Natural Product Purification of Lycopene and β-Carotene Using Flash Column Sample Clean-up Prior to High Pressure Normal Phase Chromatography on the Gilson PLC 2020

Application Note PHA0212

Keywords

PLC 2020, Natural Product Purification, Flash Chromatography, Normal Phase Chromatography, High Pressure Liquid Chromatography, Lycopene, Tea, β-Carotene, Tomatoes

Introduction

Purifying low level compounds, such as those typically isolated from natural products, is an area of growing interest for the purpose of discovering new chemical entities. Natural product purification from plants and food products is of particular interest for any pharmacological or biological activity that may be useful in drug discovery and human health. Synthesis may not be an option for all natural products where it may be too complex, expensive, or too time consuming to accomplish.

Purification of low level compounds from natural products can be challenging due to the isolation of a small compound presence among other interfering sample compounds and the sample matrix. Flash purification provides an optimal environment for low concentration compounds by allowing for a large amount of sample to be injected and purified via large particle flash columns and organic solvents that can be quickly evaporated post purification. The tedious and labor intensive process of manual fraction transfer from multiple injections into a single pooled tube requires extra rinse solvent, additional dry-down time, and can create error from transferring. All of these are eliminated by automated fraction pooling.

Figure 1. Gilson PLC 2020 Personal Purification System.

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The Gilson PLC 2020 Personal Purification System (Figure 1) purified β-Carotene and lycopene (Figure 2), naturally occurring plant carotenoid of particular interest for their antioxidant activity and possible health benefits, from tomato peel and various tea extracts using flash chromatography and solvent selection to decrease overall run time. The purified and pooled fraction was re-injected to determine percent recovery.

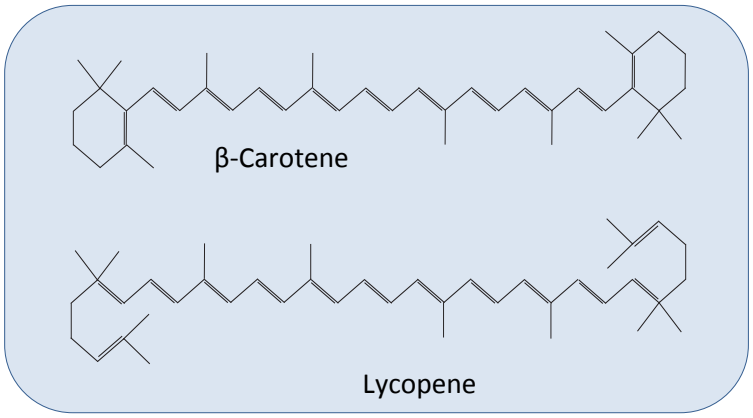

Figure 2. β-Carotene (top) and Lycopene (bottom).

Materials & Methods

Samples and Solvents

- Lycopene (VWR P/N 100369-824)
- β-Carotene (Sigma Aldrich P/N 22040-5G-F)
- Pectinase from *Aspergillus niger* (Sigma Aldrich P/N P2736-50ML)
- Pectinase from *Aspergillus aculeatus* (Sigma Aldrich P/N P2611-50ML)
- BHT (2,6-Di-tert-butyl-4-methylphenol) (Sigma Aldrich P/N B1378-100G)
- Hexane (Honeywell P/N/ AH212-4)
- Triethylamine (Fisher P/N O4884-100)
- Tetrahyrdofuran (JT Baker P/N 9441-03)
- Deionized water 18 megohm (Barnstead NANOpure® Infinity)

- Dried tomato peel from roma and cherry tomato
- Dried tea leaves (Figure 3)
    - Lipton Darjeeling Black tea (India)
    - Brooke Bond Label Special: 15% premium Darjeeling and Assam Orthodox leaves (India)
    - Hojicha green tea (Japan)
    - O'sulloc Premium green tea: Jaksulsoo (Korea)
    - Lan Bao Cha Kuding tea: Wild broad leaf holly leaf (China)

Apparatus

- Gilson PLC 2020
    - 50 SC pump heads
    - 0.2 mm detector flow cell pathlength
    - 10 mL sample loop
- Silica Flash cartridge: SiliaSep™ 12 g, 40-63 µm, 60 Å (SiliCycle P/N FLH-R10030B-ISO12) (Figure 4)
- Silica Semi-Preparative column: Luna® 5u Silica (2) 100 Å AXIA Packed, 50x21.2 mm Phenomenex P/N 00B-4274-P0-AX)

Mobile phase

- A: Hexane
- B1: Hexane : triethylamine (99.9:0.1)
- B2: Hexane : tetrahydrofuran (80:20)

Figure 3. Dried tea leaves for carotenoid extraction.

