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New Method for the Analysis of Antioxidants in Vegetable Oils using an Hybrid SFC/UHPLC System with MS Detection

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Introduction

Supercritical Fluid Chromatography (SFC) using packed columns is a complementary technique to liquid chromatography. Especially for chiral and normal phase separations, SFC has demonstrated its potential. In this study, we describe the possibility to obtain orthogonal data on analyte mixtures using a single instrument by simply 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.

Here we show the analysis of 14 antioxidants in vegetable oils using the Agilent 1260 Infinity Hybrid SFC/UHPLC system hyphenated to MS. Since the biological activities and chemical properties of tocols (tocopherols and tocotrienols) differ from each other, it is important to be able to determine each vitamin separately.

Fig. 1: Agilent Infinity 1260 SFC/UHPLC Hybrid solution with 6100 Series MSD

Experimental

Sample Preparation

Stock solutions of the individual antioxidants were prepared in methanol (1-5 mg/mL) and mixed to obtain a 14-compound test mixture (at 100 ppm). A dilution series was prepared from 0.1 – 100 ppm. For the spiked samples, the stock solution 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. The sample was then centrifuged at 5000 x g for 5 min and the supernatant was subjected to analysis.

Experimental

Table 1: Analyzed antioxidants

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

System Configuration

An Agilent 1260 Infinity Analytical SFC system can be upgraded to a hybrid SFC/UHPLC system by addition of a 2-position/10-port valve and a second pump. The system can be run in SFC (Figure 2a) or in UHPLC mode (Figure 2b). Alternating between modes is accomplished by simply switching the valve.

Table 2: System Modules

1260 Infinity Analytical Hybrid SFC/UHPLC System	
G4309A	Agilent 1260 Series Analytical SFC System
G1311B	1260 Infinity Quaternary Pump (can be replaced by G1312B, G1310B, G4220A/B, and G4204A)
G1170A	1290 Infinity Valve Drive
G4232B	2-position/10-port valve head – 600 bar
G6130B	LC/MS Single Quadrupole
G1170A	1290 Infinity Valve Drive
G4231A	2-position/6-port valve head -600 bar
AG1 AG004	Caloratherm[2] Pre-Heater[2]

Table 3: Experimental conditions

Conditions	UHPLC Mode	SFC Mode
Inj.Volume	5 µL on column	5 µL on column
Column	Poroshell 120 C18, 2.1x 100 mm	Agilent Rx-SIL, 4.6 x 250 mm, 5 µm
BPR	90 bar*	120 bar
SFC flow	-	2 mL/min
LC flow	0.4 mL/min	-
Solvents	(A) 0.1% FA, (B) MeOH 0.1% FA	(A) CO2 , (B) (MeOH)
Gradients	20-100% B in 15 min (total 25 min)	3 – 12% B (0-25 min)
Column (T)	30°C	50°C
Make-up flow		MeOH 0.1% FA at 0.8 mL/min
Post column (T)		60°C
DAD	292/10 nm, Ref. 400/50 nm	292/10 nm, Ref. 400/50 nm
APCI	Capillary V ± 4000 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 ± 4000 V, Corona I = 4.0 µA (+), 20 µA (-) Drying gas = 6.0 L/min at 325°C Nebulizer = 60 psig, Vaporizer = 350°C

