Analysis of Antioxidants in Vegetable Oils Using the Agilent 1260 Infinity Hybrid SFC/UHPLC System with MS Detection

Application Note

Food Analysis & Agriculture

Authors

Maria Rambla-Alegre,
Melissa N. Dunkle, Gerd Vanhoenacker,
Frank David, and Pat Sandra,
Research Institute for Chromatography
Kennedypark 26
B-8500 Kortrijk
Belgium

Martin Vollmer
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This Application Note demonstrates that SFC and UHPLC are complementary for the analysis of antioxidants in vegetable oil samples. The Agilent 1260 Infinity Hybrid SFC/UHPLC System combined with single quadrupole mass spectrometry detection is capable of performing both supercritical fluid chromatography (SFC) and ultrahigh performance liquid chromatography (UHPLC) by switching automatically between the two techniques.

Good MS-peak area repeatability (RSD < 5.0%) and sensitivity were achieved, allowing the system to be used for qualitative as well as quantitative analysis. The figures of merit are shown using standard solutions and vegetable oils. Using a simple methanol extraction, good recovery was obtained for all antioxidants in the oil sample.

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Introduction

The Agilent 1260 Infinity Hybrid SFC/UHPLC-MS System represents state-of-art, packed-column SFC, providing HPLC-like sensitivity, 600-bar power range, and high instrument and method robustness, all achieved on a truly modular and flexible LC-based system[1].

SFC using packed columns is a valuable complementary technique to liquid chromatography. Especially for chiral and normal phase separations, SFC has demonstrated its potential. This Application Note describes the possibility to obtain complementary data on analyte mixtures in a single sequence of runs by switching between SFC and UHPLC mode. This eliminates the need to invest in two individual systems, excludes system-to-system variability, and saves significant cost and laboratory space[1].

Vitamin E plays an important role as antioxidant. Different stereo-isomers (vitamers) are prevalent in various vegetable oils exhibiting differences in vitamin activity. This Application Note describes the analysis of 14 antioxidants in vegetable oils using the 1260 Infinity Hybrid SFC/UHPLC/MS System in the SFC/MS and LC/MS mode. Since the biological activities and chemical properties of tocols (tocopherols and tocotrienols) differ from each other, it is important to be able to determine and quantify each vitamer separately. The complete resolution of the eight tocols is only possible by using the SFC-MS mode. In this case, the separation by SFC was significantly faster than with UHPLC.

Experimental

Solutions

Stock solutions of the individual antioxidants were prepared in methanol (1–5 mg/mL, depending on solubility). These stock solutions were then mixed to obtain a 14-compound test mixture. Most experiments were performed using a 100-ppm solution; however, a dilution series was also prepared from 0.1–100 ppm. Table 1 provides peak identification, chemical name, and formula weight. For the spiked samples, a stock solution of the antioxidants in the solvent was added prior to extraction.

Oil samples were purchased from a local supermarket. The extraction of the oil and the spiked oil sample was carried out by weighing 100 mg of oil and adding 1 mL of the solvent. This mixture was vortexed for 30 seconds, allowed to stand for 2 minutes, and vortexed once more for 30 seconds.

Peak id	Chemical name	CAS	MW (g/mol)	Supplier
1	Propyl Gallate (PG)	121-79-9	212.2	Sigma
2	Tert-butyl-hydroquinone (TBHQ)	1948-33-0	166.2	Sigma
3	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX)	53188-07-1	250.3	Sigma
4	Butylated hydroxyanisole (BHA)	25013-16-5	180.2	Sigma
5	Octyl Gallate (OG)	1034-01-1	282.3	Sigma
6	Butylated hydroxytoluene (BHT)	128-37-0	220.3	Sigma
7	Lauryl Gallate (LG)	1166-52-5	338.4	Sigma
8	δ-Tocotrienol (δ-TT)	25612-59-3	396.6	Cayman Chem.
9	γ-Tocotrienol (γ-TT)	14101-61-2	410.6	Cayman Chem.
10	α-Tocotrienol (α-TT)	58864-81-6	424.7	Cayman Chem.
11	δ-Tocopherol (δ-TP)	119-13-1	402.6	Cabiochem
12	γ-Tocopherol (γ-TP)	54-28-4	416.7	Calbiochem
13	β-Tocopherol (β-TP)	148-03-8	416.7	Calbiochem
14	α-Tocopherol (α-TP)	59-02-9	430.7	Calbiochem

Table 1
Analyzed antioxidants.

