



## Determination of olive oil purity based on triacylglycerols profiling by UHPLC-CAD and principal component analysis

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### Keywords

Triacylglycerols, charged aerosol  
detector, adulteration, olive oil,  
HPLC

### Goal

The purpose of this project was to develop an adulteration detection method for olive oil based on analysis of changes in triacylglycerol (TAG) patterns using ultra-high-performance-liquid-chromatography (UHPLC)—charged aerosol detector (CAD) followed by principal component analysis (PCA).

### Application benefits

- Quickly and easily detects adulteration of olive oil at levels of 10% or greater
- Decreases cost per sample for purity determination because this one method has the potential to replace both fatty acid and sterol analyses
- Saves time in determining sample purity as there is no sample preparation needed other than dilution before analysis by UHPLC-CAD
- Uses fewer chemicals and solvents than both fatty acid and sterol analyses, providing an environmentally friendly alternative to traditional analyses
- Can be easily implemented for in-house use, thus eliminating the need for companies to send samples out for testing

## Introduction

Virgin and extra virgin olive oils are adulterated with cheaper vegetable oils and lower grade olive oils due to the high demand and price of high-grade olive oil. Not only is it important to authenticate olive oil to prevent fraud, it is also necessary to determine purity for the health and safety of consumers.<sup>1</sup> The traditional methods used for determining olive oil purity require quantification of fatty acid and sterol profiles; however, these methods involve significant time and laboratory resources to complete.<sup>2</sup> Consequently, companies send samples to professional labs for testing rather than doing so in-house, which makes testing even more expensive.

This study, an implementation of the method described by Green et al.,<sup>3</sup> uses the measurement of TAG profiles by UHPLC-CAD to determine olive oil adulteration. TAGs are used because they are the main constituent in edible oils and thus require less sample preparation and extraction for analysis. TAG analysis alone has traditionally not been used for olive oil purity determination because these compounds can be difficult to separate and analyze.<sup>4</sup> The UHPLC-CAD system is highly suitable for TAG analysis and offers several benefits over other instruments used, which include HPLC with refractive index (RI), ultraviolet absorbance (UV), and mass spectrometry (MS) detection. Recent studies demonstrate that the CAD is sensitive, has a wide dynamic range, is gradient compatible, and is simple to operate.<sup>5-8</sup> Furthermore, analytes are not required to form gas phase ions, as with MS, or have a chromophore, as with UV, for detection.

This study uses a rapid UHPLC-CAD method to measure the TAG composition of pure extra virgin olive oils, potential adulterant oils such as grapeseed, high oleic safflower, high oleic sunflower, and soybean oils, and

blends of olive oil with these adulterants. PCA, is then used to determine both the type of adulterant and the percentage of adulteration present. The details of the PCA approach are described by Abdi and Williams.<sup>9</sup> In the context of this work, PCA was used to identify pure olive from blends and other oils, based solely on their TAG profiles.<sup>6,10</sup> Compared to traditional approaches, the savings in time and cost of the UHPLC-CAD method for detecting olive oil purity will be of great benefit to the industry.

## Experimental

### Chemicals and reagents

- Acetonitrile, Fisher Scientific™ Optima™ LC/MS grade (P/N A955)
- Methanol, Fisher Scientific™ Optima™ LC/MS grade (P/N A456)
- Isopropanol, Fisher Scientific™ Optima™ LC/MS grade (P/N A461)
- Chloroform, Fisher Scientific HPLC grade (P/N C/4966)

### Consumables

- Thermo Scientific™ 9 mm Amber Glass Screw Thread Vials (P/N 60180-561)
- 9 mm Open Top Short Screw Cap, 6 mm hole (P/N 60180-729)

TAG standards were purchased from two vendors. 1,2-linolein-3-stearin (LLS), 1,2-linolein-3-olein (OLL), 1,2-olein-3-stearin (OOS), 1-palmitin-2-olein-3-linolein (POL), 1,2-palmitin-3-linolein (PPL), 1-palmitin-2-stearin-3-olein (PSO) were purchased from Larodan (Solna, Sweden). Trilinolein (LLL) and triolein (OOO) were obtained from MilliporeSigma.

## Sample preparation

Each oil sample used in this study was prepared for analysis by diluting 100  $\mu\text{L}$  of oil with methanol/chloroform (50:50, v/v) to a final concentration of 1% (by volume). Samples were capped immediately after dilution, then vortexed for 15 seconds. Samples were transferred to amber autosampler vials with screw caps for analysis.