Figure 4. Silica flash cartridge before and after tea extract injection.

Protocol

Lycopene and β-Carotene are light sensitive, so all work was performed in minimal light. Standards were dissolved in 100% hexane at 0.1 mg/mL, and stored at 4°C in amber colored vials. Standards were tested over time to observe compound degradation in solution. Because these compounds are prone to degradation in solution, BHT was used as a stabilizer. β-Carotene and lycopene have similar absorption characteristics, therefore two wavelengths (450 and 503 nM) were observed to assist in differentiation.

Dried tomato peels (8 g) and tea leaves (10 g) were digested with 100 mL 2% pectinase enzyme solution (2% each *A.niger* and *A.aculeatus*) in DI H_2O overnight at 30°C. Liquid-liquid extraction was used to extract the lipophilic carotenoids from the digested plant tissue; 100 mL of Hexane:ethanol:acetone with 0.05% BHT (2:1:1) added to the 100 mL of digested plant tissue. The liquid-liquid extraction was allowed to shake overnight at room temperature, before the top layer (hexane) was collected and evaporated to dryness. The samples were finally reconstituted in 20 mL mobile phase (A:B1) (80:20).

The flash column was conditioned for 10 minutes in A:B1 (80:20) before sample or standard (10 mL) was loaded and run (Table 1). Carotenoid fractions containing both lycopene and β- Carotene were collected, pooled and evaporated to dryness. Fractions were reconstituted in 5 mL mobile phase (A:B1) (80:20) and further purified using a Silica Semi-Preparative column. Purified lycopene and β- Carotene fractions were collected and re-injected to determine percent recovery.

Table 1. Chromatographic method for the separation of Lycopene and β-Carotene (Flow rate: 15mL/min).

0-15min	A:B1 (80:20)	isocratic
15.01min	A:B2 (80:20)	solvent selector B1 to B2
15.01-22min	A:B2 (50:50)	gradient
22.01-22.5min	A:B2 (80:20)	gradient
23min	A:B1 (80:20)	solvent selector B2 to B1
23-26min	A:B1 (80:20)	isocratic

Results

Degradation of lycopene over time was more dramatic than β-Carotene (Figure 5). After 30 days, the peak area of a 0.5 mg injection of lycopene had decreased to 30% of the day 1 peak area. After 16 days, the peak area of a 0.5 mg injection of β-Carotene had decreased to 76% of the day 1 peak area.

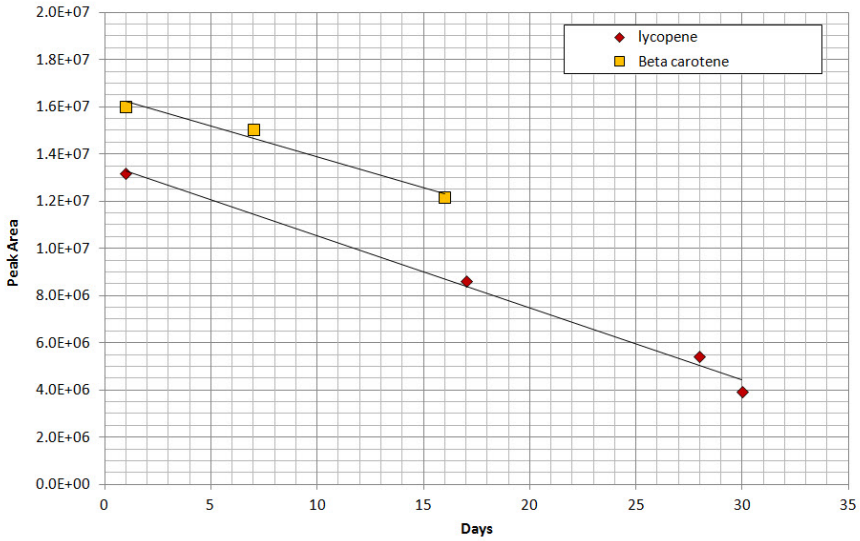

Figure 5. Degradation of β-Carotene and Lycopene over time.

