Waters™

應用手冊

Automating Calculations for Rapid Seed Oil Quality Control and Authenticity

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This technical note describes a streamlined system solution for seed oil quality control and authentication using UPLC with an Empower™ 2 Software custom field calculation function to automatically determine and report if a seed oil sample passes or fails user-set QC criteria.

Introduction

Seed oils are important components of food, cosmetics, and personal care products. They are mainly extracted from 22 oil crops around the world. Production processing, storage, transportation, and distribution are all critical to the quality of seed oils. Seed oil cross-contamination can occur accidentally or intentionally. Much legislation has been enacted, including 315/93/EEC, 2568/91/EEC, EC 333/2007, and EC 640/2008, that require authentication of seed oils and demand prevention of contamination to support public health and fair trade.[1]

Seed oil companies monitor seed oil production processes, from incoming raw materials, to the finished products in order to ensure product quality, to satisfy legislative concerns, and to protect their brand image, which is their most valuable asset. Currently, seed oil analysis mainly relies on GC and HPLC methods. GC methods require derivatization prior to analysis, which is time-consuming and laborious.[2] Conventional HPLC methods require either using halogenated solvent or non-halogenated solvent with longer runtimes in order to achieve adequate separation.[3–6] The use of halogenated solvents are restricted in most laboratories since they are known carcinogens. As a result, there is a growing demand for better analytical tools for seed oil quality control and authentication.

The ACQUITY UPLC™ System is the next generation of liquid chromatographic platforms. Using UPLC/PDA/ELSD/mass spectrometer detectors, fast screening and high-resolution methods for seed oil characterization have been developed without using halogenated solvents.[7–10] The UPLC System enables the acquisition of multiple types of data in a single injection to generate reproducible fingerprinting data, identify triglyceride components, and evaluate the degree of seed oil oxidation and decomposition. Compared to conventional HPLC, UPLC shortens analysis times, reduces solvent usage, and provides a higher resolution chromatogram with more information in a single injection. As a result, the UPLC method is more cost-effective.

This eliminates the need for manual calculations, prevents potential human errors, and delivers critical information with speed and accuracy. With accurate and timely results in hand, decision makers can deliver manufacturing efficiency and productivity, namely, reduce failed products, avoid product recalls, and minimize liability litigation. A custom field calculation example, with detailed steps, is provided in the Experimental section.

Experimental

Sample Preparation

Edible oils were bought from local grocery stores. They were diluted with 2-propanol to make a 6 mg/mL solution for the analysis.

UPLC Conditions

UPLC system:	ACQUITY UPLC with PDA Detector
Software:	Empower 2

PDA Parameters

Detection:	195 to 300 nm
Sampling rate:	20 pts/s
Filter response:	fast

UPLC Parameters:

Column:	ACQUITY BEH C_{18} 2.1 x 150 mm
Weak wash:	2-propanol (500 µL per wash)
Strong wash:	2-propanol (500 µL per wash)
Seal wash:	10% CH_3CN in H_2O (every 5 min)
Mobile phase A:	CH_3CN
Mobile phase B:	2-propanol
Column temp.:	30 °C
Injection:	2 µL (full loop)

Gradient Method

Time (min)	Flow (mL/min)	%B	Curve
0	0.15	10	—
22	0.15	90	6

Column Condition and Re-equilibration Method

Time (min)	Flow (mL/min)	%B	Curve
0	0.13	100	—
18	0.13	10	11
21.5	0.7	10	11
24.5	0.15	10	11
25	0.15	10	11

Note: A blank injection of 2-propanol was run at the beginning of the sample set and used for PDA 3D blank subtraction.

QC Criteria for Authentication of Extra Virgin Olive Oil A

For demonstration purposes, six peaks were chosen from a representative chromatogram of extra virgin olive oil A. One peak was selected as the marker peak and others were used as indicator peaks. Peak area ratio (indicator peak area divided by the marker peak area) ±3xSTDEV was used as the QC criteria for the indicator peak.

1. 1. Indicator 3O, (peak area OOO/peak area marker):

>0.84 or <0.86 = pass, otherwise = fail

2. Indicator OOL, (peak area OOL/peak area marker):
   >1.18 or <1.21 = pass, otherwise = fail
3. Indicator LLO, (peak area LLO/peak area marker):
   >0.39 or <0.41 = pass, otherwise = fail
4. Indicator LLL, (peak area LLL/peak area marker):
   >0.039 or <0.045 = pass, otherwise = fail
5. Indicator impurity, (peak area impurity peaks/peak area marker)
   <0.42 = pass, otherwise = fail.

Steps to Create a Custom Field for Peak Area Ratio Calculation:11

1. Click Configure System to open the Configuration Manager, click Projects in the tree.
2. Select and right click the working project.
3. Select Properties to open Project Properties window.
4. Click the Custom Fields tab, click New to open Data and Type Selection window (Figure 1).
5. Select Peak in field type and select Real (0.0) in Data Type, then click Next to open the Source Selection window, as shown in Figure 2.
6. Select Calculated in Data Source, select All in Sample Type and Peak Type; select Result Set Only in Search Order, click OK on the pop-up window; leave the check boxes of All or Nothing and Missing Peak un-checked; click Next to open the Formula Entry window, as shown in Figure 3.
7. Type Area/IS[Area] into the Field, click Next to open the Numeric Parameter window (use the default values).
8. Click Next to open the Name Entry window.
9. Enter a name (For example, the name “Ratio_IS” is used here); select Project in Create This Field.
10. Click Finish to create a custom field “Ratio_IS” for calculating peak area ratio, as shown in Figure 4.

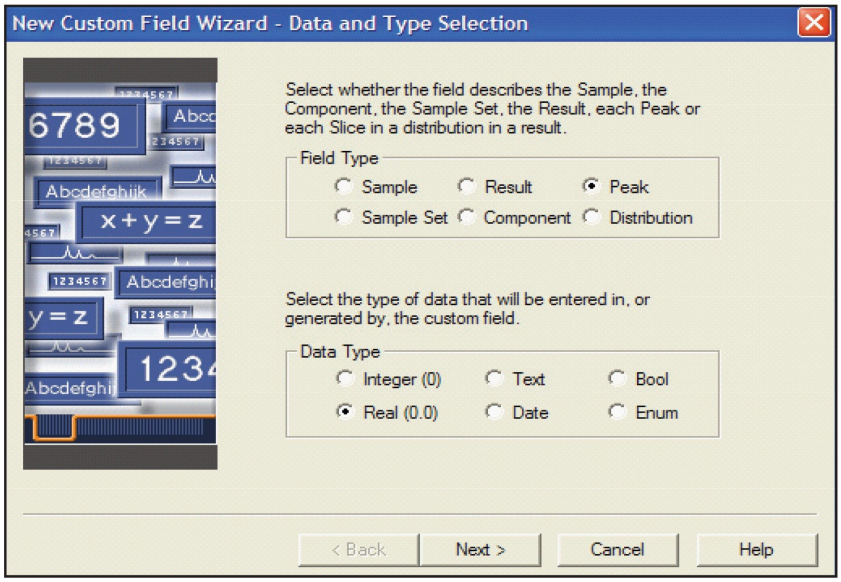


Figure 1. Data and Type Selection window.

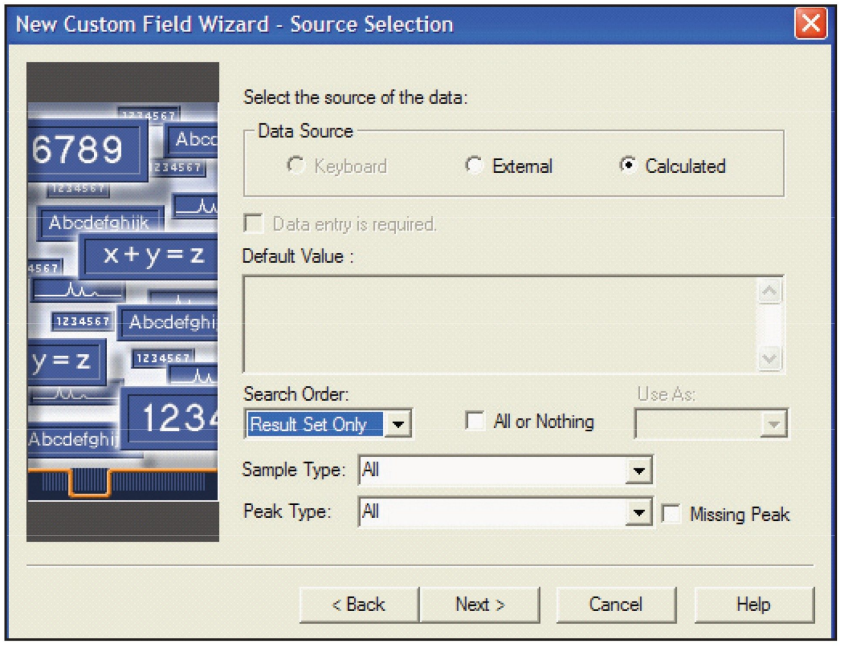


Figure 2. Source Selection window.