The sample was then centrifuged at 5,000 × g for 5 minutes and the supernatant was transferred into an autosampler vial for injection.

System Configuration

A 1260 Infinity Analytical SFC System (G4309A) can be converted into a hybrid SFC/UHPLC system by simple addition of a 2-position/10-port valve comprising universal valve drive (G1170A) with valve head (G4232B) and a second pump (G1311B). The system can be run in UHPLC mode (Figure 1a) or in SFC mode (Figure 1b). Alternating between modes is accomplished by switching the 2-position/10-port valve, which can be programmed as a method parameter at the beginning of the respective method[1]. The thermostatted column compartment is equipped with a 2-position/6-port column switching valve which enables the selection of the appropriate column for each mode.

Some modifications should be taken into account when coupling the SFC to a MS (or ELSD)[2]. A capillary heating device is installed just before the MS inlet. In SFC mode, the effluent, mainly consisting of carbon dioxide, is decompressed before entering to the MS source. The expanding CO_2 results in significant cooling which can cause freezing of the transfer line. Additionally, a make-up flow is added

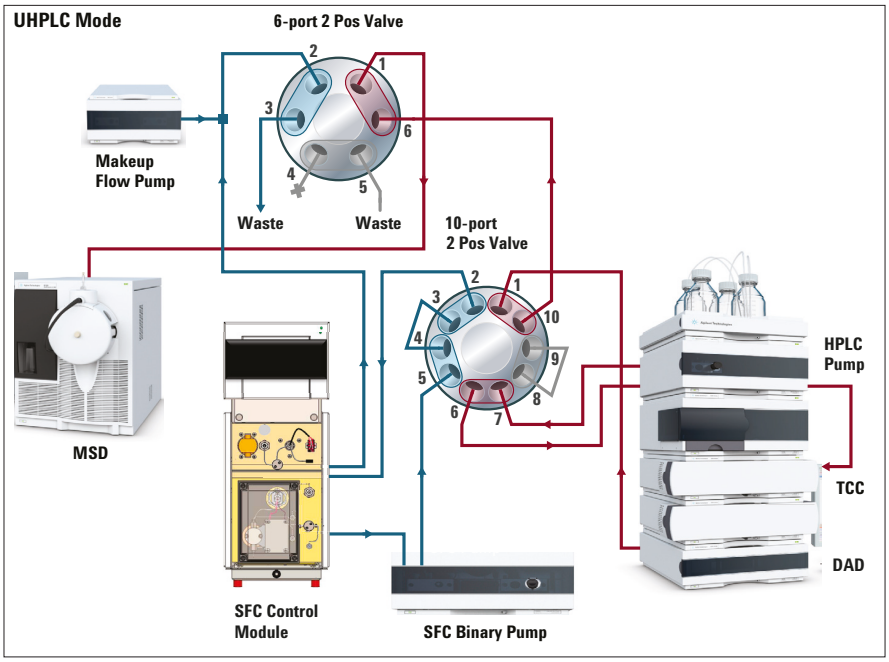

Figure 1a
Schematic of the hybrid system in UHPLC/MS mode.