Lycopene and β-Carotene were extracted from dried tomato peels and tea leaves respectively. Fractions containing a mixture of carotenoids including lycopene and β-Carotene were automatically collected using the PLC 2020 (Figure 6). The pooled fractions were evaporated to dryness and reconstituted in mobile phase. Lycopene and β-Carotene were isolated and purified using a normal phase silica Semi-Preparatory HPLC column, with automated fraction collection (Figures 7, 8, 9). A portion of the final fraction of lycopene and β-Carotene was re-injected onto the Semi-Preparatory HPLC column to determine the percent of the desired peak that was recovered. A 99.5% (standard error 2.7%) average recovery was observed in the standards and samples.

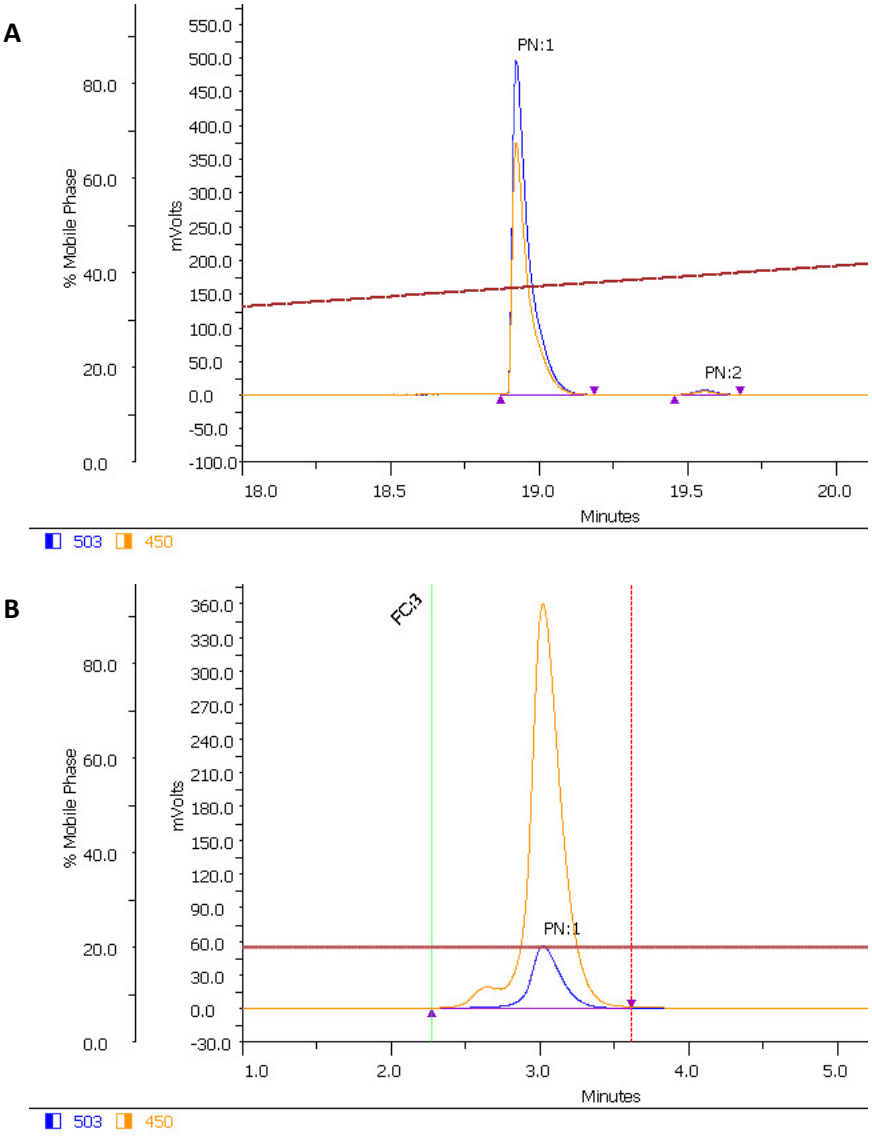

Figure 6. Lycopene from dried tomato peel (A) and β-Carotene from Lipton Darjeeling tea (B) separated on low pressure silica flash cartridge.

