Quality Analysis of Virgin Olive Oils – Part 4

Nutritive benefits - Quantification of tocopherols and tocotrienols in virgin olive oil using the Agilent 1260 Infinity Bio-inert Quaternary LC system

Application Note

Food Analysis & Agriculture

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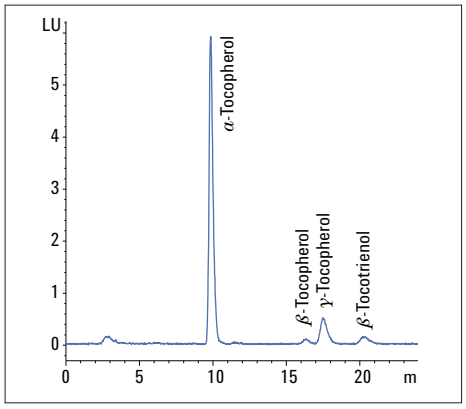


Abstract

This Application Note shows the analysis of tocopherols and tocotrienols in virgin olive oil samples using normal phase chromatography on the Agilent 1260 Infinity Bio-inert Quaternary LC system with fluorescence detection. Using the bio-inert system, huge improvements of retention time and area precision were observed in comparison to the standard stainless steel system. Very good linearity was obtained for all eight vitamin E isomers together with limits of detection (LOD) and limits of quantitation (LOQ) in the low µg/mL range. The analysis of nine olive oil samples revealed an α-tocopherol content between 100 to 390 mg/kg olive oil. No difference in the vitamin E content was detected between partly refined and virgin olive oils.

Verified for Agilent
1260 Infinity II LC
Bio-inert System

Agilent Technologies

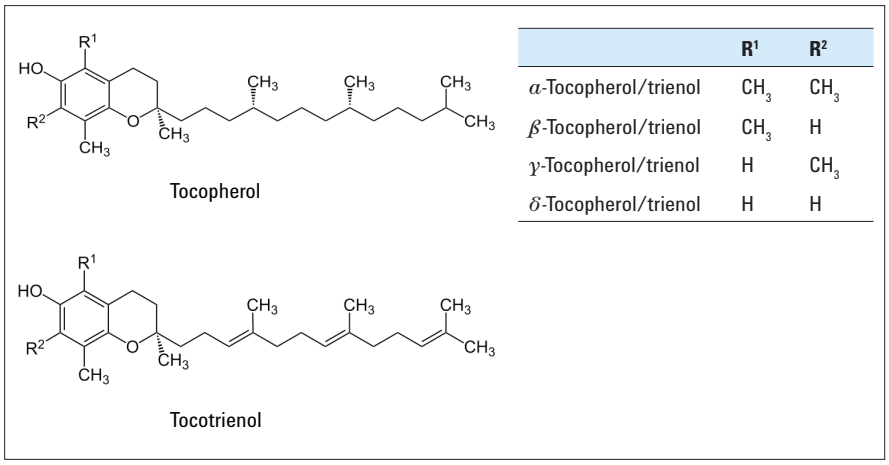
Introduction

Virgin olive oil is obtained from the fruits of the olive tree (*Olea europea L.*) only by mechanical procedures, and without the use of any thermal or chemical treatment. To ensure the authenticity of virgin olive oil regarding adulteration, mislabeling, characterization, or misleading origin, numerous methods have been developed for the evaluation of vegetable oils in the last two decades[1]. The analysis of thermally treated olive oils has been shown in previous application notes[2,3,4].

Regarding nutritive benefits, virgin olive oil is a good source of several bioactive components related to highly chemoprotective effects on human health. In addition to the high amount of mono-unsaturated and polyunsaturated fatty acids, it contains highly valuable antioxidants such as phytosterols, carotenoids, phenols, and vitamin E[5,6]. The levels of these bioactive components are dependent on genetic, agronomic, and environmental factors. The name vitamin E comprises four tocopherol (α-,β-,γ-, and δ-tocopherol) and four tocotrienol (α-,β-,γ-, and δ-tocotrienol) isomers. All eight forms contain a chromanol ring and an isoprenyl with three double bonds in tocotrienols, see Figure 1. In virgin olive oil, 95% of the total tocopherol/tocotrienol content is α-tocopherol[6].

