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Applikationsbericht

ACQUITY UPLC Analysis of Seed Oils (Part 2): Olive Oil Quality and Adulteration

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Abstract

This application note describes a 10-minute method for olive oil analysis with Waters ACQUITY UPLC/PDA System using low toxicity solvents, acetonitrile, and 2-propanol as the mobile phase.

Introduction

Market demand for olive oil worldwide has been increasing because of its reported nutritional and health benefits.[1–3] Approximately 2.8 million tons of olive oil are produced annually from Mediterranean basin countries (90% of production), as well as Australia and the United States. Factors such as genetics, climate and agronomics give olive fruits their characteristics. Extraction methods and processing techniques contribute to the quality and purity of olive oil, which ranges from extra-virgin (for direct consumption) to lampante (not fit for consumption).[1–5] European Union regulations provide guidelines for maintaining the Protected Designation of Origin (PDO) of olive oil. According to the International Olive Oil Council (IOOC), "virgin olive oil is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal, that do not lead to alterations in the oil; furthermore, these oils have not undergone any treatment other than washing, decantation, centrifugation, and filtration." Since virgin olive oil commands premium prices, adulteration through blending with lower grade of olive oil or other vegetable oils is an issue. This presents not only commercial problems but also has health implications. It has become increasingly important to analyze olive oil products for purity and to assess origin in order to satisfy legislation and public health concerns.[1,5–7]

Each type of seed oil possesses a unique composition of triglycerides (TAG), which can be used to determine purity and detect adulteration.[5,8] Analytical methods are used to characterize TAG directly (HPLC)[9–13] or indirectly (GC).[5] These methods usually require considerable time and attention from lab personnel and tie up instruments with run times of 30 to 80 minutes for a single analysis. In addition, the HPLC mobile phase typically contains a known carcinogenic, halogenated solvent.

This application note describes a 10-minute method for olive oil analysis with Waters ACQUITY UPLC/PDA System using low toxicity solvents, acetonitrile and 2-propanol as the mobile phase. The UPLC/PDA method allows fast and precise analysis of TAG to authenticate olive oil and can be used to unambiguously identify the adulteration of olive oil even when only 1% of another oil, such as soybean oil, is present. The same method can also be used to analyze oxidized and decomposed TAG, indicative of rancid product.

This sensitive testing method can improve and accelerate the quality control and authentication of olive oil products as well as screen for adulteration to protect public health worldwide.

Experimental

Sample Preparation

Edible oils were bought from local supermarkets and a chemical reagent vendor (SA). They were randomly labeled as brand B, SA, and SS. The olive oil (SS) was mixed with soybean oil and corn oil from 1 to 5w% to simulate adulterated samples. The oils were diluted with 2-propanol to make 2 mg/mL solution for the UPLC analysis.

UPLC System and Operating Conditions

System:	ACQUITY UPLC with PDA
Software:	Waters Empower 2
Detection:	PDA 195 to 300 nm
Sampling rate:	20 pts/s
Filter response:	fast
Weak wash:	2-propanol (600 µL)
Strong wash:	2-propanol (600 µL)
Seal wash:	90:10 Water: CH_3CN (5 min)
Column temp:	30 °C
Injection:	2 µL (full loop)
Mobile phase A:	CH_3CN (Fisher, Optima)
Mobile phase B:	2-propanol (Fisher, Optima)

Column:	ACQUITY UPLC 1.7 µm BEH C_{18} 2.1x 100 mm
Flow rate:	0.28 mL/min
Linear gradient:	10% to 90%B in 10 minutes

Results and Discussion

The ACQUITY UPLC high-pressure fluidic modules enable the analysis of edible oil with the ACQUITY small particle (1.7 µm) column technology using UV-detector compatible mobile phase, acetonitrile and 2-propanol, to give high resolution, sensitive and fast separations. TAG components of oils can be detected at 210 nm wavelength using a 10 minute linear gradient method. Figure 1 shows PDA extracted chromatograms at 210 nm of three different brands of olive oil revealing distinct differences. Olive oil (SS) is a supermarket brand of extra virgin oil. Olive oil (B) is an Italian brand of extra virgin oil sold in supermarkets at a premium price.