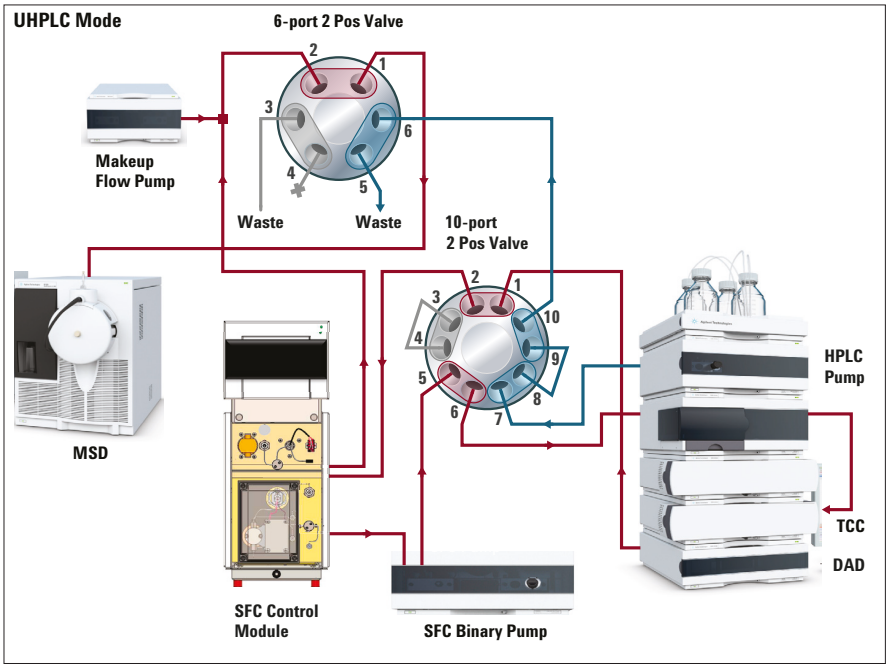

Figure 1b
Schematic of the hybrid system in SFC/MS mode

to the system between the UV detector and the BPR. This additional make-up flow is required in order to obtain the best retention time and peak area reproducibility.

From the UV detector, a 0.12 mm × 105 mm SS capillary (p/n 5021-1820) is connected to an Agilent zero dead volume T-piece (p/n 0100-0969). An Agilent G1311B Pump was used to supply the make-up flow and was connected to the T using a 0.25 mm × 800 mm SS capillary (p/n 5065-9930). A 0.12 mm x 400 mm SS capillary (p/n 5021-1823) was used to connect the T to the BPR. The Caloratherm preheater sleeve was placed over a 0.17 mm × 10 mm SS capillary (p/n 5061-3361) and the tubing containing the preheater device was connected directly to the inlet of the MS. In addition, a 2-position/6-port valve needs to be added as shown in Figures 1a and 1b to combine the flow paths from SFC and UHPLC prior to MS detection.

With the heating device and make-up flow present in the SFC configuration, freezing does not occur, and the MS reproducibility is significantly improved.

Experimental conditions

Table 3 shows the method parameters used in the separation of the 14-component antioxidant mixture and oil samples.

Agilent 1260 Infinity Hybrid SFC/UHPLC System		
G4309A	Agilent 1260 Infinity Analytical SFC System	
G1311B	Agilent 1260 Infinity Quaternary Pump (can be replaced by G1312B, G1310B, G4220A/B, and G4204A)	
G1170A	Agilent 1290 Infinity Valve Drive	
G4232B	2-position/10-port valve head – 600 bar	
G6130B	LC/MS Single Quad	
G4231A	2-position/6-port valve head -600 bar	
G1170A	Agilent 1290 Infinity Valve Drive (a second valve drive is necessary to support the 2-position/6-port valve head)	
AG1	Caloratherm[2]	Available through RIC[1]
AG004	Preheater[2]	Available through RIC[1]

[1]Contact info@richrom.com for more information.
[2]A Capillary heater can be replaced by the usage of a G1316A or G1316C heat exchanger

Table 2
System modules.

Conditions	UHPLC mode	SFC mode
Injection volume:	15 µL (5 µL on column)	15 µL (5 µL on column)
Column:	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)	Agilent ZORBAX Rx-SIL, 4.6 × 250 mm, 5 µm (p/n 880975-901)
BPR:	90 bar*	120 bar
SFC flow rate:	-	2 mL/min
UHPLC flow rate:	0.4 mL/min	1 mL/min
Supercritical fluid:	-	CO_2
Modifier:	(A) Water 0.1% FA (B) Methanol 0.1% FA	MeOH
UHPLC gradient:	20–100% B in 15 minutes (total 25 minutes)	
SFC gradient:		3–12% B (0–25 minutes)
Column temperature:	30 °C	50 °C
Make-up flow:		MeOH 0.1% FA at 0.8 mL/min
Caloratherm:		60 °C
DAD:	292/10 nm, Ref. 400/50 nm	292/10 nm, Ref. 400/50 nm
APCI:	Capillary V ± 4,000 V Corona I = 4.0 µA (+), 20 µA (-) Drying gas = 6.0 L/min at 325 °C Nebulizer = 55 psig Vaporizer = 350 °C	Capillary V ± 4,000 V Corona I = 4.0 µA (+), 20 µA (-) Drying gas = 6.0 L/min at 325 °C Nebulizer = 60 psig Vaporizer = 350 °C

* Only to maintain functioning of BPR. The pressure is not applied on the UHPLC column.

Table 3
Experimental conditions of hybrid system.