Due to the diversity of biological activity, which can be related to most of the single tocopherols and tocotrienols in cancer or infarction prevention, it is essential to gain a complete vitamin E profile[6]. Suitable chromatographic techniques that separate, identify, and quantify all vitamin E isoforms are important elements for complete vitamin E profiling. Both, reversed (RP) and normal phase (NP) high pressure liquid chromatography (HPLC) can be applied for the analysis of tocopherols and tocotrienols. This Application Note used normal phase ultra high pressure chromatography (UHPLC) due to the good separation of all vitamin E isomers[6]. The fluorescing properties of the vitamin E isomers permit detection through sensitive fluorescence detection.

Tocopherols are known for being sensitive to degradation processes into products such as, for example, tocored, tocopurple, para-tocopherol, quinone, and dimerization reactions[7]. Degradation processes occur especially by photooxidation but also by oxidation in the presence of Fe^{3+} or Cu^{2+} ions[7]. Due to the latter, liquid chromatographic analysis in stainless steel HPLC/UHPLC systems, is prone to result in very poor retention time and area precision. To compare reproducibility regarding retention time (RT) and area, both a stainless steel (Agilent 1260 Infinity Quaternary LC system) and a bio-inert system (Agilent 1260 Infinity Bio-inert Quaternary LC system) are compared in this Application Note.



	R¹	R²
α-Tocopherol/trienol	CH_3	CH_3
β-Tocopherol/trienol	CH_3	H
γ-Tocopherol/trienol	H	CH_3
δ-Tocopherol/trienol	H	H

Figure 1
Tocopherol and tocotrienol structure.

Experimental

The Agilent 1260 Infinity Bio-inert Quaternary LC system consisted of the following modules:

- Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
- Agilent 1260 Infinity Bio-inert High Performance Autosampler (G5667A)
- Agilent 1290 Infinity Thermostat (G1330B) for sample cooling
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with bio-inert solvent heat exchanger
- Agilent 1260 Infinity Fluorescence Detector (G1321B), equipped with bio-inert standard FLD flow cell

The Agilent 1260 Infinity Quaternary LC system consisted of the following modules:

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity High Performance Autosampler (G1367E)
- Agilent 1290 Infinity Thermostat (G1330B) for sample cooling
- Agilent 1290 Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Fluorescence Detector (G1321B), equipped with standard FLD flow cell

To perform normal phase chromatography on the 1260 Infinity Quaternary LC systems (stainless steel and bio-inert), the wash and piston seals have to be exchanged from reversed phase (standard) to normal phase:

- Piston seals - normal phase (p/n 0905-1420)
- Wash seals (PE) (p/n 0905-1718)

Sample

The Tocotrienol and Tocopherole Mixed Solution Standard (in Hexane) was purchased from LGC Standards, Teddington, UK. Several olive oils (virgin and partly refined olive oil) were purchased in local stores. For normal phase analysis, the olive oil samples were diluted with heptane to a concentration of 100 mg/mL before injection into the UHPLC system.

Chromatographic conditions

	Long run
Mobile phase:	tBME:Heptane (5:95)
Flow rate:	1 mL/min
Isocratic run:	Stop time – 45 minutes
Injection volume:	10 µL
Temperature thermostat:	4 °C
Temperature TCC:	35 °C
DAD:	Ex: 298 nm, Em: 330 nm
Peak width:	> 0.05 minutes (1 second resp. time) (9.26 Hz)

Table 1
Chromatographic conditions.

Solvents

N-heptane and methyl tert-butyl ether (tBME) were LC grade and purchased from Sigma-Aldrich, St. Louis, MO, USA.

Columns

Agilent LiChrospher DIOL, 4 × 250 mm, 5 µm (p/n 79925DI-584)

Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]

Results and Discussion

Figure 2 shows the successful separation of all vitamin E isomers of the tocopherol and tocotrienol standard from LGC Standards using NP chromatography. The precision of retention time and area was determined on two systems, the 1260 Infinity Quaternary LC system and the 1260 Infinity Bio-inert Quaternary LC system. Figure 2 shows an overlay of six runs on the bio-inert system with excellent relative standard deviations, regarding both retention time and area.

Table 2 summarizes the results for vitamin E isomer analysis on both systems. On the bio-inert system, the results were much better for RT precision and area due to oxidation processes of the isomers in presence of Fe^{3+} ions[7]. Regarding RT precision, the bio-inert system showed up to ten times better performance resulting in very good RT precision values under 0.07% for example for α-tocopherol in contrary to 0.68% RSD on the stainless steel system. Area precision also improved between three and ten (four times for α-tocopherol) times better on the bio-inert system.

