Ultrafast analysis of synthetic antioxidants in vegetable oils using the Agilent 1290 Infinity LC system

Application Note

Food

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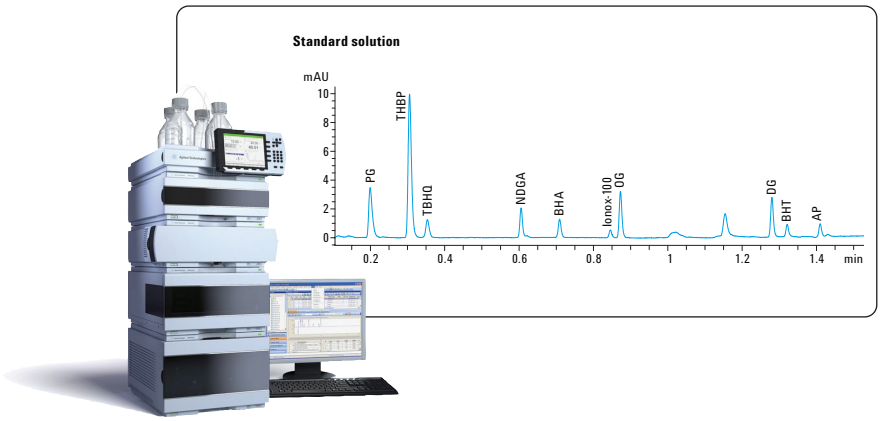

Abstract

The addition of synthetic antioxidants in edible vegetable oils is regulated in Europe and the US. The official method was translated into an ultrafast LC method using the Agilent 1290 Infinity LC equipped with an Agilent ZORBAX Rapid Resolution High Definition (RRHD) column. High throughput is obtained in 2 min with a backpressure of 1120 bar, which is below the 1200 bar upper limit of the column. Optimization of the mobile phase composition and the temperature are discussed. The figures of merit are illustrated using standard solutions and spiked vegetable oil (sunflower, rapeseed, and olive) extracts. Limits of detection are 1 mg/kg or less in the oil samples. Using a simple methanol extraction, good recovery was obtained for all antioxidants in the oil samples.

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Introduction

Lipid oxidation causes rancidity and odor problems and decreases the nutritional value of food products. Synthetic ascorbyl palmitate and phenolic antioxidants are often added to foods to prevent oxidation of unsaturated fatty acids in oils and fats. Combinations of antioxidants are commonly used to enhance the antioxidative effect. The structures and abbreviations of the investigated antioxidants are shown in Figure 1.

Regulatory agencies in Europe[1] and the US[2] have imposed maximum levels for some antioxidants while the use of others has been forbidden. The determination of antioxidants in foods and food components is therefore an important analysis. The limits are given in Table 1.