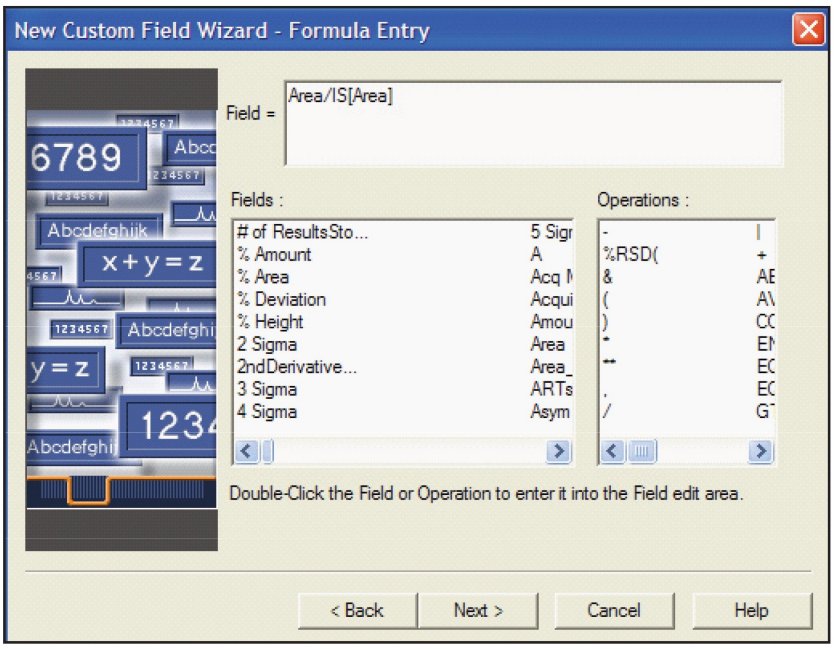


Figure 3. Formula Entry window.

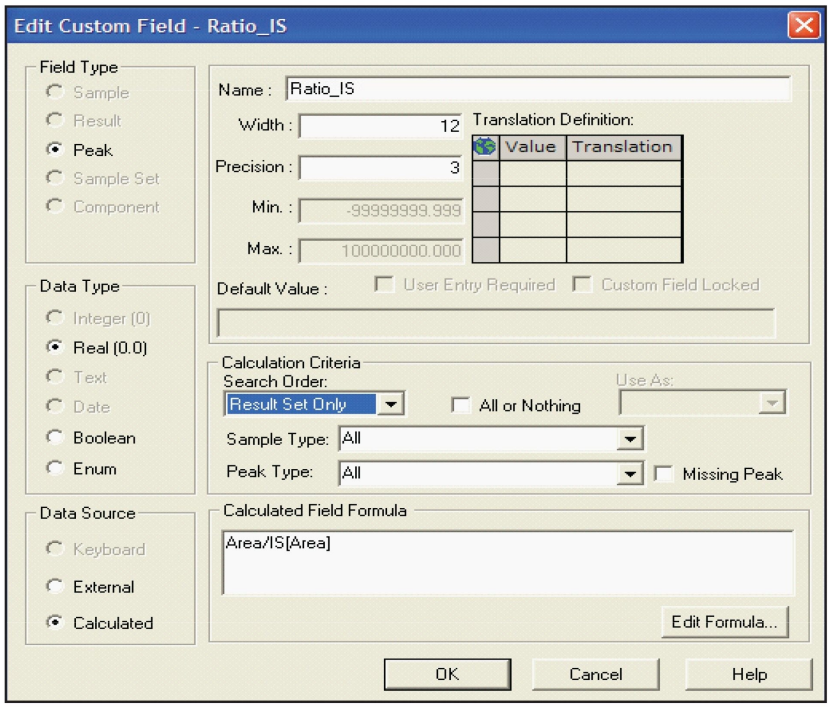


Figure 4. Custom Field "Ratio_IS" Summary window.

Note: Area is defined as the peak area of any peak observed in the chromatogram; IS[Area] is defined as the peak area of the peak named IS.

Steps to Create a Custom Field to Determine Pass or Fail According to the Specific Indicator Peak Area Ratio Criteria:

1. Click Configure System to open the Configuration Manager, click Projects in the tree.
2. Select and right click the working project.
3. Select Properties to open Project Properties window.
4. Click the Custom Fields tab; click New to open Data and Type Selection window, as shown in Figure 1.
5. Select Peak in Field Type and then Bool in Data Type; click Next to open Source Selection window.
6. Select Calculated in Data Source; select All in Sample Type and Peak Type; select Result Set Only in Search Order, click OK on the pop-up window; choose All or Nothing, click Yes on the pop-up window; then click Next

to open Formula Entry window.

7. Type the following equation into Field:
   GTE(3O[Ratio_IS],0.841)&LTE(3O[Ratio_IS],0.859]) *EQ(Name,"3O")+NEQ(Name,"3O")*-1*50000
8. Click Next to open the Translation Definition Table window, as shown in Figure 5.
9. Type Fail next to 0 and Pass next to 1, click Next to open Name Entry window.
10. Type a name (for example, "Oly_OOO" is used here), select Project in Create This Field.
11. Click Finish, a custom field "Oly_OOO" is created to examine if the peak area ratio (OOO peak divided by marker peak) meets the QC criteria for the indicator OOO, as shown in Figure 6.

Repeat Steps 1 to 8 for Other Indicators:

For indicator OOL, in Step 4, type the following equation in the Formula Entry window: GTE(OOL[Ratio_IS],1.18)&LTE(OOL[Ratio_IS],1.21]) *EQ(Name,"OOL")+NEQ(Name,"OOL")*-1*50000. In step 7, type Oly_OOL in the Name field to create a custom field Oly_OOL to examine if the peak area ratio (OOL peak divided by marker peak) meets the QC criteria.

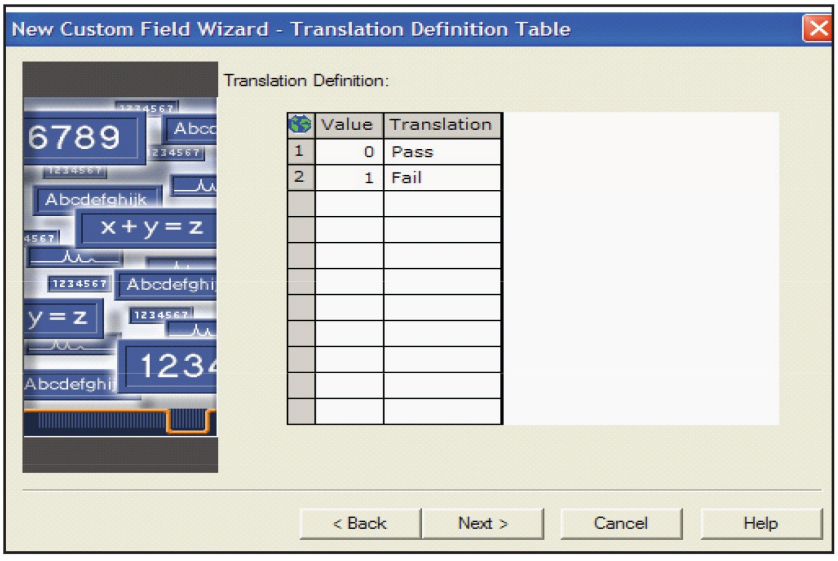


Figure 5. Translation Definition Window.

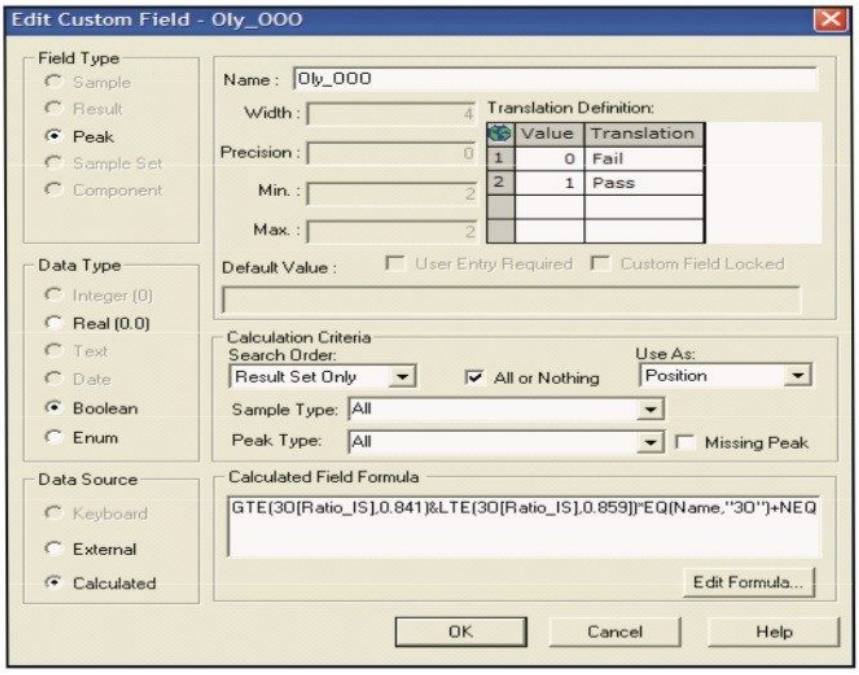


Figure 6. Custom Field "Oly_OOO" Summary window.

For indicator LLO, in Step 4, type the following equation in the Formula Entry window: GTE(LLO[Ratio_IS],0.39)&LTE(LLO[Ratio_IS],0.41])*EQ(Name," LLO")+NEQ(Name,"LLO")*-1*50000. In step 7, type Oly_LLO in the Name field to create a custom field "Oly_LLO" to examine if the peak area ratio (LLO peak divided by marker peak) meets the QC criteria.

