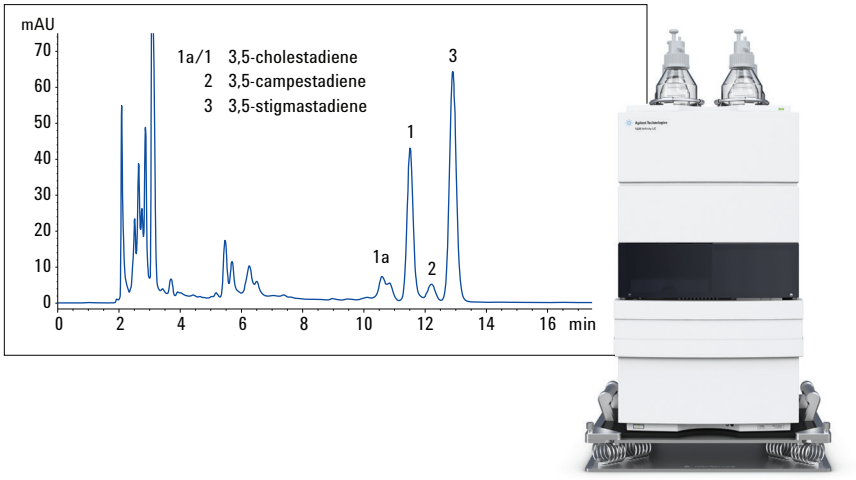
Quality analysis of virgin olive oils – Part 1

Thermal treatment analysis – determination of 3,5-stigmastadienes in olive oil using the Agilent Infinity 1220 LC System with Diode Array Detector

Application Note

Food Analysis & Agriculture



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Abstract

3,5-stigmastadienes were analyzed in seven olive oil samples using the Agilent 1220 Infinity Mobile LC Solution to differentiate virgin from refined or other thermally-treated olive oil. No 3,5-stigmastadienes were detected in all of the tested virgin oils, in contrast to the partly refined olive oil sample, where a significant amount of 3,5-stigmastadienes was found. The analysis showed excellent linearity coupled with low limits of detection and limits of quantification. The analysis time could be shortened down to 5 minutes using a 50-mm, sub-2 µm column.

Due to the robust and rugged 1220 Infinity Mobile LC Solution, it is possible to perform olive oil analysis on-site as a starting point for quality analysis of virgin olive oils.

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Introduction

Virgin olive oil can be created only by mild, cold pressing of the olives (*Olea europea* L.). Thermal or chemical treatment is not allowed in the procedure. After pressing, the virgin olive oils are only purified and filtered. The name, virgin olive oils, is only given to those oils produced by physical techniques such as pressing, filtration, decantation, and centrifugation (Regulation (EG) Nr. 1234/2007, Appendix XVI).

Refining crude vegetable oils removes impurities (for example, pigments, odors, flavoring, or bitter substances), which can have negative effects on the quality of the oils. Refining can improve flavor, stability, aroma, and color. However, during refining processes, many secondary metabolites are also removed or chemically altered.

There are different analytical methods to differentiate virgin from refined or thermally-treated olive oils. In addition to the determination of stigmastadienes and chlorophyll degradation products[1], the analysis of the concentration of polymerized triacylglycerides in olive oil is another important factor[2]. The quality of olive oils (especially regarding nutritive value) can be described in the amount of tocopherols, squalen, and fatty acid composition. Additional thermal treatment analysis and analysis regarding nutritive benefits will be addressed in upcoming Application Notes.

Virgin olive oils, obtained by cold pressing, do not contain measurable amounts of 3,5-stigmastadiene, less than 0.01 mg/kg[3]. Due to high temperatures in the bleaching and deodorizing part of the refining process, 3,5-stigmastadienes are formed by the dehydration of β-sitosterol (Figure 1), which is the most abundant steradiene found in vegetable oils[4].

The amount of stigmastadienes in commercially refined vegetable oils is dependent on the conditions applied during the refining process[4]. The determination of stigmastadienes in olive oils also detects minor amounts of refined oils in virgin olive oils and is, therefore, an important quality characteristic for virgin olive oils. Commercially refined vegetable oils normally contain a steradiene level between 1 and 100 mg/kg. The amount of 3,5-stigmastadiene in refined olive oils ranges between 0.3 and 0.9 mg/kg[4].