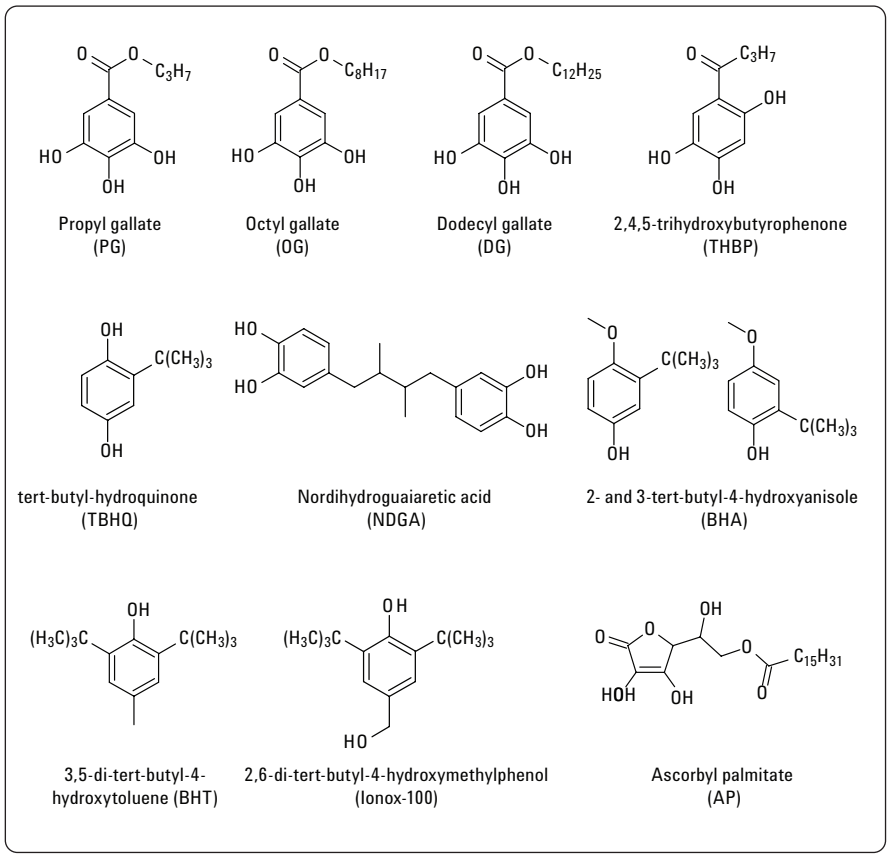

Figure 1
Structures and codes of the investigated antioxidants.

Antioxidant	Europe[1]	US[2]
AP	Quantum satis	No restriction
PG OG DG BHA	≤ 200 mg/kg, individual or combined	≤ 200 mg/kg, individual or combined
BHT	≤ 100 mg/kg	
TBHQ	Not allowed	
THBP	Not allowed	Not allowed
NDGA	Not allowed	Not allowed
Ionox-100	Not allowed	Not allowed

Table 1
Limits for antioxidants in edible oils in Europe and US.

In the official method for the determination of the antioxidants in edible oils, columns of 15 to 25 cm in length with an internal diameter of 4.6 mm, and packed with 5-µm octadecyl silica particles are used.[3] The mobile phase is composed of diluted acetic or phosphoric acid (eluent A) and methanol/acetonitrile 50/50 volume to volume (eluent B). Analysis times are between 15 to 25 min.

There are two reasons for increasing the speed of analysis for this application. First, instability of some of the targets (for example, AP) have been reported and long residence times of samples in an autosampler can already lead to significant degradation of the compounds. Perrin and Meyer could enhance the stability of sample and standard solutions by using citric and isoascorbic acid.[4] They were able to stabilize AP at room temperature for about 7 h. However, QC laboratories in edible oil and fat processing industries have a need for increased analysis speed. The presence or absence, and assay of antioxidants have to be carried out prior to loading or unloading oils and fats. A fast, accurate, and precise result is desirable for economical and practical reasons.

This Application Note describes the analysis of 10 antioxidants in vegetable oils using the Agilent 1290 Infinity LC. The original method was translated into a high throughput method by optimizing the mobile phase composition and the temperature. The figures of merit are presented for vegetable oil and spiked oil extracts.

Experimental

Instrumentation and method

An Agilent 1290 Infinity LC system with the configuration in Table 2 was used:

Solutions and samples

Sample and standard solutions were prepared according to Perrin and Meyer.[4] The solvent for the standards and extraction is a solution of citric acid (1 mg/mL) and isoascorbic acid (1 mg/mL) in methanol. For the spiked samples, a stock solution of the antioxidants in the solvent was added prior to extraction. The extraction was carried out by weighing 1 g of oil and adding 10 mL of the solvent. This mixture was vortexed for 30 s, allowed to stand for 2 min, and vortexed once more for 30 s. The sample was then centrifuged at 5000 x g for 5 min and the supernatant was transferred into an autosampler vial for injection.