For indicator LLL, in Step 4, type the following equation in the Formula Entry window: GTE(LLL[Ratio_IS],0.039)&LTE(LLL[Ratio_IS],0.045])*EQ(Name ,"LLL")+NEQ(Name,"LLL")*-1*50000. In step 7, type Oly_LL in the Name field to create a custom field "Oly_LLL" to examine if the peak area ratio (LLO peak divided by marker peak) meets the QC criteria.

For indicator Impurity, in Step 4, type the following equation in the Formula Entry window: GT(Impurity[Ratio_IS],0.42)*EQ(Name,"Impurity")+NEQ(Name," Impurity")*-1*50000. In step 7, type Oly_Impurity in the Name field to create a custom field "Oly_Impurity" to examine if the peak area ratio (impurity peaks divided by marker peak) meets the QC criteria.

The method to calculate the sum of Impurity peaks using the Timed Groups function:

1. Select Timed Groups tab from the processing method editing window, as shown in Figure 7.

2. Enter Impurity in the Name field, 3 in the Start Time field, and 13.6 in the Stop Time field.

3. Check Exclude Known Peaks field.

Label Selected Marker and Indicator Peaks in the Processing Method

1. Select the Components tab from the Processing Method Editing window.

2. Change the name of the peak having retention time of 9.81 min to IS and enter Marker in the Peak Label field, as shown in Figure 8.

3. Change the name of the peak having retention time of 13.79 min to 3L and enter LLL in the Peak Label field.

4. Change the name of the peak having retention time of 14.85 min to 2LO and enter LLO in the Peak Label field.

5. Change the name of the peak having retention time of 15.87 min to 2OL and enter OOL in the Peak Label field.

6. Change the name of the peak having retention time of 16.85 min to OOO and enter OOO in the Peak Label field.

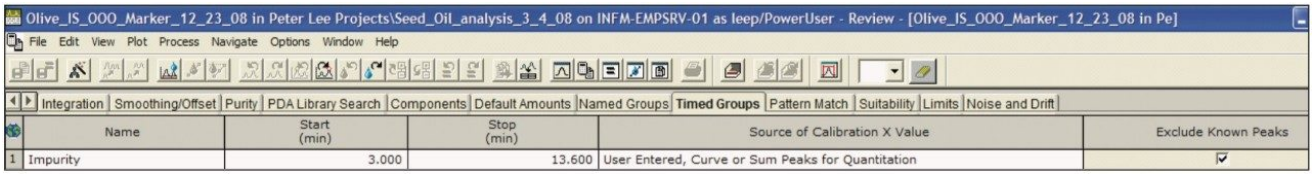
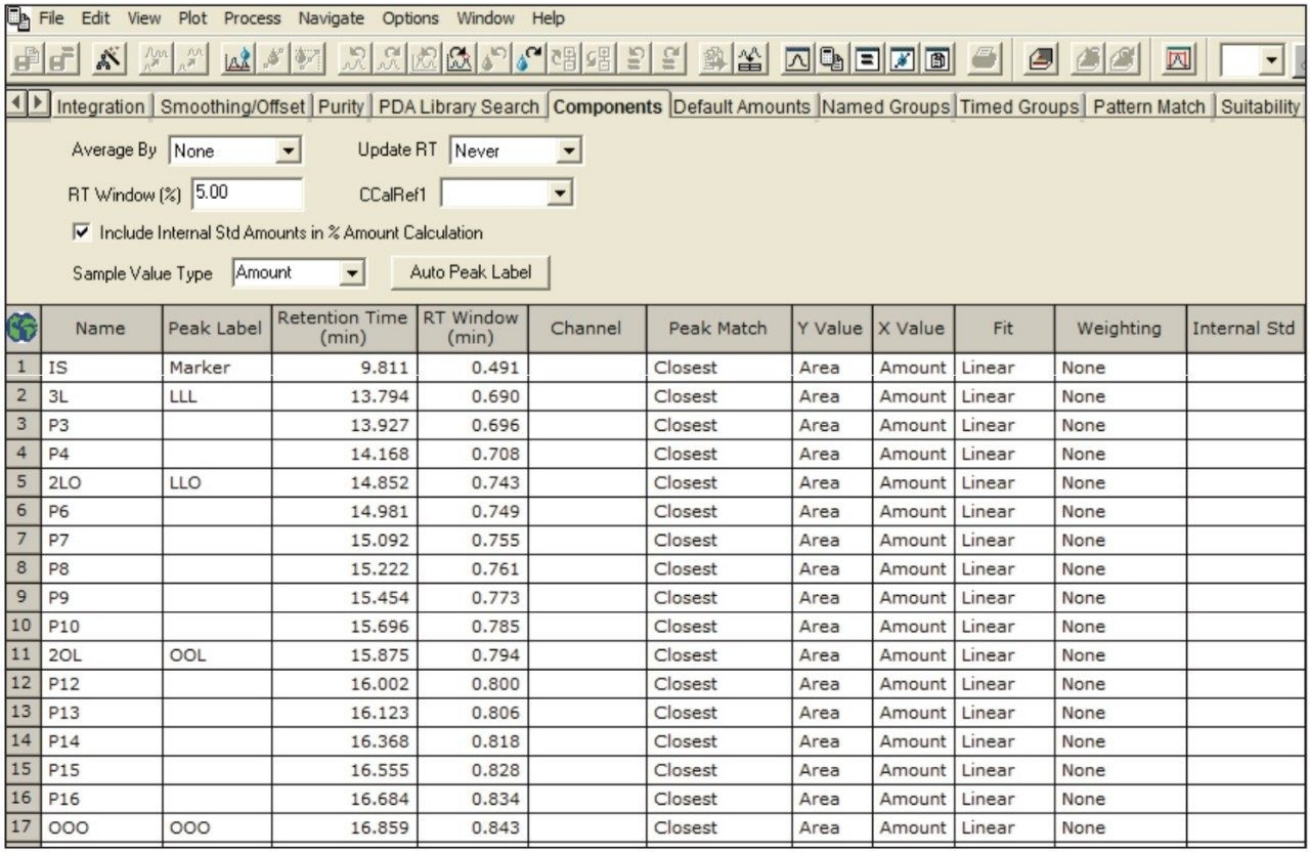


Figure 7. Timed Groups window.



	Name	Peak Label	Retention Time (min)	RT Window (min)	Channel	Peak Match	Y Value	X Value	Fit	Weighting	Internal Std
1	IS	Marker	9.811	0.491		Closest	Area	Amount	Linear	None	
2	3L	LLL	13.794	0.690		Closest	Area	Amount	Linear	None	
3	P3		13.927	0.696		Closest	Area	Amount	Linear	None	
4	P4		14.168	0.708		Closest	Area	Amount	Linear	None	
5	2LO	LLO	14.852	0.743		Closest	Area	Amount	Linear	None	
6	P6		14.981	0.749		Closest	Area	Amount	Linear	None	
7	P7		15.092	0.755		Closest	Area	Amount	Linear	None	
8	P8		15.222	0.761		Closest	Area	Amount	Linear	None	
9	P9		15.454	0.773		Closest	Area	Amount	Linear	None	
10	P10		15.696	0.785		Closest	Area	Amount	Linear	None	
11	2OL	OOL	15.875	0.794		Closest	Area	Amount	Linear	None	
12	P12		16.002	0.800		Closest	Area	Amount	Linear	None	
13	P13		16.123	0.806		Closest	Area	Amount	Linear	None	
14	P14		16.368	0.818		Closest	Area	Amount	Linear	None	
15	P15		16.555	0.828		Closest	Area	Amount	Linear	None	
16	P16		16.684	0.834		Closest	Area	Amount	Linear	None	
17	OOO	OOO	16.859	0.843		Closest	Area	Amount	Linear	None	

Figure 8. Components window.

Note: The marker and indicator peaks can be named and labeled according to user-set criteria.

Steps to Create Named Groups in the Processing Method

1. Select Named Groups tab from the processing method editing window.
2. Type 3O, LLL, LLO, OOL, and Oly in the Name column, as shown in Figure 9.
3. Drag respectively OOO, 3L, 2LO, 2OL, and IS, from Single Peak Components into each corresponding named group tree, as shown in Figure 9.

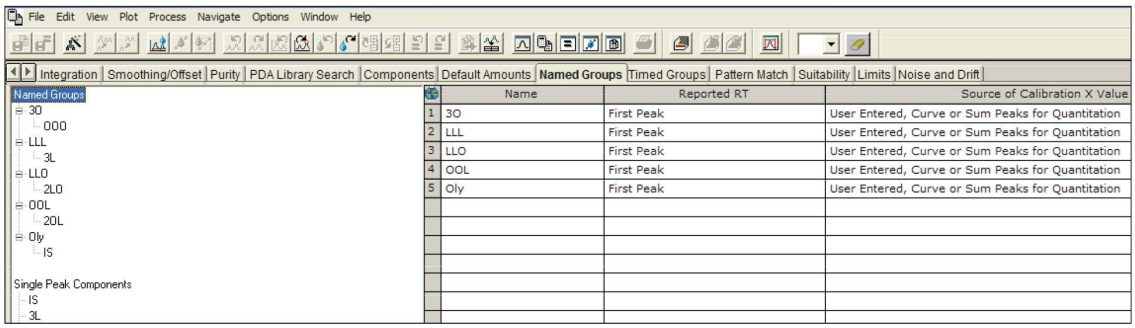


Figure 9. Named Groups window.

Note: A custom-made report template can be created to display the selected indicators only.