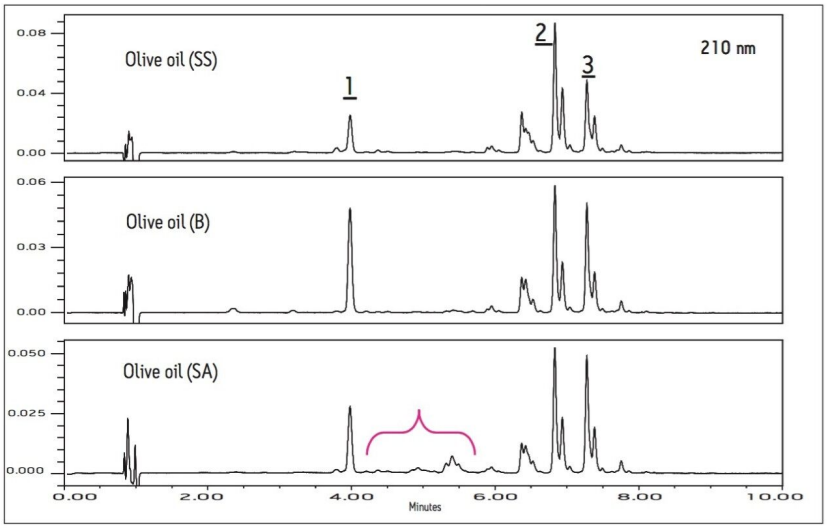

Figure 1. UPLC UV chromatograms (210 nm) of three olive oils (SS, B, SA).

Olive oil (SA) is a chemical standard. We previously reported that the separation of TAG is based on the chain length of fatty acid and the total number of double bonds.[8–10] The differences among the olive oils can

be easily recognized through the relative intensity of the peaks 1, 2, and 3. It is noteworthy that the chromatogram of olive oil (SA) shows many additional peaks with retention times between 4 to 6 minutes.

Edible oils are subject to oxidation and eventually decompose, turning rancid. The oxidized TAG (hydroperoxides) and decomposed TAG (fatty acid with three conjugated double bonds) have UV absorption wavelengths at 240 nm and 280 nm, respectively.

Figure 2 shows the PDA extracted chromatograms at 240 nm of the three olive oils (SS, B, & SA). The chromatogram of olive oil (SA) has numerous peaks with retention times between 4 to 6 minutes. Fewer peaks are apparent in the chromatograms of olive oil (SS) and (B). Figure 3 compares the PDA extracted chromatograms at 280 nm. Several peaks are evident in the chromatograms of olive oil (SS) and (SA) but there are no recognizable peaks in the chromatogram of the premium olive oil (B). The increased peak response at 240 and 280 nm shows that the oil (SA) contains more oxidized and decomposed TAG components, indicating the worst oil quality. These data indicate that UPLC can be used to provide fast and precise analysis of olive oil purity and quality.

