Thermodynamic insights into the separation of carotenoids in reversed-phase liquid chromatography

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Supplementing Material

Tracer Pulse Chromatography

According to the tracer pulse (TP) method for a binary system, operational definitions of the excess volume of the isotopically labeled i compound, V_i^{exc}, and of the thermodynamic void volume, V_0, are:

$$V_i^{exc} = (V_{R,i}^* - V_{R,j}^*)\theta_i^M \theta_j^M \tag{1}$$

and

$$V_0 = V_{R,i}^* \theta_i^M + V_{R,j}^* \theta_j^M \tag{2}$$

where $V_{R,i}^*$ and $V_{R,j}^*$ are the elution volumes for each labeled component i and j, and θ_i^M and θ_j^M are the volume fractions in the bulk mobile phase. Excess adsorption isotherms can be employed to estimate the capacity and thickness of the surface phase (absolute adsorption). The volume of i in the stationary phase, V_i^S, and the stationary phase volume, V_S, can be assessed by the linear region of the excess isotherm, being

$$V_i^{exc} = V_i^S - V_S \theta_i^M \tag{3}$$

According to eq.2, V_0 is obtained by injecting small samples of eluent with isotopically labeled solute components and determining the elution volumes (i.e., $V_{R,i}^*$ and $V_{R,j}^*$) for each labeled component. Obviously, the method can be used with a pure eluent (i.e., θ_i^M or $\theta_j^M = 1$) and this is by far the simplest way of determining V_0.

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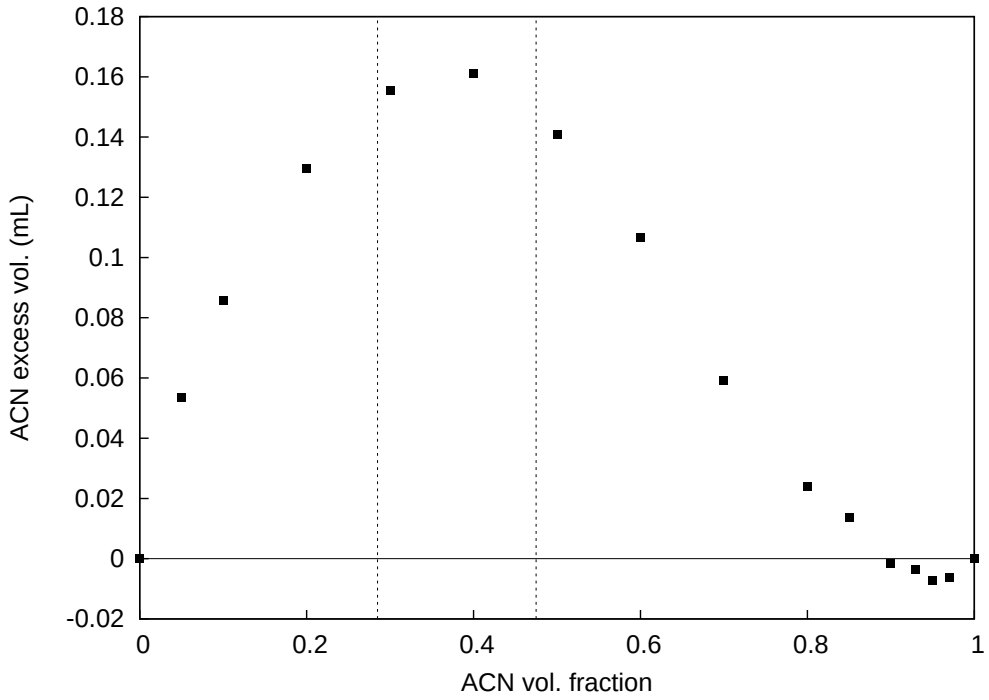

Figure S1. Acetonitrile excess isotherm. Vertical dashed lines indicate the region within ACN volume fraction are investigated.

