Facile Isolation of Carotenoid Antioxidants from *Solanum lycopersicum* using Flash Chromatography

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Abstract

Lycopenes were used as a model of other antioxidants of similar polarity to determine the best method to purify this class of compounds. Lycopene, xanthophylls, and carotenes are long chain pigment compounds that act as antioxidants and are common in nutraceuticals and natural-based dyes. These compounds are characterized as long conjugated chains that may be terminated with ring systems. The rings may contain ketones or hydroxyl groups.

A Combi*Flash*® Torrent™ large-scale Flash system is used to isolate gram quantities of carotenoids. Various column types and solvent systems are evaluated for purifying these compounds on a large scale. Non-aqueous reverse phase chromatography was determined to be the easiest method to purify these compounds.

Background

Non-polar compounds containing long hydrocarbon chains are difficult to purify using normal phase chromatography due to their weak interaction with the stationary phase. These hydrophobic compounds are also strongly retained under reverse phase conditions. Non-aqueous reverse phase is a useful means of purifying these compounds. Non-aqueous reverse phase is not commonly used because the solvents typically used reduces UV detection of compounds[1]. All-Wavelength Collection, unique to Combi*Flash* systems, suppresses the solvent absorbance and allows collection of compounds with UV spectra that overlap the solvent absorbance.

In addition, the low concentration of most compounds of interest require large medium pressure liquid chromatography systems to obtain compound from a large volume of starting material, typically tens to hundreds of grams. The large scale system is useful for purifying minor components as well. In this example, a reverse phase chromatography system is used to purify carotenoid compounds as a model for other natural products.

Experimental

Contadina tomato paste (3.14 kg/can, Contadina Foods, Inc, San Francisco, CA 94105) was used for a lycopene source. All solvents were ACS grade from BDH (VWR, Brandywine, PA). A can of paste was extracted twice with 8 L ethyl acetate. The extracts were combined and dried to yield 11.1 through 14.0 g crude extract.

Thin layer chromatography was run using silica, diol, and C18 TLC plates (Teledyne Isco, Lincoln, NE).

Method optimization was performed on a small scale with a Combi*Flash* Rf-200 system equipped with a UV-vis detector (PN 68-5230-008, Teledyne Isco, Lincoln, NE) with a 15.5 g Redi*Sep*® Rf Gold C18 column (PN 69-2203-334, Teledyne Isco) using methanol and ethyl acetate, acetone, or methylene chloride. Samples were adsorbed on 5 g C18 Solid Load Cartridges (PN 69-3873-237, Teledyne Isco) or adsorbed onto Celite and placed into empty 5 g Solid Load Cartridges (PN 69-3873-235, Teledyne Isco). Lycopene compounds were detected at 473 or 360 nm. The standard method for the C18 column was changed to start with 100% methanol (Solvent A), and end with 100% of the "B" solvent.

The large scale purification was performed on a Combi*Flash* Torrent with a UV-vis detector (PN 68-5240-004, Teledyne Isco) with a 950 g Redi*Sep* Rf Gold C18 column (PN 69-2203-492, Teledyne Isco) run as non-aqueous reverse-phase. The sample (11.1 g crude extract) was adsorbed on 62.9 g Celite 545 (Acros Organics, New Jersey, USA) and loaded into a 260 g Solid Load Cartridge. A methanol/methylene chloride gradient was employed. Fractions were collected based on detection at 470 nm and All-Wavelength Collection in the range of 200 – 360 nm. The standard C18 method was changed to start with 100% methanol and end with 100% ethyl acetate.

Purity was determined with a Isco HPLC system consisting of a model 2350 pump, 2360 Gradient Controller, and a V4 detector. A Restek Ultra C18 column was used (PA, USA) with a methanol/dichloromethane gradient. The major carotenoid collected was lycopene, determined by comparison to an authentic sample (Sigma-Aldrich, St. Louis, USA).