Results and Discussion

A 14-component antioxidant mix was analyzed to demonstrate the ease and complementary use and performance of the 1260 Infinity Hybrid SFC/UHPLC/MS System. Both UV and MS data (APCI) were collected; MS data was used to confirm the identity. Figure 2 shows the separation of the antioxidant standard mixture (10 µg/mL) in the UHPLC mode.

Calibration curves were constructed and excellent linearity was obtained for both SFC and LC mode. Table 4 summarizes the UHPLC results.

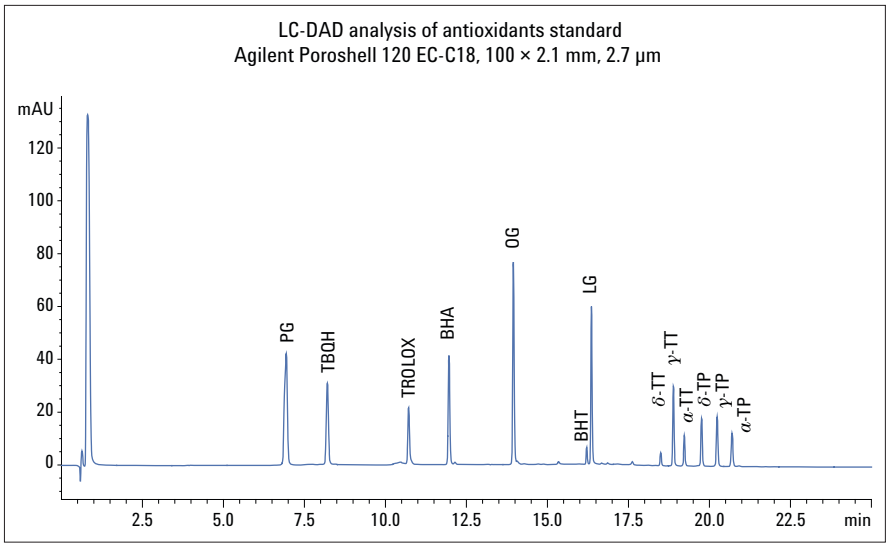

Figure 2
Analyses 14-compounds antioxidant mixture by LC-DAD (10 µg/mL).

	Linearity (R^2)[1]	Repeat. (% RSD)[2]	Repeat. (% RSD)[3]	Recovery 5 mg/kg (%)	Repeat. (% RSD)	Recovery 100 mg/kg (%)	Repeat. (% RSD)
PG	0.99977	3.7	4.11	102.8	2.0	103.3	0.7
TBHQ	0.99807	4.4	4.8	72.6	20	87.2	23
TROLOX	0.99969	5.0	4.3	94.9	17	92.6	6
BHA	0.99978	0.7	2.1	105.2	4.5	100.8	1.2
OG	0.99978	3.0	4.5	101.2	1.6	104.0	1.2
BHT	0.99981	4.9	1.7	104.7	5.6	99.4	2.0
LG	0.99974	0.8	1.4	99.97	7.2	104.4	0.9
δ-TT	0.99965	4.5	2.2				
γ-TT	0.99969	1.8	2.5				
α-TT	0.99953	2.1	2.5				
δ-TP	0.99972	1.8	2.3				
γ-TP and β-TP	0.99987	1.8	2.7				
α-TP	0.99943	1.3	2.6				

[1] 0.1, 0.5, 1, 5, 10, 25, 50, and 100 µg/mL standard solution, 1 injection/level (UV)
[2] 6 consecutive injections of 0.5 µg/mL
[3] 6 consecutive injections of 25 µg/mL
* Recoveries of tocopherols and tocotrienols were not calculated because they are already in the oil samples.

Table 4
LC mode method performance data.

The repeatability and linearity of the method were investigated using standard solutions of the antioxidant and spiked oil samples. The detection limit was equal to, or below 0.1 µg/mL for all antioxidants. This corresponds to approximately 1 mg/kg or lower in an oil or fat sample. Extracts of vegetable oil and spiked oils were analyzed to determine recovery and accuracy (Figure 3). The oil sample was spiked with 5 mg/kg and 100 mg/kg of each antioxidant, and the detected amounts in the extracts were compared to standard solutions at the same concentration.

