# Determination of Vitamin E in Olive Oil Using the Agilent 1260 Infinity Analytical SFC System

## Rapid, high-resolution separation of all isomers of tocopherol and tocotrienol

## Application Note

Food Testing and Agriculture

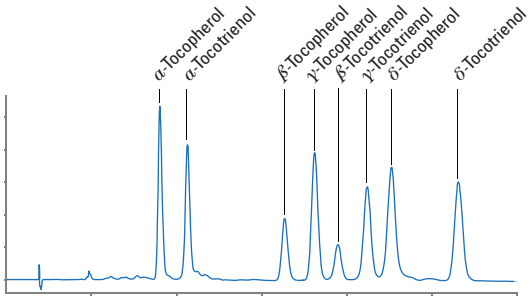

### Author

Edgar Naegele
Agilent Technologies, Inc.
Waldbronn, Germany



### Abstract

This Application Note demonstrates the separation of eight tocopherol and tocotrienol compounds by supercritical fluid chromatography (SFC) using the Agilent 1260 Infinity Analytical SFC system. Calibration curves were generated for all compounds, and a real-life virgin olive oil sample was analyzed. The limits of detection and quantification (LOD and LOQ) were determined as well as relative standard deviations (RSDs) of retention times and areas.


Agilent Technologies

## Introduction

Supercritical fluid chromatography (SFC) is a versatile tool to replace separations that to date have been preferably done on a normal-phase liquid chromatography (LC) system. The advantage of SFC is that separations are much faster and of higher precision compared to normal-phase LC. In addition, SFC does not use harmful solvents.

Fat-soluble vitamins are typical examples of compounds that can be separated by SFC. A group of such compounds is the vitamin E family. Tocopherols and their unsaturated relatives, tocotrienols, belong to this group in various isomeric forms known as $\alpha$, $\beta$, $\gamma$, $\delta$-tocopherols and -tocotrienols. These compounds have high bioactive and antioxidant potential, and are nutritionally beneficial for human health. One natural source of vitamin E, as well as other healthy substances, is virgin olive oil obtained from the fruit of the olive tree (*Olea europea* L.)[1,2]. Typically, about 95 % of the vitamin E in olive oil is $\alpha$-tocopherol[2]. Since olive oil is a commodity of important economic value for the producing countries, various methods for the analysis of olive oil have been developed to ensure authenticity and quality[3].

This Application Note describes the analysis of vitamin E compounds in olive oil by SFC that has the advantages of faster run times and higher precision compared to widely used normal-phase LC.

## Experimental

### Instrumentation

All experiments were performed with an Agilent 1260 Infinity analytical SFC solution (G4309A) comprising the following modules:

- Agilent 1260 Infinity SFC Control Module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity High-Performance Degasser
- Agilent 1260 Infinity SFC Standard Autosampler
- Agilent 1260 Infinity Diode Array Detector (DAD) with high-pressure SFC flow cell
- Agilent 1290 Infinity Thermostatted Column Compartment (TCC)

### Column

Agilent ZORBAX NH2,
4.6 × 150 mm, 5 µm (p/n 883952-708)

### Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.06

### Standards

Tocopherol and tocotrienol mixed solution standards (in hexane) were purchased from LGC Standards, Teddington, UK.

### Sample

A native olive oil was bought in a local supermarket. The olive oil was diluted in hexane 100 mg/mL, and directly used for analysis.

### Chemicals

All solvents were purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

### SFC method

| Parameter | Value |
|---|---|
| Solvent A | $CO_2$ |
| Modifier B | Ethanol |
| SFC flow | 4.5 mL/min |
| Gradient | 3 % B at 0 minutes to 4.5 % B at 6 minutes |
| Stop time | 6 minutes |
| Post time | 2 minutes |
| BPR pressure | 210 bar |
| BPR temperature | 60 °C |
| Column temperature | 50 °C |
| Injection volume | 5 µL, 3 × loop overfill, needle wash in vial with hexane |
| DAD (UV/VIS) | Wavelength 295 nm, bandwidth 4 nm<br>Reference 550 nm, bandwidth 100 nm<br>Slit 8 nm<br>Data rate 10 Hz |

## Results and Discussion

An amino phase column with a flow rate of 4.5 mL/min was used to separate the mixture of the four isomeric tocopherols and the four isomeric tocotrienol compounds. The organic composition of the gradient was close to isocratic behavior, increasing only from 3 to 4.5 % ethanol. This gradient separated the early eluting compounds, and decreased the retention of the later eluting compounds to produce sharper peaks. The optimized backpressure of 210 bar and the higher column temperature of 50 °C also helped to increase the resolution of the late eluting compounds. Figure 1 shows the chromatogram of the separation of tocopherol and tocotrienol compounds in the standard stock solution within a run time of 6 minutes.