Results and Discussion

Thin Layer Chromatography:

Thin layer chromatography was run to scout for mobile and stationary phases. Poor retention was seen with normal phase TLC (silica and diol), with lycopene compounds starting to elute with 100% hexane. The addition of a small amount of ethyl acetate caused the lycopenes to elute with the solvent front.

Using C18 TLC plates, it was determined that the carotenoids did not elute with 100% methanol. Using 1:1 methanol : dichloromethane, the carotenoids had an Rf of 0.72. Using 1:1 methanol : ethyl acetate, the carotenoids had an Rf of 0.81; indicating that a gradient of ethyl acetate or dichloromethane would elute the compounds.

Method Development:

The use of a Combi*Flash* Rf-200 allowed the method to be quickly fine-tuned. The "scale-up" feature in the Peak*Trak*™ software was used to generate the method for the Combi*Flash* Torrent module.

A sample of crude extract was run with a methanol : ethyl acetate gradient (Figure 1) with detection at 471 nm. The various carotenoids were only partially resolved. Figure 2 shows a similar separation to Figure 1 using acetone. Improved separation was observed using a methanol : dichloromethane gradient.

Figure 1: Purification of carotenoids with methanol and ethyl acetate

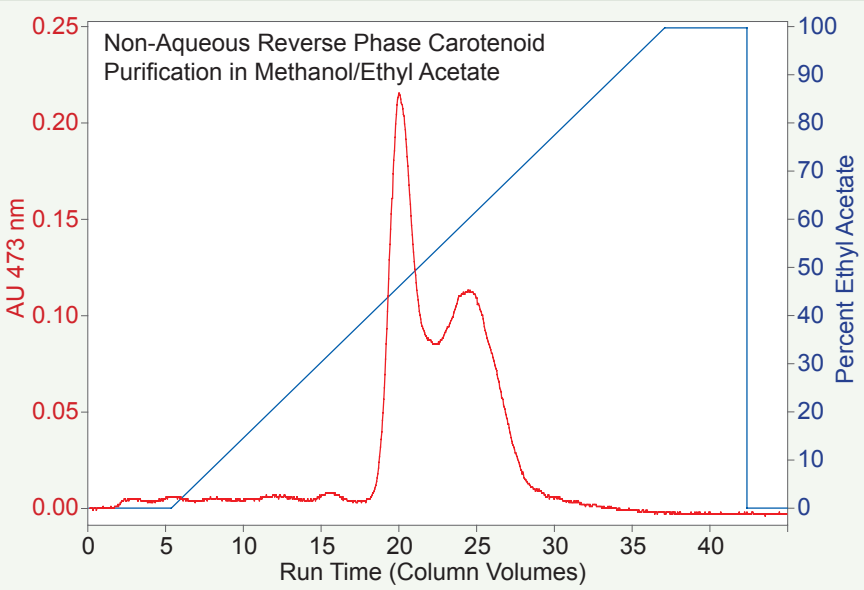


Figure 2: Purification of carotenoids with methanol and acetone

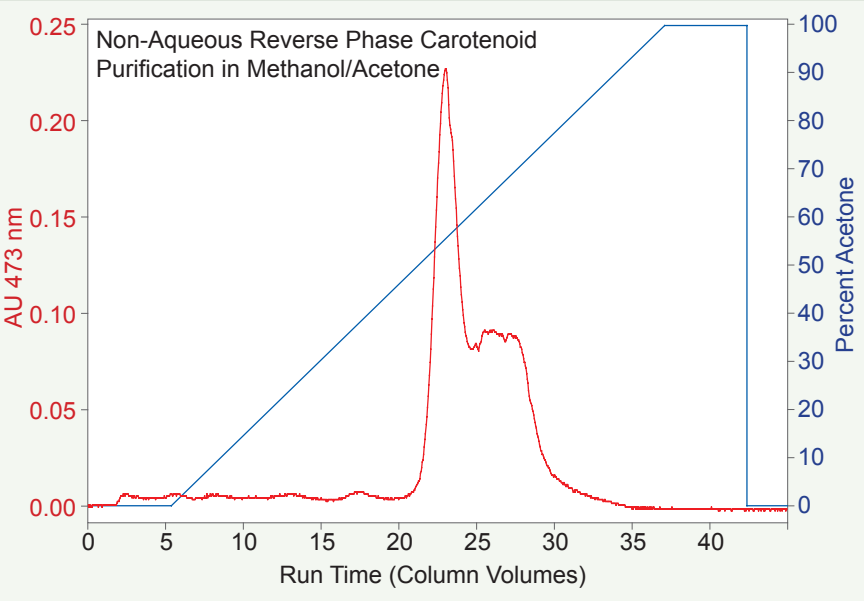


Figure 3: Purification of carotenoids with methanol and dichloromethane

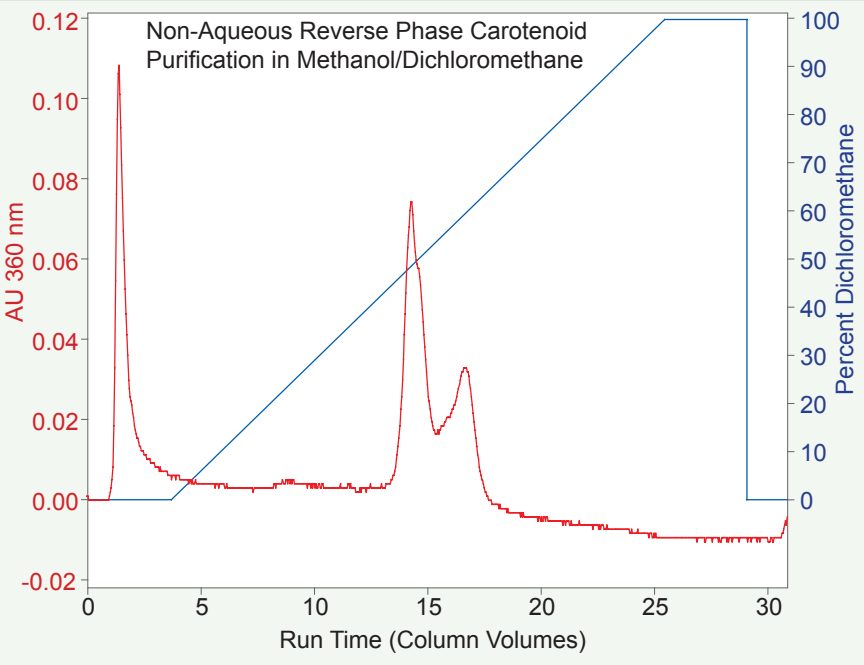


Figure 4: Purification of carotenoids with methanol/dichloromethane using All-Wavelength Collection and collection at 473 nm. Fractions collected identified by bands and numbers.