Similar resolution and peak widths for the UV and MS results were obtained and linearity was good from 0.1–100 ppm. The MSD was approximately 10 times more sensitive than UV detection for all of the components of the test mixture. Thus, APCI MS was used to confirm the identification of the peaks. It is important to note that two compounds (TBHQ and trolox) in the spiked sample were slightly decomposed during the sequence analysis, which resulted in the low recovery for these compounds.

Although the performance of the LC method was good, not all tocopherols were resolved (co-elution of β-TP and γ-TP). Additionally some other co-elutions were observed (BHA and α-tocopherol (Figure 4). Complete resolution of the eight tocols was obtained by SFC-MS mode, enabling the analysis of the individual tocols in different oils extracts (deep frying oil, sunflower, rapeseed, and tocomix). Tocomix is a commercial mixture of tocols in sunflower oil (AOMS, S.A., Argentina).

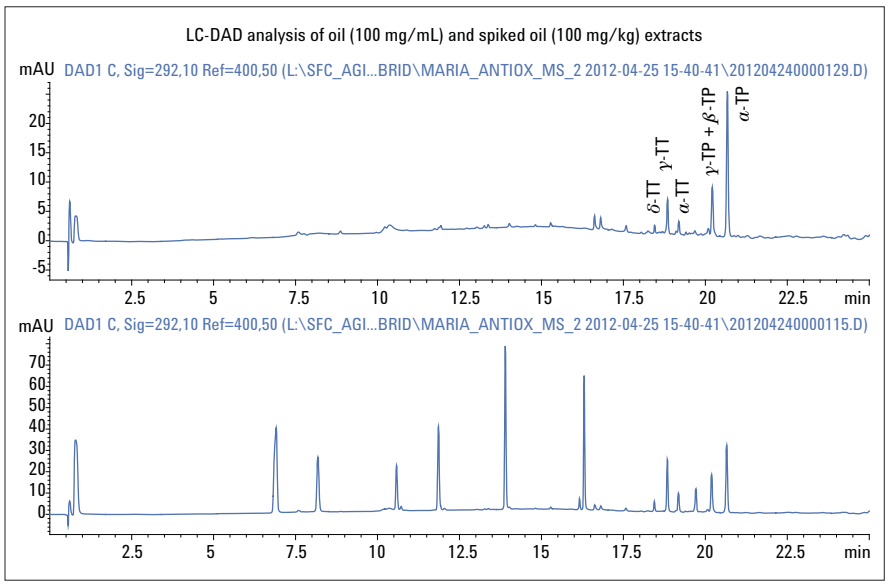

Figure 3
Analysis of oil (100 mg/mL) and spiked deep frying oil (100 mg/kg) extracts with the LC method.

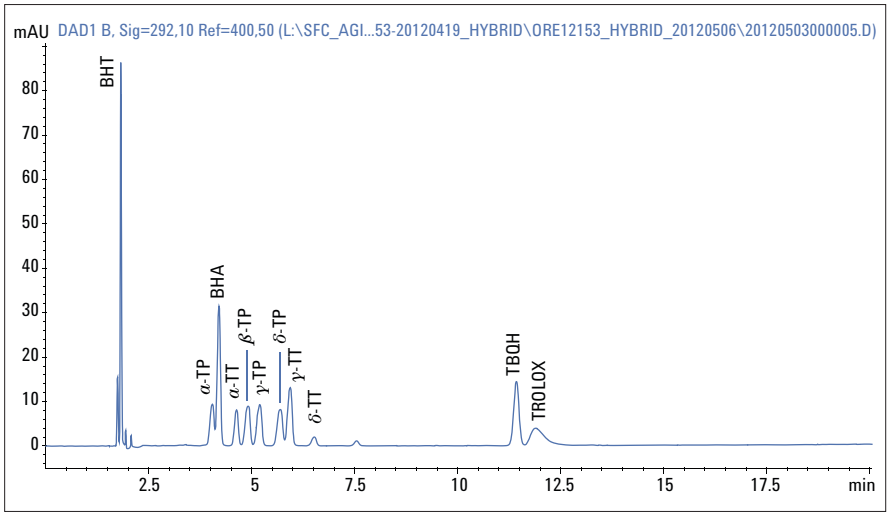

Figure 4
Analyses 14-compounds antioxidant mixture by SFC-DAD (10 µg/mL).