To calibrate all compounds, the stock solution (level 1) was diluted using a 1:2 dilution pattern over six levels (Table 1).

The peaks obtained for the dilution at level 5 showed a signal-to-noise (S/N) ratio of about 10, which was used as the lowest level in the calibration curves and to calculate the limit of quantification (LOQ). The limit of detection (LOD) was calculated from the peaks measured at level 6 with a S/N ratio of 3. The LOQ was typically less than 23 µg/mL and the LOD was less than 7 µg/mL (Table 2).

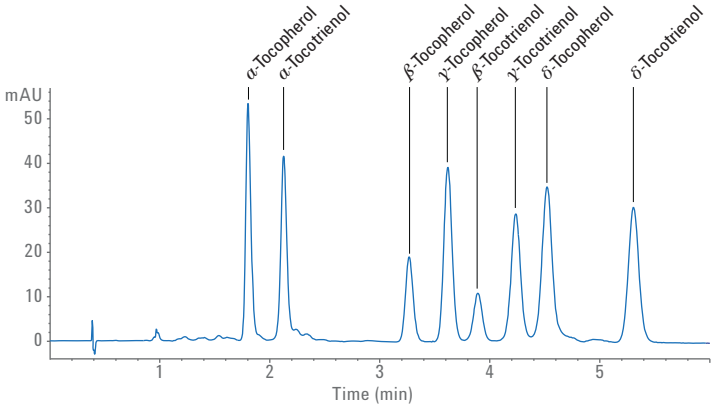



Figure 1. Separation of a mixture of α, β, γ, δ-tocopherol and α, β, γ, δ-tocotrienol by SFC.

Table 1. Concentrations of individual compounds and calibration levels using a 1:2 dilution pattern.

| | Level 1 (µg/mL) | Level 2 (µg/mL) | Level 3 (µg/mL) | Level 4 (µg/mL) | Level 5 (µg/mL) | Level 6 (µg/mL) |
|---|---|---|---|---|---|---|
| α-Tocopherol | 463.00 | 231.50 | 115.75 | 57.88 | 38.94 | 14.46 |
| α-Tocotrienol | 447.00 | 223.15 | 111.75 | 55.88 | 27.94 | 13.96 |
| β-Tocopherol | 203.00 | 101.50 | 50.75 | 25.34 | 12.69 | 4.23 |
| γ-Tocopherol | 430.00 | 215.00 | 107.50 | 53.75 | 26.88 | 13.44 |
| β-Tocotrienol | 135.00 | 67.50 | 22.50 | 11.25 | 5.65 | 2.81 |
| γ-Tocotrienol | 467.00 | 233.50 | 116.75 | 58.37 | 29.18 | 14.59 |
| δ-Tocopherol | 423.00 | 211.50 | 105.75 | 52.88 | 26.44 | 13.22 |
| δ-Tocotrienol | 414.00 | 207.00 | 103.50 | 51.75 | 25.88 | 12.94 |

Table 2. Relative standard deviations of retention times and areas, LOQ, LODs, and linearity of individual tocopherols and tocotrienols.

| | Retention time | RSD of retention times | RSD of areas | LOQ (µg/mL) | LOD (µg/mL) | $R^2$ |
|---|---|---|---|---|---|---|
| α-Tocopherol | 1.799 | 0.24 | 5.75 | 18.11 | 5.43 | 0.9997 |
| α-Tocotrienol | 2.124 | 0.31 | 4.89 | 22.88 | 6.87 | 0.9991 |
| β-Tocopherol | 3.273 | 0.23 | 5.59 | 14.58 | 4.38 | 0.9999 |
| γ-Tocopherol | 3.624 | 0.27 | 6.45 | 22.03 | 6.62 | 0.9998 |
| β-Tocotrienol | 3.884 | 0.23 | 6.27 | 14.79 | 4.44 | 0.9992 |
| γ-Tocotrienol | 4.240 | 0.16 | 5.04 | 35.58 | 10.68 | 0.9991 |
| δ-Tocopherol | 4.517 | 0.14 | 6.62 | 27.54 | 8.27 | 0.9998 |
| δ-Tocotrienol | 5.302 | 0.14 | 5.58 | 27.53 | 8.27 | 0.9999 |

Figure 2 shows an overlay of the peaks of all compounds obtained from level 1 to level 5. The linearity for all calibrations was in a good range with $R^2$ better than 0.999. For statistical evaluation, a 1:10 dilution of the stock solution was injected 10 times, and the relative standard deviation (RSD) values of the retention times and areas were calculated. The RSD of retention times was typically better than 0.3 %, and the RSD of areas was better than 6 % (Table 2). The high RSD values for areas could be explained with the known fact that tocopherols and tocotrienols degrade or adsorb on steel capillaries in LC systems[4].