Steps to Create a Template for a Pass or Fail Report

1. Click Methods tab, select a report, right click on it; and choose Open to display the Report Method Editing window.
2. Select New from the Report Method Editing window to open the New Method/Group window.
3. Select Create a New Report Method, and check Use Report Method/Group Wizard; then click OK to open the Report Method template Wizard.
4. Select Individual Report, then click Next to open the New Method Wizard window.
5. .Select Individual, then click Finish to display a report method template.
6. Right click on the chromatogram and select Properties, to open the Chromatogram Properties window (Figure 10).
7. Select Peak Labels tab and check Use Peak Label Only, then click OK.
8. Right click on Table and select Properties to open the Table Properties window.
9. Select Peaks tab and check Group Peaks.
10. Click Table tab and then click Peaks in the tree. Double-click each Indicator to add the custom field to the Result table, as shown in Figure 11.

11. Click OK, name the report template (For example, “Virgin Olive Oil QC Report” is the name shown here), click Save in the toolbar.

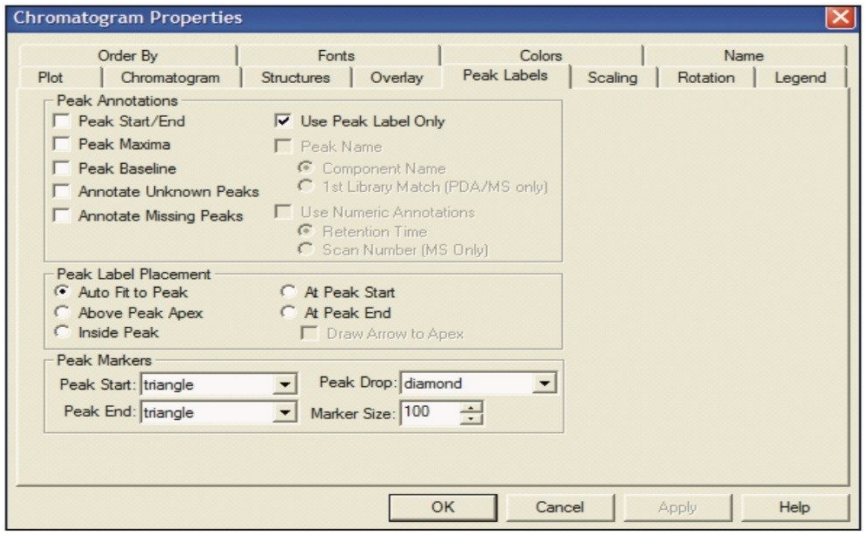


Figure 10. Chromatogram Properties window.

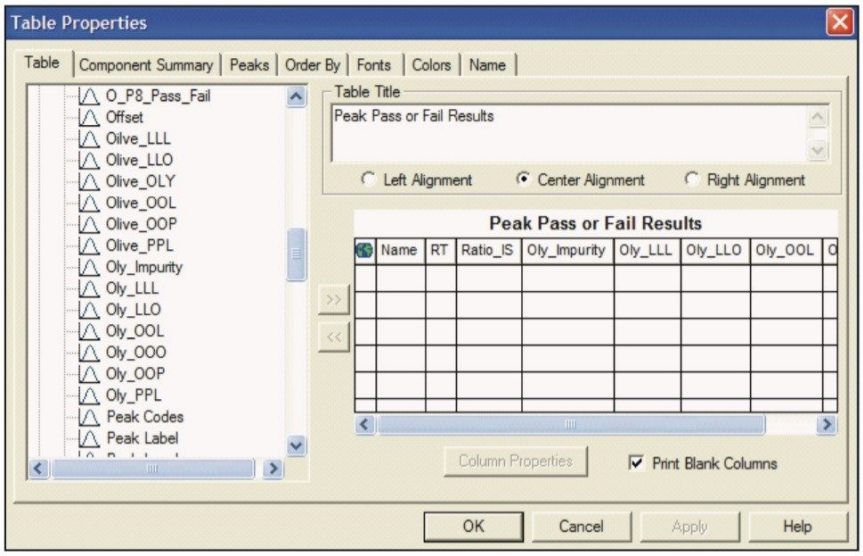


Figure 11. Table Properties window.

Results and Discussion

It is difficult to separate triglycerides, the major components of seed oils, using conventional HPLC methods without halogenated solvents. Figure 12 shows a typical ELS chromatogram of soybean oil obtained using a conventional HPLC with two 150-mm columns packed with 5-µm C_{18} particles. The separation was achieved in over 60 min using acetonitrile and methylene chloride as mobile phases. Since methylene chloride has a UV absorption up to 240 nm, which interferes with UV detection of triglycerides (λ max at approximately 210 nm), an evaporative light scattering detector (ELSD) was used for detection.

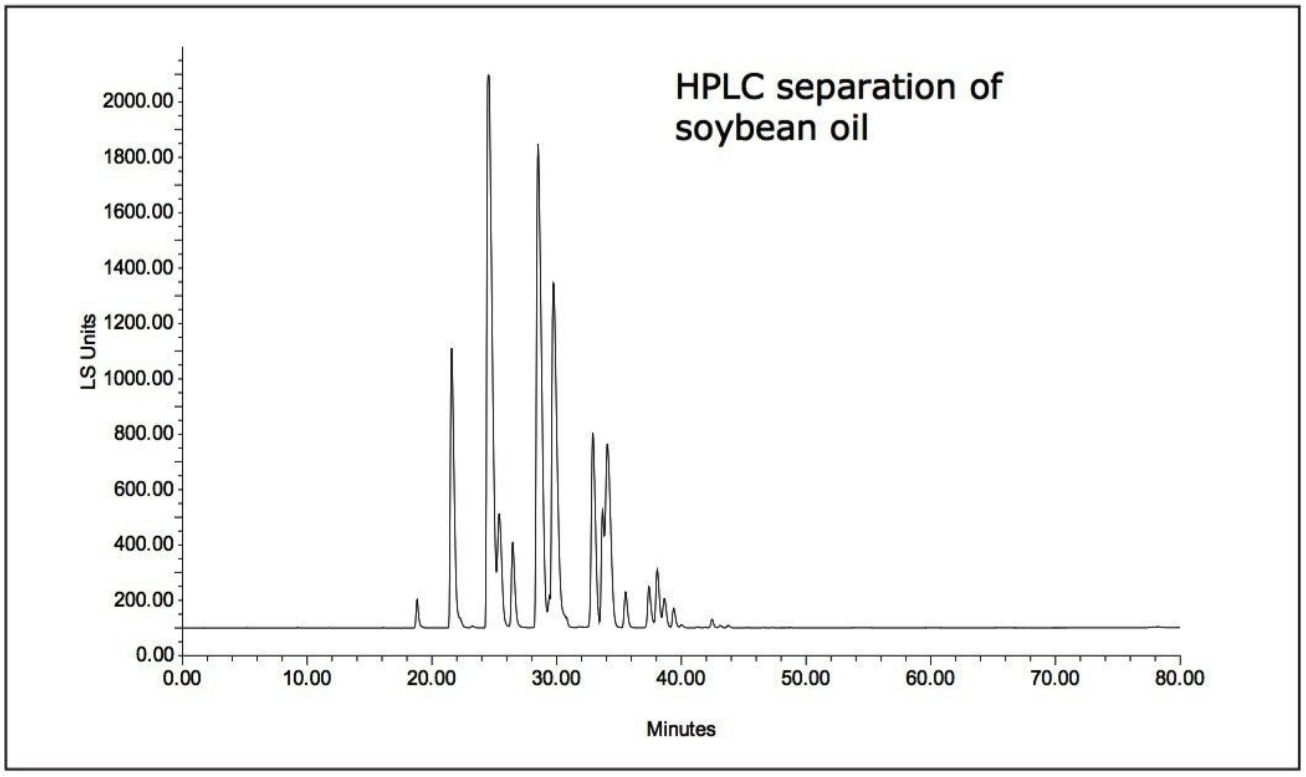


Figure 12. ELS chromatogram of a soybean oil sample: Alliance 2695 HPLC with two 3.9 x 15 cm Symmetry C_{18} Columns, Eluent A (acetonitrile), Eluent B (methylene chloride), 30 °C, flow rate at 0.57 mL/min; gradient from 20% B to 60% B in 72 min (curve 6), at 72.1 min; go to 100% B (curve 11) and hold for 10 min; then equilibrate the column with 20% B for 40 min.

The ACQUITY UPLC System design includes running high-efficiency columns packed with small particles to

perform faster, more sensitive, well-resolved separations. The UPLC solvent delivery system can sustain back pressures up to 15,000 psi enabling the use of high viscosity solvents such as 2-propanol for seed oil analysis. Since 2-propanol is good for dissolving seed oil,[12] is low in toxicity, and allows for UV detection of triglycerides due to its low limit of transparency, 2-propanol was chosen as the strong eluent. Figure 13 shows ten overlay UV chromatograms of a soybean oil sample to illustrate the reproducibility of the UPLC method. The separation was achieved in 22 minutes using a 2.1 x 150 mm UPLC Column packed with 1.7-µm BEH C_{18} particles, and acetonitrile/2-propanol as the mobile phase. In comparison, the chromatograms in Figures 12 and 13 have similar triglyceride peak patterns, but the UPLC chromatogram has higher resolution and shorter runtime. The data illustrate the advantage of using UPLC to separate seed oil components without carcinogenic solvents. The acetonitrile/2-propanol UPLC mobile phase for seed oil analysis is compatible with PDA, ELSD, and MS detectors—unlike other solvents used in conventional HPLC methods. Multiple data types can be obtained in a single injection to generate reproducible fingerprinting data,[7] identify triglyceride components by mass spectrometry,[10] and evaluate the degree of seed oil oxidation with multiple PDA wavelength channels.[8]

It is known that seed oils have characteristic ratios of triglycerides useful in fingerprinting for seed oil identification.[5–8] In Figures 14 to 16, UV chromatograms of walnut oil, grape seed oil, sesame seed oil, extra virgin olive oil A, extra virgin olive oil B, hazelnut oil, tea seed oil, corn oil, canola oil, high oleic sunflower oil, and regular sunflower oil all confirm that each oil sample has an unique chromatographic pattern, namely, relative peak intensity.