**Table 1. Separation conditions for UHPLC-CAD**

Column:	Thermo Scientific™ Accucore™ C18, 2.6 $\mu\text{m}$ , 100 mm x 2.1 mm (P/N 17126-102130)	
Column temperature:	50 °C, still air mode	
Autosampler temperature:	25 °C	
Flow rate:	0.5 mL/min	
Injection volume:	1 $\mu\text{L}$	
Mobile phase A:	Acetonitrile	
Mobile phase B:	Isopropanol	
Gradient:	<i>Time (min)</i>	<i>Mobile Phase B (%)</i>
	0	10
	2	10
	25	40
	30	60
	35	90
	40	50
	45	10
CAD evaporation temperature:	50 °C	
CAD power function value:	1.00	
CAD data collection rate:	10 Hz	

## Instrumentation

Thermo Scientific™ Vanquish™ Flex UHPLC system consisting of:

- System Base Vanquish Flex (P/N VF-S01-A-02)
- Quaternary Pump F (P/N VF-P20-A)
- Split Sampler FT (P/N VF-A10-A-02)
- Column Compartment H (P/N VH-C10-A-02)
- Vanquish Charged Aerosol Detector F (P/N VF-D20-A)

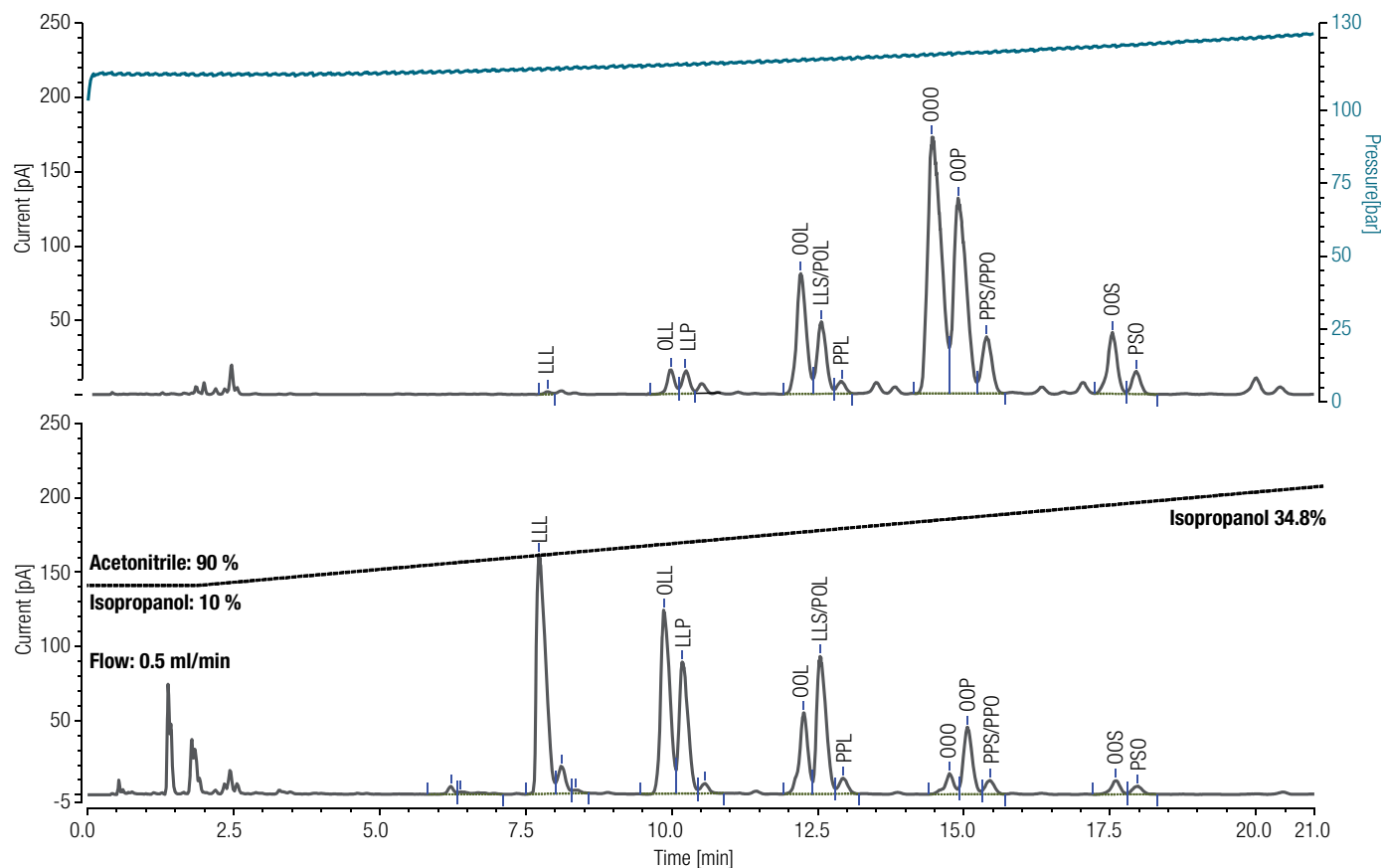
## Analytical conditions

### Data analysis

The Thermo Scientific™ Chromeleon™ 7.2.6 Chromatography Data System was used to determine peak area ratios used in PCA analysis. The percent of each TAG in the sample was calculated from a single injection by comparing each TAG peak area to the peak area total for all TAGs in the sample. The TAGs were used to create PCA plots in OriginLab Corporation software version OriginPro® 2016 Sr2. OriginLab used a correlation matrix to create the PCA and calculated