The polarity of the tocopherols and tocotrienols is mainly influenced by the number of methyl groups in the chromanol ring, and to a lesser extent by steric effects of the methyl groups and slightly increased polarity of the unsaturated side chains of tocotrienols compared to those of tocopherols. The most difficult compounds to be separated were the β- and γ- tocols (Figures 5 and 6), because they have three methyl groups in their ring structure. APCI mass to charge ratios (*m/z*) of [M-H]+ ions were 429, 415, 415, and 401 for α-, β-, γ-, and δ-tocopherols, and 423, 409, 409, and 396 for α-, β-, γ-, and δ-tocotrienols.

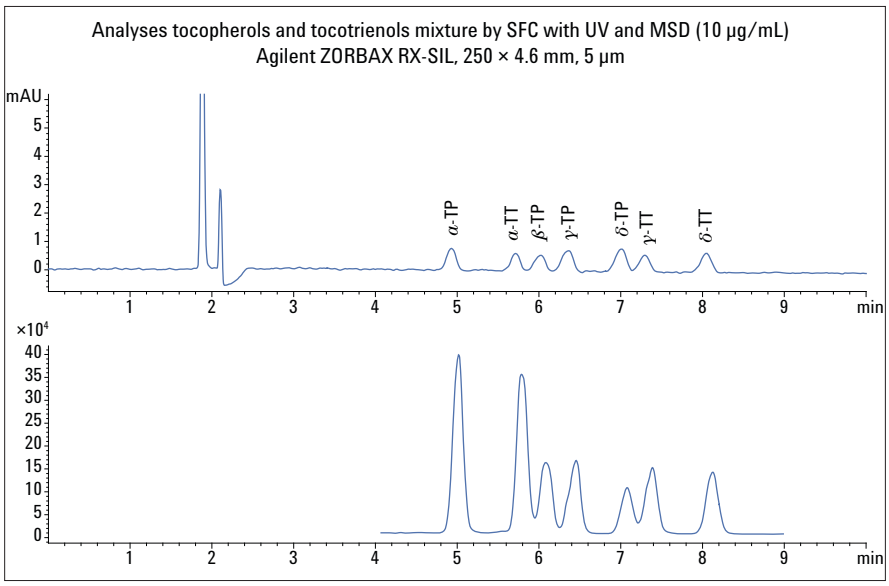

Figure 5
Analyses tocopherols and tocotrienols mixture by SFC with UV and MSD (10 µg/mL) (β-tocotrienol was not available as pure standard).

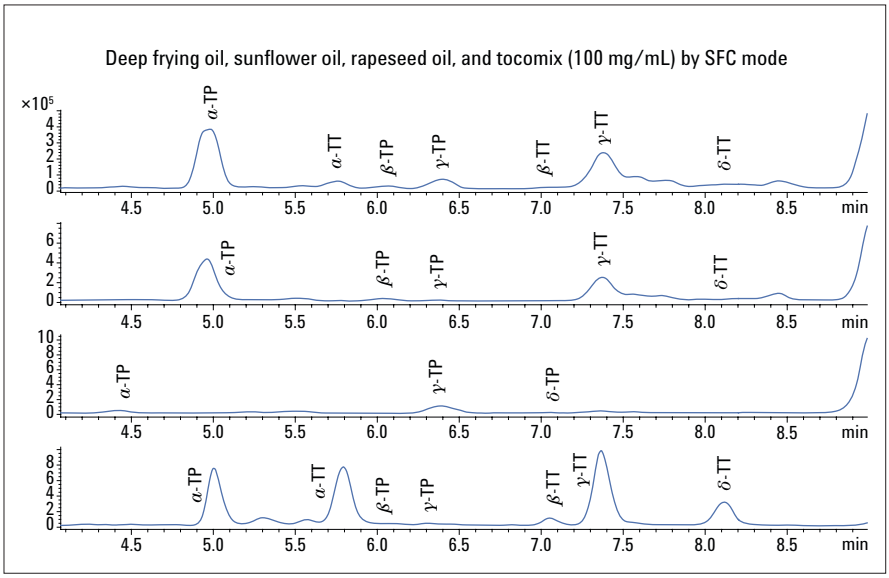

Figure 6
Deep frying oil, sunflower oil, rapeseed oil (100 mg/mL) and tocomix by SFC mode.

Figure 7 shows a Spectrum of α-tocopherol in deep frying oil sample by SFC-APCI analyses. Ion *m/z* 429 was selected for further analyses, which was attributed to [M-H]+ formed by initial protonation of α-tocopherol followed by dehydrogenation[4,5].