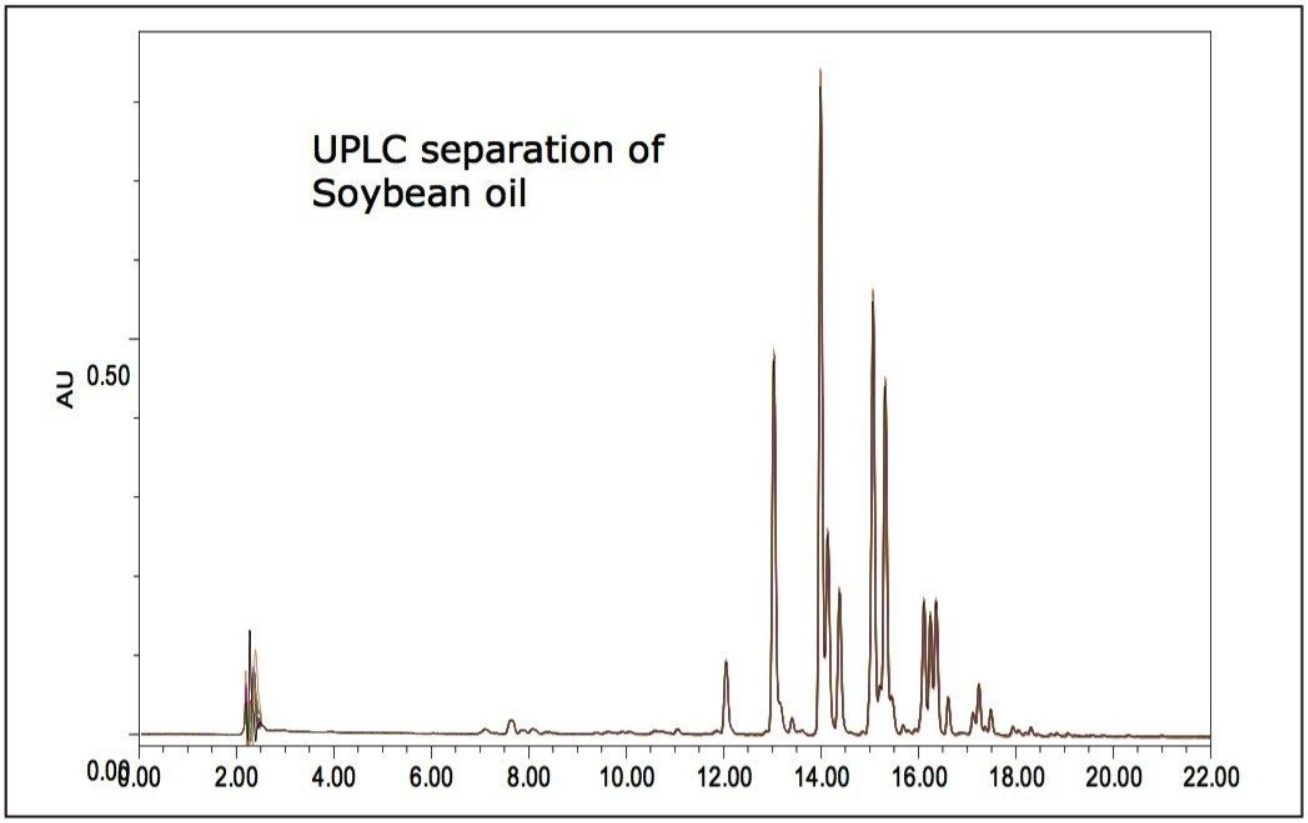


Figure 13. Overlay UV chromatograms (210 nm) of 10 replicate injections of a soybean oil sample (6 mg/mL). A blank injection of 2-propanol was run at the beginning of the sample set and used for PDA 3D blank subtraction.

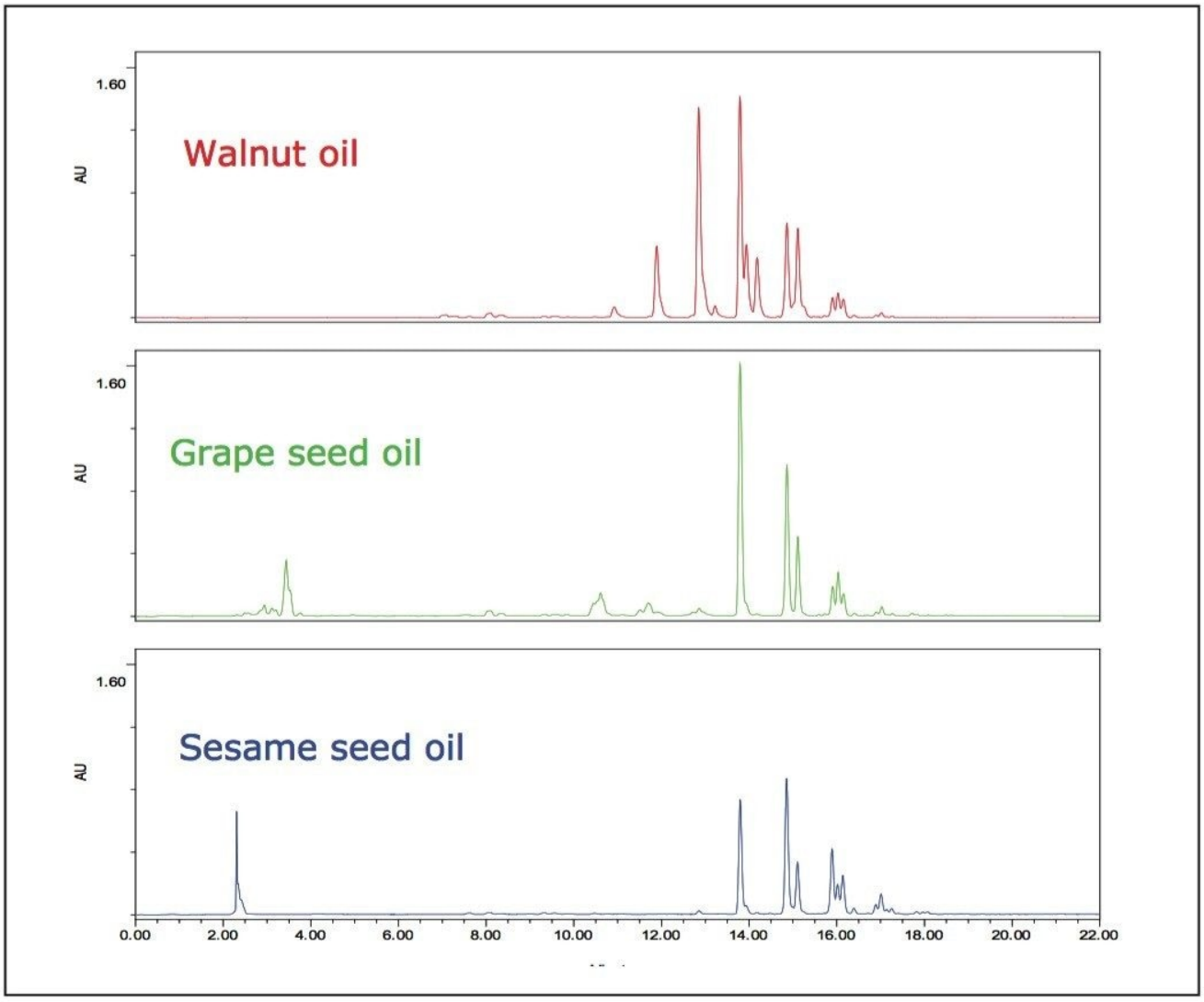


Figure 14. UV chromatograms (210 nm) of walnut oil, grape seed oil, and sesame seed oil (6 mg/mL).

