Optimization of the Separation of Tocopherols using Sub-Zero Temperature

Introduction

Vitamin E is a term used to describe compounds that contain the 6-hydroxychroman ring and possess the biological activity of α-tocopherol. There are 8 naturally occuring homologs comprised of 4 tocopherols (α-, β-, δ-, γ-) and 4 tocotrienols (α-, β-, δ-, γ-). In addition to their vitamin E activity, these compounds have been implicated in the reduced risk of many diseases including cancer, cardiovascular disease, Alzheimer's, and cataracts. Vitamin E also provides the greatest total antioxidant activity in the lipoproteins in blood.

While α-tocopherol has the greatest amount of vitamin E activity, the other homologs may be more effective at specific activities. For example, γ-tocopherol is thought to be more effective at reducing the risk of prostate cancer.

Experimental

HPLC conditions are summarized in Table 1. One separation was done at ambient temperature and one was performed at -20°C.

Results

No separations of all 8 vitamin E homologs have been previously reported using isocratic reversed-phase HPLC. Figure 1 (see next page) illustrates an injection of all 8 homologs on a C18 column using an ambient isocratic separation. Note the coelution of both β- and γ-tocotrienol and of β- and γ-tocopherol. In Figure 2, the separation is performed at –20 °C using the Polaratherm. The low temperature influences the column selectivity resulting in partial separation of the two tocotrienols and near-baseline separation (α=1.065) of the two tocopherols.

Table 1:
Conditions for Analysis of Tocopherols

Column:	Jones Chromatography Genesis C18, 150 x 4.6 mm, 4 um
Mobile Phase:	92:8 actonitrile:water (ambient temperature) 90:10 acetonitrile:THF (-20°C)
Flow Rate:	1.0 mL/min
Detection:	Absorbance at 296 nm

Conclusions

An isocratic separation of tocopherols at -20°C was achieved. The separation showed improved resolution of several isomers when compared to the analysis performed at ambient temperature.

Acknowledgement

Many thanks to Dr. Neal Craft and John Estes of Craft Technologies, Inc. in Wilson, NC, who developed this separation.

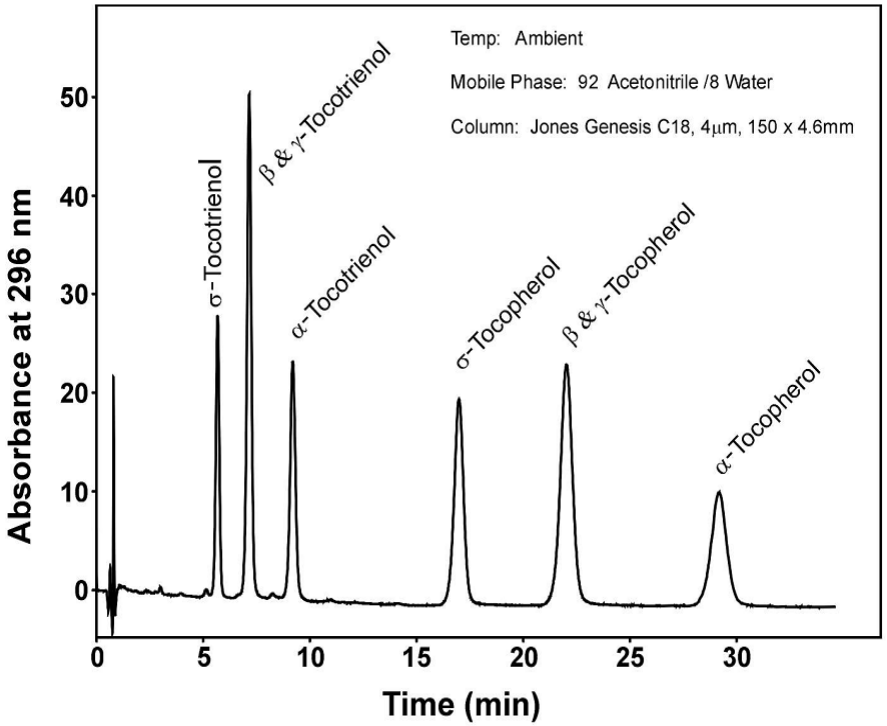

Figure 1: *Separation of tocopherols at ambient temperature. Several of the isomers coelute.*

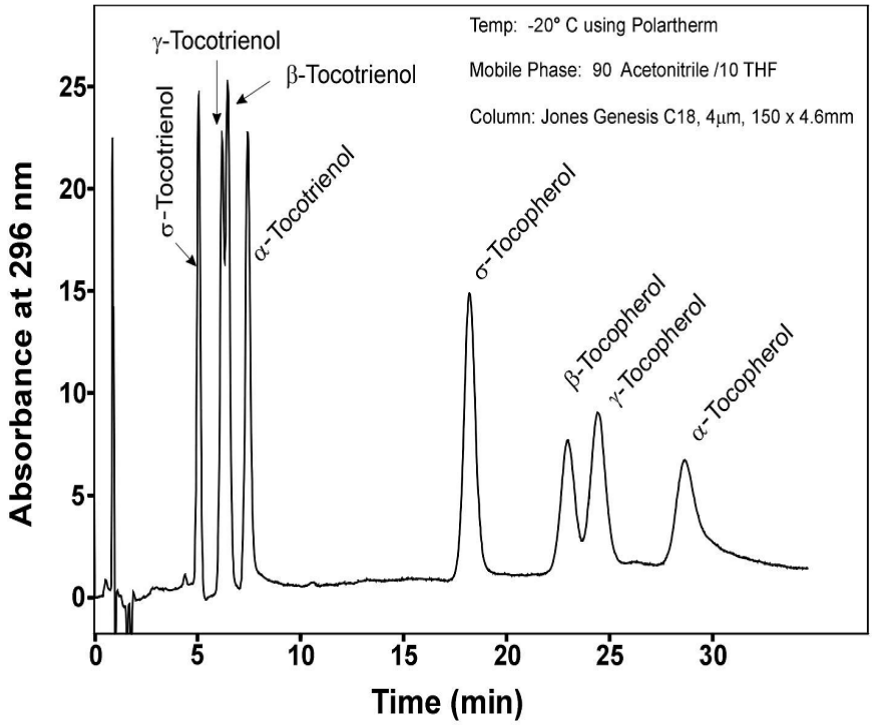

Figure 1: *Separation of tocopherols at -20°C. β-tocopherol and γ-tocopherol are now well-resolved, and β-tocotrienol and γ-tocotrienol are partially resolved.*