Experimental

Instrument Configuration of the SFC/UHPLC Hybrid System

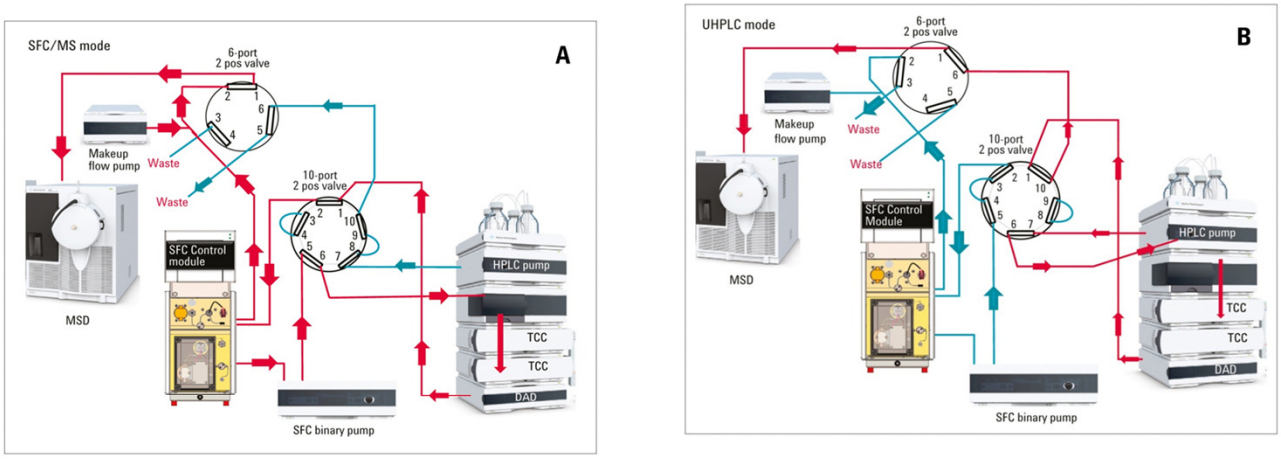

Figure 2: Instrument configuration of the SFC/UHPLC Hybrid System (Fig 2A and B)

Results and Discussion

A 14-component antioxidant mix and a spiked sample were analyzed to obtain complementary data from the Hybrid SFC/UHPLC system. Both UV and MS data (APCI) were collected; MS data was used to confirm analyte identity. UHPLC mode (10 µg/mL) resulted in excellent resolution except for the β- and γ- tocols (Fig. 3, Fig. 4).

Calibration curves were constructed and excellent linearity was obtained for both LC and SFC mode (Table 4 and 5). The repeatability and linearity of the method were investigated using standard solutions of the antioxidant and spiked oil samples. The detection limit was equal 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 4). 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 ionization was used to confirm the identification of the peaks.

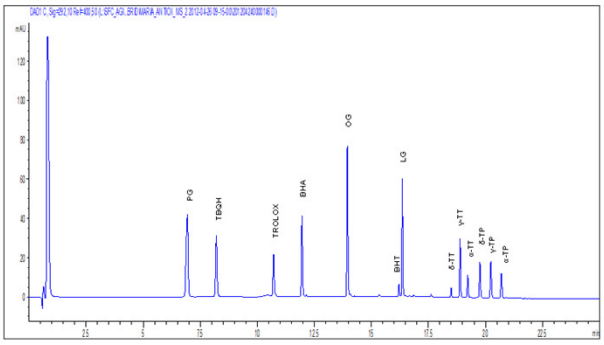

Figure 3: Analyses 14-compounds antioxidant mixture by LC-DAD (10 µg/mL)

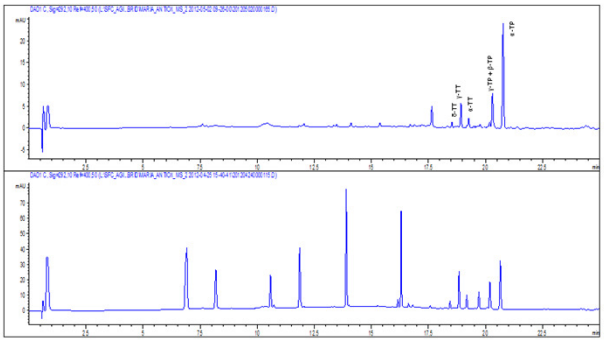

Figure 4: Analysis of oil (100 mg/mL) and spiked deep frying oil (100 mg/kg) extracts with the LC method.