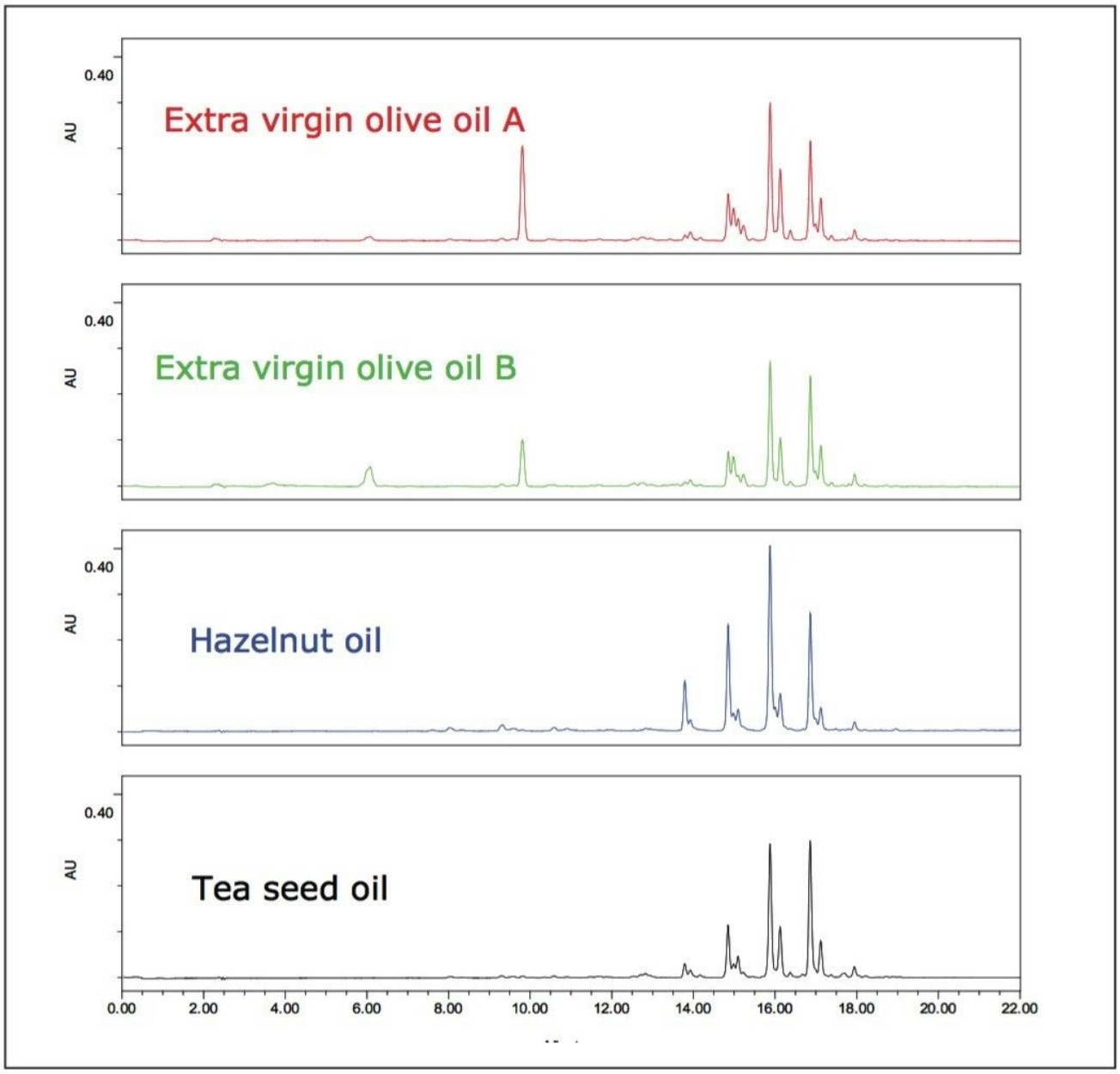


Figure 15. UV chromatograms (210 nm) of extra virgin olive oil A, extra virgin olive oil B, hazelnut oil, and tea seed oil (6 mg/mL).

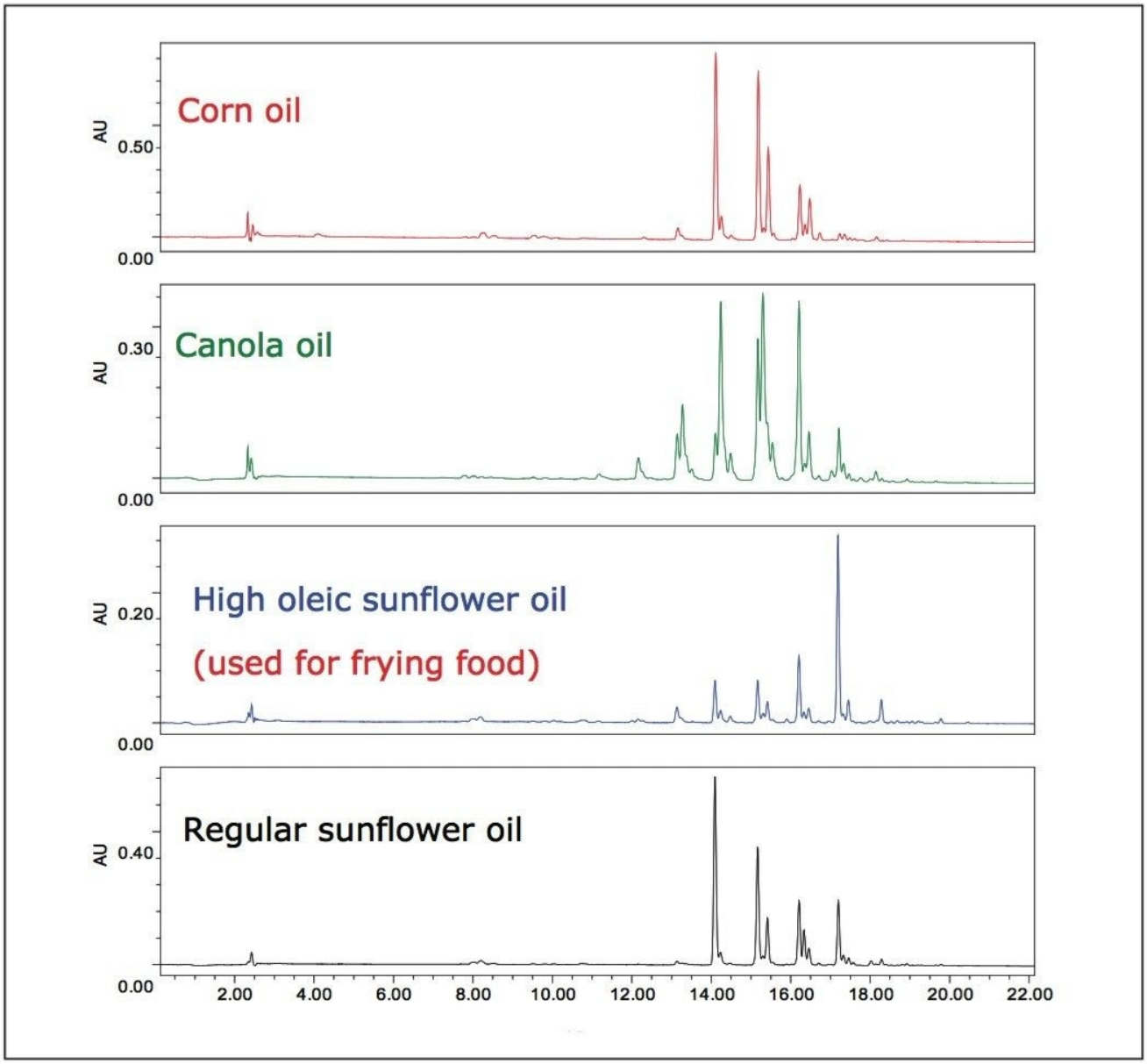
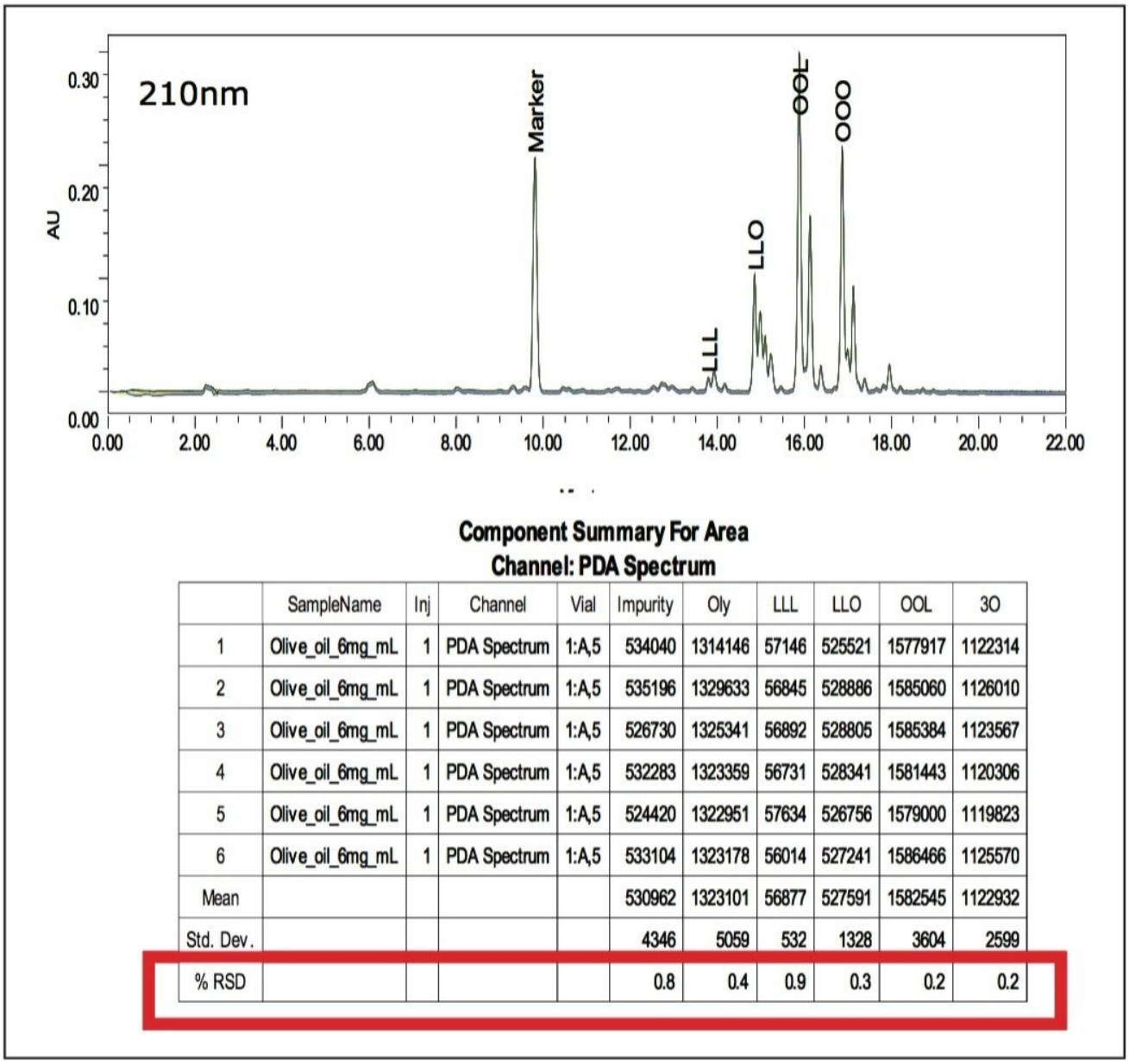


