Rapid Purification of Tocopherols

by Flash Chromatography

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Chromatography Application Note AN27

Abstract

Tocopherols (Figure 1) are fat soluble vitamins with antioxidant activity found in most vegetable oils and in many edible plants[1]. Food oil quality is determined, in part, by changes in tocopherol levels[2]. Tocopherols also represent a value-added product when purified from oils. Analysis and purification of tocopherols presents a challenge due to their relatively low concentration and chromatographic similarity to the oil matrix.

A single-step method using an automated flash chromatography system is described to remove the tocopherols from the oil matrix allowing easy analysis by HPLC or other analytical methods.

Isolation of these oils by Flash chromatography is easier than the commonly used saponification of the glycerol fatty acid esters followed by extraction into diethyl ether[3].

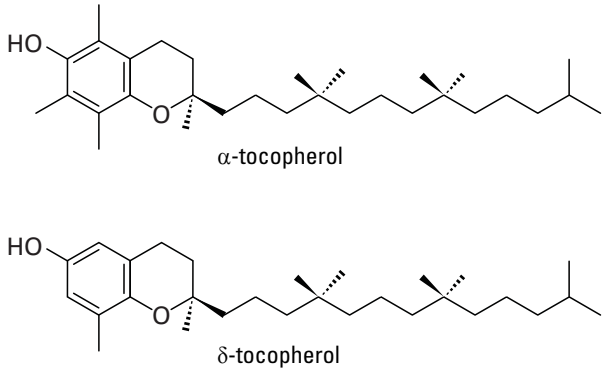

Figure 1: Structures of some common tocopherols found in vegetable oils

Materials and Methods

Weigh 16 g celite in a 500 mL round bottom flask. Weigh 4 g vegetable oil sample and dissolve it in ~20 mL methylene chloride and add this to the celite. Add enough methylene chloride to the contents of the round bottom flask to make a loose slurry. Dry the sample on the rotary evaporator to make a smooth flowing powder—this makes sure the oil is evenly distributed on the celite and allows the sample to be easily loaded onto the column.

Load a weighed portion (sample size 15 g, containing ~3 g oil) of the powder into a 25 g solid load cartridge and run on a diol column using the method in Table 2.

Table 1: Materials

Material	Quantity	Notes
Combi*Flash* Rf System	1	Part number 68-5230-006[a]
50 g Redi*Sep* Rf diol column	1	Part number 69-2203-373[a]
Empty 25 g Solid Load cartridge	1 per run	Part number 69-3873-240[a]
25 g Solid Load Cartridge Cap	1	Part number 60-5237-048[a]
Vegetable oil sample	4 g	
Celite 545	16 g	
Methylene chloride	100 mL	ACS grade
Hexane	2,000 mL	ACS grade
Isopropanol	2,000 mL	ACS grade. 2500mL needed if using a new column
500 mL round bottom flask		
Rotary evaporator		

a. Available from Teledyne Isco

Table 2: Run conditions to purify tocopherols

Column size	50 g Diol	
Load	3 g oil (6% mass load on column)	
Solvents	Hexane and isopropanol	
Equilibration	5 CV 100% hexane	
Gradient Table	Segment length	%B (isopropanol)
	Initial	0
	3 CV	0
	1 CV	5%
	7 CV	5%
	5 CV	100%
	2 CV	100%
Detection wavelength	209 nm	
Monitor wavelength	296 nm	
Flow rate	40 mL/min	

Note

The use of column volumes allows easy scale-up to larger samples if needed.

Collect the fractions containing tocopherols (see Figure 2) and dry these fractions under vacuum.

Figure 2 shows that the fractions containing the tocopherols are well resolved from the oils that elute earlier in the chromatogram. Tocopherols can be followed by the trace at 296 nm.

Fractions were evaluated by TLC, HPLC, and UV-vis spectroscopy. α-tocopherol and δ-tocopherol were used as reference compounds.

1. Ching, S.L. and Mohamed, S. *J. Agric. Food Chem.* **2001**, *49*, 3101.
2. Okogeri, O. and Tasioula-Margari, M. *J. Agric. Food Chem.* **2002**, *50*, 1077.
3. Adidi, S.L. *J. of Chrom. A.* **2000**, *881*, 197.