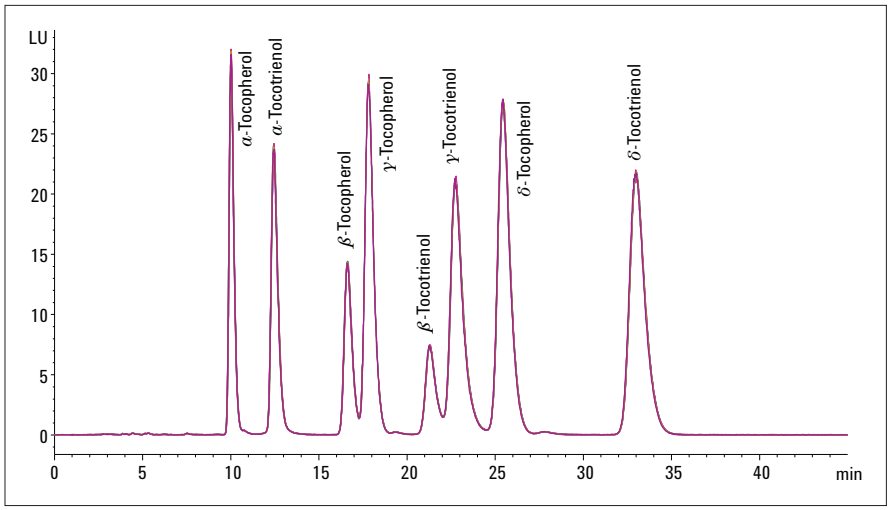


Figure 2
Overlay of six runs of all vitamin E isomers on the Agilent Infinity Bio-inert Quaternary LC system.

	Agilent 1260 Infinity Bio-inert Quaternary LC system		Agilent 1260 Infinity Quaternary LC system	
	RSD RT (%)	RSD area (%)	RSD RT (%)	RSD area (%)
α-Tocopherol	0.069	0.63	0.677	2.64
α-Tocotrienol	0.062	0.94	0.658	2.88
β-Tocopherol	0.053	0.64	0.65	2.78
γ-Tocopherol	0.103	0.61	0.691	2.73
β-Tocotrienol	0.103	0.79	0.708	3.32
γ-Tocotrienol	0.097	0.84	0.67	3.78
δ-Tocopherol	0.062	0.29	0	3.16
δ-Tocotrienol	0	0.34	0	2.74

Table 2
Precision of retention time and area on the Agilent 1260 Infinity SST Bio-inert Quaternary LC system and the Agilent 1260 Infinity Bio-inert Quaternary LC system.

To determine linearity, LOD and LOQ, the tocopherol and tocotrienol standard solution was diluted in *n*-heptane in 1:3 dilution steps, see Table 3.

Eight different concentration levels were prepared and the linear relationship was determined between the peak area and the corresponding concentrations. LOD and LOQ were defined as the signal to noise ratio of 3:1 respectively 10:1. Table 4 shows the results for linearity and LOD and LOQ for all vitamin E isomers.

Very good linearity was determined for all vitamin E isomers with correlation coefficients >0.9998. LOD and LOQ were found in the low µg/mL range where, for example in olive oil, small amounts of β-tocopherol and β-tocotrienol are found.

Analyte	Level 1 (µg/mL)	Level 2 (µg/mL)	Level 3 (µg/mL)]	Level 4 (µg/mL)	Level 5 (µg/mL)	Level 6 (µg/mL)	Level 7 (µg/mL)	Level 8 (µg/mL)
α-Tocopherol	463.00	154.33	51.44	17.15	5.72	1.91	0.64	0.21
α-Tocotrienol	447.00	149.00	49.67	16.56	5.52	1.84	0.61	0.20
β-Tocopherol	203.00	67.67	22.56	7.52	2.51	0.84	0.28	0.09
γ-Tocopherol	430.00	143.33	47.78	15.93	5.31	1.77	0.59	0.20
β-Tocotrienol	135.00	45.00	15.00	5.00	1.67	0.56	0.19	0.06
γ-Tocotrienol	467.00	155.67	51.89	17.30	5.77	1.92	0.64	0.21
δ-Tocopherol	423.00	141.00	47.00	15.67	5.22	1.74	0.58	0.19
δ-Tocotrienol	414.00	138.00	46.00	15.33	5.11	1.70	0.57	0.19

Table 3
Dilution series of the tocopherol and tocotrienol standards.