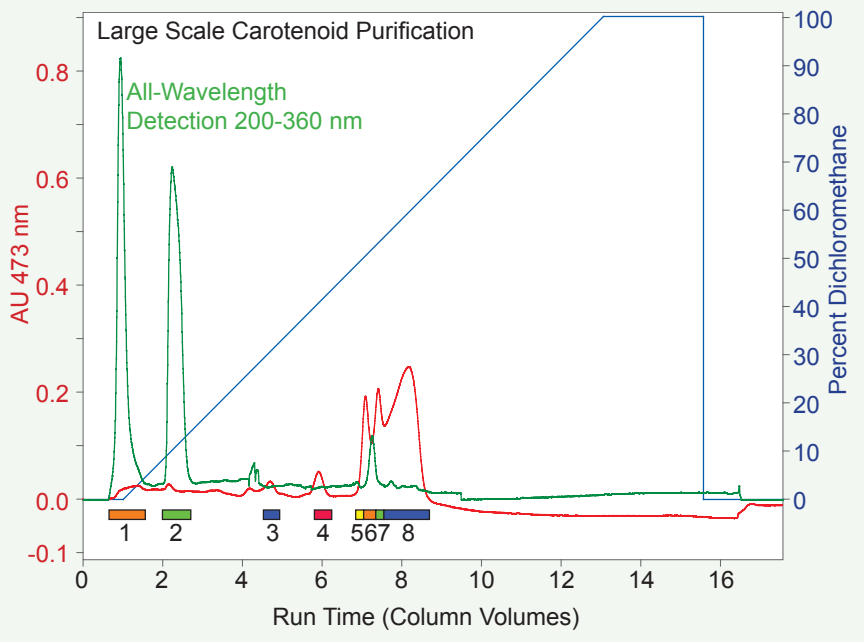


Table 1: Mass and yield for fractions collected

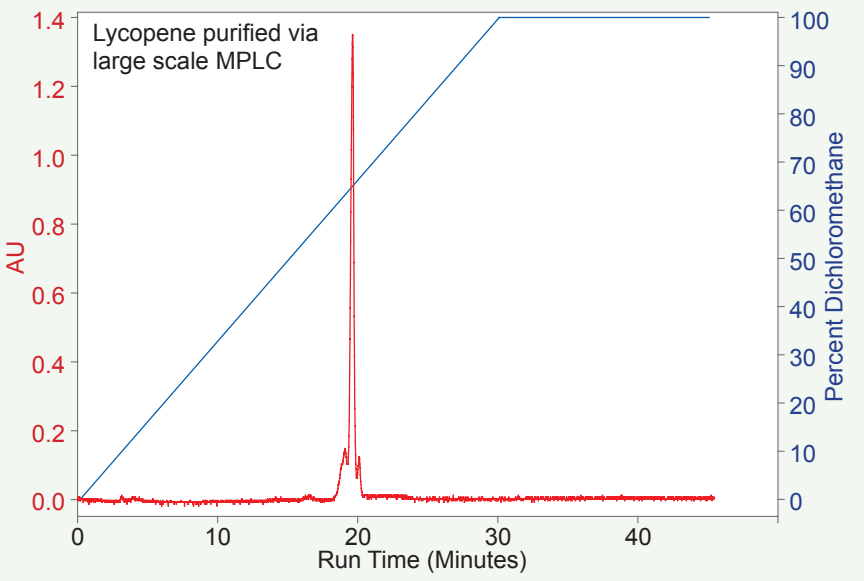
Fraction	1	2	3	4	5	6	7	8	Total
Fraction Mass (g)	1.8742	5.4886	0.271	0.0582	0.1594	0.1577	0.2757	0.8827	9.1675
% of Crude Extract	16.9%	49.4%	2.4%	0.5%	1.4%	1.4%	2.5%	8.0%	82.6%

Large Scale Purification:

The experiments on the small system indicated that the run time could be shortened from 31 to 17 column volumes while observing a usable separation on the Combi*Flash* Torrent. All-Wavelength Collection was used to look for other compounds that absorbed at other wavelengths; the range of 200 – 360 nm was used. All-Wavelength Collection uses signal processing algorithms to detect compounds over a wavelength range and suppress baseline changes due to solvent absorbance. All-Wavelength Collection presents a signal suitable for use by a fraction collector to cut peaks. In addition to the terpenes and terpenoids that eluted early in the run, All-Wavelength Collection detected an unrelated compound that eluted with the carotenoids.

Lycopene was found to be >95% pure by HPLC; overall yield for lycopene was 0.028% overall from the starting tomato paste.

Figure 5: Analytical HPLC for fraction number 8



Conclusion:

The Combi*Flash* Torrent module is an effective method to purify compounds on a large scale. Results are easily scaled from smaller Flash systems. All-Wavelength Collection works well to purify compounds and can either be used to remove impurities from compounds or indicate that these impurities are present. The Combi*Flash* Torrent module is useful to purify nutraceuticals, natural products, or other compounds on a scale of hundreds of grams.

[1] Snyder, L.R.; Kirkland, J.J.; Glajch, J.L.; *Practical HPLC Method Development*, 2nd Ed, John Wiley & Sons, 1997, p.264

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