There are two major analytical methods for steradiene analysis in vegetable oils. The AOCS Official Method Cd26-96 (1990) and the IUPAC method[4]. Both methods describe sample preparation with saponification of the triacylglycerols and extraction using silica gel solid phase extraction (SPE) with subsequent gas chromatographic separation. However, the gas chromatographic separation runs the risk of interferences with other hydrocarbons. Another method for steradiene analysis is reversed phase HPLC according to EN ISO 15788-3:2004 (D) and Fiebig (1999)[5], which was used in this Application Note.

The 1220 Infinity Mobile LC Solution is a robust and rugged system for on-site measurement. It is resistant against shocks or vibrations during transportation in a mobile van. Due to the UV detection of the stigmastadienes analysis method, the 1220 Infinity Mobile LC Solution can be used in a mobile laboratory as a starting point for olive oil quality analysis before further quality analyses are applied in a stationary lab.

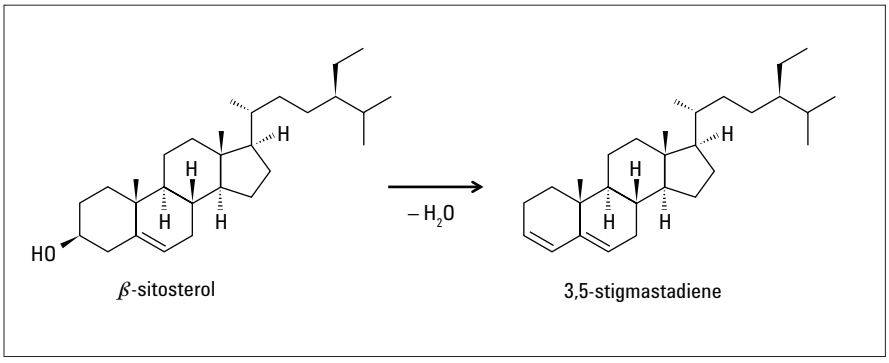

Figure 1
Formation of β-sitosterol to 3,5-stigmastadiene by dehydration.

Experimental

The Agilent 1220 Infinity Gradient LC system with a DAD (G4294B) was equipped with a dual gradient pump with integrated degasser, autosampler, column compartment, and the diode array detector. For transportation, the LC can be mounted on a transportation plate, 1220 Infinity Mobile Upgrade Kit (G4292A).

Sample

The internal standard 3,5-cholestadiene was purchased from Sigma-Aldrich, St. Louis, MO, USA and dissolved in 50% ACN and 50% methyl tert-butyl ether. Several olive oils (virgin and partly refined olive oil) were purchased in local stores. The SPE extraction was carried out using Agilent Bond Elut SI cartridges 5 g, 20 mL (p/n 14256026). Sample preparation was carried out according to EN ISO 15788-3:2004 (D) using the internal standard method.

Solvents

Acetonitrile (ACN), petroleum ether, and methyl tert-butyl ether were LC grade and purchased from Sigma-Aldrich, St. Louis, MO, USA.

Columns

Agilent LiChrospher C18, 4 × 250 mm, 5 µm (p/n 79925OD-584), Agilent ZORBAX Extend-C18 RRHT, 4.6 × 50 mm 1.8 µm (p/n 727975-902)

Software

- OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
- OpenLAB CDS 3D UV Add-On software.

Results and Discussion

The injected 3,5-cholestadien standard (10 µg/mL) showed the following chromatogram, (Figure 2) comprising two peaks for 3,5-cholestadien standard (1a and 1).

Chromatographic conditions

	Long run	Short run
Mobile phase:	ACN/methyl tert-butyl ether (70:30)	
Flow rate:	1 mL/min	
Isocratic run:	Stop time – 30 minutes	Stop time – 5 minutes
Injection volume:	10–50 µL	20 µL
Temperature TCC:	RT	
DAD:	235 nm/4 nm Ref.: off	
Peak width:	>0.05 minutes (1.0 seconds response time) (5 Hz)	

Table 1
Chromatographic conditions.