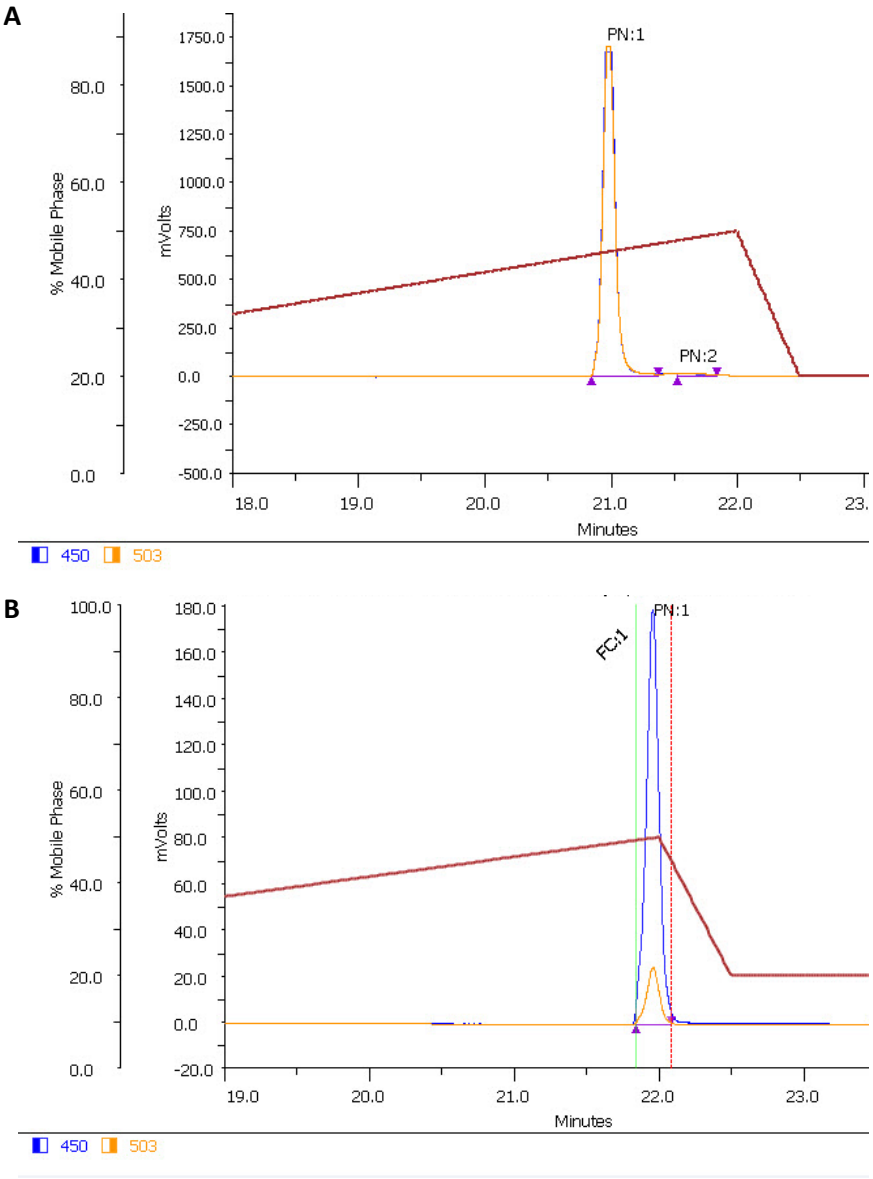

Figure 7. Lycopene (A) and β-Carotene (B) separated on silica Semi-Preparative HPLC column.

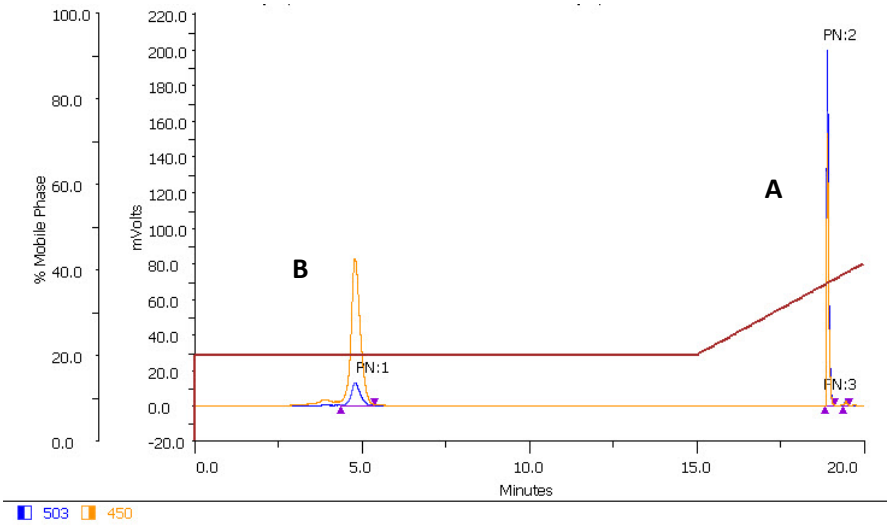

Figure 8. Lycopene (A) and β-Carotene (B) separated on silica Semi-Preparative HPLC column.

