Fast Sub-Ambient Separation of Carotenoids Using a Temperature Program

Introduction

There has been renewed interest in carotenoids like beta-carotene, lutein, zeaxanthin and lycopene because of their use as nutraceuticals. Many people believe that when taken as a nutritional supplement, they are powerful anti-oxidants that protect against heart disease and cancer, and boost the immune system. Quantitation of carotenoids by HPLC is usually done at ambient temperatures using a C_{18} or C_{30} column. Depending on the column, mobile phase, and the components being analyzed, the separation can require run times in excess of 60 minutes. Most often the analysis requires complex mobile phases with up to six components. Here we illustrate an improved separation of a mixture of carotenoids using a temperature program with a simple mobile phase, and compare it to an analysis done under isothermal conditions.

Experimental Conditions

Conditions are summarized in Table 1. The HPLC system consisted of a solvent degasser, an autosampler (AS3000) maintaining samples at 20° C, a programmable UV/visible detector (UV2000), and a computer data system (PC1000, Thermo Separations Products, San Jose, CA). A Selerity Technologies, Inc. Polaratherm Series 9000 Total Temperature Controller was used to maintain sub-ambient temperature and perform temperature gradients.

Results and Discussion

The separation uses a simple mobile phase of THF in acetonitrile. Figure 1 (see next page) shows the separation of the carotenoid mixture at 25°C. Isothermally, the analysis takes about 40 minutes and the lutein and zeaxanthin are not well resolved. Figure 2 (see next page) shows the separation of the same carotenoid mixture using a temperature program from 15°C to 35°C.

Table 1
Conditions for Analysis of Carotenoids

Column:	Jones Chromatography Genesis C_{18}, 150 x 4.6 mm
Mobile Phase:	1.25% THF in acetonitrile
Flow:	1.0 mL/min
Detection	UV at 450 nm
Temperature:	Figure 1: 25°C isothermal Figure 2: temperature program holding at 15°C for five minutes, ramp to 35°C over two minutes, hold 15 minutes

Results and Discussion (continued)

The analysis time was less than 30 minutes and lutein and zeaxanthin are nearly baseline resolved while the lycopene and beta-carotene peaks are sharper at the elevated temperature.

Conclusions

Temperature programming can simplify method development, greatly reduce analysis time and improve resolution of carotenoids using sub-ambient temperatures.

Acknowledgement

Craft Technologies, Inc. in Wilson, NC provided this separation.

References

LC/GC Application Handbook, February (2004)

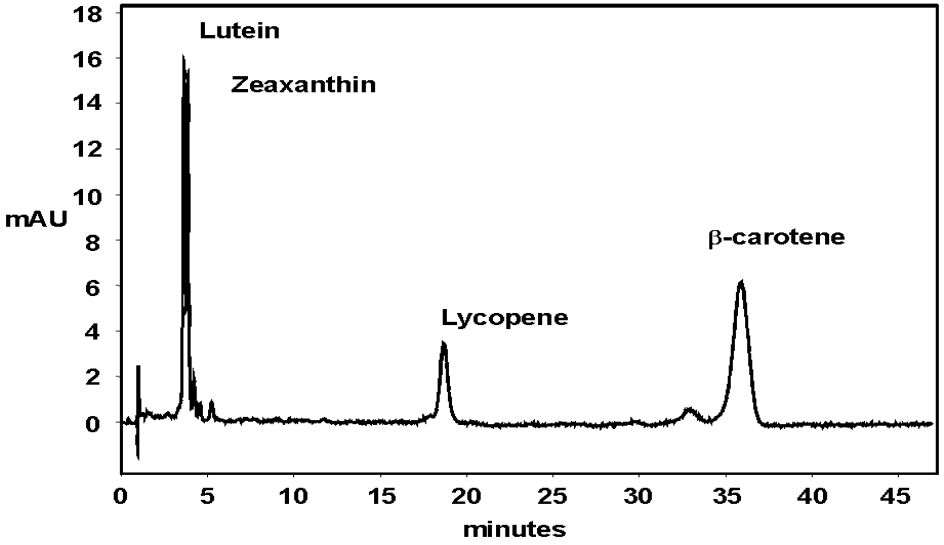

Figure 1. *Chromatogram showing the separation of carotenoid mixture isothermally at 25°C. The analysis takes about 40 minutes and the lutein and zeaxanthin are not well resolved.*

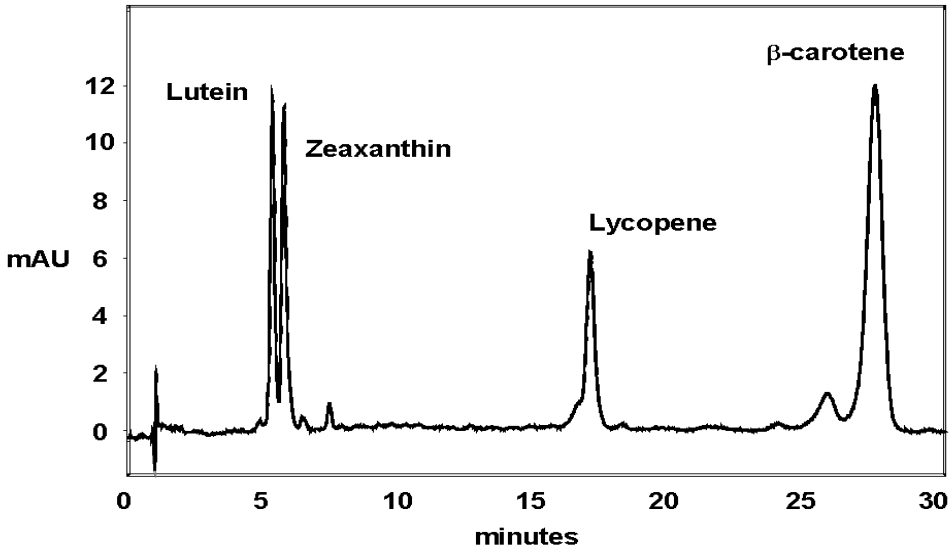

Figure 2. *Chromatogram showing the separation of carotenoid mixture using a temperature program. The analysis time was less than 30 minutes and lutein and zeaxanthin are nearly baseline resolved while the lycopene and β-carotene peaks are sharper at the elevated temperature.*