confidence ellipses and principal components for each PCA. Only the first two principal components were used for this study. The TAGs used in each PCA were optimized. This is discussed further in the results.

## Results and discussion

The UHPLC-CAD system was used to detect TAGs in 25 fresh extra virgin olive oils and five other oils that are potential olive oil adulterants: grapeseed, soybean, canola, high-oleic sunflower, and high-oleic safflower oils. Figure 1 shows two chromatograms of pure extra virgin olive oil and grapeseed oil using this method. Variability of TAG peak areas and retention times were assessed using two TAGs, one typically found in low concentrations in olive oil (LLL) and the other one of the most abundant olive oil TAGs (OOO) (Table 2).



**Figure 1. Representative chromatogram showing the TAGs LLL, OLL, LLP, OOL, LLS/POL, PPL, OOO, OOP, PPS/PPO, OOS, and PSO in an Arbosana extra virgin olive oil (top) and a grapeseed oil (bottom).** Overlaid over the top chromatogram is the pressure data (in blue), and overlaid over the bottom chromatogram is the gradient (dotted black line).

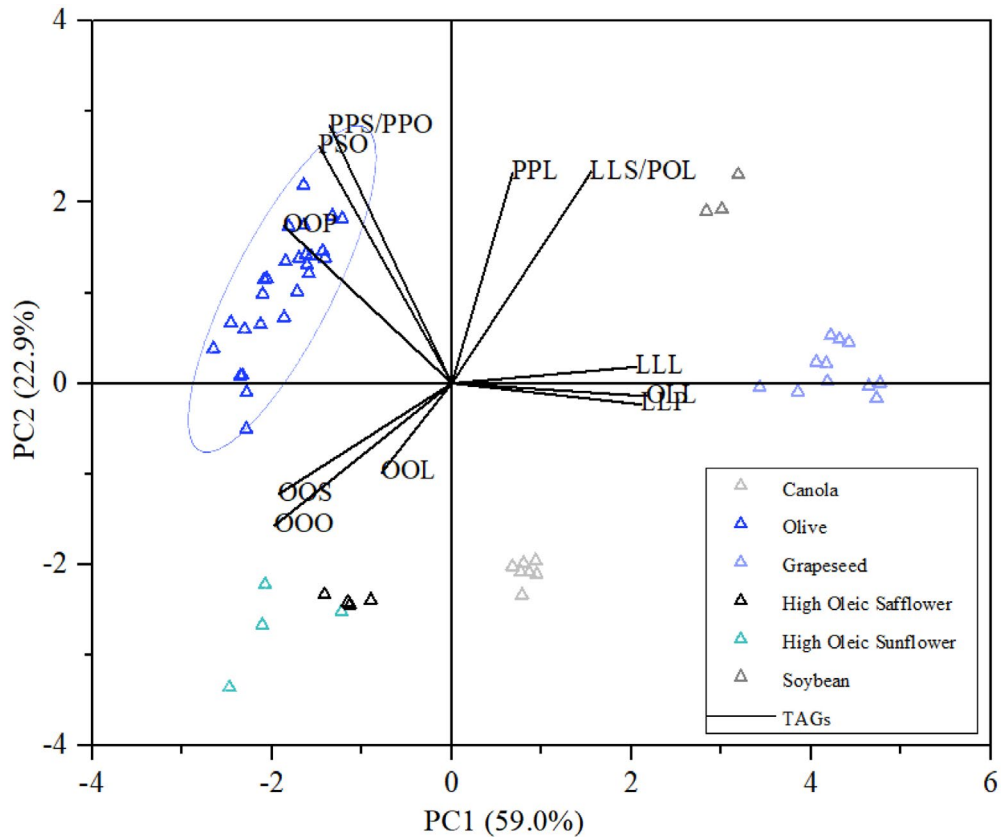
**Table 2. Variability seen in the UHPLC-CAD within the same day and different days for two selected TAGs in olive oil, LLL, and OOO.** Percent relative standard deviations (% RSD) were determined using three injections.