Part number	Description
G4220A	Agilent 1290 Infinity Binary Pump with integrated vacuum degasser
G4226A	Agilent 1290 Infinity Autosampler
G1316C	Agilent 1290 Infinity Thermostatted Column Compartment
G4212A	Agilent 1290 Infinity Diode Array Detector

Method parameters:	
Column	ZORBAX RRHD Eclipse Plus C18, 50 mm L × 2.1 mm id, 1.8 µm d_p
Mobile phase	A = 0.02% phosphoric acid in water B = Acetonitrile/methanol 50/50 or 75/25 v/v
Flow rate	Variable
Gradient	Variable
Temperature	Variable
Injection	2 µL
Detection	DAD, 40 or 80 Hz Phenolic antioxidants Signal 280/10 nm, Reference 400/50 nm Ascorbyl palmitate Signal 255/10 nm, Reference 400/50 nm

Table 2
Conditions

Results and Discussion

The analysis was first carried out with the mobile phase used in the official method. The flow rate was set at a moderate 0.4 mL/min. The analysis time was 8 min (see Figure 2A). The synthetic phenolic antioxidants are all detected at 280 nm while for ascorbyl palmitate (AP) 255 nm was used. The eluent B composition was then modified from methanol/acetonitrile 50/50 to 75/25 volume to volume to lower the viscosity and enable a faster separation. The selectivity changed considerably with this mobile phase adaptation and the temperature was optimized to obtain sufficient separation between all target antioxidants. Note that at 45 °C, the elution order of dodecyl gallate (DG) and BHT is reversed compared to Figure 2A at 30 °C. All compounds were stable at 45 °C column temperature.

An additional advantage of the increased temperature is the decrease of the backpressure. When the flow rate was increased to 1.9 mL/min the last peak eluted under 1.5 min and the pressure on the column was 1120 bar (Figure 2C).

	A	B	C
Methanol/Acetonitrile ratio (v/v)	50/50	75/25	75/25
Flow rate	0.4 mL/min	0.4 mL/min	1.9 mL/min
Gradient	0–7.5 min: 35–100% B	0–7.5 min: 30–100% B	0–1.6 min: 30–100% B
Temperature	30 °C	45 °C	45 °C
Detector speed	40 Hz	40 Hz	80 Hz
Maximum pressure	375 bar	270 bar	1120 bar