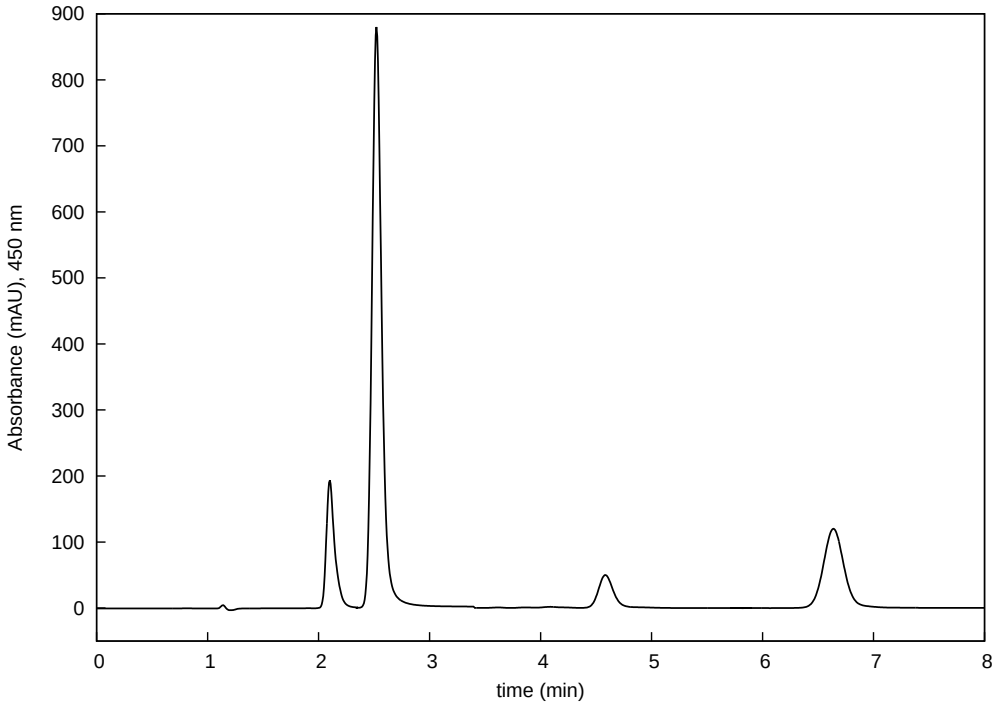

Figure S2. HPLC separation between lutein, zeaxanthin, lycopene and β-carotene. Cromatographic conditions were: column temeprature 30°C; MeOH:ACN:DCM 57:38:5.

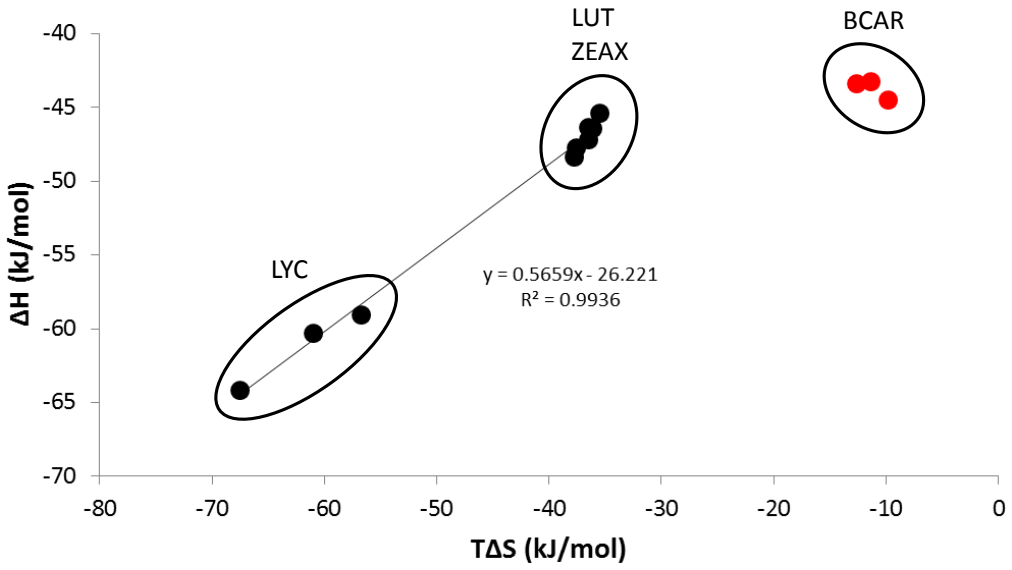

Figure S3. Evidence of enthalphy-entropy compensation for lutein, zeaxanthin and lycopene.