Results and Discussion

Table 4: LC mode method performance data

	Linearity (R^2)(1)	Repeat. (% RSD)(2)	Repeat. (% RSD)(3)	Recovery 5mg/kg (%)
PG	0.99977	3.7	4.11	102.8
TBHQ	0.99807	4.4	4.8	72.6
TROLOX	0.99969	5.0	4.3	94.9
BHA	0.99978	0.7	2.1	105.2
OG	0.99978	3.0	4.5	101.2
BHT	0.99981	4.9	1.7	104.7
LG	0.99974	0.8	1.4	99.97
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 & 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 µg/mL standard solution, 1 inj/level (MS)
(2) 6 consecutive injections of 0.5 µg/mL
(3) 6 consecutive injections of 25 µg/mL

Table 5: SFC mode method performance data

	Linearity (R^2)(1)	Repeat. (% RSD)(2)	Repeat. (% RSD)(3)
δ-TT	0.99993	3.3	2.7
β-TT*	NA	NA	NA
γ-TT	0.99975	4.6	4.4
α-TT	0.9994	3.9	2.9
δ-TP	0.99942	3.8	4.4
β-TP	0.99764	4.7	4.7
γ-TP	0.99805	3.3	4.0
α-TP	0.99692	2.1	4.5

(1) 0.1, 0.5, 1, 5, 10, 25, 50 µg/mL standard solution, 1 inj/level (MS)
(2) 6 consecutive injections of 0.5 µg/mL
(3) 6 consecutive injections of 25 µg/mL

Not all tocopherols were separated in LC mode (co-elution of β-TP and γ-TP). Complete resolution of the tocols was obtained only in SFC/MS mode (Fig 5), resulting in a method which is capable to characterize all the individual tocols in different vegetable oils (deep frying oil, sunflower, rapeseed and tocomix, Fig. 6). β- and γ- tocols are most challenging to separate, because they have three methyl groups in their ring structure and similar molecular mass. APCI mass to charge ratios (*m/z*) of $[M-H]^+$ ions are 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. Linearity was good with R^2 values of 0.99 from 0.1-50 ppm. Overall, the LODs of LC/MS mode and SFC/MS mode were in the same order of magnitude. It is important to highlight that tocopherols and tocotrienols could only be completely resolved in the SFC/MS mode.

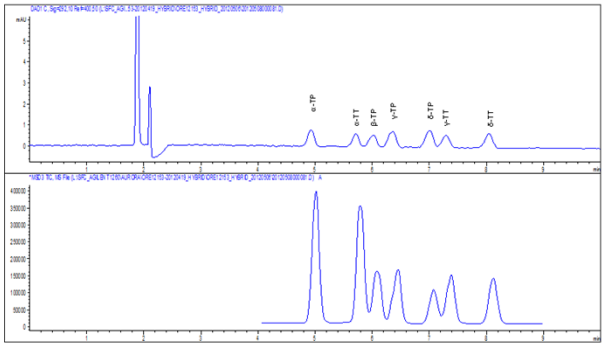

Figure 5: Analyses tocopherols and tocotrienols mixture by SFC with UV and MSD (10 µg/mL)

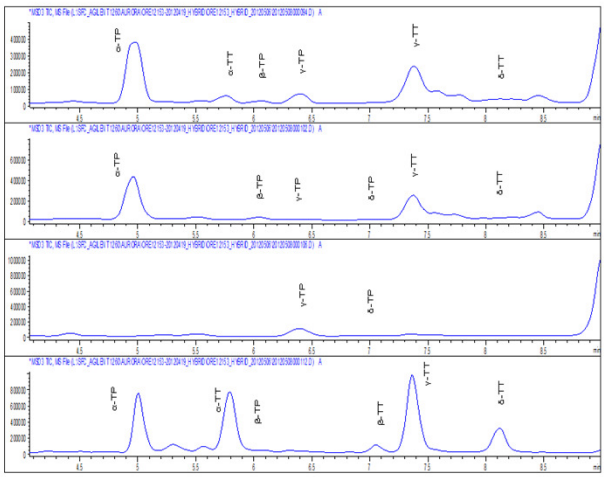

Figure 6: Deep frying oil, sunflower oil, rapeseed oil (100mg/mL) and tocomix by SFC mode.

Conclusions

The Agilent 1260 Infinity Hybrid SFC/UHPLC system provides a tool for complementary data from both SFC and UHPLC on a single instrument. Vegetable oil samples and spiked oil samples were extracted and the recoveries of the antioxidants were calculated. Phenolic antioxidants were analyzed by UHPLC. Using this mode, not all tocopherols were separated. Complete resolution of these compounds was achieved when performing SFC/MS mode. Good sensitivity and high robustness are obtained, allowing this configuration to be recommended for qualitative and quantitative vegetable oil analyses.