	LLL		OOO	
	Same-day variability	Day-to-day variability	Same-day variability	Day-to-day variability
Retention time (% RSD)	0.139	0.698	0.177	0.599
Peak area (% RSD)	0.600	2.324	0.258	5.062

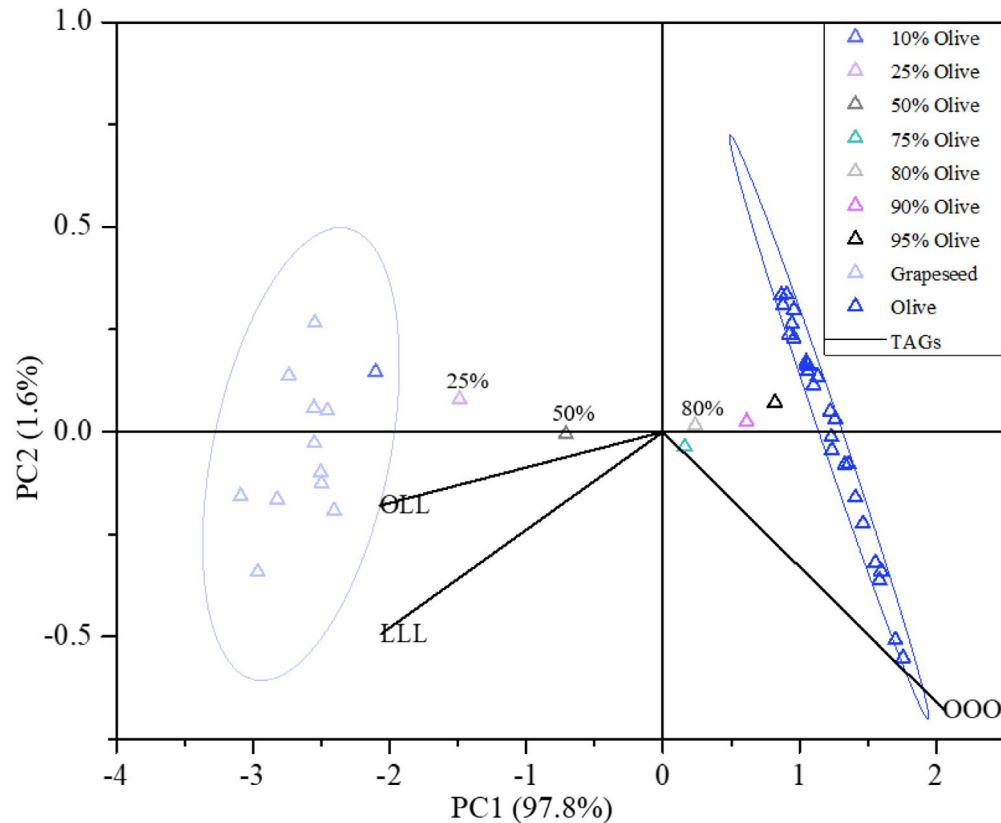
PCA biplots were created using the relative peak area values (percent) for selected TAGs as the variables and oils as the treatments. For example, Figure 2 shows that each type of oil formed a unique cluster. Furthermore, the olive oil cluster can be distinctly separated from the other oils with the 95% confidence interval indicating this method's potential for olive oil adulteration detection.

Figure 2 also shows the ability of this method to cluster oils based on their profiles. However, in order to use this method to accurately detect adulteration and, more specifically, determine the percent of adulteration

present, biplots were created to separate olive oil from blends with each adulterant oil type. Blends of olive oil and each adulterant oil in Figure 2 were made ranging from 95% olive oil to 10% olive oil. Then, the dataset from the peak areas was plotted using PCA. Figure 3 shows an example of this plot using the adulterant grapeseed oil and olive oil. Only the three TAGs contributing the most to the variance between olive oil and each adulterant oil were used to maximize separation between the olive oil cluster and the blended oils. TAGs were systematically eliminated based on their contribution to the variance between olive oil and the adulterant oil.