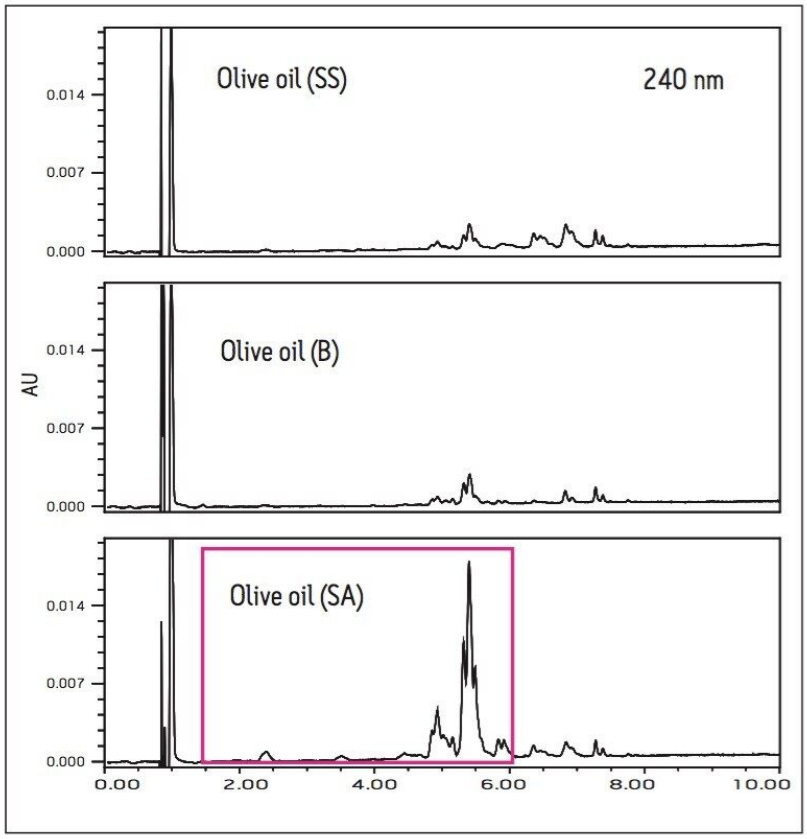

Figure 2. UPLC UV chromatograms (240 nm) of three olive oils (SS, B, SA).

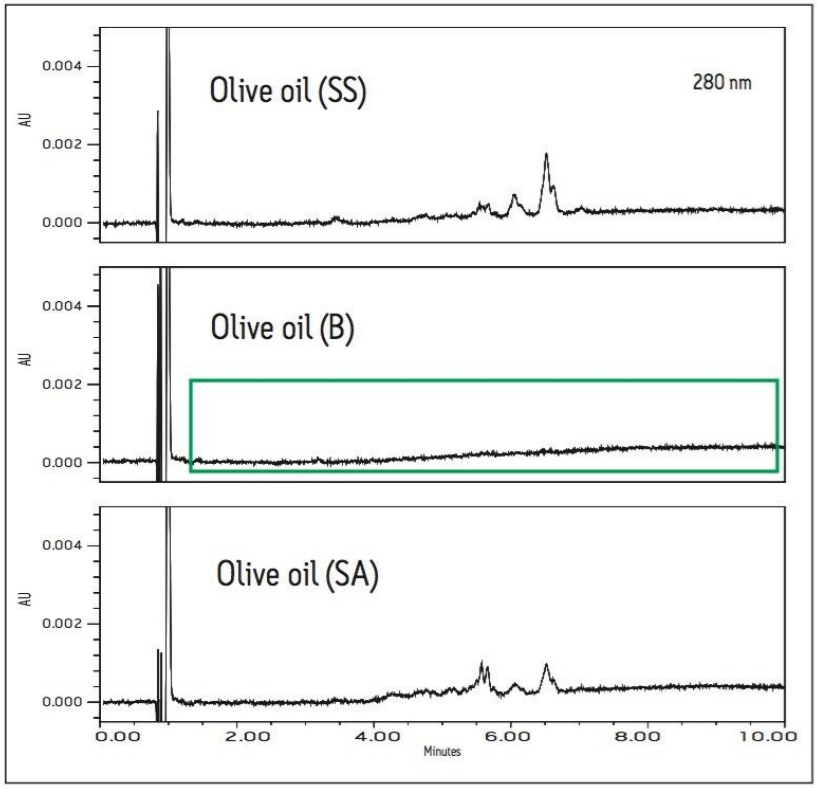

Figure 3. UPLC UV chromatograms (280 nm) of three olive oils (SS, B, SA).