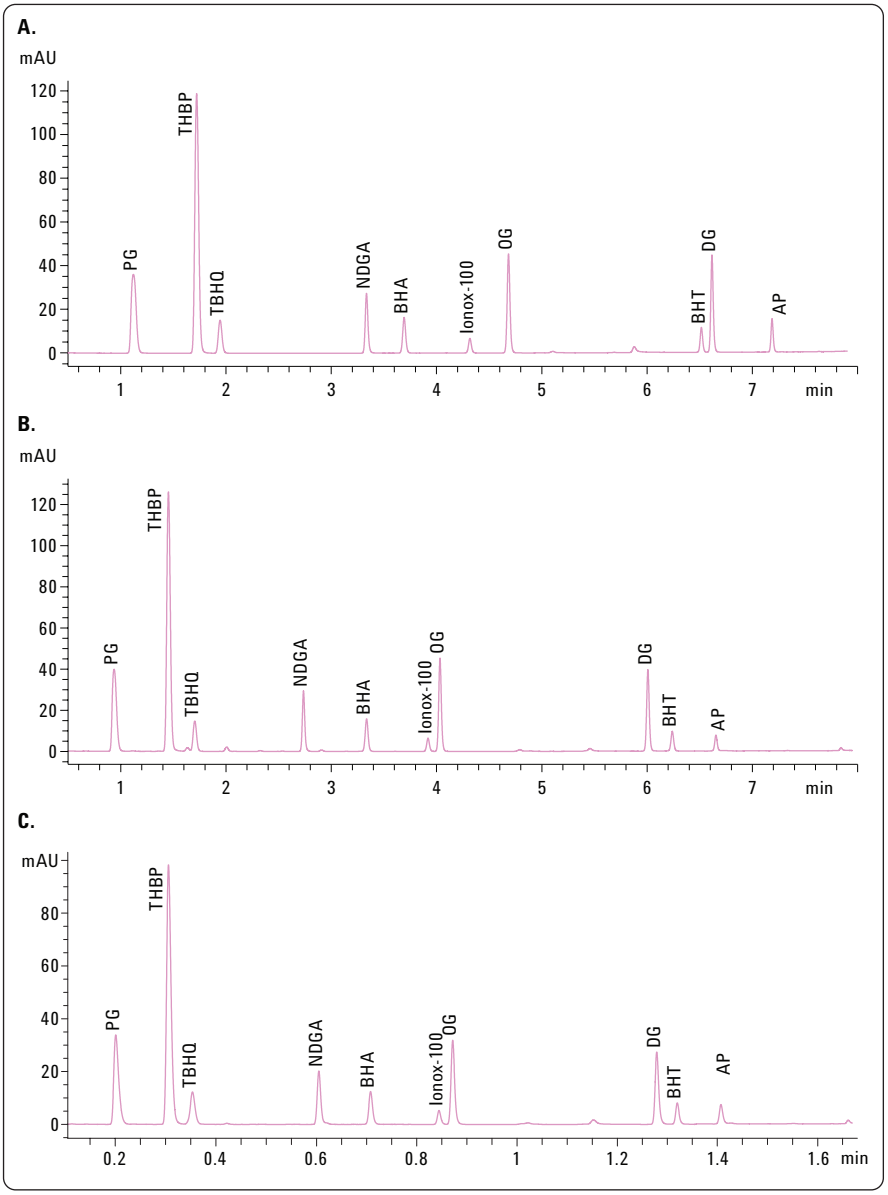

Figure 2
Analysis of 10 µg/mL standard solution under the various conditions.

The performance of the ultrafast method was evaluated and the results are summarized in Table 3. The repeatability and linearity of the method were investigated using standard solutions of the antioxidants. 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 oils and spiked oils were analyzed to determine the recovery and accuracy. The oil samples were spiked with 10 or 50 mg/kg of each antioxidant and the detected amounts in the extracts were compared to standard solutions at the same concentration.

	Repeatability (% RSD)[1]	Linearity (R^2)[2]	Recovery 10 mg/kg (%)			Recovery 50 mg/kg (%)		
			Sunflower	Rapeseed	Olive	Sunflower	Rapeseed	Olive
PG	0.27	0.99988	100.1	105.3	95.1	100.0	100.9	98.3
THBP	0.27	0.99983	97.3	99.1	105.9	98.6	99.1	99.4
TBHQ	0.99	0.99933	90.7	89.7	81.2	97.4	95.8	95.6
NDGA	0.16	0.99983	109.3	89.7	93.6	102.2	98.0	98.9
BHA	0.33	0.99983	104.8	107.0	102.0	98.5	96.4	94.4
Ionox-100	0.40	0.99974	90.7	93.8	89.7	97.5	97.5	93.3
OG	0.41	0.99985	99.3	101.0	95.9	99.7	100.3	98.8
DG	0.56	0.99985	97.8	100.1	101.9	98.4	98.9	98.7
BHT	0.54	0.99960	81.0	89.5	74.4	81.6	83.8	79.0
AP	0.67	0.99934	92.6	85.7	75.4	89.5	91.2	83.7

(1) 6 consecutive injections of 10 µg/mL standard solution
(2) 0.1, 0.2, 0.5, 1, 10 µg/mL standard solution, 1 injection/level

Table 3
Method performance data.

The chromatograms for the fast analysis of a standard solution and the spiked oil samples are shown in Figure 3. Additional peaks originating from the oil matrix are visible in the chromatograms but only a few interfere with the analysis. Most interfering peaks are present in the olive oil sample, however, the 10 mg/kg spiked oil can still be differentiated from an unspiked sample and the recovery is satisfactory (Table 3).