**Figure 2. Biplot with eleven TAGs used as variables and six oils as treatments.** The first two principal components are shown (PC1 and PC2), which account for 81.9% of sample variance. A 95% confidence ellipse is shown around the olive oil cluster.



**Figure 3. Biplot of olive oil, grapeseed oil, and olive/grapeseed oil blends ranging from 95% olive oil to 10% olive oil.** Two 95% confidence ellipses are shown around the olive and grapeseed oil clusters. Variables are three TAGs, OLL, LLL, and OOO, selected to optimize separation between olive oil and the blended samples.



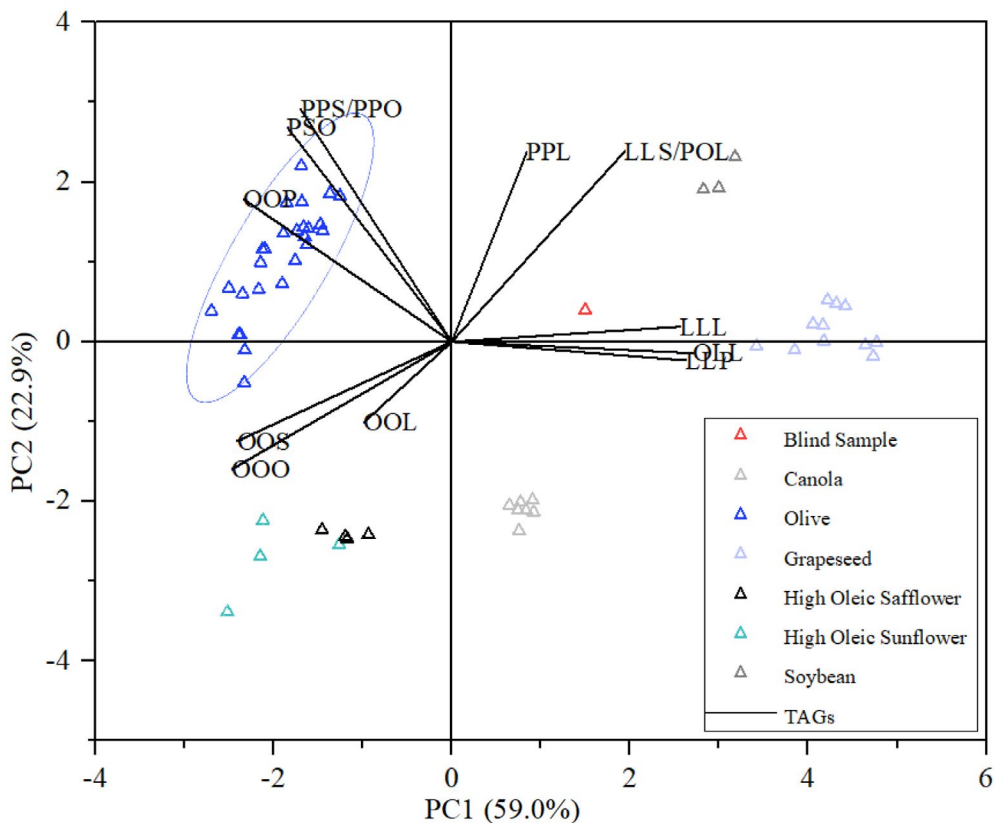
Table 3 lists the three TAGs used for each optimized plot. These optimized plots improve the adulteration detection capabilities of this method. Depending on the adulterant oil, adulteration of olive oil can be detected between approximately 5-10% using this method.

This method is intended to be used in a step-wise fashion. An unknown sample is first plotted on Figure 2 and the location of the sample on this plot can be used to predict the adulterant. Then, the unknown can be plotted on an optimized plot with the predicted adulterant. A

blind sample was tested to demonstrate this process and validate the method. The blind sample was made by a member of a lab not associated with this project by blending a verified olive oil with an unknown percent of an adulterant oil from Figure 1. An unknown sample labeled “Blind Sample” was plotted using the large TAG dataset, shown in Figure 4. The blind sample is located directly between the olive oil cluster and the grapeseed cluster. It is also well correlated with LLL, LLP, and OLL TAGs like the grapeseed oils; therefore, this sample was predicted to be olive oil adulterated with grapeseed oil.

