Separation of Retinol and Retinyl Esters Using a Sub-Ambient Temperature Program

Introduction

Vitamin A in biological samples exists as the free alcohol (retinol) and its esters with long-chain fatty acids (predominantly retinyl palmitate and retinyl stearate). The major form of vitamin A in blood plasma is retinol; high concentrations of the esters in plasma are diagnostic of hypervitaminosis A. In most other biological tissues (for example, liver and kidney), the esters dominate. Retinyl acetate does not occur naturally in biological tissues, but it is often used as an internal standard in HPLC analysis of vitamin A because it has the same absorbance characteristics and it is inexpensive and readily available. Retinyl acetate and retinyl palmitate are both used for food fortification and for vitamin A supplementation. Although all these compounds are lipophilic, the polarity range is rather large and typically requires a solvent gradient to resolve all of these forms of vitamin A in a single HPLC analysis.

Experimental

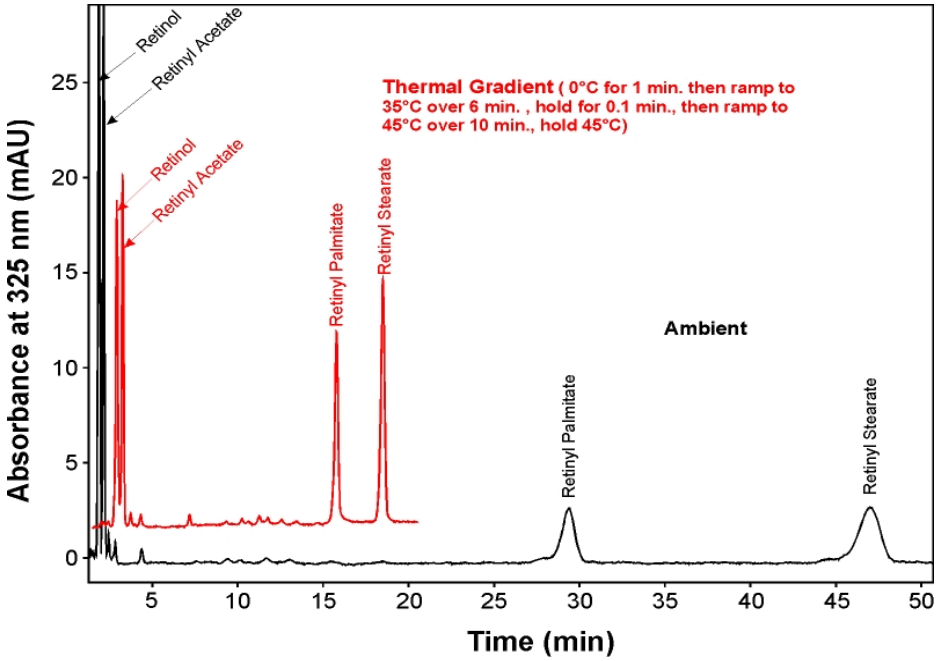
HPLC conditions are summarized in Table 1. One separation was done at ambient temperature and one was performed using a temperature program starting at 0°C, holding for one minute, ramping to 35°C over six minutes, holding for 0.1 minutes, then ramping to 45°C over ten minutes, and holding at 45°C until the end of the run.

Table 1:
Conditions for Analysis of Retinol and Retinyl Esters

Column:	Jones Chromatography Genesis C18, 150 x 4.6 mm, 4 um
Mobile Phase:	97.5:2.5 Acetonitrile:THF
Flow Rate:	1.0 mL/min
Detection:	Absorbance at 325 nm

Results

Figure 1 illustrates an overlay of two isocratic separations of retinoids using the Genesis C18 column. The lower trace was run at ambient temperature and the upper trace employed a temperature program. Using the temperature program, a 60% reduction in analysis time and much sharper peaks for all analytes were attained.



Conclusions

Speed, resolution and peak efficiency for the separation of retinol and retinyl esters were obtained using a sub-ambient temperature program.

Acknowledgement

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