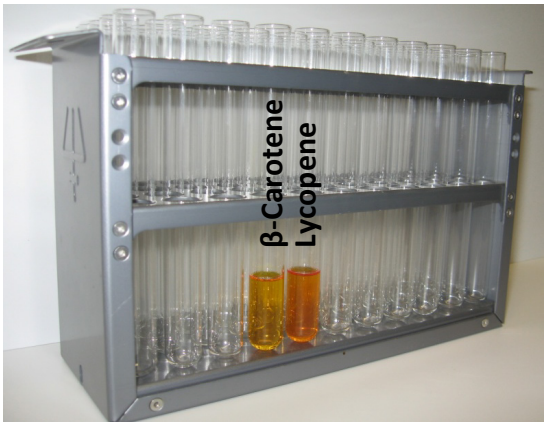

Figure 9. Purified β-Carotene (yellow) and lycopene (orange) fractions.

The dried tomato peels yielded more than 0.5 mg lycopene per 8 g extraction. The highest amount of β-Carotene came from the O'sulloc Premium Jaksulsoo green tea (Korea), and the lowest yield came from the Kudingcha Holly leaf tea (China) (Figure 10). The O'sulloc Premium Jaksulsoo green tea (Korea) yielded less than 0.5 mg β-Carotene per 10 g extraction; however the purified product was not quantified.

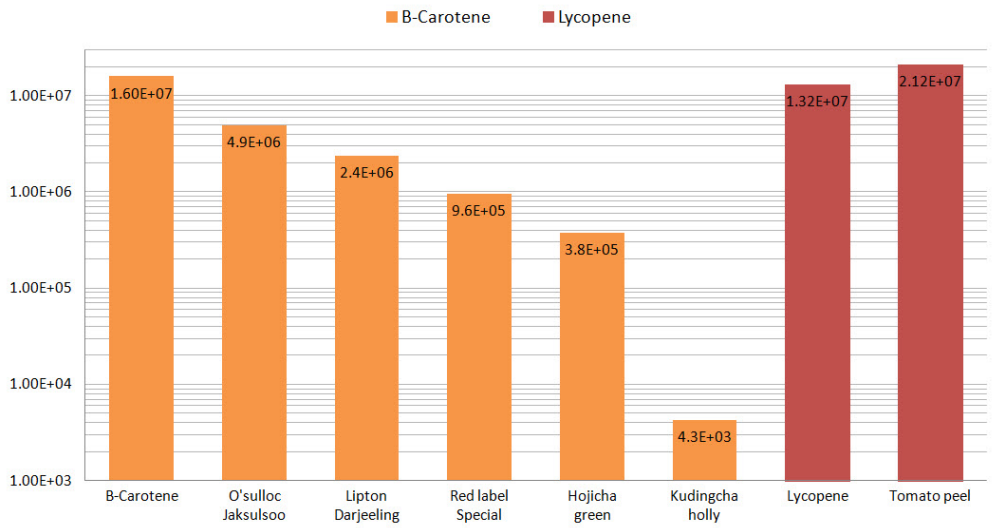

Figure 10. β-Carotene peak area for various dried tea leaf extracts (orange) and Lycopene peak area for dried tomato peel extract (red).

Summary

Many natural products are thought to have tremendous health benefits including prevention or treatment of disease. The purification of natural products from plant tissues is an area of significant interest for numerous researchers. This application presents a method for isolating and purifying lycopene and β-carotene from tomato peels and tea leaves using low pressure normal phase flash chromatography and automated fraction collection. The PLC 2020 provided hands free fraction collection with 99.5 +/-2.7 % recovery of the desired product peaks. Despite the similarities between lycopene and β-Carotene, these carotenoids were able to be separated and purified. Further method development could be used to perfect a system for the purification of these carotenoids, while modifications to this method could be employed to isolate and purify other natural products.

References

1. Xu, F., Q.P. Yuan, H.R. Dong, Determination of lycopene and β-carotene by high-performance liquid chromatography using Sudan I as internal standard. 2006, Journal of Chromatography B, 838: 44-49.