Figure 16. UV chromatograms (210 nm) of corn oil, canola oil, high oleic sunflower oil, and regular sunflower oil (6 mg/mL).



Component Summary For Area
Channel: PDA Spectrum

	SampleName	Inj	Channel	Vial	Impurity	Oly	LLL	LLO	OOL	3O
1	Olive_oil_6mg_mL	1	PDA Spectrum	1:A,5	534040	1314146	57146	525521	1577917	1122314
2	Olive_oil_6mg_mL	1	PDA Spectrum	1:A,5	535196	1329633	56845	528886	1585060	1126010
3	Olive_oil_6mg_mL	1	PDA Spectrum	1:A,5	526730	1325341	56892	528805	1585384	1123567
4	Olive_oil_6mg_mL	1	PDA Spectrum	1:A,5	532283	1323359	56731	528341	1581443	1120306
5	Olive_oil_6mg_mL	1	PDA Spectrum	1:A,5	524420	1322951	57634	526756	1579000	1119823
6	Olive_oil_6mg_mL	1	PDA Spectrum	1:A,5	533104	1323178	56014	527241	1586466	1125570
Mean					530962	1323101	56877	527591	1582545	1122932
Std. Dev.					4346	5059	532	1328	3604	2599
% RSD					0.8	0.4	0.9	0.3	0.2	0.2

Figure 17. Overlay UV chromatograms and peak areas of extra virgin olive oil A: OOO (trioleoylglycerol), OOL (dioleoyl-linoleoyl-glycerol), LLO (dilinoleoyl-oleoyl-glycerol), LLL (trilinoleoyl-glycerol), respectively; Marker = Oly, and OOO = 3O, Impurity= the sum of all the peaks except the marker peak having retention times between 3 and 13.6 min.

To effectively use the ratio of peak intensity for brand quality control and authentication, the custom field

calculation function of Empower 2 Software was utilized to automatically convert raw chromatographic data into a Pass or Fail report based on user-set QC criteria. Here, extra virgin olive oil A illustrates this streamlined method.

Figure 17 shows an overlay of UV chromatograms and peak areas of extra virgin olive oil A. The peak area RSD values (n=6) of triglyceride peaks from the strongest peak (OOL) to the weakest peak (LLL) are <0.9%. There are more than 20 observed peaks and any peak can be used as the marker or the indicator for calculating peak area ratio. For this discussion, previously identified triglyceride peaks OOO, OOL, LLO, and LLL were chosen as indicators,[10] and the strong peak with a retention time of 9.8 min observed only in olive oil products by UV detection was chosen as the marker peak.[13] Since most cheap vegetable oils and decomposed oils have many other strong peaks under 13.6 min,[9] the indicator Impurity was created using Timed Groups function (Figure 7) to monitor any occurrence of contamination. This Impurity indicator is defined as the sum of all the peaks except the marker peak having retention times between 3 and 13.6 min. By creating the custom field "Ratio_IS" (Figure 4), the peak area ratios (indicator peak area divided by the marker peak area) were automatically calculated with Empower 2 Software. Table 1 summarizes the peak area ratio results together with the STDEV values. The peak area ratio ±3xSTDEV is used as the QC criteria for each indicator. Variations for a particular type of oil exist owing to geography and other growing conditions. There is great value in comparing other seed oil samples against the QC criteria based on a particular oil.

	Impurity	LLL	LLO	OOL	30
Ratio	0.404	0.042	0.397	1.200	0.851
STDEV n=6	0.005	0.001	0.002	0.004	0.003
Ratio = indicator peak area divided by marker peak area STDEV = standard deviation					

Table 1. Indicator peak area ratios for extra virgin olive oil A.

Note: To set user criteria for any seed oil, it is important to first obtain multiple chromatograms and have the tabulated STDEV values for peak areas.

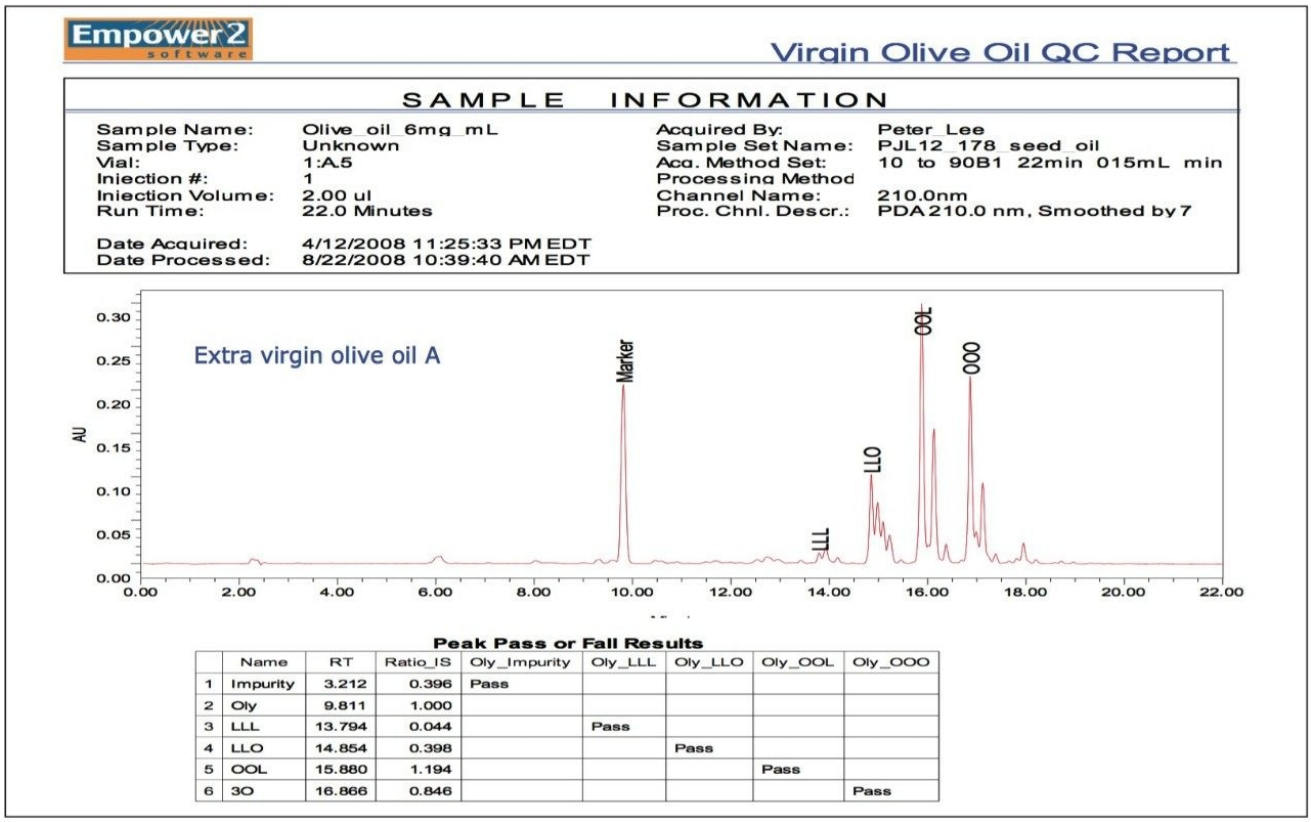


Figure 18. A QC report of extra virgin olive oil A.