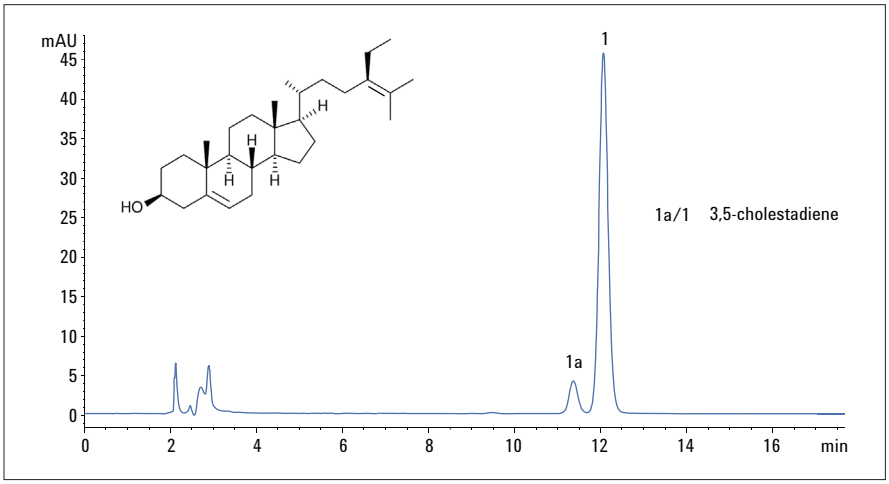

Figure 2
3,5-cholestadien standard solution separated on an Agilent LiChrospher C18, 4 × 250 mm, 5 µm.

Using a 3,5-cholestadien standard as an internal standard, the extracted sample from six different virgin olive oils revealed no additional peaks after the 3,5-cholestadien standard peaks (Figure 3). As expected, no 3,5-stigmastadienes were detected.

In contrast to virgin olive oils, 3,5-stigmastadienes were detected in partly refined olive oil (mix of refined and virgin oils), (Figure 4). The area precision for 3,5-cholestadien and 3,5-stigmastadienes was below 2.5 % for six consecutive runs of the partly refined olive oil sample.

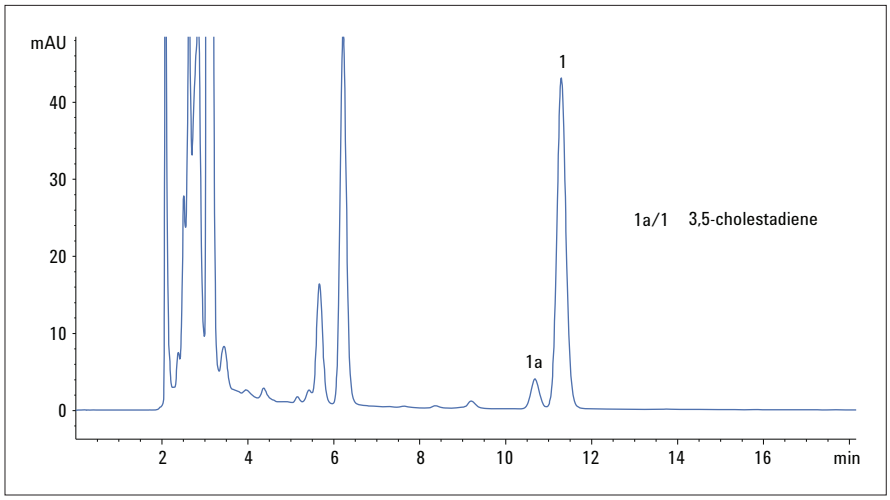

Figure 3
Virgin olive oil using the internal standard method of EN ISO 15788-3:2004 (D). No stigmastadienes were detectable.