Finally, an extra virgin olive oil sample was measured to determine the content of vitamin E compounds. The olive oil was diluted in hexane and directly injected. The chromatogram showed only $\alpha$-tocopherol at 1.79 minutes as the quantifiable main peak and a trace of $\gamma$-tocopherol at 3.6 minutes (Figure 3). The final concentration of $\alpha$-tocopherol in the measured olive oil was 184.8 mg/kg.

## Conclusion

This Application Note demonstrates the use of the Agilent 1260 Infinity analytical SFC system for quantification of vitamin E compounds such as tocopherols and tocotrienols in olive oil. The separation of four isomeric tocopherols and four isomeric tocotrienols is shown in a run time of 6 minutes, which is about five times faster than the typically used normal-phase LC methods. Another aspect is that the organic solvents used in normal-phase separations are harmful. This is in contrast to the ethanol modifier used for the SFC separation. The obtained LOQs are typically less than 23 µg/mL, LODs are less than 7 µg/mL, and $R^2$ better than 0.999.

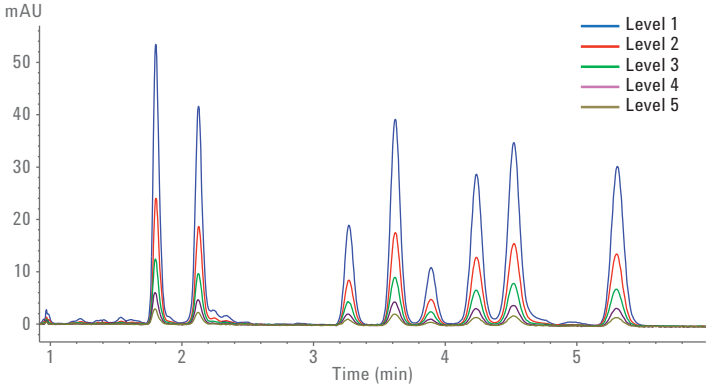



Figure 2. Overlay of calibration levels used to generate calibration curves for $\alpha$, $\beta$, $\gamma$, $\delta$-tocopherol and $\alpha$, $\beta$, $\gamma$, $\delta$-tocotrienol. (See Figure 1 for compound names, and Table 1 for the concentration of individual compounds and levels.)

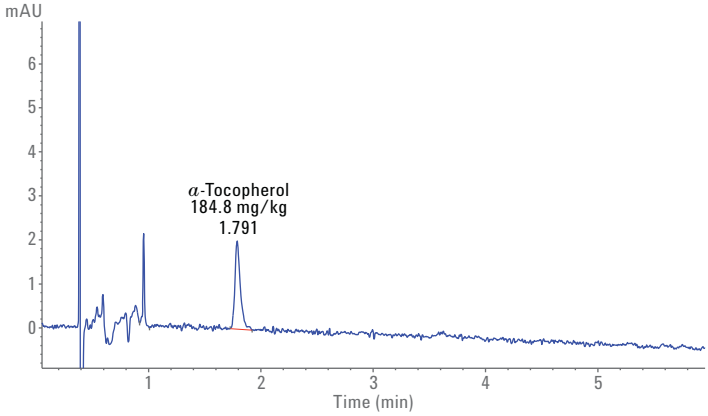



Figure 3. Measurement of an extra virgin olive oil sample and determination of the concentration of $\alpha$-tocopherol.

## References


1. Owen; *et al.* Phenolic compounds and squalene in olive oils: The concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene, *Food and Chemical Toxicology* **2000**, *38*, pp 647-659.

2. Cunha; *et al.* Quantification of Tocopherols and Tocotrienols in Portuguese olive oils using HPLC with three different detection systems, *Journal of Agricultural and Food Chemistry* **2006**, *54*, pp 3351-3356.

3. Aparicio, R; Aparicio-Ruíz, R. Authentication of vegetable oils by chromatographic techniques, *Journal of Chromatography A* **2000**, *881*, pp 93-104.

4. Cort; *et al.* Stability of Alpha- and Gamma-Tocopherol: $Fe^{3+}$ and $Cu^{2+}$ Interactions, *Journal of Food Science* **1978**, *43*, pp 797-798.


www.agilent.com/chem




Published in the USA, February 1, 2015
5991-5499EN


Agilent Technologies