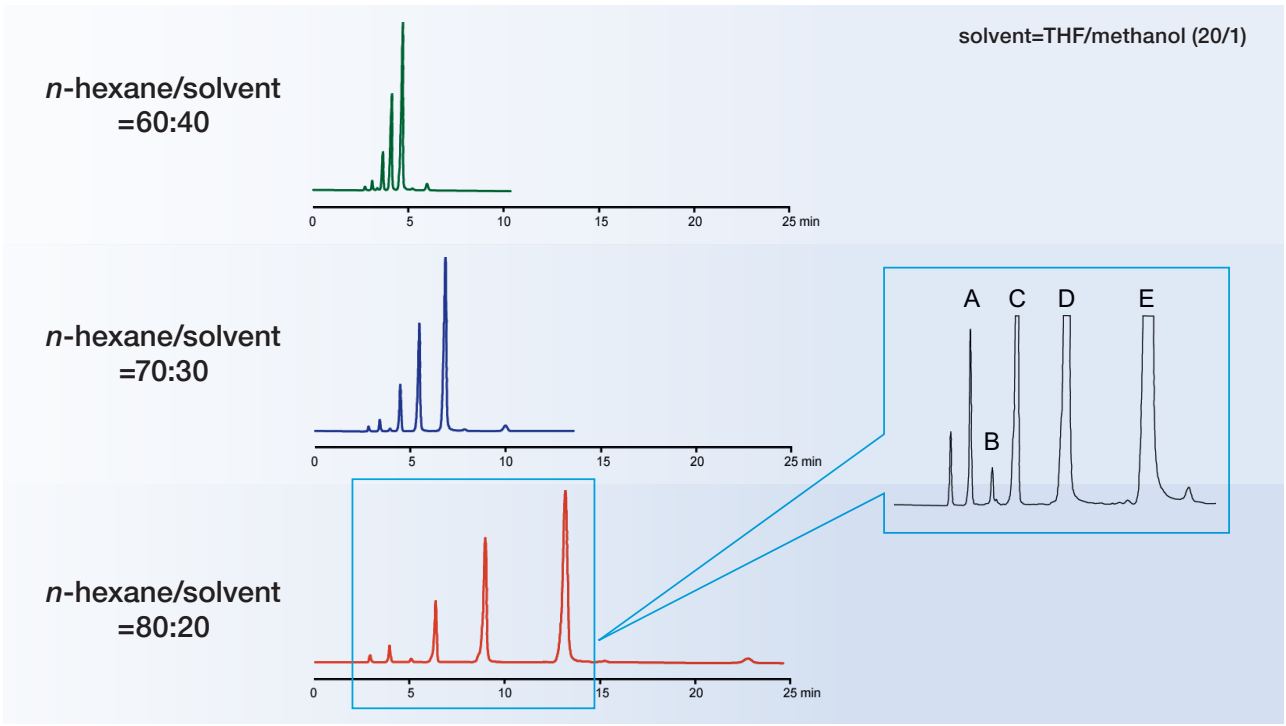
This purification was performed in three steps:

1. **Method development at analytical scale**
2. **Loadability studies**
3. **Final scale-up to the preparative process**

To perform this process development as smoothly as possible several aspects have to be taken into account.