**Table 3. Summary of the TAGs used in each optimized PCA biplot containing olive oil and an adulterant oil**

Adulterant oil	TAGs used to build PCA		
Grapeseed	LLL	OLL	OOO
Soybean	LLL	LLP	OLL
Canola	LLL	OLL	OOL
High oleic safflower	LLL	OLL	OOO
High oleic sunflower	LLL	OOO	OOS

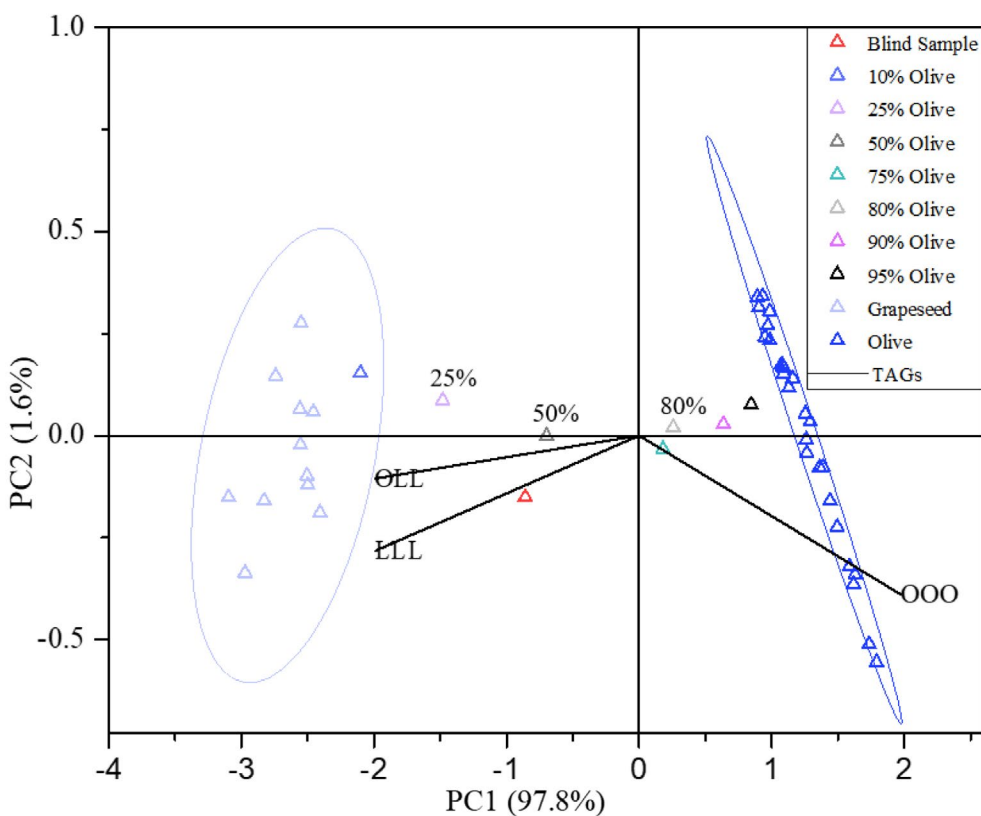


**Figure 4. Biplot of the first two components using six different pure oil types and an unknown sample, “Blind Sample”, as treatments. Variables include eleven TAGs.**

The sample was then plotted on an optimized, three-TAG plot, shown in Figure 5. “Blind #1” is located slightly beyond the 50% olive data point. Thus, it was predicted that this blind sample contained slightly less than 50% olive oil. After the analysis, the blind sample provider confirmed a 45% olive oil content in the adulterated sample and was adulterated with 55% grapeseed oil, making our prediction correct. This method has been validated with a set of 15 samples by Green et al.<sup>3</sup>

### Conclusions

Using UHPLC-CAD and PCA for analysis of TAGs offers a more time-efficient, cost-effective, and less wasteful method for olive oil adulteration detection than traditional methods. The UHPLC-CAD method offers excellent separation of TAGs. PCA analysis of TAG profiles can differentiate olive oil from cheap, lower quality oils and detect their presence in olive oil at levels between 5% and 10%, depending on the adulterant. The Thermo Scientific™ Corona™ Veo™ Charged Aerosol Detector will work in the same way as the CAD described in this study and will produce the same results.



**Figure 5. Biplot containing treatments of olive, the potential adulterant grapeseed oil, and blends of both oils.** A sample of unknown composition, “Blind Sample”, is also shown. Variables are three select TAGs were chosen to optimize separation between the olive oil cluster and the blended samples.

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