Comparable resolution in both UV and MSD were achieved when the separation was performed. Linearity was good with R^2 values of 0.99 from 0.1–50 ppm. Overall, the limits of detection (LODs) of LC/MS mode and SFC/MS mode were in the same order of magnitude. The results show that high separation power and good reproducibility were achieved with both techniques for a complex mixture of analytes. It is important to note that tocopherols and tocotrienols could only be completely resolved in the SFC-MS mode.

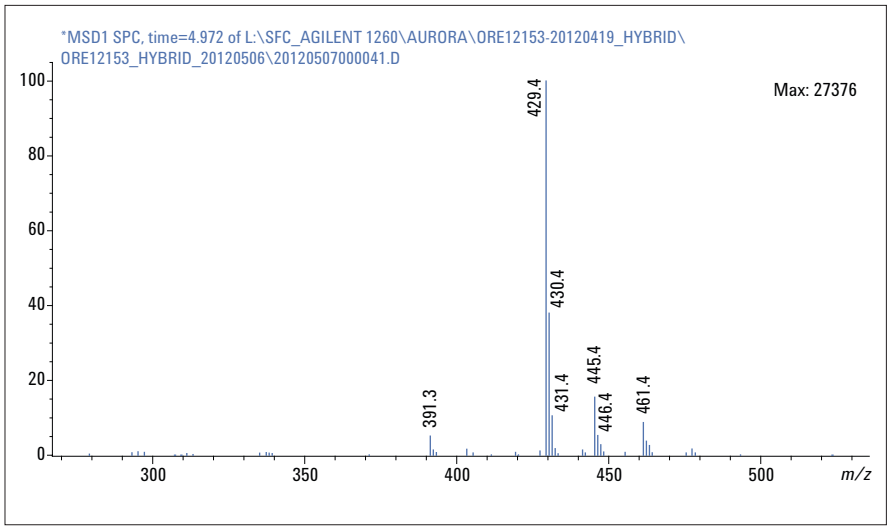

Figure 7
Spectra α-tocopherol in Deep frying oil by SFC mode.

				Deep frying[4]		Sunflower[4]		Rapeseed[4]		Tocomix	
	Linearity (R^2)[1]	Repeat. (% RSD)[2]	Repeat. (% RSD)[3]	Recov. (mg/kg)	RSD (%)	Recov. (mg/kg)	RSD (%)	Recov. (mg/kg)	RSD (%)	Recov. (ppm)	RSD (%)
δ-TT	0.99993	3.3	2.7	24	5.5	9	4.9	-	15.4	4.2	
β-TT*	NA	NA	NA	-	-	-	Detected				
γ-TT	0.99975	4.6	4.4	96	1.7	70	6.0	-	43.3	5.2	
α-TT	0.9994	3.9	2.9	19	4.3	-	-	20	4.6		
δ-TP	0.99942	3.8	4.4	-	-	0.5	5.8	-			
β-TP	0.99764	4.7	4.7	3	5.8	9	5.6	-	0.4	4.4	
γ-TP	0.99805	3.3	4.0	42	6.0	2.1	5.4	40	5.3	0.2	5.3
α-TP	0.99692	2.1	4.5	165	3.2	124	4.0	2.5	2.9	16.4	3.6

[1] 0.1, 0.5, 1, 5, 10, 25, 50 µg/mL standard solution, 1 injection/level (MS)
[2] 6 consecutive injections of 0.5 µg/mL
[3] 6 consecutive injections of 25 µg/mL
[4] 6 consecutive injections
* No quantitative data. No pure reference material available.

Table 5
SFC mode method performance data.

Conclusions

The Agilent 1260 Infinity Hybrid SFC/UHPLC/MS System provides an excellent tool to obtain complementary data from both SFC and UHPLC on a single instrument. Vegetable oil samples from different origins and spiked vegetable oil samples were extracted and the recoveries of the antioxidants were calculated. Good recovery was obtained for all antioxidants.

Phenolic antioxidants were analyzed by UHPLC. Using this mode, not all tocopherols were separated. Complete resolution of vitamers was achieved only when performing SFC-MS mode. Good sensitivity and high robustness led to the conclusion that hybrid SFC/UHPLC/MS is highly capable to separate and detect all antioxidant isomers for quantitative as well as for qualitative analyses.

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Published in the USA, October 15, 2017
5991-1546EN

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