1. Method development on analytical scale

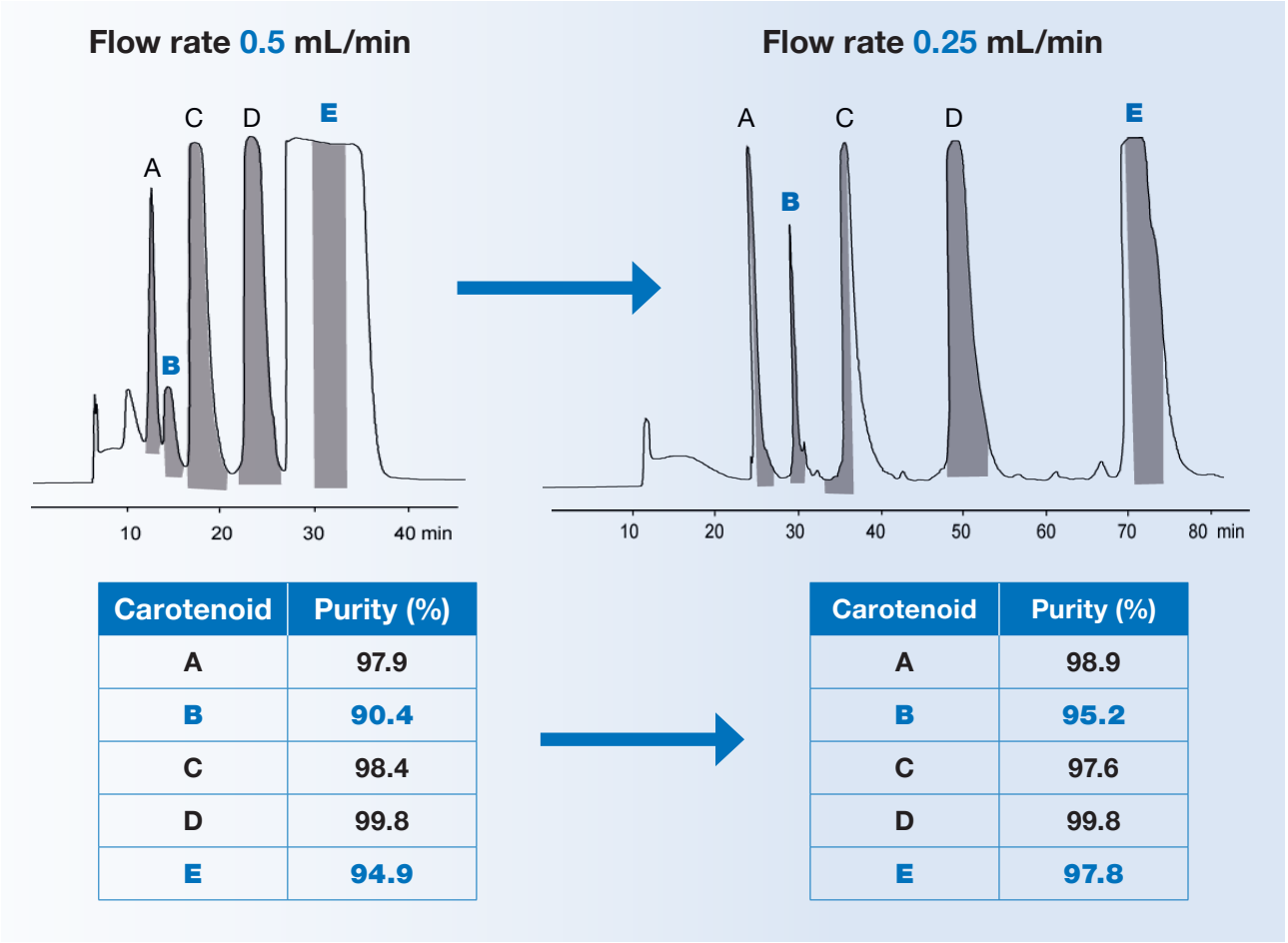
Carotenoids are easily decomposed by light, so the purification process described in this application note was conducted in darkness. Additionally, the mobile phase contained an antioxidant to avoid degradation of the carotenoids. For the method development on analytical scale an YMC-Pack SIL column (S-5 µm, 6 nm) 250 x 4.6 mm ID was used and *n*-hexane and THF/methanol (60/40) were used as an initial mobile phase. As this resulted in very rapid elution and insufficient resolution, the amount of the nonpolar solvent was increased stepwise up to 80 %, resulting in a significantly improved resolution.



Column:	YMC-Pack SIL (5 µm, 6 nm), 250 x 4.6 mm ID	Temperature:	ambient
Part No:	SL06S05-2546WT	Detection:	UV at 470 nm
Eluent:	A) *n*-hexane	Injection:	5 µL
	B) THF/methanol (20/1)	Sample:	mixture of 5 different carotenoids
Flow rate:	1 mL/min		

2. Loadability study

For the loading study using the analytical scale column, the injection volume was increased from 5 µL to 2 mL (666 µg loading). This resulted in a noticeable deterioration in the resolution which is why the flow rate was adjusted. By reducing the flow rate from 0.5 mL/min to 0.25 mL/min it was possible to increase the resolution again and obtain the desired purity.