Figures 4 and 5 are UPLC chromatograms of olive oil (SS) adulterated with soybean oil and corn oil from 1 to 5w%. The adulterated samples are easy to recognize by the increased peak intensity in the region between 4 to 6 minutes. The data show that UPLC can unambiguously identify the adulteration of olive oil even when only 1% of this vegetable oil is present. This is 5 times more sensitive than a recently published method based on the indirect analysis of TAG composition for detecting adulteration by GC.[5]

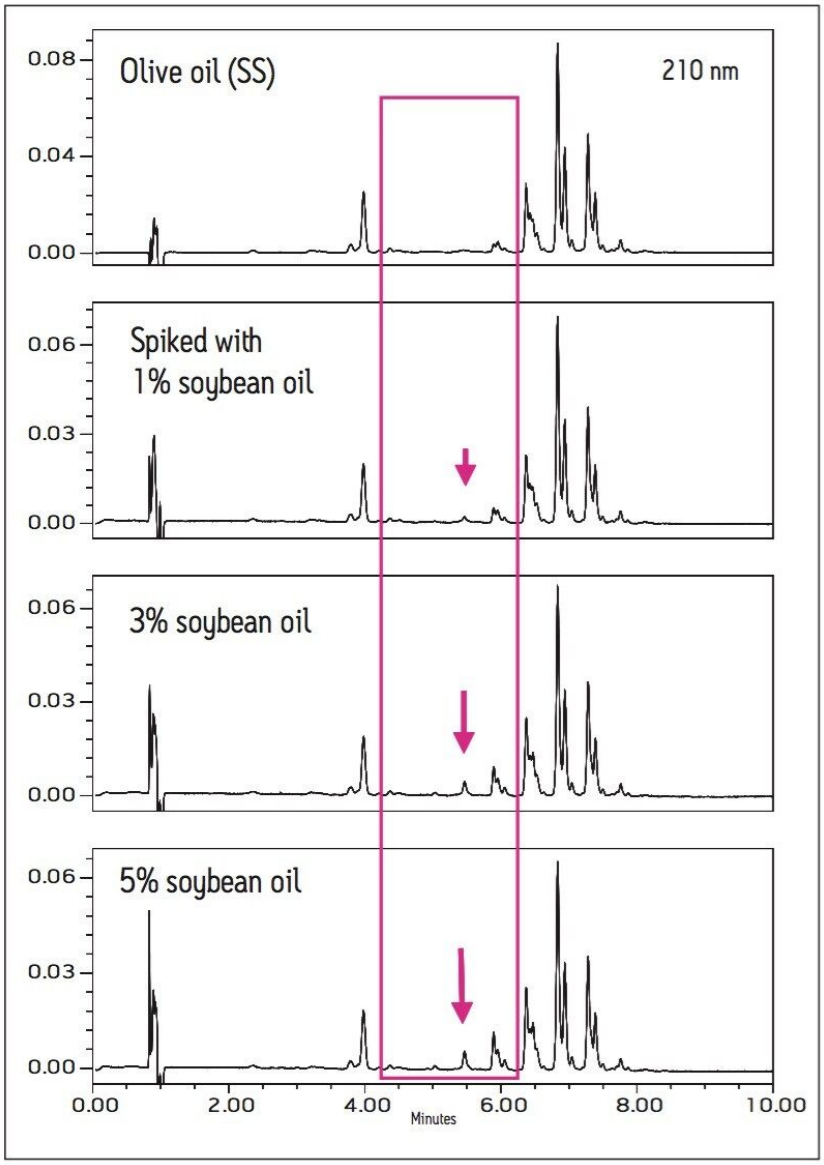

Figure 4. UV chromatograms (210 nm) of olive oil (SS) spiked with soybean oil from 0 to 5%.