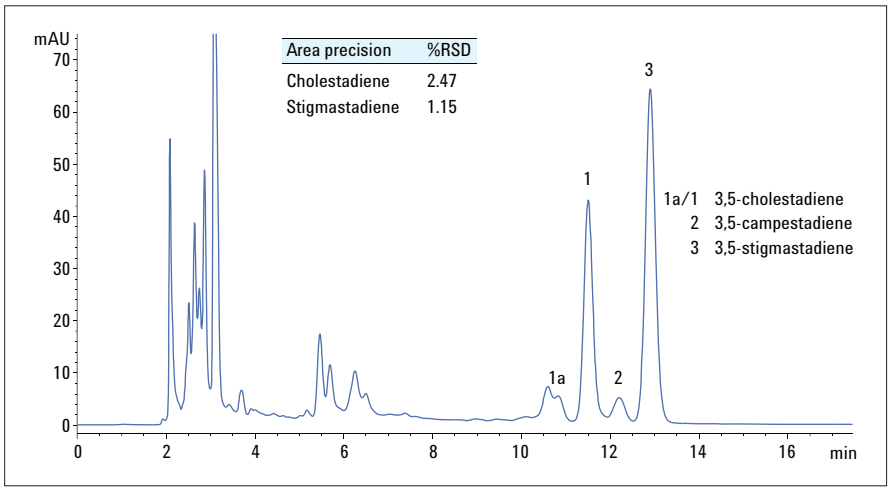

Figure 4
Detection of 3,5-stigmastadienes in partly refined olive oil.

Table 2 shows an overview for six virgin and one partly refined olive oil sample. No 3,5-stigmastadienes were detected in any of the tested virgin olive oils. The partly refined olive oil sample contained 0.63 mg 3,5-stigmastadienes per kg sample. The amount of 3,5-stigmastadienes was calculated using Formula 1 according to EN ISO 15788-3:2004 (D).

Linearity was determined using a dilution series of 3,5-cholestadiene ranging from 0.51 to 10,000 pg on column. Excellent linearity was found with a correlation factor of 0.99998.

In addition, the response factors confirmed the results except for the lowest concentration of 0.51 pg on column (data not shown). The response factors from 1.52 to 10,000 were within the ±5% range, representing excellent linearity.

The LOD and LOQ were evaluated from the concentration of 3,5-cholestadiene required to give at least a signal-to-noise ratio of 3 and 10, respectively. Table 3 displays LOD and LOQ for all for 3,5-cholestadiene, which are in the required range limit in virgin olive oils.

Olive oil	1	2	3	4	5	6	Mix of refined and virgin oils
Content of 3,5-stigmastadiene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.63 mg/kg

Table 2
Content of 3,5-stigmastadiene in different virgin and partly refined olive oil samples (n.d. not detectable).

$$w = \frac{A'_s \times M'}{A'_c \times m'}$$

w = Content of 3,5-stigmastadienes in mg per kg sample
A'_s = Peak area of 3,5-stigmastadienes
M' = Amount of injected internal standard in µg
A'_c = Peak area of 3,5-cholestadiene (both peaks)
m = Sample amount in g

Formula 1
Calculation of 3,5-stigmastadienes in mg per kg sample.

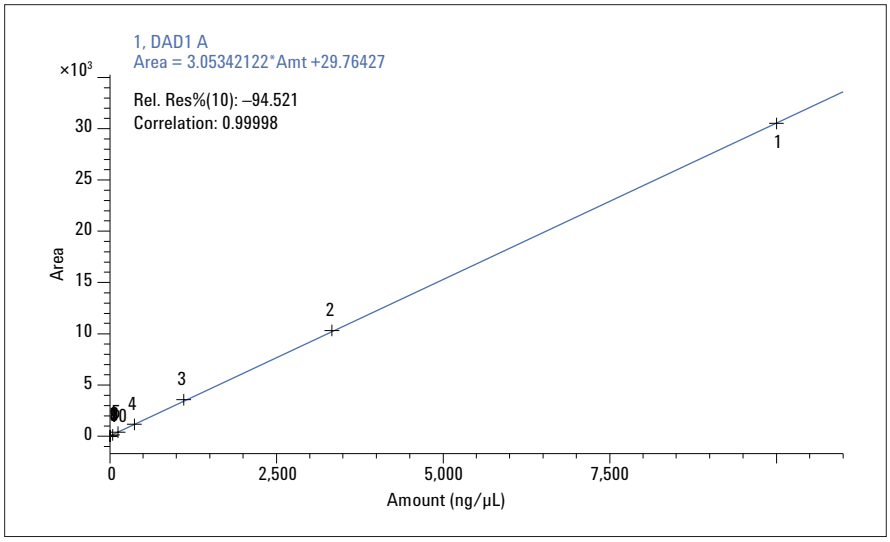

Figure 5
Linearity of 3,5-cholestadiene standard.