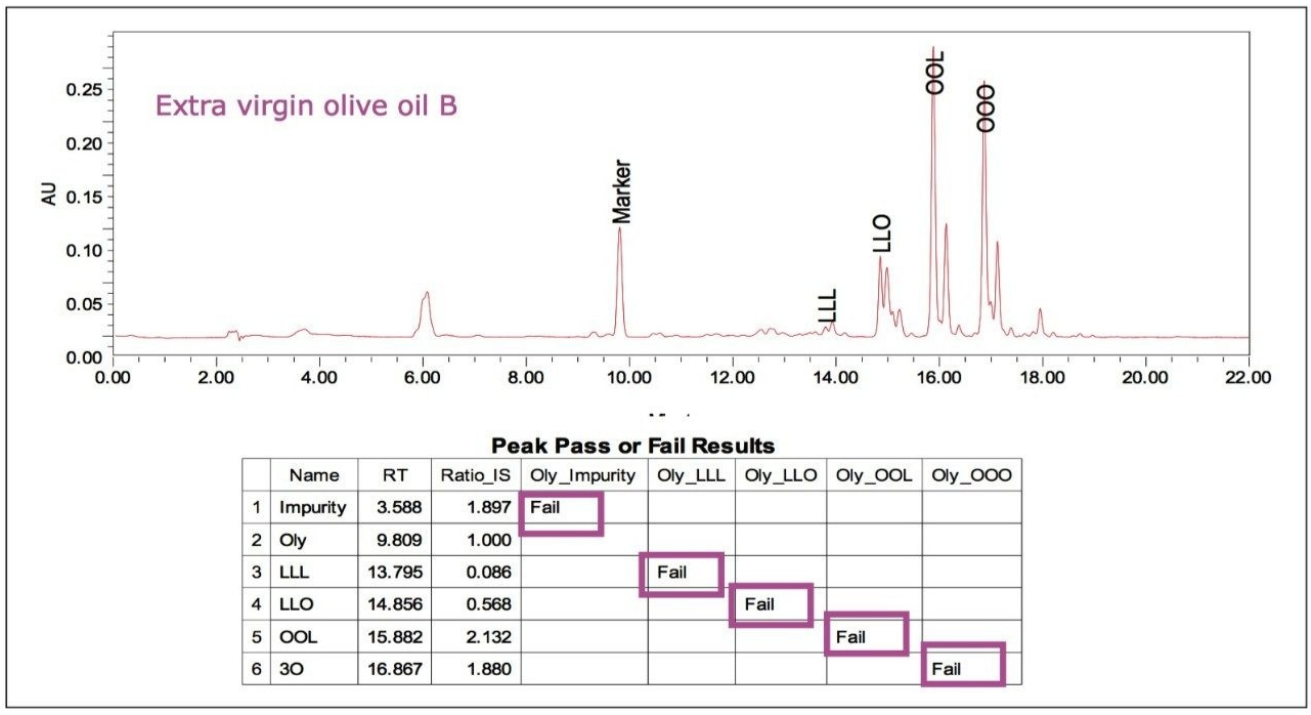
Note: Ratio_IS= peak area ratio; Oly_Impurity, Oly_LLL, Oly_LLO, Oly_OOL, and Oly_OOO are custom fields for examining if the indicator peak area ratio meets the QC criteria; Oly= Marker, 3O= OOO, RT= retention time.

Empower 2 Software can now use the Custom Field Calculation, Named Groups, Timed Groups, and Report Template, as shown in Figures 6, 7, 9, 10, and 11 to automatically calculate and report the Pass or Fail results of samples according to the QC criteria for extra virgin olive oil A. Figure 18 shows a typical Empower QC report for extra virgin olive oil A. The report shows that all the indicator peaks passed the QC criteria. These advanced functions of Empower Software eliminate the need for manual calculation, therefore, preventing potential human errors.

Expensive extra virgin olive oil is often adulterated with cheap olive oil and other seed oils such as soybean oil and hazelnut oil. Figure 19 shows a report of extra virgin olive oil B. All the indicators show that extra virgin olive oil B failed to pass the QC criteria established according to extra virgin olive oil A. Also, there are additional peaks with retention times of <13.6 min in the chromatogram. The data clearly illustrate the difference between

the two brands of olive oil sampled and confirmed that not all extra virgin olive oils on the market are the same.

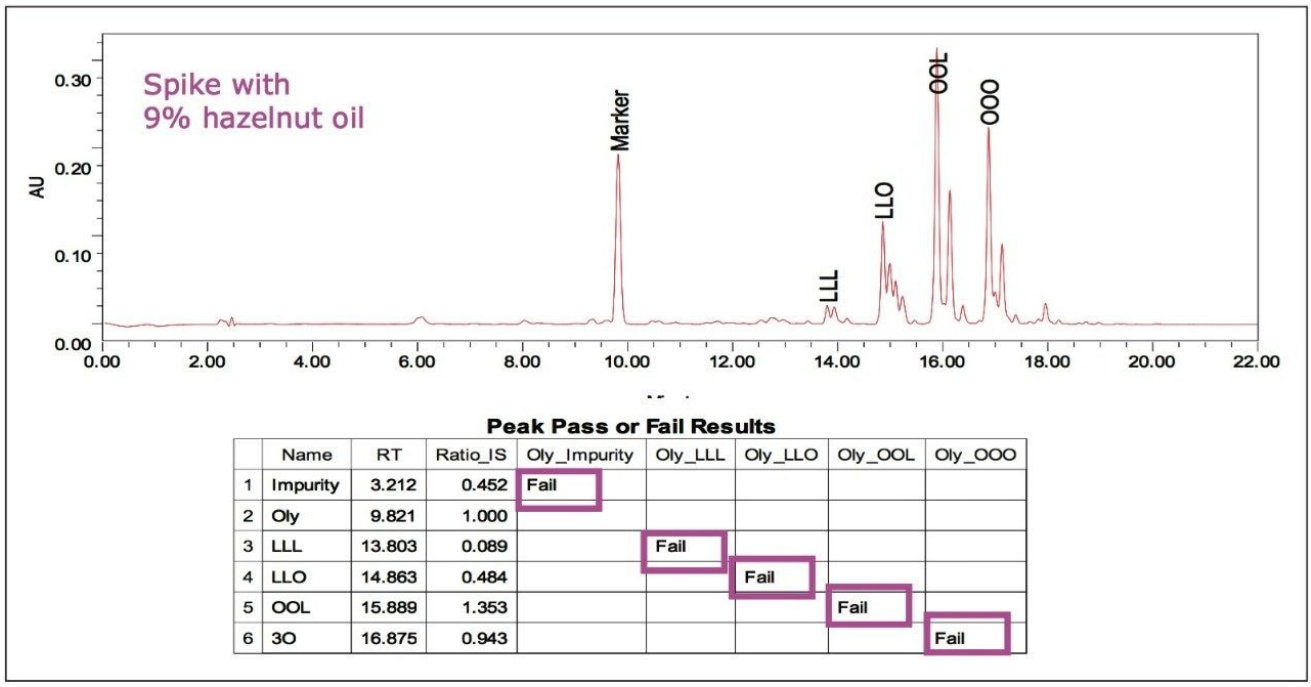
Figure 20 shows a report of extra virgin olive oil A falsified with 9% hazelnut oil. All of the indicators show that the falsified sample did not pass the QC criteria. Moreover, the same QC criteria established according to extra virgin olive oil A have been also applied in analyzing other seed oils (Figures 14 to 16), as well as extra virgin olive oil A samples falsified with 1% soybean oil, or 1% corn oil. None passed.



Peak Pass or Fail Results

	Name	RT	Ratio_IS	Oly_Impurity	Oly_LLL	Oly_LLO	Oly_OOL	Oly_OOO
1	Impurity	3.588	1.897	Fail				
2	Oly	9.809	1.000					
3	LLL	13.795	0.086		Fail			
4	LLO	14.856	0.568			Fail		
5	OOL	15.882	2.132				Fail	
6	3O	16.867	1.880					Fail

Figure 19. An analytical report of an extra virgin olive oil B.



Peak Pass or Fail Results

	Name	RT	Ratio_IS	Oly_Impurity	Oly_LLL	Oly_LLO	Oly_OOL	Oly_OOO
1	Impurity	3.212	0.452	Fail				
2	Oly	9.821	1.000					
3	LLL	13.803	0.089		Fail			
4	LLO	14.863	0.484			Fail		
5	OOL	15.889	1.353				Fail	
6	3O	16.875	0.943					Fail

Figure 20. An analytical report of an extra virgin olive oil A falsified with 9% hazelnut oil.

Previously, a chemometric method was described that utilizes UPLC-TOF with integrated software tools for detecting olive oil adulteration.[14] This Technical Note provides an alternative solution for seed oil quality control and authentication. Data are acquired and processed automatically throughout to generate unambiguous reports with Pass or Fail results.

Conclusion

The ACQUITY UPLC System with Empower 2 Software enables rapid analysis and authentication of seed oil samples without derivatization and halogenated solvents. The data illustrated great reproducibility, precision, accuracy, and simplicity of the UPLC System. The separation is three times faster than conventional HPLC methods, consumes eight times less solvents, and produces eight times less hazardous waste, resulting in cost and safety benefits. The ACQUITY PDA Detector generates data with high resolution and reproducibility, which enables easy establishment of fingerprinting data for setting QC and authentication criteria for each brand

of seed oil. With the custom field calculation function of Empower 2 Software, critical product QC information can be accurately extracted from raw data and rapidly delivered based on user-set criteria. The simple Pass or Fail report is very effective. Decision makers can use the critical information to make timely decisions, thus enhance productivity. Using this UPLC methodology, seed oil companies can certify the authenticity and quality of their products with great ease and confidence. Other industries such as cosmetics, personal care, and food companies, having vested interests in the purity of seed oil products, will also benefit from this methodology.

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13. The marker (Oly) peak at 9.8 min was well detected by UV but had weak MS response with APCI positive ionization mode. According to the SQD MS spectra, the marker peak is not a triglyceride. High resolution mass spectrometers with exact mass capabilities are needed in order to properly elucidate its chemical structure. However, it is not necessary to have peak identification for this QC and authentication methodology.

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