Carotenoid	Purity (%)
A	97.9
B	90.4
C	98.4
D	99.8
E	94.9

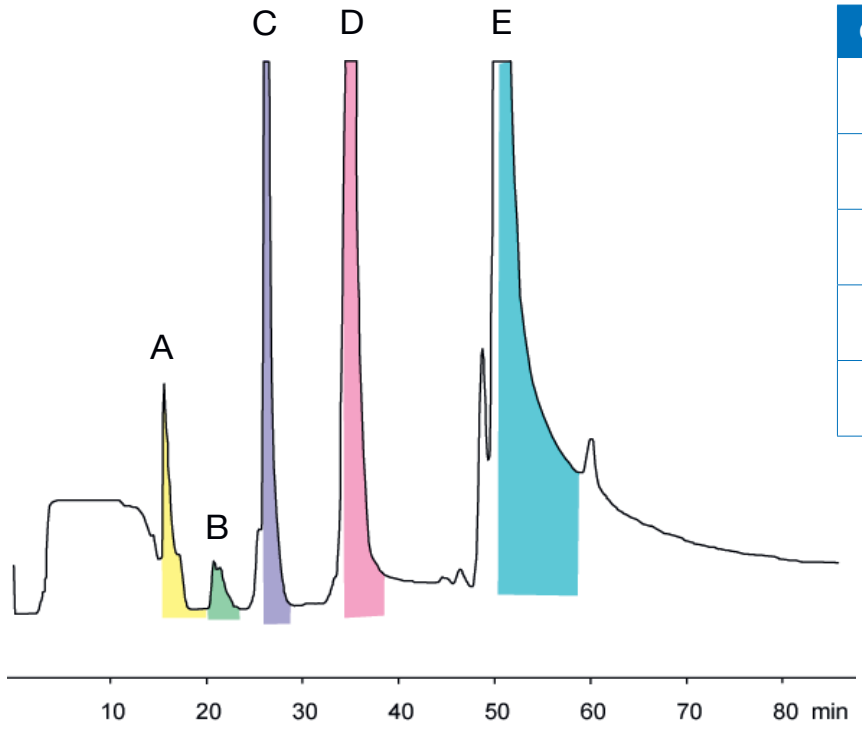
Carotenoid	Purity (%)
A	98.9
B	95.2
C	97.6
D	99.8
E	97.8

Column:	YMC-Pack SIL (5 µm, 6 nm), 250 x 4.6 mm ID
Part No:	SL06S05-2546WT
Eluent:	*n*-hexane/THF/methanol (420/100/5)
Temperature:	ambient
Detection:	UV at 470 nm
Injection:	2 mL (666 µg)
Sample:	mixture of 5 different carotenoids

3. Linear scale-up to preparative scale

The linear scale-up process enables the transfer of the lab scale method to an industrial scale process. The column length and the particle size remain the same, whilst the inner diameter is increased to maximise the loadability. During the method development the inner diameter of the column was 4.6 mm. For the preparative scale purification an inner diameter of 50 mm was chosen. By performing a linear scale-up, the chromatographic behaviour remains the same. This can also be seen in this example.

The desired purity could be obtained as well. With this optimised normal phase method, the carotenoids could be successfully separated from each other with the high purity required.



Carotenoid	Purity (%)
A	99.8
B	100
C	99.7
D	99.5
E	97.8

Column:	YMC-Pack SIL (5 µm, 6 nm), 250 x 50 mm ID
Part No:	SL06S05-2552AR
Eluent:	*n*-hexane/THF/methanol (420/100/5)
Flow rate:	30 mL/min
Temperature:	ambient
Detection:	UV at 470 nm
Injection:	80 mg
Sample:	mixture of 5 different carotenoids