LOD	LOQ
226 pg on column	754 pg on column
4.52 µg/kg oil	15.1 µg/kg oil

Table 3
LOD and LOQ of 3,5-cholestadiene.

To accelerate analysis time, the run was shortened to 5 minutes using a 50-mm, sub-2 µm column (Agilent ZORBAX Extend-C18 RRHT, 4.6 × 50 mm 1.8 µm), still obtaining good resolution of the analytes in partly refined olive oil (Figure 6).

Summary and Conclusion

Seven olive oils were analyzed for 3,5-stigmastadiene to determine refining processes or other thermal treatments according to EN ISO 15788-3:2004 (D). As expected, no 3,5-stigmastadienes were detected in any of the tested virgin oils. In contrast, in partly refined olive oil, a sample containing refined and virgin oils, the amount of 3,5-stigmastadienes found was 0.63 mg per kg sample. To determine linearity and LOD/LOQ, the internal standard 3,5-cholestadiene was diluted from 10,000 to 0.51 pg injected amount. Excellent linearity was found from 1.52 pg to 10,000 pg, confirmed by a correlation factor of 0.99998, and response factors in a ±5% range from the average value, enabling highly accurate quantification. LOD and LOQ were found in the low µg/kg oil sample. The analysis time could be shortened to 5 minutes using a 50-mm, sub-2 micron column.

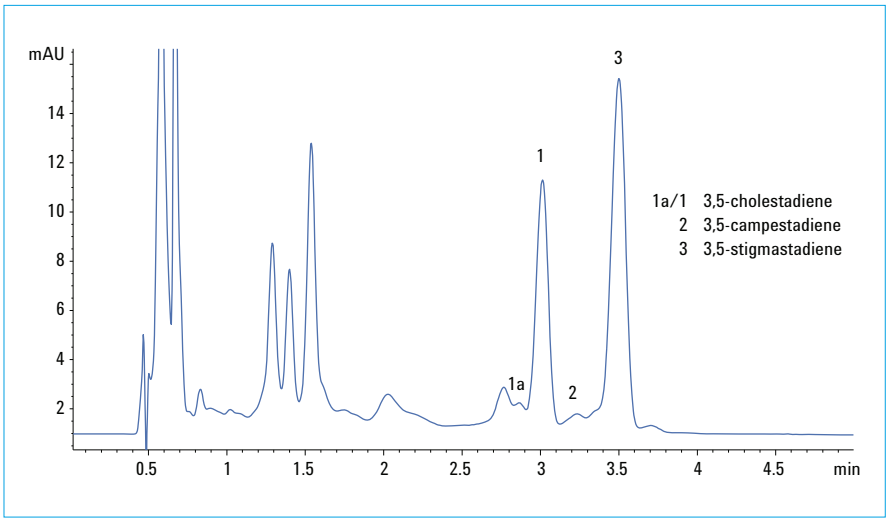

Figure 6
Short analysis of partly refined olive oil using an Agilent ZORBAX Extend-C18 RRHT, 4.6 × 50 mm 1.8 µm.

Using the Agilent 1220 Infinity Mobile LC Solution for the analysis of 3,5-stigmastadienes in olive oils, it is possible to perform olive oil analysis on-site. The 1220 Infinity Mobile LC Solution is a robust and rugged system that can be used in a mobile laboratory. The analysis of 3,5-stigmastadienes can act as a starting point for olive oil quality analysis on-site to differentiate virgin from refined olive oils. Due to the low LOD and LOQ, even small amounts of refined oil can be detected with this application. Based on those measurements, further quality analysis can then be applied in a stationary laboratory.

References

1.
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2.
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