Analyte	Correlation	LOD (µg/mL)	LOQ (µg/mL)
α-Tocopherol	0.99984	3.34	11.14
α-Tocotrienol	0.99995	2.97	9.89
β-Tocopherol	0.99998	1.70	5.66
γ-Tocopherol	0.99992	1.45	4.82
β-Tocotrienol	0.99998	1.75	5.82
γ-Tocotrienol	0.99997	1.73	5.76
δ-Tocopherol	0.99992	1.03	3.44
δ-Tocotrienol	0.99990	0.99	3.29

Table 4
Linearity of tocopherols and tocotrienols standard dilution series analyzed on the Agilent 1260 Infinity Bio-inert Quaternary LC system.

Figure 3 shows the analysis of virgin olive oil for its comprising vitamin E isomers. Like reported in literature[6], 95% of all vitamin E isomers is α-tocopherol in virgin olive oil. In addition, small amounts of γ-tocopherol, β-tocopherol and β-tocotrienol were found.

A total of nine olive oil samples were analyzed for their vitamin E content, see Table 5. The amounts of determined vitamin E isomers: α-,β-,γ-tocopherol and β-tocotrienol was correspondent to the amounts described in literature, for example, for α-tocopherol from approximately 100 to 390 mg per kg olive oil[6,8]. There was no difference found between partly refined (sample #3) and virgin olive oils (#1, 2 and #4 - #8) regarding vitamin E content.

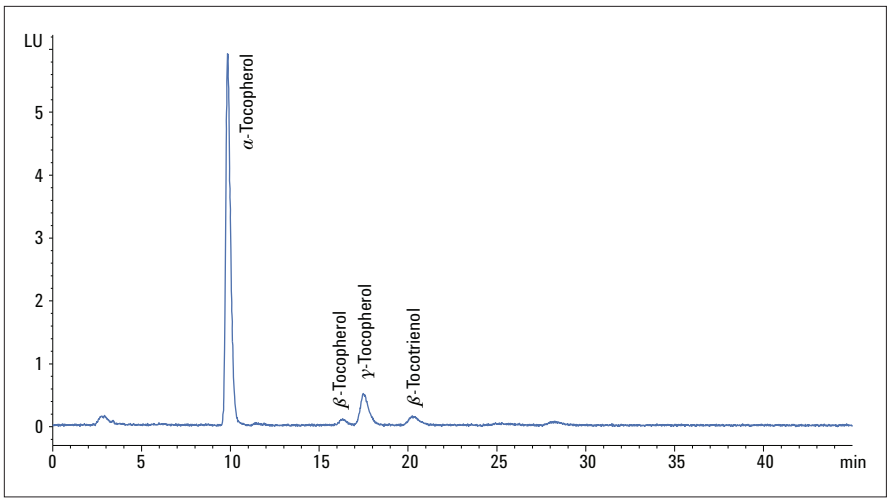


Figure 3
Analysis of virgin olive oil for vitamin E isomers.

Olive oil	#1 mg/kg	#2 mg/kg	#3 mg/kg	#4 mg/kg	#5 mg/kg	#6 mg/kg	#7 mg/kg	#8 mg/kg	#9 mg/kg
α-Tocopherol	389.7	315.8	179.8	152.1	109.8	168.2	98.9	114.2	337.3
β-Tocopherol	5.4	3.5	2.5	2.0	3.8	3.0	2.7	2.6	4.3
γ-Tocopherol	9.1	11.8	12.1	6.6	5.8	9.2	12.0	1.8	4.1
β-Tocotrienol	12.3	5.8	5.3	4.8	3.6	4.9	4.2	5.8	13.3
δ-Tocopherol	-	-	-	-	-	-	0.4	-	-

Table 5
Vitamin E content of nine analyzed olive oil samples.

Conclusion

Tocopherols are prone to degradation processes by oxidation in the presence of Fe^{3+} or Cu^{2+} ions[7]. Therefore, it is highly recommended to use the Agilent 1260 Infinity Bio-inert Quaternary LC system for the analysis of tocophenols and tocotrienols for the highest precision of retention time and area. With the bio-inert system, an up to 10x improvement of retention time precision was observed. In addition, area precision improved approximately 10x on the bio-inert system.

Using the bio-inert system together with normal phase chromatography using heptane, very good linearity was obtained for all eight vitamin E isomers together with LODs and LOQs in the low µg/mL range. The tocopherol/toctrienol amount of nine olive oil samples was found in a range between 100 to 390 mg/kg olive oil for example for α-tocopherol. No difference in the vitamin E content was detected between partly refined and virgin olive oils.

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