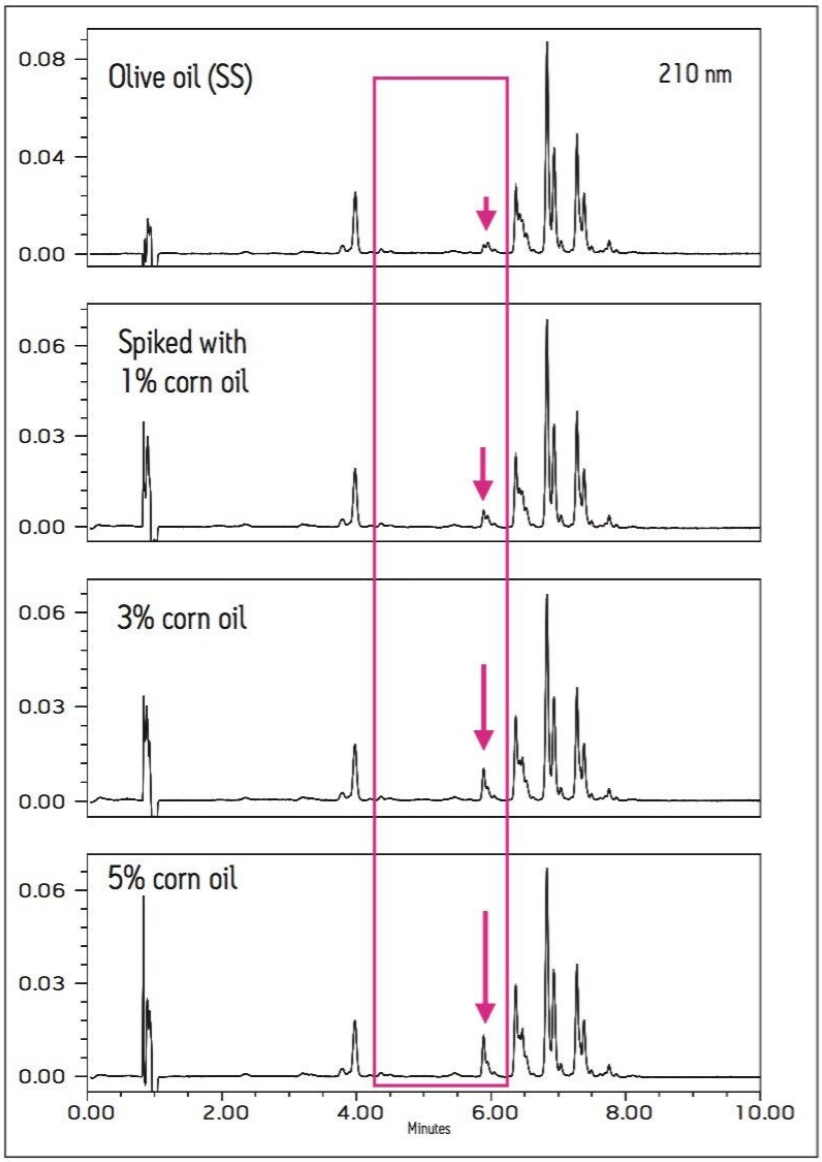

Figure 5. UV chromatograms (210 nm) of olive oil (SS) spiked with corn oil from 0 to 5%.

Conclusion

The Waters ACQUITY UPLC with PDA Detector is an ideal system for the analysis of olive oil. It enables high resolution, sensitive, rapid separations, and provides information rich data for determining origin, quality and purity of olive oil in one experiment. The separation is several times faster than conventional HPLC methods

and does not use toxic halogenated solvents. The mobile phase used in the current experiments is highly compatible with mass spectrometry detectors, if needed to obtain additional structural details.

Use of the UPLC system allows for a decrease in solvent consumption for mobile phases and in hazard waste disposal, resulting in cost and safety benefits. Olive oil producers can benefit from this UPLC methodology by certifying the authenticity and quality standards of their unique products with greater ease and confidence. Other industries with an interest in purity of seed oil products, such as cosmetic, personal care, other food applications could also benefit from this methodology.[8,14]

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