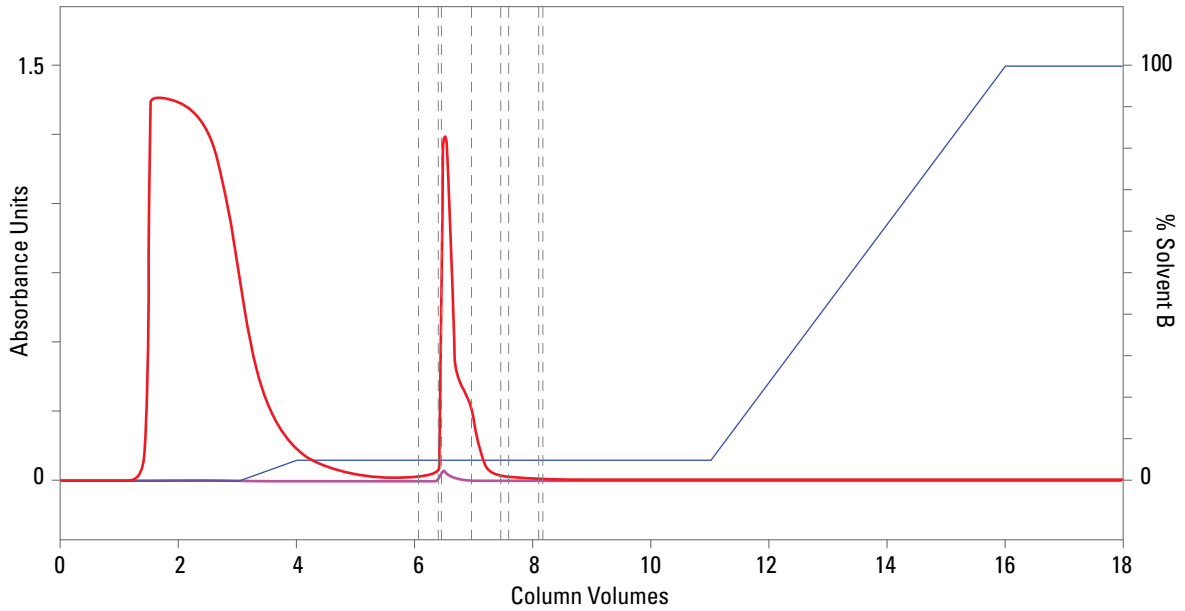

Figure 2: Purification of tocopherols from corn oil

Results and Discussion

The diol column separated the tocopherol mixture from the oil matrix (Figure 2). The ability to monitor the purification and adjust the gradient as needed during the run allowed the desired compounds to be eluted free from the oil matrix.

The purification was essentially finished by 9 column volumes (~10 minutes). The remaining run time confirmed that no other compounds are present in the sample, cleaned the column, and prepared it for storage filled with 100% isopropanol for later use with other samples. (Do not allow a reusable Redi*Sep* diol column to dry out.) The fractions can be removed within 10 to 12 minutes from the start of the run (immediately after they are collected) and analyzed, compared to the 45 minutes needed hydrolyze the glycerol-fatty acid esters so the tocopherols can be extracted in the traditional method.

Conclusion

Flash chromatography using a Combi*Flash* Rf and a Redi*Sep* Rf diol column allows a fast and easy method to purify tocopherols from vegetable oils. The method requires no heating of the sample nor extraction to prepare the sample for analysis.

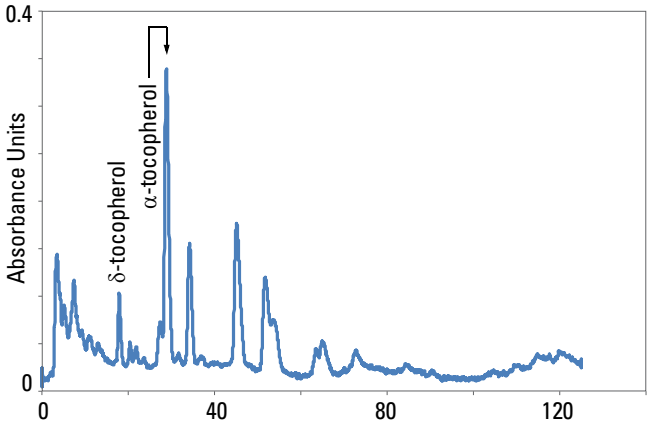

Figure 3: HPLC of Tocopherols and related compounds purified from corn oil

Table 3: Tocopherol yields from various oils

Oil	Mass of oil (g)	Yield mass (g)	Yield %
Corn	2.99	0.14575	4.9
Soybean	3.00	0.15975	5.3

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