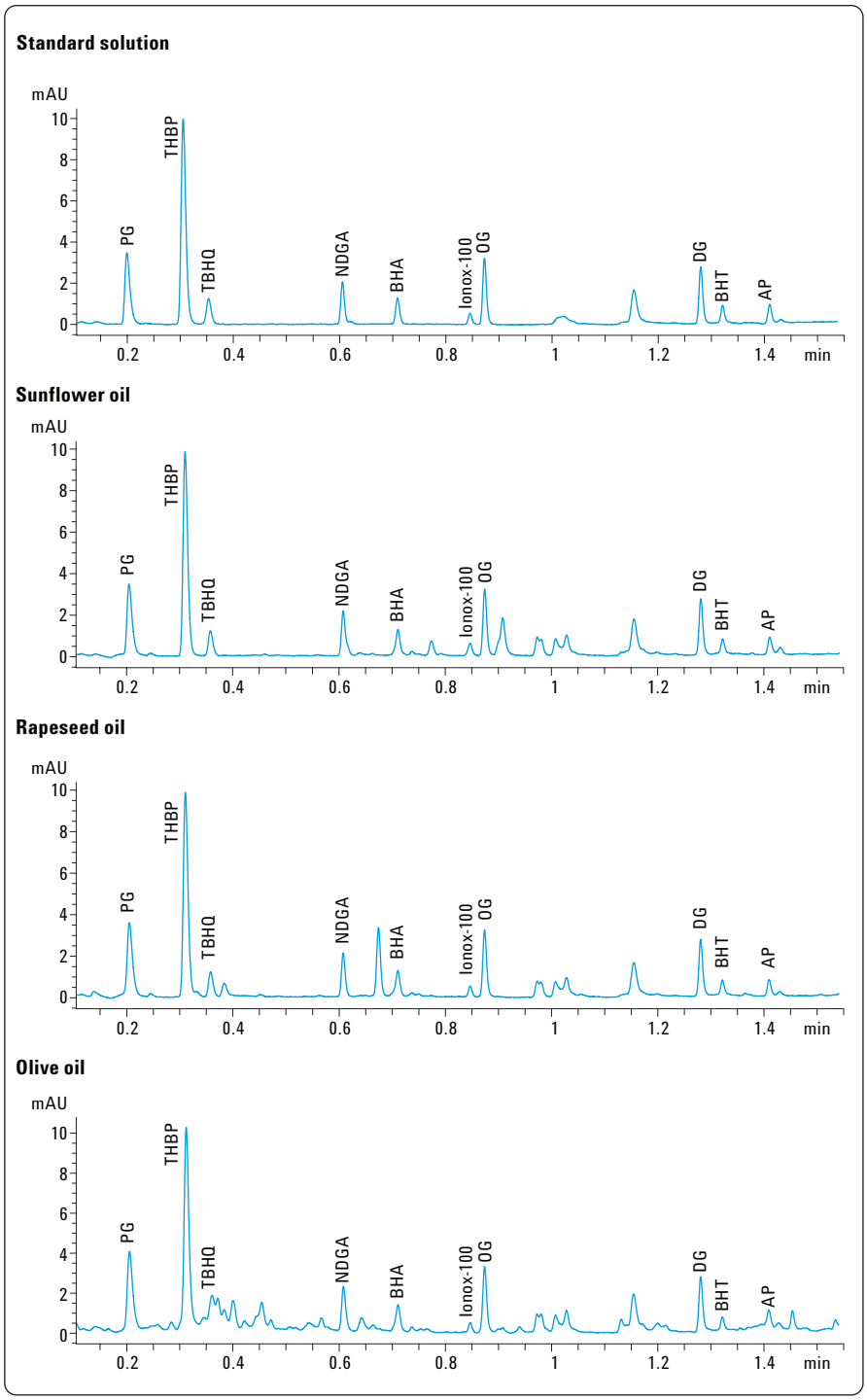

Figure 3
Analysis of standard solution (1 µg/mL) and spiked oil (10 mg/kg) extracts with the fast method.

Conclusion

Using the Agilent 1290 Infinity LC, an ultrafast analytical method could be developed for the determination of antioxidants in vegetable oils. The analysis time could be reduced to less than 2 min with a backpressure of 1120 bar. The performance of the high throughput method (repeatability, linearity, detection limits) was investigated using standard solutions. Oil samples and spiked oil samples were extracted and the recovery of the antioxidants was calculated. Satisfactory recovery was obtained for all antioxidants. The developed method is useful in laboratories where a fast result is mandatory.

References

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